Antinuclear Antibodies

Guest Editor
Falk Hiepe
Thomas Dörner
Gerd-Rüdiger Burmester

KARGER

International Archives of
Allergy and
Immunology

Antinuclear Antibodies

Guest Editor
Falk Hiepe, Berlin
Thomas Dörner, Berlin
Gerd-Rüdiger Burmester, Berlin

20 figures, 4 in color, and 27 tables, 2000

KARGER

Basel · Freiburg · Paris · London · New York ·
New Delhi · Bangkok · Singapore · Tokyo · Sydney

S. Karger
Medical and Scientific Publishers
Basel · Freiburg · Paris · London
New York · New Delhi · Bangkok
Singapore · Tokyo · Sydney

Drug Dosage
The authors and the publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accord with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new and/or infrequently employed drug.

Printed in Switzerland on acid-free paper by
Reinhardt Druck, Basel
ISBN 3–8055–7156–9

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Vol. 123, No. 1, 2000

International Archives of Allergy and Immunology

Contents

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Access to full text and tables of contents, including tentative ones for forthcoming issues:
www.karger.com/journals/iaa/iaa_bk.htm

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:4

Editorial

The *Dictionary of Immunology* by J.M. Cruse and R.E. Lewis, CRC Press, 1996, defines Immunology as a 'branch of biomedical science concerned with the response of the organism to immunogenic (antigenic) challenge, the recognition of self from non-self and all the biological *(in vivo)*, serological *(in vitro)*, physical, and chemical aspects of immune phenomena'. In this connection Clinical Immunology may be regarded as the discipline analyzing clinical manifestations in which immunological processes play a part or may need to be assessed. Therefore, all immunologic reactions responsible for clinical hypersensitivity and disease as outlined by R.R.A. Coombs and P.G.H. Gell many years ago may be covered under one headline, Clinical Immunology. This includes diseases, where infections fail to be controlled (infectious diseases, immune deficiencies), overreactions to innocuous substances in the environment (allergic diseases), reactions in which the immune system attacks self (autoimmune diseases), transplant rejection, immune response against tumours as well as manipulation of the immune response.

As outlined in our first Editorial in January 1998, the main focus of *International Archives of Allergy and Immunology* will be on molecular and cellular aspects of allergology and immunology. We thus appreciate the opportunity to publish a full issue on Antinuclear Antibodies. These antibodies are found in patients with various connective tissue disorders and, due to the knowledge of their molecular targets, they can be used as important diagnostic markers for the differentiation of autoimmune diseases. In the future, we will continue to publish such immunological papers, which combine various disciplines and thus improve the general knowledge and acceptance of Immunology research.

Rudolf Valenta, MD, Managing Editor
Dietrich Kraft, MD, Editor-in-Chief

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0004$17.50/0

Accessible online at:
www.karger.com/journals/iaa

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:5–9

Editorial Overview: Antinuclear Antibody- and Extractable Nuclear Antigen-Related Diseases

Falk Hiepe Thomas Dörner Gerd-Rüdiger Burmester

Medizinische Klinik mit Schwerpunkt Rheumatologie und Klinische Immunologie, Universitätsklinikum Charité der Humboldt-Universität zu Berlin, Berlin, Deutschland

Key Words

Antinuclear antibodies · Extractable nuclear antigens · Systemic lupus erythematosus · Sjögren's syndrome · MCTD · Scleroderma · Myositis · Autoantibodies · Pathogenesis · Connective tissue disease · Rheumatology

Abstract

In 1948, the observation of the LE cell phenomenon in a patient with systemic lupus erythematosus (SLE) began the discovery of a broad variety of autoantibodies directed to nuclear antigens called antinuclear antibodies (ANA). Nowadays, different ANA serve as important diagnostic parameters for differentiating most of the connective tissue diseases, such as SLE, neonatal lupus syndromes, Sjögren's syndrome, scleroderma, autoimmune myositis, mixed connective tissue disease and other overlaps. This overview summarizes the history of ANA and their detection methods, in part to introduce the subsequent papers dealing with special topics of ANA-related diseases in this issue. Furthermore, the pathogenic role of these autoantibodies in targeting non-organ-specific intracellular antigens as a functional important constituent of a subcellular particle or multimolecular complex is addressed. Notably, some of these autoantibodies have functioned as significant tools for cell biologists to elucidate the subcellular structures and functions of these autoantigens. In the future, we can expect further advances to answer such important questions as why these antigens are targets of autoantibodies, what is their pathogenic impact and what are the triggers of autoimmunity?

In 1948, Malcom Hargraves, Helen Richmond and the medical resident Robert Morton from the haematology laboratory of the Mayo Clinic in Rochester noted the presence of previously unknown cells in the bone marrow of a patient with acute systemic lupus erythematosus (SLE). The cells, called LE cells, were described as mature neutrophilic polymorphonuclear leukocytes which had phagocytosed Feulgen-staining nuclear material [1]. These observations marked the beginning of the history of antinuclear antibodies (ANA) and a long-lasting period of a variety of remarkable discoveries in the field. Subsequently, Haserick and Bortz [2], in 1949, made the important observation that sera from SLE patients, when incubated with normal bone marrow, were able to induce the formation of LE cells [2]. The inducing factor, the so-called LE factor, was identified as being associated with the gammaglobulin fraction of the SLE serum [3] that was suspected to be an antibody. For the next 10 years, the detection of LE cells in the peripheral blood remained the most popular laboratory test for the diagnosis of SLE. In 1953, Peter Miescher observed that sera from rabbits immunized with cell nuclei were able to induce LE cell formation using normal human

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0005$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Prof. Dr. med. Falk Hiepe
Medizinische Klinik mit Schwerpunkt Rheumatologie und Klinische Immunologie
Universitätsklinikum Charité, Humboldt-Universität
Schumannstrasse 20/21, D–10117 Berlin (Germany)
Fax +49 30 2802 8082, E-Mail falk.hiepe@charite.de

leukocytes. One year later, he was able to demonstrate that absorption of SLE sera using cell nuclei isolated from calf thymus cells eliminated the ability of the serum to induce LE cell formation [4]. Based on these experiments, the LE factor could be confirmed as an ANA. Finally, these investigations resulted in the simultaneous report of antibodies to DNA in sera from patients with SLE by at least four different groups in 1957 [5–8].

In the following decade, several efforts were undertaken to improve detection methods and to simplify antibody test systems for routine diagnostics. In this regard, George Friou was the first who applied the immunofluorescence technique for the detection of ANA [9]. Subsequently, the identification of different immunofluorescence patterns of ANA led to the detection of different antibody specificities. After 1968, the indirect immunofluorescence test was more and more routinely used in the laboratory by using different substrates, such as tissue sections, desquamated cells, chicken erythrocytes and HeLa cells. HEp-2 cells were introduced for routine ANA screening in 1975.

The different immunofluorescence patterns of ANA imply that, besides DNA and histone proteins, other nuclear proteins are targeted by autoantibodies. Using salt-soluble nuclear extracts from calf thymus [called extractable nuclear antigens (ENA)], precipitating autoantibodies could be detected in the sera of patients with SLE and Sjögren's syndrome by means of immunodiffusion (Ouchterlony technique). Among these autoantibodies, anti-Sm antibodies of the serum of Stephanie Smith, a young SLE patient, could be described for the very first time [10]. Thereafter, these antibodies could be demonstrated as a highly specific marker for SLE which were considered in the classification criteria [11]. Furthermore, antibodies to the ribonucleoprotein (RNP) antigen (originally called anti-Mo after the prototype serum) were identified in the sera of patients with SLE by Mattioli and Reichlin [12] in 1971 by immunodiffusion. The term RNP stems from the early observation that its antigenic activity could be destroyed by treatment with ribonuclease and trypsin, whereas the Sm antigen was resistant to such a digestive treatment. During the same period, Sharp et al. [13, 14] described a group of patients with a syndrome characterized by features of SLE, myositis and scleroderma, which they named mixed connective tissue disease (MCTD). The sera of these patients contained antibodies to ENA, as measured by passive haemagglutination. Subsequent studies showed that ENA reacted with RNP. Anti-RNP antibodies were found in 25–30% of patients with SLE, typically in association with anti-Sm antibodies. Isolated detection of these autoantibodies in high titres represents a good serologic parameter for MCTD.

Moreover, precipitating autoantibodies called anti-SJD and anti-SJT were described in the sera of patients with Sjögren's syndrome in 1961 [15]. Two precipitin reactions in sera from patients with SLE were designated Ro and La, based on the names of the patients in whom they were first identified [16]. In 1975, both antibodies were described again in the sera of patients with Sjögren's syndrome as designated anti-SS-A and anti-SS-B after detection by immunodiffusion using Wil2 extract [17]. The antigenic identity of Ro and SS-A, as well as of La and SS-B, was demonstrated in an interlaboratory comparison [18]. Although never confirmed by serum exchange, SJD is assumed to be identical to Ro/SS-A and SJT to La/SS-B.

In the last decades, the field of autoantibodies has developed extensively to include other diseases besides SLE and Sjögren's syndrome. It is now abundantly clear that there are multiple autoantibodies of different specificities present in several autoimmune diseases. Most systemic autoimmune diseases have a highly characteristic profile of autoantibodies to cellular antigens, both nuclear and cytoplasmic. These autoantibody profiles have been extremely useful tools for diagnostic purposes. New clinical subsets of autoimmune diseases, such as MCTD, anti-Jo-1 syndrome and certain overlaps of scleroderma/polymyositis, have been characterized on the basis of a reliable link to autoantibody specificities. On the other hand, the molecular structure and biological function of a majority of these autoantigens have been identified by using autoantibodies from patients' sera as tools. The clinical importance of antibodies, as well as the identified biological functions of the respective autoantigens of important autoantigen-autoantibody systems, are summarized in table 1. Synthesis of available data suggests that the autoimmune response is (auto)antigen driven and that the antigen is a subcellular particle or a multimolecular complex involved in important and, in part, essential cellular functions. The autoantigen appears to be presented as immunogen in its activated form, because their functional active sites are very frequently targeted by autoantibodies [19–21].

One of the central issues in the field of autoantibodies relates to the pathogenic relevance of these autoimmune phenomena. Formerly, it seemed to be almost impossible that most of the autoantibodies described are directly involved in tissue injury in different connective tissue diseases, with the exception of anti-dsDNA antibodies. The pathogenic importance of anti-dsDNA antibodies has been repeatedly demonstrated in lupus nephritis. In re-

Table 1. Characterization of autoantigen-autoantibody systems in systemic autoimmune diseases

Autoantibody to	Characterization of antigen	Biological function	Disease association
dsDNA	double-stranded, native DNA	genetic code	SLE
Histones	H1, H2A, H2B, H3, H4, H5, [H2A-H2B]-DNA dimer	organization of nucleosomes	drug-induced lupus, SLE, rheumatoid arthritis
Sm	core proteins B (28), B′ (29), D (16), E (13), F and G of U1, U2, U4, U5 and U6 snRNPs	splicing of pre-mRNA	SLE
U1-nRNP	proteins 70 kD, A (33) and C (22) of U1-snRNP	splicing of pre-mRNA	SLE, MCTD
RA33	protein A1 (34 kD) of hnRNP	splicing of pre-mRNA	rheumatoid arthritis, MCTD, SLE
Ro/SS-A	52-kD and 60-kD RNP containing small uridine-rich nucleic acids (hY1, hY3, hY4, hY5)	DNA-binding protein (52-kD Ro); quality control for 5S rRNA production/ involvement in translation of ribosomal protein mRNA (60-kD Ro)	Sjögren's syndrome, neonatal lupus. subacute cutaneous lupus, SLE
La/SS-B	phosphoprotein (48 kD) associated with a variety of small RNAs (precursors of cellular 5S RNA and tRNA, 7S RNA, viral RNAs, Ro/SS-A cytoplasmic hY RNAs)	probably transcription termination factor of RNA polymerase III	Sjögren's syndrome, neonatal lupus, subacute cutaneous lupus, SLE
PCNA	cyclin (36 kD)	auxiliary protein of DNA-polymerase δ	SLE
Ribosomal RNP	phosphoproteins P0 (15 kD), P1 (16 kD) and P2 (38 kD)	active in elongation step of protein synthesis	SLE
Scl-70	DNA topoisomerase I (100 kD)	unwinding of DNA	scleroderma (diffuse form)
Centromere	major centromere proteins A (15–16 kD), B (80 kD) and C (120 kD)	coordinated segregation of chromosomes to dividing cells	limited scleroderma (CREST)
PM-Scl	complex of 11–16 proteins ranging from 20 to 110 kD	involved in ribosome biogenesis	scleroderma, polymyositis/ scleroderma overlap
Fibrillarin	protein (34 kD) of the U3-RNP particle	role in rRNA processing and ribosome assembly	scleroderma
RNA-pol I	RNA polymerase I	transcripts rRNA precursors	scleroderma
RNA-pol II	RNA polymerase II	transcripts mRNA (hnRNA)	scleroderma
RNA-pol III	RNA polymerase III	transcripts 5S rRNA, tRNA and other small RNAs	scleroderma
Ku	heterodimer consisting of 70- and 80- to 86-kD protein subunits, DNA binding component of a 350-kD catalytic subunit with DNA-dependent kinase activity	repairs dsDNA breaks, V(D)J recombination	SLE, polymyositis/scleroderma overlap
Jo-1	histidyl-tRNA synthetase (52 kD)	catalyzes the esterification of histidine to its cognate tRNA	polymyositis (anti-Jo-1 syndrome)
Mi-2	240-kD protein (helicase/ATPase domain containing protein as part of NuRD complex)	remodelling of nucleosomes	dermatomyositis
SRP (signal recognition particle)	cytoplasmic RNP complex composed of 6 polypeptides and a tRNA-like molecule (7SL RNA)	protein translocation from the ribosome to the endoplasmic reticulum	polymyositis
NuMA (nuclear mitotic apparatus)	nuclear protein (centrophilin, 200–240 kD) associated with mitotic apparatus during mitosis	organization of chromatine architecture and role in spindle function	Sjögren's syndrome
Proteasome	cytoplasmic and nuclear-localized proteinase complex (20S), arranged in a cylindrical structure of 4 stacked rings, each composed of 7 subunits (α-type subunits form the outer rings, β-type subunits form the inner rings carrying the proteolytic sites)	involved in the ubiquitin-dependent selective degradation of short-lived and abnormal proteins; processing of antigens presented by MHC class I molecules	SLE, Sjögren's syndrome, myositis
Phospholipids	negatively charged phospholipids (e.g. cardiolipin, phosphatidylserine), phospholipid binding proteins (β2 glycoprotein I)	role in coagulation	antiphospholipid syndrome, SLE

cent years, there is increasing evidence that some ANA may be pathogenic. Besides the possibility of autoantibodies entering living cells and altering subcellular functions, which is controversial, nuclear autoantigens can appear on the cell surface. For example, apoptosis leads to apoptotic blebs on the cell surface containing several nuclear autoantigens which are enzymatically cleaved or not [22]. Moreover, other mechanisms, such as signalling pathways during immune activation, could also result in the transfer of autoantigens on the cell surface. This sur-

face expression of autoantigens might be again a trigger or further accelerator of autoantibody production. Alternatively, this accessible autoantigen might be able to induce several effector pathways after autoimmunity has been established.

The indirect immunofluorescence test using monolayers of cultured HEp-2 cells, a human laryngeal carcinoma cell line, is still recommended as the ANA screening test. Nowadays, further differentiation of ANA is performed routinely be enzyme immunoassays (EIA) using affinity-purified or recombinant antigens. A critical evaluation of EIA of different manufacturers for the detection of ANA of defined specificities demonstrated that these antibody detection systems need further improvement for certain antigen-antibody systems, especially anti-dsDNA and anti-Sm [23]. To ensure quality standards of antibody detection systems, CDC reference sera have been widely used as standards for the developement of tests and for their routine use. Additional methods for the detection of autoantibodies of patients' sera (to characterize autoimmune sera in more detail) are immunoblotting and immunoprecipitation, which have been proven to be useful as confirmation tests of EIA.

In this issue, an overview of different ANA and related diseases, as well as their role in the pathogenesis of these diseases is depicted. The current cell biological knowledge on the structure and function of subnuclear compartments as targets of ANA is summarized by Hemmerich and von Mikecz [24]. The authors emphasize that new techniques, such as confocal laser scanning microscopy, fluorescence resonance energy transfer and in vivo observation of cellular events, provide new possibilities to study targets of ANA specificities with respect to subnuclear architecture and function. Sherer and Shoenfeld [25] in their paper review the induction of autoimmune diseases via idiotype manipulation, the idiotypes of some ANA-associated antibodies (anti-DNA, anti-Ro/SS-A, anti-La/SS-B, anti-Sm), the pathogenic role of antibodies carrying idiotypes, and the clinical implications of the idiotypic network in autoimmunity. The role of autoantibodies, especially of anti-dsDNA antibodies and antinucleosome antibodies as well as immune complexes in the pathogenesis of SLE, is addressed by the article of Herrmann et al. [26]. They postulate that increased amounts and abnormal presentation of autoantigens favoured by clearance defects of apoptotic material contribute to the initiation of an autoimmune process. The importance of anti-Ro/SS-A and anti-LA/SS-B antibodies in Sjögren's syndrome is discussed in detail in the contribution from Mavragani et al. [27]. These autoantibodies are also of significant importance in the diagnosis of neonatal lupus syndromes. Additionally, these syndromes reveal that the diaplacental transfer of autoantibodies directed to nuclear antigens from the mother's circulation into the fetus is able to induce inflammatory processes, e.g. at the fetal conduction system of the heart or at the skin [28]. It is well known that ANA and related diseases can be induced by several drugs and exposure to chemical agents. Exposure to high levels of silica dust has been linked to increased risk of several autoimmune diseases, including systemic sclerosis. In a large cohort comprising 1,891 uranium miners, Conrad and Mehlhorn [29] report 390 individuals exhibiting symptoms of a connective tissue disease, including 18 patients with definite SLE and 12 patients with probable systemic sclerosis.

A subgroup of patients with SLE producing antiphospholipid antibodies manifests characteristic clinical features which can include stroke, venous thrombosis, recurrent abortion and thrombocytopenia. This was first described as antiphospholipid syndrome by Harris et al. [30]. Antiphospholipid antibodies do not belong to ANA. However, because of the increasing role of these autoantibodies in SLE and other ANA-related diseases, the current knowledge about this clinical entity, with an emphasis on clinical features and serologic tests in primary and secondary antiphospholipid syndrome, has been covered by Gromnica-Ihle and Schössler [31]. Most notably, recent studies indicate that β2-glycoprotein I is required for the binding of antiphospholipid antibodies. Another article in this issue is not directly related to classical ANA and anti-ENA. Thus, Feist [32] highlights the structure and function of proteasomes as a target of autoantibodies recently detected in several rheumatic autoimmune diseases, particularly in SLE, Sjögren's syndrome and myositis. Although these autoantibodies represent a different class of autoantibodies, they react with the proteasome complex involved in the decision of the presentation of 'self' or 'non-self' peptide that is currently only poorly understood [32].

It is hypothesized that abnormalities in the generation of the autoantibody repertoire, the processes of gene recombination, receptor editing, somatic hypermutation and/or selective influences may play a role in autoimmune disease. New approaches to the analysis of variable region genes from unstimulated individual human B cells employing single-cell polymerase chain reaction have provided new insights into the B cell repertoire of both normals and patients with systemic autoimmune diseases, as reviewed in this issue by Hansen et al. [33]. However, it remains to be elucidated whether the IgV gene usage and

the mutational pattern of the same donor at different time points of the disease, in different immune compartments as well as in particular B cell subsets, will provide new clues to understanding the development of B cells under autoimmune conditions.

During the last five decades, starting with the discovery of the LE cell phenomenon, our increasing knowledge about the diagnostic and pathogenic role of ANA and other autoantibodies has been extended enormously and is partially reflected in this issue. Moreover, several autoantibodies have proven to be important tools for cell biologists to study subcellular structures and functions. In the future, we can expect further advances to answer such important questions as why these subcellular structures are targets of autoantibodies, what are the true triggers of autoimmunity and what is their pathogenic impact? Based on clear answers to these questions, we could expect the development of specific therapies targeting autoantibody-producing cells or their precursors, including a potential specific suppression of autoantibody production by the induction of tolerance.

References

1 Hargraves MM, Richmond H, Morton RJ: Presentation of two bone marrow elements: The 'tart' cell and the 'L.E.' cell. Proc Mayo Clin 1948;23:25–28.
2 Haserick JR, Bortz DW: Normal bone marrow inclusion phenomena induced by lupus erythematosus plasma. J Invest Dermatol 1949;13: 47–49.
3 Haserick JR, Lewis LA, Bortz DW: Blood factor in acute disseminated lupus erythematosus. I. Determination of γ-globulin as specific plasma fraction. Am J Med Sci 1950;210:660–663.
4 Miescher P, Fouconnet M: L'absorption du facteur 'LE' par des noyaux cellulaires isolés. Experimentia 1954;10:252–254.
5 Holman HR, Kunkel HG: Affinity between the LE factor and cell nucleic acid nucleoprotein. Science 1957;126:162–163.
6 Miescher P, Straessle R: New serological methods for the detection of the LE factor. Vox Sang 1957;2:283–287.
7 Ceppellini R, Polli E, Celada FA: DNA-reacting factor in serum of a patient with lupus erythematosus diffuses. Proc Soc Exp Biol 1957; 96:572–574.
8 Seligmann M: Mise en evidence dans le serum de malades atteints de lupus erythemateux dissemine diune substance determinant une reaction de precipitation avec liacide desoxyribonucleique. CR Acad Sci (Paris) 1957;245:243–245.
9 Friou CJ: Clinical application of lupus serum nucleoprotein reaction using fluorescent antibody technique. J Clin Invest 1957;36:890–897.
10 Tan EM, Kunkel HG: Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J Immunol 1966;96:464–471.
11 Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ: The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–1277.
12 Mattioli M, Reichlin M: Characterization of a soluble nuclear ribonucleoprotein antigen reactive with SLE sera. J Immunol 1971;107:1281–1290.
13 Sharp GC, Irvin WS, LaRoque RL, Velez C, Daly V, Kaiser AD, Holman HR: Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. J Clin Invest 1971; 50:350–359.
14 Sharp GC, Irvin WS, Tan EM, Gould RG, Holman HR: Mixed connective tissue disease – an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). Am J Med 1972;52:148–159.
15 Anderson JR, Gray KG, Beck JS, Kinnear WF: Precipitating autoantibodies in Sjögren's disease. Lancet 1961;ii:456–460.
16 Clark G, Reichlin M, Tomasi TBJ: Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythematosus. J Immunol 1969;102:117–122.
17 Alspaugh MA, Tan EM: Antibodies to cellular antigens in Sjogren's syndrome. J Clin Invest 1975;55:1067–1073.
18 Alspaugh M, Maddison P: Resolution of the identity of certain antigen-antibody systems in systemic lupus erythematosus and Sjogren's syndrome: An interlaboratory collaboration. Arthritis Rheum 1979;22:796–798.
19 Peter JB, Shoenfeld Y: Autoantibodies. Amsterdam, Elsevier, 1996.
20 van Venrooij WJ, Maini RN: Manual of Biological Markers of Disease. Dordrecht, Kluwer, 1999.
21 von Mühlen C, Tan EM: Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum 1995;24:323–358.
22 Rosen A, Casciola-Rosen L: Autoantigens as substrates for apoptotic proteases: Implications for the pathogenesis of systemic autoimmune disease. Cell Death Differ 1999;6:6–12.
23 Tan EM, Smolen JS, McDougal JS, Butcher BT, Conn D, Dawkins R, Fritzler MJ, Gordon T, Hardin JA, Kalden JR, Lahita RG, Maini RN, Rothfield NF, Smeenk R, Takasaki Y, van Venrooij WJ, Wiik A, Wilson M, Koziol JA: A critical evaluation of enzyme immunoassays for detection of antinuclear autoantibodies of defined specificities. I. Precision, sensitivity, and specificity. Arthritis Rheum 1999;42:455–464.
24 Hemmerich P, van Mikecz A: Antinuclear autoantibodies (ANA): Fluorescent highlights on structure and function in the nucleus. Int Arch Allergy Immunol 2000;123:16–27.
25 Sherer Y, Schoenfeld Y: The idiotypic network in ANA-associated diseases. Int Arch Allergy Immunol 2000;123:10–15.
26 Herrmann M, Winkler T, Gaipl U, Lorenz HM, Geiler T, Kalden JR: Etiopathogenesis of systemic lupus erythematosus. Int Arch Allergy Immunol 2000;123:28–35.
27 Mavragani CP, Tzioufas AG, Moutsopoulos HM: Sjögren's syndrome: Autoantibodies to cellular antigens. Clinical and molecular aspects. Int Arch Allergy Immunol 2000;123:46–57.
28 Dörner T, Feist E, Pruss A, Chaoui R, Göldner B, Hiepe F: Significance of autoantibodies in neonatal lupus erythematosus. Int Arch Allergy Immunol 2000;123:58–66.
29 Conrad K, Mehlhorn J: Diagnostic and prognostic relevance of autoantibodies in uranium miners. Int Arch Allergy Immunol 2000;123: 77–91.
30 Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young CG, Loizou S, Hughes GR: Anticardiolipin antibodies: Detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983;ii:1211–1214.
31 Gromnica-Ihle E, Schössler W: Antiphospholipid syndrome. Int Arch Allergy Immunol 2000; 123:67–76.
32 Feist E: Diagnostic importance of anti-proteasome antibodies. Int Arch Allergy Immunol 2000;123:92–97.
33 Hansen A, Dörner T, Lipsky PE: Use of immunoglobulin variable-region genes by normal subjects and patients with systemic lupus erythematosus. Int Arch Allergy Immunol 2000; 123:36–45.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:10–15

The Idiotypic Network in Antinuclear-Antibody-Associated Diseases

Yaniv Sherer Yehuda Shoenfeld

Department of Medicine 'B' and the Research Unit of Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Key Words

Antinuclear antibodies · Double-stranded DNA antibodies · Idiotypes · Idiotypic network · SS-A antibodies · SS-B antibodies · Sm antibodies · Systemic lupus erythematosus

Abstract

Antinuclear antibodies (ANA) entail a large group of autoantibodies (Abs) that bind certain nuclear antigens. The ANA test is a useful screening test for many autoimmune diseases and the presence of a specific binding pattern directs secondary testing for specific Abs associated with the suspected disease. Idiotypes (Ids) are the antigenic constitution of the variable region of an Ab, and they are recognized by anti-Ids Abs. The Id network is composed of interacting Abs in which the Id determinants of each Ab are complemented by those of another. It has a role in both physiologic and pathologic conditions. In this communication, we review the induction of autoimmune diseases via Id manipulation, the Ids of some ANA-associated Abs (DNA, SS-A, SS- B, Sm Abs), the pathogenic role of Abs carrying Ids, and the clinical implications of the Id network in autoimmunity.

This study was supported by the Freda and Leon Schaller grant for research in autoimmunity.

Introduction

Antinuclear antibodies (ANA) entail a large group of autoantibodies (Abs) that bind certain nuclear antigens. These are classified according to patterns observable by indirect immunofluorescence that can predict the proteins that bind the Ab, but since immunofluorescence patterns do not provide definite identification of Abs, secondary testing is necessary for identification of the specific autoantigen reactive with the Abs [1]. The ANA test is a useful screening test for many autoimmune diseases, especially for systemic lupus erythematosus (SLE). The presence of a specific binding pattern directs secondary testing for specific Abs associated with the suspected disease (table 1). Nonetheless, ANA can be found in healthy individuals as well, mainly in the speckled, homogeneous, and mixed homogeneous and speckled pattern [2]. As such, ANA is considered one of the natural Abs which exist in healthy individuals [3]. Additionally, ANA can be

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0010$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Yehuda Shoenfeld
Department of Medicine 'B'
Tel-Hashomer 52621 (Israel)
Tel. +972 3 530 2652, Fax +972 3 535 2855
E-Mail Shoenfel@post.tau.ac.il

Table 1. Association between ANA immunofluorescence pattern, autoantigen and autoimmune diseases [6]

Immunofluorescence pattern	Antigen	Disease
Cytoplasmic	Jo-1	PM + ILD (70)
	ribosomal P	SLE (10)
	M2	PBC (97), CREST (15)
Mitotic spindle apparatus	Numa, 250	SLE, SS (rare)
Homogeneous nuclear	dsDNA	SLE (60)
	histon	SLE (60), drug-induced SLE (95)
	topoisomerase-1	PSS (15–70)
Speckled nuclear	hnRNP	MCTD (100)
	U1 snRNP 70, 33, 22	MCTD (100), SLE (25)
	Sm snRNP core 29, 28, 16	SLE (20)
	Ki 66, 86	SLE (10)
	SS-A	SS (60), SLE (35)
	SS-B	SS (40), SLE (15)
	Cyclin	SLE (2)
	CENP 17, 80, 160	CREST (80), PBC (15)
Nuclear membrane	Gp 120	PM (rare)
Nucleolar	PM/Scl	PM/PSS (50), PM (8), PSS (3)
	Pol 1, 2, 3	PSS (2–43)
	fibrillarin	PSS (8)

Antibody frequency, expressed in percentages, is shown in parentheses. CREST = Calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly, teleangiectasia; ILD = interstitial lung disease; MCTD = mixed connective-tissue disease; PBC = primary biliary cirrhosis; PM = polymyositis; PSS = progressive systemic sclerosis; SLE = systemic lupus erythematosus; SS = Sjögren's syndrome.

found in certain other conditions, such as among elderly persons [4], in persons with chronic abscesses, tuberculosis, subacute bacterial endocarditis and malaria [5], and in patients treated with drugs such as procainamide, hydralazine and isoniazide [6]. In this communication, we review the idiotypic network in some of the Abs and diseases associated with ANA.

The Idiotypic Network in Autoimmunity

Idiotypes – General Considerations

Antibodies can be characterized by the antigens with which they bind, and by the isotypic variation of their constant regions. Nevertheless, the variable regions of the antibodies are immunogenic, and thus can be used to generate a set of Abs that recognize them. The antigenic constitution of the variable region of an antibody is known as its idiotype (Id), and it is recognized by anti-idiotypic antibodies [7]. The Ids may be composed of amino acid sequences located on either light or heavy chains alone, or in combination (conformational idiotypes). They can also be located within the antigen-binding hypervariable segments, or within the intervening framework sequences. Private Ids are those that react only with the immunizing immunoglobulin, and define Ids specific for the individual antibody clone. Conversely, Ids that are shared between separate antibody clones from different individuals are termed common or cross-reactive Ids [8]. These are believed to result either from inheritance of antibody genes among related individuals, or from preservation and sharing of certain germline genes by unrelated individuals within a species.

Induction of Autoimmune Diseases via Idiotypic Manipulation

In 1974, Jerne [9] presented his theory of the idiotypic network. Briefly, as all individuals possess thousands of Ids reflecting the infinite possibilities of foreign antigen structure, any antigenic stimulation leads to the produc-

Table 2. Anti-DNA idiotypes (partial list) [25]

Name of idiotype	Source of idiotype	Idiotypic site
31	monoclonal mouse	L lambda
16/6	polyclonal rabbit	VH(VH_{III}) (CDR1, CDR2)
MIV-7	polyclonal rabbit	VH1
18/2	polyclonal rabbit	light chain CDR3
21/28	rabbit antipeptide, monoclonal mouse	heavy chain
4.6.3	polyclonal rabbit	VL (VL1)
PR4	polyclonal rabbit	conformational heavy and light
BEG-2	polyclonal rabbit	light chain
SA1	polyclonal	VH1
8.12	monoclonal mouse	L
F4	monoclonal mouse	VH
AM	polyclonal rabbit	conformational
TOF	polyclonal rabbit	close to DNA binding site
9G4	polyclonal rabbit	VH
B3	polyclonal rabbit	lambda chain

tion of Abs carrying Ids (Ab1), anti-Ids (Ab2) and anti-anti-Ids (Ab3) as a network of interacting Abs in which the Id determinants of each Ab are complemented by those of another. Whereas the idiotypic network plays a crucial physiologic role in regulating the immune response to nonself antigens and in preventing the development of pathogenic Abs, it also can be manipulated either naturally or by in vivo experiments that lead to the development of autoimmune diseases [10].

Models of induction of autoimmune diseases in animals via idiotypic manipulation share common principles [11]: immunization of naive mice with a specific Ab (Ab1) leads to the generation of anti-Ab (e.g. anti-Id = Ab2) directed against the Id on the immunizing Ab. A follow-up of the mice for a longer period reveals the de novo generation of anti-anti-Abs (Ab3) by the mice, which may simulate the original Ab in its binding characteristics. The phenomenon of naive mice producing specific Abs is associated with the emergence of the full-blown serological, immunohistochemical, and clinical manifestations of the respective disease. Examples for such models include induction of SLE, antiphospholipid syndrome, vasculitis, Goodpasture's syndrome, thyroiditis, and even atherosclerosis, in which the immune system has an important role in both prevention and acceleration [12–24]. A representative example would be the induction of SLE: immunization of mice with monoclonal or polyclonal human or murine anti-DNA antibodies in an adjuvant (active induction) led to the appearance of SLE in the mice with characteristic Abs (anti-DNA, SS-A, histones, Sm) and clinical presentations (proteinuria, alopecia, increased erythrocyte sedimentation rate, paralysis, immune complexes in kidneys, short survival time).

Idiotypes in ANA-Associated Abs

A discussion about the large number of Abs associated with positive staining of ANA (table 1) is beyond the scope of this paper. Specific Abs, on the other hand, are discussed in detail.

Anti-DNA Abs

Over 30 Ids of anti-DNA Abs have been described [reviewed in ref. 25]. Most of them were described on human hybridoma-derived monoclonal Abs from the peripheral blood lymphocytes of lupus or leprosy patients, while some were identified on monoclonal anti-DNA Abs derived from normal individuals. There are two general classes of anti-DNA Abs: germline gene segments encode one group, while the other is encoded by genes that have undergone mutations [26, 27]. Some of these Ids are presented in table 2.

Anti-SS-A and Anti-SS-B Abs

The sera of precipitants of 13 individuals positive for anti-SS-A was used to prepare a heterologous rabbit anti-Id to polyclonal anti-SS-A [28]. The resulting anti-Id, anti-Ro1, was specific for anti-SS-A F(ab′)2 immunogen, but did not bind to human IgG. The anti-Id was blocked by anti-SS-A IgG and F(ab′)2 but not by normal human IgG. The location of the Id Ro1 was on the Ab heavy chain, in or close to the antigen binding site of anti-SS-A [29]. Moreover, 3 out of 12 additional anti-SS-A positive women showed varying degrees of reactivity with the anti-Id.

Similarly, the sera of 3 unrelated patients was used to first prepare rabbit anti-Id Abs against affinity-purified anti-SS-B Abs [30]. Each anti-Id recognized private Id expressed only on the immunizing anti-SS-B, located in the hypervariable regions either in or near the antigen binding site. The expression of private Ids on the Abs may reflect their respective restricted antigenic specificity, in contrast to the diversity of antigens that are recognized by anti-DNA Abs [31].

Idiotypic manipulation with anti-SS-A and anti-SS-B Abs failed to induce Ab3, which is mouse anti-anti-Id [32]. However, active immunization with mouse monoclonal anti-SS-B Abs generated from a 16/6 Id immunized mice, led to the induction of experimental SLE in the

mice [33]. It is possible that this Ab3 carried a parallel pathogenic Id to the 16/6 anti-DNA Id.

Anti-Sm Abs

As are anti-dsDNA Abs, anti-Sm Abs are specific for SLE, but are found only in 25–30% of lupus patients [34]. It is not surprising, therefore, that anti-dsDNA Abs in patients correlated closely with Abs to Sm A and D subunits [35], and many of the anti-dsDNA Abs cross-reacted with the Sm A and D subunits. Lupus anti-Sm Abs express interspecies cross-reactive Id: a monoclonal Ab called Y2 derived from MRL mice has activity against the Sm ribonucleoprotein; specific rabbit antiserum against the cross-reactive Id of Y2 was used to probe SLE sera for this Id. Consequently, 25 of 51 SLE patients seropositive for anti-Sm Abs had elevated levels of the Y2 Id compared to a normal control group [36]. The anti-Y2 serum also inhibited the ability of 12 of the 25 anti-Sm positive sera to bind Sm [37]. Moreover, 41% of SLE patients and 27% of their relatives showed increased serum levels of the Y2 Id compared to only 6% in normal control group [38]. A monoclonal Ab carrying the Y2 Id, termed 4B4, was used successfully in the induction of a SLE-like syndrome in BALB/c mice [39].

Pathogenic Role of Abs Carrying Ids

The importance of identifying Id of Abs is their relation to the disease pathogenesis and clinical manifestations. The detection of immunoglobulin carrying the anti-dsDNA 16/6 Id in the skin, kidneys and brain of SLE patients favors a pathogenic role for this Id [40]. Similarly, the anti-dsDNA Id GN1 and GN2 were found in 38% and 75% of the biopsy specimens from 32 kidneys of patients with SLE, respectively, whereas they were found in only 6% of 19 patients with non-lupus immune glomerulonephritis [41]. Regarding a possible pathogenic role for anti-Sm Abs, it has been shown that anti-dsDNA Abs cross-reacting with the Sm A and D subunits are cytotoxic to cultured kidney cells [42]. There are also a few examples for the pathogenic role of anti-SS-A Abs: these Abs are enriched in acid eluates of saline extract of affected organs from SLE and Sjögren's syndrome patients [43], and human IgG containing anti-SS-A Abs can both induce repolarization abnormalities in neonatal rabbit hearts, and induce conduction abnormalities in adult rabbit hearts [44, 45]. With respect to anti-SS-B, the serum activity of these Abs correlates with the degree of salivary gland lymphocytic activity [46], and like the SS-A antigen, the SS-B antigen is also present on the surface of the fibers of affected hearts, suggesting a pathogenic role for anti-SS-B in heart block too [47].

Clinical Implications of the Idiotypic Network in Autoimmunity

Identification of certain Ids on Abs provides some measures to treat or prevent autoimmune diseases. These include injection of anti-Ids, injection of anti-Ids conjugated to a cytotoxic agent, direct injection of a common Id with the subsequent production of anti-Ids, passage of plasma over an anti-Id column, treatment with Id-specific T-suppressor cells, and intravenous immunoglobulin (IVIg) [48]. Whereas most of these are still experimental, the use of anti-Id against certain Abs in autoimmune diseases is practically a fact. When a genetically susceptible host is exposed to an environmental agent such as a virus, anti-viral antibodies are generated (Ab1) followed by the generation of anti-Id (Ab2) and anti-anti-Id (Ab3). When the normal cascade of Abs generation may be lost, leading to the emergence of self-reacting Abs, there is a relative shortage of anti-Ids directed against Ab3 (the pathogenic anti-anti-Id). IVIg, a therapeutic agent widely used in various autoimmune diseases, is composed of a pool of immunoglobulins from numerous donors. One of its mechanisms of action is manipulation of the idiotypic network, by providing anti-Ids present within the IVIg preparation that bind to the Ids found on the patients' pathogenic Abs [49]. The list of Abs known to be inhibited by IVIg in vitro include Abs to factor VIII, cardiolipin, platelet, endothelial cells, C3 convertase, acetylcholine receptor, mitochondrial antigens, intrinsic factor, erythroblast, retinal S antigen, DNA, thyroglobulin and neutrophil cytoplasmic antigens [50]. Whereas IVIg preparations may contain some of the Ids and anti-Ids involved in autoimmunity [51], they do not contain other Abs [52]. Hence, as IVIg is not specific enough, future aims of immunotherapy in autoimmune diseases would be to provide disease-specific and even patient-specific therapy by means of infusions of one or only few anti-Id in sufficient concentrations. Since this 'super-IVIg' will be specific, it will probably have higher efficacy than the current preparations.

References

1 Homburger HA: Laboratory medicine and pathology: Cascade testing of autoantibodies in connective tissue diseases. Mayo Clin Proc 1995;70:183–184.
2 Fritzler MJ, Pauls JD, Kinsella TD, Bowen TJ: Antinuclear, anticytoplasmic, and anti-Sjögren's syndrome antigen A (SS-A/Ro) antibodies in female blood donors. Clin Immunol Immunopathol 1985;36:120–128.
3 George J, Shoenfeld Y: Natural autoantibodies; in Peter JB, Shoenfeld Y (eds): Autoantibodies. Amsterdam, Elsevier, 1996, pp 534–539.
4 Tomer Y, Shoenfeld Y: Aging and autoantibodies. Autoimmunity 1988;1:141–149.
5 Reichlin M: ANAs and antibodies to DNA: Their use in clinical diagnosis. Bull Rheum Dis 1993;42:3–5.
6 Hollingsworth PN, Pummer SC, Dawkins RL: Antinuclear Antibodies; in Peter JB, Shoenfeld Y (eds): Autoantibodies. Amsterdam, Elsevier, 1996, pp 74–90.
7 Tomer Y, Shoenfeld Y: The idiotypic network in autoimmunity. Giorn It Allergol Immunol Clin 1991;1:3–15.
8 Shoenfeld Y, Isenberg D: The Mosaic of Autoimmunity. Amsterdam, Elsevier, 1989.
9 Jerne NK: Towards a network theory of the immune system. Ann Immunol 1974;125:373–389.
10 Shoenfeld Y: Idiotypic induction of autoimmunity: A new aspect of the idiotypic network. FASEB J 1994;8:1296–1301.
11 Shoenfeld Y, Sherer Y: The Idiotypic Network in Autoimmune Diseases – Induction and Treatment; in Conrad K, Humbel RL, Meurer M, Shoenfeld Y, Tan EM (eds): Pathogenic and Diagnostic Relevance of Autoantibodies. Langerich, Pabst, 1998, pp 124–134.
12 Mendlovic S, Brocke S, Shoenfeld Y, Ben-Bassat M, Meshorer A, Bakimer R, Mozes E: Induction of SLE-like disease in mice by a common anti-DNA idiotype. Proc Natl Acad Sci USA 1988;85:2260–2264.
13 Rombach E, Stetler DA, Brown JC: Rabbits produce SLE-like anti-RNA polymerase I and anti-DNA autoantibodies in response to immunization with either human or murine SLE anti-DNA antibodies. Autoimmunity 1992;13: 291–302.
14 Dang H, Ogawa N, Takei M, Lazaridis K, Talal N: Induction of lupus- associated autoantibodies by immunization with naive and recombinant Ig polypeptides expressing a cross-reactive idiotype 4B4. J Immunol 1993;151:7260–7267.
15 Krause I, Blank M, Shoenfeld Y: Treatment of systemic lupus erythematosus and antiphospholipid syndrome: From experimental models to patients' bedside. Int Arch Allergy Immunol 1996;111:355–361.
16 Ziporen L, Blank M, Shoenfeld Y: Animal models for antiphospholipid syndrome in pregnancy. Rheum Dis Clin North Am 1997;23: 99–117.
17 Blank M, Cohen J, Toder V, Shoenfeld Y: Induction of antiphospholipid syndrome by passive transfer of anticardiolipin antibodies. Proc Natl Acad Sci USA 1991;88:3069–3073.
18 Bakimer R, Fishman P, Blank M, Sredni B, Djaldetti M, Shoenfeld Y: Induction of primary antiphospholipid syndrome in mice by immunizing with a human monoclonal anticardiolipin antibodies (H3). J Clin Invest 1992;89: 1558–1563.
19 Pierangeli SS, Harris EN: Induction of phospholipid-binding antibodies in mice and rabbits by immunization with β_2-glycoprotein I or anticardiolipin antibodies alone. Clin Exp Immunol 1993;93:269–272.
20 Blank M, Tomer Y, Stein M, Kopolovic J, Wiik A, Meroni PL, Conforti G, Shoenfeld Y: Immunization with anti-neutrophil cytoplasmic antibody (ANCA) induces the production of mouse ANCA and perivascular lymphocyte infiltration. Clin Exp Immunol 1995;102:120–130.
21 Damianovich M, Gilburd B, George J, Del Papa N, Afek A, Goldberg I, Kopolovic Y, Roth D, Barkai G, Meroni PL, Shoenfeld Y: Pathogenic role of antiendothelial cell antibodies (AECA) in vasculitis: An idiotypic experimental model. J Immunol 1996;156:4946–4951.
22 Shoenfeld Y, Gilburd B, Hojnic M, Damianovich M, Hacham S, Kopolovic Y, Polak-Charcon M, Goldberg I, Afek A, Hun-Chi L, Peter JB: Induction of Goodpasture's antibodies to noncollagenous domain (NC1) of type IV collagen in mice by idiotypic manipulation. Hum Antibody Hybridomas 1995;6:122–128.
23 Tomer Y, Gilburd B, Sack J, Davies TF, Meshorer A, Burek CL, Rose NR, Shoenfeld Y: Induction of thyroid antibodies in naive mice by idiotypic manipulation. Clin Immunol Immunopathol 1996;78:180–187.
24 George J, Afek A, Gilburd B, Levy Y, Blank M, Kopolovic J, Harats D, Shoenfeld Y: Atherosclerosis in LDL-receptor knockout mice is accelerated by immunization with anticardiolipin antibodies. Lupus 1997;6:723–729.
25 Buskila D, Abu-Shakra M, Shoenfeld Y: Idiotypes of Anti-DNA antibodies; in Shoenfeld Y, Kennedy RC, Ferrone S (eds): Idiotypes in Medicine: Autoimmunity, Infection and Cancer. Amsterdam, Elsevier, 1997, pp 75–88.
26 Baccala R, Quang TV, Gilbert M, Ternynck T, Avrameas S: Two murine natural polyreactive autoantibodies are encoded by nonmutated germ-line genes. Proc Natl Acad Sci USA 1989; 86:4624–4628.
27 Marion TN, Bothwell ALM, Briles DE, Janeway CA Jr: IgG anti-DNA autoantibodies within an individual autoimmune mouse are the products of clonal selection. J Immunol 1989; 142:4269–4272.
28 Gaither KK, Harley JB: A shared idiotype among human anti-Ro/SSA autoantibodies. J Exp Med 1989:1583.
29 Bar-Dayan Y, Amital H, Shoenfeld Y: Idiotypes of anti-Ro and anti-La; in Shoenfeld Y, Kennedy RC, Ferrone S (eds): Idiotypes in Medicine: Autoimmunity, Infection and Cancer. Amsterdam, Elsevier, 1997, pp 95–97.
30 Horsfall AC, Venables PJW, Mumford PA, Maini RN: Idiotypes of antibodies to La (SS-B) antigen are restricted and associated with the antigen binding site. Clin Exp Immunol 1986; 63:395.
31 Eilat D, Hochberg M, Fischel R, Laskov R: Antibodies to RNA from autoimmune NZB/NZW mice recognize a similar antigenic determinant and show a large idiotypic diversity. Proc Natl Acad Sci USA 1982;79:3818.
32 Shoenfeld Y: Common infections, idiotypic dysregulation, autoantibody spread and induction of autoimmune diseases. J Autoimmunity 1996;9:235–239.
33 Fricke H, Offen D, Mendlovic S, Shoenfeld Y, Bakimer R, Sperling J, Mozes E: Induction of experimental SLE in mice by immunization with a monoclonal anti- La autoantibody. Int Immunol 1989;2:225–230.
34 Fields M, Williams DG, Charles R, Maini RN: Specificity of anti-Sm antibodies by ELISA for systemic lupus erythematosus: Increased sensitivity of detection using purified peptide antigens. Ann Rheum Dis 1988;47:820–825.
35 Reichlin M, Martin A, Taylor-Albert E, Tsuzaka K, Zhang W, Reichlin M, et al: Lupus autoantibodies to native DNA cross-react with the A and D SnRNP polypeptides. J Clin Invest 1994;93:443–449.
36 Takei M, Dang H, Talal N: A common idiotype expressed on a murine anti-Sm monoclonal antibody and antibodies in SLE sera. Clin Exp Immunol 1987;70:546–554.
37 Dang H, Talal N: Idiotypes of anti-Sm antibodies; in Shoenfeld Y, Kennedy RC, Ferrone S (eds): Idiotypes in Medicine: Autoimmunity, Infection and Cancer. Amsterdam, Elsevier, 1997, pp 89–93.
38 Dang H, Takei M, Isenberg D, Shoenfeld Y, Bakimer R, Rauch J, Talal N: Expression of an interspecies idiotype in sera of SLE patients and their first-degree relatives. Clin Exp Immunol 1988;71:445–450.
39 Blank M, Drup M, Mendlovic S, Fricke H, Mozes E, Talal A, Coates A, Shoenfeld Y: The importance of the pathogenic 16/6 idiotype of anti-DNA antibodies in the induction of SLE in naive mice. Scand J Immunol 1990;31:45–52.
40 Isenberg DA, Collin C: Detection of cross-reactive anti-DNA antibody idiotypes on renal tissue bound immunoglobulins from lupus patients. J Clin Invest 1985;76:287–294.
41 Kalunian K, Panosin-Sahakian N, Ebling FM, Cohen AH, Louie JS, Kaine J, Hahn BH: Idiotypic characteristics of immunoglobulins associated with systemic lupus erythematosus: Studies of antibodies deposited in glomeruli of humans. Arthritis Rheum 1989;32:513–522.

42 Koren E, Koscec M, Wolfson-Reichlin M, Ebling FM, Tsao B, Hahn BH, Reichlin: Murine and human antibodies to native DNA that cross-react with the A and D SnRNP polypeptides cause direct injury of cultured kidney cells. J Immunol 1995;154:4857–4864.
43 Reichlin M, Scofield RH: SS-A (Ro) Autoantibodies; in Peter JB, Shoenfeld Y (eds): Autoantibodies. Amsterdam, Elsevier, 1996, pp 783–788.
44 Alexander E, Buyon JP, Provost TT, Gaurneri T: Anti-Ro/SSA antibodies in the pathophysiology of congenital heart block in neonatal lupus syndrome, an experimental model. In vitro electrophysiologic and immunocytochemical studies. Arthritis Rheum 1992;35:176–189.
45 Garcia S, Nascimento JH, Bonfa E, Olivera SF, Tavares AV, de Carvalho AC: Cellular mechanisms of the conduction abnormalities induced by serum from anti-Ro/SSA-positive patients in rabbit hearts. J Clin Invest 1994;93:718–724.
46 Atkinson JC, Travis WD, Slocum L, Ebbs WL, Fox PC: Serum anti-SS-B/La and IgA rheumatoid factor are markers of salivary gland disease activity in primary Sjögren's syndrome. Arthritis Rheum 1992;35:1368–1372.
47 Horsfall AC, Venables PJ, Taylor PV, Maini RN: Ro and La antigens and maternal anti-La idiotype on the surface of myocardial fibers in congenital heart block. J Autoimmun 1991;4:165–176.
48 Buskila D, Shoenfeld Y: Manipulation of anti-DNA idiotypes: A possible treatment approach to autoimmune diseases; in Cruse JM, Lewis RE Jr (eds): Clinical and Molecular Aspects of Autoimmune Diseases. Concepts Immunopathol. Basel, Karger, 1992, vol 8, pp 114–128.
49 Ballow M: Mechanisms of action of intravenous immunoglobulin: Clinical implication in autoimmune and inflammatory diseases; in Kazatchkine MD, Morell A (eds): Intravenous Immunoglobulin Research and Therapy. New York, Parthenon Publishing, 1996, pp 123–128.
50 Spalter SH, Kaveri S, Kazatchkine MD: Anti-idiotypes to autoantibodies in therapeutic preparations of normal polyspecific human IgG (intravenous immunoglobulin, IVIg); in Shoenfeld Y, Kennedy RC, Ferrone S (eds): Idiotypes in Medicine: Autoimmunity, Infection and Cancer. Amsterdam, Elsevier, 1997, pp 217–225.
51 Krause I, Blank M, Shoenfeld Y: Anti-DNA and antiphospholipid antibodies in IVIG preparations: In vivo study in naive mice. J Clin Immunol 1998;18:52–60.
52 Krause I, Hacham S, Gilburd B, Damianovitch M, Blank M, Shoenfeld Y: Absence of anti-idiotypic antibodies in IVIG preparations to autoantibodies of rare autoimmune diseases. Clin Immunol Immunopathol 1995;77:229–235.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:16–27

Antinuclear Autoantibodies: Fluorescent Highlights on Structure and Function in the Nucleus

Peter Hemmerich[a] Anna von Mikecz[b]

[a]Institute of Molecular Biotechnology, Jena, and [b]Medical Institute of Environmental Hygiene, Düsseldorf, Germany

Key Words

Autoantibodies · Autoantigen · Systemic autoimmune diseases · Nuclear structure · Confocal microscopy · Fluorescence

Abstract

The eukaryotic nucleus is dynamically organized with respect to particular activities, such as RNA transcription, RNA processing or DNA replication. The spatial separation of metabolic activities is best reflected by the identification of functionally related proteins, in particular substructures of the nucleus. In a variety of human diseases, the integrity of such structures can be compromised, thus underlining the importance of a proper nuclear architecture for cell viability. Besides their clinical relevance, antinuclear autoantibodies (ANAs) have contributed to a large extent to the identification of subnuclear compartments, the isolation and cloning of their components (the autoantigens), as well a the characterization of their function. Although sophisticated techniques, such as confocal laser scanning microscopy (CLSM), fluorescence resonance energy transfer (FRET) and in vivo observation of cellular events have recently been established as valuable tools to study subnuclear architecture and function, cell biologists will continue to appreciate the specificity and power of ANAs for their research.

Dedicated to Eng M. Tan.

Introduction

A hallmark of eukaryotic cells is their separation into compartments. The nucleus contains many internal nuclear domains including the nucleolus, nuclear envelope (NE), nuclear speckles, coiled bodies, PML nuclear bodies, and gems. This organization most likely reflects the requirement for spatial and temporal coordination of many nuclear processes. Nuclear proteins with related functions, such as DNA and chromatin replication, transcription of RNA or subsequent RNA splicing are often assembled in multiprotein/nucleic acid complexes and colocalize at cytological level. They form a dynamic framework that is able to change its functional organization during the cell cycle in order to fulfill altered requirements. Over the past 25 years, characterization of antinuclear autoantibodies (ANA) has helped identify many nuclear proteins by their subcellular localization. Epi-

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Peter Hemmerich
Institute of Molecular Biotechnology, Beutenbergstrasse 11
D–07745 Jena (Germany)
Tel. +49 3641 656262, Fax +49 3641 656272
E-Mail phemmer@imb-jena.de

fluorescence microscopy is a valuable technique for both the clinician identifying certain autoantibody specificities, thereby diagnosing subsets of systemic autoimmune diseases, and the research biologist analyzing the structure and function of nuclear autoantigens. It enables not just visualization, but also identification of structures within cells and tissues. The emitted signal is viewed against a black background providing high contrast. In addition, fluorescence imaging can provide superb selectivity.

This review summarizes current cell biological knowledge on the structure, function and dynamics of subnuclear compartments that are frequent targets of ANA produced by patients with systemic rheumatic diseases.

