Autoantibodies in Connective Tissue Diseases: Clinical Significance and Analysis of Target Autoantigens

Tsuneyo MIMORI

Systemic connective tissue diseases are characterized by the production of a number of autoantibodies directed against various cellular constituents. These autoantibodies are closely associated with certain diseases and clinical manifestations, and are therefore useful for clinical practice such as to diagnose diseases and to predict clinical subsets, disease activity and prognosis. To understand the etiology and pathogenic mechanisms of connective tissue diseases, it is particularly important to elucidate the structure and function of target autoantigens recognized by these disease-specific autoantibodies. In recent years, the nature of many target autoantigens have been identified using molecular biology approaches. Most of them are intracellular enzymes and regulatory factors necessary for important biological function involved in gene replication, transcription, RNA processing and protein translation. Thus, the studies of autoantibodies are useful not only in clinical medicine but also in basic cellular and molecular biology. (Internal Medicine 38: 523-532, 1999)

Key words: ribonucleoprotein, deoxyribonucleoprotein, systemic lupus erythematosus, scleroderma, polymyositis/dermatomyositis

Introduction

Autoantibodies directed against various cellular components are found in sera from connective tissue diseases. These autoantibodies have been demonstrated to be associated with certain diseases and clinical manifestations, and provide us useful information for clinical practice. The development of new technologies for detecting autoantibodies has facilitated identification of more than 50 autoantibodies and their target autoantigens. Most autoantigen molecules are complexes with proteins and RNA (ribonucleoprotein) or DNA (deoxyribonucleoprotein), and act as intracellular enzymes or regulatory factors necessary for important biological function involved in gene replication, transcription, RNA processing and protein translation. Thus, the studies of autoantibodies are useful not only in clinical medicine but also in basic cellular and molecular biology.

The measurement of autoantibodies offers numerous clinical utilities:

1. Marker of diseases: most autoantibodies have high disease specificity and therefore a diagnostic value if they are positive.
2. Marker of disease subsets: most autoantibodies are associated with certain subsets or clinical symptoms of each disease (i.e. anti-dsDNA and lupus nephritis, anti-Scl-70 and diffuse

scleroderma, and anti-Jo-1 and myositis with interstitial lung disease).

3. Marker of disease activity: some, but not all, autoantibodies are closely correlated to the disease activity (i.e. anti-dsDNA in SLE, and C-ANCA in Wegener's granulomatosis).

4. Marker of prognosis: certain autoantibodies can be useful to predict the prognosis or severity of diseases (i.e. anti-SRP and severe polymyositis, and anti-RNA polymerases and renal crisis in scleroderma).

Autoantibodies detected in connective tissue diseases are summarized in Table 1. In this review, the clinical significance of these autoantibodies in connective tissue diseases and the nature of their target autoantigens will be discussed.

Autoantibodies in systemic lupus erythematosus (SLE)

LE factor

LE factor is autoantibodies against DNA-histone complex and is a serum factor necessary to produce LE cells (1, 2). LE cells are observed *in vitro* when denatured leukocyte nuclei bound to LE factor and complement are ingested by living phagocytic leukocytes. LE factor can also be measured by latex agglutination test, although both specificity and sensitivity

From the Department of Internal Medicine, Keio University School of Medicine, Tokyo

Reprint requests should be addressed to Dr. Tsuneyo Mimori, the Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582

Table 1. Autoantibodies in Connective Tissue Diseases and Their Clinical Significance

Antibodies	Nature of target antigens	Frequency %	Clinical significance
1. Autoantibodies in SLE			
LE factor	DNA-histone complex, histone	40–50%	Active SLE, drug-induced lupus
Anti-dsDNA	dsDNA	50–70%	Lupus nephritis, active SLE
Anti-Sm	U1, U2, U4/U6, U5-snRNP (mRNA-splicing factor)	15–30%	CNS lupus, delayed proteinuria
Anti-ribosomal P	P0, P1, P2 (60S subunit)	10%	CNS lupus
Anti-Ki/SL	34 kDa nuclear protein	10%	Sicca syndrome, myopathy
Anti-PCNA	DNA polymerase δ auxiliary protein	<5%	Thrombocytopenia, nephropathy
2. Autoantibodies in SSc			
Anti-Scl-70 (Topo I)	DNA topoisomerase I	20–30%	Diffuse scleroderma
Anti-centromere	Centromere proteins A, B, C	20–30%	Limited scleroderma
Anti-U3RNP	U3RNP (32 kDa fibrillarin)	5–10%	Diffuse scleroderma
Anti-7-2/8-2RNP(Th/To)	RNase P & RNase MRP	5–10%	Limited scleroderma
Anti-RNA polymerases	RNA polymerase I, II, III	6–24%	Diffuse scleroderma, renal crisis
3. Autoantibodies in PM/DM			
Anti-ARS			
Anti-Jo-1	Hystidyl-tRNA synthetase (50 kDa)	20–30%	Anti-synthetase syndrome (pulmonary fibrosis, polyarthritis, inflammatory myopathy)
Anti-PL-7	Threonyl-tRNA synthetase (80 kDa)	<5%	
Anti-PL-12	Alanyl-tRNA synthetase (110 kDa)	<5%	
Anti-EJ	Glycyl-tRNA synthetase (75 kDa)	<5%	
Anti-OJ	Isoleucyl-tRNA synthetase (multienzyme complex)	<5%	
Anti-AS	Asparaginyl-tRNA synthetase	?	
Anti-SRP	Signal recognition particle	5%	Severe, refractory PM DM
Anti-Mi-2	helicase family protein (218 k/240 kDa)	5–10%	
4. Autoantibodies in overlap syndrome			
Anti-U1RNP	U1-snRNP (mRNA splicing factor)	100% of MCTD	MCTD, non-renal SLE, Raynaud's phenomenon
Anti-U2RNP	U2-snRNP (mRNA splicing factor)	10–20%	SSc-PM overlap
Anti-Ku	DNA-PK regulatory subunit (70 k/80 kDa)	20–30%	SSc-PM overlap in Japanese
Anti-DNA-PKcs	DNA-PK catalytic subunit (460 kDa)	<5%	PM-overlap
Anti-PM-Scl	Nucleolar protein complex (110–20 kDa)	8–10%	SSc-PM overlap in Caucasian
5. Autoantibodies in Sjögren's syndrome			
Anti-SS-A/Ro	Y1-Y5RNP (60 k/52 kDa, Y1-Y5RNA)	50–70%	Neonatal lupus, SCLE
Anti-SS-B/La	RNA polymerase III termination factor (48 kDa)	20–30%	Recurrent annular erythema
6. Autoantibodies in vascular diseases			
C-ANCA	Neutrophil proteinase-3	50–90%	Wegener's granulomatosis
P-ANCA	Neutrophil myeloperoxidase	30–50%	Microscopic polyangiitis, crescentic gromeluronephritis, Churg-Strauss syndrome, normotensive scleroderma kidney
Anti-phospholipid	β 2-glycoprotein I	10–20% of SLE etc	Anti-phospholipid syndrome (recurrent thrombosis, abortion)
7. Autoantibodies in rheumatoid arthritis			
Anti-calpastatin	Inhibitor of calpain (72 kDa)	45–57%	

SLE: systemic lupus erythematosus, SSc: systemic sclerosis, PM/DM: polymyositis/dermatomyositis, ARS: aminoacyl-tRNA synthetase, snRNP: small nuclear ribonucleoprotein, DNA-PK: DNA-dependent protein kinase, MCTD: mixed connective tissue disease, CNS: central nervous system, SCLE: subacute cutaneous lupus erythematosus.

are lower than the classical LE cell phenomenon. In some reports, anti-histone antibodies are thought to also act as LE factor.

