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

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Internal Medicine Vol. 38, No. 7 (July 1999) 523
Table 1. Autoantibodies in Connective Tissue Diseases and Their Clinical Significance

<table>
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<tr>
<th>Antibodies</th>
<th>Nature of target antigens</th>
<th>Frequency %</th>
<th>Clinical significance</th>
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<tr>
<td>1. Autoantibodies in SLE</td>
<td></td>
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</tr>
<tr>
<td>LE factor</td>
<td>DNA-histone complex, histone</td>
<td>40–50%</td>
<td>Active SLE, drug-induced lupus</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>dsDNA</td>
<td>50–70%</td>
<td>Lupus nephritis, active SLE</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>U1, U2, U4/U6, U5-snRNP (mRNA-splicing factor)</td>
<td>15–30%</td>
<td>CNS lupus, delayed proteinuria</td>
</tr>
<tr>
<td>Anti-ribosomal P</td>
<td>P0, P1, P2 (60S subunit)</td>
<td>10%</td>
<td>CNS lupus</td>
</tr>
<tr>
<td>Anti-Ki/SL</td>
<td>34 kDa nuclear protein</td>
<td>10%</td>
<td>Sicca syndrome, myopathy</td>
</tr>
<tr>
<td>Anti-PCNA</td>
<td>DNA polymerase δ auxiliary protein</td>
<td>&lt;5%</td>
<td>Thrombocytopenia, nephropathy</td>
</tr>
<tr>
<td>2. Autoantibodies in SSc</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Anti-Scl-70 (Topo I)</td>
<td>DNA topoisomerase I</td>
<td>20–30%</td>
<td>Diffuse scleroderma</td>
</tr>
<tr>
<td>Anti-centromere</td>
<td>Centromere proteins A, B, C</td>
<td>20–30%</td>
<td>Limited scleroderma</td>
</tr>
<tr>
<td>Anti-U3RNP</td>
<td>U3RNP (32 kDa fibrillarin)</td>
<td>5–10%</td>
<td>Diffuse scleroderma</td>
</tr>
<tr>
<td>Anti-7-2/8-2RNP(Th/To)</td>
<td>RNase P &amp; RNase MRP</td>
<td>5–10%</td>
<td>Limited scleroderma</td>
</tr>
<tr>
<td>Anti-RNA polymerases</td>
<td>RNA polymerase I, II, III</td>
<td>6–24%</td>
<td>Diffuse scleroderma, renal crisis</td>
</tr>
<tr>
<td>3. Autoantibodies in PM/DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-ARS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Jo-1</td>
<td>Hystidyl-tRNA synthetase (50 kDa)</td>
<td>20–30%</td>
<td>Anti-synthetase syndrome</td>
</tr>
<tr>
<td>Anti-PL-7</td>
<td>Threonyl-tRNA synthetase (80 kDa)</td>
<td>&lt;5%</td>
<td>(pulmonary fibrosis, polyarthritis, inflammatory myopathy)</td>
</tr>
<tr>
<td>Anti-PL-12</td>
<td>Alanyl-tRNA synthetase (110 kDa)</td>
<td>&lt;5%</td>
<td></td>
</tr>
<tr>
<td>Anti-EJ</td>
<td>Glycyl-tRNA synthetase (75 kDa)</td>
<td>&lt;5%</td>
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<tr>
<td>Anti-OJ</td>
<td>Isoleucyl-tRNA synthetase (multi-enzyme complex)</td>
<td>&lt;5%</td>
<td></td>
</tr>
<tr>
<td>Anti-AS</td>
<td>Asparaginyl-tRNA synthetase</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Anti-SRP</td>
<td>Signal recognition particle</td>
<td>5%</td>
<td>Severe, refractory PM</td>
</tr>
<tr>
<td>Anti-Mi-2</td>
<td>helicase family protein (218 k/240 kDa)</td>
<td>5–10%</td>
<td>DM</td>
</tr>
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<td>4. Autoantibodies in overlap syndrome</td>
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<td></td>
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<tr>
<td>Anti-U1RNP</td>
<td>U1-snRNP (mRNA splicing factor)</td>
<td>100%</td>
<td>MCTD, non-renal SLE, Raynaud’s phenomenon</td>
</tr>
<tr>
<td>Anti-U2RNP</td>
<td>U2-snRNP (mRNA splicing factor)</td>
<td>10–20%</td>
<td>SSc-PM overlap</td>
</tr>
<tr>
<td>Anti-Ku</td>
<td>DNA-PK regulatory subunit (70 k/80 kDa)</td>
<td>20–30%</td>
<td>SSc-PM overlap in Japanese</td>
</tr>
<tr>
<td>Anti-DNA-PKcs</td>
<td>DNA-PK catalytic subunit (460 kDa)</td>
<td>&lt;5%</td>
<td>PM-overlap</td>
</tr>
<tr>
<td>Anti-PM-Scl</td>
<td>Nucleolar protein complex (110–20 kDa)</td>
<td>8–10%</td>
<td>SSc-PM overlap in Caucasian</td>
</tr>
<tr>
<td>5. Autoantibodies in Sjögren’s syndrome</td>
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<td></td>
</tr>
<tr>
<td>Anti-SS-A/Ro</td>
<td>Y1-Y5RNP (60 k/52 kDa, Y1-Y5RNA)</td>
<td>50–70%</td>
<td>Neonatal lupus, SCLE</td>
</tr>
<tr>
<td>Anti-SS-B/La</td>
<td>RNA polymerase III termination factor (48 kDa)</td>
<td>20–30%</td>
<td>Recurrent anular erythema</td>
</tr>
<tr>
<td>6. Autoantibodies in vascular diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-ANCA</td>
<td>Neutrophil proteinase-3</td>
<td>50–90%</td>
<td>Wegener’s granulomatosis</td>
</tr>
<tr>
<td>P-ANCA</td>
<td>Neutrophil myeloperoxidase</td>
<td>30–50%</td>
<td>Microscopic polyangitis, crescentic gromeluronephritis, Churg-Strauss syndrome, normotensive scleroderma kidney</td>
</tr>
<tr>
<td>Anti-phospholipid</td>
<td>β2-glycoprotein I</td>
<td>10–20%</td>
<td>Anti-phospholipid syndrome (recurrent thrombosis, abortion)</td>
</tr>
<tr>
<td>Anti-calpastatin</td>
<td>Inhibitor of calpain (72 kDa)</td>
<td>45–57%</td>
<td></td>
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</table>