Nuclear Envelope

Autoantibodies to NE antigens were found in 52% of patients with chronic fatigue syndrome, mainly nuclear lamins. Combination of nuclear rim staining observed in indirect immunofluorescence microscopy and immunoblot analysis of highly purified proteins provided initial characterization of these autoantibodies. The occurrence of autoantibodies to a conserved intracellular protein, such as lamin B1, provided new laboratory evidence for an autoimmune component in chronic fatigue syndrome [Konstantinov et al., 1996; von Mikecz et al., 1997, reviewed in Bennet, 1998]. In addition, between 10 and 42% of patients with primary biliary cirrhosis have been reported to have antibodies against gp210, a glycoprotein of the nuclear pore complex (NPC) [Wesierska-Gadek et al., 1995; Bandin et al., 1996; Courvalin and Worman, 1997].

The NE is a double-membrane system consisting of an inner and outer nuclear membrane enclosing a lumen called perinuclear space. Hence, the NE physically separates the genetic machinery residing in the nucleus from protein synthesis occurring in the cytoplasm. Ultrastructural and diffusion analyses have documented that the membranes of the endoplasmatic reticulum (ER) form an interconnected boundary that includes the outer nuclear membrane of the NE [reviewed in Gant et al., 1998]. Recent studies have shown that the lumen of the ER and NE of resting cells is not compartmentalized by mechanical barriers, suggesting that the free calcium concentration in the lumen of the ER and NE can equilibrate throughout the cell [Peterson et al., 1998].

In most eukaryotic cells, the nucleoplasmic face of the NE is lined by a highly dynamic, fibrous meshwork, called the nuclear lamina. The major molecular constituents of the nuclear lamina are the nuclear lamins, members of the intermediate filament protein family. Lamins build a polymer of four intermediate filament type proteins, lamins A, B1, B2, and C, as well as integral membrane proteins specific to the inner nuclear membrane (LAP1, LAP2, LBR) [reviewed in Gant and Wilson, 1997; Stuurman, 1998]. Results from a number of studies suggest that lamins may be involved in nuclear events such as DNA replication through interaction with specific proteins [i.e. elongation factors; Laskey et al., 1996]. The NE system of higher eukaryotic cells undergoes complete breakdown in prometaphase and reassembles in the late anaphase of the cell cycle [Fields and Thompson, 1995]. However, during interphase, lamins physically interact with the inner nuclear membrane via integral membrane proteins. Moreover, the nuclear lamina interacts with chromatin and is also physically associated with NPCs [Goldberg and Allen, 1995]. NPCs are the major gateways for transport of cargo, like ions, small molecules, proteins, RNAs and RNP particles shutteling between the cytoplasm and the nucleus. NPCs are highly conserved supramolecular assemblies with a mass of ~ 125 MD, which are built from about 100 different polypeptides, many of them autoantigens [Gant et al., 1998]. An ever increasing number of these 'nucleoporins' are being identified, cloned, and their role in nucleo-cytoplasmic transport explored [Pemberton et al., 1998; Wozniak et al., 1998], since translocation through the NPC requires transport factors that transiently associate with nucleoporins en route [Ohno et al., 1998]. Import of most nuclear proteins is a signal-mediated, energy-requiring process. Different classes of nuclear localization signals (NLS) have been identified, because translocation of karyophilic proteins through NPC usually requires specific recognition of its NLS by a corresponding transport factor, such as importin or transportin, and their interaction with nucleoporins [Izaurralde and Adam, 1998; Cole and Hammel, 1998].

By means of confocal laser scanning microscopy, it became possible to localize single NPCs and analyze their distribution [Kubitscheck and Peters, 1998]. Specific labeling of individual nucleoporins detected by laser confocal or electron microscopy will further reveal the three-dimensional and molecular architecture of NPCs and elucidate molecular mechanisms of intracellular trafficking [Görlich, 1998; Nigg, 1997]. In addition, the relative rates of NLS-green fluorescent fusion protein (NLS-GFP) import and passive export can now be measured directly in living cells [Roberts and Goldfarb, 1998].

Histones

Autoantibodies to chromatin are most prevalent in systemic lupus erythematosus (SLE) but also occur in drug-induced autoimmunity, rheumatoid arthritis, and an undifferentiated form of connective tissue disease [Robitaille and Tan, 1973; Burlingame et al., 1994]. While in SLE antichromatin antibodies target naked DNA, histones or histone/DNA complexes, the main target of histone antibodies in drug-induced lupus is the H2A-H2B/DNA complex [reviewed in Burlingame and Rubin, 1996]. Histones are the building units of eukaryotic nucleosomes which are the primary repeating units of chromatin. Packaging of nearly 2 m of DNA within the confines of the eukaryotic nucleus represents a mechanistic and logistic problem of enormous proportions. In addition, the DNA has to be organized in a manner that allows the essential processes of DNA replication, repair and transcription while maintaining a considerable degree of condensation. Arrays of nucleosomes and their higher-order complexes, rather than naked DNA, are the substrate for DNA-processing enzymes and DNA-binding transcription factors [Beato and Eisfeld, 1997; Felsenfeld, 1996]. Recent studies imply that chromatin is highly dynamic [reviewed in Luger and Richmond, 1998]. The propensity for folding and refolding of chromatin stems from the so-called histone fold, an architectural motif mediating DNA compaction and protein dimerization of histones and some transcription factors [Arents and Moudrianakis, 1995].

The three-dimensional structure of the nucleosome core has been solved, showing the histone proteins and DNA in atomic detail [Luger et al., 1997]. A nucleosomal core contains about 140 bp of DNA, wrapped around a protein octamer containing two copies each of histones H2A, H2B, H3, and H4, designated core histones [Wolffe, 1992]. In nucleosome assembly, binding of the H3/H4 tetramer to DNA is followed by assembly of H2A/H2B dimers. Additionally, histone H1 proteins serve as linker histones, deriving their name from their association with the linker DNA which connects nucleosomal cores. Histone H1 locks the two helical turns of the DNA around the nucleosome, thus maintaining higher-order chromatin structures [Boulikas, 1993]. One copy of histone H1 per nucleosome promotes a higher-order chromatin structure: the 1- to 72-amino-acid domain of histone H1 interacts with three core histones of neighboring nucleosomes, whereas the 73- to 106-amino-acid segment of H1 contacts histone H2A of its 'own' nucleosome and locks the two ends of DNA around the particle into a closed conformation [Fletcher and Hansen, 1995]. Thus removal of histone H1 might constitute the first step in chromatin unfolding and unlocking of nucleosomes.

Despite its similarity to a bead on a string, the building block of the chromosome is dynamic. The histone amino termini extend from the core, where they can be modified posttranslationally by acetylation, phosphorylation and methylation, which affects their charge and function [Svaren and Horz, 1996]. Of such modifications, acetylation and deacetylation have generated most interest, since gene activity was first correlated with histone acetylation [Hebbes et al., 1988; reviewed in Grunstein, 1997]. As acetylation neutralizes the positively charged lysine residues of the histone N-termini, decreasing their affinity for DNA, this might allow the termini to be displaced from the nucleosome, causing the nucleosomes to unfold. This in turn increases access to transcription factors and leads to initiation of transcription [Lee et al., 1993]. How acetylation and deacetylation targets specific genes or chromosomal domains has long been enigmatic. Euchromatin and heterochromatin exhibit different acetylation patterns. Euchromatin is the chromatin that decondenses during interphase of cell cycle and which contains most of the genes coding for cellular proteins. Heterochromatin, however, is condensed even in interphase, often contains repetitive DNA, and is generally silent. Histone acetylation involves histone acetylases (HATs), which are nuclear and transcription related. Transcription factors that can activate or inhibit transcription may do so by associating with HATs or deacetylases [reviewed in Pazin and Kadonaga, 1997; Wade et al., 1997]. For example, nuclear hormone receptor function, with and without ligand, may require binding to HATs (e.g. p300/CBP) and to deacetylases, respectively [Jenster et al., 1997]. How these events regulate gene-specific repression, which sites on which histones they affect and whether they affect all promotors or chromosomal domains equally remains to be explored. However, it is certain that histones are important regulators of gene activity.

Centromeres

The centromere (or kinetochor) is an integral part of human chromosomes and an essential actor in mitotic cell division. In interphase cells centromeres are evenly distributed throughout the nucleoplasm (fig. 1). However, a characteristic feature of centromere distribution is the formation of prominent clusters organized around the nucleoli [Ochs and Press, 1992]. A centromere is com-

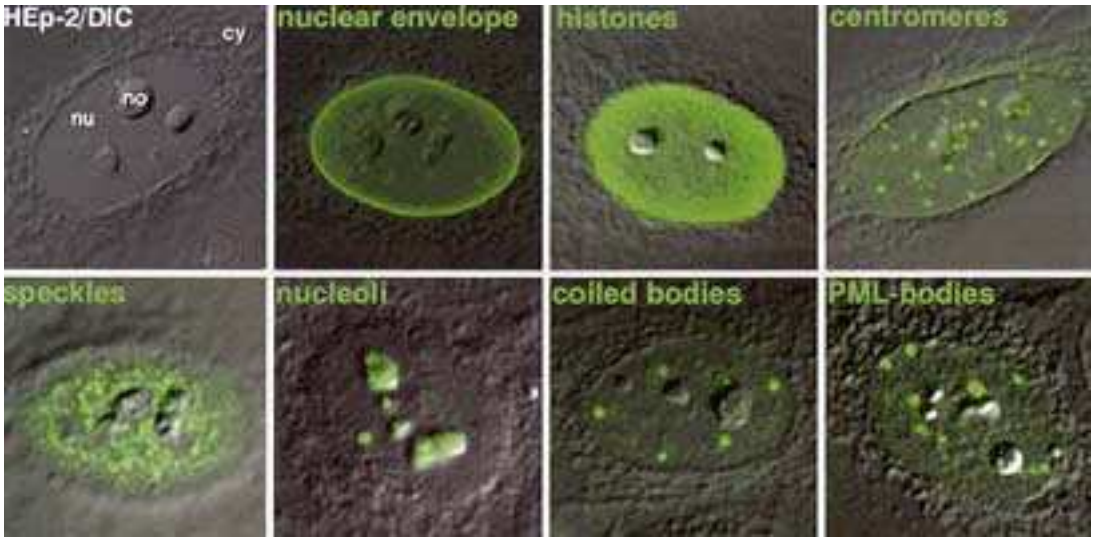


Fig. 1. Staining patterns of nuclear compartments. Confocal microscopy images of HEp-2 cells stained with specific antibodies decorating the indicated nuclear subcompartments (green). Differential interference contrast (DIC) images (gray) were taken from each cell at the same time and merged with the respective fluorescence image. Due to differences between the refractive indices of cellular compartments, DIC reveals the cytoplasm (cy), nucleoplasm (nu), and nucleoli (no) in HEp-2 cells (HEp-2).

posed of a variety of proteins associated with alphoid DNA. Alphoid DNA belongs to the family of highly repetitive sattelite DNA and is specific to centromeres of all human chromosomes [Mitchell et al., 1985; reviewed in Csink and Henikoff, 1998]. The centromere modulates the association of sister chromatids, represents the major site for the association of the chromosome with the spindle, captures microtubules growing from the spindle pole, and participates in the regulation of chromosome movement through interaction with the motor-protein complex [reviewed in Mitchel, 1996; Pluta et al., 1995].

Strategies to examine centromere function and chromosome dynamics include fluorescence in situ hybridization (FISH) [Durm et al., 1998], expression of GFP fused to centromere proteins [Shelby et al., 1996], or yeast two-hybrid analyses using centromere proteins (CENPs) as baits (see later). However, initial studies on the structure and function of centromere and kinetochore components were aided by the discovery that sera of scleroderma patients with CREST syndrome contain autoantibodies against CENPs [Moroi et al., 1980]. Anti-centromere antibodies (CENPs A, B, and C) are typically associated with Raynaud's phenomena, telangiectasias, lung involvement, and a significantly younger age of disease onset [Fritzler, 1993]. Not less than fourteen different autoantigens have been identified to be associated with the human centromere. Their clinical correlations have recently been reviewed [Rattner et al., 1998].

cDNAs encoding CENP-A, -B, and -C have been cloned and the proteins have been studied in detail. CENP-A is a centromere-specific core histone related to histone H3 [Sullivan et al., 1994]. CENP-B is an alpha-satellite-binding protein that is localized throughout the centromeric heterochromatin located beneath the kinetochor [Earnshaw et al., 1987]. Assembly studies of the CENP-B/alpha-satellite DNA show that the dimeric structure of CENP-B is sufficiently stable to bundle together two 3.5-kbp DNA fragments in vitro when each DNA contains a CENP-B DNA-binding motif [Yoda et al., 1998]. The authors propose that CENP-B functions as a structural factor in the centromere region in order to establish a unique, centromere-specific pattern of nucleosome positioning. CENP-C is also a DNA-binding protein and is located at the interface between the centromeric heterochromatin and the innermost region of the kinetochor [Saitoh et al., 1992]. A yeast interaction trap identified protein HDaxx as a specific CENP-C interactor [Pluta et al., 1998]. HDaxx is homologous to murine Daxx, a protein identified through its ability to bind Fas, a central mediator of apoptosis. The specific interaction of HDaxx with CENP-C suggests that centromeres may play as yet unsuspected roles in cell cycle progression and, possibly,

the modulation of cellular responses to apoptotic stimuli [Pluta et al., 1998]. Monoclonal antibodies against CENP-E specifically stain the centromere region of mitotic human chromosomes. Interestingly, in cells progressing through different parts of the cell cycle, the localization of CENP-E differs markedly from that observed for CENP-A, CENP-B, CENP-C and CENP-D. In contrast to these antigens, anti-CENP-E staining is not detected during interphase, and staining first appears at the centromere region of chromosomes during prometaphase [Yen et al., 1991]. Recently, a 350-amino-acid domain of CENP-E has been identified that specifies kinetochore binding in mitosis but not during interphase [Chan et al., 1998]. This domain was used in a yeast two-hybrid screen to isolate interacting proteins that included the kinetochore proteins, CENP-F and hBUBR1. hBUBR1 is related to BUB1, a kinase that was found to be mutated in some colorectal carcinomas [Cahill et al., 1998]. Chan et al., 1998 [reviewed in Grancell and Sorger, 1998] found that CENP-F, hBUBR1, and CENP-E assemble onto kinetochores in sequential order during late stages of the cell cycle and suggest that this complex is responsible for defining discrete steps along the kinetochore assembly pathway and to function as a motor-kinase complex at kinetochores. Recently, a new centromere-specific protein (CENP-G) has been identified as a result of its recognition as an autoantigen by serum from a patient with gastric antral vascular ectasia disease [He et al., 1998]. The localization of this new centromere protein and its alpha-1 DNA-specific association suggest that CENP-G may play a role in kinetochore organization and function.

In the future, successful construction of artificial chromosomes will be an important step for studies to elucidate the DNA and protein elements necessary for chromosome structure and function. The development of human artificial chromosome systems should also facilitate investigation of the DNA and chromatin requirements for active centromere assembly [reviewed in Willard, 1998].

Nuclear Speckles

Splicing of pre-mRNA occurs in a large RNA-protein complex, the spliceosome. Spliceosomes consist of small nuclear ribonucleoprotein particles (snRNPs), assemble on pre-mRNA molecules, catalyze the removal of introns, and subsequently dissociate from the mature mRNA [Maniatis and Reed, 1987; Tarn and Steitz, 1997]. The immunological and biochemical features of snRNP antigens were elucidated by the important studies by Lerner and Steitz [1979], who introduced the tools of molecular biology into this field and showed that these autoantigens were subcellular particles composed of a species of small nuclear RNAs (UsnRNAs) complexed with protein. Autoantibodies against snRNPs occur predominantly in sera from patients with SLE or mixed connective tissue disease [reviewed in Tan, 1989; van Venrooij and Sillekens, 1989]. Many studies have addressed the question of where in the interphase nucleus the splicing components are localized. Initial work made use of autoimmune sera that contained antibodies against snRNPs [Spector, 1984; Nyman et al., 1986; Verheijen et al., 1986]. Recently, antibodies against the non-snRNP-splicing factor SC35 have become the general marker for the analysis of the distribution of the splicing machinery in nuclei [Fu and Maniatis, 1990]. Immunocytochemical studies have shown that pre-mRNA splicing is localized in a speckled nuclear pattern (fig. 1) that corresponds to interchromatin granule clusters and perichromatin fibrils [reviewed in Puvion and Puvion-Dutilleul, 1998]. While perichromatin fibrils are thought to represent RNA transcripts, little to no transcription is associated with the majority of the interchromatin granule clusters [Spector, 1996]. One function of interchromatin granule clusters is to provide pre-mRNA splicing factors to sites of transcription. Autoantibodies stain snRNPs showing a speckled labeling against a more diffuse background of nucleoplasmic staining [Spector, 1993]. The diffuse staining pattern is largely due to snRNPs interacting with nascent RNA and disappears when transcription is inhibited [Bauren and Wieslander, 1994]. Cells showing high transcription exhibit more widespread nucleoplasmic localization of RNA-processing factors and less speckled staining, whereas reduced transcription is often accompanied by increased speckled staining [Zeng et al., 1997]. The mRNAs from some highly transcribed genes have been shown to colocalize with snRNP/SC35 speckles, whereas inactive genes do not, suggesting that speckles can be actively involved in splicing [Huang and Spector, 1991; Zhang et al., 1995]. However, the spatial organization of transcription and splicing in the mammalian nucleus, especially the importance of speckles is still under debate [Singer and Green, 1997; Lamond and Earnshaw, 1998].

Recent findings provide interesting new insights into structure/function relationships of mRNA processing with respect to nuclear speckles: a differential display assay used to identify cellular genes differentially expressed during human immunodeficiency virus 1 (HIV-1) infection revealed that the expression of SC35 is altered by HIV infection [Maldarelli et al., 1998]. These findings

indicate that an essential splicing factor is induced after HIV infection, suggesting that the consequences of HIV infection include alterations in relative levels of a splicing factor [Maldarelli et al., 1998]. A new class of splicing factors has recently been identified in yeast that are members of the DEAD box family of ATP-dependent putative RNA helicases and which are required for pre-mRNA splicing in *Saccharomyces cerevisiae* [reviewed in Hamm and Lamond, 1998]. A human homolog has been shown to be involved in splicing events and to localize to speckles in human cell lines [Ortlepp et al., 1998]. Since RNA helicases can change RNA conformation, the newly discovered splicing factors containing such domains are believed to be essential for spliceosome assembly [Hamm and Lamond, 1998]. Schul et al. [1998] have found that a subset of poly(A) polymerase is concentrated at sites of RNA synthesis and domains enriched in splicing factors, indicating that there is a spatial and structural relationship between the 3′ processing of mRNAs and the nuclear speckles. Finally, a study performed in living cells has shown that upon transcriptional activation of an integrated viral genome, the splicing factors present in speckles are rapidly recruited to the sites of active viral transcription, clearly demonstrating that one function of speckles is to supply splicing factors to neighbor active genes [Misteli et al., 1997]. Phosphorylation of splicing factors seems to be instrumental for the recruitment of these proteins to active sites of transcription in vivo [Misteli et al., 1998]. The picture that emerges is that the nuclear distribution of splicing components is highly dynamic and might reflect an arrangement optimally suited to provide transcripts with splicing factors anywhere in the nucleus.

Nucleoli

Ribosomal RNA (rRNA) gene expression and assembly of mature ribosomes take place in a cytologically distinct subcompartment of the nucleus, the nucleolus [Perry et al., 1961; Hadjiolov, 1985]. Nucleolus formation is dependent both on transcription and cell cycle: in most eukaryotic cells, the entire structure breaks down and reforms during each mitotic cycle. Thus, the nucleolus is a dynamic structure that forms in response to the requirement of new ribosome synthesis [Melese and Xue, 1995]. Ribosomal RNA in eukaryotes is synthesized as a 40S precursor that is subsequently cleaved to give rise to mature 18S, 5.8S and 28S ribosomal RNA which in turn is assembled with ribosomal proteins to preribosomal particles. Those preribosomes built up to the ribosomal subunits and have to be transported out of the nucleus into the cytoplasm where they serve in protein biosynthesis [Hadjiolov, 1985]. To this day, a number of nonribosomal nucleolar proteins have been identified, some of which are autoantigens in systemic sclerosis [Bunn et al., 1988; reviewed in Lee and Craft, 1995]. The transcription of ribosomal DNA is mediated by RNA polymerase I (RNA pol I). Autoantibodies to RNA pol I are present in at least 4% of patients with scleroderma [Reimer et al., 1987]. A molecular mechanism by which RNA pol I activity can be regulated was found recently. Milkereit and Tschochner [1998] showed that RNA pol I can form a functional complex with the pol-I-specific initiation factor Rrn3 in yeast. Formation and disruption of the pol I-Rrn3p complex seem to reflect a molecular switch for regulating rRNA synthesis and its growth-rate-dependent regulation. Autoantibodies against NOR-90 (identical to hUBF, human upstream binding factor) were first identified using scleroderma sera that stained nucleoli in multiple discrete dots and the nucleolar organizer region (NOR) on several chromosomes [Rodriguez-Sanchez et al., 1987]. hUBF together with selectivity factor SL1 are transcription factors required for RNA-pol I function in mammalian cells [Bazett-Jones et al., 1994; Schnapp and Grummt, 1991]. In *Xenopus laevis* transcription of ribosomal genes by RNA pol I is directed by a stable transcription complex that forms on the gene promoter. As part of this complex, UBF has been shown to be a transcriptional enhancer [Sullivan and McStay, 1998]. Anti-PM-Scl antibodies were first described in patients with polymyositis, and scleroderma [Lee and Craft, 1995 and references therein]. PM-Scl is believed to be involved in ribosome biogenesis. The yeast homolog of human PM-Scl, rRNA-processing protein 6 (Rrp6), has been shown to be essential for 5.8S rRNA maturation [Briggs et al., 1998]. PM-Scl may also have functions outside the nucleolus, since it is also localized to distinct nucleoplasmic foci and can activate transcription through specific interaction with transcription factors E12 and E47 in vivo [Kho et al., 1996]. Other autoantigens with nucleoplasmic and/or nucleolar function include topoisomerase I, Th snoRNP, and Ku [reviewed in Lee and Craft, 1995]. Fibrillarin is a highly conserved nucleolar autoantigen of 34 kD, which localizes to the dense fibrillar component of the nucleolus [Ochs et al., 1985, fig. 1]. Autoantibodies to fibrillarin serve as specific markers for scleroderma. A recent study found anti-fibrillarin antibodies in 8% of 335 systemic sclerosis sera and significantly more common in blacks (16%) than whites (5%), in males (33%) than females (14%), and in patients

with cardiac, renal, or intestinal involvement [Arnett et al., 1996]. Fibrillarin seems to be involved in many nucleolar processes, such as the first steps of rRNA processing, pre-rRNA modification and ribosome assembly. Several small nucleolar RNAs (snoRNAs) involved in pre-rRNA cleavage (U3, U8, U14 and U22) or rRNA methylation (U14, U18 and U24-U63) physically associate with fibrillarin [Tyc and Steitz, 1984]. Fibrillarin localizes to the transcription sites in nucleoli and to coiled bodies, suggesting an interrelationship between these structures (fig. 1). Nucleolin and B23/NO38 have also been shown to localize to both, coiled bodies and nucleoli [Raska et al., 1991]. In addition, mutant forms of p80 coilin induced nucleolar disorganization upon transfection into cell lines, supporting the idea of an intimate connection between coiled bodies and the nucleolus [Bohmann et al., 1995]. Recent findings from the laboratory of Tom Meier show that nucleolar phosphoprotein NOPP140 may function as a molecular link between the two prominent nuclear organelles. Exogenous NOPP140 accumulated rapidly in the nucleolus, but only after a lag phase in the coiled bodies, suggesting a pathway between the two organelles [Meier and Blobel, 1992]. Moreover, its association with major nucleolar proteins (e.g. fibrillarin) suggests that NOPP140 may serve as a chaperone in nucleoplasmic transport.

Recently, essential non-rRNA processing events have been identified to be associated with the nucleolus, including the export of a subset of mRNAs, processing of the signal recognition particle, telomerase RNP assembly, and transfer RNA maturation. These new data reveal the nucleolus as a multifunctional organelle and add a new perspective to our current picture of the spatial-functional design of the cell nucleus [Pederson, 1998].

Coiled Bodies

Coiled bodies are small nuclear organelles, generally 0.15–1.5 μm in diameter [Monneron and Bernhard, 1969], found in a variety of animal and plant cells. They disassemble during mitosis and reform during G_1 phase after transcription is initiated [Ferreira et al., 1994]. Coiled bodies are characterized by the presence of a unique protein, p80 coilin, an autoantigen in patients with diverse autoimmune features [Andrade et al., 1991], along with nucleolar proteins, fibrillarin and NOPP140 and components involved in three different RNA-processing pathways: splicing, pre-rRNA processing, and histone pre-mRNA processing [reviewed by Lamond and Carmo-Fonseca, 1993; Lamond and Earnshaw, 1998; Matera, 1998]. However, their protein composition might differ slightly in different cell lines [Alliegro and Alliegro, 1998]. Coiled bodies vary in number from 1 to 10 per nucleus being most prominent in rapidly growing cells [Andrade et al., 1993]. Coiled bodies are often seen adjacent to nucleoli in the nucloplasm. Electron-microscopic studies have shown fibers connecting coiled bodies to the nucleolar periphery which may serve as nuclear highways for snRNP traffic [Puvion-Dutilleul et al., 1995]. They not only contain high concentrations of snRNPs, as judged by anti-Sm antibodies, but also colocalize with specific DNA loci which include histone and snRNA genes [Matera and Ward, 1993; Smith et al., 1995]. The function of coiled bodies is still under debate, but it has been speculated that coiled bodies may be sites of splicing factor assembly and/or recycling, or may play a role in histone mRNA 3′ processing [Wu and Gall, 1993; Frey and Matera, 1995]. It has recently been shown that coiled bodies are involved in processing or transport of snoRNA precursors [Gerbi and Borovjagin, 1997]. They may also act as nuclear transport or sorting structures. Coiled-body-like structures called 'gems' (gemini of coiled body) have recently been shown to be associated with coiled bodies [Liu and Dreyfuss, 1996]. Gems contain the survival of motor neuron (SMN) protein, encoded by the gene responsible for a severe inherited form of human muscular atrophy [reviewed in Levebvre et al., 1998]. The SMN protein interacts with Sm class snRNP proteins, plays an essential role in cytoplasmic snRNP biogenesis [Fischer et al., 1997] and binds to single-stranded nucleic acids [Lorson and Androphy, 1998]. Defects in spliceosomal snRNP assembly may be involved in spinal muscular atrophy, and an intranuclear snRNP trafficking pathway may involve interactions between gems and coiled bodies.

PML Nuclear Bodies

The nucleus is separated into largely nonoverlapping chromosomal territories. Additional specific nuclear domains may permeate these territories or exist as interchromosomal or interchromatinic domains subdividing the nuclear space structurally and functionally. PML nuclear bodies also known as nuclear dots or nuclear domain 10 (ND10) correspond to nuclear bodies, some of which are ultrastructurally recognizable because they displace chromatin. They display as discrete punctate structures (fig. 1) and appear to be firmly attached to the nuclear matrix.

A typical mammalian nucleus has ~10–20 PML bodies, which vary in size from ~0.3 to 1 μm. PML nuclear bodies were first recognized with antibodies against SP100, an autoantigen in primary biliary cirrhosis. In primary biliary cirrhosis ANA against 5–15 nuclear dots are found in 10–40% of cases. Most of these autoantibodies recognize Sp100 [Szostecki et al., 1990]. However, autoantibodies targeted against another component of PML bodies, namely PML protein, occur frequently in patients with primary biliary cirrhosis and at a low frequency in patients with SLE [Andre et al., 1996]. Anti-Sp100 and anti-PML antibodies are directed against proteins which share the same subnuclear localization: PML nuclear bodies. Proteins within the same functional complex or organelles are often common targets of autoimmune responses, such as Sm and U1snRNP in SLE. Failure of immune tolerance toward both Sp100 and PML proteins may derive from the fact that both can be induced by estrogen and interferons [Koken et al., 1994]. These features are remarkable as ANA-associated diseases, are predominantly found in women and proposed to be triggered by viruses.

The PML protein forms part of the oncogenic PML-RARα (RARα = retinoic acid receptor alpha) hybrid protein associated with acute promyelocytic leukemia. In acute promyelocytic leukemia, nuclear bodies are disrupted and replaced by a micropunctate pattern in which PML, PML-RARα, and the steroid receptor RXR associate in many nucleoplasmic foci [Dyck et al., 1994]. Remarkably, retinoic acid and aresenic trioxide, both of which are used in clinical treatment of patients with acute promyelocytic leukemia induce reformation of PML bodies and trigger degradation of PML-RARα in acute promyelocytic leukemia cells in culture [Müller et al., 1998]. This points to an intimate relationship between nuclear organization in acute promyelocytic leukemia blasts and the malignant phenotype.

The role of PML bodies is unknown. They are not major sites of transcription, they lack snRNPs and protein splicing factors and contain little or no replication DNA during S phase. Interferon treatment upregulates PML and Sp100 and several viral proteins also associate with PML bodies. Infection with herpes simplex virus type I, adenovirus and human cytomegalovirus disrupts PML bodies, suggesting that they may play some role in antiviral defense [Everett and Maul, 1994]. The PML protein exists in two forms: in a 'free' form that is dispersed throughout the nucleoplasm and as a conjugate with the ubiquitin-like protein SUMO1 (small ubiquitin-like modifier) [Sternsdorf et al., 1997; Müller et al., 1998]. The PML-SUMO1 conjugate is exclusively localized in PML bodies, suggesting that linkage of PML to SUMO1 may either stabilize or promote the assembly of PML bodies. Conjugation to SUMO appears to direct protein localization rather than degradation. Posttranscriptional conjugation with SUMO1, and perhaps with other members of the ubiquitin-related protein family, may thus be a general mechanism directing protein assembly into specific nuclear structures [Lamond and Earnshaw, 1998]. Kamatini et al. [1998, and references therein] have shown that wild-type PML, but not PML-RARα, can be covalently modified by the sentrin family of ubiquitin-like proteins. These studies demonstrate that sentrinization of PML can regulate nuclear body formation providing novel insight into the pathobiochemistry of acute promyelocytic leukemia, the sentrinization pathway, and regulatory mechanisms of PML body formation.

Conclusion/Outlook

New techniques to study ANA specificities or structure and function of corresponding nuclear autoantigens emerge: immunofluorescence is generally applied to fixed specimens, but recently there have been a number of fluorescent techniques that can be applied to living specimens. Purified proteins can be derivatized with a fluorophore yet still retain their function. These derivatized proteins may be injected back into a cell, thereby enabling the dynamic behavior of the protein to be observed by fluorescence microscopy. Cells and organisms may be transfected with the gene for the naturally fluorescent GFP [Chalfie et al., 1994]. An autoantigen under investigation may be cloned by fusion with the GFP gene and the distribution of the chimeric protein within a cell observed by GFP fluorescence. Fluorescence in turn may be visualized by confocal microscopy. Confocal microscopes differ from conventional (wide-field) microscopes because they do not 'see' out-of-focus objects. In a confocal microscope, most of the out-of-focus light is excluded from the final image, greatly increasing the contrast, and hence the visibility of fine details in the specimen. Thus, confocal microscopy may lead to a refined analysis of nuclear autoantigens, thereby providing new opportunities for diagnosis of systemic rheumatic diseases and identification of (new) autoantigens which may have been missed by conventional immunofluorescence. Moreover, new techniques may promote the discovery of novel nuclear organelles. The recently defined nuclear bodies called gems are an example for such a discovery. Gems are simi-

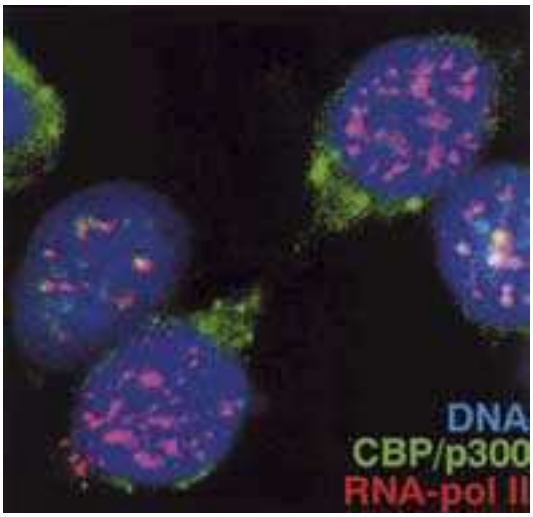


Fig. 2. Triple staining of cellular structures. HEp-2 cells were processed for immunofluorescence staining using antibodies against RNA-polymerase II (red), CBP/p300 (green), and DNA (blue). Images for each fluorescent dye were taken with a confocal laser microscope (Zeiss LSM 510) at the same time and merged digitally. In mitotic cells (along the diagonal axis) nuclear RNA-pol II speckles are spatially separated from CBP/p300 staining in the cytoplasm. In interphase cells, however, RNA-pol II colocalizes with CBP/p300 in some of the speckles (yellow, or white if DNA colocalizes) [von Mikecz and Hemmerich, in preparation].

lar in number and size to coiled bodies and are commonly found associated with them. Among other proteins, gems contain SMNs which are directly linked to spinal muscular atrophy, one of the most common human genetic diseases. SMN is involved in the nuclear organization of snRNPs and in pre-mRNA splicing [Fischer et al., 1977], suggesting a functional relationship between gems, coiled bodies and the splicing machinery.

In dual immunofluorescence labeling experiments it is possible to determine the colocalization of nuclear structures (fig. 2), yet this does not prove an intimate physical relationship between the stained antigens. The solution to this problem might be the fluorescence resonance energy transfer technique (FRET). FRET is used for quantifying the distance between two molecules conjugated to different fluorophores [Szollosi et al., 1998]. By combining optical microscopy with FRET it is possible to obtain quantitative temporal and spatial information about the binding and interaction of proteins, lipids, enzymes, DNA and RNA in vivo [Gordon et al., 1998]. In conjunction with the recent development of a variety of mutant GFPs, FRET microscopy provides the potential to measure the interaction of intracellular molecular species in intact living cells where the donor and acceptor fluorophores are actually part of the molecules themselves [Day, 1998]. Future studies will illuminate the basic principles underlying nuclear organization and will increase our understanding of how disruptions of this organization contributes to human disease, and notably to systemic autoimmune diseases.

Despite these technical advances, the events that initially trigger autoantibody production in systemic rheumatic diseases are not yet known. It seems likely that they do not merely represent epiphenomena of systemic autoimmune syndromes, because ANA segregate by syndrome: anti-nucleolar ANA in systemic sclerosis, anti-DNA/histone ANA in SLE. Rather, it is logical to assume that the origin of autoantibodies against nuclear components is linked to the etiology of systemic autoimmunity. Alteration of structure and change in the molecular context (= subcellular redistribution) of autoantigens may permit the efficient processing/presentation of previously cryptic determinants, thus breaking the tolerance of the immune system to self-determinants. The unique autoantibody response observed in the different autoimmune diseases may therefore be viewed as the time-averaged immunologic memory of the altered circumstances that initially revealed this cryptic structure and subsequently exposed these autoantigens to drive the ongoing immune response [as cited in Rosen et al., 1997].

Acknowledgments

We thank Bianca Voigt for excellent technical assistance preparing confocal images, Dr. Peter Ullmann (Carl-Zeiss AG Jena, Germany) for help with three-color imaging on a Zeiss LSM 510 confocal microscope, and Dipl.-Ing. Jakob Risch (Rheinisch-Westfälische-Technische Hochschule, Aachen, Germany) for visualization. Research cited from the authors' laboratories was supported in part by NIH grants to Eng Tan (Scripps Research Institute, La Jolla, Calif., USA) and by the Deutsche Forschungsgemeinschaft through SFB grant 503 to A.v.M.

References

Alliegro MC, Alliegro MA: Protein heterogeneity in the coiled body compartment. Exp Cell Res 1998;239:60–68.

Andrade LEC, Chan EKL, Raska I, Peebles CL, Roos G, Tan EM: Human autoantibody to a novel protein of the nuclear coiled body: Immunological characterization and cDNA cloning of p80 coilin. J Exp Med 1991;173:1406–1419.

Andrade LEC, Tan EM, Chan EKL: Immunocytochemical analysis of the coiled body in the cell cycle and during cell proliferation. Proc Natl Acad Sci USA 1993;90:1947–1951.

Andre C, Guillemin M-C, Zhu J, et al: The PML and PML/RARα domains: From autoimmunity to molecular oncology and from retinoic acid to arsenic. Exp Cell Res 1996;229:253–260.

Arents G, Moudrianakis EN: The histone fold: A ubiquitous architectural motif utilized in DNA compaction and protein dimerization. Proc Natl Acad Sci USA 1995;92:11170–11174.

Arnett FC, Reveille JD, Goldstein R, Pollard KM, Leaird K, Smith EA, Leroy EC, Fritzler MJ: Autoantibodies to fibrillarin in systemic sclerosis (scleroderma). An immunogenetic, serologic, and clinical analysis. Arthritis Rheum 1996;39:1151–1160.

Bandin O, Courvalin JC, Poupon R, Dubel L, Homberg JC, Johanet C: Specificity and sensitivity of pg210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. Hepatology 1996;23:1020–1024.

Bauren G, Wieslander L: Splicing of Balbiani ring 1 gene pre-mRNA occurs simultaneously with transcription. Cell 1994;76:183–192.

Bazett-Jones DP, Leblanc B, Herfort M, Moss T: Short-range DNA looping by the Xenopus HMG-box transcription factor, xUBF. Science 1994;264:1134–1137.

Beato M, Eisfeld K: Transcription factor access to chromatin. Nucleic Acids Res 1997;25:3559–3563.

Bennett R: Fibromyalgia, chronic fatigue syndrome, and myofascial pain. Curr Opin Rheumatol 1998;10:95–103.

Berezney R, Coffey DS: Nuclear matrix. Isolation and characterization of a framework structure from rat liver nuclei. J Cell Biol 1977;73:616–637.

Bohmann K, Ferreira JA, Lamond AI: Mutational analysis of p80 coilin indicates a functional interaction between coiled bodies and the nucleolus. J Cell Biol 1995;131:817–831.

Boulikas T: Poly(ADP-ribosyl)ation, DNA strand breaks, chromatin and cancer. Toxicol Lett 1993;67:129–150.

Briggs MW, Burkard KT, Butler JS: Rrp6p, the yeast homologue of the human PM-Scl 100-kDa autoantigen, is essential for efficient 5.8 S rRNA 3′ end formation. J Biol Chem 1998;273:13255–13263.

Bunn CC, Denton CP, Schi-Wen X, Knight C, Black CM: Anti-RNA polymerases and other autoantibody specificities in systemic sclerosis. Br J Rheumatol 1998;37:15–20.

Burlingame RW, Boey ML, Starkebaum G, Rubin RL: The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erthematosus. J Clin Invest 1994;94:184–192.

Burlingame RF, Rubin RL: Autoantibody to the nucleosome subunit (H2A-H2B)-DNA is an early and ubiquitous feature of lupus-like conditions. Mol Biol Rep 1996;23:159–166.

Cahill DP, Lengauer C, Yu J, Riggins GJ, Wilson JK, Markowitz SD, Kinzler KW, Vogelstein B: Mutations of mitotic checkpoint genes in humans cancers. Nature 1998;392:300–303.

Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC: Green fluorescent protein as a marker for gene expression. Science 1994;263:802–805.

Chan GK, Schaar BT, Yen TJ: Characterization of the kinetochore binding domain of CENP-E reveals interactions with the kinetochore proteins CENP-F and hBUBR1. J Cell Biol 1998;143:49–63.

Cole CN, Hammell CM: Nucleocytoplasmic transport: Driving and directing transport. Curr Biol 1998;8:368–372.

Courvalin JC, Wormann HJ: Nuclear envelope protein autoantibodies in primary biliary cirrhosis. Semin Liver Dis 1997;17:79–90.

Csink AK, Henikoff S: Something from nothing: The evolution and utility of satellite repeats. Trends Genet 1998;5:200–204.

Day RN: Visualization of Pit-1 transcription factor interactions in the living cell nucleus by fluorescence resonance energy transfer microscopy. Mol Endocrinol 1998;12:1410–1419.

Durm M, Sorokine-Durm I, Haar FM, Hausmann M, Ludwig H, Voisin P, Cremer C: Fast-FISH technique for rapid, simultaneous of all human centromeres. Cytometry 1998;31:153–162.

Dyck JA, Maul GG, Miller WH Jr, Chen JD, Kakizuka A, Evans RM: A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. Cell 1994;76:333–343.

Earnshaw WC, Ratrie H, Stetten G: Visualization of centromere proteins CENP-B and CENP-C on a stable dicentric chromosome in cytological spreads. Chromsoma 1989;98:1–12.

Earnshaw WC, Sullivan KF, Machlin PS, Cooke CA, Kaiser DA, Pollard TD, Rothfield NF, Cleveland DW: Molecular cloning of cDNA for CENP-B, the major human centromere autoantigen. J Cell Sci 1987;104:817–829.

Everett RD, Maul GG: HSV-1 IE protein Vmw110 causes redistribution of PML. EMBO J 1994;13:5062–5069.

Felsenfeld G: Chromatin unfolds. Cell 1996;86:13–19.

Ferreira JA, Carmo-Fonseca M, Lamond AI: Differential interaction of splicing snRNPs with coiled bodies and interchromatin granules during mitosis and assembly of daughter cell nuclei. J Cell Biol 1994;126:11–23.

Fields AP, Thompson LJ: The regulation of mitotic nuclear envelope breakdown: A role for multiple lamin kinases. Prog Cell Cycle Res 1995;1:271–286.

Fischer U, Liu X, Dreyfuss G: The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell 1997;90:1023–1029.

Fletcher TM, Hansen JC: Core histone tail domains mediate oligonucleosome folding and nucleosomal DNA organization through distinct molecular mechanisms. J Biol Chem 1995;270:25359–25362.

Fomproix N, Gebrane-Younes J, Hernandez-Verdun D: Effects of anti-fibrillarin antibodies on building of functional nucleoli at the end of mitosis. J Cell Sci 1998;111:359–372.

Frey MR, Matera AG: Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. Proc Natl Acad Sci USA 1995;92:5915–5919.

Fritzler MJ: Autoantibodies in scleroderma. J Dermatol 1993;20:257–268.

Gant TM, Wilson KL: Nuclear assembly. Annu Rev Cell Dev Biol 1997;13:669–695.

Gant TM, Goldberg MW, Allen TD: Nuclear envelope and nuclear pore asembly: Analysis of assembly intermediates by electron microscopy. Curr Opin Cell Biol 1998;10:409–415.

Gordon GW, Berry G, Liang XH, Levine B, Herman B: Quantitative fluorescence energy transfer measurements using fluorescence microscopy. Biophys J 1998;74:2702–2713.

Gordon GW, Gerbi SA, Borovjagin A: U3 snoRNA may recycle through different compartments of the nucleolus. Chromosoma 1997;105:401–406.

Goldberg MW, Allen TD: Structural and functional organization of the nuclear envelope. Curr Opin Cell Biol 1995;7:301–309.

Görlich D: Transport into and out of the cell nucleus. EMBO J 1998;17:2721–2727.

Grancell A, Sorger PK: Chromosome movement: Kinetochores motor along. Curr Biol 1998;11:382–385.

Grunstein M: Histone acetylation in chromatin structure and transcription. Nature 1997;389:349–352.

Hadjiolov AA: The nucleolus and ribosome biogenesis; in Cell Biology Monographs. Wien, Springer, 1985, vol 12.

Hamm J, Lamond AI: Spliceosome assembly: The unwinding role of DEAD-box proteins. Curr Biol 1998;8:532–534.

He D, Zeng C, Woods K, Zhong L, Turner D, Busch RK, Brinkley BR, Busch H: CENP-G: A new centromeric protein that is associated with the alpha-1 satellite DNA subfamily. Chromosoma 1998;107:189–197.

Hebbes TR, Thorne AW, Crane-Robinson C: A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J 1988;7:1395–1402.

Huang S, Spector DL: Nascent pre-mRNA transcripts are associated with nuclear regions enriched in splicing factors. Genes Dev 1991;5:2288–2302.

Izaurralde E, Adam S: Transport of macromolecules between the nucleus and the cytoplasm. RNA 1998;4:351–364.
Jenster G, Spencer TE, Burcin MM, Tsai SY, Tsai MJ, O'Malley BW: Steroid receptor induction of gene transcription: A two-step model. Proc Natl Acad Sci USA 1997;94:7879–8784.
Kamitani T, Kito K, Nguyen HP, Wada H, Fukuda-Kamitani T, Yeh ET: Identification of three major sentrinization sites in PML. J Biol Chem 1998;273:26675–26682.
Kho CJ, Huggins GS, Endege WO, Patterson C, Jain MK, Lee ME, Haber E: The olymyositis-scleroderma autoantigen interacts with the helix-loop-helix proteins E12 and E47. J Biol Chem 1997;272:13426–13431.
Koken MHM, Puvion-Dutilleul F, Guillemin M-C, et al: The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. EMBO J 1994;13:1073–1083.
Konstantinov K, von Mikecz A, Buchwald D, Gerace L, Tan EM: Autoantibodies to nuclear envelope antigens in chronic fatigue syndrome. J Clin Invest 1996;98:1888–1896.
Kubitscheck U, Peters R: Localization of single nuclear pore complexes by confocal laser scanning microscopy and analysis of their distribution. Methods Cell Biol 1998;53:79–98.
Lamond AI, Carmo-Fonseca, M: The coiled body. Trends Cell Biol 1993;3:198–204.
Lamond AI, Earnshaw WC: Structure and function in the nucleus. Science 1998;280:547–553.
Laskey RA, Gorlich D, Madine MA, Makkerh JPS, Romanowski P: Regulatory roles of the nuclear envelope. Exp Cell Res 1996;229:204–211.
Lee B, Craft JE: Molecular structure and function of autoantigens in systemic sclerosis. Int Rev Immunol 1995;12:129–144.
Lee DY, Hayes JJ, Pruss D, Wolffe AP: A positive role for histone acetylation in transcription factor access to DNA. Cell 1993;72:73–84.
Lefebvre S, Burglen L, Frezal J, Munnich A, Melki J: The role of the SMN gene in proximal spinal muscular atrophy. Hum Mol Genet 1998;7: 1531–1536.
Lerner MR, Steitz JA: Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. Proc Natl Acad Sci USA 1979;76:5495–5497.
Liu Q, Dreyfuss G: A novel nuclear structure containing the survival of motor neurons protein. EMBO J 1996;15:3555–3565.
Lorson CL, Androphy EJ: The domain encoded by exon 2 of the survival motor neuron protein mediates nucleic acid binding. Hum Mol Genet 1998;7:1269–1275.
Luger K, Maeder AW, Richmond RK, Sargent DF, Richmond TJ: X-ray structure of the nucleosome core particle at 2.8Å resolution. Nature 1997;389:251–259.
Luger K, Richmond TJ: DNA-binding within the nucleosome core. Curr Opin Struct Biol 1998; 8:33–40.
Maldarelli F, Xiang C, Chamoun G, Zeichner SL: The expression of the essential nuclear splicing factor SC35 is altered by human immunodeficiency virus infection. Virus Res 1998;53:39–51.
Maniatis T, Reed R: The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing. Nature 1987;325:673–678.
Meier UT, Blobel G: Nopp140 shuttles on tracks between nucleolus and cytoplasm. Cell 1992; 70:127–138.
Matera AG: Of coiled bodies, gems, and salmon. J Cell Biochem 1998;70:181–192.
Matera AG, Ward DC: Nucleoplasmic organization of small nuclear ribonucleoproteins in cultured human cells. J Cell Biol 1993;121:715–727.
Melese T, Xue Z: The nucleolus: An organelle formed by the act of building a ribosome. Curr Opin Cell Biol 1995;7:319–324.
Milkereit P, Tschochner H: A specialized form of RNA polymerase I, essential for initiation and growth-dependent regulation of rRNA synthesis, is disrupted during transcription. EMBO J 1998;17:3692–3703.
Misteli T, Caceres JF, Spector DL: The dynamics of a pre-RNA splicing factor in living cells. Nature 1997;387:526–527.
Misteli T, Caceres JF, Clement JQ, Krainer AR, Wilkinson MF, Spector DL: Serine phosphorylation of SR proteins is required for their recruitment to sites of transcription in vivo. J Cell Biol 1998;143:297–307.
Mitchel AR: The mammalian centromere: Its molecular structure. Mut Res 1996;372:153–162.
Mitchel AR, Jeppesen P, Hanratty D, Gosdon J: The organization of repetitive DNA sequences on human chromosomes with respect to the kinetochor using a combination of oligonucleotide primers and CREST anticentromere serum. Chromosoma 1985;101:333–341.
Monneron A, Bernhard W: Fine structural organization of the interphase nucleus in some mammalian cells. J Ultrastruct Res 1969;27:266–288.
Moroi Y, Peebles C, Fritzler MJ, Steigerwald J, Tan EM: Autoantibody to centromere (kinetochore) in scleroderma sera. Proc Natl Acad Sci USA 1980;77:1627–1631.
Müller S, Matunis MJ, Dejean A: Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. EMBO J 1998:17:61–70.
Nigg EA: Nucleocytoplasmic transport: Signals, mechanisms and regulation. Nature 1997;386: 779–787.
Nyman U, Hallman H, Hadlaczky G, Pettersson I, Sharp G, Ringertz NR: Intranuclear localization of snRNP antigens. J Cell Biol 1986;102: 137–144.
Ochs RL, Lischwe MA, Spohn WH, Busch WH: Fibrillarin: A new protein of the nucleolus identified by autoimmune sera. Biol Cell 1985; 54:123–134.
Ohno M, Fornerod M, Mattaj IW: Nucleocytoplasmic transport: The last 100 nanometers. Cell 1998;92:327–336.
Ortlepp D, Laggerbauer B, Mullner S, Achsel T, Kirschbaum B, Lührmann R: The mammalian homologue of Prp16p is overexpressed in a cell line tolerant to Leflunomide, a new immunoregulatory drug effective against rheumatoid arthritis. RNA 1998;4:1007–1018.
Pazin MJ, Kadonga JT: What's up and down with histone deacetylatin and transcription? Cell 1997;89:325–328.
Pemberton LF, Blobel G, Rosenblum JS: Transport routes through the nuclear pore complex. Curr Opin Cell Biol 1998;10:392–399.
Ochs RL, Press RI: Centromere autoantigens are associated with the nucleolus. Exp Cell Res 1992;200:339–350.
Pederson T: The plurifunctional nucleolis. Nucl Acids Res 1998;17:3871–3876.
Penman S: Rethinking cell structure. Proc Acad Sci USA 1995;92:5251–5257.
Perry RP, Hell A, Errera M, Durwald H: The role of the nucleolus in ribonucleic acid and protein synthesis. I. Incorporation of cytidine in normal and nucleolar inactivated HeLa cells. Biochim Biophys Acta 1961;49:47–57.
Petesen OH, Gerasimenko OV, Gerasimenko JV, Mogami H, Tepikin AV: The calcium store in the nuclear envelope. Cell Calcium 1998;23: 87–90.
Pluta AF, Mackay AM, Ainztain AM, Goldberg IG, Earnshaw WC: The centromere: Hub of chromosomal activities. Science 1995;270:1591–1594.
Pluta AF, Earnshaw WC, Goldberg IG: Interphase-specific association of intrinsic centromere protein CENP-C with HDaxx, a death domain-binding protein implicated in Fas-mediated cell death. J Cell Sci 1998;111:2029–2041.
Puvion E, Puvion-Dutilleul F: Ultrastructure of the nucleus in relation to transcription and splicing: Roles of perichromatin fibrils and interchromatin granules. Exp Cell Res 1996;229: 217–225.
Puvion-Dutilleul F, Besse S, Chan EK, Tan EM, Puvion E: p80-coilin: A component of coiled bodies and interchromatin granule-associated zones. J Cell Sci 1995;108:1143–1153.
Raska I, Andrade LEC, Ochs RL, Chan EKL, Chang C-M, Roos G, Tan EM: Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. Exp Cell Res 1991;195:127–137.
Rattner JB, Mack GJ, Fritzler MJ: Autoantibodies to components of the mitotic apparatus. Mol Biol Rep 1998;25:143–155.
Reimer G, Rose KM, Scheer U, Tan EM: Autoantibody to RNA-polymerase I in scleroderma sera. J Clin Invest 1987;79:65–72.
Roberts PM, Goldfarb DS: In vivo nuclear transport kinetics in *Saccharomyces cerevisiae.* Methods Cell Biol 1998;53:545–557.
Robitaille P, Tan EM: Relationship between deoxyribonucleoprotein and deoxyribonucleic acid antibodies in systemic lupus erythematosus. J Clin Invest 1973;52:316–323.
Rodriguez-Sanchez JL, Gelpi C, Juarez C, Hardin JA: A new autoantibody in scleroderma that recognizes a 90-kDa component of the nucleolus organizing region of chromatin. J Immunol 1987;123:2579–2584.
Rosen A, Casciola-Rosen L, Wigley F: Role of metal-catalized oxidation reactions in the early pathogenesis of scleroderma. Curr Opin Rheumatol 1997;9:538–543.

Saitoh H, Tomkiel J, Cooke CA, Ratrie H III, Maurer M, Rothfield NF, Earnshaw WC: CENP-C, an autoantigen in scleroderma, is a component of the human inner kinetochore plate. Cell 1992;70:115–125.

Schnapp G, Grummt I: Transcription complex formation at the mouse rDNA promoter involves the stepwise association of four transcription factors and RNA polymerase I. J Biol Chem 1991;266:24588–24595.

Schul W, van Driel R, de Jong L: A subset of poly(A) polymerase is concentrated at sites of RNA synthesis and is associated with domains enriched in splicing factors and poly(A) RNA. Exp Cell Res 1998;238:1–12.

Shelby RD, Hahn KM, Sullivan KF: Dynamic elastic behavior of alpha-satellite DNA domains visualized in situ in living human cells. J Cell Biol 1996;135:545–557.

Singer RH, Green MR: Compartmentalization of eukaryotic gene expression: Causes and effects. Cell 1997;91:291–294.

Smith KP, Carter KC, Johnson CV, Lawrence JB: U2 and U1 snRNA gene loci associate with coiled bodies. J Cell Biochem 1995;59:473–485.

Spector DL: Colocalization of U1 and U2 small nuclear RNPs by immunocytochemistry. Biol Cell 1984;51:109–112.

Spector DL: Macromolecular domains within the cell nucleus. Annu Rev Cell Biol 1993;9:265–315.

Spector DL: Nuclear organization and gene expression. Exp Cell Res 1996;229:189–197.

Sternsdorf T, Jensen K, Will H: Evidence for covalent modification of the nuclear dot-associated proteins PML and Sp100 by PIC1/SUMO-1. J Cell Biol 1997;139:1621–1634.

Stuurman N, Heins S, Aebi U: Nuclear lamins: Their structure, assembly, and interactions. J Struct Biol 1998;122:42–66.

Sullivan GJ, McStay B: Dimerization and HMG box domains 1-3 present in Xenopus UBF are sufficient for its role in transcriptional enhancement. Nucleic Acids Res 1998;26:3555–3561.