LE factor is detected in 40–50% of active SLE patients and is associated with disease activity. However, LE factor is also detected in other connective tissue diseases, rheumatoid arthritis and chronic active hepatitis, although the frequency is much lower.

Anti-DNA antibodies

Anti-DNA antibodies are detected in 50–70% of SLE patients and are correlated to lupus nephritis in particular. Although low-titers of anti-DNA antibodies are sometimes detected in various connective tissue diseases, anti-DNA of high titer (>50 IU/ml) can be found only in active SLE. Since the titer of anti-DNA is closely correlated to the disease activity, the measurement is very useful to predict the lupus activity and to indicate the treatment.

Both double-strand DNA (dsDNA) and single-strand DNA (ssDNA) are targets of anti-DNA antibodies. However, only anti-dsDNA antibodies are a specific marker of SLE.

Anti-ssDNA can be widely detected in various connective tissue diseases. High-affinity anti-dsDNA antibodies are particularly associated with lupus nephritis. Anti-ssDNA and anti-dsDNA should be measured simultaneously. Since anti-dsDNA recognizes the phosphate-deoxyribose structure of the DNA molecule, anti-dsDNA can bind to both dsDNA and ssDNA.

It has been reported that anti-DNA antibodies are cross-reactive against various cellular proteins such as cell surface proteins, vimentin, A and D polypeptides of U1RNP and ribosomal S1 proteins (3, 4). This fact suggests a possibility that anti-DNA antibodies are the polyreactive autoantibodies not only to DNA but also to various antigens, and that the immunogen triggering the production of anti-DNA antibodies is not necessary for DNA itself. Moreover, it has been pointed out that the hypervariable region of anti-DNA shows homology with that of antibodies to certain bacteria, suggesting the possibility of some bacterial infection for anti-DNA production.

It has been widely accepted that circulating DNA-anti-DNA immune complexes are deposited into renal glomeruli and cause inflammation by the activation of complement. However, an alternative hypothesis is proposed in that lupus nephritis may develop by direct interaction of anti-DNA with intrinsic glomerular antigens such as heparan sulfate and laminin which are negatively charged and are cross-reactive with DNA (5, 6).

Anti-Sm antibodies

Anti-Sm antibodies, a specific marker for SLE, are detected in 15–30% of SLE patients. RNase-resistant anti-ENA antibodies are the same as anti-Sm. The target molecules of anti-Sm are U1, U2, U4/U6 and U5 small nuclear ribonucleoproteins (snRNP) that play an important role in splicing of pre-messenger RNA molecules (7). Patient sera with anti-Sm always contain antibodies to U1-RNP, probably because the U1-RNP antigen is a part of the Sm antigen and both target molecules have a common structure. Polypeptides termed B'/B,

D, E, F and G are the common protein components among U1-U6 (Sm) snRNPs. Anti-Sm antibodies recognize specifically the B'/B and D polypeptides (8).

Anti-Sm antibodies have been reported to be associated with delayed proteinuria, central nervous system involvement and a poor SLE prognosis (9).

Other SLE-specific autoantibodies

Anti-ribosomal P antibodies

Autoantibodies to ribosome recognize mainly a common epitope among P0 (38 kDa), P1 (19 kDa) and P2 (17 kDa) proteins of 60S ribosomal subunit (10), therefore termed anti-ribosomal P antibodies. Sera with anti-ribosomal P usually contain antibodies to 28S-ribosomal RNA. In immunofluorescence, nucleolus and cytoplasm are stained by these antibodies. Anti-ribosomal P are detected in 10% of SLE patients. It is noteworthy that they are associated with central nervous system involvement (11).

Anti-Ki/SL antibodies

Anti-Ki/SL antibodies target an unknown 32 kDa nuclear protein and are found in 10% of SLE patients. Although the clinical significance is still unclear, an association with sicca syndrome and myopathy have been reported. The function of the Ki/SL antigen is unknown, but a sequence homology between the Ki/SL and SV-40 large antigen has been described (12).

Anti-PCNA (proliferating cell nuclear antigen) antibodies

PCNA is an antigen that is present only in proliferating cells (late G1-S phase of cell cycle) (13). Therefore, autoantibodies to this antigen show a variegated speckled pattern on immunofluorescence, in which only some of the nuclei show positive staining. PCNA is an auxiliary protein necessary for the activation of DNA polymerase δ (14). Although anti-PCNA is found specifically in SLE, the prevalence is only 1–2% of SLE patients. Patients with anti-PCNA frequently show renal disease and thrombocytopenia.

Autoantibodies in scleroderma (systemic sclerosis: SSc)

Anti-Scl-70 (DNA topoisomerase I) antibodies

Anti-Scl-70 antibodies were first described as autoantibodies to a 70 kDa nuclear protein that were specifically found in scleroderma (15). Later, the target molecule of anti-Scl-70 was elucidated to be DNA topoisomerase I (topo I), an intranuclear enzyme that alters the topology of (or relaxes) supercoiled DNA molecules (16).

Anti-topo I are specifically found in 20–30% of SSc patients, and are closely associated with diffuse cutaneous involvement and severe pulmonary fibrosis. Respiratory failure due to lung fibrosis is the main cause of death in SSc patients with anti-topo I antibodies.

Anti-centromere antibodies

Centromere or kinetocore of chromosomes is the target of autoantibodies in SSc. Anti-centromere antibodies can be detected on immunofluorescence as a discrete speckled pattern of interphase cells and chromosome-associated staining in mitotic cells (17). The main target molecules are three centromere proteins termed CENP-A (17 kDa), CENP-B (80 kDa) and CENP-C (140 kDa) (18).

Anti-centromere antibodies are detected in 20–30% of SSc patients, but are also found in primary Raynaud's disease, Sjögren's syndrome and primary biliary cirrhosis. Anti-centromere antibodies are associated with limited cutaneous involvement or CREST syndrome and have been reported to be associated with pulmonary hypertension in Caucasian patients.

Anti-nucleolar antibodies

Anti-nuclear antibodies that show nucleolar staining on immunofluorescence are relatively specific for SSc. Recently, some target nucleolar antigens were identified.

Anti-RNA polymerase antibodies

Autoantibodies to RNA polymerase I were first described as a rare anti-nucleolar antibody system in SSc (19). Recently, it was demonstrated that these autoantibodies recognized not only RNA polymerase I, but also RNA polymerases II and III. These autoantibodies show heterogeneous reactivity against each class of polymerases such as polymerases I+II+III, polymerase II and polymerase III (20–22). These autoantibodies can be detected by only the protein-immunoprecipitation assay using ³⁵S-methionine-labeled HeLa cells, in which a characteristic set of proteins (13 polypeptides ranging from 210 k to 14 kDa) can be seen.