Autoantibodies in Collagen Diseases

Anti-DNA antibodies

Anti-DNA antibodies are detected in 50–70% of SLE patients and are correlated with 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 trigger the production of anti-DNA antibodies. Since the presence of antibodies to certain bacteria, suggesting the possibility of some bacterial infection for anti-DNA production.

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 proteinurina, central nervous system involvement and a poor SLE prognosis (9).

Other SLE-specific autoantibodies

Anti-riboosomal 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-riboosomal P antibodies. Sera with anti-riboosomal P usually contain antibodies to 28S-riboosomal RNA. In immunofluorescence, nucleolus and cytoplasm are stained by these antibodies. Anti-riboosomal 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-nuclear 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-nuclear 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 autoantibodies 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 (glyclyl), anti-OJ (isoleucyl) and anti-KS (asparaginyl). Patients with these autoantibodies reveal a similar clinical feature of polymyositis, polymyopathy 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 (tRNAHis) 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 the Jo-1 antigen (29) and a 50 kDa protein from HeLa cell extracts. The immunoprecipitation assay using 35S-methionine-labeled HeLa cells, in which sera containing anti-Jo-1 specifically inhibited the binding of histidine to the corresponding tRNA (29).

 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, polyarthritis, and pulmonary fibrosis. These syndromes are characterized by autoantibodies to ARS are designated as “anti-SRP antibodies”. 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 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 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 synthetase. 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 HFBSDK47) 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), B7/B (29 k/28 kDa), C (22 kDa), D (16 kDa), E (13 kDa), F (12 kDa) and G...
Anti-Ku antibodies were first described in patients with poly-
later separated from several specificities, are closely associ-
ted 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 Japa-
nese 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 nucleolus 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 au-
toantibodies 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 pro-
tein 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 es-
timated that the SS-B/La protein is the termination factor of RNA polymerase III that is involved in terminating the poly-
merase 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 syn-
drome 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 neo-
natal lupus syndrome, and are thought to be developed by ma-
ternal (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 SS-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 nécrotizing vasculitides such as polyarteritis nodosa. However, once ANCA (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 angiitis 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 β2-glycoprotein I (β2-GPI) is one of the serum factors. β2-GPI changes its conformation and the epitope is exposed when β2-GPI binds to phospholipids (79). Such APA is now called β2-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-1α 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-antigen. 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

Autoantibodies in Collagen Diseases


80) Mimori T, Suganuma K, Tanari Y, et al. Autoantibodies to calpastatin (an endogenous inhibitor for calcium-dependent neutral protease, calpain)