Sullivan KF, Hechenberger M, Masri K: Human CENP-A contains a histone H3 related histone fold domain that is required for targeting to the centromere. J Cell Biol 1994;127:581–592.

Svaren J, Horz W: Regulation of gene expression by nucleosomes. Curr Opin Genet Dev 1996;6:164–170.

Szollosi J, Damjanovich S, Matyus L: Application of fluorescence resonance energy transfer in the clinical laboratory: Routine and research. Cytometry 1998;34:159–179.

Szostecki C, Guldner HH, Netter HJ, Willi H: Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. J Immunol 1990;145:4338–4347.

Tan EM: Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989;44:93–151.

Tarn WY, Steitz JA: Pre-mRNA splicing: The discovery of a new spliceosome doubles the challenge. Trends Biochem Sci 1997;22:132–137.

Tyc K, Steitz J: U3, U8 and U13 comprise a new class of mammalian snRNPs localized in the cell nucleolus. EMBO J 1989;8:3113–3119.

Van Venrooij WJ, Sillekens PTG: Small nuclear RNA associated proteins: Autoantigens in connective tissue diseases. Clin Exp Rheumatol 1989;7:635–645.

Verheijen R, Kuijpers H, Vooijs P, van Venrooij W, Ramaekers F: Distribution of the 70k U1 RNA-associated protein during interphase and mitosis. J Cell Sci 1986;86:173–190.

von Mikecz A, Konstantinov K, Buchwald D, Gerace L, Tan EM: High frequency of autoantibodies to insoluble cellular antigens in patients with chronic fatigue syndrome. Arthritis Rheum 1997;40:295–305.

Wade PA, Pruss D, Wolffe AP: Histone acetylation: Chromatin in action. Trends Biochem Sci 1997;22:128–132.

Wesierska-Gadek J, Hohenauer H, Hitchmann E, Penner E: Autoantibodies from patients with primary biliary cirrhosis preferentially react with the amino-terminal domain of nuclear pore complex glycoprotein gp210. J Exp Med 1995;182:1159–1162.

Willard HF: Centromeres: The missing link in the development of human artificial chromosomes. Curr Opin Genet Dev 1998;8:219–225.

Wolffe A: Chromatin structure and function. London, Academic Press, 1992.

Wozniak RW, Rout MP, Aitchison JD: Karyopherins and kissing cousins. Trends Cell Biol 1998;8:184–188.

Wu CHH, Gall JG: U7 small nuclear RNA in C snurposomes of the Xenopus germinal vesicle. Proc Natl Acad Sci USA 1993;90:6257–6259.

Yen J, Compton DA, Wise D, Zinkowski RP, Brinkley BR, Earnshaw WC, Cleveland DW: CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. EMBO J 1991;10:1245–1254.

Yoda K, Ando S, Okuda A, Kikuchi A, Okazaki T: In vitro assembly of the CENP-B/alpha-satellite DNA/core histone complex: CENP-B causes nucleosome positioning. Genes Cells 1998;8:533–548.

Zeng CQ, Kim E, Warren SL: Dynamic relocation of transcription and splicing factors dependent upon transcriptional activity. EMBO J 1997;16:1401–1412.

Zhang GH, Taneja KL, Singer RH, Green MR: Localization of pre-mRNA splicing in mammalian nuclei. Nature 1994;372:809–812.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:28–35

Etiopathogenesis of Systemic Lupus Erythematosus

Martin Herrmann Thomas Winkler Udo Gaipl Hanns-Martin Lorenz
Thomas Geiler Joachim R. Kalden

Institute of Clinical Immunology and Rheumathology, Medical Department III,
University Erlangen-Nuremberg, Erlangen, Germany

Key Words
Systemic lupus erythematosus · Etiopathogenesis · Apoptosis · Phagocytosis · B cells

Abstract
Systemic lupus erythematosus is an autoimmune disease of unknown etiology. Research efforts of the last few years have mainly focused on basic molecular and cellular pathogenetic processes of the disease. Consequently, this paper reviews the etiopathogenetic hallmarks, such as impaired amount and presentation of nuclear antigens, production of antinuclear antibodies by T-cell-dependent B cell stimulation and organ damage by anti-dsDNA antibodies or immune complexes that are discussed at the present time. In summary, the hypothesis of a dysregulation of apoptotic cell clearance is strongly supported and broadly discussed.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease clinically characterized by a broad diversity of different symptoms. The incidence of this disease in Europe is roughly 1 in 10,000 persons. Based on novel therapeutic principles, most of the patients will experience a remission and more than 50% of SLE patients are still alive after a 5-year follow-up [1]. The serological hallmark, the appearance of anti-dsDNA antibodies associated with a lot of other autoantibody specificities, suggest polyclonal B cell activation.

The etiopathogenesis of SLE, although not yet fully understood, is doubtless a multifactorial event. Environmental factors, including viruses and other infectious agents, drugs, chemicals as well as occupational exposure and food [2], in association with a defined genetic background [3], might lead to profound alterations of the immune system. Changes in the immune system include the appearance of different autoantibodies with different specificity, altered T cell function, as well as a defective phagocytosis and changes in oncogens [4]. Based on intensive research during the last decades, three main mechanisms might contribute to the development of SLE: (1) increased amounts and abnormal presentation of potential autoantigens including nuclear antigens; (2) T-cell-dependent stimulation of B cells for the production of antinuclear antibodies, and (3) anti-dsDNA as well as immune-complex-mediated organ damage.

In the present review, we discuss how these mechanisms might be initiated and contribute to the induction and maintenance of SLE (fig. 1).

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0028$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Martin Herrmann
Institute of Clinical Immunology and Rheumatology
Medical Department III
University Erlangen-Nürnberg
D–91054 Erlangen (Germany)

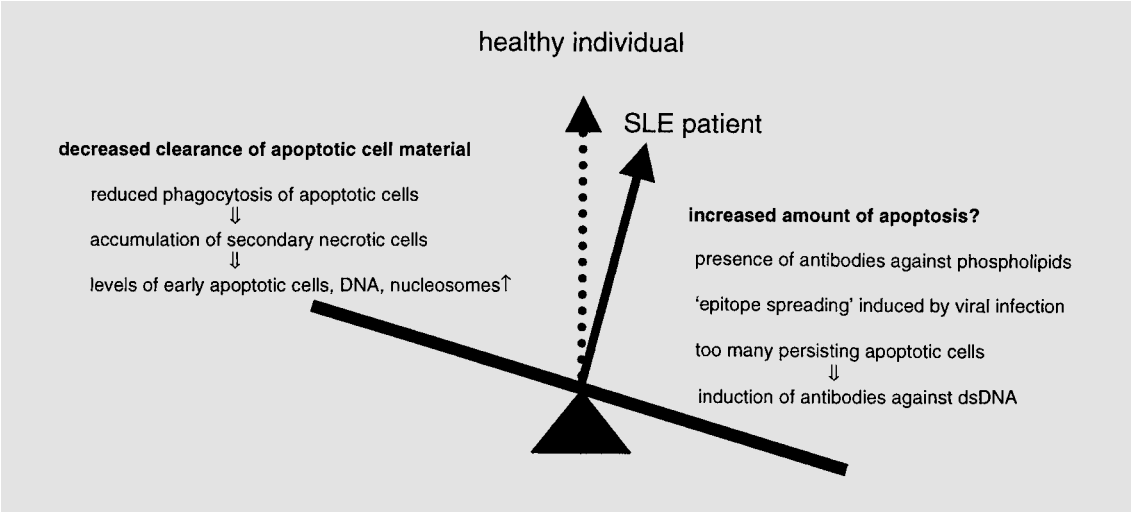


Fig. 1. Does dysregulation of apoptosis lead to SLE?

Increased Amounts and Abnormal Presentation of Nuclear Antigens

A dysregulation of apoptosis (programmed cell death) might be responsible for the induction of nuclear antibodies frequently found in SLE. This hypothesis is partly based on experiments with MRL/lpr mice, an animal model for SLE. In this mouse strain, the mean mechanism leading to an SLE-like disease was a genetic defect defined in a deficient expression of the membrane molecule Fas/Apo-1 (CD95). Ligation of this molecule was shown to induce apoptosis in various cells. Insufficient elimination of lymphocytes was observed in Fas/Apo-1-deficient animals, leading to the assumption that autoreactive lymphocytes could survive and consequently cause autoimmune phenomena [5]. However, in human SLE, the Fas/Apo-1-dependent apoptosis pathway was shown to be unaffected [6]. Patients with a defect in the Fas/Apo-1 molecule develop non-malignant lymphoproliferation accompanied by hemolytic anemia and other autoimmune symptoms, referred to as Canale-Smith syndrome [7–9].

In contrast to MRL/lpr mice or patients with a Canale-Smith syndrome, showing prolonged survival of potential autoreactive lymphocytes (due to a defective clearance of these cells by apoptosis), reports have been published that discussing a clearance defect of apoptotic cells as a possible major event in the etiopathogenesis of this disease entity. This hypothesis is based on the observation that in the execution phase of apoptosis, nuclear autoantigens can be detected in surface blebs [10, 11]. Thus, potential autoantigens might be sequestered during apoptosis and become accessible to immune competent cells. There might be two major mechanisms that could lead to an increased amount of circulating nuclear antigens in SLE: increased apoptosis or decreased clearance of apoptotic cell material as an important etiopathogenic event in SLE.

Various studies have demonstrated increased apoptosis of in-vitro-cultured blood mononuclear cells of SLE patients. However, this effect was reported not to be specific to SLE patients, since similar data were obtained in patients with systemic vasculitis or patients with mixed connective tissue disease; however, these observations are in contrast to a normal rate of apoptosis in peripheral blood mononuclear cells in rheumatoid arthritis patients [12]. The increased rate of apoptosis of peripheral blood mononuclear cells in SLE patients could be diminished by feeding the cultures with IL-2. This finding suggests that the increased apoptosis in peripheral blood mononuclear cells and specifically in lymphocytes of SLE patients is due to an increased number of circulating activated lymphocytes rather than to a disease-specific increased apoptosis. Interestingly, lymphocytes of SLE patients with bacterial infections, as indicated by increased CRP, exhibit increased spontaneous apoptosis of peripheral blood mononuclear cells [12] which could account for the clinical observation that a flare of SLE could follow an infectious episode [13, 14]. Thus, in summary, there is no evidence for increased spontaneous apoptosis of peripheral blood mononuclear cells in SLE patients, not suffering from a concomitant bacterial infection.

Concerning the clearance of apoptotic cells, we were able to demonstrate that in vitro differentiated macrophages from SLE patients show a significantly reduced phagocytosis of apoptotic cells, leading to the accumulation of secondary necrotic cells [15]. This finding might explain the increased levels of early apoptotic cells, DNA and nucleosomes observed in the circulation of SLE patients [16–19]. Since keratinocytes show an increased rate of apoptosis after UV irradiation [20], keratinocytes might also contribute to the presence of potential nuclear autoantigens in the skin after sun exposure.

Since components of the cellular nucleus are self-antigens, the immune system usually does not respond to these antigens. In this context, it is of interest that also double-stranded (ds) DNA is not immunogenic per se [21]. The mechanism of the immune response against nuclear particles in patients with SLE is not completely understood. One possible explanation would be that phagocytosis and degradation of apoptotic cells by macrophages are usually fast and efficient. Therefore nuclear antigens do not come into contact with cells of the immune system. In the case of SLE patients, the impaired clearance of apoptotic cells resulting in an accumulation of late apoptotic and secondary necrotic cells, including oligonucleosomes, might lead to activation of autoreactive T cells with subsequent anti-dsDNA antibody production [22]. However, increased amounts of nucleosomes were found in sera of patients undergoing hemodialysis, chemotherapy or irradiation treatment without leading to induction of an immune response [23–25]. This shows that an increased amount of nuclear antigen alone is not sufficient to induce an SLE-like autoimmune response.

The hypothesis that structural changes in DNA or in nucleosomes may induce an autoimmune response is based on experiments with drugs known to cause a transient form of SLE [26]. In addition, it was reported that some DNA molecules with low cytidine methylation display increased immunogenicity and induce autoimmune phenomena, including the synthesis of antibodies against dsDNA as well as immune-complex glomerulonephritis [13, 26]. There are many reports on modifications of nuclear antigens that may lead to increased immunogenicity of nuclear antigens, e.g. phosphorylation or dephosphorylation [25, 27], citrullization [28], oxidative stress in combination with heavy metals [29], mercury intoxication [30–32], the activation of transglutaminases [33], or acetylation [25]. In addition, Rosen et al. [11] were able to show that in Sindbis-virus-induced apoptosis of keratinocytes viral and nuclear cellular antigens were colocalized in the apoptotic surface blebs. Thus it can be speculated that an immune response that is initiated against viral antigens may extend and target adjoining components of the cellular nucleus. This phenomenon, called 'epitope-spreading', could be observed in mice and rabbits infected with BK virus. In this animal model it was shown that autologous dsDNA can be rendered immunogenic through complex formation with viral DNA binding protein(s), such as structural protein VP1. The formation of autoantibodies specific to dsDNA and histones could be observed [34, 35]. Immunization studies on rabbits and mice with fragments of the autoantigen Sm-B typical of SLE confirmed the possibility to induce autoantibodies recognising dsDNA by DNA protein complexes [36].

Meanwhile at least 39 proteins are known which are proteolytically cleaved during apoptosis and thereby probably modified in their immunogenicity. Seventeen of these proteins – many of them components of complex particles – are frequently targeted by autoantibodies of SLE patients [25]. Since minor changes in protein structures (e.g. proteolytic cleavage) may dramatically change the epitope hierarchy for antigen presentation, caspase activity in apoptosing cells may render cryptic epitopes immunodominant and lead to antigen presentation of epitopes to which the immune system has not achieved tolerance [37, 38].

T-Cell-Dependent Stimulation of B Cells for the Production of Antinuclear Antibodies

Characteristics of Monoclonal Human Anti-dsDNA Autoantibodies

The fact that serum titers of anti-dsDNA antibodies might mirror the disease activity of SLE has prompted intensive research on the pathogenic role as well as on the origin of anti-dsDNA antibodies which were first described more than 25 years ago [39]. With regard to mechanisms inducing anti-dsDNA antibodies, several reviews have been published [40, 41].

The establishment of anti-DNA hybridomas from SLE patients based on modern technologies and the analysis of the immunoglobulin variable genes have contributed important information on possible induction mechanisms. Initially, it was reported that even normal individuals are able to express anti-dsDNA antibodies with a specificity similar to that of SLE patients. However, recently published studies clearly indicate that the anti-dsDNA antibodies demonstrated in healthy people are mainly of the

IgM isotype and have low affinity for DNA, and particularly for dsDNA. These antibodies are so-called 'natural autoantibodies' and are characterized by wide cross-reactivity and germline-encoded immunoglobulin gene sequences. In the mouse, at least part of these natural autoantibodies are produced by CD5+ or Ly1+ B cells, respectively. The physiological role of naturally occurring autoantibodies is still under discussion. One possibility is that these antibodies present first-line defense mechanisms against invading microorganisms. In addition, it is of interest that this B cell subset was reported to be of importance for antigen presentation to T cells. This was shown for rheumatoid factor-secreting B cells [42] and more recently in a transgenic mouse model [43]. Natural antibodies secreting B lymphocytes constitute a large proportion of the fetal B cell repertoire and contribute to natural immunity in the developing immune system and may participate in shaping the adult B cell repertoire [44].

In contrast to naturally occurring antibodies, anti-dsDNA antibodies involved in the pathogenesis of SLE patients are of the IgG isotype, with a high affinity for dsDNA. They are only seldom detectable in normal sera. Detailed studies of hybridomas reflecting pathogenic anti-dsDNA antibodies established from lupus-prone mice [45] and from SLE patients [46, 47] revealed that the IgG-anti-dsDNA antibody response bears all the characteristics of an antigen-driven, T-cell-dependent immune response. Molecular analyses of human anti-dsDNA antibody clones revealed somatic mutations in the complementarity-determining regions (CDRs), the site of antigen contact. It is of special interest that a significant exchange towards the amino acids arginine and asparagine occurs in the CDRs; this exchange is particularly important for dsDNA binding, most likely due to electrostatic interaction. The bias towards arginine and asparagine had been generated by typical reading frame usage of the D elements, by frameshifts in the Vκ-J-κ junction, or by somatic mutations.

A review on human anti-dsDNA monoclonal antibodies has recently been published [48], indicating that there is no VH restriction within IgM antibodies. In contrast, IgG anti-DNA antibodies appear to use mainly members of the VH3 or VH4 family. Whether this restricted usage is only a reflection of a limited number of established IgG monoclonals remains open to discussion. κ and λ light gene isotypes are represented normally, no specific Vκ or Vλ members were shown to be prominent.

The nature of epitopes recognized by anti-dsDNA antibodies is still a matter of debate. When SLE sera and human monoclonal anti-dsDNA antibodies were employed to analyze their preferential recognition site on DNA molecules, anti-dsDNA antibodies derived from SLE patients preferentially selected sequences expected to form nonrandom B-DNA structures. In addition, competition studies applying the Farr assay confirmed the increased affinity of selected epitopes for anti-dsDNA antibodies as compared to random B-DNA. From these data it can be concluded that bent DNA with in-phase adenosine triplets might be implicated in the induction and maintenance of anti-dsDNA antibody responses [49].

Role of T Cell Induction of Anti-dsDNA Antibodies

It is well established that affinity maturation, memory formation and isotype switch are T-cell-dependent immune processes. The similarity of anti-dsDNA response in murine and human SLE strongly support the hypothesis of the involvement of T helper cells in this pathogenesis [50–53]. In addition, histone-specific T cell clones were established from healthy donors and SLE patients, which were able to induce autologous cultured B cells to secrete anti-dsDNA antibodies [22, 54]. Since autoreactive T cell clones and T cell lines can also be established from nonautoimmune individuals, the question of the nature of the antigen or peptides stimulating the proliferation of T cell remains elusive. In addition, the specificity of help for anti-dsDNA antibody production appears not yet established, since in vivo activation of anti-dsDNA reactive B cells or simply a higher precursor frequency of anti-DNA reactive B cells in autoimmune individuals might result in in vitro anti-dsDNA production mediated by the secretion of certain lymphokines providing nonspecific help by T cells. In this context, the recent report by Naiki et al. [52] is of interest, indicating that T helper clones from autoimmune mice, which were able to provide help for anti-dsDNA production, produced IL-4 but not interferon-gamma and could therefore be classified as belonging to the TH2 phenotype [52].

Using either autologous apoptotic cells or isolated histones, we were able to induce T cell proliferation in peripheral blood mononuclear cells isolated from normal healthy donors or patients with SLE [22]. Cloning of these T cells confirmed that histones were targeted by some of these T cell clones. These results show that increased concentrations and/or an abnormal presentation of nuclear antigens are able to stimulate autoreactive peripheral T cells in vitro. In coincubation experiments we were able to demonstrate that coincubation of the histone-specific T cells with autologous B cells induces the production of anti-dsDNA autoantibodies. These results were con-

firmed in similar assays with human cells [51, 55, 56] as well as in experiments on different mouse strains [50, 52, 54, 57, 58].

These in vitro experiments suggest that an excess of persisting apoptotic cells may lead to the induction of SLE-specific antibodies against dsDNA. Experiments with thymectomized [59], T-cell-receptor-depleted [60] or anti-CD4-antibody-treated mice [61] confirmed the contribution of T cells in the etiopathogenesis of murine SLE. In humans the efficiency of the T-cell-specific immunosuppresive cyclosporin A suggests a T cell involvement in the etiopathogenesis of SLE.

Anti-dsDNA- and Immune-Complex-Mediated Organ Damages

Tissue Damage by Anti-dsDNA Antibodies

Just as the origin of anti-dsDNA antibodies, the mechanisms by which these antibodies could cause tissue damage remain unclear. Suzuki et al. [53] suggested that cationic anti-dsDNA antibodies binding to heparan-sulfate, a major glucosaminoglycan in the glomerular basement membrane, locally form immune complexes with the subsequent development of lupus nephritis [53]. Although, several other communications have postulated that anti-heparan-sulfate antibodies might be directly involved in the development of renal disease in SLE [62, 63], clear evidence is still missing. Morioka et al. [64] recently provided evidence that antibodies from sera of SLE patients can form soluble histone-DNA- anti-DNA immune complexes that bind to rat glomerular capillary wall in vivo, indicating yet another mechanism by which kidney injury might occur. Raz et al. [65] postulated a more direct role of anti-dsDNA antibodies in disease pathology. Their work complementing earlier studies that had shown that murine monoclonal anti-DNA antibodies bind to renal tissue demonstrated that murine anti-DNA monoclonal antibodies, but not monoclonal anti-RNA or anti-histone antibodies bind weakly to normal epithelium and strongly to various human tumor cell lines, indicating the possibility of a direct pathogenic effect on cell surfaces.

Generating 4 human anti-dsDNA hybridomas from 1 SLE patient, we were able to demonstrate that 3 out of 4 anti-dsDNA monoclonal antibodies had high affinity for dsDNA. The antibody with low affinity cross-reacted with cardiolipin. Biological studies of the monoclonals revealed different interesting characteristics. Thus, the monoclonal antibody 33.C9 was demonstrated in a SCID mouse model to produce proteinuria by depositing in the glomeruli, the mesangium, and capillary wall of kidneys [66]. Furthermore, antibody 33.H11 cross-reacted with the ribosomal protein S1 and suppressed in vitro protein synthesis. In addition, 33.H11 inhibited in vitro translation of globulin mRNA, which was enhanced when the reticulocyte lysate was treated with DNAse. From these data it might be speculated that suppression of protein synthesis could be regarded as a pathogenic mechanism of anti-dsDNA antibodies, since it had previously been shown that some anti-dsDNA antibodies were able to penetrate living cells in culture [67, 68]. The different biological activities of monoclonal anti-dsDNA antibodies obtained from one patient give rise to the hypothesis that at least one possible mechanism leading to a specific organ targeting by anti-dsDNA antibodies in SLE could be due to a cross-reactivity of anti-dsDNA antibody clones with organ-specific antigens. Further studies are presently in progress to substantiate this hypothesis. Forthcoming research will doubtless reveal new and important mechanisms with regard to pathogenic tissue-destructive activities of anti-dsDNA antibodies which might additionally serve as a basis for the development of new therapeutic principles.

Organ Damage Mediated by Immune Complexes

Deposition of glomerular immune complexes could be observed in mice injected with anti-dsDNA antibodies. Deposition or in situ formation of immune complexes led to structural and functional kidney alterations [69]. Structural differences, such as charge, isotype or kryoprecipitation, influence the pathogenicity of ds-DNA antibodies. Glomerular binding is caused by a charge-dependent interaction of DNA, histones or nucleosomes with elements of the glomerular basement membrane such as laminin or heparan sulfate [70, 71]. An alternative pathway is the formation of nucleosome containing immune complexes and their deposition at anionic parts of the basement membrane [72–76]. Immune complex deposition may then activate the complement system with consecutive complement consumption. This illustrates why decreased concentrations of C3 and C4 are established as activation parameters of SLE. Activation of the complement system leads to stimulation of the cellular immune system with granulocyte and lymphocyte infiltration [71]. The excretion of lysosomal enzymes and oxygen radicals may finally cause tissue damage resulting in proteinuria and kidney failure. Similar pathogenetic mechanisms

may also work in SLE vasculitis and other organ damage. Another important pathogenetic factor in the pathological cascade might be the defective clearance of immune complexes by an unfavorable expression of Fc receptors capable of binding the Fc part of IgG. An important consequence could be that immune complexes are not adequately cleared. We have been able to show that SLE patients with a certain Fcγ type II receptor genotype show proteinuria, hemolytic anemia, hypocomplementemia and snRNP antibodies at a significantly higher level [77]. In addition, dysfunction of nucleosome receptors [78, 79], Fc receptors [80], C3b receptors [81, 82], or defective resolution of DNA-nucleosome complexes have been discussed as pathogenetic factors [83–86].

Antibodies against phospholipids (e.g. cardiolipin or phosphatidylserine) are often associated with systemic autoimmunopathies, such as SLE. Phosphatidylserine is deposited on dying cells as an early sign of apoptotic cell death and serves as a phagocyte recognition molecule for apoptotic cells. This again shows the close relationship between autoantibodies and apoptosis. Although there are many questions to be asked and answered, some of the arguments mentioned in this review support the hypothesis of a contribution of a 'dysregulation' of apoptosis in the etiopathogenesis of SLE. The answers to the open questions will help establish a specific therapy for the SLE.

References

1 Swaak AJ, Nossent JC, Bronsveld W, Van Rooyen A, Nieuwenhuys EJ, Theuns L, Smeenk RJ: Systemic lupus erythematosus. I. Outcome and survival: Dutch experience with 110 patients studied prospectively. Ann Rheum Dis 1989;48:447–454.

2 Love LA: New environmental agents associated with lupus-like disorders. Lupus 1994;3: 467–471.

3 Hartung K, Baur MP, Coldewey R, Fricke M, Kalden JR, Lakomek HJ, Peter HH, Schendel D, Schneider PM, Seuchter SA, et al: Major histocompatibility complex haplotypes and complement C4 alleles in systemic lupus erythematosus. Results of a multicenter study. J Clin Invest 1992;90:1346–1351.

4 Kalden JR, Winkler TH, Herrmann M, Krapf F: Pathogenesis of SLE: Immunopathology in man. Rheumatol Int 1991;11:95–100.

5 Watson ML, Rao JK, Gilkeson GS, Ruiz P, Eicher EM, Pisetsky DS, Matsuzawa A, Rochelle JM, Seldin MF: Genetic analysis of MRL-lpr mice: Relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci. J Exp Med 1992;176: 1645–1656.

6 Mysler E, Bini P, Drappa J, Ramos P, Friedman SM, Krammer PH, Elkon KB: The apoptosis–1/Fas protein in human systemic lupus erythematosus. J Clin Invest 1994;93:1029–1034.

7 Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, Strober W, Lenardo MJ, Puck JM: Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 1995;81:935–946.

8 Drappa J, Vaishnaw AK, Sullivan KE, Chu JL, Elkon KB: Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity (see comments). N Engl J Med 1996;335:1643–1649.

9 Rieux-Laucat F, Le Deist F, Hivroz C, Roberts IA, Debatin KM, Fischer A, de Villartay JP: Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science 1995;268:1347–1349.

10 Casciola-Rosen LA, Anhalt G, Rosen A: Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes (see comments). J Exp Med 1994;179:1317–1330.

11 Rosen A, Casciola-Rosen L, Ahearn J: Novel packages of viral and self-antigens are generated during apoptosis. J Exp Med 1995;181: 1557–1561.

12 Lorenz HM, Grunke M, Hieronymus T, Herrmann M, Kuhnel A, Manger B, Kalden JR: In vitro apoptosis and expression of apoptosis-related molecules in lymphocytes from patients with systemic lupus erythematosus and other autoimmune diseases (see comments). Arthritis Rheum 1997;40:306–317.

13 Krieg AM: CpG DNA: A pathogenic factor in systemic lupus erythematosus? J Clin Immunol 1995;15:284–292.

14 Tsai YT, Chiang BL, Kao YF, Hsieh KH: Detection of Epstein-Barr virus and cytomegalovirus genome in white blood cells from patients with juvenile rheumatoid arthritis and childhood systemic lupus erythematosus. Int Arch Allergy Immunol 1995;106:235–240.

15 Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR: Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. Arthritis Rheum 1998;41:1241–1250.

16 Perniok A, Wedekind F, Herrmann M, Specker C, Schneider M: High levels of circulating early apoptic peripheral blood mononuclear cells in systemic lupus erythematosus. Lupus 1998;7: 113–118.

17 Raptis L, Menard HA: Quantitation and characterization of plasma DNA in normals and patients with systemic lupus erythematosus. J Clin Invest 1980;66:1391–1399.

18 McCoubrey-Hoyer A, Okarma TB, Holman HR: Partial purification and characterization of plasma DNA and its relation to disease activity in systemic lupus erythematosus. Am J Med 1984;77:23–34.

19 Steinman CR: Circulating DNA in systemic lupus erythematosus. Isolation and characterization. J Clin Invest 1984;73:832–841.

20 Golan TD, Elkon KB, Gharavi AE, Krueger JG: Enhanced membrane binding of autoantibodies to cultured keratinocytes of systemic lupus erythematosus patients after ultraviolet B/ultraviolet A irradiation. J Clin Invest 1992; 90:1067–1076.

21 Krapf F, Herrmann M, Leitmann W, Kalden JR: Antibody binding of macromolecular DNA and RNA in the plasma of SLE patients. Clin Exp Immunol 1989;75:336–342.

22 Voll RE, Roth EA, Girkontaite I, Fehr H, Herrmann M, Lorenz HM, Kalden JR: Histone-specific Th0 and Th1 clones derived from systemic lupus erythematosus patients induce double-stranded DNA antibody production. Arthritis Rheum 1997;40:2162–2171.

23 Rumore P, Muralidhar B, Lin M, Lai C, Steinman CR: Haemodialysis as a model for studying endogenous plasma DNA: Oligonucleosome-like structure and clearance. Clin Exp Immunol 1992;90:56–62.

24 Itescu S: Rheumatic aspects of acquired immunodeficiency syndrome. Curr Opin Rheumatol 1996;8:346–353.

25 Utz PJ, Hottelet M, van Venrooij WJ, Anderson P: Association of phosphorylated serine/arginine (SR) splicing factors with the U1-small ribonucleoprotein (snRNP) autoantigen complex accompanies apoptotic cell death. J Exp Med 1998;187:547–560.

26 Yung RL, Quddus J, Chrisp CE, Johnson KJ, Richardson BC: Mechanism of drug-induced lupus. I. Cloned Th2 cells modified with DNA methylation inhibitors in vitro cause autoimmunity in vivo. J Immunol 1995;154:3025–3035.
27 Utz PJ, Hottelet M, Schur PH, Anderson P: Proteins phosphorylated during stress-induced apoptosis are common targets for autoantibody production in patients with systemic lupus erythematosus. J Exp Med 1997;185:843–854.
28 Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ: Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998; 101:273–281.
29 Rosen A, Casciola-Rosen L, Wigley F: Role of metal-catalyzed oxidation reactions in the early pathogenesis of scleroderma. Curr Opin Rheumatol 1997;9:538–543.
30 Enestrom S, Hultman P: Does amalgam affect the immune system? A controversial issue. Int Arch Allergy Immunol 1995;106:180–203.
31 Pollard KM, Lee DK, Casiano CA, Bluthner M, Johnston MM, Tan EM: The autoimmunity-inducing xenobiotic mercury interacts with the autoantigen fibrillarin and modifies its molecular and antigenic properties. J Immunol 1997;158:3521–3528.
32 Takeuchi K, Turley SJ, Tan EM, Pollard KM: Analysis of the autoantibody response to fibrillarin in human disease and murine models of autoimmunity. J Immunol 1995;154:961–971.
33 Melino G, Piacentini M: 'Tissue' transglutaminase in cell death: A downstream or a multifunctional upstream effector? FEBS Lett 1998; 430:59–63.
34 Fredriksen K, Osei A, Sundsfjord A, Traavik T, Rekvig OP: On the biological origin of anti-double-stranded (ds) DNA antibodies: Systemic lupus erythematosus-related anti-dsDNA antibodies are induced by polyomavirus BK in lupus-prone (NZB × NZW) F1 hybrids, but not in normal mice. Eur J Immunol 1994;24:66–70.
35 Rekvig OP, Fredriksen K, Brannsether B, Moens U, Sundsfjord A, Traavik T: Antibodies to eukaryotic including autologous, native DNA are produced during BK virus infection but not after immunization with non-infectious BK DNA. Scand J Immunol 1992;36:487–495.
36 James JA, Gross T, Scofield RH, Harley JB: Immunoglobulin epitope spreading and autoimmune disease after peptide immunization: Sm B/B′-derived PPPGMRPP and PPPGIRGP induce spliceosome autoimmunity. J Exp Med 1995;181:453–461.
37 Moudgil KD, Sercarz EE, Grewal IS: Modulation of the immunogenicity of antigenic determinants by their flanking residues. Immunol Today 1998;19:217–220.
38 Benichou G, Fedoseyeva E, Olson CA, Geysen HM, McMillan M, Sercarz EE: Disruption of the determinant hierarchy on a self-MHC peptide: Concomitant tolerance induction to the dominant determinant and priming to the cryptic self-determinant. Int Immunol 1994;6: 131–138.
39 Tan EM, Schur PH, Carr PI, Kunkel HG: Desoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. J Clin Invest 1966;45: 1732–1738.
40 Winkler TH, Kalden JR: Origin of anti-DNA autoantibodies in SLE (editorial). Lupus 1994; 3:75–76.
41 Rahman MA, Isenberg DA: Autoantibodies in systemic lupus erythematosus. Curr Opin Rheumatol 1994;6:468–473.
42 Roosnek E, Lanzavecchia A: Efficient and selective presentation of antigen-antibody complexes by rheumatoid factor B cells. J Exp Med 1991;173:487–489.
43 Tighe H, Chen PP, Tucker R, Kipps TJ, Roudier J, Jirik FR, Carson DA: Function of B cells expressing a human immunoglobulin M rheumatoid factor autoantibody in transgenic mice. J Exp Med 1993;177:109–118.
44 Coutinho A, Freitas AA, Holmberg D, Grandien A: Expression and selection of murine antibody repertoires. Int Rev Immunol 1992;8: 173–187.
45 Shlomchik M, Mascelli M, Shan H, Radic MZ, Pisetsky D, Marshak-Rothstein A, Weigert M: Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J Exp Med 1990;171:265–292.
46 van Es JH, Gmelig Meyling FH, van de Akker WR, Aanstoot H, Derksen RH, Logtenberg T: Somatic mutations in the variable regions of a human IgG anti-double-stranded DNA autoantibody suggest a role for antigen in the induction of systemic lupus erythematosus. J Exp Med 1991;173:461–470.
47 Winkler TH, Fehr H, Kalden JR: Analysis of immunoglobulin variable region genes from human IgG anti-DNA hybridomas. Eur J Immunol 1992;22:1719–1728.
48 Isenberg DA, Ehrenstein MR, Longhurst C, Kalsi JK: The origin, sequence, structure, and consequences of developing anti-DNA antibodies. A human perspective. Arthritis Rheum 1994;37:169–180.
49 Herrmann M, Winkler TH, Fehr H, Kalden JR: Preferential recognition of specific DNA motifs by anti-double-stranded DNA autoantibodies. Eur J Immunol 1995;25:1897–1904.
50 Ando DG, Sercarz EE, Hahn BH: Mechanisms of T and B cell collaboration in the in vitro production of anti-DNA antibodies in the NZB/NZW F1 murine SLE model. J Immunol 1987; 138:3185–3190.
51 Shivakumar S, Tsokos GC, Datta SK: T cell receptor alpha/beta expressing double-negative (CD4–/CD8–) and CD4+ T helper cells in humans augment the production of pathogenic anti-DNA autoantibodies associated with lupus nephritis. J Immunol 1989;143:103–112.
52 Naiki M, Chiang BL, Cawley D, Ansari A, Rozzo SJ, Kotzin BL, Zlotnik A, Gershwin ME: Generation and characterization of cloned T helper cell lines for anti-DNA responses in NZB.H-2bm12 mice. J Immunol 1992;149: 4109–4115.
53 Suzuki N, Harada T, Mizushima Y, Sakane T: Possible pathogenic role of cationic anti-DNA autoantibodies in the development of nephritis in patients with systemic lupus erythematosus. J Immunol 1993;151:1128–1136.
54 Datta SK, Patel H, Berry D: Induction of a cationic shift in IgG anti-DNA autoantibodies. Role of T helper cells with classical and novel phenotypes in three murine models of lupus nephritis. J Exp Med 1987;165:1252–1268.
55 Theocharis S, Sfikakis PP, Lipnick RN, Klipple GL, Steinberg AD, Tsokos GC: Characterization of in vivo mutated T cell clones from patients with systemic lupus erythematosus. Clin Immunol Immunopathol 1995;74:135–142.
56 Murakami M, Kumagai S, Sugita M, Iwai K, Imura H: In vitro induction of IgG anti-DNA antibody from high density B cells of systemic lupus erythematosus patients by an HLA DR-restricted T cell clone. Clin Exp Immunol 1992;90:245–250.
57 Mohan C, Shi Y, Laman JD, Datta SK: Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. J Immunol 1995;154:1470–1480.
58 Sobel ES, Kakkanaiah VN, Kakkanaiah M, Cheek RL, Cohen PL, Eisenberg RA: T-B collaboration for autoantibody production in lpr mice is cognate and MHC-restricted. J Immunol 1994;152:6011–6016.
59 Steinberg AD, Roths JB, Murphy ED, Steinberg RT, Raveche ES: Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. J Immunol 1980;125:871–873.
60 Mihara M, Ohsugi Y, Saito K, Miyai T, Togashi M, Ono S, Murakami S, Dobashi K, Hirayama F, Hamaoka T: Immunologic abnormality in NZB/NZW F1 mice. Thymus-independent occurrence of B cell abnormality and requirement for T cells in the development of autoimmune disease, as evidenced by an analysis of the athymic nude individuals. J Immunol 1988;141:85–90.
61 Wofsy D, Seaman WE: Reversal of advanced murine lupus in NZB/NZW F1 mice by treatment with monoclonal antibody to L3T4. J Immunol 1987;138:3247–3253.
62 Pirner K, Rascu A, Nurnberg W, Rubbert A, Kalden JR, Manger B: Evidence for direct anti-heparin-sulphate reactivity in sera of SLE patients. Rheumatol Int 1994;14:169–174.
63 Fillit H, Shibata S, Sasaki T, Spiera H, Kerr LD, Blake M: Autoantibodies to the protein core of vascular basement membrane heparan sulfate proteoglycan in systemic lupus erythematosus. Autoimmunity 1993;14:243–249.

64 Morioka T, Fujigaki Y, Batsford SR, Woitas R, Oite T, Shimizu F, Vogt A: Anti-DNA antibody derived from a systemic lupus erythematosus (SLE) patient forms histone-DNA-anti-DNA complexes that bind to rat glomeruli in vivo. Clin Exp Immunol 1996;104:92–96.
65 Raz E, Ben-Bassat H, Davidi T, Shlomai Z, Eilat D: Cross-reactions of anti-DNA autoantibodies with cell surface proteins. Eur J Immunol 1993;23:383–390.
66 Ehrenstein MR, Katz DR, Griffiths MH, Papadaki L, Winkler TH, Kalden JR, Isenberg DA: Human IgG anti-DNA antibodies deposit in kidneys and induce proteinuria in SCID mice. Kidney Int 1995;48:705–711.
67 Tsuzaka K, Leu AK, Frank MB, Movafagh BF, Koscec M, Winkler TH, Kalden JR, Reichlin M: Lupus autoantibodies to double-stranded DNA cross-react with ribosomal protein S1. J Immunol 1996;156:1668–1675.
68 Tsuzaka K, Winkler TH, Kalden JR, Reichlin M: Autoantibodies to double-stranded (ds)DNA immunoprecipitate 18S ribosomal RNA by virtue of their interaction with ribosomal protein S1 and suppress in vitro protein synthesis. Clin Exp Immunol 1996;106:504–508.
69 Vlahakos DV, Foster MH, Adams S, Katz M, Ucci AA, Barrett KJ, Datta SK, Madaio MP: Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. Kidney Int 1992;41:1690–1700.
70 Mohan C, Datta SK: Lupus: Key pathogenic mechanisms and contributing factors. Clin Immunol Immunopathol 1995;77:209–220.
71 Belmont HM, Abramson SB, Lie JT: Pathology and pathogenesis of vascular injury in systemic lupus erythematosus. Interactions of inflammatory cells and activated endothelium. Arthritis Rheum 1996;39:9–22.
72 Termaat RM, Assmann KJ, Dijkman HB, van Gompel F, Smeenk RJ, Berden JH: Anti-DNA antibodies can bind to the glomerulus via two distinct mechanisms. Kidney Int 1992;42:1363–1371.
73 Madaio MP, Carlson J, Cataldo J, Ucci A, Migliorini P, Pankewycz O: Murine monoclonal anti-DNA antibodies bind directly to glomerular antigens and form immune deposits. J Immunol 1987;138:2883–2889.
74 Morioka T, Woitas R, Fujigaki Y, Batsford SR, Vogt A: Histone mediates glomerular deposition of small size DNA anti-DNA complex. Kidney Int 1994;45:991–997.
75 Kramers C, Hylkema MN, van Bruggen MC, van de Lagemaat R, Dijkman HB, Assmann KJ, Smeenk RJ, Berden JH: Anti-nucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane in vivo. J Clin Invest 1994;94:568–577.
76 Faaber P, Rijke TP, van de Putte LB, Capel PJ, Berden JH: Cross-reactivity of human and murine anti-DNA antibodies with heparan sulfate. The major glycosaminoglycan in glomerular basement membranes. J Clin Invest 1986;77:1824–1830.
77 Manger K, Repp R, Spriewald BM, Rascu A, Geiger A, Wassmuth R, Westerdaal NA, Wentz B, Manger B, Kalden JR, van de Winkel JG: Fcgamma receptor IIa polymorphism in Caucasian patients with systemic lupus erythematosus: Association with clinical symptoms. Arthritis Rheum 1998;41:1181–1189.
78 Bennett RM, Peller JS, Merritt MM: Defective DNA-receptor function in systemic lupus erythematosus and related diseases: Evidence for an autoantibody influencing cell physiology. Lancet 1986;1:186–188.
79 Bennett RM, Kotzin BL, Merritt MJ: DNA receptor dysfunction in systemic lupus erythematosus and kindred disorders. Induction by anti-DNA antibodies, antihistone antibodies, and antireceptor antibodies. J Exp Med 1987;166:;850–863.
80 Frank MM, Hamburger MI, Lawley TJ, Kimberly RP, Plotz PH: Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. N Engl J Med 1979;300:518–523.
81 Taylor RP, Horgan C, Buschbacher R, Brunner CM, Hess CE, O'Brien WM, Wanebo HJ: Decreased complement mediated binding of antibody/3H-dsDNA immune complexes to the red blood cells of patients with systemic lupus erythematosus, rheumatoid arthritis, and hematologic malignancies. Arthritis Rheum 1983;26:736–744.
82 Wilson JG, Fearon DT: Altered expression of complement receptors as a pathogenetic factor in systemic lupus erythematosus. Arthritis Rheum 1984;27:1321–1328.
83 Rynes RI: Inherited complement deficiency states and SLE. Clin Rheum Dis 1982;8:29–47.
84 Robey FA, Jones KD, Steinberg AD: C-reactive protein mediates the solubilization of nuclear DNA by complement in vitro. J Exp Med 1985;161:1344–1356.
85 Morgan BP, Walport MJ: Complement deficiency and disease. Immunol Today 1991;12:301–306.
86 Christiansen FT, Zhang WJ, Griffiths M, Mallal SA, Dawkins RL: Major histocompatibility complex (MHC) complement deficiency, ancestral haplotypes and systemic lupus erythematosus (SLE): C4 deficiency explains some but not all of the influence of the MHC. J Rheumatol 1991;18:1350–1358.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:36–45

Use of Immunoglobulin Variable-Region Genes by Normal Subjects and Patients with Systemic Lupus Erythematosus

A. Hansen[a] T. Dörner[b] P.E. Lipsky[c]

[a]Outpatients Clinic, [b]Clinic of Rheumatology of the University Hospital Charité, Berlin, Germany;
[c]Harold C. Simmons Arthritis Research Center, University of Texas, Southwestern Medical Center at Dallas, Dallas, Tex., USA

Key Words

IgV gene use · Natural antibody · Systemic lupus erythematosus · Pathogenic autoantibody

Abstract

Antibodies to specific autoantigens are serological hallmarks of systemic autoimmune diseases. These autoantibodies are thought to represent a consequence of immune dysregulation in these conditions, and, in part, have been shown to be involved in their pathologic consequences. However, the mechanisms that lead to the production of autoantibodies are still unknown. The observation that certain autoantibodies are frequently encoded by a limited number of immunoglobulin (Ig) variable-region gene segments suggested that a bias in the development of the Ig repertoire might play a role in the tendency to develop autoimmunity. Whether the use of these individual gene segments is random or different in normal subjects and patients with systemic autoimmune disorders remains a matter of controversy. New approaches for the analysis of variable-region genes from unstimulated individual human B cells employing the single-cell polymerase chain reaction have provided new insights in the B cell repertoire of both normal subjects and patients with systemic autoimmune diseases. Using this approach, the analysis of nonproductive and productive Ig variable-region gene rearrangements made it possible to distinguish molecular processes, as manifested in the nonproductive repertoire, from subsequent selection influences. An initial study in a patient with systemic lupus erythematosus has led to the hypothesis that the molecular generation of the B cell repertoire is similar in patients and normal subjects but subsequent influences and, most notably, extensive mutations and receptor editing differ significantly in shaping the peripheral IgV gene use by persons with autoimmune diseases.

Introduction

The specificity of an antibody for an antigen is determined by three complementarity determining regions (CDRs) in the variable (V) region of both heavy (H) and light (L) chains. Each B lymphocyte displays a unique set of CDRs that is interposed in the tertiary structure of the protein to form the classical antigenic binding site [1]. At the molecular level, a high degree of diversity of the immunoglobulin (Ig) variable region (IgV) is generated during early B cell development by the somatic recombi-

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. med. Arne Hansen
Medizinische Universitätspoliklinik Charité
Schumannstrasse 20/21, D–10117 Berlin (Germany)
Tel. +49 30 2802 2012, Fax +49 30 2802 1308
E-Mail ahansen@charite.de

nation process [2] that assembles functional genes by successive rearrangements of one of a number of joining (J), diversity (D) and variable (V) minigene elements of the heavy chain, followed by V-J rearrangement of the light chain. During this recombination, further diversity can be introduced by exonuclease activity as well as the addition of nontemplated N-terminal (N) nucleotides at the joining sites. Moreover, the association of heavy and light chain genes is random [3, 4]. Somatic hypermutation and, occasionally, secondary rearrangement of upstream localized V gene segments (receptor replacement or editing) may further diversify the IgV gene repertoire following antigen exposure [3]. These various mechanisms are able to generate a highly diversified array of IgV gene products.

However, studies in the mouse have indicated that the B cell repertoire is limited and developmentally regulated. Thus, certain V_H, D and J_H segments are preferentially used during neonatal development [5, 6]. Moreover, diversification mechanisms, such as N-terminal addition and somatic hypermutation, do not appear to play an important role during early development of the murine B cells [7]. Similar restrictions in the use of individual IgV gene elements have also been shown for human fetal and neonatal B cells [8–14]. In contrast to observations in the mouse [5–7], however, the chromosomal order of human V_H gene segments does not appear to play an important role for the frequency of V_H use in V_HDJ_H rearrangements [10, 11]. Of note and similar to the developing murine repertoire, a limited number of individual human IgV gene segments has been found to be frequently used in naturally occurring (auto)antibodies in both fetuses and adults [12–22] as well as in putatively pathogenic autoantibodies [20–27]. These observations have led to the concept of 'autoantibody-associated IgV genes' [for reviews, see ref. 27, 28]. Moreover, this 'autoantibody-associated' IgV repertoire has been thought to recapitulate that expressed by CD5+ B cell lines [12] and in certain B cell malignancies [27].

More recent studies [28–36] have revealed that the entire peripheral B cell repertoire in normal human adults is limited in its IgV gene representation as well. Of note, combining molecular and serological/anti-idiotypic findings, a strong overlap has been noted between this biased IgV gene repertoire in normal adults and that used in fetal Igs, autoantibodies and B cell malignancies [8–43]. For example, it could be clearly demonstrated that V_H gene use by patients with chronic lymphoid leukemia is very similar as that found in normal subjects [38]. Maturationally regulated and molecular mechanisms (e.g., enhancer-like sequences, D proximity, insertion polymorphism, gene duplication) may account for some of the limited use of V_H genes by B cell repertoires in both normal and abnormal conditions. However, the regulation of these limitations has not been completely elucidated. Thus, studies are under way to determine whether genetic polymorphisms, abnormalities in ontogeny, the molecular mechanisms underlying somatic recombination and hypermutation processes (within and outside the IgV gene loci), clonal selection or defects in selection processes as well as receptor editing can contribute to the tendency to produce autoantibodies in autoimmune diseases.

IgV Gene Use by Normal Individuals and Naturally Occurring (Auto)antibodies

In order to evaluate the B cell repertoire in pathologic conditions, a comprehensive data base of the IgV gene use by B cells of normal individuals is necessary. Until recently, most information available on the human B cell repertoire derived from Epstein-Barr virus transformed cell lines, hybridomas, anti-idiotype analyses, and molecular biological techniques, such as in situ hybridization and, especially, the analysis of cDNA libraries. These data include the Ig genes encoding naturally occurring polyreactive (auto)antibodies that make up a considerable fraction of the total repertoire in healthy humans, and are thought to be involved in diverse physiological functions at different stages of development of the immune system [44–46]. Reactivity to more than two structurally unrelated antigens, low-affinity binding, high idiotypic connectivity and a biased use of germline genes with little or no somatic mutations are main features of these, mostly IgM antibodies [44–46] which must be distinguished from pathogenic autoantibodies [47].

The expression of a rather restricted set of individual IgV gene segments during the intrauterine and neonatal phases of development is well established [8–14, 37]. Of note, most if not all of these developmentally expressed IgV genes have been found to encode naturally occurring (auto)antibodies in human fetuses and adults with little or no somatic mutations [12–22]. This finding suggests that the natural (auto)antibody repertoire could simply reflect the expression of a subset of developmentally related, conserved IgV genes. However, Ig chain shuffling studies in transfectomas have indicated that (auto)polyreactivity is often dependent on the somatically generated CDR3. Therefore, these autoantibodies are candidates for selection into the repertoire [41].

Since a substantial part of the fetal B cell repertoire expresses Ig molecules capable of binding to a variety of structurally unrelated antigens, including self-determinants, the hypothesis has been suggested that repertoire selection in the physiologically germfree intrauterine environment may be initiated by binding of surface Ig molecules to developmentally related antigens (e.g., self-determinants, Ig idiotypes) [48]. Therefore, the fetal repertoire may be limited by cellular selection rather than by molecular processes, e. g., restricted or biased V(D)J recombination [10, 11]. The function of the (auto)polyreactive repertoire in fetal life is not clear, but it may act to opsonize and dispose of apoptotic material and other cellular debris produced to the rapid organ remodeling of the fetus. One result of this process, however, is that a 'primitive' broadly specific (polyreactive) repertoire is generated, which then may assume different functions in postnatal life [44–46].

It should be noted that a significant portion of the polyreactive (auto)antibodies from the germfree fetal environment have been found to react with bacterial components [13], as has been reported for the adult polyreactive B cell repertoire [44]. These cross-reactivities generated in utero, encoded by conserved and developmentally regulated (most likely autoantigen/idiotype-selected) IgV genes [13, 14], may act as a 'first-line defense' of the neonate against bacterial infections when placentally transferred maternal IgG is exhausted [13].

During maturation and exposure to exogenous antigens, the poly/self-reacting B cell pool may diminish in size, whereas the non-self-reacting cell pool expands, and, by somatic hypermutation and other diversification mechanisms, produces classical high-affinity antibodies [48, 49]. In contrast to the B cell depletion/anergy induced by B cell receptor binding to conventional autoantigens [50], it seems possible that B cells expressing surface-bound naturally occurring (auto)antibodies may be stimulated by diverse, polyvalent, T-cell-independent (auto)antigens to proliferate in a manner that may render these cells more susceptible to abnormal clonal expansion and malignant transformation. This contention is supported by the finding that there is a close relationship of the Ig repertoire expressed by certain B cell malignancies, particularly IgM bearing chronic lymphocytic leukemia B cells, to the natural antibody repertoire [42, 43]. Similarities, strongly indicative for this linkage, include a biased IgV gene use, expression of common Ig idiotypes, a similar pattern of binding specificities [27, 37–43, 51] as well as a rather low affinity for the recognized antigens [40]. Moreover, autoreactive B cell precursors in the human bone marrow were frequently found to be in a proliferative state [52], presumably reflecting the nonspecific nature of their activation signals. Whether pathogenic high-affinity autoantibodies could arise by somatic hypermutation from the polyreactive cell pool [24, 25], e.g., following a breakdown of T cell tolerance, or whether polyreactive B cells may be involved in normal and/or abnormal antigen processing is still unclear.

In adults, in situ hybridization experiments and studies in EBV-transformed B cell lines revealed inconsistent results with regard to whether the V_H gene family use approximated the complexity of individual families [30, 53–55]. However, when assessing individual IgV gene use of peripheral B cells in normal humans by analyzing cDNA libraries [28–31], there was strong evidence that some individual genes were overexpressed in the adult repertoire. Moreover, these studies have indicated that the pattern of IgV gene use may vary between individuals and with time, putatively because of environmental influences [28]. Interestingly, Huang and Stollar [29] demonstrated that a majority of IgH chain cDNA of normal human adult blood lymphocytes resembled cDNA for fetal Ig and naturally occurring (auto)antibodies. Accordingly, examples of V_H gene segments found to be overexpressed in adult normals included the V_H3 family member 3-23/DP-47/V_H26/30p1 [31], the V_H4 family member 4-34/DP-63/V_H4.21 [21, 28], and the V_H1 family member 1-69/DP-10/51p1 [28], all three belonging to the restricted fetal IgV gene repertoire and expressed frequently in B cell malignancies [27, 37–43]. More recently, using a single-cell polymerase chain reaction (PCR) methodology to assess the Ig repertoire, potential problems of biased sampling have been avoided. By this means, it is possible to analyze B cell subpopulations by using specific antibodies for cell sorting. Because of the small numbers of cells needed for this analysis, different subsets from the same donor can be evaluated and, moreover, analysis of the H/L chain pairing of single cells can be determined [4]. In addition, analysis of genomic DNA amplifies both the functionally and nonfunctionally rearranged Ig genes and, thereby, can provide definitive information about the possible basis of any biases detected (fig. 1). Based on these critical assumptions, molecular events and/or selective influences that might influence the expressed repertoire can be differentiated.