An ethnic background has been described. Anti-RNA polymerases are frequently found in Caucasian patients with SSc (24%) but are rare in Japanese patients (6%). However, patients with these antibodies in both ethnic groups reveal diffuse cutaneous involvement, a low prevalence of lung fibrosis and a high prevalence of renal crisis.

Anti-U3RNP (fibrillarin) antibodies

U3RNP is a complex of U3RNA and a 34 kDa protein termed "fibrillarin" that is localized in the nucleolus and is thought to be involved in the processing of ribosomal RNA (23). The RNA-immunoprecipitation assay is necessary to detect anti-U3RNP antibodies.

Autoantibodies to U3RNP are detected in 5–10% of Japanese patients with SSc. African American and Latin American patients are reported to reveal a higher reactivity (20–40%) to U3RNP. Anti-U3RNP are associated with diffuse cutaneous scleroderma but not with obvious internal organ involvements (24).

Anti-7-2/8-2RNP (Th/To) antibodies

Autoantibodies that immunoprecipitate two classes of small nucleolar RNAs, 7-2RNA (or 7SM-RNA) and 8-2RNA (or H1-RNA), are found in patients with scleroderma. These autoanti-

bodies are also called anti-Th or anti-To antibodies. 8-2RNA is RNase P that is involved in the processing of transfer RNAs (25), and 7-2RNA is identical with RNase MRP that is involved in the processing of mitochondria primer RNA (26). The 40 kDa protein, a common protein component of these ribonucleoprotein complexes is the main target of autoantibodies.

Anti-7-2/8-2RNP antibodies are detected in 5% of scleroderma patients and are correlated with limited cutaneous involvement. Some patients with only Raynaud's phenomenon may develop sclerodermatous skin changes if they have anti-7-2/8-2RNP antibodies (24).

Autoantibodies in Polymyositis/Dermatomyositis (PM/DM)

Autoantibodies to aminoacyl-tRNA synthetases

Aminoacyl-tRNA synthetases (ARS) are the enzymes that catalyze the binding of amino acids to their corresponding tRNAs under the presence of ATP. Aminoacyl-tRNA synthetases are the major targets of autoimmunity in PM/DM. Six classes of autoantibodies reacting with ARS have been recognized to date; anti-Jo-1 (histidyl), anti-PL-7 (threonyl), anti-PL-12 (alanyl), anti-EJ (glycyl), anti-OJ (isoleucyl) and anti-KS (asparaginyl). Patients with these autoantibodies reveal a similar clinical feature of polymyositis, polyarthropathy and pulmonary fibrosis (so-called "anti-synthetase syndrome") despite their different immunological specificities.

Anti-Jo-1 (histidyl-tRNA synthetase) antibodies

Anti-Jo-1 antibodies are the first well-characterized autoantibodies in PM/DM, and are the most common myositis-specific autoantibodies found in 20–30% of PM/DM patients in all ethnic and geographic populations (27, 28). Although anti-Jo-1 is detected by standard Ouchterlony double immunodiffusion, it can also be detected by immunoprecipitation assay in which sera containing anti-Jo-1 immunoprecipitate a distinct tRNA for histidine (tRNA^{His}) and a 50 kDa protein from HeLa cell extracts. The identification of the Jo-1 antigen as histidyl-tRNA synthetase was demonstrated by the evidence that anti-Jo-1 antibodies immunoprecipitated tRNA for histidine (tRNA containing anticodon for histidine) along with a 50 kDa immunoreactive protein dimer (29), and IgG from sera containing anti-Jo-1 specifically inhibited the binding of histidine to the corresponding tRNA (30).

Patients with anti-Jo-1 reveal the characteristic clinical manifestations. These features include polymyositis, chronic pulmonary fibrosis, polyarthritis, and although less frequent, Raynaud's phenomenon (28, 31, 32). Pulmonary fibrosis is likely to precede myositis in some patients, and vice versa in other patients (31). Therefore, the presence of anti-Jo-1 appears to be a predicting marker of the disease.

Other autoantibodies to aminoacyl-tRNA synthetases

Five classes of autoantibodies reacting with ARS, as well as anti-Jo-1, are identified. Although the frequency of these autoantibodies in myositis patients is less than that of anti-Jo-1

(2–5% of PM/DM respectively), patients with these autoantibodies reveal similar clinical manifestations which are found in patients with anti-Jo-1 antibodies, such as myositis, polyarthropathy and pulmonary fibrosis. These syndromes characterized by autoantibodies to ARS are designated as “anti-synthetase syndrome”. However, recent clinical studies also show that the clinical manifestations seem to be slightly different among patient groups having these autoantibodies.

Anti-PL-7 (threonyl-tRNA synthetase): Anti-PL-7 antibodies are the second anti-ARS that immunoprecipitate tRNA for threonine (tRNA^{Thr}) and a 80 kDa protein of threonyl-tRNA synthetase (33). The clinical features of patients with anti-PL-7 are quite similar to those with anti-Jo-1 (34).

Anti-PL-12 (alanyl-tRNA synthetase): Sera with anti-ARS immunoprecipitate distinct tRNAs but most sera do not recognize directly the tRNA itself. However, anti-PL-12 antibodies are an exception to this point, since such patient sera also contain antibodies that recognize directly the tRNA for alanine (tRNA^{Ala}) along with a 110 kDa protein of alanyl-tRNA synthetase (35). Although it was believed that anti-PL-12 is associated with myositis and pulmonary fibrosis (36), a recent report suggests that anti-PL-12 appears to correlate more strongly to interstitial lung disease without myositis (37).

Anti-OJ (isoleucyl-tRNA synthetase): The major target antigen of anti-OJ antibodies are isoleucyl-tRNA synthetase, but anti-OJ antibodies also recognize other synthetases. Although other autoantibodies to five classes of ARS recognize independent single proteins, only anti-OJ react with a multi-enzyme complex organized by 10 polypeptides that appear to include ARS activities for 9 amino acid systems (38).

Anti-EJ (glycyl-tRNA synthetase): Anti-EJ antibodies recognize glycyl-tRNA synthetases. Most sera containing anti-EJ immunoprecipitate a 75 kDa protein of glycyl-tRNA synthetase along with 4 associated tRNAs (39).

Anti-KS (asparaginyl-tRNA synthetase): A new class of autoantibodies to aminoacyl tRNA synthetases, termed anti-KS that recognized asparaginyl-tRNA synthetase, was recently described (40). Unlike other anti-synthetases, anti-KS antibodies were detected only in patients with idiopathic interstitial pneumonitis without myositis (40).

Autoantibodies to signal recognition particle (SRP)

Signal recognition particle (SRP) is a cytoplasmic small RNA-protein complex that consists of 7SL-RNA and 6 polypeptides of 72 k, 68 k, 54 k, 19 k, 14 k, and 9 kDa. The biological function of SRP is to recognize signal sequences in N-termini of secretory proteins or membrane proteins via binding to the 54 kDa subunit and to regulate the translocation of newly synthesized proteins across the endoplasmic reticulum membrane.

Some patients (about 5%) of PM/DM produce autoantibodies to SRP (27, 41). Anti-SRP antibodies recognize mainly the 54 kDa subunit that is involved in binding to signal sequences of newly synthesized proteins as well as the SRP receptor on the endoplasmic reticulum. However, the 72 k and 9 kDa subunits are also recognized by sera containing anti-SRP antibodies.

It is noted that patients with anti-SRP reveal severe myositis with a relatively acute onset. These patients are usually resistant to standard treatment by corticosteroids and show frequent exacerbation (41). Unlike anti-ARS, skin rashes of DM, pulmonary disease, arthritis and Raynaud's phenomenon are infrequent in patients with anti-SRP.

Anti-Mi-2 antibodies

Anti-Mi-2 antibodies are more common in DM than in PM and therefore appear to be a marker for DM. Anti-Mi-2 antibodies are detected in 8% of all myositis patients and in 15–20% of DM patients (42). Unlike other myositis-specific autoantibodies, anti-Mi-2 antibodies are also found in juvenile DM.

Several recent reports have clarified the nature of the Mi-2 antigen. Seelig et al demonstrated by molecular cloning that the target antigen was a 218 kDa nuclear protein encoded on chromosome 12 and belonged to the SNF/RAD 54 helicase family (43). They isolated a cDNA clone encoding 6.4 kb that hybridized the 6.8 kb poly(A)⁺ RNA. This cDNA encoded a polypeptide of 1,912 amino acids with a calculated molecular weight of 217,989. This cDNA had a sequence homology at the amino acid level to various helicases. Helicases are the enzymes that are thought to be involved in genomic replication, expression, repair and chromosome segregation.

Ge et al reported independently the molecular cloning of the Mi-2 antigen (44). They isolated a cDNA clone containing a 1,589 bp insert that hybridized to a single 7.5–8.0 kb mRNA of HeLa cells by Northern blot. The sequence contained four potential zinc-finger motifs and several charged regions. However, only one of five anti-Mi-2 sera reacted with the recombinant protein in vitro expressed from the cDNA, suggesting that a major common epitope might be conformational in nature. They claimed that the 240 kDa Mi-2 protein was a novel protein, and showed similarity to a sequence tag from a cDNA derived from human brain (EST03869, clone HFBDK47) of unknown function.

It has been recently identified that both cDNAs for the Mi-2 major antigen reported by the two investigators are different, but they belong to the same group of the human helicase family.

Autoantibodies in overlap syndrome

Anti-U1RNP antibodies

Antibodies to U1RNP, formerly known as anti-RNP or anti-nRNP antibodies, are widely detected in various connective tissue diseases and even in unclassified connective tissue disease. However, patients with overlapping SLE, scleroderma and PM/DM most frequently have anti-U1RNP antibodies. In particular, patients with a high titer of anti-U1RNP antibodies but not with anti-Sm are diagnosed as having mixed connective tissue disease (MCTD).

U1RNP is a complex of U1 small nuclear RNA and 9 polypeptides termed 70 K (70 kDa), A (34 kDa), B'/B (29 k/28 kDa), C (22 kDa), D (16 kDa), E (13 kDa), F (12 kDa) and G

(11 kDa), which is involved in the splicing of pre-mRNA (7). B'/B and D-G polypeptides are the common components of Sm snRNPs (U1-U6RNP). The 70 K, A and C polypeptides are the main targets of anti-U1RNP antibodies (8).

Anti-U2RNP antibodies

Autoantibodies to U2RNP that recognize U2RNP-specific A' and B'' polypeptides have been identified (45). Since the B'' polypeptide has a common amino acid sequence with the U1RNP-A polypeptide, autoantibodies reactive with the B'' epitope also recognize U1RNP molecule (46).

Both anti-U2RNP (A') and anti-U1/U2RNP (B'') antibodies are specifically found in patients with scleroderma and elevated serum myogenic enzymes (47). These autoantibodies can only be detected by RNA-immunoprecipitation assay.

Autoantibodies to Ku(p70/p80) and DNA-dependent protein kinase (DNA-PK)

Anti-Ku antibodies were first described in patients with polymyositis-scleroderma overlap syndrome in the Japanese population (48). However, the same autoantibodies were described later in the Caucasian population in patients with mainly SLE.

The target antigen of anti-Ku antibodies is a heterodimer of 70 kDa and 80 kDa proteins (p70/p80) that binds selectively to the ends of double-strand DNA (49, 50). Recently, the Ku protein was identified as a regulatory subunit of DNA-dependent protein kinase (DNA-PK) (51, 52). DNA-PK consists of an 460 kDa catalytic subunit (p460 or DNA-PKcs, formerly known as p350) and the regulatory component Ku (p70/p80), and catalyzes phosphorylation of many nuclear proteins including nuclear enzymes and transcription factors under the presence of DNA fragments. Once bound to double-strand DNA, the Ku protein creates a binding site for DNA-PKcs and assembles the DNA-PK holoenzyme. More recently, it has been demonstrated that this enzyme complex is involved in DNA repair and V(D)J recombination (53–55). It has also been suggested that the defect of the gene encoding DNA-PKcs may be a cause of SCID mouse (56, 57). The cDNA encoding for DNA-PKcs was isolated by molecular cloning, and was identified to have structural similarities to the phosphatidylinositol-3-kinase and the product of the ataxia-telangiectasia gene (58).

New autoantibodies directed against the 460 kDa DNA-PKcs have been described. Suwa et al reported autoantibodies to DNA-PKcs in 10 patients that could be detected by protein immunoprecipitation technique (59). Six out of ten sera with anti-DNA-PKcs simultaneously contained anti-Ku antibodies, suggesting that relatively intact forms of the DNA-PK complex may act as autoantigenic particles to trigger the autoimmune response in selected patients. Autoantibodies to DNA-PKcs were found mainly in patients with PM or PM-scleroderma overlap syndrome.

Anti-PM-Scl antibodies

Anti-PM-Scl antibodies, initially referred to anti-PM-1 and later separated from several specificities, are closely associated with Caucasian patients with myositis-scleroderma over-

lap syndrome (60). It has been reported that almost 50% of patients with anti-PM-Scl have myositis-scleroderma and about 25% of the patients with this syndrome have the antibodies (61). However, anti-PM-Scl antibodies are not found in Japanese patients with myositis overlap syndrome (28). One of the reasons for the ethnic discrepancy of anti-PM-Scl appears to be attributed to the genetic background. Anti-PM-Scl are strongly associated with HLA-DR3 haplotype that is found in 75–100% of patients with anti-PM-Scl but in 30% of the normal Caucasian population (61). In Japanese, HLA-DR3 is only found in less than 1% of the normal population.