Studies of the V_H gene use in normal individuals [32, 33] analyzed the distribution of V_H gene families, and found that the frequency of occurrence is a function of germline complexity, although a bias towards V_H3 and some of its members was found. At the level of individual

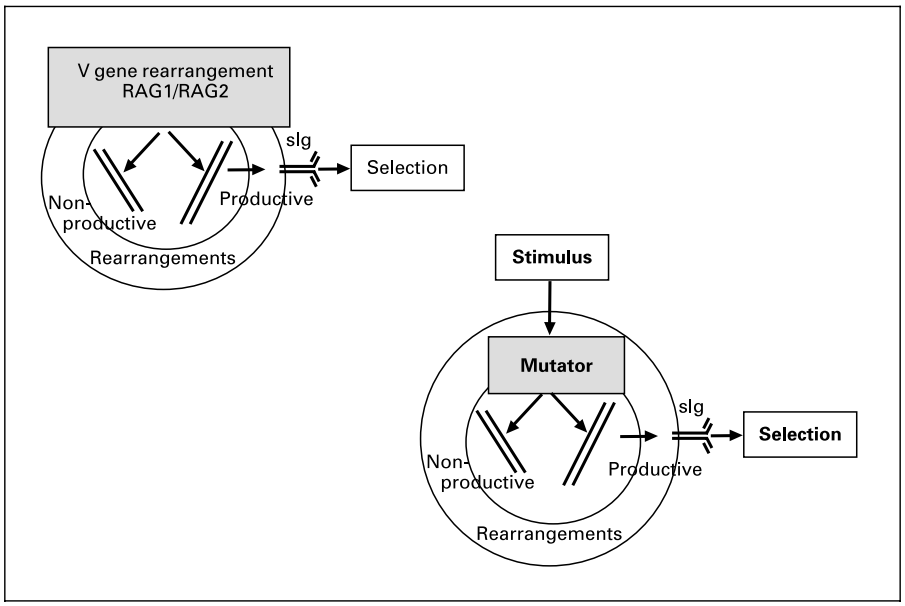


Fig. 1. Differentiation of molecular processes, such as recombination and somatic hypermutation, represented by nonproductive rearrangements and superimposed selective influences that shape the productive V gene repertoire.

genes, preferential use of some gene segments, as suggested by analyses of cDNA libraries [28–31], has been verified. In detail, ten V_H genes (3-23, 4-59, 4-39, 3-07, 3-30, 1-18, 3-30.3, DP-58, 4-34, and 3-09) were shown to be used by approximately 60% of normal peripheral blood B cells. One particular V_H3 family member, 3-23/DP-47/V_H26 was used by about 15% of unselected B cells [31–33]. This bias was noted in the productive but not in the nonproductive repertoire, indicating that it resulted from selection. Previous studies [56] had suggested that overrepresentation of V_H3-23 occurred at the pre-B cell stage of development. Selection of V_H3-23 was independent of the D_H or J_H segment employed, the length or charateristics of the CDR3 and the pairing of Vκ chains, consistent with the possibility that this selection may have occurred as a result of a B cell superantigen-like influence [57, 58]. In contrast to V_H3-23, V_H4-59 was overexpressed in the nonproductive repertoire and its frequency was not altered in the productive and nonproductive repertoires, consistent with the conclusion that molecular biases in the VDJ recombination process accounted for its overrepresentation. Evidence for a negative selection of certain V_H3 and V_H4 family members was noted in that they were found less often in the productive than the nonproductive VDJ repertoire. In addition, evidence for a positive selection based on CDR3 of the V_H rearrangements was obtained, in that J_H6 and DXP′1 were found at a higher frequency in the productively compared to the nonproductively rearranged repertoire. A much higher frequency of mutations was noted in the nonproductively rearranged V_H genes from individual B cells [33, 59]. Although V_H utilization by CD5+ and CD5– B cells was comparable, a statistically significant difference in the frequency of somatic mutation between these subpopulations was noted [33, 36]. These results from healthy individuals demonstrating the influences of molecular and selective events in shaping the peripheral B cell repertoire provided the basis for a comparison with the IgV gene repertoire in different autoantibody-associated autoimmune diseases.

IgV Gene Use in Systemic Lupus Erythematosus

Initial studies have demonstrated that the single-cell PCR technique is a powerful tool to gain insight into the differential impact of molecular and selective influences in autoimmune diseases, most notably in SLE [60]. SLE is an autoimmune disease serologically characterized by the production of multiple autoantibodies, especially those to double-stranded DNA (dsDNA) [61]. It is likely that anti-dsDNA antibodies are pathogenic in that they are able to induce renal damage, as documented by perfusion experiments in rodents [62, 63] and by the presence of such antibodies in human lupus kidneys [64]. Of note, however, anti-single-stranded DNA (ssDNA), a common specificity of naturally occurring (auto)antibodies, has also been

shown to induce severe diffuse proliferative nephritis in mice [65].

In contrast to the naturally occurring (auto)antibodies found in healthy humans and normal nonimmunized animals, pathogenic antibodies are not polyreactive in that they do not react with a wide spectrum of unrelated antigens, are often cationic, are usually IgG, and, when sequenced, are often highly mutated, suggesting that they result from a T-cell-dependent immune response [24, 25, 47, 66–71]. Remarkably, the notion that pathogenic anti-DNA antibodies are characteristically of high affinity has been challenged, since it appears that the charge and affinity for dsDNA are not predictors of the pathogenic capacity of anti-DNA antibodies [72]. Thus, the fine specificity of autoantibodies rather than avidity alone appears to determine whether they are pathogenic or nonpathogenic [72]. In addition, the potential of autoantibodies to enter living cells may contribute to their pathogenic consequence [73].

There is some evidence that potentially pathogenic autoantibodies may arise by somatic hypermutation during the immune response to foreign antigens from antibodies that have no reactivity to autoantigens in their germline configuration [72, 74]. Consistent with this, high-titer expression of anti-dsDNA antibody-associated idiotypes can be found within SLE kindreds in clinically unaffected family members who express no anti-dsDNA antibodies [75].

Despite intensive study, the factors that lead to the production of pathogenic autoantibodies in SLE remain largely unknown. The possibility that there are diffuse abnormalities in the Ig repertoire of patients with SLE has not been completely examined. It is not known whether interindividual genetic polymorphisms of the IgV gene loci, abnormalities in the V(D)J recombination process or in the somatic hypermutation and/or subsequent selective influences underlie the generation of autoantibodies. There is a suggestion that a distinct V_H gene haplotype may predispose to both rheumatoid arthritis and SLE in humans and may be involved in the generation of an aberrant idiotypic network [76]. Other studies have demonstrated that the major part of human anti-dsDNA is encoded by V_H3 family members, although there is no restriction to a certain V_H family or an individual IgV gene segment [25, 70], in contrast to the absolute V_H gene restriction found in cold agglutinins [23]. Of note, anti-dsDNA-antibody-associated idiotypes encoded by distinct IgV gene segments have been described, although idiotype-related gene use is not absolutely restricted to anti-dsDNA autoantibodies [75, 77–81]. In addition, certain features of the CDR3, such as use of an uncommon reading frame of the D segment, D-D fusions or the frequent presence of arginine, an amino acid implicated in DNA binding, have been found in anti-dsDNA antibodies [24, 25, 67, 68]. Furthermore, it has been suggested that an abnormality in the 'mutator' mechanism may predispose to increased somatic mutations, since most pathogenic anti-DNA antibodies are heavily mutated [82]. This remains controversial, however, since it has also been reported that there are no significant differences in the mutational activity directed towards murine antibodies to exogeneous antigens and autoantibodies [83]. Although the precise mechanism remains unclear, the aggregate test results suggest that diffuse abnormalities in one or more of the processes governing the generation of the B cell repertoire could contribute to the tendency to produce pathogenic autoantibodies in SLE.

In a more recent study of the IgV gene repertoire in peripheral B cells of an untreated SLE patient employing a single-cell PCR technique [60, Dörner et al. submitted], the V_LJ_L recombination process was found to be comparable to normal individuals, as judged by analysis of the distribution of nonproductive V_LJ_L rearrangements. However, striking differences in the productive V_L gene repertoire of this patient were noted, with increased use of the J_L distal V_L genes and a marked increase in the use of Jκ5 and Jλ7, the most V_L distal J_L genes. It is noteworthy that these differences from normal were found despite the fact that the entire B cell population was sampled, consistent with the conclusion that there was a global B cell abnormality in this SLE patient, rather than a defect limited to a subset of B cells or Ig genes. These data suggested that the replacement of primary V_LJ_L rearrangements by subsequent rearrangements (receptor replacement or editing) was more frequent in this SLE patient than had been observed in normal individuals [34, 35]. Despite this, the mechanisms of receptor editing of Vκ and Vλ genes appeared to be different. The data from this patient suggested that Vκ receptor editing in SLE occurs in the periphery after somatic hypermutation has been initiated, based on the higher mutational frequency of productive rearrangements using Jκ1-4 compared to those using Jκ5 [60]. In contrast, analysis of the mutational pattern of Vλ rearrangements suggested that the dominant influence was central before the mutational machinery had been activated, and therefore, most likely in the bone marrow during B cell ontogeny. Thus, there was an increased use of 5′ Vλ genes and the 3′ Jλ7 segment, but there was no decrease in the mutational frequency of productive Vλ rearrangements using these elements nor of the entire pro-

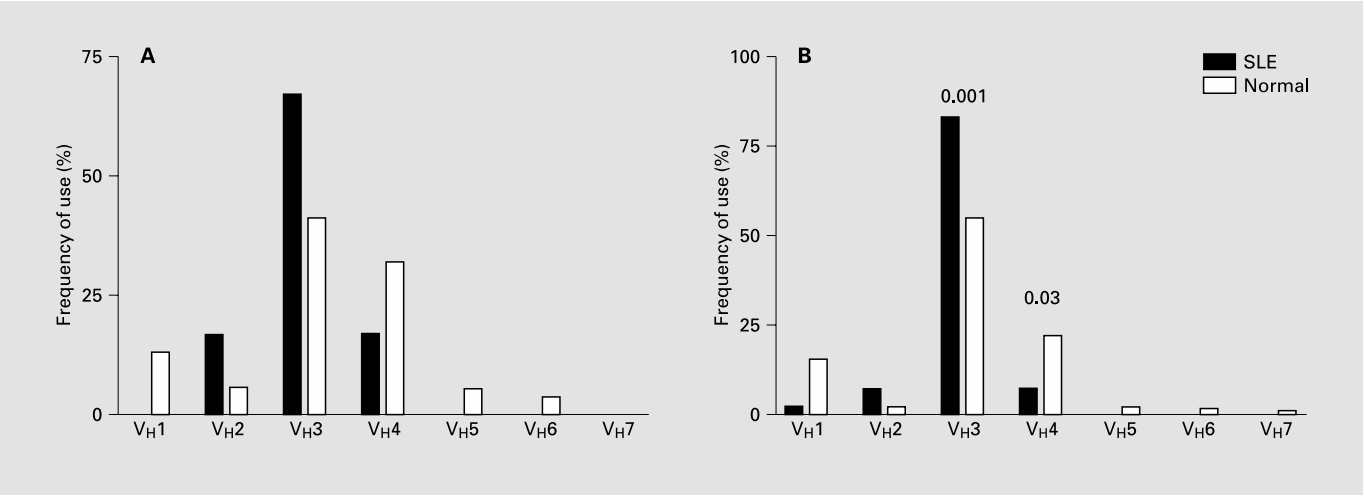


Fig. 2. Comparison of V_H gene use in the nonproductive (**A**) and productive (**B**) repertoire of an SLE patient and normal subjects showed a significantly different usage of V_H3 ($p < 0.001$) and V_H4 ($p < 0.03$) in the productively rearranged genes.

ductive Vλ repertoire. Data from transgenic mice have shown that central receptor editing can operate to replace light chains of B cells expressing autoantibodies [84], although there are no previous examples of central receptor editing of Vλ chains. The results of the repertoire analysis of the SLE patient suggest that emergence of Vλ-containing autoantibodies during B cell ontogeny may have been the stimulus for central Vλ receptor editing in this SLE patient. In this context, Vλ genes have been shown to be critical parts of a number of human autoantibodies, including those to dsDNA [69, 70, 77].

It is noteworthy that certain genes (Vλ4B/Jλ2/3) were found exclusively in the nonproductive repertoires of both normal individuals and the SLE patient, suggesting that they were similarly eliminated from the productive gene repertoire of each. This implies that some elements of negative selection or receptor editing operated normally in the SLE patient. Similarly, A30/Jκ2 was exclusively found in the nonproductive repertoire of this SLE patient [60]. Productively rearranged A30/Jκ2 genes have been shown to bind dsDNA in their germline configuration [66, 71]. Although the binding specificity of 4B/Jλ2/3 gene rearrangements has not been delineated, it was detected only in the nonproductive repertoire, suggesting the possibility that it might bind an autoantigen. Its elimination from the productive repertoire of normal individuals and the SLE patient might, therefore, result from negative selection and/or receptor editing. Whatever the mechanism of elimination, this process appeared to be intact in this SLE patient and comparable to normal individuals.

In contrast to analysis of V_L gene use by this patient, examination of V_H gene use revealed no evidence of increased receptor editing, but other differences in the V_H gene repertoire that could contribute to autoantibody formation. Thus, the comparison of the productive V_H repertoires between the SLE patient and the normal individuals revealed a striking overrepresentation of V_H3 family members and underrepresentation of the V_H4 family (fig. 2). The possibility that the patient manifested a generalized enhancement in positive selection of V_H3-expressing B cells was suggested by the analysis of the entire V_H3 family, as well as of the V_H3-23 (DP-47) gene – the most frequently used V_H3 family member in healthy adults [31–33, 56, 85]. In this SLE patient, V_H3-23 was even more frequently used than in normal individuals. Of importance, V_H3-23 has previously been noted to encode anti-DNA antibodies, especially the 16/6 idiotype [81]. Whether an abnormal mechanism, such as B cell superantigen stimulation [57, 58], causes expansion of V_H3 expressing B cells in SLE will require careful analysis of other patients. Further comparison on the level of individual V_H gene use by the productive repertoire revealed that the V_H3-11 gene segment was also found significantly more often in this SLE patient than in normal subjects. Of note, negative selection of V_H3-11 in normal subjects has previously been suggested in other studies [56, 85] regardless of the donor's genetic background. This mandates analy-

sis of other SLE patients to determine whether the V_H3-11 segment is overrepresented in the expressed repertoire.

One of the remarkable findings of this analysis was the identification of a clone of B cells that expressed V_H3-11/Vλ1G. Previous analysis demonstrated no comparably expanded B cell clones in normal peripheral blood B cells [33–36]. The use of VH3-11 and Vλ1G gene segments by this clone requires emphasis, since both genes have been reported to be negatively selected in normal subjects [32, 86]. Although proof of autoreactivity of these resulting receptors is lacking, these data are consistent with the conclusion that clonal expansion of B cells can occur in the initial stages of SLE, suggesting an overwhelming antigenic stimulus. Studies in mice have also documented that clonal expansion of autoreactive B cells occurs in early lupus [87, 88]. Thus, the expansion of a B cell clone in the initial stages of SLE is consistent with findings noted in autoimmune-prone mice [88, 89].

Analysis of mutations provided further insights into the generation of diversity in this SLE patient. Markedly increased somatic hypermutation of the V_H rearrangements of this untreated SLE patient was apparent. Thus, mutational frequencies of nonproductive and productive rearrangements in CD19+ B cells from the SLE patient were significantly greater than those found in normal subjects [36, 90]. Since mutational activity in general is induced in response to T-dependent antigens [49] and the frequency of mutations in the nonproductive repertoire reflects the activity of the mutational machinery without subsequent selection [90], the B cells of this patient appear to have been stimulated in a T-cell-dependent manner more intensively or more persistently than in normal subjects. Whether this reflects the intensity or persistence of stimulation or a defect in apoptosis of B cells expressing mutated receptors, as has been suggested [50, 74], remains to be determined.

The difference in the frequency of mutations in the productive and nonproductive repertoires reflects the influence of selection, with elimination of mutation-generated defective B cell receptors normally more evident than positive selection of those with increased avidity [33, 36, 90, Foster et al., submitted]. This process seems to be generally intact in this SLE patient, even though the overall resulting frequency of mutations in the productive repertoire is much greater than normal.

Taken together, (1) receptor editing of V_L gene rearrangements, (2) skewing of the V_H gene repertoire towards utilization of V_H3 genes, (3) clonal expansion of B cells, and (4) a generalized increase in somatic hypermutation may all contribute to the emergence of autoimmunity in this SLE patient. Whether these findings reflect a reactive pattern based upon extensive stimulation or represent intrinsic abnormalities in the entire B cell population predisposing to the emergence of autoimmunity remains to be elucidated. The data are most consistent, however, with the conclusion that extreme T-cell-dependent B cell overactivity is found in the initial stages of SLE leading to remarkable changes in peripheral IgV gene use and despite extensive light-chain receptor editing permits to the emergence of autoimmunity.

Conclusion

In conclusion, the single-B-cell PCR methodology makes it possible to analyze IgV gene use by unstimulated individual B cells without bias. The use of this technique has permitted an analysis of the distribution of light and heavy chain use, assessment of differences between nonproductive and productive rearrangements that imply influences of molecular versus selective mechanisms in shaping the repertoire and also an examination of the impact of somatic mutations. Finally, experiments are currently in progress to determine the IgV gene use in other systemic autoimmune diseases. It remains to be elucidated whether the IgV gene use and the mutational pattern of the same donor at different time points of the disease, in different immune compartments as well as in particular B cell subsets will provide new clues in understanding B cell autoimmunity. Altogether these studies should provide a unique opportunity to test the hypothesis that abnormalities in the generation of the Ig repertoire, the process of somatic hypermutation and/or selective influences play a role in autoimmune disease.

Acknowledgment

This work was supported by Deutsche Forschungsgemeinschaft Grants Do 491/2-1, 4-1, and National Institutes of Health Grant AI 31229.

References

1 Kabat EA, Wu TT, Perry HM, Gottesmann KS, Foeller C: Sequences of Proteins of Immunological Interest, ed 5. Bethesda, National Institutes of Health, 1991, NIH Publ. 91–3242.
2 Tonegawa S: Somatic generation of antibody diversity. Nature 1983;302:575–581.
3 Rolink A, Melchers F: Molecular and cellular origins of B lymphocyte diversity. Curr Opin Immunol 1993;5:207–217.
4 Brezinschek HP, Foster SJ, Dörner T, Brezinschek RI, Lipsky PE: Pairing of variable heavy and variable kappa chains in individual naive and memory B cells. J Immunol 1998;160: 4762–4767.
5 Yancopoulos GD, Desiderio SV, Paskin M, Kearney JF, Baltimore D, Alt FW: Preferential utilization of the most VH proximal VH gene segments in pre-B cell lines. Nature 1984; 311: 727–733.
6 Perlmutter RM, Kearney J, Chang SP, Hood LE: Developmentally controlled expression of immunoglobulin VH genes. Science 1985;227: 1597.
7 Kofler R, Geley S, Kofler H, Helmberg A: Mouse variable-region gene families: Complexity, polymorphism and use in non-autoimmune responses. Immunol Rev 1992;128:5.
8 Schroeder HW Jr, Hillson JL, Perlmutter RM: Early restriction of the human antibody repertoire. Science 1987;238:791–793.
9 Schroeder HW Jr, Wang JY: Preferential utilization of conserved immunoglobulin heavy chain variable gene segments during human fetal life. Proc Natl Acad Sci USA 1990;87: 6146–6150.
10 Willems van Dijk K, Milner LA, Sasso EH, Milner ECB: Chromosomal organization of the heavy chain variable region gene segments comprising the human fetal antibody repertoire. Proc Natl Acad Sci USA 1992;89:10430–10434.
11 Pascual V, Verkruyse L, Casey ML, Capra JD: Analysis of IgH chain gene segment utilization in human fetal liver. Revisiting the 'proximal utilization hypothesis'. J Immunol 1993;151: 4164.
12 Schutte MEM, Ebeling SB, Akkermans KE, Gmelig-Meyling FHJ, Logtenberg T: Antibody specificity and immunoglobulin VH gene utilization of human monoclonal CD5+ B cell lines. Eur J Immunol 1991;21:1115–1121.
13 Hansen A, Jahn S, Lukowsky A, Grütz G, Bohn J, von Baehr R, Settmacher U: VH/VL gene expression in polyreactive-antibody-producing human hybridomas from the fetal B cell repertoire. Exp Clin Immunogenet 1994;11:1–16.
14 Settmacher U, Jahn S, Siegel P, von Baehr R, Hansen A: An anti-lipid A antibody obtained from the fetal B cell repertoire is encoded by VH6/Vlambda1 genes. Mol Immunol 1993; 30: 953–954.
15 Sanz I, Casali P, Thomas JW, Nokins AL, Capra JD: Nucleotide sequences of eight human natural autoantibody VH regions reveals apparent restricted use of VH families. J Immunol 1989;142:4054.
16 Hansen A, Roggenbuck D, Porstmann T, von Baehr R, Franke L: Selection and characterization of an human anti-P24 IgM hybridoma (abstract). Immunobiology 1993;189:175.
17 Leibiger H, Hansen A, Schoenherr G, Seifert M, Wüstner D, Stigler R, Marx U: Glycosylation analysis of a polyreactive human monoclonal IgG antibody derived from a human-mouse hybridoma. Mol Immunol 1995;32: 595–602.
18 Dersimonian H, Schwartz RS, Barrett KJ, Stollar BD: Relationship of human variable region heavy chain germline genes to genes encoding anti-DNA autoantibodies. J Immunol 1987; 139:2496.
19 Cairns EP, Kwong C, Misener V, Ip P, Bell DA, Siminovitch LA: Analysis of variable region genes encoding a human anti-DNA antibody of normal origin. J Immunol 1989;143:685–691.
20 Harindranath N, Goldfarb IS, Ikematsu H, Burastero SE, Wilder RL, Notkins AL, Casali P: Complete sequence of the genes encoding the VH and VL regions of low- and high-affinity monoclonal IgM and IgA1 rheumatoid factors produced by CD5+ B cells from a rheumatoid arthritis patient. Int Immunol 1991;3:865–875.
21 Pascual V, Capra JD: VH4–21, a human VH gene segment overrepresented in the autoimmune repertoire. Arthritis Rheum 1992;35:11–18.
22 Logtenberg T, Schutte MEM, Ebeling SB, Gmelig-Meyling FHJ, van Es JH: Molecular approaches to the study of human B cell and (auto)antibody repertoire generation and selection. Immunol Rev 1992;128:23–46.
23 Pascual V, Victor K, Spellenberg M, Hamblin TJ, Stevenson FK, Capra JD: VH restriction among human cold agglutinins. The VH4-21 gene segment is required to encode anti-I and anti-i specificities. J Immunol 1992;149:2337–2344.
24 van Es JH, Gmelig-Meyling FHJ, van de Akker WRM, Aanstoot H, Derksen RHWM, Logtenberg T: Somatic mutations in the variable regions of a human IgG anti-double-stranded DNA autoantibody suggest a role for antigen induction of systemic lupus erythematosus. J Exp Med 1991;173:461–470.
25 Winkler TH, Fehr H, Kalden JR: Analysis of immunoglobulin variable region genes from human IgG anti-DNA hybridomas. Eur J Immunol 1992;22:1719.
26 Pascual V, Randen I, Thompson K, Mouldy S, Forre O, Natvig J, Capra D: The complete nucleotide sequences of the heavy chain variable regions of six monospecific rheumatoid factors derived from Epstein-Barr-virus-transformed B cells isolated from the synovial tissue of patients with rheumatoid arthritis. J Clin Invest 1990;86:1320–1328.
27 Chen PP, Olsen NJ, Yang PM, Soto-Gil RW, Olee T, Siminovitch KA, Carson DA: From human autoantibodies to the fetal antibody repertoire to B cell malignancy: It's a small world after all. Intern Rev Immunol 1990;5: 239–251.
28 Stewart AK, Huang C, Long AA, Stollar BD, Schwartz RS: VH-gene representation in autoantibodies reflects the normal human B-cell repertoire. Immunol Rev 1992;128:101–122.
29 Huang C, Stollar BD: A majority of IgH chain cDNA of normal human adult blood lymphocytes resembles cDNA for fetal Ig and natural autoantibodies. J Immunol 1993;151:5290.
30 Huang C, Stewart AK, Schwartz RS, Stollar BD: Immunoglobulin heavy chain gene expression in peripheral blood B lymphocytes. J Clin Invest 1992;89:1331.
31 Stewart AK, Huang C, Stollar B, Schwartz RS: High-frequency representation of a single VH gene in the expressed human B cell repertoire. J Exp Med 1993;177:409.
32 Brezinschek HP, Brezinschek RI, Lipsky PE: Analysis of the heavy chain repertoire of human peripheral blood B cells using single-cell polymerase chain reaction. J Immunol 1995; 155:190–202.
33 Brezinschek HP, Foster SJ, Brezinschek RI, Dörner T, Domiati-Saad R, Lipsky PE: Analysis of the human VH gene repertoire. Differential effects of selection and somatic hypermutation on peripheral CD5+/IgM+ and CD5–/IgM+ B cells. J Clin Invest 1997;99:2488–2501.
34 Farner NL, Dörner T, Lipsky PE: Molecular mechanisms and selection influence the generation of the human VlambdaJlambda repertoire. J Immunol 1999;162:2137–2145.
35 Foster SJ, Brezinschek HP, Brezinschek RI, Lipsky PE: Molecular mechanisms and selective influences that shape the kappa gene repertoire of IgM+ B cells. J Clin Invest 1997; 99:1614–1627.
36 Dörner T, Brezinschek HP, Foster SJ, Brezinschek RI, Farner NL, Lipsky PE: Comparable impact of mutational and selective influences in shaping the expressed repertoire of peripheral IgM+/CD5– and IgM+/CD5+ B cells. Eur J Immunol 1998;28:657–668.
37 Kipps TJ, Robbins BA, Carson DA: Uniform high frequency expression of autoantibody-associated crossreactive idiotypes in the primary B cell follicles of human fetal spleen. J Exp Med 1990;171:189–196.
38 Fais F, Ghiotto F, Hashimoto S, Sellers B, Valetto A, Allen SC, Schulmann P, Vinciquerra VP, Rai K, Rassenti CZ, Kipps TJ, Dighiero G, Schroeder HW Jr, Ferrarini M, Chiorazzi N: Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest 1998;102:1515–1525.

39 Bahler DW, Miklos JA, Swerdlow SH: Ongoing Ig gene hypermutation in salivary gland mucosa-associated lymphoid tissue lymphomas. Blood 1997;89:3335–3344.
40 Böhme H, Seifert M, Roggenbuck D, Döcke W, von Baehr R, Hansen A: Characterization of a B-CLL derived IgM-lambda antibody expressing typical features of a NPAB. Immunol Lett 1994;41:261–266.
41 Martin T, Duffy SF, Carson DA, Kipps TJ: Evidence for somatic selection of natural autoantibodies. J Exp Med 1992;175:983–991.
42 Schroeder HW Jr, Dighiero G: The pathogenesis of chronic lymphocytic leukemia: Analysis of the antibody repertoire. Immunol Today 1994;15:288–294.
43 Brezinschek HP, Brezinschek RI, Dörner T, Lipsky PE: Similar characteristics of the CDR3 of VH1–69/DP10 rearrangements in normal human peripheral blood and chronic lymphocytic leukaemia B cells. Br J Haematol 1998; 102:516–521.
44 Casali P, Notkins A: Probing the human B-cell repertoire with EBV: Polyreactive antibodies and CD5+ B cells. Annu Rev Immunol 1989;7: 513–535.
45 Guigou V, Guilbert B, Moinier D, Tonelle C, Boubli L, Avrameas S, Fougereau M, Fumoux F: Ig repertoire of human polyspecific antibodies and B-cell ontogeny. J Immunol 1991; 146:1368–1374.
46 Avrameas S: Natural autoantibodies: From 'horror autotoxicus' to 'gnothi seauton'. Immunol Today 1991;12:154–159.
47 Tron F, Bach JF: Molecular and genetic characteristics of pathogenic autoantibodies. J Autoimm 1989;2:311–320.
48 Couthino A, Grandien A, Faro-Rivas J, Mota-Santos TA: Idiotypes, tailers and networks. Ann Inst Pasteur Immunol 1988;139:599–607.
49 Kocks C, Rajewsky K: Stable expression and somatic hypermutation of antibody V regions in B cell developmental pathways. Annu Rev Immunol 1989;7:537–559.
50 Fang W, Weintraub BC, Dunlap B, Garside P, Pape KA, Jenkins MK, Goodnow CC, Mueller DL, Behrens TW: Self-reactive B lymphocytes overexpressing bcl-xL escape negative selection and are tolerized by clonal anergy and receptor editing. Immunity 1998;9:35.
51 Jahn S, Schwab J, Hansen A, Heider H, Schroeder C, Lukowsky A, Achtmann M, Matthes H, Kiessig ST, Volk HD, Krüger DH, von Baehr R: Human hybridomas derived from CD5+ B lymphocytes of patients with chronic lymphocytic leukemia (B-CLL) produce multispecific natural IgM(kappa) antibodies. Clin Exp Immunol 1991;84:413–417.
52 Fong S, Gilbertson TA, Hueniken RJ, Singhal SK, Vaughan JH, Carson DA: IgM rheumatoid factor autoantibody and immunoglobulin-producing precursor cells in the bone marrow of humans. Cell Immunol 1985;95:157–172.
53 Guigou V, Cuisinier AM, Tonnelle C, Moinier D, Fougereau M, Fumoux F: Human immunoglobulin VH and Vk repertoire revealed by in situ hybridization. Mol Immunol 1990;27: 935.
54 Zouali M, Theze J: Probing VH gene-family utilization in human peripheral B cells by in situ hybridization. J Immunol 1991;146:2855–2864.
55 Pascual V, Capra JD: Human immunoglobulin heavy-chain variable region genes: Organization, polymorphism, and expression. Adv Immunol 1991;49:1.
56 Rao SP, Huang SC, Milner ECB: Analysis of the VH3 repertoire among genetically disparate individuals. Exp Clin Immunogenet 1996; 13:131.
57 Silverman GJ: B cell superantigens: Possible roles in immunodeficiency and autoimmunity. Semin Immunol 1998;10:43.
58 Domiati-Saad R, Lipsky PE: Staphylococcal enterotoxin A induces survival of VH3-expressing human B cells by binding to the VH region with low affinity. J Immunol 1998;161: 1257.
59 Dörner T, Brezinschek HP, Brezinschek RI, Foster SJ, Domiati-Saad R, Lipsky PE: Analysis of the frequency and pattern of somatic mutations within nonproductively rearranged human variable heavy chain genes. J Immunol 1997;158:2779–2789.
60 Dörner T, Foster SJ, Farner NL, Lipsky PE: Immunoglobulin kappa chain receptor editing in systemic lupus erythematosus. J Clin Invest 1998;102:688–694.
61 Tan EM: Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989;44:93.
62 Raz E, Brezis M, Rosenmann E, Eilat D: Anti-DNA antibodies bind directly to renal antigens and induce kidney dysfunction in the isolated perfused rat kidney. J Immunol 1989 142: 3076.
63 Tsao BP, Ebling FM, Roman C, Panosian-Sahakian N, Calame K, Hahn BH: Structural characteristics of variable regions of immunoglobulin genes encoding a pathogenic autoantibody in murine lupus. J Clin Invest 1990;85: 530.
64 Koffler D, Schur PW, Kunkel HG: Immunological studies concerning the nephritis of systemic lupus erythematosus. J Exp Med 1967; 126:607.
65 Swanson PC, Yung RL, Blatt NB, Eagan MA, Norris JM, Richardson BC: Ligand recognition by murine anti-DNA autoantibodies, II. Genetic analysis and pathogenicity. J Clin Invest 1996;97:1748–1760.
66 Harada T, Suzuki N, Mizushima Y, Sakane T: Usage of a novel class of germline immunoglobulin variable region genes for cationic anti-DNA autoantibodies in human lupus nephritis and its role for the development of the disease. J Immunol 1994;1153:4806.
67 Shlomchik M, Mascelli M, Shan H, Radic MZ, Pisetsky D, Marshak-Rothstein A, Weigert M: Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J Exp Med 1990;171:265.
68 Eilat D, Webster DM, Rees AR: V region sequences of anti-DNA and anti-RNA autoantibodies from NZB/NZW F1 mice. J Immunol 1988;141:1745.
69 Ravirajan CT, Rahman MA, Papdaki L, Griffiths MH, Kalsi J, Martin ACR, Ehrenstein MR, Latchman DS, Isenberg DA: Genetic, structural and functional properties of an IgG DNA-binding monoclonal antibody from a lupus patient with nephritis. Eur J Immunol 1998;28:339.
70 Roben P, Barbas SM, Sandoval L, Lecerf JM, Stollar BD, Solomon A, Silverman GJ: Repertoire cloning of lupus anti-DNA autoantibodies. J Clin Invest 1996;98:2827.
71 Suzuki N, Harada T, Mihara S, Sakane T: Characterization of a germline encoding cationic anti-DNA antibody and role of receptor editing for development of the autoantibody in patients with systemic lupus erythematosus. J Clin Invest 1996;98:1843.
72 Putterman C, Limpanasithkul W, Edelman M, Diamond B: The double edge sword of the immune response: Mutational analysis of a murine anti-pneumococcal anti-DNA antibody. J Clin Invest 1996;97:2251–2259.
73 Zack DJ, Stempniak M, Wong AL, Taylor C, Weisbart RH: Mechanisms of cellular penetration and nuclear localization of an anti-double strand DNA antibody. J Immunol 1996;157: 2082–2088.
74 Ray SK, Putterman C, Diamond B: Pathogenic autoantibodies are routinely generated during the response to foreign antigen: A paradigm for autoimmune disease. Proc Natl Acad Sci USA 1996;93:2019.
75 Livneh A, Halpern A, Perkins D, Lazo A, Halpern R, Diamond B: A monoclonal antibody to a cross-reactive idiotype on cationic human anti-DNA antibodies expressing lambda light chains: A new reagent to identify a potentially differential pathogenic subset. J Immunol 1987;139:123.
76 Olee T, Yang PM, Siminovitch KA, Olsen NJ, Hillson J, Wu J, Kozin F, Carson DA, Chen PP: Molecular basis of an autoantibody associated restriction fragment length polymorphism that couples susceptibility to autoimmune diseases. J Clin Invest 1991;88:193.
77 Paul E, Iliev AA, Livneh A, Diamond B: The anti-DNA-associated idiotype 8.12 is encoded by the V lambda II gene family and maps to the vicinity of L chain CDR1. J Immunol 1992; 149:3588.
78 Hirabayashi Y, Munakata Y, Sasaki T, Sano H: Variable regions of a human anti-DNA antibody O-81 possessing lupus nephritis-associated idiotype. Nucleic Acids Res 1992;20:2601.
79 Manheimer-Lory AJ, Davidson A, Watkins D, Hannigan NR, Diamond B: Generation and analysis of clonal IgM- and IgG-producing human B cell lines expressing an anti-DNA-associated idiotype. J Clin Invest 1991;87:1519.

80 Stevenson FK, Longhurst C, Chapman CJ, Ehrenstein M, Spellerberg MB, Hamblin TJ, Ravirajan CT, Latchman D, Isenberg D: Utilization of the VH4-21 gene segment by anti-DNA antibodies from patients with systemic lupus erythematosus. J Autoimmun 1993;6:809–825.
81 Buskila D, Shoenfeld Y: Manipulation of anti-DNA idiotypes: A possible treatment approach to autoimmune diseases. Concepts Immunopathol 1992;8:114.
82 Manheimer-Lory AJ, Zandman-Goddard G, Davidson A, Aranow C, Diamond B: Lupus-specific antibodies reveal an altered pattern of somatic mutation. J Clin Invest 1997;100: 2538–2546.
83 Smith DS, Creadon G, Jena PK, Portanova JP, Kotzin BL, Wysocki LJ: Di- and trinucleotide target preferences of somatic mutagenesis in normal and autoreactive B cells. J Immunol 1996;156:2642.
84 Radic MZ, Erikson J, Litwin S, Weigert M: B lymphocytes may escape tolerance by revising their antigen receptors. J Exp Med 1993;177: 1165.
85 Huang SC, Jiang R, Glas AM, Milner ECB: Nonstochastic utilization of Ig V region genes in unselected human peripheral B cells. Mol Immunol 1996;33:553.
86 Farner NL, Dörner T, Lipsky PE: Distribution of Vλ and Jλ gene usage in normal individuals. J Immunol 1999;162:2137–2145.
87 Ash-Lerner A, Ginsberg-Strauss M, Pewzner-Jung Y, Desai DD, Marion TN, Eilat D: Expression of an anti-DNA-associated VH gene in immunized and autoimmune mice. J Immunol 1997;159:1508–1519.
88 Portanova JP, Creadon G, Zhang X, Smith DS, Kotzin BL, Wysocki LJ: An early postmutational event directs expansion of autoreactive B cells in murine lupus. Mol Immunol 1995;32: 117.
89 Wloch MK, Alexander AL, Pippen AM, Pisetsky DS, Gilkeson GS: Molecular properties of anti-DNA induced in preautoimmune NZB/W mice by immunization with bacterial DNA. J Immunol 1997;158:4500.
90 Dörner T, Foster SJ, Brezinschek HP, Lipsky PE: Analysis of the targeting of the hypermutational machinery and the impact of subsequent selection on the distribution of nucleotide changes in human VHDJH rearrangements. Immunol Rev 1998;162:161.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:46–57

Sjögren's Syndrome: Autoantibodies to Cellular Antigens

Clinical and Molecular Aspects

Clio P. Mavragani Athanasios G. Tzioufas Haralampos M. Moutsopoulos

Department of Pathophysiology, School of Medicine, National University of Athens, Greece

Key Words

Anti-Ro/SSA · Anti-La/SSB · Antinuclear antibodies · Extractable nuclear antigen · Sjögren's syndrome

Abstract

Autoantibodies to cellular autoantigens are usually found in sera of patients with systemic autoimmune rheumatic diseases. Patients with Sjögren's syndrome (SS) frequently present autoantibodies to both organ and non-organ-specific autoantigens. The most commonly detected autoantibodies are those directed against the ribonucleoproteins Ro/SSA and La/SSB. The presence of the antibodies in SS is associated with early disease onset, longer disease duration, parotid gland enlargement, higher frequency of extraglandular manifestations and more intense lymphocytic infiltration of the minor salivary glands. Over the past several years, the structure and function of these autoantigens have been extensively studied. Several centers, using different techniques, have investigated the B cell epitopes on the protein components Ro 60 kD, Ro 52kD, and La 48 kD. Finally, increased evidence of direct involvement of anti-Ro/SSA and anti-La/SSB autoantibodies in the pathogenesis of tissue injury has been contributed by several studies.

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease, which affects primarily salivary and lacrimal glands leading to dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia). The prominent histopathological lesion in this disorder consists of a round cell infiltrate, which initially surrounds the ducts, extending later to the acinar epithelium leading to diminished glandular secretion through apoptosis of these cells [1, 2]. Other organs, which may be involved, include the bronchial tree, kidneys, liver, blood vessels, peripheral nerves and the pancreas. Of particular interest is the dual presentation of SS: either alone as primary disorder in women of the fourth and fifth decades (primary SS) or in the context of other autoimmune diseases (secondary SS); glandular (sicca symptoms) and systemic (extraglandular) clinical manifestations may be present. Therefore, this syndrome could represent a classical model of autoimmune disease where both features of organ-specific and systemic diseases are expressed. Serological evidence of the autoimmune response in SS provides the concomitant presence of circulating organ- and non-organ-specific autoantibodies in the serum of these patients. In the current review several aspects of the non-organ-specific autoantibodies found in SS are discussed and data suggestive of their potential involvement in disease pathogenesis are provided.

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0046$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Prof. H.M. Moutsopoulos, MD, FACP, FRCP (Edin)
75, M. Asias
GR–115 27 Athens (Greece)
Tel. +30 1 7771 095, +30 1 7789 480, Fax +30 1 7703 876

A View of Autoantibodies in SS

In the late fifties, in almost simultaneously appearing reports, the presence of rheumatoid factors, antinuclear and precipitating autoantibodies in sera of patients with SS was claimed [reviewed in 3]. Rheumatoid factors are directed against the Fc region of IgG showing significant affinity only with aggregated IgG molecules. Antibodies that bind the cell nucleus (antinuclear antibodies) are present in approximately 90% of patients with SS as revealed by immunofluorescence techniques. However, no specific fluorescent pattern seems to characterize the syndrome, since homogeneous or speckled patterns can be seen. The exact antigens, which account for the positive fluorescent pattern noted, are not clearly identified; among the various autoantigens-targets, the cytoplasmic/nuclear ribonucleoprotein particles (Ro/SSA and La/SSB) appear to have a prominent role in the autoimmune response of SS providing predominantly a finely speckled nucleoplasmic pattern when the substrate used is Hep-2 cells.

The history of these antigens begins in 1958 when Jones first reported a serum factor in patients with SS that gave a precipitate with extracts of salivary and lacrimal glands. Subsequent reports revealed two immunologically distinct precipitating antibody systems from patients with SS (SjT and SjD) and systemic lupus erythematosus (Ro and La). From the similarity in physical and serological properties, it was deduced that SjD corresponded to Ro and the SjT to the La antigen. In 1975, Alspaugh and Tan detected two 'new' antigenic specificities in sera of patients with SS termed SS-A and SS-B. By serum exchange, thanks to an interlaboratory cooperation, the immunological identity of Ro with SSA and La with SSB was demonstrated and since then these antigen systems are referred to as Ro/SSA and LA/SSB [reviewed in 4].

Other antigens involved in the positive nuclear pattern by immunofluorescence include the following: Ku, NOR-90 (nucleolar organizing region), p-80 coilin, HMG-17 (high-mobility group), Ki/SL. However, autoantibodies to the above antigens are found in low frequencies and are not disease specific [reviewed in 5]. Furthermore, organ-specific autoantibodies are also recognized including antithyroglobulin, antierythrocyte and antisalivary gland epithelium antibodies [reviewed in 4]. Recently, a 120-kD organ-specific autoantigen was purified from salivary gland tissues of an NFS/sld mouse model of human SS. The amino-terminal residues were identical to those of the human cytoskeletal protein α-fodrin. Sera from patients with SS reacted positively with purified antigen and recombinant human α-fodrin protein in contrast to controls with rheumatoid arthritis and systemic lupus erythematosus (SLE) [6]. Since the main antibodies found in sera of patients with SS are directed against Ro/SSA and La/SSB antigens and their presence is suggested as one of the criteria for disease classification [7], the present report will be focused mainly on clinical, functional and pathogenetic aspects of these autoantibodies.

Structure of Autoantigens

The major target antigens Ro/SSA, La/SSB and their cognate antibodies have been extensively defined at the molecular level. In 1981, it was described that Ro/SSA is a ribonucleoprotein containing small, cytoplasmic RNAs [8]. In human cells, four small cytoplasmic RNAs called hY1, hY3, hY4 and hY5 (h for human and Y for cYtoplasmic), of a length ranging from 84 to 112 nucleotides, have been recognized. These are uridine rich, present at about 10^5 copies/cell and transcribed by RNA polymerase III.

In 1984, the protein component of Ro/SSA antigen was investigated: a 60-kD protein (60-kD Ro/SSA, Ro60) was bound to one of several small cytoplasmic RNA molecules. This protein is phylogenetically conserved since it has been described in several other species including mammalians and nematodes. Later on, it was proposed that a 52-kD peptide is another component of Ro/SSA antigen (52-kD Ro/SSA; Ro52) [reviewed in 9].

La/SSB antigen is composed of a polypeptide consisting of 408 amino acids and a calculated molecular mass of 46.7 kD in conjunction with RNA polymerase III transcripts. These include the precursors of cellular 5S RNA and tRNA, human cytoplasmic RNA, U6RNA, viral RNAs (adenovirusencoded VA-RNA, Epstein-Barr-encoded EBER). U1RNA transcribed by RNA polymerase III and vesicular stomatitis leader RNA may also participate in the formation of the ribonucleoproteinic complexes. A 3′ uridine stretch common to all RNA-polymerase-III-transcribed RNAs constitutes the La/SSB-binding region of the RNA. Both 60-kD Ro/SSA and La/SSB proteins are members of a family of RNA-binding proteins that contain a sequence of 80 amino acids known as the RNA recognition motif (RNP). Based on the protein/RNA interactions a putative structure of the Ro RNP particle has been proposed; the hY RNAs are bound to the 60-kD Ro/SSA via the lower stem of the RNA forming by basepairing their 5′ and 3′ ends while La/SSB binds to the 3′ oligonucleotide residues of hY RNAs in a transient

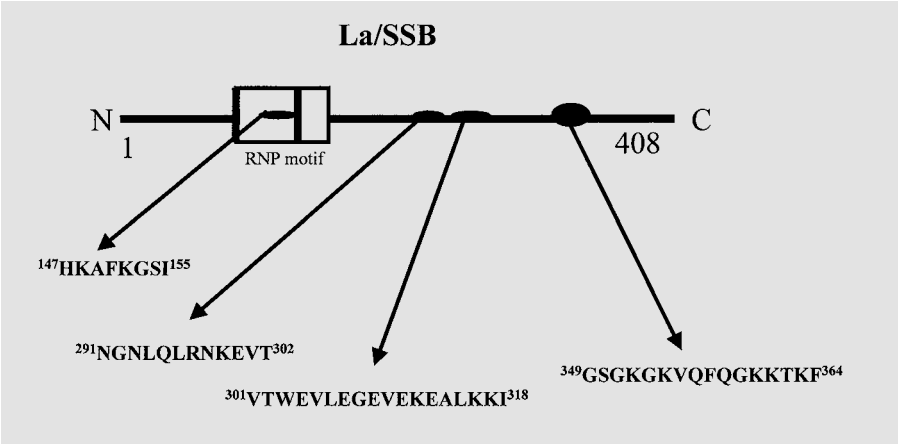


Fig. 1. The linear sequence of La/SSB and its four linear epitopes as they are defined by peptide mimotope scanning.

Table 1. Molecular and functional characteristics of Ro/SSA and La/SSB antigens

	Ro52		Ro60		La/SSB
	Ro52a	Ro52b	Ro60a	Ro60b	
Gene location	chromosome 11		chromosome 1		chromosome 2
Number of amino acids	475	398	538	525	408
Calculated molecular weight, kD	54.1	45	60.6	59.3	46.7
Function	DNA-binding protein		quality control for 5S rRNA production/involvement in translation of ribosomal protein mRNA		initiation, termination factor for RNA polymerase III transcription/viral replication

manner since this 3′ sequence motif is mostly lost upon maturation of the transcripts [reviewed in 10]. The molecular characteristics of 60-kD Ro/SSA, 52-kD Ro/SSA and La/SSB autoantigens appear in table 1 [11–14].

In order to elucidate the precise targets of the autoimmune response, research has focused on the identification of antigenic determinants recognized by anti-Ro/SSA and anti-La/SSB antibodies. B cell epitope mapping of 60-kD Ro/SSA, 52-kD Ro/SSA and La/SSB molecules using several strategies (overlapping synthetic peptides or recombinant proteins tested in ELISA or in Western blotting) have revealed specific epitopes in several studies. However, the results obtained in different reports are conflicting due possibly to different assays used, to the presence of conformational epitopes or to patients' sera selection. The identification of the fine specificity of these autoantibodies has soon become an important issue because of its relationship to possible mechanisms of autoantibody production and the potential use of synthetic peptides as diagnostic tools for the detection of the autoantibodies.

B cell epitopes of 60-kD Ro/SSA autoantigen appear to be located in the central region and the carboxy-terminal part of the molecule. Scofield et al. [15] reported that the C-terminal 13-kD fragment of the 60-kD Ro/SSA protein was recognized by 28 of 45 anti-Ro/SSA sera (62%). Later on, Barakat et al. [16] using five selected synthetic peptides revealed an oligopeptide (21–41) with considerable reactivity with anti-Ro/SSA sera [16]. Wahren et al. [17] using recombinant fusion protein of 60-kD Ro/SSA has shown that all anti-Ro/SSA sera recognized an epitope in the central region 181–396. Routsias et al. [18] using 22-mer, synthetic peptides overlapping by 8 residues covering the entire sequence of the 60-kD Ro/SSA autoantigen, revealed two disease-specific epitopes: the TKYKQRNGWSHKDLLRSHLKP (169–190) and the ELYKEKALSVETEKLLKYLEAV (211–232) region recognized by sera from SLE and SS patients, respectively (fig. 1). In a subsequent study it was shown that the epitope 169–190 possessed sequence similarity with the peptide RPDAEYWNSQKDLLEQKRGR, shared in the β-chain of different HLA-DR molecules [19].

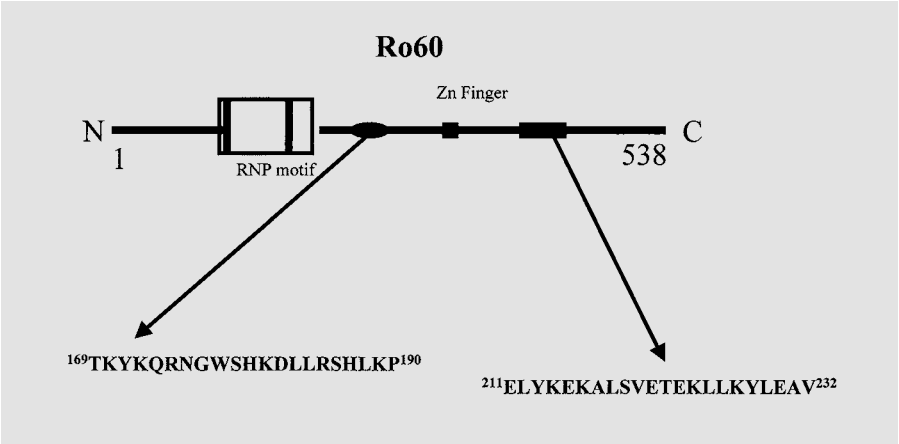


Fig. 2. The linear sequence of 60-kD Ro/SSA and its linear epitopes as they are defined by peptide mimotope scanning.

Boire et al. [20] have reported that a significant portion of the humoral autoimmune response to the 60-kD Ro/SSA antigen is directed against conformational antigenic determinants. Recently it was revealed that multiple conformational epitopes can be bound simultaneously by polyclonal anti-Ro/SSA sera from patients with SLE [21, 22].

In their study Scofield et al. [23] have shown that immunization with short peptides from the sequence of the SLE associated 60-kD Ro/SSA autoantigen results in an autoimmune response to the entire 60-kD Ro/SSA antigen while in about 20% of these animals, autoimmunity also spreads to the La/SSB antigen.

The antigenic determinants of 52-kD Ro/SSA protein are mainly linear and are found in the central part of the molecule [24, 25]. Ricchiuti et al. [25] using 39 overlapping synthetic peptides spanning the entire sequence of 52-kD Ro/SSA protein reported that four peptides (amino acids 2–11, 107–126, 277–292 and 365–382) were recognized by anti-Ro/SSA sera. Bozic et al. [26] have shown that the region 1–292 was the smallest C-terminal deletion fragment, which reacted with anti-Ro/SSA sera as the full length 52-kD Ro/SSA. In a subsequent report, Frank et al. [27] revealed an immunodominant epitope of 11 amino acids (197–207) on the NH_2-terminal side of this protein's putative leucine zipper, while Kato et al. [28] supported that the presence of antigenicity of the molecule at the leucine zipper region could explain the preference of anti-52-kD SSA antibodies to denatured forms of the molecule.

The detection of antigenic determinants of La/SSB autoantigen has also been considered in several studies. Initially, Chan et al. [29] using controlled proteolytic degradation with *Staphylococcus aureus* V8 protease of La/SSB protein identified two antigenically distinct sets of protease-resistant peptides termed X and Y. Sturgess et al. [30] identified a major antigenic epitope within the 103 amino acids of the C-terminal portion of the protein, while St. Clair et al. [31] using fusion proteins encoded by La/SSB cDNA fragments defined three antigenic regions comprising the sequences 1–107, 111–242 and 242–408. More recently four highly reactive peptides with purified IgG, spanning the regions 145–164, 289–308, 301–320 and 349–368 of the La/SSB protein, have been reported (fig. 2). [32].

Recently, two conformational antigenic determinants on hY5 RNA recognized by anti-Ro/SSA antibodies were defined [33]. These epitopes were distinct from regions bound by the 60-kD Ro/SSA and La/SSB proteins suggesting a direct role of human Ro/SSA hY5 particles in the induction of the immune response. T cell epitopes of both autoantigens have not yet been defined in humans.

Cellular Localization – Function

The cellular localization of the Ro/SSA complex has remained a controversial issue since the initial description of the molecule; in fact, Ro antigen was primarily considered as a cytoplasmic antigen in contrast to SSA that was identified as a nuclear autoantigen [4]. In immunofluorescence studies with anti-Ro/SSA antibodies, the 52-kD Ro/SSA and 60-kD Ro/SSA protein have been demonstrated to reside either only in the nucleus, in both nucleus and cytoplasm or predominantly in the cytoplasm [reviewed in 10]. Both proteins are transported to the nucleus in an energy-dependent manner suggesting that for import of both proteins a nuclear localization signal is

required. After the association with Y RNAs, 60-kD Ro/SSA and possibly also 52-kD Ro/SSA are likely to be exported again to the cytoplasm [34].

The La/SSB protein is primarily located in the nucleus [35]. However, it is reported that under stress conditions it is transported in the cytoplasm [36]. Based on the above observations La/SSB was proposed to shuttle between nucleus and cytoplasm and to be involved in nucleocytoplasmic transport of RNA polymerase III transcripts [37]. Consistent with the latter finding is the involvement of La/SBB protein in the initiation and termination of RNA-polymerase-III-dependent transcription [38, 39]. Moreover recent data suggest that human autoantigen La/SSB facilitates viral replication since it accelerates herpes simplex virus type 1 replication in transfected mouse 3T3 cells [40].

Conversely, data regarding the role of Ro/SSA antigen are still unclear. However, a recent study claimed that Ro/SSA autoantigen may function as part of a novel quality control or discard pathway for 5S rRNA production since it was found to be associated with defective 5S rRNA precursors in *Xenopus* oocytes [41]. Furthermore, similarity of the 60-kD Ro/SSA antigen with the p80 subunit of telomerase was shown; telomerase is a ribonucleoprotein complex involved in mechanisms of senescence of mammalian cells, adding hexameric repeats (telomeres) to the growing ends of chromosomal DNA [42]. More recent data suggest that the *Xenopus laevis* 60-kD Ro/SSA autoantigen may be implicated in the regulation of translation of ribosomal protein mRNAs [43] while 52-kD Ro/SSA protein, a DNA-binding protein, seems to participate in transcription regulation [44]. The functional role of the above autoantigens is summarized in table 1.

Methods of Detection

Several methods have been developed for the detection of anti-Ro/SSA and anti-La/SSB antibodies. Among them double immunodiffusion and counterimmunoelectrophoresis are mainly used in clinical diagnosis while Western blot, immunoprecipitation and ELISA were mainly reserved for research purposes. Western blot permits the direct visualization of the Ro/SSA and La/SSB antigenic polypeptides while RNA precipitation detects radiolabeled small Y RNAs. Finally, the use of ELISA provides information regarding quantitative levels, isotype and complement-fixing ability of the antibody.

Meilof et al. [45] reported that the RNA precipitation and counterimmunoelectrophoresis have shown high specificity and sensitivity for anti-Ro/SSA detection, whereas the Ro/SSA ELISA or HeLa immunoblot showed lower sensitivities (96 and 80%, respectively).