The PM-Scl antigen is a nucleolar and nuclear antigen complex that consists of at least 11 polypeptides (60) or, in other study, 16 polypeptides ranging from 110 kDa to 20 kDa (62). The biological function of the PM-Scl is not known. Since the PM-Scl antigen is mainly located in the granular component of nucleoli which is known to be the site of ribosomal assembly and packaging, this antigen complex may be involved in pre-ribosomal particle assembly (60).

Autoantibodies in Sjögren's Syndrome

Anti-SS-B/La antibodies

Anti-SS-B/La antibodies are the highly specific marker autoantibodies found in 20–30% of primary Sjögren's syndrome patients. Although anti-SS-B/La can be also detected in 5–10% of SLE patients, such patients always reveal sicca symptoms (secondary Sjögren's syndrome). Recently, an association with recurrent annular erythema has been reported in patients with anti-SS-B/La antibodies (63). Patient sera with anti-SS-B/La consistently contain anti-SS-A/Ro antibodies.

The target antigen of anti-SS-B/La is a 48 kDa nuclear protein bound to various small RNAs such as tRNA precursors, ribosomal 5S-RNA precursor, 7S-RNAs and Y1-Y5RNAs (64). All of these SS-B/La-associated RNAs are the transcription products of RNA polymerase III, and therefore it has been estimated that the SS-B/La protein is the termination factor of RNA polymerase III that is involved in terminating the polymerase reaction and increasing the efficacy of transcription (65).

Anti-SS-A/Ro antibodies

Anti-SS-A/Ro antibodies are detected in 50–70% of patients with primary Sjögren's syndrome. However, since anti-SS-A/Ro can be widely found in patients with other connective tissue diseases, they are not as highly specific for Sjögren's syndrome as anti-SS-B/La antibodies. Anti-SS-A/Ro are rather important as a marker of neonatal lupus and subacute cutaneous lupus erythematosus. Neonatal babies whose mothers have anti-SS-A/Ro may develop rash, cytopenia, liver dysfunction and congenital heart block. These conditions are known as neonatal lupus syndrome, and are thought to be developed by maternal (IgG-type) anti-SS-A/Ro antibodies that pass through the placenta during pregnancy (66).

The target antigen of anti-SS-A/Ro is 60 kDa and 52 kDa proteins associated with five cytoplasmic small RNAs (Y1-Y5RNA) (67–69). Since Y-RNAs are the transcription prod-

ucts of RNA polymerase III and are bound to La/SS-B protein, at least some of SS-B/La and SS-A/Ro antigens are located on the same complex molecule via Y-RNAs. The biological function of SS-A/Ro antigen has been unknown, but recently it was proposed that the SS-A/Ro protein might be involved in the quality control or discard pathway of 5S-ribosomal RNA (70).

Autoantibodies in Vasculitis Syndrome and Vascular Diseases

Anti-neutrophil cytoplasmic antibodies (ANCA)

It was thought long time that autoantibodies were not found in patients with necrotizing vasculitides such as polyarteritis nodosa. However, once ANCAs (anti-neutrophil cytoplasmic antibodies) were discovered (71), they were recognized as marker antibodies of certain vasculitis syndromes (72). In indirect immunofluorescence using ethanol-fixed human granulocytes as antigen substrates, C-ANCA (stains whole cytoplasm) and P-ANCA (stains only perinuclear cytoplasm) are distinguished by their staining patterns.

C-ANCA (PR3-ANCA) is highly specific for Wegener's granulomatosis. C-ANCA can be detected in 50–80% of the patients with acute phase Wegener's granulomatosis, and the titer is correlated to the disease activity (73, 74). The target molecule of C-ANCA is proteinase-3 (PR-3), the 23 kDa serine proteinase localized in alpha granules of neutrophils.

P-ANCA (MPO-ANCA) is detected in patients with microscopic polyarteritis, idiopathic crescentic glomerulonephritis and, less frequently, allergic angitis and granulomatosis (Churg-Strauss syndrome) (74, 75). Recently, P-ANCA was also reported to be associated with normotensive renal crisis in SSc patients (76). Myeloperoxidase (MPO) in neutrophils is the main target molecule of P-ANCA in these vasculitides. However, atypical P-ANCAs are sometimes detected in patients with rheumatoid arthritis and inflammatory bowel diseases. In these cases, neutrophil-cytoplasmic antigens such as cathepsin-G, lactoferrin and elastase, and nuclear antigens such as HMG-1 and 2 have been reported to be their target molecules (77).

Anti-phospholipid antibodies (APA)

Anti-phospholipid antibodies (APA) are a generic term of autoantibodies that react negative-charged phospholipids such as cardiolipin and phosphatidyl serine. Lupus anticoagulant and BFP are included in the category of APA. Patients with APA frequently reveal repetitive thrombotic events of the artery and vein, such as deep vein thrombosis, cerebral infarction, pulmonary embolism and habitual abortion by placental infarction. These conditions are known as "anti-phospholipid syndrome (APS)" (78). APS is sometimes found in patients with SLE (secondary APS), but are also found in apparently "healthy" individuals (primary APS).

Recently, it was noted that a serum factor is necessary for the binding between APA and phospholipid, and that β 2-glycoprotein I (β 2-GPI) is one of the serum factors. β 2-GPI changes its conformation and the epitope is exposed when β 2-

GPI binds to phospholipids (79). Such APA is now called β 2-GPI-dependent APA.

Autoantibodies in Rheumatoid Arthritis

Anti-calpastatin antibodies

Calpastatin is an endogenous inhibitor protein of the calcium-dependent neutral proteinase, calpain. We as well as Canadian investigators described independently the presence of autoantibodies to calpastatin in patients with systemic rheumatic diseases (80, 81).

There have been several reports suggesting that calpain may be involved in activating inflammatory processes and pathogenic mechanisms of rheumatic disorders. That is; 1) calpain is increased in synovial cells and is secreted into synovial fluid of rheumatic patients, 2) calpain degrades cartilage proteoglycan, 3) calpain activates and secretes IL-1 α through processing its precursor molecules, 4) autodigestion of calpain generates an oligopeptide which acts as a chemotactic factor, and 5) calpain irreversibly activates protein kinase C, a key enzyme of signal transduction. We demonstrated that IgG from patient sera containing anti-calpastatin antibodies specifically inhibited the biological function of calpastatin and therefore increased the proteolytic activity of calpain (80). This finding supports the hypothesis that autoantibodies to calpastatin may play a role in tissue destruction and activation of inflammation through increasing calpain activity in tissues.

Anti-calpastatin antibodies were detected in 45–57% of patients with rheumatoid arthritis in both reports. However, Despres et al described that the antibodies are exclusively found in RA patients (81), whereas we found that the antibodies were also detected in 20–30% of other systemic rheumatic diseases (80). The true disease specificity and clinical significance of anti-calpastatin antibodies remain to be determined.