In a comparative study of Manoussakis et al. [46], five methods used for the detection of anti-Ro/SSA antibodies are compared in 93 sera deriving from unselected patients with autoimmune disease. In this study, it was shown that the RNA precipitation assay showed the highest sensitivity and was selected as reference method. Counterimmunoelectrophoresis exhibited a specificity of 100% and a sensitivity of 89%. ELISA showed a comparable specificity (95%) but lower sensitivity. Finally, Western blot used for the detection of 52-kD and 60-kD antigenic specificities demonstrated a high specificity (95 and 97%, respectively) but very low sensitivity (36 and 17%, respectively). However, the preferred method for detection of anti-Ro/SSA antibodies in clinical practice remains counterimmunoelectrophoresis because of ease of performance and reliability. ELISA and immunoprecipitation methods are more analytically sensitive methods, which detect even small amounts of anti-Ro/SSA antibodies leading to a decrease in diagnostic specificity. On the other side, studies for the detection of anti-La/SSB antibodies have revealed that immunoblotting of recombinant La/SSB protein or whole cell extract is the most sensitive and specific method for the detection of anti-La/SSB antibodies. Counterimmunoelectrophoresis – conversely to what was reported for anti-Ro/SSA antibodies – does not seem to be a sensitive method for the detection of anti-La/SSB since it yields false-negative results because of the presence of nonprecipitating anti-La/SSB [47].

The identification of linear epitopes of Ro/SSA and La/SSB antigens has led to the development of immunoassays based on the use of synthetic peptides. However, the use of synthetic epitope analogues of 60-kD Ro/SSA for the detection of anti-Ro/SSA seems to be of limited diagnostic value. In their study, Routsias et al. [19] found that the anti-60-kD-Ro/SSA reactivity of 60-kD Ro/SSA epitopes is rather small (table 2). On the other side, recombinant 52-kD Ro/SSA ELISA was proved to be a sensitive method for the detection of anti-Ro/SSA in primary SS [48].

In contrast to anti-Ro/SSA, the synthetic peptide epitope analogues of La/SSB exhibit high sensitivity and specificity for the detection of anti-La/SSB antibodies in ELISA and dot blot techniques. The prevalence of antibodies against several La/SSB epitopes in Greek autoimmune sera is shown in table 2. In the same study it was also demonstrated that the most sensitive and specific peptide (amino acids 349–364) attached on a tetramer

Table 2. Prevalence of antibodies against several epitopes of 60-kD Ro/SSA and La/SSB in Greek patients with SLE and SS

a Ro60

	Amino acids	
	169–190	211–232
SLE (n = 31)	55	35
SS (n = 30)	33	53
Normals (n = 25)	0	0

b La/SSB

	Amino acids			
	147–154	291–302	301–318	349–368
SLE (n = 24)	50	37.5	79	100
SS (n = 39)	18	20	69	82
Disease controls (n = 35)	0	0	2.9	11.4

Table 3. Prevalence (% positive) of anti-Ro/SSA and anti-La/SSB in Greek patients with systemic autoimmune rheumatic diseases

Disease	Anti-Ro/SSA	Anti-La/SSB
Primary SS (n = 101)	63	40
SLE (n = 112)	52	10
Rheumatoid arthritis (n = 350)	10	1
Normals (n = 200)	0.5	0

sequential oligopeptide SOC_4 has the same sensitivity for the detection of anti-La/SSB antibodies as the recombinant protein [49].

Clinical Associations

Anti-Ro/SSA is detected in a variety of connective tissue disorders in various frequencies ranging from 30 to 50% in patients with SLE, 50 to 80% in SS, 95 to 100% in neonatal lupus erythematosus, 60% in subacute cutaneous lupus erythematosus and from 3 to 14% in patients with rheumatoid arthritis [50]. The frequencies for anti-La/SSB antibodies in various disorders depend on the method of detection used, since they are considerably higher when they are measured by ELISA and immunoblotting [47]. In primary SS, anti-La/SSB antibodies are found in 60% of patients [51]. In sera of patients with SLE the prevalence of the above antibody ranges between 6 and 15%, while the correspondent figure in subacute cutaneous lupus erythematosus ranges from 25 to 35% [52, 53]. The prevalence of anti-Ro/SSA and anti-La/SSB in Greek patients with systemic autoimmune diseases is reported in table 3 [51].

In primary SS, anti-Ro/SSA is linked with early disease onset, long disease duration, parotid or major gland enlargement and intensive lymphocytic infiltration of the minor salivary glands. Furthermore, it is considered a marker of extraglandular involvement since it is associated with lymphadenopathy, splenomegaly and vasculitis [54, 55].

Neonatal lupus erythematosus is invariably associated with the occurrence of anti-Ro/SSA and/or anti-La/SSB antibodies in maternal sera. This is a clinical syndrome, affecting primarily the newborn of anti-Ro/SSA-positive mothers regardless of their clinical status; it is characterized by the presence of cutaneous lesions resembling subacute lupus erythematosus, congenital heart block, hepatitis hemolytic anemia and thrombocytopenia. This syndrome represents a classical model of passively acquired autoimmunity since it is considered to result from transplacental passage of IgG anti-Ro/SSA and anti-La/SSB antibodies starting around the twentieth week of gestation causing histopathological lesions in the developing tissues. Among the clinical manifestations of this syndrome irreversible congenital heart block appears to be the more serious feature, in contrast to the remainder of clinical manifestations which resolve usually at about 6 months of life with the disappearance of maternal antibodies from the neonatal circulation [56]. In a recent study, it was shown that anti-Ro/SSA antibody, although it does not adversely effect pregnancy outcome in SLE patients, appears to be associated with recurrent pregnancy loss in non-SLE patients suggesting the heterogeneous nature of the immune response to Ro/SSA antigen [57].

The presence of anti-La/SSB in SLE patients has mainly been associated with sicca symptoms [58]. SLE anti-Ro/SSA-positive patients frequently present with non-fixed nonscarring cutaneous lesions of subacute cutaneous lupus erythematosus or evidence of sicca syndrome (SS/lupus erythematosus overlap) [59]. In these patients the presence of the autoantibody is correlated with late-onset disease (after the age of 55 years) and lupus-like syndrome associated with C2 or C4 deficiency [60, 61].

In a more recent study, Simmons-O'Brien et al. [62] have explored the clinical relevance of anti-Ro/SSA anti-

bodies in 100 patients with unselected connective tissue disorders revealing their correlation with systemic features as interstitial pulmonary disease, central nervous involvement and vasculitis. Cutaneous manifestations, particularly photosensitivity, malar dermatitis and discoid lesions, were also reported [62].

Greek patients with rheumatoid arthritis and anti-Ro/SSA positivity constitute a distinct clinical subgroup presenting with erosive symmetric polyarthritis, a high incidence of histopathological evidence of SS and intolerance to *D*-penicillamine treatment [63].

Immunogenetic Associations

Several studies have so far reported the association between anti-Ro/SSA and anti-La/SSB responses with certain major histocompatibility complex (MHC) class II alleles suggesting the MHC-dependent nature of the immune response to these autoantigens. HLA-DR8 and -DR3 were correlated with anti-Ro/SSA and anti-La/SSB responses in patients with SS and SLE, while HLA-DR2 is found to be associated with anti-Ro/SSA responses not accompanied by anti-La/SSB [64–66]. A strong gene interaction between HLA-DQ1 and -DQ2 alleles has been associated with higher levels of anti-Ro/SSA and anti-La/SSB antibodies in patients with SS [67]. In a subsequent study of Fujisaku et al. [68], restriction fragment length polymorphisms of the DQa and DQβ genes have been related to Ro/SSA precipitins in patients with SLE suggesting a gene complementation mechanism involved in the generation of the autoimmune response. In another study, Reveille et al. [69] found that almost all of the anti-Ro/SSA patients had a glutamine at amino acid sequence position 34 of a DQA1 chain and a leucine at position 26 of DQB1 chain. It is noteworthy that the DQA1 0501 gene possesses glutamine at position 34 and it is found in the majority of SS patients, across racial and ethnic boundaries, suggesting its involvement in the predisposition to primary SS [70].

On the other side, Miyagawa et al. [71] have shown that HLA class II allele distributions differ between anti-Ro/SSA-positive Japanese patients according to the presence or absence of coexisting anti-La/SSB antibodies. The presence of both anti-Ro/SSA and anti-La/SSB responses, but not those to anti-Ro/SSA alone, were associated with DRB1 alleles that shared the same amino acid residues at positions 14–31 and 71 of the hypervariable regions of the DRB1 chain [71].

Rischmueller et al. [72] provided a model of HLA-restricted presentation of La/Ro peptide determinants showing that the HLA DR3-DQA1*0501-DQB1*02 (DR3-DQ2) haplotype was primarily associated with a diversified La/Ro RNP response containing precipitating autoantibodies to La/SSB, whereas the haplotype HLA DR2-DQA1*0102-DQB1*0602 (DR2-DQ1) was associated with a less diversified La/Ro RNP response involving nonprecipitating anti-La/SSB autoantibodies. Scofield et al. [73] also report that antibodies binding the 13-kD fragment of 60-kD Ro/SSA autoantigen are more likely to be found in the sera of patients with particular DQA1 and DQB1 alleles, while antibodies binding the epitope at 480–494 are found almost exclusively in the sera of patients with a Bg/II 9.8-kb polymorphism of the T cell receptor β gene.

Finally, specific maternal MHC class II genes seem to correlate with specific neonatal outcomes in neonatal lupus syndrome. In fact, a recent study in Japanese women reveals that the maternal HLA-DR5 haplotype DRB1*1101-DQA1*0501-DQB1*0301 was significantly associated with neonatal cutaneous lupus but not congenital heart block in contrast to maternal HLA-DQB1*0602 carried on HLA-DR2 haplotypes which was correlated with congenital heart block but not cutaneous neonatal lupus [74].

Pathogenetic Role

Increasing evidence of direct involvement of anti-Ro/SSA and anti-La/SSB antibodies in the pathogenesis of tissue injury is supported by several studies. In fact, their correlation with distinct clinical features as it is mentioned above, the local production of anti-La/SSB antibodies in the salivary glands of patients suffering from the syndrome [75], the detection of anti-Ro/SSA in skin biopsies of patients with subacute cutaneous lupus erythematosus [76] and the strong association with congenital heart block in neonates of anti-Ro/SSA-positive mothers [56] are examples of their putative pathogenetic potential. Meanwhile, in a recent study a co-variation of autoantibody levels and disease activity in 11 of 14 patients examined was demonstrated [77]. However, their precise role remains unknown.

The study of the immune response to Ro/SSA and La/SSB autoantigens could provide some clues for the generation of autoimmune responses towards the cellular components. At this point the question is how intracellular antigens such Ro/SSA and La/SSB are presented to the

Table 4. Molecular mimicry of Ro60 and La/SSB autoantigens

Ro60		La/SSB	
^{175}NGWSHKDLLR^{184}	^{485}EYRKK*MD^{491}	^{147}HKAFKGSI^{154}	^{93}PSKLPEV^{109}
HLA class II β-chain (human) YWNSQKDLLQ	N-protein VSV ^{151}-EYRKKLMD^{158}	human myelin basic protein ^{139}HKGFKGVD^{146} human DNA topoisomerase II YKNFKGTI	gag polyprotein FSV ^{32}PSKLSEV^{39}

VSV = Vesicular stomatitis virus; FSV = feline sarcoma virus.

immune system. It is known that the presentation of an antigen to the immune system occurs either by tissue damage and release of the cellular components to the circulation or by active translocation of autoantigens from the nucleus to the cytoplasm and selective presentation in the cell surface. The first possibility seems to be weak in the case of anti-Ro/SSA antibodies. In fact, in the study of Blann et al. [78], no correlation between the presence of anti-Ro/SSA and anti-La/SSB with endothelium damage (estimated by von Willebrand levels) in SS was observed. On the other side, several reports demonstrate the aberrant membrane localization of Ro/SSA and La/SSB autoantigens in disease states and in vitro experiments under stressful conditions for the cell stimuli (UV light, TNF-α, viruses). Studies in SS patients have shown cytoplasmic/surface staining of conjunctival epithelium and increased expression of cytoplasmic La/SSB in the salivary epithelium compared to control individuals [79]. Furthermore, Ro/SSA can be found on the membrane of skin keratocytes of patients with SLE [80].

LeFeber et al. [81] have shown surface expression of an antigen reactive with anti-Ro/SSA-positive sera after irradiation of keratinocytes with UV light, while Dorner et al. [82] reported that TNF-α mediates the surface expression of 52-kD Ro/SSA and La/SSB autoantigens on human keratinocytes of patients with SLE and SS. In another report, Baboonian et al. [83] demonstrated that infection of epithelial cells with adenovirus 2 promotes the translocation of La/SSB antigen from the nucleus to the cytoplasm and the cell membrane.

Programmed cell death (apoptosis) seems to be a mechanism, which can explain the above findings. In the study of Casciola-Rosen et al. [84], it was demonstrated that during apoptosis of keratinocytes in response to UV radiation, autoantigens are clustered in two different populations in the apoptotic blebs. One population consisted of Ro/SSA, fragmented endoplasmic reticulum and ribosomes, while the other contained DNA surrounded by ribonucleoprotein antigens including Ro/SSA and Sn RNPs.

The second major question raised in autoimmunity is how these self-autoantigens break the immune tolerance. There are several hypotheses accounting for autoantibody production: molecular mimicry as a possible mechanism of induction of autoimmune response has been so far implicated; i.e. antibodies for foreign antigens would cross-react with homologous self-proteins. Using protein databases, sequence similarities of antigenic determinants of Ro/SSA and La/SSB with other molecules have been reported. Initially, it was shown that 60-kD Ro/SSA contains a sequence homology of 7 of 8 amino acids with vesicular stomatitis virus [85]. However, only a minority of sera from anti-Ro/SSA-positive patients present autoantibodies directed to that region [86]. Subsequent studies of antigenic epitope of 60-kD Ro/SSA antigen ^{175}NGNSHKDLLD^{184} rvealed sequence similarity with a conserved region on human HLA class II β-chain [19]. Similarly, Tzioufas et al. [32] reported that the La/SSB epitope HKAFKGSI presented sequence similarity with fragments of human myelin basic protein and DNA topoisomerase II. Another antigenic La/SSB sequence (residues 88–105) demonstrated a sequence homology with a retroviral gag protein suggesting a possible role for retroviral infection in the induction of anti-La/SSB response [87]. Data regarding molecular mimicry of Ro/SSA and La/SSB antigens are shown in table 4. Accumulated information suggests that anti-Ro/SSA and anti-La/SSB responses are antigen driven rather than products of a nonspecific polyclonal B cell activation: the presence of a selective and coordinated antibody response to polypeptides on the same particle provides such an evidence. Topfer et al. [88] demonstrated intramolecular and intermolecular spreading of the anti-La/SSB response. Immunization of healthy nonautoimmune mice with recombi-

nant mouse La/SSB was initially directed against the La C subfragment (amino acids 111–242) but rapidly spread to involve the La A (amino acids 1–107) and La F (amino acids 243–345) regions of the La/SSB antigen. Moreover immunization with 60-kD Ro/SSA produced a high-titer anti-Ro/SSA antibody response and anti-La/SSB autoantibodies [88].

Scofield et al. [89] have shown that the initiation of the immune response to the Ro/SSA ribonucleoprotein in a patient with SLE was similar to that following the invasion of a foreign antigen. IgM anti-Ro/SSA appeared shortly before IgG anti-Ro/SSA and disappeared as IgG anti-Ro/SSA increased in titer and affinity [89].

On the basis of this theoretical model, Ro/SSA antigen is processed and presented by molecules of the MHC as a foreign antigen. This assumption is further supported by the association of anti-Ro/SSA responses with HLA and T cell receptor genes in SLE patients [90] suggesting that anti-Ro/SSA production is antigen driven, a notion further solidified by data revealed from epitope mapping studies. In fact, it seems that activated B cells specific for a certain epitope play an antigen-presenting role leading to activation of specific T cells and induction of a T cell help autoimmune response [91].

The pathogenic impact of anti-Ro/SSA and anti-La/SSB in neonatal lupus syndromes seems to be considerable. However, although several hypotheses have been proposed, the exact mechanism through which these autoantibodies bind the fetal conduction system and elicit a local inflammatory response is unclear. Maternal IgG anti-La/SSB and anti-Ro/SSA have been isolated from affected fetal hearts, while animal studies revealed possible arrhythmogenic effects of these antibodies [92]. Alexander et al. [93] reported that superinfusion of newborn rabbit ventricular papillary muscles with IgG-enriched fractions from sera containing anti-La/SSB and anti-Ro/SSA antibodies reduced the plateau phase of the action potential leading to alteration in Ca^{2+} influx. Garcia et al. [94] using adult rabbit hearts showed that IgG-enriched fractions from anti-La/SSB- and anti-Ro/SSA-positive women induced conduction abnormalities and reduction of the peak slow inward current. More recent data have shown that IgG-enriched fractions and anti-52-kD-Ro/SSA antibodies affinity purified from sera of mothers whose children have congenital heart block induce complete atrioventricular block in the human heart perfused by the Langendorff technique [95]. The above data suggest a direct pathogenetic role of these autoantibodies in congenital heart block. IgG antibodies against a 57-kD protein, found in 10% of SLE patients almost always with the presence of anti-Ro/SSA antibodies are also proposed as an additional risk factor in the pathogenesis of neonatal lupus syndromes [86].

Therapeutic Perspectives

During the past decade, the molecular structure of Ro/SSA and La/SSB antigens was elucidated and disease-specific epitopes, against which anti-Ro/SSA and anti-La/SSB antibodies are directed, were identified. As long as the mechanism of the elicited immune response in autoimmune disorders is better conceived as antigen driven, new implications even at the therapeutic level are emerging. It is well known that prior oral administration of antigen can lead to a state of specific immunological unresponsiveness known as oral tolerance. Thus, the oral use of Ro/SSA and La/SSB antigens and particularly of peptides corresponding to immunodominant T cell epitopes is proposed as a future intervention in patients with SS inducing oral tolerance to the above autoantigens [97].

References

1 Moutsopoulos HM: Sjögren's synrome: Autoimmune epithelitis. Clin Immunol Immunopathol 1994;72:162–165.
2 Polychronis M, Tapinos N, Theocharis S, Economou A, Kittas C, Moutsopoulos HM: Modes of epithelial cell death in Sjögren's syndrome. Clin Exp Immunol 1998;114:485–490.
3 Tzioufas AG, Moutsopoulos HM: Sjögren's syndrome; in Belch JJF, Zurier R (eds): Textbook of Connective Tissue Diseases. London, Chapman & Hall, 1995, pp 103–130.
4 Harley J: Autoantibodies in Sjögren's syndrome; in Talal N, Moutsopoulos HM, Kassan S (eds): Sjögren's Clinical and Immunological Aspects. Berlin, Springer, 1987.
5 Von Muhlen CA, Tan EM: Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum 1995;24:323–358.
6 Haneji N, Nakamura T, Takio K, Yanagi K, Higashiyama H, Saito I, Noji S, Sugino H, Hayashi Y: Identification of alpha-fodrin as a candidate autoantigen in primary Sjögren's syndrome. Science 1997;276:604–607.
7 Vitali C, Bombardieri S, Moutsopoulos HM, et al: Preliminary criteria for the classification of Sjögren's syndrome. Arthritis Rheum 1993;36:340–347.
8 Hendrick JP, Wolin SL, Rinke J, Lerner MR, Steitz JA: Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: Further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. Mol Cell Biol 1981;1:1138–1149.
9 Chan EK, Buyon JP: The SSA/Ro antigen; in Van Venrooij W, Maini RN (eds): Manual of Biological Markers of Disease, Dordrecht, Kluwer Academic Publishers, 1994, B4.1, pp 1–18.
10 Van Venrooij WJ, Slobbe RL, Pruijn GJ: Structure and function of La and Ro RNPs. Mol Biol Rep 1993;18:113–119.

11 Frank MB, Itoh K, Fujisaku A, Pontarotti P, Mattei MG, Neas BR: The mapping of the human 52-kD Ro/SSA autoantigen gene to human chromosome 11, and its polymorphisms. Am J Hum Genet 1993;52:183–191.
12 Chan EK, Di Donato F, Hamel JC, Tseng CE, Buyon JP: 52-kD SS-A/Ro: Genomic structure and identification of an alternatively spliced transcript encoding a novel leucine zipper-minus autoantigen expressed in fetal and adult heart. J Exp Med 1995;182:983–992.
13 Frank MB, Mattei MG: Mapping of the human 60,000 M(r) Ro/SSA locus: The genes for three Ro/SSA autoantigens are located on separate chromosomes. Immunogenetics 1994;39:428–431.
14 Chambers JC, Kenan D, Martin BJ, Keene JD: Genomic structure and amino acid sequence domains of the human La autoantigen. J Biol Chem 1988;263:18043–18051.
15 Scofield RH, Dickey WD, Jackson KW, James JA, Harley JB: A common autoepitope near the carboxyl terminus of the 60-kD Ro ribonucleoprotein: Sequence similarity with a viral protein. J Clin Immunol 1991;11:378–388.
16 Barakat S, Meyer O, Torterotot F, Youinou P, Briand JP, Kahn MF, Muller S: IgG antibodies from patients with primary Sjögren's syndrome and systemic lupus erythematosus recognize different epitopes in 60-kD SSA/Ro protein. Clin Exp Immunol 1992;89:38–45.
17 Wahren M, Rudén U, Andersson B, Ringertz NR, Pettersson I: Identification of antigenic regions of the human Ro60kDa protein using recombinant antigen and synthetic peptides. J Autoimmun 1992;5:319–332.
18 Routsias JG, Tzioufas AG, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM: Epitope mapping of the Ro/SSA60kD autoantigen reveals disease-specific antibody-binding profiles. Eur J Clin Invest 1996;26:514–521.
19 Routsias JG, Sakarellos-Daitsiotis M, Tsikaris V, Sakarellos C, Moutsopoulos HM, Tzioufas AG: Structural, molecular and immunological properties of linear B-cell epitopes of Ro60kD autoantigen. Scand J Immunol 1998;47:280–287.
20 Boire G, Lopez-Longo F, Lapointe S, Menard HA: Sera from patients with autoimmune disease recognize conformational determinants on the 60-kD Ro/SSA protein. Arthritis Rheum 1991;34:722–730.
21 Scofield RH, Zhang FC, Kurien BT, Harley JB: Anti-Ro fine specificity defined by multiple antigenic peptides identifies components of tertiary epitopes. Clin Exp Immunol 1997;109:480–487.
22 Huang SC, Scofield RH, Kurien BT, Harley JB: Human anti-Ro autoantobodies bind multiple conformational epitopes of 60-kD Ro autoantigen. J Clin Immunol 1997;17:212–219.
23 Scofield RH, Henry WE, Kurien BT, James JA, Harley JB: Immunization with short peptides from the sequence of the systemic lupus erythematosus-associated 60-kDa Ro autoantigen results in anti-Ro ribonucleoprotein autoimmunity. J Immunol 1996;156:4059–4066.
24 Dorner T, Feist E, Wagenmann A, Kato Y, Yamamoto K, Nishioka K, Burmester GR, Hiepe F: Anti-52kDa Ro (SSA) autoantibodies in different autoimmune diseases preferentially recognize epitopes on the central region of the antigen. J Rheumatol 1996;23:462–468.
25 Ricchiuti V, Briand JP, Meyer O, Isenberg DA, Pruijn G, Muller S: Epitope mapping with synthetic peptides of 52-kD SSA/Ro protein reveals heterogeneous antibody profiles in human autoimmune sera. Clin Exp Immunol 1994;95:397–407.
26 Bozic B, Pruijn GJ, Rozman B, van Venrooij WJ: Sera from patients with rheumatic diseases recognize different epitope regions on the 52-kD Ro/SS-A protein. Clin Exp Immunol 1993;94:227–235.
27 Frank MB, Itoh K, McCubbin V: Epitope mapping of the 52-kD Ro/SSA autoantigen. Clin Exp Immunol 1994;95:390–396.
28 Kato T, Sasakawa H, Suzuki S, Shirako M, Tashiro F, Nishioka K, Yamamoto K: Autoepitopes of the 52-kD SS-A/Ro molecule. Arthritis Rheum 1995;38:990–998.
29 Chan EK, Francoeur AM, Tan EM: Epitopes, structural domains, and asymmetry of amino acid residues in SS-B/La nuclear protein. J Immunol 1986;136:3744–3749.
30 Sturgess AD, Peterson MG, McNeilage LJ, Whittingham S, Coppel RL: Characteristics and epitope mapping of a cloned human autoantigen La. J Immunol 1988;140:3212–3218.
31 St Clair EW, Pisetsky DS, Reich CF, Keene JD: Analysis of autoantibody binding to different regions of the human La antigen expressed in recombinant fusion proteins. J Immunol 1988;141:4173–4180.
32 Tzioufas AG, Yiannaki E, Sakarellos-Daitsiotis M, Routsias JG, Sakarellos C, Moutsopoulos HM: Fine specificity of autoantibodies to La/SSB: Epitope mapping, and characterization. Clin Exp Immunol 1997;108:191–198.
33 Granger D, Tremblay A, Boulanger C, Chabot B, Menard HA, Boire G: Autoantigenic epitopes on hY5 Ro RNA are distinct from regions bound by the 60-kD Ro and La proteins. J Immunol 1996;157:2193–2200.
34 Pruijn GJ, Simons FH, van Venrooij WJ: Intracellular localization and nucleocytoplasmic transport of Ro RNP components. Eur J Cell Biol 1997;74:123–132.
35 Habets WJ, den Brok JH, Boerbooms AM, van de Putte LB, van Venrooij W: Characterization of the SS-B (La) antigen in adenovirus-infected and uninfected HeLa cells. EMBO J 1983;2:1625–1631.
36 Bachmann M, Falke D, Schroder HC, Muller WE: Intracellular distribution of the La antigen in CV-1 cells after herpes simplex virus type 1 infection compared with the localization of U small nuclear ribonucleoprotein particles. J Gen Virol 1989;70:881–891.
37 Bachmann M, Pfeifer K, Schroder HC, Muller WE: The La antigen shuttles between the nucleus and the cytoplasm in CV-1 cells. Mol Cell Biochem 1989;85:103–114.
38 Gottlieb E, Steitz JA: Function of the mammalian La protein: Evidence for its action in transcription termination by RNA polymerase III. EMBO J 1989;8:851–861.
39 Maraia R: Transcription termination factor La is also an initiation factor for RNA polymerase III. Proc Natl Acad Sci USA 1996;93:3383–3387.
40 Bachmann M, Deister H, Pautz A, Laubinger J, Schmitz M, Falke D, Podlech J, Grolz D: The human autoantigen La/SS-B accelerates herpes simplex virus type 1 replication in transfected mouse 3T3 cells. Clin Exp Immunol 1998;112:482–489.
41 O'Brien CA, Wolin SL: A possible role for the 60-kD Ro autoantigen in a discard pathway for defective 5S rRNA precursors. Gene Dev 1994;8:2891–2903.
42 Ramakrishnan S, Sharma HW, Farris AD, Kaufman KM, Harley JB, Collins K, Pruijn GJ, van Venrooij WJ, Martin ML, Narayanan R: Characterization of human telomerase complex. Proc Natl Acad Sci USA 1997;94:10075–10079.
43 Pellizzoni L, Lotti F, Rutjes SA, Pierandrei-Amaldi P: Involvement of the *Xenopus laevis* Ro60 autoantigen in the alternative interaction of La and CNBP proteins with the 5′UTR of L4 ribosomal protein mRNA. J Mol Biol 1998;281:593–608.
44 Frank MB, McCubin VR, Heldermon C: Expression and DNA binding of the human 52 kD Ro/SSA autoantigen. Biochem J 1995, 305:359–362.
45 Meilof JF, Bantjes I, De Jong J, Van Dam AP, Smeenk RJT: The detection of anti-Ro (SSA) and anti-La (SSB) antibodies: A comparison of counter immunoelectrophoresis with immunoblot, ELISA and RNA-precipitation assays. J Immunol Methods 1990;133:215–226.
46 Manoussakis MN, Kistis KG, Liu X, Aidinis V, Guialis A, Moutsopoulos HM: Detection of anti-Ro (SSA) antibodies in autoimmune diseases: Comparison of five methods. Br J Rheumatol 1993;32:449–455.
47 Keeth CL, McCluskey, Gordon TP: SSB (La) autoantibodies; in Peter JB, Shoefeld Y (eds): Autoantibodies. Amsterdam, Elsevier Science BV, 1996, pp 789–797.
48 McCauliffe DP, Wang L, Satoh M, Reeves WH, Small D: Recombinant 52kDa Ro (SSA) ELISA detects autoantibodies in Sjögren's syndrome sera that go undetected by conventional serological assays. J Rheumatol 1997;24:860–866.
49 Yiannaki EE, Tzioufas AG, Bachmann M; Hantoumi J, Tsikaris V, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM: The value of synthetic linear epitope analogues of La/SSB for the detection of autoantibodies to La/SSB: Specificity, sensitivity and comparison of methods. Clin Exp Immunol 1998;112:152–158.
50 Tan EM: Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989;44:93–151.

51 Tzioufas AG, Moutsopoulos HM: Clinical Significance of Antibodies to Ro/SSA and La/SSB. Autoantibody Manual. Dordrecht, Kluwer Academic Publishers, 1996, vol C4.1, pp 1–14.
52 Venalbes PJ, Shattles W, Pease CT, Ellis JE, Charles PJ, Maini RN: Anti-La (SS-B): A diagnostic criterion for Sjögren's syndrome? Clin Exp Rheumatol 1989;7:181–184.
53 Sontheimer RD, McCauliffe DP: Pathogenesis of anti-Ro/SS-A autoantibody-associated cutaneous lupus erythematosus. Dermatol Clin 1990;8:751–758.
54 Manoussakis MN, Tzioufas AG, Pange PJ, Moutsopoulos HM: Serological profiles in subgroups of patients with Sjögren's syndrome. Scand J Rheumatol Suppl 1986;61:89–92.
55 Molina R, Provost TT, Alexander EL: Peripheral inflammatory vascular disease in Sjögren's syndrome: Association with nervous system complications. Arthritis Rheum 1985;28: 1341–1347.
56 Buyon JP: Neonatal lupus: Bedside to bench and back. Scand J Rheumatol 1996;25:271–276.
57 Mavragani CP, Dafni UG, Tzioufas AG, Moutsopoulos HM: Pregnancy outcome and anti-Ro/SSA in autoimmune diseases: A retrospective cohort study. Br J Rheumatol 1998; 37:740–745.
58 Andonopoulos AP, Skopouli FN, Dimou GS, Drosos AA, Moutsopoulos HM: Sjögren's syndrome in systemic lupus erythematosus. J Rheumatol 1990;17:201–204.
59 Sontheimer RD, Maddison PJ, Reichlin M, Jordon RE, Stastny P, Gilliam JN: Serologic and HLA associations in subacute cutaneous lupus erythematosus, a clinical subset of lupus erythematosus. Ann Intern Med 1982;97:664–671.
60 Catoggio LJ, Skinner RP, Smith G, Maddison PJ: Systemic lupus erythematosus in the elderly: Clinical and seerological characteristics. J Rheumatol 1984;11:175–181.
61 Provost TT, Arnett FC, Reichlin M: Homozygous C2 deficiency, lupus erythematosus, and anti-Ro (SSA) antibodies. Arthritis Rheum 1983;26:1279–1282.
62 Simmons-O'Brien E, Chen S, Watson R, Antoni C, Petri M, Hochberg M, Stevens MB, Provost TT: One hundred anti-Ro (SS-A) antibody positive patients: A 10-year follow-up. Medicine (Baltimore) 1995;74:109–130.
63 Moutsopoulos HM, Giotaki H, Maddison PJ, Mavridis AC, Drosos AA, Skopouli FN: Antibodies to cellular antigens in Greek patients with autoimmune rheumatic diseases: Anti-Ro (SSA) antibody, a possible marker of penicillamine-D intolerance. Ann Rheum Dis 1984; 43:285–287.
64 Harley JB, Alexander EL, Bias WB, Fox OF, Provost TT, Reichlin M, Yamagata H, Arnett FC: Anti-Ro (SS-A) and anti-La (SS-B) in patients with Sjögren's syndrome. Arthritis Rheum 1986;29:196–206.
65 Wilson RW, Provost TT, Bias WB, Alexander EL, Edlow DW, Hochberg MC, Stevens MB, Arnett FC: Sjögren's syndrome: Influence of multiple HLA-D region alloantigens on clinical and serologic expression. Arthritis Rheum 1984;27:1245–1253.
66 Hamilton RG, Harley JB, Bias WB, Roebber M, Reichlin M, Hochberg MC, Arnett FC: Two Ro (SS-A) autoantibody responses in systemic lupus erythematosus: Correlation of HLA-DR/DQ specificities with quantitative expression of Ro (SS-A) autoantibody. Arthritis Rheum 1988;31:496–505.
67 Harley JB, Reichlin M, Arnett FC, Alexander EL, Bias WB, Provost TT: Gene interaction at HLA-DQ enhances autoantibody production in primary Sjögren's syndrome. Science 1986; 232:1145–1147.
68 Fujisaku A, Frank MB, Neas B, Reichlin M, Harley JB: HLA-DQ gene complementation and other histocompatibility relationships in man with the anti-Ro/SSA autoantibody response of systemic lupus erythematosus. J Clin Invest 1990;86:606–611.
69 Reveille JD, Macleod MJ, Whittington K, Arnett FC: Specific amino acid residues in the second hypervariable region of HLA-DQA1 and DQB1 chain genes promote the Ro (SS-A)/La (SS-B) autoantibody responses. J Immunol 1991;146:3871–3876.
70 Roitberg-Tambur A, Friedmann A, Safirman C, Markitziu A, Ben-Chetrit E, Rubinow A, Moutsopoulos HM, Stavropoulos E, Skopouli FN, Margalit H, et al: Molecular analysis of HLA class II genes in primary Sjögren's syndrome: A study of Israeli Jewish and Greek non-Jewish patients. Hum Immunol 1993;36: 235–242.
71 Miyagawa S, Shinohara K, Nakajima M, Kidoguchi K, Fujita T, Fukumoto T, Yoshioka A, Dohi K, Shirai T: Polymorphisms of HLA class II genes and autoimmune responses to Ro/SS-A-La/SS-B among Japanese subjects. Arthritis Rheum 1998;41:927–934.
72 Rischmueller M, Lester S, Chen Z, Champion G, Van Den Berg R, Beer R, Coates T, McCluskey J, Gordon T: HLA class II phenotype controls diversification of the autoantibody response in primary Sjögren's syndrome (pSS). Clin Exp Immunol 1998;111:365–371.
73 Scofield RH, Dickey WD, Hardgrave KL, Neas BR, Horowitz RM, McArthur RA, Fujisaku A, Frank MB, Harley J: Immunogenetics of epitopes of the carboxyl terminus of the human 60-kD Ro autoantigen (published erratum appears in Clin Exp Immunol 1995;100:377). Clin Exp Immunol 1995;99:256–261.
74 Miyagawa S, Shinohara K, Kidoguchi K, Fujita T, Fukumoto T, Yamashina Y, Hashimoto K, Yoshioka A, Sakurai S, Nishihara O, Shirai T: Neonatal lupus erythematosus: HLA-DR and -DQ distributions are different among the groups of anti-Ro/SSA-positive mothers with different neonatal outcomes. J Invest Dermatol 1997;108:881–885.
75 Horsfall AC, Rose LM, Maini RN: Autoantibody synthesis in salivary glands of Sjögren's syndrome patients. J Autoimmun 1989;2:559–568.
76 Bennion SD, Ferris C, Lieu TS, Reimer CB, Lee LA: IgG subclasses in the serum and skin in subacute cutaneous lupus erythematosus and neonatal lupus erythematosus. J Invest Dermatol 1990;95:643–646.
77 Wahren M, Tengner P, Gunnarsson I, Lundberg I, Hedfors E, Ringertz NR, Pettersson I: Ro/SS-A and La/SS-B antibody level variation in patients with Sjögren's syndrome and systemic lupus erythematosus. J Autoimmune 1998;11:29–38.
78 Blann AD, Wainwright AC, Emery P: Damage to the endothelium in Sjögren's syndrome: Lack of correlation with antinuclear antibody titre and presence of antibodies to SSA or SSB autoantigens. Ann Rheum Dis 1992;51:389–390.
79 Yannopoulos DI, Roncin S, Lamour A, Pennec YL, Moutsopoulos HM, Youinou P: Conjunctival epithelial cells from patients with Sjögren's syndrome inappropriately express major histocompatibility compex molecules, La (SSB) antigen, and heat-shock proteins. J Clin Immunol 1992;12:259–265.
80 Golan TD, Elkon KB, Gharavi AE, Krueger JG: Enhanced membrane-binding of autoantibodies to cultured keratinocytes of systemic lupus erythematosus patients after ultraviolet B/ultraviolet A irradiation. J Clin Invest 1992; 90:1067–1074.
81 LeFeber WP, Norris DA, Ryan SR, Huff JC, Lee LA, Kubo M, Boyce ST, Kotzin BL, Weston WL: Ultraviolet light induces binding of antibodies to selected nuclear antigens on cultured human keratinocytes. J Clin Invest 1984; 74:1545–1551.
82 Dorner T, Hucko M, Mayet WJ, Trefzer U, Burmester GR, Hiepe F: Enhanced membrane expression of the 52 kDa Ro (SS-A) and La (SS-B) antigens by human keratinocytes induced by TNF alpha. Ann Rheum Dis 1995;54:904–909.
83 Baboonian C, Venables PJ, Booth J Williams DG, Roffe LM, Maini RN: Virus infection induces redistribution and membrane localization of the nuclear antigen La (SS-B): A possible mechanism for autoimmunity. Clin Exp Immunol 1989;78:454–459.
84 Casciola-Rosen LA, Anhalt G, Rosen A: Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J Exp Med 1994;179:1317–1330.
85 Scofield RH, Harley JB: Autoantigenicity of Ro/SSA antigen is related to a nucleocapsid protein of vesicular stomatitis virus. Proc Natl Acad Sci USA 1991;88:3343–3347.
86 Routsias JG, Detsikas A, Sakarellos-Daitsiotis M, Tzioufas AG, Sakarellos C, Moutsopoulos HM: Antibodies to EYRKK vesicular stomatitis virus associated peptide account only for a minority of anti Ro60kD antibodies. Clin Exp Immunol 1994;98:414–418.
87 Kohsaka H, Yamamoto K, Fujii H, Miura H, Miyasaka N, Nishioka K, Miyamoto T: Fine epitope mapping of the human SS-B/La protein: Identification of a distinct autoepitope homologous to a viral gag polyprotein. J Clin Invest 1990;85:1566–1574.

88 Topfer F, Gordon T, McCluskey J: Intra- and intermolecular spreading of autoimmunity involving the nuclear self-antigens La (SS-B) and Ro (SS-A). Proc Natl Acad Sci USA 1995;92: 875–879.
89 Scofield RH, Zhang F, Kurien BT, Anderson CJ, Reichlin M, Harley JB, Stafford HA: Development of the anti-Ro autoantibody response in a patient with systemic lupus erythematosus. Arthritis Rheum 1996;39:1664–1668.
90 Frank MB, McArthur R, Harley JB, Fujisaku A: Anti-Ro (SSA) autoantibodies are associated with T cell receptor beta genes in systemic lupus erythematosus patients. J Clin Invest 1990;85:33–39.
91 Lanzavecchia A: Antigen-specific interaction between T and B cells. Nature 1985;314:537–539.
92 Horsfall AC, Venables PJ, Taylor PV, Maini RN: Ro and La antigens and maternal anti-La idiotype on the surface of myocardial fibres in congenital heart block. J Autoimmune 1991;4: 165–176.
93 Alexander E, Buyon JP, Provost TT, Guarnieri T: Anti-Ro/SS-A antibodies in the pathophysiology of congenital heart block in neonatal lupus syndrome, an experimental model: In vitro electrophysiologic and immunocytochemical studies. Arthritis Rheum 1992;35: 176–189.
94 Garcia S, Nascimento JH, Bonfa E, Levy R, Oliveira SF, Tavares AV, de Cavalho AC: Cellular mechanism of the conduction abnormalities induced by serum from anti-Ro/SSA-positive patients in rabbit hearts. J Clin Invest 1994;93:718–724.
95 Boutjdir M, Chen L, Zhang ZH, Tseng CE, DiDonato F, Rashbaum W, Morris A, el-Sherif N, Buyon JP: Arrhythmogenicity of IgG and anti-52-kD SSA/Ro affinity-purified antibodies from mothers of children with congenital heart block. Circ Res 1997;80:354–362.
96 Maddison PJ, Lee L, Reichlein M, Sinclair A, Wasson C, Schemmer G, Reichlein M: Anti-p57: A novel association with neonatal lupus. Clin Exp Immunol 1995;99:42–48.
97 Moutsopoulos HM: Sjögren's syndrome therapy: Future directions. J Rheumatol 1997; 24(suppl 50):33–34.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:58–66

Significance of Autoantibodies in Neonatal Lupus Erythematosus

Thomas Dörner[a] Eugen Feist[a] Axel Pruss[b] Rabija Chaoui[c]
Burkhard Göldner[d] Falk Hiepe[a]

[a]Department of Medicine (Rheumatology and Clinical Immunology), [b]Institute for Transfusion-Medicine, [c]Department of Gynecology & Obstetrics, and [d]Department of Pediatry (Cardiology), Charité Medical School, Humboldt University, Berlin, Germany

Key Words
Autoantibodies · Neonatal lupus erythematosus · Congenital heart block · Systemic lupus erythematosus · Sjögren's syndrome

Abstract
Autoantibodies produced by the mother and transported into the fetal circulation are of significant importance in the diagnosis of neonatal lupus syndromes. These humoral autoimmune findings provide an unique opportunity to assess the pathogenic role of autoantibodies against the Ro(SS-A)/La(SS-B) complex, most notably for congenital heart block. Current knowledge about the involved autoantibody-autoantigen systems, including recent therapeutic concepts of these autoimmune syndromes, is summarized.

Introduction

Neonatal lupus erythematosus (NLE) includes a variety of syndromes characterized by evidence of maternal autoantibodies against the RNA protein complex Ro(SS-A)/La(SS-B). Since these IgG antibodies have been produced by the mother and actively transported into the fetal circulation, they can potentially lead to typical clinical manifestations in the fetus and newborn. Therefore, these entities are also called maternal-fetal autoimmune syndromes. Although these diseases occur infrequently, they provide a unique opportunity to investigate the pathogenic importance of autoantibodies involved in fetal tissue injury.

While isolated congenital heart block (CHB), as one of the clinical syndromes, has been described in 1901 [1] for the very first time, associated maternal diseases became apparent in 1928 [2] and 1954 [3], respectively. Finally, the typical association of maternal antibodies to Ro(SS-A) has been described for CHB in 1983 [4, 5], whereas another syndrome, cutaneous NLE, has been described in 1981 [6, 7] (table 1).

Table 1. Neonatal lupus syndromes – History

Year	Finding	Ref.
1901	First description of isolated CHB	1
1928	CHB in 2 children whose mother suffered from Mikulicz's disease	2
1954	Discoid LE in a newborn infant with subsequent development of SLE in the mother	3
1981	Association of NLE with antibodies to Ro/SS-A	6, 7
1983	Association of CHB with anti-Ro/SS-A antibodies	4, 5

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0058$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Priv.-Doz. Dr. med. Thomas Dörner
Department of Medicine Charité/Rheumatology and Clinical Immunology, Schumannstrasse 20/21
D–10117 Berlin (Germany)
Fax +49 30 2802 8300, E-Mail thomas.doerner@charite.de

Table 2. Clinical features of NLE

Rash
Erythematous, scaly
Photosensitive
Annular or elliptical
Face and scalp
Cytopenias
Hemolytic anemia
Thrombocytopenia
Leukopenia
Hepatosplenomegaly
Myocarditis/pericarditis
Pneumonitis?

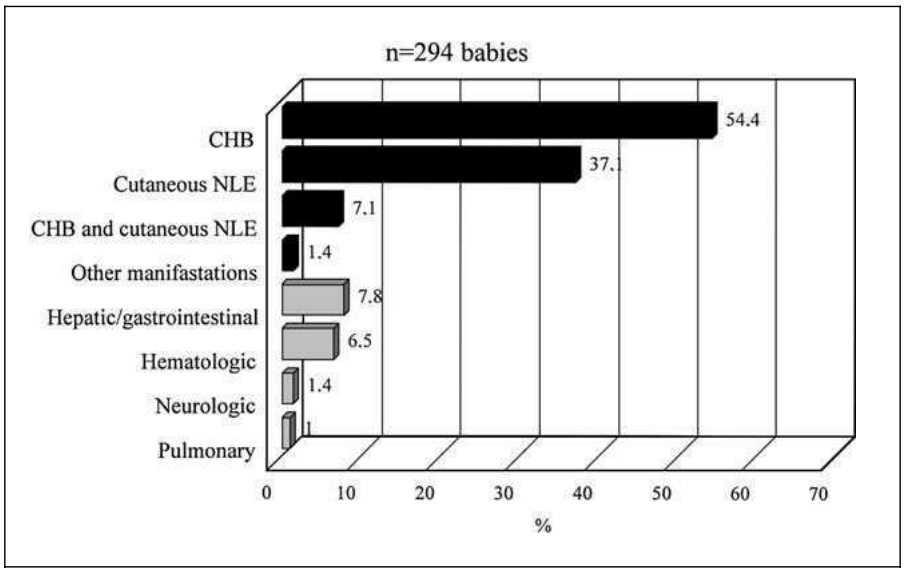


Fig. 1. Distribution of clinical manifestations of NLE.

Subtypes of NLE Syndromes

In general, the neonatal lupus syndromes can be differentiated into a permanent manifestation, such as CHB, and transient syndromes, such as cutaneous, hepatic and hematologic involvement in NLE (fig. 1, table 2). Of note, only 7–10% of all cases exhibit an association of CHB and features of NLE. This indicates that factors other than autoantibodies are involved in the manifestation of the disease. CHB is the permanent or irreversible manifestation of NLE. Isolated CHB detected in utero is strongly associated with autoantibodies reactive with the intracellular soluble ribonucleoproteins, Ro(SS-A) (52 kD), Ro(SS-A) (60 kD), and La(SS-B) (48 kD). Although the majority of complete CHB occurs in conjunction with maternal autoantibodies against the Ro(SS-A)/La(SS-B) complex, it should be noted that not all CHB are mediated by these autoantibodies. These last cases account for about 10% and occur frequently in infants suffering from major anatomic lesions or mesotheliomas of the atrioventricular node. Moreover, a neonatal heart block, evident shortly after birth in the absence of maternal antibodies, has to be differentiated and does not belong to autoimmune-associated CHB. Most notably, most CHB are third- and second-degree atrioventricular heart blocks [8, 9]. The association of low-titer maternal anti-Ro(SS-A)/La(SS-B) antibodies and first-degree atrioventricular heart block as well as sinus bradycardia have been documented by our group [10, 11] and confirmed by other reports [12].

Permanent cardiac and transient cutaneous disease are the most common manifestations of NLE [9]. The transient NLE syndromes are characterized by a variety of clinical manifestations (table 2). Most notably, an erythematous skin rash with a predilection for the scalp and periorbital area is most often apparent in the first few postnatal months and also highly associated with maternal antibodies against the Ro(SS-A) and La(SS-B) complex. The disappearance of all these clinical manifestations within 6 months after birth coincides with the disappearance of maternal autoantibodies. This observation is of outstanding importance for the pathogenic relevance of these autoantibodies in NLE.

Immunopathogenic Aspects

Fetal and neonatal injury is presumed to be due to the transplacental passage of IgG autoantibodies into the fetal circulation from the mother, who may have systemic lupus erythematosus (SLE) or Sjögren's syndrome or be entirely asymptomatic. In contrast to the adult heart, the fetal heart appears to be uniquely vulnerable. Although we were able to detect first-degree atrioventricular blocks in a variety of mothers with affected children [10], only few reports state that atrioventricular heart blocks coincided with the detection of specific autoantibodies in adults [13, 14]. However, the unusual occurrence of heart rhythm disorders in mothers with affected children, despite exposure to identical circulating levels of autoanti-

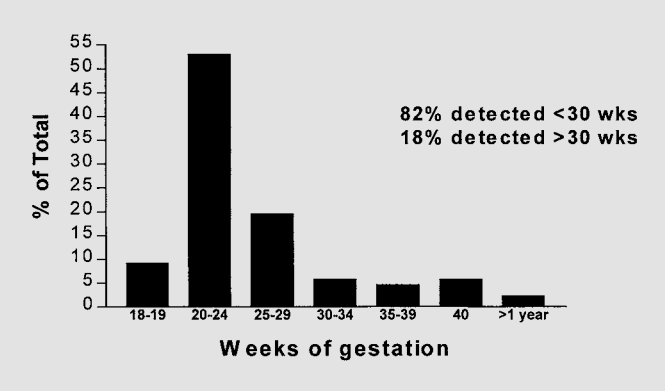


Fig. 2. Detection of CHB during pregnancy [adapted from 12].

Table 3. Gender bias in children with CHB compared to newborns with other congenital heart rhythm disorders [17]

Group	Females	Males	Ratio
CHB infants	19	8	2.38[1]
Nonautoimmune heart blocks	14	18	0.77[1]
Healthy children from SLE patients	22	23	0.96

[1] Odds ratio 3.054, 95% CI, $p < 0.04$.

bodies, is remarkable and points towards the specific vulnerability of the fetal heart.

Extensive studies have focused on the molecular characterization of maternal autoantibody responses and cognate antigens that might be involved in transcriptional regulation. Although the precise pathogenetic mechanism of autoantibody-mediated tissue injury is not defined, it has been demonstrated in adult rabbit and human fetal hearts that sera containing anti-Ro(SS-A) antibodies induce atrioventricular block and inhibit L-type calcium currents in isolated ventricular myocytes [15]. Since this L-type calcium channel is expressed preferentially in early life and is targeted by autoantibodies, it might explain that the adult cardiac conduction system is not affected to the same degree as that of the fetus and neonate.

However, several clinical observations in CHB remain unexplained. Permanent disease does not occur in other fetal organs, and abnormalities are not detectable in maternal cardiac function. Recent data from an US national registry suggest that the timing of CHB is not randomly distributed throughout gestation. Bradycardia (identified by auscultation and ultrasound and confirmed by fetal echocardiogram) is most often detected during the mid to late second trimester (fig. 2) [12, 16].

Another unexplained observation is that not all mothers with diseases associated with these antibodies have affected offsprings, implying involvement of as yet unknown factors, some of which are likely to be fatal. In this context, a recent study could demonstrate a female to male ratio of about 3:1 among children with CHB (table 3) [17]. Since data of a national US registry have reported a 1:1 ratio [12], careful further analysis is needed to make some final conclusions whether gender is one of the predisposing factors for CHB. However, it is well known that NLE without cardiac manifestations affects girls more often than boys [9].

There may be differences in expression or accessibility of Ro(SS-A) or La(SS-B) antigens in fetal compared to adult hearts. An alternative 52-kD Ro/SS-A mRNA derived from the splicing of exon 4 encoding amino acids 168- 245 including a leucine zipper has been identified [18]. This isoform was recognized by 26 of 30 mothers with CHB children. Additional studies provided evidence that this mRNA is expressed in fetal but not adult hearts. On the other hand, a variety of studies have shown that the leucine zipper represents the major autoantigenic epitope of this protein [19–26]. Therefore, it raises the question to which degree alternative splicing products play a significant immunopathogenic role. Most recently, Miranda-Carus et al. [27] have shown that immunization with different 52-kD Ro(SS-A) proteins independent of the mRNA splicing product can lead to conduction defects in BALB/c mice. These observations suggest a strong arrhythmogenic effect of the autoantibodies.

Despite studies on the major autoantigen complex, other groups have focused on different autoantigenic systems. Thus, Li et al. [28] suggested that a candidate fetal factor may be endogenous retrovirus-3 (ERV-3) that encodes an open reading frame for an 68-kD envelope protein. This protein is expressed at high levels in placental syncytiotrophoblast and fetal hearts. Most recently, Horsfall et al. [29] could provide strong evidence for a pathogenic role of anti-ERV-3 antibodies by analysis of one newborn with CHB and concomitant antibodies followed by a healthy child without detectable anti-ERV-3 antibodies. Maddison et al. [30] described an additional antigen of 57 kD recognized by 8/21 (38%) mothers of affected neonates. Sequencing of p57 DNA demonstrates that this antigen is clearly distinct from Ro(SS-A) and La(SS-B). In addition, sera from paired serum samples from mothers with CHB children provided evidence that

calreticulin represents an autoantigenic target in CHB [31]. Most remarkably, this molecule is involved in the cellular calcium transport system and may also be part of the Ro(SS-A)/La(SS-B) complex under certain circumstances [32]. Further experimental work is needed to establish whether these autoantibodies can influence the calcium transport of myocytes and subsequently lead to heart rhythm disorders.

The mechanism by which antibodies might interrupt critical intracellular events in the fetal myocyte or cells of the specialized conducting system is largely unknown. For antibodies to Ro(SS-A) and La(SS-B) to be causal in the development of NLE, a variety of requirements must be fulfilled (table 4).

One of the major questions concerns the accessibility of the intracellular antigens to their autoantibodies (table 5). Casciola-Rosen et al. [33] have shown that Ro(SS-A) and La(SS-B) antigens are present on small and large surface blebs during the process of apoptosis in neonatal keratinocytes, as demonstrated by immunofluorescence staining using confocal microscopy. Reichlin et al. [34] provided evidence for the accessibility of the candidate antigens to their cognate antibodies by the finding of antibodies to 60-kD Ro(SS-A) and 52-kD Ro(SS-A) in acid eluates of a heart from a fetus with CHB who died at 34 weeks of gestation. The enrichment of these antibodies was not demonstrated in eluates from the brain, kidney, or skin. Our group [35] reported an enhanced membrane expression of 52-kD Ro(SS-A) and 48-kD La(SS-B) on keratinocytes after exposure to tumor necrosis factor-α. Kinetic analysis revealed that 52-kD Ro(SS-A) was maximal after 2 h of exposure and remained increased, whereas 48-kD La(SS-B) peaked by 1 h and rapidly decreased to baseline values within 3 h. In general, UV irradiation, estradiol and tumor necrosis factor-α have been shown to induce surface expression of intracellular antigens, whereas apoptosis constitutes one of the major pathways of membrane expression (table 5). Alternatively, several lines of evidence suggest that autoantibodies can penetrate living cells, subsequently alter function, and cause cell death [36]. These data are consistent with the conclusion that autoantigens can shuttle between different subcellular compartments and, finally, appear accessible on the cell surface under certain circumstances (table 4). Induction of apoptosis in cultured cardiocytes resulting in surface accessibility of the Ro(SS-A)/La(SS-B) antigens has been demonstrated recently [37]. The data of this study suggest a role of apoptotic cardiocytes leading to subsequent leukocyte infiltration and tissue damage.

Table 4. Requirements for anti-Ro/SS-A and anti-La/SS-B to be involved in the development of NLE

Maternal autoantibodies must be present in the fetal circulation
Putative antigens (Ro/SS-A or La/SS-B) must be present in the target fetal tissues
Putative antigens (Ro/SS-A or La/SS-B) must be accessible to the autoantibodies

Table 5. Accessibility of autoantigens in the target fetal tissue

Detection of Ro/SS-A and La/SS-B on the surface of the fibers of the affected heart	[50]
Induction of Ro/SS-A and La/SS-B membrane expression by	
UV irradiation	[51]
Estradiol	[52, 53]
TNF-α	[26]
Detection of Ro/SS-A and La/SS-B in apoptotic blebs on the cell surface of	
Cultured keratinocytes after UVirradiation	[33]
Apoptotic cardiocytes in culture	[37]

Cross-reactivity between maternally derived autoantibodies and fetal cardiac antigens has been postulated as a major mechanism involved in the pathogenesis of CHB. Li et al. [28] reported that affinity-purified anti-La(SS-B) antibodies but not anti-52-kD Ro(SS-A) antibodies recognize laminin, a high-molecular-weight noncollagenous structural glycoprotein present on the sarcolemmal membrane of cardiocytes. Several anti- La(SS-B) antibodies bound to the sarcolemmal membrane of human fetal cardiocytes at 9–15 weeks of gestation, as demonstrated by immunofluorescence, whereas binding to adult hearts was not observed. These results support the hypothesis that 'molecular mimicry' between laminin and La(SS-B) may account, in part, for the vulnerability of the fetal heart.

The timing of heart block is not random. Bradycardia is most often identified between 18 and 24 weeks of gestation. During this period of time, echocardiographic examination is able to identify atrioventricular heart blocks higher than 1st degree. The development of CHB during the same time coincides with a period of remarkably increased passage of maternal IgG autoantibodies into the fetal circulation [38, 39]. It is accepted that maternal autoantibodies bind preferentially to the fetal cardiac conduction system. Subsequently, immunopathogenic mechanisms have the potential of enhancing the inflam-

Table 6. Potential arrhythmogenic effects of anti-Ro/SS-A and anti-La/SS-B autoantibodies

Perfusion of newborn rabbit ventricular papillary muscles with IgG-enriched fractions results in reduction of cardiac repolarization consistent with an alteration of calcium transport [54, 55]
Induction of AV block in whole young adult rabbit hearts perfused with IgG fractions [15]
AV block in the human fetal heart perfused with affinity-purified anti-52-kD Ro/SS-A [40]
Inhibition of L-type Ca^{2+} currents [15, 40]
Induction of conduction defects in BALB/c mice after immunization with 52-kD Ro(SS-A) proteins [27]

mation of the atrioventricularnode that results into calcification, fibrosis, and fatty degeneration of the cardiac conduction system, especially the atrioventricular node, evident around the end of gestation. Most notably, a variety of studies could demonstrate an arrhythmogenic effect of autoantibodies against Ro(SS-A)/La(SS-B) proteins [15, 40] (table 6).

Clinical studies have especially focused on the maternal autoantibody profile, but a unique pattern could not be identified. Anti-52-kD Ro(SS-A) and La(SS-B) responses detected by enzyme-linked immunosorbent assay (ELISA) and immunoblot represent the most frequently occurring findings in mothers whose children have NLE and CHB, respectively. The presence of anti-U1 RNP in the absence of anti-Ro(SS-A)/La(SS-B) antibodies occurs only in cases of isolated cutaneous disease and not in mothers of infants with cardiac manifestations [39, 41].

The Central Autoantigenic Complex Ro(SS-A)/La (SS-B)

Antibodies directed towards this RNA-protein complex are not only associated with neonatal lupus syndromes; they can also be detected at lower frequencies in patients with SLE, subacute cutaneous LE, Sjögren's syndrome and in other autoimmune diseases.

In a study of sera from 31 mothers of children with CHB, Julkunen et al. [42] demonstrated by ELISA that 97% reacted with 52-kD Ro(SS-A), 77% reacted with 60-kD Ro(SS-A), and 39% reacted with La(SS-B). In earlier studies, our group [38] used an ELISA to investigate quantitative and qualitative differences of anti-Ro(SS-A) and anti-La(SS-B) antibodies in sera from 16 infants with CHB and their mothers compared with 8 healthy anti-Ro(SS-A)-positive infants born to mothers with SLE. No serum sample contained IgM autoantibodies. All 16 (100%) infants with CHB had anti-52-kD Ro(SS-A) antibodies, 14 (88%) had anti-La(SS-B), and 9 (56%) had anti-60-kD Ro(SS-A) compared with 6 (75%) control infants with anti-52-kD, 3 (38%) with anti-48-kD, and 2 (25%) with anti-60-kD. The anti-52-kD Ro(SS-A) and anti-La(SS-B) antibody levels were significantly higher in infants with CHB than in the controls, whereas the anti-60-kD Ro(SS-A) IgG levels of sera from infants and mothers from the CHB and control groups were similar. Detection of the anti-60-kD response depends remarkably on the properties of the antigen and the test since only the native form apparently represents the autoantigen. In this context, several groups [9, 34, 41] could demonstrate that immunoprecipitation may be the optimal assay for evaluating the anti-60-kD Ro(SS-A) response, whereas other detection systems may underestimate the frequency of these autoantibodies.

Previous work suggested that there was no 'unique' high-risk profile that predicted the development of neonatal lupus [43]. However, other studies [9, 38–40, 44–46] have proposed that a low-risk profile consisted of anti-Ro(SS-A) antibodies of low titer without anti-La(SS-B) antibodies. Antibodies to 52-kD Ro(SS-A) in association with the anti-48-kD La(SS-B) were less frequently detected in mothers of healthy compared with affected children but significantly dominated the group of mothers with CHB children.

Silverman el al. [45] evaluated the maternal antibody profile in two groups of sera, 41 obtained from mothers whose children had manifestations of neonatal lupus (21 with CHB and 20 with skin manifestations) and 19 from lupus patients known to have anti-Ro(SS-A) and/or anti-La(SS-B) antibodies and healthy children. Significantly higher levels of anti-La(SS-B) and anti-52kD Ro(SS-A) antibodies were demonstrated in the mothers of affected children.

Although it is not known how maternal antibodies influence the development of cardiac versus cutaneous manifestations of neonatal lupus, to date antibodies to U1-RNP in the absence of anti-Ro(SS-A) and/or La(SS-B) occur preferentially in cutaneous NLE but not in CHB. The segregation of anti-U1-RNP antibodies with cutaneous disease may be a useful maternal marker and is consistent with our observations in mothers with CHB children in whom these antibodies were not found [39]. Thus, these antibodies are most likely characteristics of a different entity that apparently does not bear a high risk for CHB.

Maternal Characteristics

The immunogenetic features of mothers of affected children appear to be closer to Sjögren's syndrome than to SLE [9, 41, 42]. Early evidence for that came from a variety of studies typing HLA loci of mothers of affected children [42]. The assocciation of HLA-DR3 and HLA-B8 and absence of HLA-DR2 in mothers with CHB offspring are strikingly similar to the HLA association found in Sjögren's syndrome. The autoantibody profile that normally lacks high-titer anti-DNA antibodies in mothers of affected children provides further substantial evidence. Based on long-term follow-up studies, asymptomatic mothers do not invariably become ill, and if an asymptomatic mother does develop lupus, it is not likely to be life-threatening. As shown in table 7, most mothers of CHB children do not have systemic autoimmune or other diseases. Moreover, CHB is associated with substantial morbidity and mortality. Only about 10–25% of mothers actually fulfill the criteria for the diagnosis of SLE.

Table 7. Maternal diagnosis of mothers with CHB children

	Literature	Charité
Asymptomatic mothers	38%	23 (79%)
SLE	39.5%	5 (17%)[1]
Sjögren's syndrome	13.2%	1 (3%)
Other diagnoses	9.3%	

[1] 2 with secondary Sjögren's syndrome.

Treatment

We need to differentiate between therapeutic options for the mother and the fetus during pregnancy and treatment for the fetus after delivery. Guidelines for these situations are not well established and are empirically based on a variety of case reports.

Treatment during Pregnancy

Although the morbidity of CHB is greatly enhanced and the pathophysiologic mechanisms of maternal autoantibodies appear to play a major role, evidence of therapeutic approaches based on large controlled studies is still missing. Numerous anecdotal cases support the use of dexamethasone for the treatment of effusions and possibly incomplete block [46]. Although treatment of affected fetuses with dexamethasone and in some case reports in conjunction with plasmapheresis has successfully diminished effusions [9, 41, 46–48a], this therapy has not been shown to reverse established third-degree block. It should be noted that prevention, other than serial echocardiographic evaluation, is not supported by any study. However, combined therapy using plasmapheresis and dexamethasone has led to marked reduction in maternal autoantibodies during pregnancy and represents a safe treatment [48b]. Treatment with sympathomimetics may be beneficial in fetuses with hydropic changes [10, 49]. One study [49] evaluated the effects of two sympathomimetic agents, isoprenaline and salbutamol. While no firm conclusion can be drawn, a beneficial effect of isoprenaline is becoming established.

Although prospective clinical trials of fluorinated steroids in women with anti-Ro(SS-A) and/or anti-La(SS-B) antibodies are still needed to evaluate for this therapy, dexamethasone is administered frequently in low dosages after CHB has been diagnosed. Given the low rate of CHB, it is recommended to monitor these pregnancies in specialized centers.

Figure 3 shows a current approach modified from Tseng and Buyon [41] to identify high- and low-risk pregnancies in mothers with autoimmune diseases based on expressed autoantibody specificities. However, healthy mothers with pregnancies complicated by CHB cannot be identified by this means. In general, the clinical approach to cardiac manifestations of neonatal lupus includes obstetric and rheumatologic management of fetuses with CHB and fetuses with a normal heart beat but at high risk of developing CHB (mothers with antibodies to Ro(SS-A)/La(SS-B) antigens plus previous sibling with neonatal lupus). Unfortunately, there is currently no possibility of identifying asymptomatic women since there is no reason to screen all pregnant women for anti-Ro(SS-A) or anti-La(SS-B) antibodies. In women known to have these autoantibodies, we recommend close follow-up with echocardiograms between 16 and 29 weeks of gestation, because this is the most prevalent time for detection of fetal bradyarrhythmia. These recommendations are based on observations that incomplete or unstable block or evidence of myocarditis might benefit from treatment with dexamethasone in an attempt to prevent permanent fibrosis. Complete atrioventricular block is not reversible, but some investigators might argue that if it is complete but identified days after a normal rate, there might be some chance for reversibility.

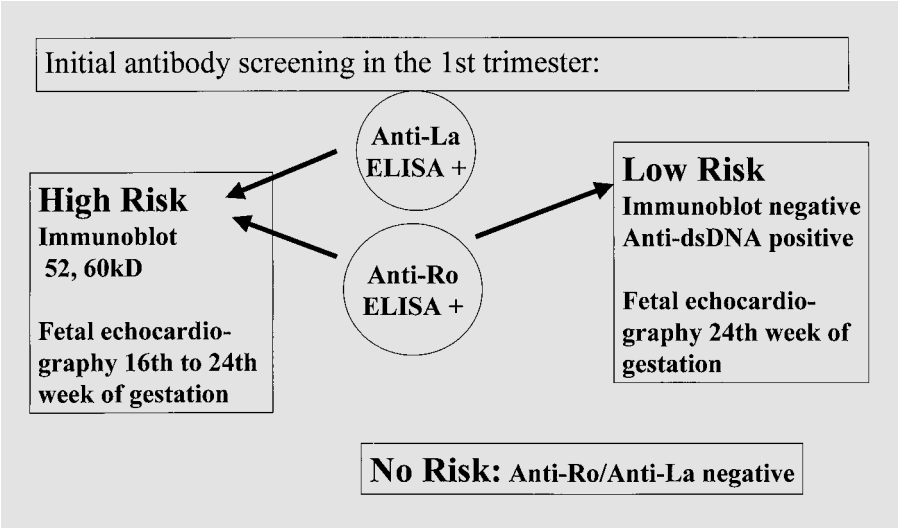


Fig. 3. Management of pregnancies at risk for CHB modified according to Buyon et al. [12].

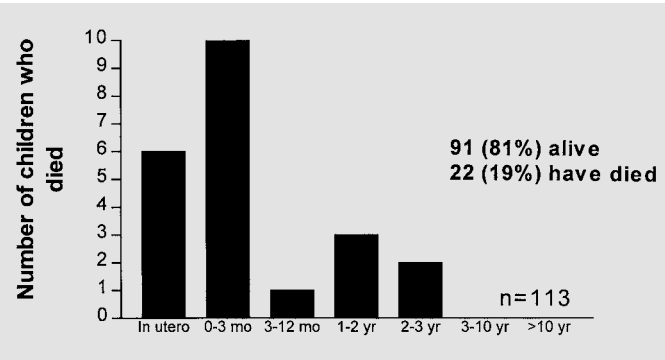


Fig. 4. Mortality of congenital heart block in relation to the time of gestation [adapted from 12].

Table 8. Recurrence rate of anti-Ro/SS-A-associated CHB

Julkunen et al. [42]	2/26 (8%)
McCune et al. [57]	2/12 (17%)
Buyon et al. [12]	8/49 (16%)
Charité Berlin	3/5 (60%)

Table 9. Mortality of CHB

Julkunen et al. [2]	5/34 (15%)
McCune et al. [57]	3/14 (21%)
Buyon et al. [12]	22/113 (19%)
Charité Berlin	4/29 (14%)

Therapy after Birth

Most fetuses require administration of glycosides and/or sympathomimetics to improve cardiac function and to maintain a sufficient heart rhythm [10], respectively. In some rare cases with associated cardiomyopathy, heart transplantation has been performed successfully [9]. Two thirds of children require permanent pacing within 3 years after birth, whereas almost 100% have pacemakers at the age of 18. Pacemaker therapy is well accepted for CHB and apparently leads to a normal life expectancy [9].

Recurrence Rate, Mortality and Morbidity

The recurrence rate of CHB has been reported to be low at approximately 8–18% (table 8). However, we have reported a mother with a series of CHB girls only interrupted by one healthy boy [17]. But this is nearly three times higher than the risk for CHB in a primigravida with the putative antibodies. CHB carries a significant mortality (14–22%, table 9) and morbidity. A recent study by Buyon et al. [12] demonstrated that mortality is highest within the first 3 months (fig. 4).

The identification of the gestational period in which the fetal heart might be most vulnerable is one of the central problems of the understanding of the immunopathogenesis and of therapeutic approaches. Bradycardia (identified by auscultation and ultrasound and confirmed by echocardiogram) is most often detected during the mid to late second trimester [16].

Conclusions

The neonatal lupus syndromes represent a unique challenge for rheumatologists, dermatologists, obstetricians, perinatologists, and pediatric cardiologists to identify pregnancies at risk for these entities and to care for the patients. There are several unresolved problems in understanding the pathogenicity of antibodies to components of the Ro(SS-A)/La(SS-B) system in NLE. Fetal factors are being sought that could be involved in cardiac antigen processing during the second trimester. Finally, we need to concentrate resources on this rare disease to perform additional basic scientific as well as controlled therapeutic studies.

References

1 Morquio L: Sur une maladie infantile caracterisée par des modifications des pouls, des attaques syncopales et épileptiformes et la mort subite. Arch Méd Enfants 1901;4:467.
2 Alyward RD: Congenital heart block. Br Med J 1928;1:943.
3 McCuiston CH, Schoch EP Jr: Possible discoid lupus erythematosus in newborn infant. Report of case with subsequent development of acute systemic lupus erythematosus in mother. Arch Dermatol 1954;70:782–785.
4 Scott, JS, Maddison PJ, Taylor PV, Esscher E, Scott O, Skinner RP: Connective tissue disease, antibodies to ribonucleoprotein and congenital heart block. N Engl J Med 1983;309:209–212.
5 Reed DR, Lee LA, Harmon C: Autoantibodies to SS-A/Ro in infants with congenital heart block. J Pediatr 1983;103:889–891.
6 Franco HL, Weston WL, Peebles C, Forstot SL, Pheanuphak P: Autoantibodies directed against sicca syndrome antigens in neonatal lupus. J Am Acad Dermatol 1981;4:67–72.
7 Kephart DC, Hood AF, Provost TT: Neonatal lupus erythematosus: New serological findings. J Invest Dermatol 1981;77:331–333.
8 Buyon JP: Neonatal lupus syndromes. Curr Opin Rheumatol 1994;6:523–529.
9 Silverman ED, Laxer RM: Neonatal lupus erythematosus. Rheum Dis Clin North Am 1997; 23:599–618.
10 Dörner T, Hiepe F, Göldner B, Apostoloff E: Investigations into Ro-specific antibody-associated congenital cardiac conduction defects. Clin Invest 1992;70:492–496.
11 Dörner T, Trebeljahr G, Göldner B, Yamamoto K, Apostoloff E, Hiepe F: Detection of autoantibodies to Ro(SS-A), La(SS-B) and U1RNP in different congenital heart rhythm disorders using immunoblot and enzyme immunoassay. J Autoimmun 1994;7:93–106.
12 Buyon JP, Hiebert R, Copel J, Craft J, Friedman D, Katholi M, Lee LA, Provost TT, Reichlin M, Rider L, Rupel A, Saaleb S, Weston WL, Skovron ML: Autoimmune-associated congenital heart block: Demographics, mortality, morbidity and recurrence rates obtained from a national neonatal lupus registry. J Am Coll Cardiol 1998;31:1658–1666.
13 Baumgart DC, Gerl H, Dörner T: Panhypopituitarism and Sjögren's syndrome. Ann Rheum Dis 1998;57:635.
14 Lee LA, Pickrell MB, Reichlin M: Development of complete heart block in an adult patient with Sjögren's syndrome and anti-Ro/SS-A autoantibodies. Arthritis Rheum 1996;39: 1427–1429.
15 Garcia S, Nascimento JHM, Bonfa E, Levy R, Oliveira SF, Tavares AV, Campos Decarvalho AC: Cellular mechanism of the conduction abnormalities induced by serum from anti-Ro/SSA-positive patients in rabbit hearts. J Clin Invest 1994;93:713–724.
16 Buyon JP, Waltuck J, Kleinman C, Copel J: In utero identification and therapy of congenital heart block (CHB). Lupus 1995;4:116–121.
17 Dörner T, Feist E, Chaoui R, Hiepe F: Increased recurrence rate of congenital heart block in female offsprings. Rheumatology (Oxford) 1999;38:382–384.
18 Chan EK, DiDonato F, Hamel JC, Tsang CE, Buyon JP: 52-kD SS-A/Ro: Genomic structure and identification of an alternatively spliced transcript encoding a novel leucine zipper-minus autoantigen expressed in fetal and adult heart J Exp Med 1995:182:983–992.
19 Frank MB, Itoh K, McCubbin V: Epitope mapping of the 52-kD Ro/SSA autoantigen. Clin Exp Immunol 1994;95:390–396.
20 Buyon JP, Slade SG, Reveille JD, Hamel JC, Chan EK: Autoantibody responses to the 'native' 52-kDa SS-A/Ro protein in neonatal lupus syndromes, systemic lupus erythematosus, and Sjögren's syndrome. J Immunol 1994;152: 3675–3684.
21 Ricchiuti V, Briand JP, Meyer O, Isenberg DA, Pruijn G, Muller S: Epitope mapping with synthetic peptides of 52-kD SSA/Ro protein reveals heterogeneous antibody profiles in human autoimmune sera. Clin Exp Immunol 1994;95:397–407.
22 Bozic B, Pruijn GJ, Rozman B, van Venrooij WJ: Sera from patients with rheumatic diseases recognize different epitope regions on the 52-kD Ro/SS-A protein. Clin Exp Immunol 1993; 94:227–235.
23 McCauliffe DP, Yin H, Wang LX, Lucas L: Autoimmune sera react with multiple epitopes on recombinant 52 and 60 kDa Ro(SSA) proteins. J Rheumatol 1994;21:1073–1080.
24 Kato T, Sasakawa H, Suzuki S, Shirako M, Tashiro F, Nishioka K, Yamamoto K: Autoepitopes of the 52-kd SS-A/Ro molecule. Arthritis Rheum 1995;38:990–998.
25 Dörner T, Feist E, Wagenmann A, Kato T, Yamamoto K, Nishioka K, Burmester GR, Hiepe F: Anti-52 kD Ro(SS-A) autoantibodies in different autoimmune diseases preferentially recognize epitopes on the central region of the antigen. J Rheumatol 1996;23:462–468.
26 Dörner T, Feist E, Held C, Conrad K, Burmester GR, Hiepe F: Differential recognition of the 52 kD Ro(SS-A) antigen by sera from patients with primary biliary cirrhosis and primary Sjögren's syndrome. Hepatology 1996;24: 1404–1407.
27 Miranda-Carus ME, Boutjdir M, Tseng CE, DiDonato F, Chan EK, Buyon JP: Induction of antibodies reactive with SSA/Ro-SSB/La and development of congenital heart block in a murine model. J Immunol 1998;161:5886–5892.
28 Li JM, Horsfall AC, Maini RN: Anti-La (SS-B) but not anti-Ro(SS-A) antibodies cross-react with laminin: A role in the pathogenesis of congenital heart block? Clin Exp Immunol 1995; 99:316–324.
29 Horsfall AC, Neu E, Forrest G, Venables PJW, Field M: Maternal autoantibodies and congenital heart block: Clues from two consecutive pregnancies, one in which there was congenital heart block and one in which the fetus was healthy. Arthritis Rheum 1998;43:2079–2080.
30 Maddison PJ, Lee L, Reichlin M, Sinclair A, Wasson C, Schemmer G: Anti-p57: A novel association with neonatal lupus. Clin Exp Immunol 1995;99:42–48.
31 Orth TRU, Dörner T, Meyer zum Büschenfelde KH, Mayet WJ: Complete congenital heart block is associated with increased autoantibody titers against calreticulin. Eur J Clin Invest 1996;26:205–215.
32 McCauliffe DP, Zappi E, Lieu TS, Capra JD: A human Ro/SS-A autoantigen is the homologue of calreticulin. J Clin Invest 1990;86:332–335.
33 Casciola-Rosen LA, Anhalt G, Rosen A: Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J Exp Med 1994;179:1317–1330.
34 Reichlin M, Brucato A, Frank MB, Maddison PJ, McCubbin, Wolfson-Reichlin M, Lee L: Concentration of autoantibodies to native 60-kd Ro/SS-A and denatured 52-kd Ro/SS-A in eluates from the heart of a child who died with congenital complete heart block. Arthritis Rheum 1994;37:1698–1703.

35 Dörner T, Hucko M, Mayet WJ, Trefzer U, Burmester GR, Hiepe F: Enhanced membrane expression of 52 kDa Ro(SS-A) and La(SS-B) antigens by human keratinocytes induced by TNF-α. Ann Rheum Dis 1995;54:904–909.
36 Alarcon-Segovia D, Ruiz-Arguelles A, Llorente L: Broken dogma: Penetration of autoantibodies into living cells. Immunol Today 1995;17:163–164.
37 Miranda ME, Tseng CE, Rashbaum W, Ochs RL, Casiano CA, DiDonato F, Chan EK, Buyon JP: Accessibility of SSA/Ro and SSB/La antigens to maternal autoantibodies in apoptotic human fetal cardiac myocytes. J Immunol 161:5061–5069.
38 Dörner T, Chaoui R, Feist E, Göldner B, Yamamoto K, Hiepe F: Significantly increased maternal and fetal IgG autoantibody levels to 52 kD Ro(SS-A) and La(SS-B) in complete congenital heart block. J Autoimmun 1995;8:675–684.
39 Dörner T, Trebeljahr G, Göldner B, Yamamoto K, Apostoloff E, Hiepe F: Detection of autoantibodies to Ro(SS-A), La(SS-B) and U1RNP in different congenital heart rhythm disorders using immunoblot and enzyme immunoassay. J Autoimm 1994;7:93–106.
40 Boutjdir M, Chen L, Zhang ZH, Tseng CE, DiDonato F, Rashbaum W, Morris A, El-Sherif N, Buyon JP: Arrhythmogenicity of IgG and anti-52 kD SSA/Ro affinity-purified antibodies from mothers of children with congenital heart block. Circ Res 1997;80:354–362.
41 Tseng CE, Buyon JP: Neonatal lupus syndromes. Rheum Dis Clin North Am 1997;23:31–54.
42 Julkunen H, Siren MK, Kaaja R, Kurki P, Friman C, Koskimies S: Maternal HLA antigens and antibodies to SS-A/Ro and SS-B/La: Comparison with systemic lupus erythematosus and primary Sjögren's syndrome. Br J Rheumatol 1995;34:901–907.
43 Meilof JF, Frohn-Muldner IM, Stewart PA, Szatmari A, Hess J, Veldhoven CH, Smeenk RJ, Swaak AJ: Maternal autoantibodies and congenital heart block: No evidence for the existence of a unique heart block-associated anti-Ro/SS-A autoantibody profile. Lupus 1993;2:239–246.
44 Buyon JP, Winchester RJ, Slade SG, Arnett F, Copel J, Friedman D, Lockshin MD: Identification of mothers at risk for congenital heart block and other neonatal lupus syndromes in their children. Arthritis Rheum 1993;36:1263–1273.
45 Silverman ED, Buyon J, Laxer RM, Hamilton R, Bini P, Chy JL, Elkon KB: Autoantibody response to the Ro/La particle may predict outcome in neonatal lupus erythematosus. Clin Exp Immunol 1995;100:499–505.
46 Press J, Uziel Y, Laxer R, Luy L, Hamilton RM, Silverman E: Long-term outcome of mothers of children with complete congenital heart block. Am J Med 1996;100:328–332.
47 Copel J, Buyon J, Kleinman C: Successful in utero therapy of fetal heart block: Am J Obstet Gynecol 1995;173:1384–1390.
48a Silverman ED: Congenital heart block and neonatal lupus erythematosus: Prevention is the goal. J Rheumatol 1993;20:1101–1104.
48b Feist E, Dörner T, Wagenmann R, Chaoui R, Burmester G, Hiepe F: Erfolgreiches therapeutisches Management einer Risikoschwangerschaft bei primärem Sjögren-Syndrom mit Plasmapherese und Dexamethason. Z Rheumatol 1996;55:127–132.
49 Groves AMM, Allan LD, Rosenthal E: Therapeutic trial of sympathomimetics in three cases of complete heart block in the fetus. Circulation 1995;92:3394–3396.
50 Horsfall AC, LM Rose: Cross-reactive maternal autoantibodies and congenital heart block. J Autoimmunity 1992;5:479–493.
51 Golan TD, Elkon KB, Gharavi AE, Krueger JG: Enhanced membrane binding of autoantigens to cultured keratinocytes of systemic lupus erythematosus patients after UVB/UVA irradiation. J Clin Invest 1992;90:1067–1076.
52 Furukawa F, Lyons MB, Lee LA, Coulter SN, Norris DA: Estradiol enhances binding to cultured human keratinocytes of antibodies specific for SS-A/Ro and SS-B/La. J Immunol 1988;141:1480–1488.
53 Wang D, Chan EKL:17-β-Estradiol increases expression of 52-kDa and 60-kDa SS-A/Ro autoantigens in human keratinocytes and breast cancer cell line MCF-7. J Invest Dermatol 1996;107:610–614.
54 Alexander EL, Buyon JP, Lane J, Lafond Walker A, Provost TT, Guarnieri T: Anti-SS-A/Ro SS-B/La antibodies bind to neonatal rabbit cardiac cells and preferentially inhibit in vitro cardiac repolarization. J Autoimmun 1989;2:463–469.
55 Alexander EL, Buyon JP, Provost TT, Guarnieri T: Anti-Ro/SS-A antibodies in the pathophysiology of congenital heart block in neonatal lupus syndrome, an experimental model. Arthritis Rheum 1992;35:176–189.
56 Waltuck J, Buyon JP: Autoantibody-associated congenital heart block: Outcome in mothers and children. Ann Intern Med 1994;120:544–551.
57 McCune AB, Weston WL, Lee LA: Maternal and fetal outcome in neonatal lupus erythematosus. Ann Intern Med 1987;106:518–523.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:67–76

Antiphospholipid Syndrome

Erika Gromnica-Ihle[a] W. Schössler[b]

[a]Medical Center for Rheumatology and [b]IMTEC Immundiagnostika GmbH, Berlin, Germany

Key Words

Antiphospholipid syndrome · Hughes' syndrome · Antiphospholipid antibodies · Thromboses · Recurrent abortions

Abstract

Antiphospholipid syndrome (APS) is a disease characterized by venous and arterial thromboses or spontaneous abortions and the repeated detection of antiphospholipid antibodies (aPL). APS may be associated with another autoimmune disease (secondary APS), particularly systemic lupus erythematosus (SLE), or unrelated to an underlying disease (primary APS). APS affects almost all organs. In addition to the clinical criteria, lupus anticoagulant testing and immunological aPL determinations are required to establish the diagnosis of APS.

Antiphospholipid syndrome (APS) is characterized by venous/arterial thrombosis and/or recurrent fetal loss and the repeated detection of antiphospholipid antibodies (aPL) directed against phospholipid-protein complexes. APS may be associated with another autoimmune disease (secondary APS), particularly systemic lupus erythematosus (SLE), or unrelated to an underlying disease (primary APS/PAPS). The clinical syndrome of thrombosis, recurrent abortions and neurological symptoms in conjunction with the detection of aPL was first described by Hughes [1] in 1983. It was therefore proposed that the syndrome be named after him [2]. The history of this syndrome (table 1) dates back to William Osler, who described stroke as a symptom of lupus.

Epidemiology and Classification

APS may be one of the most common autoimmune diseases, i.e., one of the major causes of organ damage in autoimmune diseases. A clear classification system is needed to properly assess the frequency of occurrence of a syndrome. The most common criteria for classification of APS (table 2) differ from other classification criteria in rheumatology because they have not been tested in large numbers of patients. Hence, no data on their specificity and sensitivity are available.

New preliminary criteria for the classification of APS (table 3) have recently been proposed. They clearly define the obstetric complications of APS and provide clear-cut data on aPL and their isotypes. After the manuscript had been accepted the preliminary criteria for the classification of the APS were published in a modified form [48]. Many subsets of APS are encountered in practice (ta-

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0067$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Prof. Dr. Erika Gromnica-Ihle
Medical Center for Rheumatology
Zepernicker Strasse 1
D–13125 Berlin (Germany)
Tel. +49 30 9401 2650, Fax +49 30 949 7139, E-Mail gromnica-ihle@berlin.snafu.de

Table 1. History of antiphospholipid syndrome (Hughes' syndrome)

Authors	Year	Discovery
Conley and Hartmann [3]	1952	circulating anticoagulant in SLE
Moore and Mohre [4]	1952	BFP-STS especially in SLE
Bowie et al. [5]	1963	thrombosis in SLE despite circulating anticoagulant
Feinstein and Rapaport [6]	1972	introduction of the term 'lupus anticoagulant'
Nilsson et al. [7]	1975	SLE associated with lupus anticoagulant and intrauterine death
Thiagarajan et al. [8]	1980	nature of lupus anticoagulant: aPL
Hughes [1]	1983	anticardiolipin syndrome
Harris et al. [9]	1983	aCL – detection by radioimmunoassay
Loizou et al. [10]	1985	aCL – detected by ELISA
Galli et al. [11]	1990	β_2-GP1-cofactor
McNeil et al. [12]	1990	
Matsuura et al. [13]	1990	
Gharavi et al. [14]	1998	classification criteria of workshop on APS

Table 2. Suggested criteria for the APS [15]

Clinical	Laboratory
Venous thrombosis	lupus anticoagulant
Arterial thrombosis	IgG anticardiolipin (>20 GPL units)
Pregnancy loss (unexplained)	IgM anticardiolipin (>20 MPL units)
Thrombocytopenia	

Patients should have at least one clinical and one laboratory feature to make the diagnosis. The laboratory test should be positive on at least two occasions, 8 weeks apart.

Table 3. Preliminary international criteria for the classification of APS [14]

1 aPL documented on at least two occasions 6 weeks apart and occurring at the same time as:
2 One or more clinical features that have previously been associated with aPL in serum

ad 1. aPL: aCL IgG, IgM or IgA
or
LA

ad 2: Thrombosis criteria
- a One or more episodes of arterial or venous thrombosis, confirmed by imaging or Doppler studies or histopathology.
- b Three or more unexplained consecutive miscarriages or one or more unexplained deaths of a morphologically normal fetus at or after the 10th week of gestation or one or more premature births at or before the 34th week of gestation, associated with severe preeclampsia or placental insufficiency
- c Two or more episodes of reversible cerebral ischemia
- d Multiple sclerosis-like syndrome or focal neurologic deficit, otherwise unexplained

Associated features – not criteria
1 Thrombocytopenia $<100{,}000/mm^3$
2 Hemolytic anemia with reticulocytosis and positive Coombs test, not induced by drugs
3 Transverse myelopathy, otherwise unexplained
4 Livedo reticularis
5 Unexplained mitral or aortic valve thickening and/or regurgitation of blood, demonstrated by echocardiography
6 Unexplained chorea, observed by a physician
7 Migraine, onset within 1 year of a positive test for aPL in blood

ble 4). There seems to be a genetic basis for APS since a familial occurrence of primary APS is observed in 10% of the cases. The HLA loci DR4, DR7 and DRw53 were also found to be associated with APS.

Etiology and Pathogenesis

aPL are a group of heterogeneous antibodies directed against phospholipid-binding proteins such as β_2-glycoprotein I (β_2-GPI) (or apolipoprotein H), prothrombin, protein C, protein S, thrombomodulin, annexin V and kininogen. The term 'antiphospholipid antibody' is therefore incorrect, since the antibody is actually directed against a phospholipid-protein complex, but the name has been kept for historical reasons. Although the negatively charged phospholipid cardiolipin plays the most impor-

Table 4. Classification of APS into various subsets [16]

'Primary' vs. 'secondary' APS
Other clinical subsets
Patients characteristics
Sex
Age
Sporadic vs. familial forms
Nature of the vessels involved
Veins vs. arteries
Macro- vs. microvasculature
Preferential clustering of symptoms
Cerebral ischemia/left-sided heart valve lesions
Cerebral ischemia/livedo reticularis (Sneddon's syndrome)
Disease course
One-shot vs. multiple-shot
Timing of events
Catastrophic APS
Purely obstetrical APS

tant role, phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine may also form part of the complex. The target epitope is still not fully explained. The reactions between cardiolipin and β_2-GPI are the best known. β_2-GPI was first described in 1961, and its amino acid sequence has been decoded. This glycoprotein acts as a natural inhibitor of blood coagulation at various sites of the coagulation cascade. The binding site for cardiolipin on β_2-GPI is also known [reviewed in ref. 17].

aPL have an effect on all phospholipid-dependent hemostasis processes. Phospholipid-dependent hemostasis tests are delayed in vitro due to the inhibition of prothrombin activation. Thrombophilia in these patients can be attributed to the interactions between aPL and natural inhibitors of blood clotting such as activated protein C, protein S, and thrombomodulin. This suggests that aPL are of pathogenetic importance for the development of thrombosis. This pathogenetic role is supported by evidence from animal experiments. During their passive transmission or active immunization, aPL induce thrombocytopenia, fetal resorption (equates to spontaneous abortion in humans), and increased coagulation.

Thrombosis does not develop in all patients with aPL. Additional pathogenetic mechanisms must contribute to the development of thrombosis (secondary 'hit'). Endothelial vessel damage (infection?) is a very important developmental factor. Thrombosis is the primary symptom of both primary and secondary APS. Veins and arteries of all calibers located throughout the entire body may be involved.

Clinical Manifestations

Thromboses

Recurrent thromboses are the major clinical manifestation of APS. 2.5–3.5% of all patients with APS – whether primary or secondary – are affected by thrombosis each year. Venous thrombosis (65–70%) is more common than arterial thrombosis. aPL can be detected in 8–10% of all patients with venous thrombosis. Based on data from retrospective studies, the risk of phlebothrombosis in patients who test positive for aPL was estimated to be 2.7–11.9 [see review in ref. 18]. Venous thromboses are usually localized in the lower extremities and are frequently multiple and bilateral. Around one third of the cases are complicated by pulmonary embolism, which can lead to pulmonary hypertension and right heart failure. Primary thromboses in the pulmonary circulation can also result in pulmonary hypertension. aPL are detected in around 10% of all patients with pulmonary hypertension. Postthrombotic symptoms associated with ulcers, frequently on the medial malleolus, are further complications of leg and pelvic vein thrombosis. Thromboses of the axillary veins, subclavian vein, inferior vena cava, superior vena cava and veins of various organs may also occur together with the corresponding clinical symptoms (table 5).

Among the great vessels, arterial thrombosis most commonly involves the cerebral arteries, but may also affect the coronary arteries and those in the extremities and other organs (table 5). aPL can be detected in 18–20% of all stroke patients under 50 years of age. The risk of stroke development in aPL-positive patients was determined to be 2.33–10.6. General risk factors such as smoking, hypertension, metabolic disease, heart disease and the use of oral contraceptives increase the risk of thrombosis.

One third of all APS patients experience only one thrombotic event; the other two thirds have recurrent thromboses. A history of thrombosis is the greatest risk factor for future occlusions. Eighty percent of the patients have either recurrent venous thromboses or recurrent arterial thromboses, whereas only 20% have both arterial and venous thromboses [19]. The time between the initial occurrence and the recurrence may be several days to several years. The reason why some patients develop venous thrombosis (approximately 48%), whereas others develop

Table 5. Thromboses and clinical manifestations of APS

Venous		Arterial	
vessel	clinical finding	vessel	clinical finding
Extremities	phlebothrombosis thrombophlebitis	Brain	stroke, transient ischemic attack multi-infarction dementia
Skin	livedo reticularis, ulcers pulmonary embolism		valvular vegetatins myocardial infarction
Lungs	pulmonary hypertension	Heart	thrombus, cardiomyopathy
Liver	Budd-Chiari syndrome	Kidney	renal infarction microangiopathy
Adrenal glands	Addison's disease		
CNS	sinus vein thrombosis sinus cavernosus thrombosis	Liver Skin Eyes	hepatic infarction finger gangrene retinal artery occlusion

arterial thrombosis (approximately 38%) or recurrent abortions is still unexplained.

Disseminated clot formation in numerous vessels associated with multiple organ failure and thrombocytopenia is called 'catastrophic antiphospholipid syndrome' [20]. This form of the disease can be lethal.

Complications during Pregnancy and the Puerperium

Over half of all thromboses in women with APS occur during pregnancy, the puerperium, and during oral contraceptive use. Two prospective studies found that 5 and 12% of pregnant women with APS developed thrombosis or stroke, despite prophylactic heparin administration in a portion of cases [21, 22]. Furthermore, the occurrence of preeclampsia and gestational hypertension is higher in women with APS.

The complications of APS that may involve the fetus are recurrent miscarriage, intrauterine fetal death, premature delivery (30%) and fetal growth impairment (30%). In patients with APS, fetal loss is more common after the 10th gestational week than in early pregnancy. Some investigators even doubt whether early abortion should be considered as the sole clinical criterion for APS [23]. It is difficult to assess the risk of fetal loss in healthy women with aPL. Additional factors that increase the risk of spontaneous abortion in healthy aPL-positive pregnant females have not yet been identified. Genetic factors, infections, medications, and subclinical autoimmune disease or environmental factors have been discussed as possible factors. High aPL titers in pregnant women with SLE are predictive of spontaneous abortion, with rates of 50–85% reported in the literature. Eighty percent of all untreated healthy women with high aPL titers and previous fetal loss have another miscarriage in a subsequent pregnancy.

Thrombosis and infarction of the placental vessels are considered to be responsible for the complications in pregnancy but, since these placental pathologies do not occur in all cases, other mechanisms have also been proposed [see review in ref. 24].

Hemocytopenia

Thrombocytopenia, hemolytic anemia and, less frequently, leukocytopenia are observed in patients with APS, whereas thrombocytopenia is usually mild and does not cause severe bleeding. The prevalence was estimated to be 30–50% for mild thrombocytopenia (50–100 $\times$ 10^9/l) and 5–10% for severe thrombocytopenia ($<50 \times 10^9$/l) [25]. The cause of thrombocytopenia is complex and the details are not fully understood. Binding of aPL to the platelet membrane apparently occurs in the presence of β_2-GPI.

Thirty percent of patients with idiopathic thrombocytopenic purpura are aPL-positive, and they probably form a special subset.

Hemolytic anemia in APS may occur as isolated hemocytopenia or in conjunction with thrombocytopenia. Hemolytic anemia is frequently associated with anticardiolipin antibodies (aCL) of the IgM isotype.

A connection between APS and thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome is assumed as well.

Neurological Manifestations

The most common neurological manifestations of APS are ischemic stroke and transitory ischemic attack. aPL-positive stroke patients are usually younger than 50 years of age and have a very high rate of recurrence. The recurrence usually takes place within 1 year after the initial

event. aPL are also an independent risk factor for development of cerebral infarction in the elderly.

Cardiac etiologies such as valvular defects, intracardiac thrombus and other risk factors must also be taken into consideration. Multiple infarction events can result in dementia.

A number of other neurological symptoms are associated with aPL detection, but a causal relationship cannot be identified in every case (table 6). A possible connection between APS and Sneddon's syndrome, which is characterized by symptoms of cerebrovascular disease, hypertension and livedo racemosa, has been suggested. aPL can be detected in at least a portion of patients with Sneddon's syndrome.

There is also a significant correlation between aPL positivity and CNS manifestations in patients with SLE. It therefore seems justified to perform aPL testing as well as magnetic resonance imaging and single-photon computed emission tomography (SPECT) in addition to the conventional neurological work-up in all SLE patients with suspected neurolupus. These are currently the best studies for identifying clinically silent CNS manifestations of APS.

Table 6. Neurologic syndromes associated with aPL [26]

Cerebrovascular ischemia
Stroke
Transient ischemic attack
Sinus thrombosis
Ocular ischemia
Dementia
Acute ischemic encephalopathy
with Sneddon's syndrome
without Sneddon's syndrome
Atypical migraine-like events
Seizures
Chorea
Transverse myelopathy
Guillain-Barré syndrome
Transient global amnesia
Psychiatric disorders
Orthostatic hypotension

Cardiac Manifestations

The potential cardiac manifestations of APS are valvular heart disease (usually involving the mitral valve), coronary artery occlusion, intracardiac thrombus, pericardial effusion, dilatative cardiomyopathy due to occlusion of smaller vessels, and early bypass occlusion after coronary surgery.

High aPL titers are an independent risk factor for myocardial infarction and sudden cardiac death. The risk is even higher in patients who also have antibodies against oxidized low-density lipoproteins (ox LDL). aPL and antibodies to ox LDL were found to cross-react [27]. Since the latter antibodies play a role in the progression of arteriosclerosis, a connection with arteriosclerotic complications is being debated.

Morphological studies of pathological changes in the heart valves in patients with APS have revealed fibrin deposits and subsequent valvular damage in the absence of an inflammatory process. Hence, the way in which aPL antibodies are involved in the pathogenesis of endocardiac lesions remains unclear.

Skin Manifestations

The skin manifestations of APS are multifarious. Thrombotic occlusions of the cutaneous vessels are not always the underlying cause. Livedo reticularis, a reticular and cyanotic circular discoloration of the skin with a pale central area, and skin ulcers are the most common skin manifestations of APS. Livedo reticularis may form a fine, closed pattern caused by anastomoses between the very small skin vessels that result in reduced blood flow, or it may have a larger, lightening-like shape with small areas of necrosis. The latter type is classified as livedo racemosa in the European literature. Livedo reticularis is observed in 20–50% of patients with APS.

The skin ulcers most commonly associated with APS are small ulcers secondary to necrotizing purpura. They are usually 0.5–3 cm in diameter and occur on the ankles, feet, calves and leave a white atrophic scar (atrophie blanche) after healing. Other large ulcers are reminiscent of pyoderma gangrenosum. Postthrombotic ulcers may also occur.

Large areas of skin necrosis, finger and toe gangrene, malignant atrophic papulosis-like lesions, multiple subungual splinter hemorrhages, anetoderma and melanoderma are also associated with APS.

Renal Manifestations

In APS clots may form in the arteries and veins as well as in the glomerular capillaries, which can result in proteinura, hypertension, renal cortical infarction, and renal failure. A correlation between vessel transplant thrombosis in chronic dialysis patients and aPL has also been reported.

Various Other Organ Manifestations

Addison's disease due to thrombosis of the adrenal vessels, intestinal vessel occlusion with intestinal necrosis, and Budd-Chiari syndrome with hepatic vein thrombosis may also occur in APS. Hepatic artery occlusion with liver infarction, fibrin thrombi in the lesser liver vessels with hepatomegaly and increased enzyme activity, splenic infarction and occlusion of other organ vessels are less common.

aPL in Infectious Diseases

There is a strong correlation between aPL and infectious diseases. Syphilis was the first infectious disease in which anticardiolipin antibodies were identified as the 'reagin'. AIDS, malaria, borreliosis, leprosy, tuberculosis, and streptococcal, mycoplasmal, salmonella and coli infections as well as measles, mumps and infectious mononucleosis are associated with aPL at various rates of frequency. In infectious diseases, aPL do not need β_2-GPI for binding in enzyme-linked immunosorbent assays (ELISA), nor are they associated with the development of the clinical symptoms of APS. Hence, the pathogenetic importance of aPL associated with infectious diseases is fundamentally different from that in SLE and PAPS.

Laboratory Diagnosis

aPL can be detected using the lupus anticoagulant (LA) test or immunological tests.

Lupus Anticoagulant

The LA test was originally used in patients with SLE [3]. By definition, LA consists of immunoglobulins that interfere with one or more in vitro phospholipid-dependent coagulation tests. Several methods of LA testing have been developed because of the heterogeneity of LA, among other things. Since none of these methods cover all variants of LA, the SSC Subcommittee for Standardization of Lupus Anticoagulants published a set of guidelines and revised criteria for testing [28, 29]. In accordance with these guidelines, multi-step LA testing is recommended according to the following scheme:

(1) abnormal screening test;

(2) demonstration that the abnormality of the screening test is due to an inhibitor;

(3) demonstration that the inhibitor is PL dependent, and

(4) confounding coagulopathies (e.g., FVIII, FIX) ruled out.

LA Screening Tests. The most commonly used screening tests for the detection of LA are the activated partial thromboplastin time (APTT), kaolin clotting time (KCT), and diluted Russell viper venom time (dRVVT) tests. These tests may exhibit extremely variable differences in sensitivity and specificity depending on the manufacturer. It is therefore recommended to perform two independent screening tests in doubtful cases. Additional screening tests include the tissue thromboplastin inhibition (TTI) test, plasma clotting time (PCT) test, and the textarin clotting time test [review in ref. 29, 30].

Identification of LA as Inhibitor. The identification of LA as the inhibitor is achieved using a plasma exchange technique. In the presence of LA, the extended clotting time cannot be corrected by addition of normal plasma. These plasma exchange techniques are poorly standardized, and they are affected by a number of variables.

Confirmation Tests. The most commonly used confirmation tests include the platelet neutralization test and various modifications of the dRVVT, TTI and APTT tests [review in ref. 29, 30].

Immunological Tests

In addition to the aforementioned coagulation tests, aPL diagnosis should always include immunological determination of aPL. Since the introduction of the radioimmunoassay by Harris et al. [9] and the introduction of the ELISA shortly thereafter [10], immunological determination of aPL is almost always performed using the ELISA (table 1).

For the sake of completeness, we also mention the use of FACS analysis for the determination of aCL [31].

The principle of the ELISA is based on the binding of cardiolipin or other phospholipids (e.g., phosphatidylserine, phosphatidic acid, phosphatidylethanolamine) to the ELISA plate and subsequent binding of aPL and detection of the bound antibodies with an enzyme-labeled isotype-specific anti-human antibody.

Although the principles of these tests are the same as those of any ELISA test, many modifications specific to anticardiolipin tests have been introduced. The sensitivity and specificity of the anticardiolipin test and other antiphospholipid tests depend on a few factors enumerated below and the experience of the tester.

Pre-Analytical Considerations. Immunological aPL tests can be performed in serum as well as in plasma. Lipemic sera should not be used in testing. Repeated freezing and thawing of the sera can lead to false-positive test results.

Purity of the Antigen. The purity of the antigen (>98%) is of immense significance for the specificity of the test.

Antigen Presentation. Besides the phosphodiester groups, the glyceride portion including the fatty acid chains is important for antibody binding [32–34]. ELISA plates are often coated with cardiolipin or phosphatidylserine in ethanol, then evaporating the ethanol to dryness (either by exposure to air or nitrogen).

As Rauch and Janoff [35] showed using the example of phosphatidylethanolamine, the phospholipid bilayer that forms in these methods has only limited reactivity with aPL. Only the hexagonal structure (H_{II}) of phospholipid generates epitopes that react with aPL.

Blocking Medium. As already mentioned, the anticardiolipin test originally had many shortcomings. The use of fetal calf serum (FCS) or adult bovine serum instead of gelatin to block the plates increased the sensitivity of the ELISA.

Dilution Buffer. In 1984 and 1985, some investigators [10, 36] observed a sharp increase in the readings of positive samples relative to negative controls when about 10% (v/v) FCS or adult bovine serum in phosphate-buffered saline was used as the serum diluent. aPL require a cofactor for phospholipid binding. In 1990, several groups [11–13] reported that β_2-GPI is such a cofactor, and that the aPL are directed against a complex of phospholipid and β_2-GPI. This explains why the assay was more sensitive with 10% FCS or bovine serum. The amount of β_2-GPI used as the blocking medium and in the dilution buffer has a decisive effect on the sensitivity and specificity of ELISA aPL determinations. The concentration of β_2-GPI in FCS is approximately 600 μg/ml. Hence, the concentration of β_2-GPI is approximately 60 μg/ml in dilution buffers containing 10% FCS. The β_2-GPI concentration is only 2 μg/ml at the 1:100 dilution generally used in ELISA systems. Therefore, the serum concentration of β_2-GPI is not high enough to achieve sufficient sensitivity or, especially, specificity. For reasons of standardization, defined amounts of human β_2-GPI should be added to the dilution buffer. The use of Tween 20 in the sample dilution buffer is a controversial issue in the literature. Last but not least, the ion strength of the dilution buffer has a great effect on the signal-to-noise ratio [34].

Incubation Time. The incubation time must be long enough (approximately 1 h) to ensure solid-phase saturation and, hence, the binding of even low-avidity antibodies [37]. Incubation of the plates at 37°C seems to yield nonvalid results.

Isotypes. Determination of the IgG, IgM, and IgA isotypes is of great clinical importance (see above) and is dependent on the specificity of the conjugates used for determination.

Quantitative Analysis. A quantitative analysis of aPL using standards should be performed, first, because the curve is sigmoidal and nonlinear and, second, because the aPL titer is important for establishing the diagnosis as well as for assessing the course of the disease (see above). A minimum of five standard points should be used. Despite numerous efforts, the standardization of the available ELISA still leaves a lot to be desired. Lyophilized standards, first for IgG and IgM antibodies and later for IgA antibodies, have been introduced as a result of international workshops [38, 39]. One GPL unit was thereby defined as 1 μg/ml of IgG antibody and MPL and APL units were similarly defined as 1 μg/ml IgM and IgA antibodies, respectively. Hence, these antibodies obtained by affinity chromatography were standardized according to mass units, not immunological activity. Because of nonuniform cut-off computations among other things, the aPL prevalences given in the literature tend to vary. According to accepted criteria of the signal-to-noise theory, the cut-off value should be calculated as 'x + 3s', where 'x' is the mean of a sufficiently large group of healthy controls and 's' represents the standard deviation [40].

β_2-Glycoprotein I Antibodies. In addition to the role of β_2-GPI as a cofactor for aPL binding, several independent research groups [11–13] have shown that (at least a portion of) 'aPL' are directed against β_2-GPI and that they can be detected in vitro if the antigen is coated on negatively charged surfaces. Matsuura et al. [41] showed that strong aPL binding occurs when β_2-GPI is coated on electron or gamma-irradiated plates in the absence of anionic phospholipids. It is assumed that the β_2-GPI undergo a conformational change that exposes the cryptic epitope that is the target for aPL.

β_2-GPI antibodies are detected using only human β_2-GPI isolated from human plasma by precipitating it with perchloric acid followed by heparin affinity chromatography and other chromatographic techniques if necessary. The initial perchloric acid precipitation step seems to be of importance for preventing proteolytic cleavage of β_2-GPI, which is functionally inactive. The coating concentration of β_2-GPI should range between 3 and 10 μg/ml. Absolutely lipid-free inert proteins such as bovine serum albumin should be used as the blocking medium. FCS is therefore not recommended as a blocking medium. Isotype determination is performed using monospecific conjugates purified by affinity chromatography. In addition to the IgG and IgM antibodies, determination of IgA antibodies also appears to play an important role [42].

Table 7. Exclusion criteria for primary APS [43]

Malar rash
Discoid rash
Oral or pharyngeal ulceration, excluding nasal septum ulceration or perforation
Frank arthritis
Pleuritis, in the absence of pulmonary embolism or left-sided heart failure
Pericarditis, in the absence of myocardial infarction or uremia
Persistent proteinuria >0.5 g/day, due to biopsy-proven immune-complex-related glomerulonephritis
Lymphopenia <1,000/μl
Antibodies to native DNA, by radioimmunoassay or Crithidia fluorescence
Anti-extractable nuclear antigen antibodies
Antinuclear antibodies of > 1:320
Treatment with drugs known to induce aPL

In summary, it can be concluded that, according to the classification criteria for APS at least one laboratory parameter must be positive on two occasions at least 8 weeks apart in addition to the clinical criteria.

Diagnosis and Differential Diagnosis

The overlap of APS symptoms with those of other autoimmune diseases, especially systemic lupus erythematosus, is of particular importance. The criteria in table 7 were therefore developed for exclusion of SLE and PAPS.

Therapy

The optimal therapy for APS is still being debated because of the lack of large-scale treatment studies. Patients with acute vessel occlusion may receive fibrinolytics if there are no contraindications. After the first incidence of phlebothrombosis, the patient must receive sufficient continuous anticoagulation with coumarin derivatives. Rethrombosis often occurs within 1 year after discontinuation of anticoagulants.

Recurrent thrombotic complications are also to be expected in arterial thrombosis (particularly stroke) associated with high titers of aCL-IgG or LA. Anticoagulation therapy with platelet aggregation inhibitors should also be considered in this case.

The concomitant occurrence of thrombocytopenia and thrombosis is of major importance. These patients require especially careful monitoring. Khamashta [44] recommended the following procedure: anticoagulation with an international normalized ratio of 2.0–3.0 in platelet counts of 50–100 GPT/l, and additional corticosteroid administration in platelet counts of less than 50 GPT/l. Dapsone, danazol and chloroquine were reported to have positive effects, even in corticosteroid-resistant thrombocytopenia. Potential interactions with azathioprine and warfarin must be taken into account if administered simultaneously. When azathioprine is discontinued, the anticoagulant effect of warfarin is increased, and bleeding may occur. Fibrinolysis and plasmapheresis have been tried in 'catastrophic' antiphospholipid syndrome.

Various therapies have been used for treatment of recurrent abortion: low-dose aspirin, prednisone, low-dose heparin, intravenous high-dose immunoglobulin, and various combinations.

Based on the data of the available studies [45], the following recommendations can currently be made regarding prophylaxis in women with a history of abortions and positive aPL tests:

- 75 mg of aspirin per day after diagnosis of pregnancy;
- After detection of fetal heart sounds, additional dosage of unfractionated heparin (e.g., 5,000 units every 12 h) or
- low-molecular heparin (5,000–10,000 units per day);
- therapy can be discontinued in the 34th gestational week; if there is a history of thrombosis, anticoagulation can be continued for 6 weeks or, better yet, 2 months postpartum;
- due to the risk of heparin-induced osteoporosis, calcium supplements, vitamin D_3, and 1 h of fast walking or other physical activity are also recommended.