Etiology and Pathogenic Mechanisms of Autoantibodies

The production of disease-specific autoantibodies may closely correlate to etiopathogenic mechanisms of connective tissue diseases. Neonatal lupus syndrome may be a good example to demonstrate that autoantibodies can be pathogenic in a certain condition, although the mechanism is still unclear. Another example is anti-synthetase syndrome. Patients with antibodies to different aminoacyl tRNA synthetases (ARS) reveal the same clinical symptoms known as anti-synthetase syndrome, despite the fact that each ARS molecule is immunologically distinct. This fact may suggest that the immune response to molecules with analogous functions leads a similar clinical syndrome. However, there has been no evidence that anti-ARS autoantibodies penetrate into living cells and directly inhibit the function of ARS *in vivo*. One possible hypothesis is that several myogenic viruses might interact with ARS and that different virus-ARS complexes act as antigens to disrupt the tolerance to self-antigens. In fact, certain RNA virus genomes have tRNA-like structures and can be aminoacylated by ARS

(30). There are many findings suggesting that picornaviruses (particularly Coxsackie B virus) might be the causative agents of myositis in children (82). However, this hypothesis appears to have a disadvantage since anti-ARS are found in adults but are rarely found in child myositis.

An alternative hypothesis is that molecular mimicry between autoantigen molecules and infectious agents might produce cross-reactive autoantibodies. Many instances of molecular mimicry between myositis-related autoantigens and viral proteins have been reported (83–89). Once anti-viral antibodies cross-reacting with an autoantigen epitope are produced, B cells which have immunoglobulin receptor to the common epitope bind autoantigen molecules and act as antigen-presenting cells against autoreactive T cells. The activated autoreactive T cells in turn stimulate autoantibody-producing B cells. Thus, epitopes on autoantigen molecules will be expanding (90, 91).

References

- Hargraves MM, Richmond H, Morton R. Presentation of two bone marrow elements. The "tart" cell and the "LE" cell. *Proc Staff Meet Mayo Clin* **23**: 23–28, 1948.
- Holman H, Deicher HR. The reaction of the lupus erythematosus (L.E.) cell factor with deoxyribonucleoprotein of the cell nucleus. *J Clin Invest* **38**: 2059–2072, 1959.
- Reichlin M, Martin A, Taylor-Albert E, et al. Lupus autoantibodies to native DNA cross-react with the A and D snRNP polypeptides. *J Clin Invest* **93**: 443–449, 1994.
- Tsuzaka K, Leu AK, Frank MB, et al. Lupus autoantibodies to double stranded DNA cross-react with ribosomal protein S1. *J Immunol* **156**: 1668–1675, 1996.
- Faaber P, Rijke TPM, van de Putte LBA, 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* **77**: 1824–1830, 1986.
- Sabbaga J, Line SRP, Potocnjak P, Madaio MP. A murine nephritogenic monoclonal anti-DNA antibody binds directly to mouse laminin, the major non-collagenous protein component of the glomerular basement membrane. *Eur J Immunol* **19**: 137–143, 1989.
- 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* **76**: 5495–5499, 1979.
- Pettersson I, Hinterberger M, Mimori T, Gottlieb E, Steitz JA. The structure of mammalian small nuclear ribonucleoproteins. Identification of multiple protein components reactive with anti-(U1) ribonucleoprotein and anti-Sm autoantibodies. *J Biol Chem* **259**: 5907–5914, 1984.
- Homma M, Mimori T, Takeda Y, et al. Autoantibodies to the Sm antigen: immunological approach to clinical aspects of systemic lupus erythematosus. *J Rheumatol* **14**(Suppl 13): 188–193, 1987.
- Francoeur A-M, Peebles CL, Heckman KJ, Lee JC, Tan EM. Identification of ribosomal protein autoantigens. *J Immunol* **135**: 2378–2384, 1985.
- Bonfa E, Golombek SJ, Kaufman LD, et al. Association between lupus psychosis and anti-ribosomal P protein antibodies. *N Engl J Med* **317**: 265–271, 1987.
- Yamanaka K, Takasaki Y, Nishida Y, Shimada K, Shibata M, Hashimoto H. Detection and qualification of anti-Ki antibodies by enzyme-linked immunosorbent assay using recombinant Ki antigen. *Arthritis Rheum* **35**: 667–671, 1992.
- Miyachi K, Fritzler MJ, Tan EM, et al. Autoantibody to a nuclear antigen in proliferating cells. *J Immunol* **121**: 2228–2234, 1978.
- Prelish G, Tan CK, Kostura M, et al. Functional identity of proliferating nuclear antigen and a DNA polymerase- δ auxiliary protein. *Nature* **326**: 517–520, 1987.
- Douvas AS, Achten M, Tan EM. Identification of a nuclear protein (Scl-70) as a unique target of human antinuclear antibodies in scleroderma. *J Biol Chem* **254**: 10514–10522, 1979.
- Shero JH, Bordwell B, Rothfield NF, Earnshaw WC. High titers of autoantibodies to topoisomerase I (Scl-70) in sera from scleroderma patients. *Science* **231**: 737–740, 1986.
- Moroi Y, Peebles C, Fritzler MJ, Steigerwald J, Tan EM. Autoantibody to centromere (kinetocore) in scleroderma sera. *Proc Natl Acad Sci USA* **77**: 1627–1631, 1980.
- Earnshaw WC, Bordwell B, Marino C, Rothfield N. Three human chromosomal autoantigens are recognized by sera from patients with anticentromere antibodies. *J Clin Invest* **77**: 426–430, 1986.
- Reimer G, Rose KM, Scheer U, Tan EM. Autoantibodies to RNA polymerase I in scleroderma sera. *J Clin Invest* **79**: 65–72, 1987.
- Kuwana M, Kaburaki J, Mimori T, Tojo T, Homma M. Autoantibody reactive with three classes of RNA polymerases in sera from patients with systemic sclerosis. *J Clin Invest* **91**: 1399–1404, 1993.
- Hirakata M, Okano Y, Pati U, et al. Identification of autoantibodies to RNA polymerase II. Occurrence in systemic sclerosis and association with autoantibodies to RNA polymerases I and III. *J Clin Invest* **91**: 2665–2672, 1993.
- Okano Y, Steen VD, Medsger TA Jr. Autoantibody reactive with RNA polymerase III in systemic sclerosis. *Ann Intern Med* **119**: 1005–1013, 1993.
- Lischwe MA, Ochs RL, Reddy R, et al. Purification and partial characterization of a nucleolar scleroderma antigen (Mr=34,000; pI, 8.5) rich in N^G, N^G-dimethylarginine. *J Biol Chem* **260**: 14304–14310, 1985.
- Kuwana M, Mimori T, Hama N, Kaburaki J, Okano Y, Tojo T. Clinical significance of anti-nucleolar antibodies detected by immunoprecipitation assays in sera from patients with systemic sclerosis. *Jpn J Rheumatol* **4**: 265–275, 1993.
- Gold HA, Craft J, Hardin JA, Bartkiewicz M, Altman S. Antibodies in human serum that precipitate ribonuclease P. *Proc Natl Acad Sci USA* **85**: 5483–5487, 1988.
- Gold HA, Topper JN, Clayton DA, Craft J. The RNA processing enzyme RNase MRP is identical to the Th RNP and related to RNase P. *Science* **245**: 1377–1380, 1989.
- Nishikai M, Reichlin M. Heterogeneity of precipitating antibodies in polymyositis and dermatomyositis. Characterization of the Jo-1 antibody system. *Arthritis Rheum* **23**: 881–888, 1980.
- Hirakata M, Mimori T, Akizuki M, Craft J, Hardin JA, Homma M. Autoantibodies to small nuclear and cytoplasmic ribonucleoproteins in Japanese patients with inflammatory muscle disease. *Arthritis Rheum* **35**: 449–456, 1992.
- Rosa MD, Hendrick JP Jr, Lerner MR, Steitz JA, Reichlin M. A mammalian tRNA^{His}-containing antigen is recognized by the polymyositis-specific antibody anti-Jo-1. *Nucleic Acid Res* **11**: 853–870, 1983.
- Mathews MB, Bernstein RM. Myositis autoantibody inhibits histidyl-tRNA synthetase: a model for autoimmunity. *Nature* **304**: 177–179, 1983.
- Yoshida S, Akizuki M, Mimori T, Yamagata H, Inada S, Homma M. The precipitating antibody to an acidic nuclear protein antigen, the Jo-1, in connective tissue diseases. A marker for a subset of polymyositis with interstitial pulmonary fibrosis. *Arthritis Rheum* **26**: 604–611, 1983.
- Oddis CV, Medsger TA Jr, Cooperstein LA. A subluxing arthropathy associated with the anti-Jo-1 antibody in polymyositis/dermatomyositis. *Arthritis Rheum* **33**: 1640–1645, 1990.
- Mathews MB, Reichlin M, Hughes GRV, Bernstein RM. Anti-threonyl-tRNA synthetase, a second myositis-related autoantibody. *J Exp Med* **160**: 420–434, 1984.
- Targoff IN, Arnett FC, Reichlin M. Antibody to threonyl-transfer RNA synthetase in myositis sera. *Arthritis Rheum* **31**: 515–524, 1988.
- Bunn CC, Bernstein RM, Mathews MB. Autoantibodies against alanyl-tRNA synthetase and tRNA^{Ala} coexist and are associated with myositis. *J Exp Med* **163**: 1281–1291, 1986.
- Targoff IN, Arnett FC. Clinical manifestations in patients with antibody to PL-12 antigen (alanyl-tRNA synthetase). *Am J Med* **88**: 241–251, 1990.
- Hirakata M, Nakamura K, Okano Y, et al. Anti-alanyl tRNA synthetase