If the recommended therapy fails to achieve satisfactory results or if thrombocytopenia is corticosteroid-resistant, intravenous high-dose immunoglobulin administration (very expensive) in conjunction with low-dose aspirin can also be considered in isolated cases [review in ref. 46]. Women with aPL should not take estrogen-containing contraceptives, because they significantly increase the risk of thrombosis in these patients.

In patients with underlying SLE, treatment of the underlying disease depending on its activity level and organ manifestations is also recommended in addition to all measures against hemostasis (the prophylactic effects of chloroquine and hydroxychloroquine have also been discussed in recent years). Immunosuppressive therapy alone is not sufficient in patients with thrombotic complications.

Prophylactic treatment of patients with persistent aPL or LA but no clinical symptoms of APS remains questionable. We still do not understand why some of these patients develop thrombosis and others do not. It is absolutely essential to eliminate all additional risk factors for the development of thrombosis (e.g., hypertension, hypercholesterolemia, smoking, or estrogen-containing contraceptives) in patients with LA and/or aPL. Khamashta and Hughes [47] recommend a dose 75 mg of aspirin per day in patients with persistent LA and/or moderate or high IgG-aCL titers, even in those without a history of thrombosis. Careful clinical monitoring of this patient population is also necessary in light of their very high risk of thrombosis.

References

1 Hughes GRV: Thrombosis, abortion, cerebral disease and lupus anticoagulant. Br Med J 1983;187:1088–1089.
2 Gharavi AE, Wilson WA: The syndrome of thrombosis, thrombocytopenia and recurrent abortions associated with antiphospholipid antibodies: Hughes syndrome. Lupus 1996;5: 343–344.
3 Conley CL, Hartmann RC: A haemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematodes. J Clin Invest 1952;31:621–622.
4 Moore JE, Mohre CF: Biologically false-positive serological tests for syphilis. Type, incidence, and cause. JAMA 1952;150:467–473.
5 Bowie WEF, Thompson JH, Pascuzzi CA, Owen GA: Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. J Lab Clin Med 1963;62:416–430.
6 Feinstein DI, Rapaport SI: Acquired inhibitors of blood coagulation. Prog Hemost Thromb 1972;1:75–95.
7 Nilsson IM, Astedt B, Hedner V, Berezin D: Intrauterine death and circulating anticoagulant (antithromboplastin). Acta Med Scand 1975;197:153–159.
8 Thiagarajan P, Shapiro SS, DeMarco L: Monoclonal immunoglobulin M lambda coagulation inhibitor with phospholipid specificity: Mechanisms of a lupus anticoagulant. J Clin Invest 1980;66:397–405.
9 Harris EN, Gharavi AE, Boyle ML, et al: Anticardiolipin antibodies: Detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983;ii: 1211–1214.
10 Loizou S, McCrea JD, Rudge AC, et al: Measurement of anticardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): Standardization and quantitation of results. Clin Exp Immunol 1985;62:738–742.
11 Galli M, Comfurius P, Maassen C, Hemker HC, de Baets MH, van Breda-Vriesmann PJ, Barbui T, Zwaal RF, Bevers EM: Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet 1990;335:1544–1547.
12 McNeil HP, Simpson RJ, Chesterman CN, Krilis SA: Antiphospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: β_2-Glycoprotein I. Proc Natl Acad Sci USA 1990; 87:4120–4124.
13 Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Koike T: Anticardiolipin co-factor(s) and differential diagnosis of autoimmune disease. Lancet 1990;336:177–178.
14 Gharavi AE, Koike T, Wilson WA: Classification Criteria Workshop on Antiphospholipid Syndrome, Sapporo 1998.
15 Harris EN: Antiphospholipid syndrome; in Klippel JH, Dieppe PA: Rheumatology, ed 2. London, Mosby, 1998.
16 Piette JC: 1996 diagnostic and classification criteria for the antiphospholipid/cofactors syndrome: A 'mission impossible'? Lupus 1996;5: 354–363.
17 Koike T, Ichikawa K, Kasahara H, Atsumi T, Tsutsumi A, Matsuura E: Epitopes on β_2-GPI recognized by anticardiolipin antibodies. Lupus 1998;7(suppl 2):14–17.
18 Ordi-Ros J, Selva-O'Callaghan A, Vilardell-Tarrés M: Thrombotic manifestations in the antiphospholipid syndrome; in Asherson RA, Cervera R, Piette JC, Shoenfeld Y (eds): The Antiphospholipid Syndrome. Boca Raton, CRC Press, 1996, pp 107–115.
19 Khamashta MA: Clinical features and outcome of patients with aPL syndrome: A 10-year follow-up study. 57th Annual Meeting American College of Rheumatology, San Antonio 1993.
20 Asherson RA: The catastrophic antiphospholipid syndrome. J Rheumatol 1992;19:508–512.
21 Branch DW, Silver RM, Blackwell JL, Reading JC, Scott JR: Outcome of treated pregnancies in women with anti-phospholipid syndrome: An update of the Utah experience. Obstet Gynecol 1992;80:614–620.
22 Lima F, Khamashta MA, Buchanan NM, Kerslake S, Hung BJ, Hughes GR: A study of sixty pregnancies in patients with the anti-phospholipid syndrome. Clin Exp Rheumatol 1996;14: 131–136.
23 Welsch S, Branch DW: Anti-phospholipid syndrome in pregnancy. Rheum Dis Clin North Am 1997;23:71–84.
24 Salafia CM, Parke AL: Placental pathology in systemic lupus erythematosus and phospholipid antibody syndrome. Rheum Dis Clin North Am 1997;23:85–97.
25 Italian Registry of Antiphospholipid Antibodies (IR-APA): Thrombosis and thrombocytopenia in antiphospholipid syndrome (idiopathic and secondary to SLE): First report from the Italian Registry. Haematologic 1993;78: 313–318.
26 Levine SR, Brey RL: Neurological aspects of antiphospholipid antibody syndrome. Lupus 1996;5:347–353.
27 Vaarala O, Alfthan G, Jauhiainen M, et al: Crossreaction between antibodies to oxidised low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. Lancet 1993; 341:923–925.
28 Exner T, Triplett D, Taberner D, Machin SJ: Guidelines for testing and revised criteria for lupus anticoagulants. SSC Subcommittee for the Standardization of Lupus Anticoagulants. Thromb Haemost 1991;65:320–322.
29 Triplett DA: Assay for detection of antiphospholipid antibodies. Lupus 1994;3:281–287.
30 Triplett DA: Many faces of lupus anticoagulants. Lupus 1998;suppl 2:18–22.
31 Stewart MW, Etches WS, Russell AS, Percy JS, Johnston CA, Chew CK, Gordon PA: Detection of antiphospholipid antibodies by flow cytometry: Rapid detection of antibody isotype and phospholipid specificity. Thromb Haemostas 1993;70:603–607.
32 Faure M, Coulon-Morelec M: Entre la structure chimique du cardiolipide et son activité sérologique. Conservation du cardiolipide. Ann Inst Pasteur Immunol 1963;104:246–250.
33 Inoue K, Nojima S: Immunochemical studies of phospholipids. IV. The reactivities of antisera against natural cardiolipin and synthetic cardiolipin analogues-containing antigens. Chem Phys Lipids 1969;3:70–74.
34 Franz J: Dissertation, Humboldt University, Berlin 1992.
35 Rauch J, Janoff AS: The nature of antiphospholipid antibodies. J Rheumatol 1992;19: 1782–1785.
36 Koike T, Seuishi M, Funaki M, et al: Antiphospholipid antibodies and biological false positive serological test for syphilis in patients with systemic lupus erythematosus. Clin Exp Immunol 1984;56:193–199.
37 Binder WL: The anticardiolipin ELISA test. Am Clin Lab 1992;11:24–25.

38 Harris EN, Charavi AE, Patel SP, Hughes GRV: Evaluation of the anticardiolipin antibody test: Report of an international workshop held 4 April 1986. Clin Exp Immunol 1987;68: 215–219.
39 Harris EN: The Second International Anticardiolipins Standardization Workshop/the Kingston Anti-Phospholipid Antibody Study (KAPS) Group. Am J Clin Pathol 1990;94: 476–480.
40 Schoessler W: Zur Bestimmung der Nachweisgrenze im Enzymimmunoassay. Z Med Lab Diagn 1990;31:349–350.
41 Matsuura E, Igarashi Y, Yasuda T, et al: Cardiolipin antibodies recognized β_2-glycoprotein I structure altered by interaction with an oxygen-modified solid phase surface. J Exp Med 1994;179:457–461.
42 Tsutsumi A, Ichikawa K, Matsuura E, Koike T: Anti-β_2-glycoprotein I antibodies. Lupus 1998; suppl 2:98–102.
43 Piette JC, Wechsler B, Frances C, Papo T, Godeau P: Exclusion criteria for primary antiphospholipid syndrome. J Rheumatol 1993;20: 1802–1804.
44 Khamashta MA: Management of thrombosis in the antiphospholipid syndrome. Lupus 1996;5:463–466.
45 Khamashta MA: Management of thrombosis and pregnancy loss in the antiphospholipid syndrome. Lupus 1998;suppl 2:162–165.
46 Clark AL, Gall SA: Clinical uses of intravenous immunoglobulin in pregnancy. Am J Obstet Gynecol 1997;176:241–253.
47 Khamashta MA, Hughes GRV: Das Antiphospholipid-Syndrom. Rheumatol Europa 1994; 23:23–26.
48 Wilson WA, Gharavi AE, Koike T et al.: International consensus statement on preliminary classification criteria for definite Antiphospholid syndrome. Arthritis Rheum 1999;42: 1309–1311.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:77–91

Diagnostic and Prognostic Relevance of Autoantibodies in Uranium Miners

Karsten Conrad Jürgen Mehlhorn

Institute of Immunology, Medical Faculty of the Technical University of Dresden and
Medical Opinion Community, Niederdorf, Germany

Key Words

Autoantibodies · Uranium miners · Systemic sclerosis · Systemic lupus erythematosus

Abstract

Uranium miners exposed to silica dust have a higher risk of developing systemic sclerosis (SSc) and systemic lupus erythematosus (SLE). Sera of 1976 former uranium miners were analysed for autoantibodies typical of connective tissue disease. The frequency of some of these antibodies (anti-centromere, -topoisomerase I, -nucleolar, -dsDNA, -Ro/SSA, -La-SSB and U1-RNP antibodies) was significantly higher compared to a gender- and age-matched control group and was associated with the intensity of exposure as well as with clinical symptoms of SSc or SLE. It was also shown that SSc-associated autoantibodies may serve as an early indicator of disease development. Some differences in the autoantibody production between silica-dust-associated and idiopathic SLE/SSc were observed that might be caused by environmental factors in the population of uranium miners.

The complex pathogenesis of autoimmune diseases is poorly understood. Endogenous and exogenous factors are involved in the 'mosaic' of autoimmune disease development. Environmental factors operating in a genetically susceptible host may initiate the pathological immune process, often leading to the expression of specific autoimmune parameters. Indeed, many disease-specific autoantibodies (AAb) are detectable prior to the manifestation of the corresponding autoimmune disease. Early diagnosis or the prediction of a risk of developing a disease is becoming more and more important for the elaboration of new prophylactic and therapeutic strategies as has been shown for type 1 diabetes [1]. The combined analyses of ICA, IAA, GAD and IA-2/IA-2β antibodies have improved the ability to predict diabetes in risk groups as well as in the general population based on the level and number of AAb markers [2]. Viruses, toxins and dietary factors are discussed as putative environmental factors in type 1 diabetes [3]. In systemic autoimmune diseases, especially systemic sclerosis (SSc), crystalline silica (SiO_2) is one factor probably involved in the pathogenesis in some cases.

Silica and Systemic Autoimmune Diseases

Exposure to high levels of silica dust has been linked to an increased risk of developing several systemic autoimmune diseases, including SSc (scleroderma), rheumatoid arthritis and systemic lupus erythematosus (SLE). As early as 1914, SSc was described in a group of Scottish stonemasons [4]. Subsequently, an association between silica dust exposure and SSc was reported in the occupational cohorts of South African gold miners and North American hard coal miners [5, 6]. In the last 30 years, an increased incidence of scleroderma has been observed in

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0077$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. med. Karsten Conrad
Institute of Immunology
Medical Faculty, Carl Gustav Carus Technical University of Dresden
Karl-Marx-Strasse 3, D–01109 Dresden (Germany)
Tel. +49 351 8882 767, Fax +49 351 8832 778, E-Mail k_conrad@rcs.urz.tu-dresden.de

East-German uranium miners heavily exposed to quartz dust [7, 8]. In a prospective (historical) cohort study, a high relative risk compared to the general population was found (RR >7.8; 95% CI 6.5–9.5), with a nearly 100-fold increased incidence in miners with diagnosed silicosis [9]. An association between SLE and exposure to quartz dust has only recently been recognized [10, 11]. A study of a cohort of workers handling silica in a Spanish scouring powder factory suggests that highly exposed workers have at least a 10-fold increased risk of having SLE [12]. A similar risk was estimated in the cohort of uranium miners heavily exposed to dust [13]. Both studies showed that the kind and intensity of the exposure to quartz dust are significant for the development of SLE or other connective tissue diseases (CTD). A review of human studies presenting evidence for the association between silica and autoimmune diseases has recently been published [14]. Summarizing these studies, one can conclude that strong exposure to quartz dust with a high content of silica may predispose to or initiate the development of systemic autoimmune diseases. Therefore, occupational risk groups should provide ideal models for comparing patients who may suffer from the same disease, but differ in their exposure to a specific environmental trigger. Studying risk groups may give answers to the following questions: (1) Is there a difference in symptoms, AAb responses and immunogenetic markers between silica-associated CTD and idiopathic CTD? (2) What are the frequencies and the clinical relevance of AAb typical of CTD in different clinical and exposure groups within the risk group? (3) Can such AAb predict the development of CTD in these persons? (4) What conclusions regarding the induction of specific autoimmunity and development of autoimmune diseases can be drawn from these results?

In this paper we will focus on AAb specificities and their probable predictive value in uranium miners.

Occupational Situation in the Uranium-Mining Industry of the Former German Democratic Republic

In the south of the former German Democratic Republic, the Soviet-German company SDAG Wismut exploited uranium ore by underground mining in Western Saxony and Eastern Thuringia. Shortly after the second world war and up to the reunification of West and East Germany, approximately 300,000 underground miners were employed. Exposure to respirable dust containing 10–30% of quartz was extremely high in the so-called 'wild years' from 1946/47 until the mid-fifties due to very poor working conditions (e.g., no or inadequate mechanical ventilation). Respirable dust levels as high as 90–100 mg/m^3 air were produced during dry-drilling [15]. Fresh-broken quartz particles were regarded as crucial, because of their biologically reactive crystalline surfaces [16]. Even after the fifties, the possibility of inhaling, ingesting, or dermal incorporation [17] of large amounts of fresh-broken dust occurred, for example, through dry-drilling. For these reasons, the intensity and kind of exposure to silica cannot be compared with those in ore mines of the West European countries. Other components of the dust may play a role in triggering autoimmune disease processes. There was significant exposure through inhalation to alpha-radiating substances. Exposure to noxious substances, such as arsenic (in some mines) or heavy metals, was also possible. Furthermore, long-term hand-arm vibrations may play a pathogenic role in the development of Raynaud's phenomenon ('vibration-induced white fingers'; VWF) and scleroderma [18].

Material and Methods

Patients and Subjects

The different exposures and clinical groups of uranium miners and control subjects are listed in table 1. Uranium miners were recruited by control examinations for occupational lung diseases (silicosis, lung cancer) at the Center for Occupational Diseases, Niederdorf/Saxony from 1985 to 1990 and at the Medical Opinion Community Niederdorf from 1991 to 1998. The exposure data were obtained by case histories and by occupational exposure reports [15]. The case histories revealed that all of the miners included in this study were potentially exposed through inhaling, ingesting and skin incorporation of fresh-broken silica dust. The highly exposed miners worked underground mainly as drillers and conveyors starting from 1946 up to 1954/1955, the years with the highest dust levels. The miners were divided into different groups according to the degree of exposure and CTD symptoms (table 1). SSc and SLE were diagnosed according to criteria of the American Rheumatism Association [19, 20]. Probable SSc cases totaled 3–4 points of the score of Metzger and Masi [21]. In cases of Raynaud's phenomenon or VWF or diffuse interstitial lung fibrosis, possible SSc development was assumed. Possible CTD or SLE cases met one of the clinical criteria of the American College of Rheumatology (ACR), formerly American Rheumatism Association for SLE and/or at least two 'minor signs' of possible CTD development (episodes of unexplained fever, sicca symptoms, long-lasting very high erythrocyte sedimentation rates or lymph node swelling of unknown cause). Furthermore, three different degrees of silicosis were diagnosed [23].

Determination of AAb

All sera were screened for non-organ-specific AAb, especially for antinuclear antibodies (ANA) by indirect immunofluorescence (IIF) on acetone-fixed self-produced HEp-2 cells. AAb typical of CTD were analyzed by various methods (table 2) (a) in patients positive for

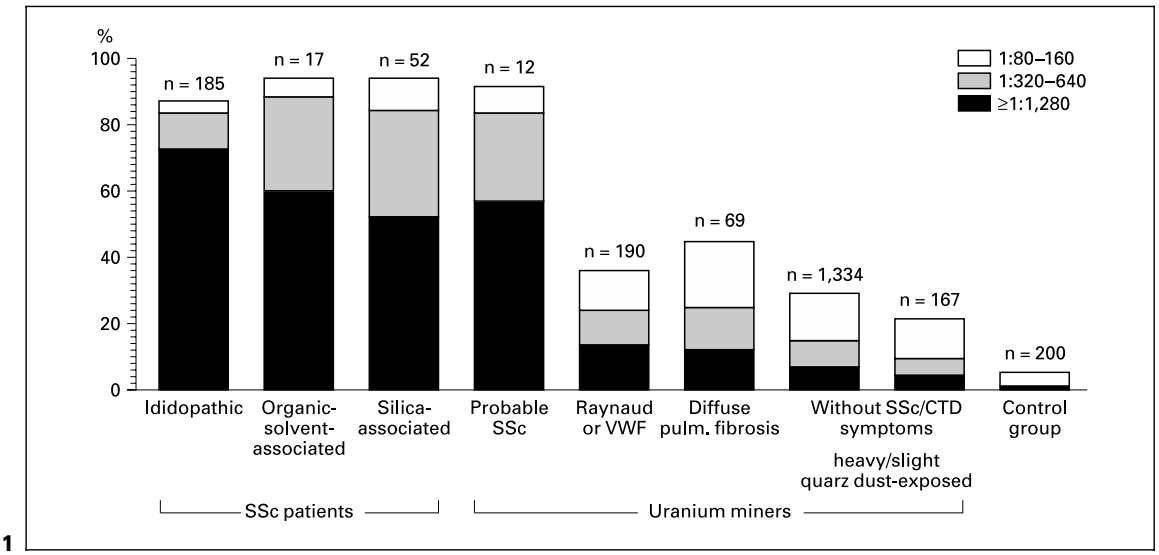
Table 1. Patients with CTD and different clinical and exposure groups of uranium miners

Patients/subjects	n	Criteria	Ref.
Silica-associated definite SSc (sSSc)	52	The major criterion or at least two of the minor criteria Exposed to quartz dust in (n = 38) or outside (n = 14) uranium mines	18
Organic solvent-associated definite SSc	17		21
Uranium miners		Miners working for more than 1 month in uranium mines	
With symptoms of CTD	437		
Probable SSc	12	3–4 points of the score of Medsger and Masi	21
Raynaud's phenomenon or VWF	190		
Diffuse lung fibrosis	69	Radiographically definite or suspected	
Definite SLE (sSLE)	18	4 or more ACR criteria	19
Probable SLE	11	1 or 3 clinical ACR criteria	19
Possible CTD	90	1 clinical ACR criterion and/or 2 or more 'minor criteria'	19
Without symptoms of CTD	1,501		
Heavily exposed	1,334	>20 mg respirable dust/m^3 (up to 100 mg dust/m^3)	15
Slightly exposed	167	<20 mg dust/m^3	15
Patients with idiopathic CTD	561		
Definite SSc (iSSc)	185	The major criterion or at least 2 of the minor criteria	18
Definite SLE (iSLE)	194	4 or more ACR criteria	19
Probable SLE	82	2 or 3 clinical ACR criteria	19
Control group	200	Gender- and age-related persons of the same geographical region and the same ethnicity, but without silica exposure and without CTD symptoms	

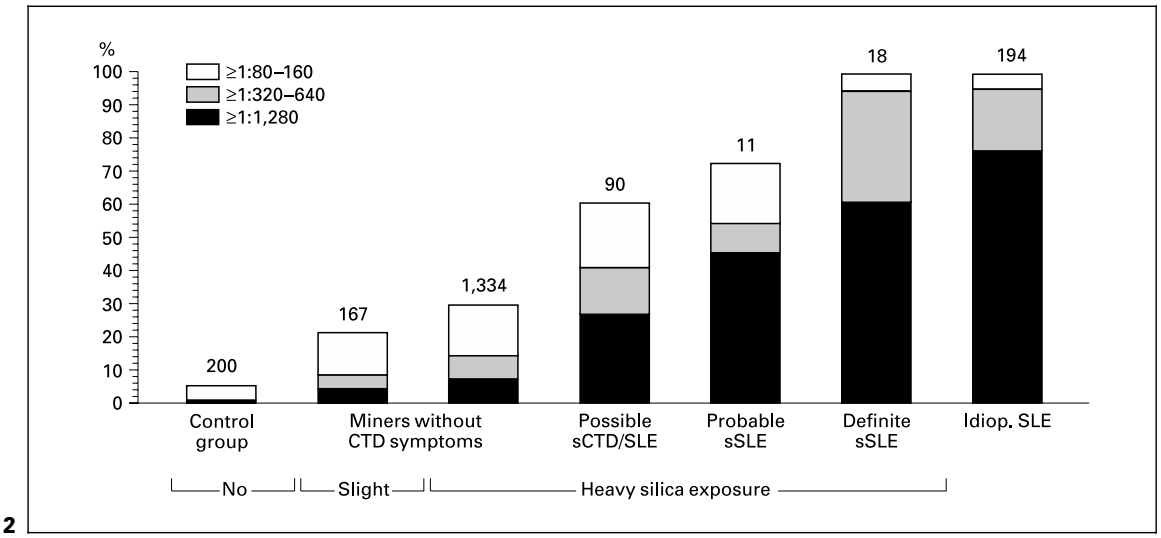
Table 2. Methods used for the determination of SSc and SLE/CTD typical AAb specificities

Autoantibody against	Indirect immuno-fluorescence	Immuno-diffusion	EIA rec. protein (eukaryot.)	EIA rec. protein (prokaryot.)	EIA nat. protein	Immunoblot
Nucleolar antigens (NUC)	HEp-2 liver	×	× (PM-Scl-100)			×
Centromere proteins (ACA)	HEp-2		× (CENP-B)	× (CENP-B)		× (CENP-A, -B, -C)
Topoisomerase I (ATA)	HEp-2	×	×	×	×	×
tRNA synthetases (Jo-1)	HEp-2	×	×		×	×
dsDNA	HEp-2 Crithidia				× (3 diff. assays)	
ssDNA					×	
Sm-proteins	HEp-2	×	×		×	×
U1-RNP-proteins	HEp-2	×	×		×	×
Ro/SS-A-proteins	HEp-2	×	× (Ro52, Ro60)		×	× (Ro52, Ro60)
La/SS-B	HEp-2	×	×		×	×
PCNA	HEp-2	×				×

ANA or anticytoplasmic antibodies (ribosomal or tRNA-synthetase pattern) or (b) in patients negative for ANA or anticytoplasmic antibodies, but with symptoms of probable or possible CTD development (table 1). AAb against DNA topoisomerase I (ATA), histidyl-tRNA synthetase (Jo-1), snRNP proteins (Sm and U1-RNP), La/SS-B and Ro/SS-A proteins were determined by at least four different methods: Ouchterlony technique, enzyme immunoassays (EIA) with recombinant (Pharmacia & Upjohn, Elias Division, Freiburg, Germany) and natural autoantigens (ORGenTec, Mainz and IMTEC, Berlin, Germany) and immunoblot with HEp-2 proteins. For immu-



1



2

Fig. 1. Frequencies of ANA in different groups of patients with SSc and different groups of uranium miners.
Fig. 2. Frequencies of ANA in patients with SLE and different groups of uranium miners.

nodiffusion (Ouchterlony technique), a mixture of soluble proteins from rabbit thymus and calf spleen was used. Independent of the IIF results, a further 1,000–1,200 uranium miners without CTD symptoms were tested for ATA, CENP-B, Jo-1, Ro52 and Ro60 AAb by EIA using human eukaryotically expressed autoantigens (Pharmacia & Upjohn, Elias Division, Freiburg).

Criteria for the Evaluation of AAb Findings

(1) ANA: According to the results in 1,200 blood donors, findings were classified into low (1:80–1:160), middle (1:320–1:640) and high titers (≥1:1,280). Middle and high ANA titers were found in less than 1% and 0.1% in blood donors, respectively.

(2) The reactivities of AAb typical of CTD were classified into three groups: low to middle titers in only one assay, middle titers in at least two assays, middle to high titers in two or more assays including positivity on the Ouchterlony assay (ATA, Jo-1, Sm, U1-RNP, Ro/SS-A, La/SS-B antibodies) or *Crithidia luciliae* IF test (dsDNA antibodies).

Non-Organ-Specific AAb Detected by IIF on HEp-2 Cells

Antinuclear Antibodies

The frequencies of low, middle and high ANA titers in different exposure and clinical groups of uranium miners are shown in figures 1 and 2. The results can be summa-

rized as follows: (1) In uranium miners without CTD symptoms, the ANA frequencies were significantly higher compared to the nonexposed control group ($p < 0.0001$). Furthermore, the heavily exposed miners had a higher frequency of ANA compared to the slightly exposed (29.4 vs. 21.6%, in the case of the more relevant middle to high ANA titers 14.3 vs. 9.0%). (2) In uranium miners with possible SSc (fig. 1) or CTD/SLE development (fig. 2), the ANA frequencies were again significantly higher compared to miners without CTD symptoms: 36.3% (23% middle to high titers, $p = 0.0004$) in patients with Raynaud's phenomenon or VWF, 44.9% (24.6% middle to high titers, $p = 0.018$) in patients with diffuse pulmonary fibrosis, 61.1% (41.1% middle to high titers, $p < 10^{-7}$) in patients with possible CTD/SLE development. (3) The ANA frequencies in silica-associated definite and probable SSc were similar to those of patients with idiopathic or organic-solvent-associated SSc (fig. 1). Furthermore, no difference between silica-associated and idiopathic SLE was observed, but the ANA frequency in probable SLE was lower (fig. 2). (4) Comparing miners with silicosis with those without silicosis, no difference in ANA frequency could be found: 32.0 vs. 32.7% [23]. However, within different silicosis groups, a higher frequency in miners with high-degree silicosis with opacities was observed compared to miners with low-degree silicosis: 38.7% (in 24.3% middle to high titers) vs. 29.4% (in 13.7% middle to high titers) [23].

In summary, there is a slight association of ANA frequencies (especially the middle to high titers of ANA) with the intensity of silica exposure and with the degree of silicosis, but a high association with symptoms of possible or probable SSc/CTD/SLE development.

Anticytoplasmic Antibodies

The frequencies of antibodies showing homogeneous, lysosomal and cytoskeletal patterns were similar to those found in the control group, but AAb with fine or middle granular patterns were found more often. The highest frequency of 8.7% was found in miners with diffuse pulmonary fibrosis in the absence of other CTD symptoms. 3.2% (1.6% of high titers) of the miners with Raynaud's phenomenon or VWF, and 2.2% (all high titers) of the miners with possible CTD/SLE development were positive. Again, there was an association with the intensity of exposure:3.4% (1.3% of high titers) in heavily exposed vs. 0.7% (no high titers) in slightly exposed miners. A Golgi-like pattern was seen in 2 highly exposed miners, 1 had Raynaud's phenomenon.

Autoantibodies against Mitotic Structures

Autoantibodies against mitotic structures were found in 0.5% of the heavily exposed miners. Two miners had NuMA/centrophilin antibodies, 3 had antibodies against mitotic spindle apparatus (MSA/tubulin pattern), 1 had antibodies against centriole and 1 had antibodies against midbody antigens.

AAb Typical of SSc

Antitopoisomerase antibodies, anticentromere antibodies (ACA) and some specificities of antinucleolar antibodies are diagnostic markers for SSc. Furthermore, U1-RNP, Ro/SS-A antibodies and ANA of unknown specificities can be found in patients with SSc. The frequencies of these AAb in different groups of uranium miners compared to SSc patients and controls of the same ethnicity and geographical region but without silica exposure are shown in table 3 and figure 3. The following results are highlighted: (1) In comparison to idiopathic and organic-solvent-associated SSc, the frequency of SSc-specific AAb was even higher due to the higher prevalence of antitopoisomerase antibodies (table 3). Antinucleolar antibodies were less frequently detected. Anticentromere antibodies showed the same frequency in the different exposure groups of SSc. (2) Similar to the ANA frequencies, SSc-specific AAb were associated with symptoms of SSc. Of the uranium miners with probable SSc, 41.7% were positive compared to 10.1, 6.4 and 2.2% of miners with diffuse lung fibrosis, miners with Raynaud's phenomenon and miners without SSc symptoms, respectively (table 3). (3) In comparison to the nonexposed and nondiseased control group, ACA, ATA and antinucleolar antibodies were significantly ($p < 0.0001$) more frequent even in the group of miners without CTD/SSc symptoms (table 3, fig. 3). There were only small differences between highly and slightly exposed miners. Unlike the findings for ANA frequencies, ATA were more prevalent in slightly exposed compared to heavily exposed miners. (4) After 2–10 years of follow-up of nearly 500 miners, disease progression or the development of SSc only occurred in miners positive for AAb typical of SSc (fig. 4). Of the ACA-positive miners without SSc symptoms, 2 developed Raynaud's phenomenon 3 and 4 years later, 1 developed probable and another definite SSc within 3 years. Furthermore, one ATA-positive miner developed SSc within 3 years. Altogether, 15.2% of the miners without SSc symptoms but with SSc typical AAb developed SSc symptoms or disease. Anticentromere-antibody- and ATA-positive miners with

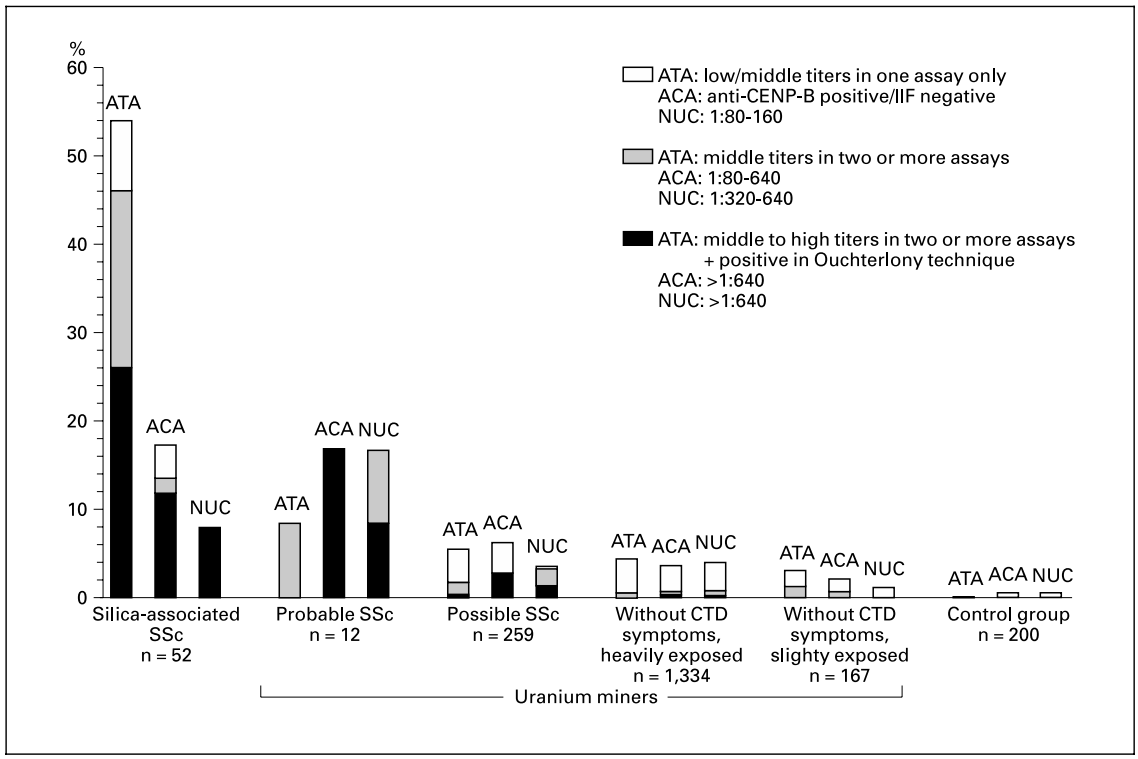


Fig. 3. Frequencies of the SSc typical ANA specificities ATA, ACA and NUC in patients with silica-associated SSc and in different groups of uranium miners (probable SSc, miners with possible SSc development, heavily and slightly exposed miners without CTD symptoms).

Table 3. SSc relevant ANA specificities in SSc patients and different clinical and exposure groups of uranium miners

Patients/miners	n	ACA[1]	ATA[2]	NUC[3]	Ro/SS-A Ab[2]	U1-RNP Ab[2]
Patients with SSc						
Idiopathic SSc	185	27 (14.6)	65 (35.1)	25 (13.5)	4 (2.2)	8 (4.3)
Silica-associated SSc	52	7 (13.4)	28 (53.9	4 (7.7)	1 (1.9)	0
Organic solvent-associated SSc	17	2 (11.8)	6 (35.3)	2 (11.8)	0	0
Uranium miners with SSc symptoms						
Probable SSc	12	2 (16.7)	1 (8.3)	2 (16.7)	1 (8.3)	2 (16.7)
Raynaud / VWF	190	7 (3.7)	2 (1.1)	3 (1.6)	5 (2.6)	0
Diffuse lung fibrosis	69	0	2 (2.9)	5 (7.2)	4 (5.8)	0
Uranium miners without SSc symptoms	1,501	11 (0.7)	10 (0.7)	12 (0.8)	25 (1.7)	3 (0.2)
Heavily exposed	1,334	10 (0.8)	8 (0.6)	12 (0.9)	24 (1.8)	3 (0.2)
Slightly exposed	167	1 (0.6)	2 (1.2)	0	1 (0.6)	0
Control group	200	0	0	0	1 (0.5)	0

Figures in parentheses are percentages.
1 Anti-CENP-B positive / ACA on HEp-2 cells negative results were not included.
2 Low / middle-titred results in only one assay were not included.
3 Nucleolar fluorescence at titres ≥ 1:320.

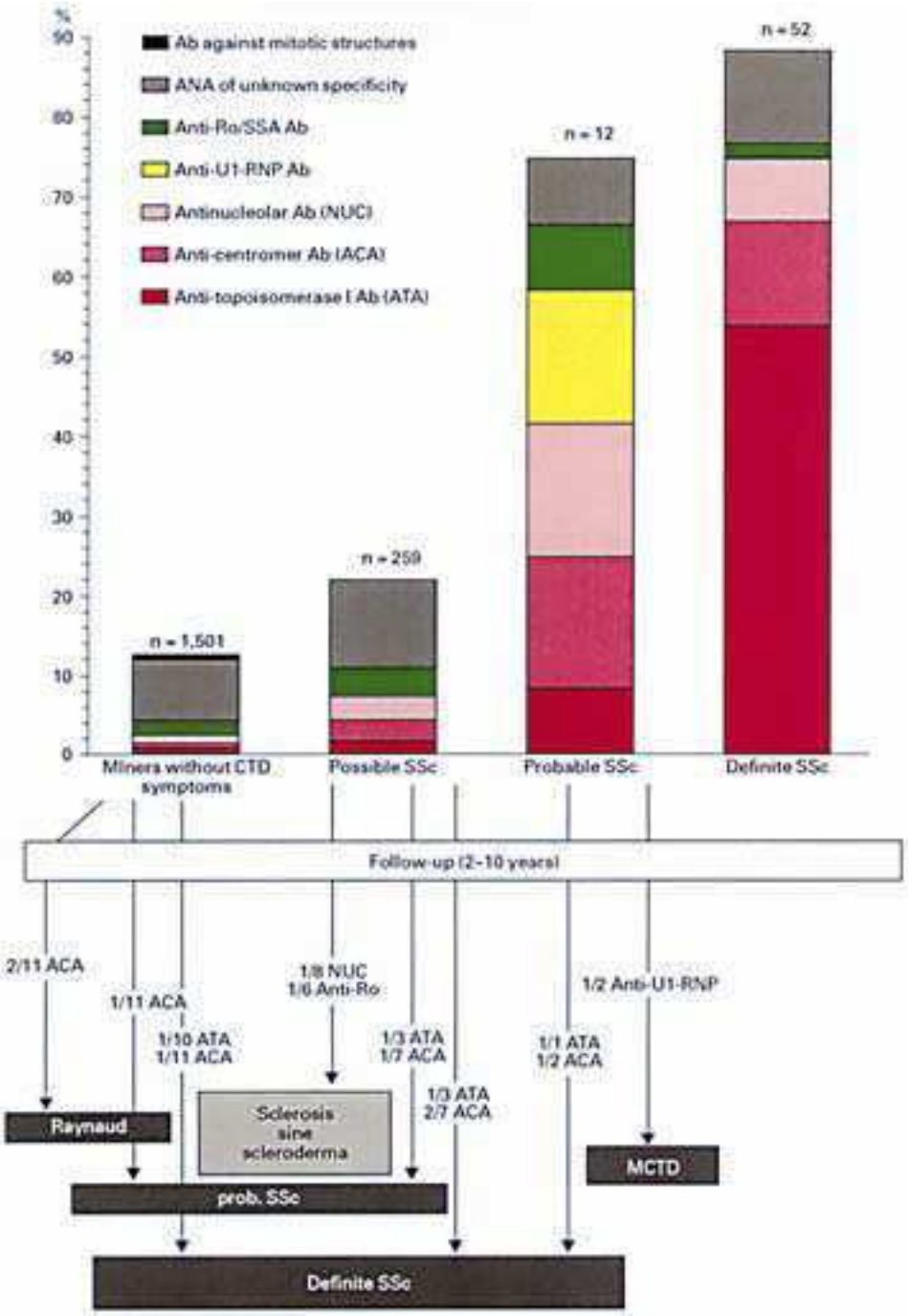


Fig. 4. Diagram of the results of AAb testing in uranium miners with definite and probable SSc, miners with possible SSc development and miners without CTD symptoms. The clinical development of AAb-positive miners within 2–10 years is indicated.

Raynaud's phenomenon developed probable or definite SSc (within 2–4 years) more often: 42.9 and 33.3%, respectively. Furthermore, 2 patients with diffuse lung fibrosis, 1 with antinucleolar and 1 with anti-Ro/SS-A antibodies, were diagnosed post mortem by histology as having 'sclerosis sine scleroderma' (SSc without skin manifestation). Taken together, 21.8% of the miners positive for SSc-specific autoantibodies developed SSc symptoms (Raynaud's phenomenon), probable or definite SSc, in particular 40% of the ACA-positive, 28.5% of the ATA-positive and 4.8% of the antinucleolar-antibody-positive miners.

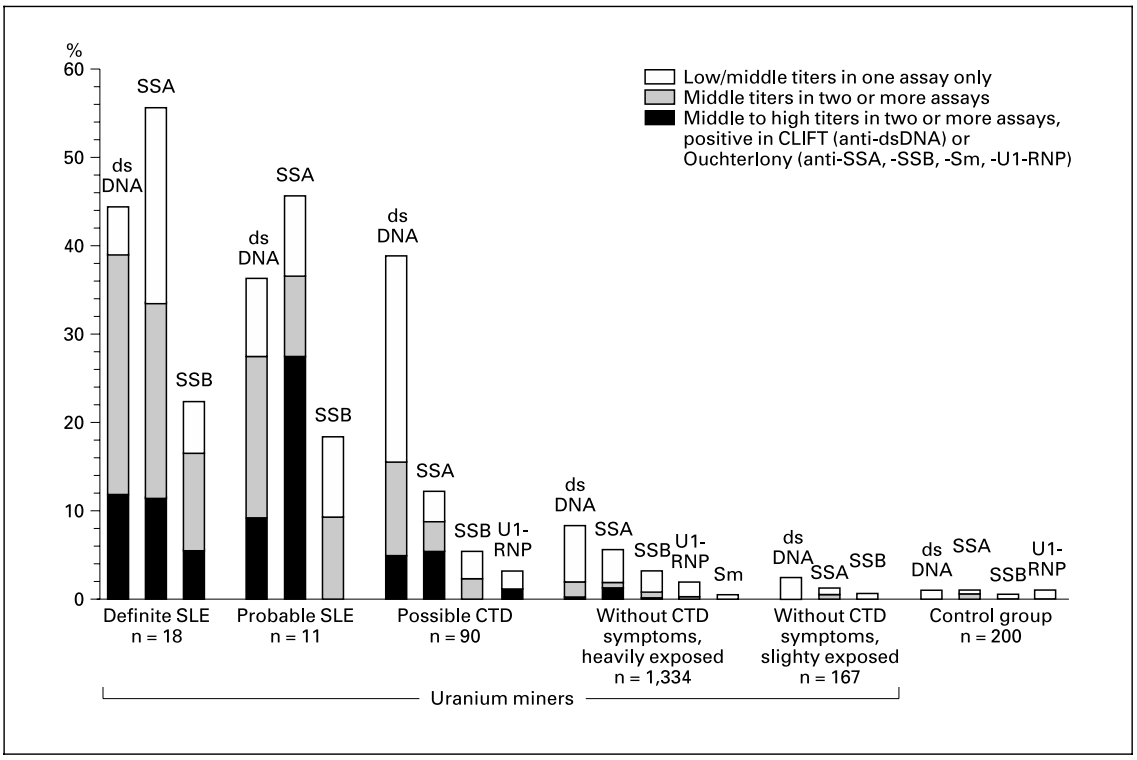


Fig. 5. Frequencies of the SLE marker antibodies anti-dsDNA and anti-Sm as well as other SLE-associated autoantibodies (anti-Ro/SS-A, -La/SS-B, -U1-RNP) in different groups of uranium miners (miners with definite and probable SLE, miners with possible CTD/SLE development, heavily and slightly exposed miners without CTD symptoms).

Antihistidyl tRNA Synthetase (Jo-1) Antibodies

In patients with SLE, probable SLE and probable SSc no anti-Jo-1 reactivities could be found. In all other groups of uranium miners, only low-titer results by enzyme immunoassay were seen in frequencies between 2.3 and 5.1%, similar to the results of the negative controls (5.5%). In patients with diffuse lung fibrosis, the frequency of low-titer reactivities was 3.3%. No associations were observed between a granular cytoplasmic pattern on HEp-2 cells and anti-Jo-1 reactivities.

AAb Typical of SLE

The SLE marker antibodies against dsDNA, Sm and PCNA as well as other AAb often associated with SLE (Ro/SS-A, La/SS-B, U1-RNP antibodies) were analyzed. The frequencies of SLE-relevant ANA specificities in different groups of uranium miners are shown in figure 5. Clearly positive results were assumed for middle to high titers in at least two different assays. Those results can be summarized as follows: (1) 38.9% of silica-exposed definite SLE cases had anti-dsDNA, 33.3% anti-Ro/SS-A (in 5/6 cases together with anti-dsDNA) and 16.7% anti-La/SSB antibodies. AAb to proteins of U-snRNP complexes (anti-Sm, -U1-RNP, U2-RNP) or to proliferating cell nuclear antigen could not be detected by the methods used in any of the 18 SLE patients examined. By immunoblot, various reactivities not related to the known relevant ANA specificities were seen in 9 patients. Of these, 4 were negative for all AAb typical of CTD. (2) The AAb profiles of probable SLE were similar, with a slightly lower prevalence of antibodies to dsDNA, Ro/SS-A and La/SS-B. (3) Similarily to ANA frequencies, dsDNA, Ro/SS-A and La/SS-B antibodies were associated with CTD/SLE symptoms and intensity of exposure. There was a highly significant difference in dsDNA and Ro/SS-A frequency between miners without CTD symptoms and those with possible CTD/SLE development ($p < 10^{-8}$ and 0.0001, respectively) as well as a difference between heavily and slightly exposed miners without CTD symptoms ($p = 0.06$

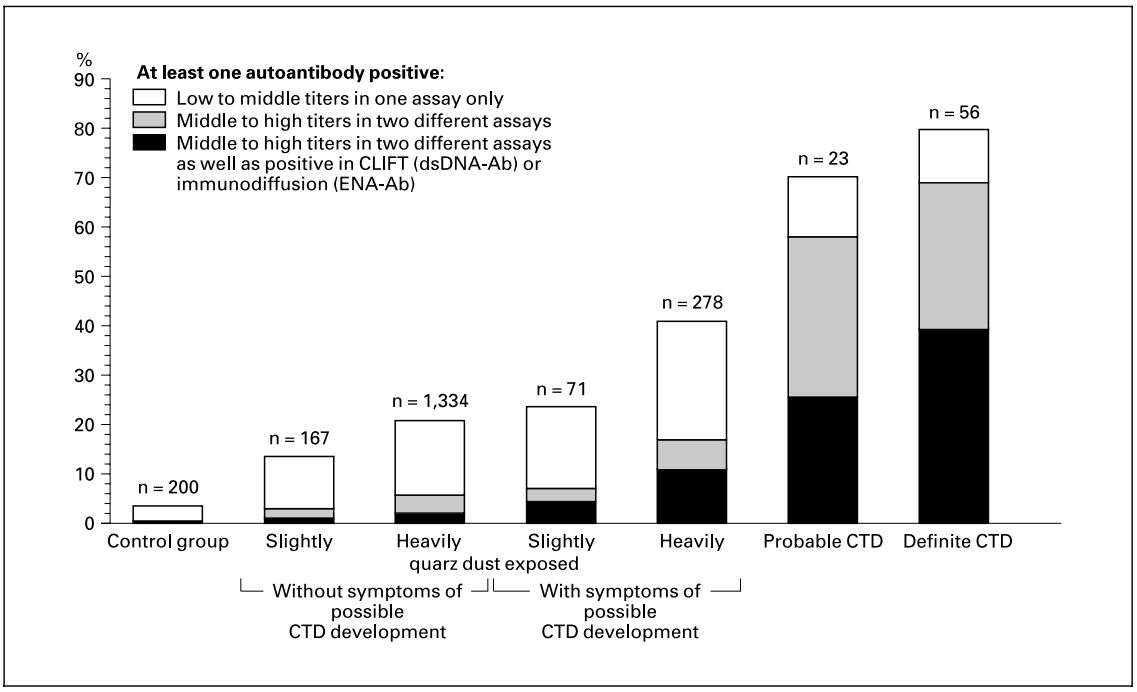


Fig. 6. Summary of the frequencies of CTD typical autoantibodies (ACA, ATA, antinucleolar, anti-dsDNA, anti-Ro/SS-A, anti-La/SS-B and anti-U1-RNP antibodies) in different groups of uranium miners (miners with definite CTD = SLE + SSc, miners with probable CTD = probable SLE + probable SSc, miners with possible CTD development, heavily and slightly exposed miners without CTD symptoms).

and 0.35, respectively). (4) In uranium miners without SLE, AAb against U-snRNP proteins were rarely detectable; their titers were mostly low.

Of the miners with dsDNA antibodies, 32 could be followed for 1–5 years after the first serum analysis. One patient with neutropenia and elevated erythrocyte sedimentation rate at the time of the first serum sample and analysis developed kidney manifestation (cellular casts and proteinuria >0.5 g/day) 1 year later. Furthermore, in the group of anti-dsDNA-antibody-positive miners without CTD symptoms, progression to possible CTD with kidney involvement and elevated erythrocyte sedimentation rates were seen in 1 miner. No clinical progression have been observed in dsDNA-antibody-negative miners up to now.

Diagnostic and Prognostic Relevance of AAb Typical of CTD in Uranium Miners

The frequencies of AAb typical of CTD in different clinical and exposure groups of uranium miners are summarized in figure 6. As was shown for the frequencies of ANA (fig. 1, 2), ATA, ACA, antinucleolar, anti-dsDNA and anti-Ro/SS-A antibodies (fig. 3, 5), there was a significant to highly significant association with clinical symptoms of CTD and to a lesser degree an association to the intensity of exposure to silica. These associations are more important regarding the clearly positive results (marked as black and dark gray in the figures). Except for the slightly exposed miners without CTD symptoms, all other groups of uranium miners had significantly higher frequencies of clearly positive CTD AAb ($p = 0.0054$ to $<10^{-8}$) compared to the nonexposed control group. The production of AAb typical of CTD was also significantly different ($p = 0.023$) in the group of miners with symptoms of possible CTD development if we compare the heavily and slightly exposed miners. The difference of AAb frequency in highly exposed miners with possible CTD development and those without CTD symptoms was highly significant ($p < 0.0001$). Taken together, the CTD AAb frequencies are rising in the following sequence: nonexposed controls, slightly exposed miners without CTD symptoms, heavily exposed miners without CTD symptoms, slightly exposed miners with possible CTD development, heavily exposed miners with possible

CTD development, miners with probable CTD, miners with definite CTD (SSc, SLE). Standing alone, these results suggest that clearly positive AAb typical of CTD or CTD-specific AAb may be predictive of CTD development in the risk group of uranium miners. This hypothesis is strengthened by the results of the follow-up studies, as shown in figure 4. Unfortunately, the real predictive value cannot be determined because of some limitations in the follow-up of CTD AAb-positive patients: Primarily the majority of miners of interest were heavily exposed in the so-called 'wild years' from 1946/47 until the mid-fifties. Most of these miners are older than 65 years and age-related diseases (e.g., chronic ischemic heart diseases) play a role in mortality. Furthermore, the consequences of severe silicosis and alpha-radiation (lung cancer and other tumors) are important causes of mortality. In addition, some patients are no longer available for follow-up studies.

Questions arising from this study include: What is the relevance of low- to middle-titer reactivities in highly sensitive assays in the absence of positivity in other assays? Are there additional parameters for predicting the development of systemic autoimmune diseases? In the following discussion, data and suggestions regarding defined AAb specificities will be presented, some of which have already been published [13, 22–25].

Anticentromere Antibodies

ACA are diagnostic markers of SSc, especially of the CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) or related scleroderma variants. They bind to the centromeric regions of the chromosomes in all phases of the cell cycle, giving the typical discrete speckled staining of the interphase nuclei and the chromatin region of mitotic cells by IFF on tumor cell monolayers. These AAb may be detectable years before disease manifestation [27, 28] and can therefore serve as an early indicator of the development of SSc. This was also shown by our study on uranium miners: 40% of the 20 ACA-positive miners without definite SSc developed SSc symptoms (2 miners), probable (2 miners) or definite SSc (4 miners) within 2–4 years (fig. 4). Of the other ACA-positive miners only 5 could be followed up and no disease progression to SSc was seen. Of these, 1 developed lung cancer and 1 died of cardiac infarction.

The main targets of ACA have been identified as the centromere-specific proteins CENP-A (17 kD), CENP-B (80 kD) and CENP-C (140 kD) by immunoblotting [29]. It has been shown by Earnshaw et al. [30] that all tested sera from patients with CREST syndrome reacted with the CENP-B protein. For the detection of a possible early immune response to centromere proteins not visible as ACA by IFF on HEp-2 cells, we used a quantitative enzyme immunoassay with a eukaryotically expressed full-length (amino acids 18–599) human recombinant CENP-B protein [31]. We tested sera of 1,750 uranium miners including all sera with positive ACA immunofluorescence and all sera of miners with SSc symptoms. Of the 27 miners positive for ACA by immunofluorescence, 26 were positive or strongly positive for CENP-B. Therefore, CENP-B is also the main target protein in ACA-positive uranium miners as has been shown for CREST patients and ACA-positive patients with other diseases, e.g. other CTD, pulmonary hypertension, primary biliary cirrhosis [24, 30, 32]. In the group of ACA-negative miners, 48 were slightly (5–20 U/ml) and 7 clearly positive (>20 U/ml) for CENP-B antibodies. The frequency of anti-CENP-B-positive/ACA-negative reactivities was highest in miners with Raynaud's phenomenon (4.7%), followed by SSc patients (3.8%), heavily exposed miners without CTD symptoms (2.8%) and slightly exposed miners without CTD symptoms (1.8%). Patients with diffuse pulmonary fibrosis alone had no elevated levels of anti-CENP-B antibodies. By analyzing a greater cohort of miners with Raynaud's phenomenon in this study, it has been shown that the frequency of CENP-B reactivities is lower than described in an earlier study [24]. Nevertheless, the prevalence is significantly higher in these patients ($p = 0.009$) as well as in heavily exposed miners ($p = 0.05$) compared to the nonexposed control group. Therefore one may suggest the importance of anti-CENP-B response in predicting SSc development in risk groups even if ACA were not detectable by immunofluorescence or masked by a high-titer ANA of other specificity [24]. This may be confirmed by the results on nonexposed patients with possible (mainly Raynaud's phenomenon) and probable SSc development showing a frequency of 7% [24]. The follow-up of anti-CENP-B-positive patients and miners should reveal if (a) these AAb are early indicators for the development of CREST or related scleroderma variants and/or (b) whether there are hints of an epitope spreading. Anti-CENP-B antibodies in the absence of the typical IFF pattern may represent the primary autoimmune response with a reaction to only one epitope. Later on, more epitopes could be involved thus allowing the exact diagnosis of ACA by IFF. To investigate this hypothesis 22 anti-CENP-B positive, ACA-negative miners were tested at intervals of 2 years. In 16 of the 21 miners with low-titer CENP-B antibodies, the immune responses disappeared

within 2–6 years. Only 5 showed a continuous low-titer response, 2 over a period of 6 years. The only available miner with a clearly positive anti-CENP-B response (26.6 U/ml) but without ACA fluorescence (this negativity was confirmed by an independent investigator) developed positive ACA with a titer of 1:640 (CENP-B: >100 U/ml) after 6 years. Therefore epitope spreading may be possible in rare cases. The clinical relevance of this is not yet clear and requires a further follow-up of additional ACA-negative/CENP-B-antibody-positive patients. In the clinical follow-up, no progression to scleroderma could be seen in the 22 miners. One miner with a continuous low-titer anti-CENP-B response over a 6-year period died of lung cancer. Interestingly, there was a significantly ($p = 0.009$) higher prevalence of lung cancer in the group with CENP-B antibodies (10.9%) compared to the group without such reactivity (3.7%).