- (PL-12) antibodies are associated with interstitial lung diseases in Japanese patients. *Arthritis Rheum* **38**: S321, 1995 (Abstract).
- 38) Targoff IN, Trieu EP, Miller FW. Reaction of anti-OJ autoantibodies with components of the multi-enzyme complex of aminoacyl-tRNA synthetases in addition to isoleucyl-tRNA synthetase. *J Clin Invest* **91**: 2556–2564, 1993.
- 39) Targoff IN, Trieu EP, Plotz PH, Miller FW. Antibodies to glycyl-transfer RNA synthetase in patients with myositis and interstitial lung disease. *Arthritis Rheum* **35**: 821–830, 1992.
- 40) Hirakata M, Suwa A, Nagai S, et al. Anti-KS. Identification of autoantibodies to asparaginyl-transfer RNA synthetase associated with interstitial lung disease. *J Immunol* **162**: 2315–2320, 1999.
- 41) Targoff IN, Johnson AE, Miller FW. Antibody to signal recognition particle in polymyositis. *Arthritis Rheum* **33**: 1361–1370, 1990.
- 42) Targoff IN, Reichlin M. The association between Mi-2 antibodies and dermatomyositis. *Arthritis Rheum* **28**: 796–803, 1985.
- 43) Seelig HP, Moosbrugger I, Ehrfeld H, Fink T, Renz M, Genth E. The major dermatomyositis-specific Mi-2 autoantigen is a presumed helicase involved in transcriptional activation. *Arthritis Rheum* **38**: 1389–1399, 1995.
- 44) Ge Q, Nilasena DS, O'Brien CA, Frank MB, Targoff IN. Molecular analysis of a major antigenic region of the 240-kD protein of Mi-2 autoantigen. *J Clin Invest* **96**: 1730–1737, 1995.
- 45) Mimori T, Hinterberger M, Pettersson I, Steitz JA. Autoantibodies to the U2 small nuclear ribonucleoprotein in a patient with scleroderma-polymyositis overlap syndrome. *J Biol Chem* **259**: 560–565, 1984.
- 46) Habets WJ, Sillescu PTG, Hoet MH, McAllister G, Lerner MR, van Venrooij WJ. Small nuclear RNA-associated proteins are immunologically related as revealed by mapping of autoimmune reactive B-cell epitopes. *Proc Natl Acad Sci USA* **86**: 4674–4678, 1989.
- 47) Craft J, Mimori T, Olsen TL, Hardin JA. The U2 small nuclear ribonucleoprotein particle as an autoantigen. Analysis with sera from patients with overlap syndromes. *J Clin Invest* **81**: 1716–1724, 1987.
- 48) Mimori T, Akizuki M, Yamagata H, Inada S, Yoshida S, Homma M. Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. *J Clin Invest* **68**: 611–620, 1981.
- 49) Mimori T, Hardin JA, Steitz JA. Characterization of the DNA-binding protein antigen Ku recognized by autoantibodies from patients with rheumatic disorders. *J Biol Chem* **261**: 2274–2278, 1986.
- 50) Mimori T, Hardin JA. Mechanism of interaction between Ku protein and DNA. *J Biol Chem* **261**: 10375–10379, 1986.
- 51) Dvir A, Peterson SR, Knuth MW, Lu H, Dynan WS. Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II. *Proc Natl Acad Sci USA* **89**: 11920–11924, 1992.
- 52) Gottlieb TM, Jackson SP. The DNA-dependent protein kinase. Requirement for DNA ends and association with Ku antigen. *Cell* **72**: 131–142, 1993.
- 53) Rathmell WK, Chu G. Involvement of the Ku autoantigen in the cellular response to DNA double-strand breaks. *Proc Natl Acad Sci USA* **91**: 7623–7627, 1994.
- 54) Taccioli GE, Gottlieb TM, Blunt T, et al. Ku 80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. *Science* **265**: 1442–1445, 1994.
- 55) Smider V, Rathmell WK, Lieber MR, Chu G. Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. *Science* **266**: 288–291, 1994.
- 56) Kirchgessner CU, Patil CK, Evans JW, et al. DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science* **267**: 1178–1183, 1995.
- 57) Blunt T, Finnie NJ, Taccioli GE, et al. Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. *Cell* **80**: 813–823, 1995.
- 58) Hartley KO, Gell D, Smith GCM, et al. DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. *Cell* **82**: 849–856, 1995.
- 59) Suwa A, Hirakata M, Takeda Y, et al. Autoantibodies to DNA-dependent protein kinase. Probes for the catalytic subunit. *J Clin Invest* **97**: 1417–1421, 1996.
- 60) Reimer G, Scheer U, Peters JM, Tan EM. Immunolocalization and partial characterization of a nucleolar autoantigen (PM-Scl) associated with polymyositis/scleroderma overlap syndromes. *J Immunol* **137**: 3802–3808, 1986.
- 61) Oddis CV, Okano Y, Rudert WA, Trucco M, Duquesnoy RJ, Medsger TA Jr. Serum autoantibody to the nucleolar antigen PM-Scl. Clinical and immunogenetic associations. *Arthritis Rheum* **35**: 1211–1217, 1992.
- 62) Gelpi C, Alguero A, Angeles Martinez M, Vidal S, Juarez C, Rodriguez-Sanchez JL. Identification of protein components reactive with anti-PM-Scl autoantibodies. *Clin Exp Immunol* **81**: 59–64, 1990.
- 63) Hoshino Y, Hashimoto T, Mimori T, Akizuki M, Nishikawa T. Recurrent annular erythema associated with anti-SS-B/La antibodies. Analysis of the disease-specific epitope. *Br J Dermatol* **127**: 608–613, 1992.
- 64) Rinke J, Steitz JA. Precursor molecules of both human 5S ribosomal RNA and transfer RNAs are bound by a cellular protein reactive with anti-La lupus antibodies. *Cell* **29**: 149–159, 1982.
- 65) Gottlieb E, Steitz JA. Function of the mammalian La protein. Evidence for its action in transcription termination by RNA polymerase III. *EMBO J* **8**: 851–861, 1989.
- 66) Scott JS, Maddison PJ, Taylor PV, Esscher E, Scott O, Skinner RP. Connective tissue disease, antibodies to ribonucleoprotein, and congenital heart block. *New Engl J Med* **309**: 209–212, 1983.
- 67) Wolin SL, Steitz JA. The Ro small cytoplasmic ribonucleoproteins. Identification of the antigenic protein and its binding site on the Ro RNAs. *Proc Natl Acad Sci USA* **81**: 1996–2000, 1984.
- 68) Ben-Chetrit E, Chan EKL, Sullivan KF, Tan EM. A 52-kD protein is a novel component of the SS-A/Ro antigenic particle. *J Exp Med* **167**: 1560–1571, 1988.
- 69) Lerner MR, Boyle JA, Hardin JA, Steitz JA. Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science* **211**: 400–402, 1981.
- 70) O'Brien CA, Wolin SL. A possible role for the 60-kD Ro autoantigen in a discard pathway for defective 5S rRNA precursors. *Genes & Development* **8**: 2891–2903, 1994.
- 71) Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotizing glomerulonephritis with antineutrophil antibody. Possible arbovirus aetiology? *Br Med J (Clin Res Ed)* **285**: 606, 1982.
- 72) Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* **37**: 187–192, 1994.
- 73) Cohen TJW, van der Woude FJ, Fauci AS, et al. Association between active Wegener's granulomatosis and anticytoplasmic antibodies. *Arch Intern Med* **149**: 2461–2465, 1989.
- 74) Gross WL. Antineutrophil cytoplasmic autoantibody testing in vasculitides. *Rheum Dis Clin North Am* **21**: 987–1011, 1995.
- 75) Falk RJ, Jennette CJ. Antineutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med* **318**: 1651–1657, 1988.
- 76) Endo H, Hosono T, Kondo H. Antineutrophil cytoplasmic autoantibodies in 6 patients with renal failure and systemic sclerosis. *J Rheumatol* **21**: 864–870, 1994.
- 77) Sobajima J, Ozaki S, Osakada F, et al. Novel autoantigens of perinuclear anti-neutrophil cytoplasmic antibodies (P-ANCA) in ulcerative colitis. Non-histone chromosomal proteins, HMG1 and HMG2. *Clin Exp Immunol* **107**: 135–140, 1997.
- 78) Hughes GR, Harris NN, Gharavi AE. The anticardiolipin syndrome. *J Rheumatol* **13**: 486–489, 1986.
- 79) Matsuura E, Igarashi Y, Yasuda T, Triplett DA, Koide T. Anticardiolipin antibodies recognize β 2-glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. *J Exp Med* **179**: 457–462, 1994.
- 80) Mimori T, Suganuma K, Tanami Y, et al. Autoantibodies to calpastatin (an endogenous inhibitor for calcium-dependent neutral protease, calpain)

- in systemic rheumatic diseases. *Proc Natl Acad Sci USA* **92**: 7267–7271, 1995.
- 81) Despres N, Talbot G, Plouffe B, Boire G, Menard HA. Detection and expression of a cDNA clone that encodes a polypeptide containing two inhibitory domains of human calpastatin and its recognition by rheumatoid arthritis sera. *J Clin Invest* **95**: 1891–1896, 1995.
 - 82) Christensen ML, Pachman LM, Schneiderman R, Patel DC, Friedman JM. Prevalence of Coxsackie B virus antibodies in patients with juvenile dermatomyositis. *Arthritis Rheum* **29**: 1365–1370, 1986.
 - 83) Query CC, Keene JD. A human autoimmune protein associated with U1 RNA contains a region of homology that is cross-reactive with retroviral p30^{gag} antigen. *Cell* **51**: 211–220, 1987.
 - 84) Maul GG, Jimenez SA, Riggs E, Ziemnicka-Kotula D. Determination of an epitope of the diffuse systemic sclerosis marker antigen DNA topoisomerase I. Sequence similarity with retroviral p30^{gag} protein suggests a possible cause for autoimmunity in systemic sclerosis. *Proc Natl Acad Sci USA* **86**: 8492–8496, 1989.
 - 85) Reeves WH, Stoecker ZM. Molecular cloning of cDNA encoding the p70 (Ku) lupus autoantigen. *J Biol Chem* **264**: 5047–5052, 1989.
 - 86) Kohsaka H, Yamamoto K, Fujii H, et al. Fine epitope mapping of the human SS-B/La protein. Identification of a distinct autoepitope homologous to a viral gag polyprotein. *J Clin Invest* **85**: 1566–1574, 1990.
 - 87) Walker EJ, Jeffrey PD. Polymyositis and molecular mimicry, a mechanism of autoimmunity. *Lancet* **2**: 605–607, 1986.
 - 88) Walker EJ, Jeffrey PD. Sequence homology between encephalomyocarditis virus protein VP1 and histidyl-tRNA synthetase supports a hypothesis of molecular mimicry in polymyositis. *Med Hypotheses* **25**: 21–25, 1988.
 - 89) Ge Q, Trieu EP, Targoff IN. Primary structure and functional expression of human glycyl-tRNA synthetase, an autoantigen in myositis. *J Biol Chem* **269**: 28790–28797, 1994.
 - 90) Fatenejad S, Mamula MJ, Craft J. Role of intermolecular/intrastructural B- and T-cell determinants in the diversification of autoantibodies to ribonucleoprotein particles. *Proc Natl Acad Sci USA* **90**: 12010–12014, 1993.
 - 91) Dong X, Hamilton KJ, Satoh M, Wang J, Reeves WH. Initiation of autoimmunity to the p53 tumor suppressor protein by complexes of p53 and SV40 large T antigen. *J Exp Med* **179**: 1243–1252, 1994.
-