It is not clear why anti-CENP-B antibodies can be identified in sera not showing the typical ACA fluorescence. Perhaps in those cases, the primary autoimmune response is not polyclonal but induced by molecular mimicry to one (to the main?) epitope on CENP-B. To detect anti-CENP-B, Verheijen [33] used an ELISA with a cloned C-terminal CENP-B fragment of 60 amino acid residues that seems to be identical with the CE1 epitope described by Earnshaw et al. [34]. Both have shown that this region is the main CENP-B epitope recognized by virtually all ACA-positive patient sera. Since some ACA-negative sera recognize this epitope [33], the C-terminal region of CENP-B (CE1) may be the candidate epitope for the ACA-fluorescence-pattern-negative, CENP-B-antibody-positive sera described in this study. In some cases, the primary autoimmune response may be directed to the main CENP-B epitope, which is not detectable by IFF on cell lines. It remains to be elucidated whether this is induced by molecular mimicry. An antigen-driven process may lead to an intra- and intermolecular 'determinant spreading' of the autoimmune response resulting in a polyclonal reaction to various independent epitopes of centromeric proteins and in a positive immunofluorescence typical of ACA.

Antitopoisomerase Antibodies

ATA are marker antibodies for SSc variants with a poorer prognosis than ACA-positive SSc. ATA-positive patients more often have internal manifestations, especially lung fibrosis. The prevalence of ATA in silica-associated SSc was higher than in idiopathic SSc (table 3) in part due to the very high frequency (71.4%) observed in the group of SSc patients who were exposed to quartz dust outside uranium mines. This again may be the result of selecting the more serious forms of SSc for evaluation as an occupational disease at the Center for Occupational Diseases (since 1990 at the Medical Opinion Community Niederdorf). Nevertheless, among the 38 uranium miners with definite SSc, the ATA frequency is still higher (42.1%) compared to idiopathic SSc. This difference may not be gender-related (most of the patients with idiopathic SSc are women) since ATA was found equally among males and females in other studies [35, 36]. Variations in genetic determinants may result in different responses to different triggering mechanisms. Indeed numerous immunogenetic differences were found between silica-associated and idiopathic SSc, the most significant differences were among the ATA-positive patients [26, 37]: HLA-DPB1 alleles with glutamic acid residue at position 69 (HLA-DPB1*0601, *1301, *1701): 29% in silica-associated SSc vs. 71% in idiopathic SSc ($p = 0.018$), HLA-DRB1*0301: 69% in silica-associated SSc vs. 5% in idiopathic SSc ($p = 0.0002$), HLA-DQB1*0201: 77% in silica-associated SSc vs. 15% in idiopathic SSc ($p = 0.001$), TNFα2: 84.6% in silica-associated SSc vs. 20% in idiopathic SSc ($p = 0.001$), TNF-308A: 77% in silica-associated SSc vs. 15% in idiopathic SSc ($p = 0.001$). Different epitope recognition on DNA topoisomerase I may account for the described discrepancies in HLA alleles. However, no major differences in immunoblot with three overlapping recombinant topoisomerase I fragments between ATA-positive sera of idiopathic SSc ($n = 19$) and silica-associated SSc ($n = 13$) could be found. The N-terminal (amino acid residues 1–362), middle (amino acid residues 231–483) and C-terminal (amino acid residues 364–765) fragments gave positive results in 12, 11 and 14 sera in idiopathic SSc and in 7, 3 and 7 sera in silica-associated SSc, respectively. The analysis of the sera positive for more than one fragment also showed no significant differences. This issue can only be resolved by performing an epitope analysis. Nevertheless, the immunogenetic differences in ATA responders among idiopathic and silica-associated SSc patients suggest that the mechanisms that lead to the production of this AAb are distinct. The dichotomy in different HLA alleles should mean that different peptide motifs were chosen for HLA class II molecule loading and T helper cell/B cell activation [38]. Since uranium miners were also exposed to several metals (Cd, Ni, Se, As, Co, Cu, Fe, Mn, Bi, Ag, Pb, Zn, Sn, Li, B, Sb), abnormal cell accumulation of metals together with reactive oxygen species generated under the influence of silica [39] might be of critical pathogenic importance in the

development of ATA. As has been shown by Casciola-Rosen et al. [40], scleroderma autoantigens, including topoisomerase I, are uniquely fragmented by metal-catalyzed oxidation reactions. They were cleaved at highly specific sites in a reaction that required metal binding (Fe, Cu) and the generation of reactive oxygen species. This cleavage might have permitted the efficient presentation of previously cryptic determinants with the potential for breaking T cell tolerance. Differences in metal exposure depending on the mines may in part explain why, unlike all other AAb typical of CTD, ATA were more prevalent in slightly exposed compared to highly exposed miners (table 3).

As has been shown for ACA, the clearly positive ATA response may also indicate a higher risk of SSc development because of the association of its frequency with SSc symptoms (fig. 3) and because of the observed disease progression in 28.5% of the ATA-positive miners (fig. 4). One patient with probable SSc developed definite SSc within 4 years. Two of three ATA-positive miners with diffuse interstitial pulmonary fibrosis progressed to probable and definite SSc within 3 and 2 years, respectively. This suggests that in some uranium miners diffuse lung fibrosis may be an early manifestation of SSc or a form of sclerosis sine scleroderma [41], as has been shown for 2 miners with other AAb specificities (fig. 4). The higher frequency of ATA in miners with diffuse lung fibrosis compared to miners with Raynaud's phenomenon or miners without SSc symptoms also supports this theory. Considering the potentially predictive importance of ATA, we looked further for reactivities against human eukaryotically expressed topoisomerase I as possible early autoimmune responses even in miners without ANA. Only low-titer reactivities could be observed in ANA-negative miners. Neither significant associations with clinical symptoms nor progression of the autoimmune ATA responses or development of SSc could be observed. Furthermore, the HLA and TNF allele associations described for the clear ATA responses were not found in these miners. Therefore, only clearly positive ATA seem to be relevant for SSc development. Interestingly, the prevalence of lung cancer was higher in miners with ATA responses compared to those without ATA responses (6.7 vs. 3.8%).

Antinucleolar Antibodies

AAb against the nucleolar antigens fibrillarin, RNA polymerase I, II and III, 7-2/8-2 RNP (To/Th antigen), NOR-90 and 20- to 110-kD proteins (PM-Scl antigens) are more or less SSc specific or are markers for SSc-myositis overlap syndrome (anti-PM-Scl antibodies). The nucleolar fluorescence pattern found with sera of uranium miners were mainly speckled or homogeneous. The speckled pattern resembles the pattern observed for antifibrillarin antibodies, whereas the homogeneous pattern resembles that observed for PM-Scl antibodies [42]. We have only tested for PM-Scl specificity by enzyme immunoassay with recombinant PM-Scl-100 protein and found no reactivity in sera with antinucleolar antibodies. Therefore, the antinucleolar specificities remain to be determined. The frequencies of antinucleolar antibodies in different groups of uranium miners (table 3) and the results of the follow-up study (fig. 4) suggest that antinucleolar antibodies may also be important for the diagnosis of early manifestations of SSc or sclerosis sine scleroderma in some uranium miners with diffuse lung fibrosis, as suggested for ATA. The diagnostic and prognostic relevance may become more important if the specificities of these AAb are known. Not all of the antinucleolar AAb seem to be relevant for SSc because various specificities against nucleolar antigens are also detectable in patients with tumors [43]. Indeed, the prevalence of lung cancer was higher in antinucleolar-AAb-positive miners (11.8%) compared to antinucleolar-AAb-negative miners (3.6%, $p = 0.0006$).

Anti-snRNP Antibodies

These AAb are directed against various proteins of small nuclear ribonucleoprotein complexes. The *anti-Sm antibodies* recognize the core proteins, especially B, B′ and D proteins, of U1-, U2- and U4- to U6-RNPs. They are highly specific for SLE (American Rheumatism Association criterion!) and are detectable in 20–35% of the patients with idiopathic SLE. In silica-associated definite and probable SLE as well as in miners with possible CTD development no anti-Sm reactivities could be found. In miners without CTD symptoms low-titer reactivities were measured in only 0.5% (fig. 5). Therefore, anti-Sm antibodies do not appear relevant for the diagnosis of SLE or for predicting a higher risk of SLE development in uranium miners. Furthermore, this discrepancy in AAb response between idiopathic and silica-associated SLE may indicate different mechanisms in AAb and probably disease induction.

Anti-U1-RNP antibodies are directed against the U1-RNP-specific proteins 70K, A and C. They are markers for mixed CTD (MCTD or Sharp syndrome). Furthermore, they are detectable in SSc patients (often developing MCTD) and anti-Sm-positive SLE patients. In uranium miners, low-titer U1-RNP antibodies could be found

in slightly higher frequencies than anti-Sm antibodies (fig. 5). Clearly positive results were obtained only in 6 miners, in 3 with positive results by immunodiffusion against 'extractable nuclear antigens'. Two patients were diagnosed as probable SSc. Of these, one developed MCTD within 5 years. The other developed lung fibrosis in addition to Raynaud's phenomenon, sclerodactyly, proteinuria and elevated erythrocyte sedimentation rates. Therefore, a diagnosis of MCTD is likely also in this patient. A clearly positive U1-RNP reactivity was also seen in one miner with possible CTD development. His serum reacted strongly positive in EIA and showed reactivities in immunoblot against 70K protein (weak), protein A (strong) and protein C. This patient had elevated erythrocyte sedimentation rates, lymphopenia, arthralgia, Raynaud's phenomenon and a progressive restrictive ventilation disturbance but no radiographic signs of diffuse pulmonary fibrosis. In the three other miners with clearly positive anti-U1-RNP results, no CTD symptoms were seen at the time of the first serum analysis. One miner developed Raynaud's phenomenon within 3 years. In conclusion, the follow-up showed that miners with clearly positive anti-U1-RNP antibodies may have a high risk of developing MCTD or SSc with lung fibrosis. This relationship has already been described in nonexposed patients [44]. In 1 miner, the autoimmune response was seen after a follow-up of 9 years. In 1987, he was negative for anti-U1-RNP; in 1994, his serum showed a low-titer response in EIA and 2 years later, he was clearly positive, showing a high-titer reactivity in different EIAs, positivity in immunodiffusion and immunoblot. Therefore, miners with low-titer reactivities should also be followed up.

In summary, anti-U1-RNP antibodies are only rarely detectable in uranium miners. But if they are found at higher titers, development of MCTD or SSc is possible.

Anti-dsDNA Antibodies

AAb against double-stranded DNA are marker antibodies for SLE and are considered as an ACR criterion for the diagnosis of SLE [20]. However, the diagnostic sensitivity and specificity for SLE depend on the techniques used for the determination of these antibodies. We found these antibodies in medium to high titers in the sera of uranium miners with definite SLE (38.9%), with probable SLE (27.3%), with signs of a possible SLE/CTD development (15.5%) and even in 2% of the miners without CTD symptoms. The frequency in silica-associated SLE was lower compared to 61.4% in the group of nonexposed SLE patients from the same geographical region and with the same ethnicity, but a female predominance. Since all of the uranium miners with SLE are men with mainly a late onset of disease, the differences may be related to age or gender. Some authors described a lower prevalence of anti-dsDNA as well as -U1-RNP and -Sm antibodies [45, 46] and a higher prevalence of anti-Ro and -La antibodies [45–47], which corresponds to the serological findings in the silica-associated SLE group. However, this was not confirmed in other groups of later-onset SLE [48–50] which may reflect ethnic [48, 50] or methodical [49] differences.

Little is known about the predictive value of anti-dsDNA AAb in risk persons for CTD/SLE development. To look for further parameters for a risk assessment we investigated the prevalence of the idiotype 16/6 (16/6 Id), a major cross-reactive idiotype of anti-DNA antibodies involved in the pathogenesis of experimental lupus, in different groups of uranium miners [25]. The prevalence of 16/6 Id was higher in all groups compared to those in healthy blood donors. It was 18.5% in miners with SLE (definite and probable) and 22.2–26.5% in miners with clinical and/or serological signs for CTD development. All 16/6-Id-positive miners were positive for anti-dsDNA antibodies and were mostly associated with the production of other CTD-typical autoantibodies. The prevalence of the idiotype 16/6 in anti-dsDNA-positive miners correlated slightly with CTD/SLE symptoms: 55.6% in SLE patients, 47.4% in miners with possible CTD/SLE development and 22.2% in miners without CTD symptoms. Furthermore, in a short-time follow-up, a progression of the disease state was seen in two miners of the 16/6-Id-positive group, but not in 16/6-Id-negative miners. In conclusion, the detection of 16/6 Id in quartz dust-exposed miners may indicate a higher risk for the development of SLE. On the other hand, it can be suggested that disturbances of the idiotypic/anti-idiotypic network through chronic stimulation of the immune system by quartz particles and/or infections (which are more often seen in uranium miners compared to the normal population) may play a pathogenic role in this risk group.

Anti-Ro/SS-A Antibodies

These AAb are directed against proteins of Y-snRNP complexes (Ro52, Ro60). They are diagnostic markers of primary and secondary Sjögrens' syndrome and SLE subtypes. Furthermore, anti-Ro/SS-A antibodies are rarely found in SSc patients. In uranium miners with definite and probable SLE they were found in similar frequencies as anti-dsDNA antibodies (fig. 5) and were often associated with these. This suggests that some antiRo/SS-A antibodies are anti-idiotypic anti-dsDNA antibodies as de-

scribed by Zhang and Reichlin [51]. One miner with anti-Ro/SS-A antibodies died by progressive diffuse pulmonary fibrosis. The histological findings led to the diagnosis of 'sclerosis sine scleroderma' (fig. 4). Anti-Ro/SS-A antibodies were also detectable in miners without CTD symptoms. There was a negligible association of the anti-Ro/SS-A frequency with the intensity of exposure in these miners as well as in miners with Raynaud's phenomenon. All anti-Ro/SS-A-positive patients of the other groups of uranium miners were highly exposed to silica dust. An antigen-driven induction of anti-Ro/SS-A antibodies in uranium miners dependent on the intensity of silica exposure can be suggested by the following points: (1) Silica can lead to an upregulation of the TNF-α expression by influencing the TNF gene promoter [52], (2) TNF-α itself may induce a translocation of the intracytoplasmically localized Ro52 and La proteins to the cell surface [53].

Summary and Conclusions

The quartz dust-exposed uranium miners, especially the highly exposed, have a higher risk of developing systemic autoimmune diseases as has been shown by epidemiological studies [8, 9]. The data on AAb presented here show that the risk of developing CTD is even higher if the miners have disease-specific AAb. The following ANA specificities should be determined for a risk assessment in uranium miners: (1) ACA, ATA and antinucleolar antibodies for SSc, (2) anti-U1-RNP antibodies for MCTD/SSc, and (3) anti-dsDNA antibodies (carrying 16/6 Id) for SLE. From our data it can be concluded that it is not necessary to determine anti-Sm and anti-Jo-1 antibodies. The highest predictive value was seen for ACA and ATA regarding SSc development. The risk for SLE development in subjects positive for anti-Ro/SSA, -La/SSB and/or -dsDNA antibodies remains to be established in larger groups. The higher prevalence of these AAb in quartz dust-exposed uranium miners compared to various control groups and in relation to clinical symptoms of CTD may be a sign of a developing autoimmune disease. Alternatively, they may be produced as a result of the exposure to silica not resulting in the development of CTD. This question can be addressed through a long-term follow-up of all uranium miners with such AAb.

Acknowledgments

The authors wish to thank Dr. Karl-Heinz Frank (Institute of Immunology, Technical University Dresden) and Christine Gibson Parks (Department of Epidemiology, University of North Carolina at Chapel Hill) for helpful suggestions; Dr. Laszlo Czirják (University Medical School Pésc, Hungary) for providing sera of patients with organic solvent-associated scleroderma; Mrs. Uta Kiessling, Christine Streller and Bärbel Naumann (Institute of Immunology, Technical University Dresden) for expert technical assistance.

This work was supported by research grants of the Sächsisches Staatsministerium für Wissenschaft und Kunst, Freistaat Sachsen (AZ 7541.82-0450/405) and by the Federal Institute for Occupational Safety and Health, Department of Occupational Health, Berlin, Project Number 1267.

References

1 Bosi E, Bottazzo GF: Autoimmunity in insulin-dependent diabetes mellitus. Implications for prediction and therapy. Clin Immunother 1995;3:125–135.
2 Bonifacio E, Bingley PJ: Islet autoantibodies and their use in predicting insulin-dependent diabetes. Acta Diabetol 1997;34:185–193.
3 Lo SSS, Tun RYM, Leslie RDG: Non-genetic factors causing type 1 diabetes. Diabetic Med 1991;8:609–618.
4 Bramwell B: Diffuse scleroderma – its frequency, its occurrence in stone-masons, its treatment by fibrinolysin – elevations of temperature due to fibrinolysin injections. Edinburgh Med J 1914;12:387–401.
5 Erasmus LD: Scleroderma in gold-miners on the Witwatersrand with particular reference to pulmonary manifestations. S Afr J Lab Clin Med 1957;3:209–231.
6 Rodnan GP, Benedek TG, Medsger TA, Cammarata RJ: The association of progressive systemic sclerosis (scleroderma) with coal miners' pneumoconiosis and other forms of silicosis. Ann Intern Med 1967;66:323–334.
7 Mehlhorn J: Sklerodermie auch ohne Silikose eine BK? Z Erkr Atmungsorgane 1984;163:65–69.
8 Ziegler V, Haustein UF: Die progressive Sklerodermie – eine quarzinduzierte Berufskrankheit? Dermatol Monatsschr 1992;178:34–43.
9 Mehlhorn J, Enderlein G, Conrad K, Ziegler V: Analysis for the association between progressive systemic sclerosis (scleroderma), exposure to quartz dust and silicosis in East German uranium mining. Zentralbl Arbeitsmed 1999;49:134–147.
10 Koeger AC, Lang T, Alcaix D, Milleron B, Rozenberg S, Chaibi P, Arnaud J, Mayaud C, Camus JP, Bourgeois P: Silica-associated connective tissue disease: A study of 24 cases. Medicine (Baltimore) 1995;74:221–237.
11 Masson C, Audran M, Pascaretti C, Chevailler A, Subra JF, Tuchais E, Kahn MF: Silica-associated systemic erythematosus lupus or mineral dust lupus? Lupus 1997;6:1–3.
12 Sanchez-Roman J, Wichmann I, Salaberri J, Varela JM, Nunez-Roldan A: Multiple clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica. Ann Rheum Dis 1993;52:534–538.
13 Conrad K, Mehlhorn J, Lüthke K, Dörner T, Frank KH: Systemic lupus erythematosus after heavy exposure to quartz dust in uranium mines: Clinical and serological characteristics. Lupus 1996;5:62–69.

14 Parks CG, Conrad K, Cooper GS: Occupational exposure to crystalline silica and autoimmune disease. Environ Health Persp 1999; 107(suppl 5):793–802.
15 Bauer H-D, Schulze U: Staub- und Schwermetallbelastung; in: Untersuchungen zur Staub- und Schwermetallbelastung sowie zur Strahlenbelastung durch Radionuklide in Stäuben in der Gang-Erzlagerstätte Schlema-Alberoda der Wismut. Bochum, Bergbau-Berufsgenossenschaft, 1995, pp 30–88.
16 Vallyathan V, Xianglin S, Dalal NS, Castranova V: Generation of free radicals from freshly fractured silica dust. Potential role in acute silica-induced lung injury. Am Rev Respir Dis 1988;138:1213–1219.
17 Mehlhorn J: Quarzkristalle in der Haut als Auslöser der Sklerodermie. Teil I. Dtsch Dermatologe 1992;11:1563–1572.
18 Pelmear PL, Roos JO, Maehle WM: Occupationally-induced scleroderma. J Occup Med 1992;34:20–25.
19 Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee: Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980; 23:581–590.
20 Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ: The revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–1277.
21 Medsger TA, Masi AT: Epidemiology of systemic sclerosis (scleroderma). Ann Intern Med 1971;74:714–721.
22 Czirják L, Bokk Á, Csontos G, Lörincz G: Clinical findings in 61 patients with progressive systemic sclerosis. Acta Derm Venereol (Stockh) 1989;69:533–536.
23 Conrad K, Frank KH, Mehlhorn J: Kollagenosetypische Autoantikörper in Seren von quarzstaubexponierten Uranerzbergarbeitern. Diagnostische, prognostische und ätiopathogenetische Bedeutung in Bezug auf die Entwicklung systemischer Autoimmunerkrankungen; in Conrad K (ed): Autoantikörper. Update: Clinical Immunology. Lengerich, Pabst Science Publishers, 1998, vol 8, pp 250–288.
24 Conrad K, Stahnke G, Liedvogel B, Mehlhorn J, Barth J, Blasum C, Altmeyer P, Sönnichsen N, Frank KH: Anti-CENP-B response in sera of quartz dust-exposed uranium miners and patients with possible development of systemic sclerosis (scleroderma). J Rheumatol 1995;22: 1286–1294.
25 Conrad K, Levy Y, Blank M, Mehlhorn J, Frank KH, Roch B, Shoenfeld Y: The pathogenic idiotype 16/6 in patients with silica-associated systemic lupus erythematosus (SLE) and uranium miners with an increased risk for the development of SLE. J Rheumatol 1997;24: 660–666.
26 Frank KH, Füssel M, Conrad K, Rihs HP, Koch R, Gebhardt B, Mehlhorn J: Different distribution of HLA class II and tumor necrosis factor alleles (TNF-308.2, TNFα_2 microsatellite) in anti-topoisomerase I responders among scleroderma patients with and without exposure to quartz/metal dust. Arthritis Rheum 1998;41:1306–1311.
27 Tramposch HD, Smith CD, Senécal JL, Rothfield NF: A long-term longitudinal study of anticentromere antibodies. Arthritis Rheum 1984;27:121–124.
28 Weiner ES, Hildebrandt S, Senécal JL, Daniels L, Noell S, Joyal F, Roussin A, Earnshaw W, Rothfield NF: Prognostic significance of anticentromere antibodies and anti-topoisomerase I antibodies in Raynaud's disease. Arthritis Rheum 1991;34:68–77.
29 Earnshaw WC, Rothfield NF: Identification of a family of human centromere proteins using autoimmune sera from patients with scleroderma. Chromosoma (Berl) 1985;91:313–321.
30 Earnshaw WC, Bordwell B, Marino C, Rothfield NF: Three human chromosomal autoantigens are recognized by sera from patients with anti-centromere antibodies. J Clin Invest 1986; 77:426–430.
31 Stahnke G, Meier E, Scanarini M, Northemann W: Eukaryotic expression of recombinant human centromere autoantigen and its use in a novel ELISA for diagnosis of CREST syndrome. J Autoimmun 1994;7:107–118.
32 Lee SL, Tsay GJ, Tsai RT: Anticentromere antibodies in subjects with no apparent connective tissue disease. Ann Rheum Dis 1993; 52:586–589.
33 Verheijen R, de Jong BAW, Oberyé EHH, van Venrooij WJ: Molecular cloning of a major CENP-B epitope and its use for the detection of anti-centromere autoantibodies. Mol Biol Rep 1992;16:49–59.
34 Earnshaw WC, Machlin PS, Bordwell BJ, Rothfield NF, Cleveland DW: Analysis of anticentromere autoantibodies using cloned autoantigen CENP-B. Proc Natl Acad Sci USA 1987;84:4979–4983.
35 Picillo U, Migliaresi S, Vatti M, Marcialis MR, Ferruzzi AM, Tirri G: Demographic differences in the frequency of scleroderma-related autoantibodies. Arthritis Rheum 1993;36: 1332–1234.
36 Maddison PJ, Stephens C, Briggs D, Welsh KI, Harvey G, Whyke J, McHugh N, Black CM: Connective tissue disease and autoantibodies in the kindred of 63 patients with systemic sclerosis. The United Kingdom Systemic Sclerosis Study Group. Medicine (Baltimore) 1993;72: 103–112.
37 Rihs HP, Conrad K, Mehlhorn J, May-Taube K, Welticke B, Frank KH, Baur X: Molecular analysis of HLA-DPB1 alleles in idiopathic systemic sclerosis patients and uranium miners with systemic sclerosis. Int Arch Allergy Immunol 1996;109:216–222.
38 Kuwana M, Medsger TA, Wright TM: T cell proliferative responses induced by DNA topoisomerase I in patients with systemic sclerosis and healthy controls. J Clin Invest 1995;96: 586–596.
39 Gossart S, Cambon C, Orfila C, Seguelas MH, Rami J, Carre P, Pipy B: Reactive oxygene intermediates as regulators of TNF-alpha production in rat lung inflammation induced by silica. J Immunol 1996;156:1540–1548.
40 Casciola-Rosen L, Wigley F, Rosen A: Scleroderma autoantigens are uniquely fragmented by metal-catalyzed oxidation reactions: Implications for pathogenesis. J Exp Med 1997;185: 71–79.
41 Giordano M, Ara M, Capelli L, Tirri G, Vatti M: Vorschlag zu einer neuen Klassifizierung und Nomenklatur der progredienten generalisierten Sklerodermie (PGS). Verh Dtsch Ges Rheumatol 1980;6:379–382.
42 Reimer G, Steen VD, Penning CA, Medsger TA, Tan EM: Correlation between autoantibodies to nucleolar antigens and clinical features in patients with systemic sclerosis (scleroderma). Arthritis Rheum 1988;31:525–532.
43 Imai H, Ochs RL, Kiyosawa K, Furuta S, Nakamura RM, Tan EM: Nucleolar antigens and autoantibodies in hepatocellular carcinoma and other malignancies. Am J Pathol 1992;140: 859–870.
44 Steen VD, Powell DL, Medsger TA: Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. Arthritis Rheum 1988;31:196–203.
45 Jonsson H, Nived O, Sturfelt G: The effect of age on clinical and serological manifestation in unselected patients with systemic lupus erythematosus. J Rheumatol 1988;15:505–509.
46 Catoggio LJ, Skinner RP, Smith G, Maddison PJ: Systemic lupus erythematosus in the elderly: Clinical and serological characteristics. J Rheumatol 1984;11:175–181.
47 Hochberg MC, Boyd RE, Ahearn JM, Arnett FC, Bias WB, Provost TT, Stevens MB: Systemic lupus erythematosus: A review of clinicolaboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets. Medicine (Baltimore) 1985;64: 285–295.
48 Koh ET, Boey ML: Late onset lupus: A clinical and immunological study in a predominantly Chinese population. J Rheumatol 1994;21: 1463–1467.
49 Bell DA, Rigby R, Stiller CR, Clark WF, Harth M, Ebers G: HLA antigens in systemic lupus erythematosus: Relationship to disease severity, age at onset, and sex. J Rheumatol 1984;11: 475–479.
50 Hashimoto H, Tsuda H, Hirano T, Takasaki Y, Matsumoto T, Hirose S: Differences in clinical and immunological findings of systemic lupus erythematosus related to age. J Rheumatol 1987;14:497–501.
51 Zhang W, Reichlin M: Some autoantibodies to Ro/SS-A and La/SS-B are antiidiotypes to antidouble-stranded DNA. Arthritis Rheum 1996; 39:522–531.
52 Savici D, He B, Geist LJ, Monick MM, Hunninghake GW: Silica increases tumor necrosis factor (TNF) production, in part by upregulating the TNF promoter. Exp Lung Res 1994;20: 613–625.
53 Dörner T, Hucko M, Mayet WJ, Trefzer U, Burmester GR, Hiepe F: Enhanced membrane expression of the 52 kDa Ro(SS-A) and La (SS-B) antigens by human keratinocytes induced by TNF alpha. Ann Rheum Dis 1995;54:904–909.

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2000;123:92–97

Diagnostic Importance of Anti-Proteasome Antibodies

Eugen Feist[a] Thomas Dörner[a] Ulrike Kuckelkorn[b] Sonja Scheffler[a]
Gerd-R. Burmester[a] Peter-M. Kloetzel[b]

[a]Department of Rheumatology and Clinical Immunology, [b]Institute of Biochemistry,
Charité Medical School, Humboldt University, Berlin, Germany

Key Words
Autoantibodies · Proteasome · Autoimmune myositis · Systemic lupus erythematosus · Primary Sjögren's syndrome

Abstract
20S proteasome represents the proteolytic core complex for cytoplasmic protein degradation that is involved in the activation and regulation of the immune response. In this context, proteasome generates antigenic peptides for the MHC class I pathway and activates NF-κB. In a recent analysis, we could identify a frequent humoral autoimmune response directed against specific proteasomal subunits in patients with autoimmune myositis, systemic lupus erythematosus and primary Sjögren's syndrome. The outer ring subunit HC9(α3) was identified as the predominant target of the anti-proteasome response in these entities. In addition to the reactivity against HC9(α3), patients with primary Sjögren's syndrome expressed a more polyspecific recognition pattern of proteasomal subunits involving the active inner ring proteins. In follow-up analysis, anti-proteasome antibody titers revealed a correlation with disease activity in patients with autoimmune myositis and systemic lupus erythematosus. The current review summarizes recent data providing evidence that the 20S proteasome represents an important target of the humoral autoimmune response in systemic autoimmune diseases and extends insight into pathogenic aspects of these diseases.

Structure and Function of the 20S Proteasome

20S proteasome is an abundant and highly conserved protease complex with N-terminal nucleophile hydrolase activity throughout the evolution of eukaryotics and archebacteria [1, 2]. In mammals, it represents the major cytoplasmatic machinery for protein degradation consisting of its catalytic core, the 20S proteasome, in interaction with 19S regulator caps or the 11S proteasomal activator (PA28) [3, 4]. The 20S proteasome itself is arranged in a cylindrical structure of four staggered rings, each composed of seven different but evolutionary related subunits (fig. 1) [5]. The proteasomal proteins of the outer rings belong to one protein family named α-type, while the inner rings, which carry the active sites, are members of the so called β-type family (table 1).

Former investigations revealed the fundamental role of proteasome for cell homeostasis as a multicatalytic cytoplasmatic protease by degradation of polyubiquitinylated proteins into short peptides [6]. Moreover, it became clear that the proteasome is not only a selective machinery for the clearance of misfolded proteins: it also

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/00/1231–0092$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Eugen Feist
Department of Rheumatology and Clinical Immunology
Charité Medical School, Humboldt University of Berlin
Schumannstrasse 20/21
D–10117 Berlin (Germany)

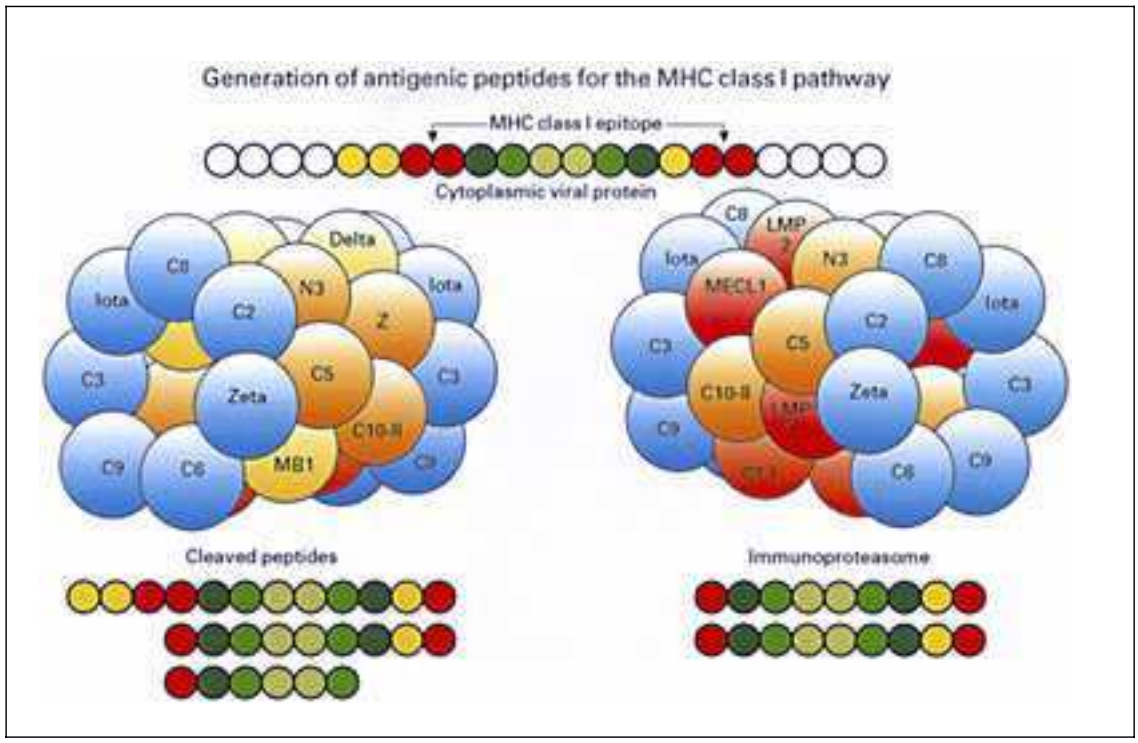


Fig. 1. Structure and function of the 20S proteasome. The house-keeping catalytic subunits MB-1, delta and Z are replaced by the interferon-γ-inducible subunits LMP-7, LMP-2 and MECL-1 increasing the generation of antigenic peptides.

Table 1. Subunits of the human 20S proteasome [5, 23–31, 33]. The inducible by interferon-γ-subunits are indicated as β1i, β2i and β5i. Four of the α-subunits carry putative nuclear localization sequences (NLS). Subunits undergoing posttranslational processing are indicated

Subunit	Amino acids	Molecular weight	Isoelectric point	Function and interaction
α1-IOTA	246	27.4	6.35	NLS (nuclear transport?), RNAse activity
α2-HC3	233	25.7	7.12	NLS (nuclear transport?), phosphorylated
α3-HC9	261	29.5	7.58	NLS (nuclear transport?), binds HTLV-1 TAX, phosphorylated
α4-HC6	248	27.9	8.60	NLS (nuclear transport?), binds HBV HBx
α5-ZETA	241	26.4	4.69	RNAse activity, phosphorylated
α6-HC2	263	29.6	6.16	binding site for PA28
α7-HC8	254	28.3	5.20	phosphorylated
β1-δ[1]	205	21.7	4.91	catalytic
β1i-LMP2[1]	199	21.3	4.80	catalytic
β2-Z[1]	277	25.2	5.54	catalytic
β2i-MECL1[1]	234	24.6	6.07	catalytic
β3-HC10-II	205	22.9	6.15	
β4-HC7-I	201	22.8	6.52	
β5-MB1[1]	204	22.5	8.67	catalytic
β5i-LMP7[1]	204	22.6	7.59	catalytic
β6-HC5[1]	241	26.5	8.27	phosphorylated
β7-HN3[1]	219	24.3	5.47	binds HTLV-1 TAX?

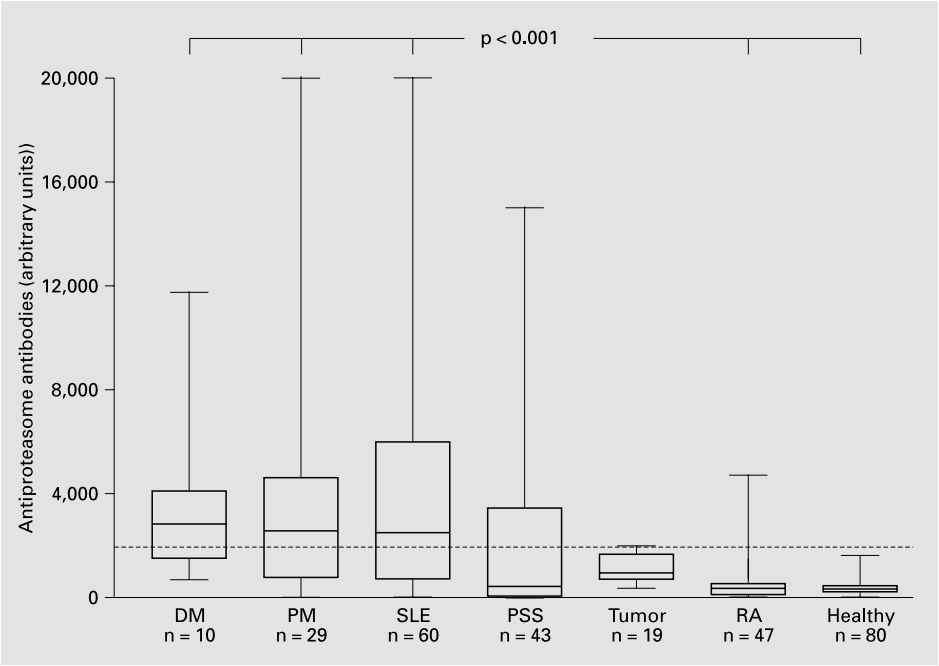


Fig. 2. Autoimmune response against the proteasome in sera from patients with autoimmune myositis (DM 6/10; PM 17/29), systemic lupus erythematosus (SLE 34/60), primary Sjögren's syndrome (pSS 14/43), rheumatoid arthritis (RA 1/47), tumor patients and normal healthy controls. ELISA reactivities and median values of patients and healthy control sera to purified 20S proteasome are shown. The respective cut-off value (2,000 arbitrary units) is indicated (– – –). Statistics were performed using the non-parametric Kruskal-Wallis test.

activates some transcriptional factors such as NFκB and controls cell cycle via processing of cyclin. Most interestingly for immunologists, proteasome represents the main machinery for the production of antigenic peptides with a high affinity for the MHC-class-I binding domain [7, 8]. In this way, foreign proteins (e.g. proteins of viral origin) are cleaved to peptides and subsequently transported via TAP-1 and TAP-2 into the endoplasmic reticulum, where they bind to the MHC class I molecules. Under the influence of the inflammatory cytokine interferon-γ, the proteolytical properties of proteasome change remarkably [9–12]. In this context, the three subunits of the β-type LMP-7, LMP-2 and MECL-1 are overexpressed and integrated into the proteasomal complex replacing the house keeping catalytic subunits MB-1, delta and Z, respectively. This different composition of the proteasomes leads to effective usage of the cleavage sites and results in enhanced generation of some antigenic peptides with an affinity for the MHC class-1 binding domain [13]. While the proteolytic activity of proteasome is restricted to three β-sites, the α-type subunits interact with regulatory proteins, such as the 19S regulator or the PA28 activator of the proteasome.

The subunits of the proteasome vary in their molecular weight and isoelectric points, allowing a differentiation of the respective protein in two-dimensional gel electrophoresis (table 1). Furthermore, sequencing of all proteasomal subunits revealed no sequence homologies to other so far known proteins [14]. Interestingly, two of the interferon-γ-inducible subunits of the proteasome, LMP-7 and LMP-2 (low-molecular-weight proteins) are encoded on chromosome 6 within the MHC class II locus.

The cellular localization of proteasome is not restricted to the cytoplasm. A remarkable amount of proteasome is detectable in the nucleus of normal cells and especially of tumor cells. Four of the mammalian α-type subunits of the proteasome carry putative nuclear localization signals (table 1). These sequences may enable the entrance of the whole complex into the nucleus, while investigations regarding the function and possible interactions of proteasome with other nuclear proteins are in progress.

Detection of Antiproteasome Autoantibodies

The first description of a humoral antiproteasomal response in patients with systemic lupus erythematosus was performed by Arribas et al. [15] in 1991. Their analysis revealed a reactivity against different proteasomal proteins in 35% of patients with systemic lupus erythematosus as judged by immunoblotting. The detection of antiproteasome antibodies of IgG type has been confirmed by our group, revealing that this phenomenon is not unique to systemic lupus erythematosus but can occur also in

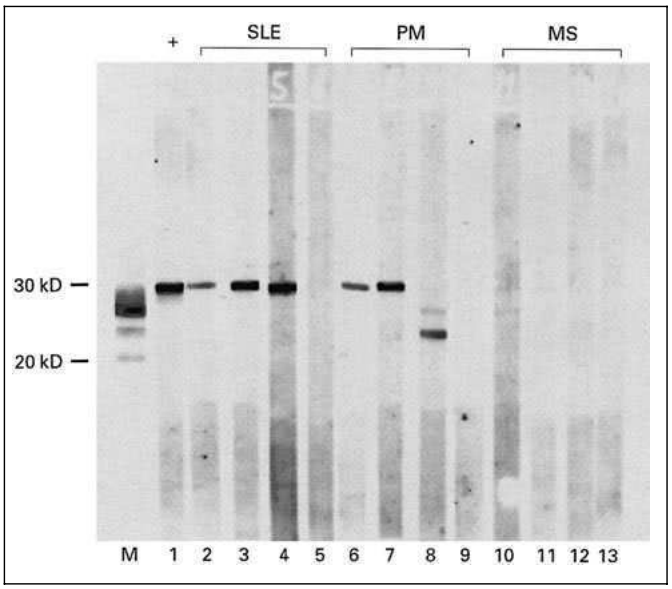


Fig. 3. Antiproteasome recognition pattern by sera from patients with systemic lupus erythematosus (SLE) and polymyositis (PM) using immunoblot analysis. Patients with multiple sclerosis (MS) served as controls. Lane M: rabbit anti-proteasome antibody MP1 recognized proteasomal subunits of 30–20 kD. Lane 1: positive control.

patients with other systemic autoimmune diseases, such as autoimmune myositis and primary Sjögren's syndrome [16, 32]. Moreover, the sensitivity and specificity of this autoantibodies were evaluated in different rheumatic diseases and controls using ELISA technigue (fig. 2).

The detection of antiproteasomal antibodies in ELISA requires a highly purified fraction of the 20S proteasome complex, especially cleared from a heat shock protein with a molecular weight of about 90 kD. This protein could be potentially copurified after ion exchange chromatography on DEAE-Sephacel, density gradient centrifugation in a 10–40% sucrose gradient for 16 h at 40,000 rpm and even after ion-exchange chromatography on ResourceQ and MonoQ-columns (FPLC, Pharmacia). To exclude reactivities against other proteins, a sandwich-assay using a monoclonal antiproteasomal antibody avoids these problems providing a sensitive and specific detection method. The performance of this assay has been described in detail elsewhere [16].

To characterize the targets of the antiproteasomal response, it was necessary and of major importance to differentiate between the recognized subunits of the complex. Therefore, we investigated purified proteasome in immunoblotting as well as in two-dimensional gel electrophoresis experiments. SDS-PAGE alone in its technical variations is not sufficient to differentiate between all subunits of the proteasome, because some subunits have almost the same molecular weight. However, immunoblotting is able to detect the antibody recognition of more than one subunit of the proteasome. Moreover, signals against proteins with a molecular weight between 26 and 30 kD represent α-type subunits, whereas molecular weights in the range of 21–25 kD correspond to β-type subunits of the proteasome in their processed form.

Immunoblot analysis of sera from patients with systemic lupus erythematosus and autoimmune myositis revealed that proteasomal proteins with a molecular weight of about 28 kD are autoimmune targets. Of note, in most cases the sera recognized only one single band in immunoblotting [16].

In order to identify the target proteins, an analysis of proteasome has been performed in two-dimensional electrophoresis and the sera were subsequently tested by Western blotting. As a result, the α-type subunit HC9 has been identified as the major antigen in patients with systemic lupus erythematosus and autoimmune myositis [16] (fig. 3). Less frequent reactivities were observed against other α-proteasomal subunits, such as HC8 and HC2, and β-type subunits, such as the active subunits MECL-1, LMP-7 and Z. Furthermore, the detection of antiproteasomal antibodies directed against specific subunits is possible employing recombinant subunits of the complex such as HC9.

Further analysis sought to evaluate the findings obtained in patients with systemic lupus erythematosus and autoimmune myositis. In these studies, patients with primary Sjögren's syndrome, 39% of whom reacted against proteasome, revealed a more polyspecific recognition pattern of proteasomal proteins involving subunits of both families in immunoblotting and two-dimensional gel electrophoresis. Taken together, almost all patient's sera that were positive in ELISA showed reactivity in immunoblotting.

Diagnostic Importance of Anti-Proteasome Antibodies

Autoantibodies are a hallmark of systemic autoimmune diseases that provides important diagnostic and prognostic information [17]. Antiproteasomal antibodies were detected in different diseases of rheumatic origin and, therefore, their diagnostic specificity is limited. Despite the fact that in systemic lupus erythematosus anti-dsDNA antibodies represent the major diagnostic and

pathogenetic autoantibody, the description of antiproteasomal antibodies complete our understanding of B cell autoimmunity in this entity. On the other hand, autoimmune myositis can be differentiated histologically and clinically into two entities, poly- and dermatomyositis, while no reliable serological marker exists. Distinct autoantibodies occur in about 20% of these cases, characterizing the clinical manifestations of the anti-tRNA synthetase syndrome with arthritis, Raynaud's phenomenon and pneumonitis [18, 19]. Therefore, of antiproteasomal antibodies, which are defected in about 60% of patients with autoimmune myositis, are the most frequent autoantibody phenomenon in this entity described so far. Furthermore, our data provide evidence that the antiproteasomal response is related to disease activity in systemic lupus erythematosus as well as in autoimmune myositis. However, comparison of the clinical data obtained from the antibody-positive patients with those of the antibody-negative patients showed no direct correlation to clinical manifestations in systemic lupus erythematosus and autoimmune myositis.

Pathogenetic Mechanisms in Systemic Autoimmune Diseases Targeting Proteasomes

The mechanisms initiating autoimmune processes and breaking tolerance are of special interest in immunology as well as rheumatology. Here, the proteasome stands at a cross-point of two pathways. Naturally, it represents a machinery for the production of antigens of foreign or self origin, which are able to prime the cytotoxic T cell immune response. On the other hand, subunits of the 20S proteasome itself are targets of systemic B cell autoimmunity.

In eukaryotes, determination of self or nonself is provided by the presentation of processed protein fragments via MHC class I or II molecules. Antigenic peptides are either produced by proteasome for the MHC class I pathway or are degraded within the lysosomal compartment for uploading of MHC class II molecules. Subsequently, effector cells, such as cytotoxic CD8+ T cells for the MHC class I and CD4+ T-helper cells for MHC class-II response, initiate the amplification of the autoimmune response.

Current investigations provide data of a low linkage rate for antigens between both presentation pathways [20–22]. Furthermore, overexpression of both MHC class molecules occurs under the influence of the inflammatory cytokine interferon-γ, which is secreted by CD4+ T cells and simultaneously activates CD8+ T cells. However, the proteasome is preferentially involved in the initiation and perpetuation of autoimmune cytotoxic T cell response.

Further studies are needed to establish how the B cell autoimmune response initiates autoimmunity against such antigens as proteasome. One possible mechanism is a cross-reactivity of a primary response against exogenous proteins. This pathway would require sequence and/or epitope homologies between the targets and, therefore, seems to be unlikely for the unique sequences of proteasomal proteins. Probably, the proteasome itself drives its own autoimmune response as suggested for other known autoantigens. A break of tolerance is possible after immunization with a large amount of autoantigen in vivo, as demonstrated by a variety of studies. In the case of proteasome, our group was able to measure elevated levels of the antigen (25–560 ng/ml) in the circulation of patients with autoimmune myositis, primary Sjögren's syndrome and systemic lupus erythematosus compared to healthy controls, patients with rheumatoid arthritis and solid tumors. Moreover, follow-up analysis of 2 patients has documented that the free circulating proteasome is enhanced prior to elevated antiproteasome antibody reactivities. Recognition of different proteasomal subunits in immunoblotting and two-dimensional gel electrophoresis raised the question whether this phenomenon is due to sequence homologies between the respective subunits or mechanisms of intermolecular spreading of the autoimmune response against proteasome. Most recently, we obtained data from a follow-up analysis of a patient with polymyositis revealing that intramolecular spreading mechanism occur. In detail, after initial reactivity against 20S proteasome by ELISA a signal was observed against a β-type subunit with a molecular weight of about 22 kD by immunoblotting. After a period of 3 months, the immune response switches against a proteasomal α-type subunit with a molecular weight of about 28 kD.

Together with the data on correlating disease activity and anti-proteasome reactivity, these data suggested that the proteasome apparently drives its own autoimmune response with enhanced autoantibody titers during immunologically active disease stages.

The current data on an autoimmune response to the proteasome complex are most consistent with the conclusion that autoantigens are substantially involved in the induction and maintenance of their autoimmune function. However, the initial mechanisms of breaking tolerance as well as the pathogenic importance of antiproteasomal autoantibodies need to be addressed in further studies.

References

1 Coux O, Tanaka K, Goldberg AL: Structure and function of the 20S and 26S proteasomes. Ann Rev Biochem 1996;65:801–847.

2 Seemüller E, Lupas A, Stock D, Löwe J, Huber R, Baumeister W: Proteasome from *Thermoplasma acidophilum:* A threonine protease. Science 1995;268:579–582.

3 Rivett AJ, Mason GG, Murray RZ, Reidlinger J: Regulation of proteasome structure and function. Mol Biol Rep 1997;24:99–102.

4 Ahn JY, Tanahashi N, Akiyama K, Hisamatsu H, Noda C, Tanaka K, Chung CH, Shibmara N, Willy PJ, Mott JD: Primary structures of two homologous subunits of PA28, a gamma-interferon-inducible protein activator of the 20S proteasome. FEBS Lett 1995;366:37.

5 Groll M, Ditzel L, Löwe J, Stock D, Bochtler M, Bartunik HD, Huber R: Structure of 20S proteasome from yeast at 2.4 Å resolution. Nature 1997;386:463–471.

6 Seufert W, Jentsch S: In vivo function of the proteasome in the ubiquitin pathway. EMBO J 1992;11:3077–3080.

7 Groettrup M, Soza A, Kuckelkorn U, Kloetzel PM: Peptide antigen production by the proteasome: Complexity provides efficiency. Immunol Today 1996;17:429–435.

8 Rammensee HG, Falk K, Rötzschke O: Peptides naturally presented by MHC class I molecules. Annu Rev Immunol 1993;11:213–244.

9 Boes B, Hengel H, Ruppert T, Multhaup G, Koszinowski UH, Kloetzel PM: Interferon gamma stimulation modulates the proteolytic activity and cleavage site preference of 20S mouse proteasomes. J Exp Med 1994;179:901–909.

10 Aki M, Shimbara N, Takashina M, Akiyama K, Kagawa S, Tamura T, Tanahashi N, Yoshimura T, Tanaka K, Ichihara A: Interferon-γ induces different subunit organizations and functional diversity of proteasomes. J Biochem 1994;115:257–269.

11 Stohwasser R, Standera S, Peters I, Kloetzel PM, Groettrup M: Molecular cloning of the mouse proteasome subunits MC14 and MECL-1: Reciprocally regulated tissue expression of interferon-γ-modulated proteasome subunit. Eur J Immunol 1997;27:1182–1187.

12 Nandi D, Jiang H, Monaco JJ: Identification of MECL-1 (LMP10) as the third IFN-γ-inducible proteasome subunit. J Immunol 1996;156:2361–2364.

13 Kuckelkorn U, Frentzel S, Kraft R, Kostka S, Groettrup M, Kloetzel PM: Incorporation of major histocompatibility complex-encoded subunits LMP2 and LMP7 changes the quality of the 20S proteasome polypeptide processing products independent of interferon-γ. Eur J Immunol 1995;25:2605.

14 Monaco JJ, Nandi D: The genetics of proteasomes and antigen processing. Annu Rev Genet 1995;29:729–754.

15 Arribas J, Luz Rodriguez M, Alvarez Do Forno R, Castano JG: Autoantibodies against the multicatalytic proteinase in patients with systemic lupus erythematosus. J Exp Med 1991;173:423.

16 Feist E, Dörner T, Kuckelkorn U, Schmidtke G, Micheel B, Hiepe F, Burmester GR, Kloetzel PM: The proteasome α-type subunit C9 is a primary target of autoantibodies in sera of patients with myositis and systemic lupus erythematosus. J Exp Med 1996;184:1313–1318.

17 von Mühlen CA, Tan EM: Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum 1995;5:323–358.

18 Targoff IN: Immune manifestations of inflammatory muscle disease. Rheum Dis Clin North Am 1994;20:857.

19 Bernstein RM, Morgan SH, Chapman J, Bunn CC, Mathews MB, Turner Warwick M, Hughes GR: Anti-Jo-1 antibody: A marker for myositis with interstitial lung disease. Br Med J Clin Res Ed 1984;289:151.

20 Kovacsovics-Bankowski M, Rock KL: A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. Science 1995;267:243–246.

21 Chicz RM, Urban RG, Gorga JC, Vignali DAA, Lane WS, Strominger JS: Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. J Exp Med 1993;178:27–47.

22 Malnati MS, Marti M, LaVaute T, Jaraquemada D, Biddison W, DeMars R, Long EO: Processing pathways for presentation of cytosolic antigen to MHC-II-restricted T-cells. Nature 1992;357:702–704.

23 Hendil KB, Kristensen P, Uerkvitz W: Human proteasomes analysed with monoclonal antibodies. Biochem J 1995;305:245–252.

24 Kania MA, Demartino GN, Baumeister W, Goldberg AL: The proteasome subunit, C2, contains an important site for binding of the PA28 (11S) activator. Eur J Biochem 1996;236:510.

25 Nederlof PM, Wang HR, Baumeister W: Nuclear localization signals of human and Thermoplasma proteasomal α-subunits are functional in vitro. Proc Natl Acad Sci USA 1995;92:12060–12064.

26 Knuehl C, Seelig A, Brecht B, Henklein P, Kloetzel PM: Functional analysis of eukaryotic 20S proteasome nuclear localization signal. Exp Cell Res 1996;225:67–74.

27 Rousset R, Desbois C, Bantignies F, Jalinot P: Effects on NF-kappa B1/p105 processing of the interaction between the HTLV-1 transactivator Tax and the proteasome. Nature 1996;381:328–331.

28 Huang J, Kwong J, Sun EC, Liang TJ: Proteasome complex as a potential cellular target of hepatitis B virus X protein. J Virol 1996;70:5582–5591.

29 Fischer M, Runkel L, Schaller H: Hbx protein of hepatitis B virus interacts with the C-terminal portion of a novel human proteasome alpha-subunit. Virus Genes 1995;10:1:99–102.

30 Petit F, Jarrousse AS, Dahlmann B, Sobek A, Hendil KB, Buri J, Briand Y, Schmid HP: Involvement of proteasomal subunits zeta and iota in RNA degradation. Biochem J 1997;326:93–98.

31 Wehren A, Meyer HE, Sobek A, Kloetzel PM, Dahlmann B: Phosphoamino acids in proteasome subunits. Biol Chem 1996;377:497–503.

32 Feist E, Kuckelkorn U, Dörner T, Dönitz H, Scheffler S, Hiepe F, Kloetzel PM, Burmester GR: Autoantibodies in Primary Sjögren's syndrome are directed against proteasomal subunits of the α and β-type. Arthritis and Rheumatism 1999;42/4:697–702.

33 Tamura T, Hee Lee D, Osaka F, Fujiwara T, Shin S, Ha Chung C, Tanaka K, Ichihara A: Molecular cloning and sequence analysis of cDNAs for five major subunits of human proteasomes (multi-catalytic proteinase complexes). Biochem Biophys Acta 1991;1089:95–102.

Author Index Vol. 123, No. 1, 2000

International Archives of Allergy and Immunology

Subject Index Vol. 123, No. 1, 2000

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/journals/iaa