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Interactions between Autoimmunity and Molecular and Cell Biology

Bridges between Clinical and Basic Sciences

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A group of systemic autoimmune diseases, including systemic lupus erythematosus (SLE),[1] scleroderma, Sjogren's syndrome, dermatomyositis/polymyositis, and mixed connective tissue disease (MCTD), are characterized by a distinctive immune response which is manifested as the spontaneous production of autoantibodies that react with intracellular proteins and nucleic acids (reviewed in reference 1). A finding that has had an important impact on clinical medicine has been the demonstration that each of the diseases described above has its own individual set of autoantibodies, so that autoantibody profiles have been useful in differential diagnosis. In molecular and cell biology, autoantibodies have been used to clarify the molecular structure of intracellular molecules and their role in the biological function of important intracellular processes. The interactions between clinical and basic sciences in this field of research have been especially active in the past decade and are a good example of bridges being built between these disciplines.

Most of the earlier studies on autoantibodies focused on those reactive with DNA and nucleoproteins, the latter now recognized as the histone components of deoxyribonucleoprotein. These studies demonstrated the important role of autoantibody to DNA in the pathogenesis of tissue inflammation via immune complex formation (2, 3) and stimulated continued investigations into the relationship of autoantibodies with disease mechanisms. A nonhistone nuclear protein antigen called Sm was defined in 1966 by immunoprecipitation in agarose gel double diffusion analysis (4). The Sm antigen was shown to be a protein that was nuclear in location and antibodies were found to be highly specific for SLE. Shortly thereafter, another nuclear antigen was identified with antibodies present in sera of patients with lupus and MCTD, and since immunological studies showed that integrity of both RNA and protein was required for antigenicity, the antigen was designated nuclear ribonucleoprotein (RNP) (5, 6). In addition to cell fractionation experiments, immunofluorescence helped to confirm the nuclear location of both antigens. When isolated from homogenized cell preparations, the two antigens were shown to be in close physical association, with the nuclear RNP antigen appearing to be always coisolated with Sm, but with a fraction of Sm that could be isolated free of nuclear RNP antigen (5–7).

Autoantibodies in molecular and cell biology

It is of interest but perhaps coincidental that the molecular structures of these two antigens were the first nuclear nonhistone protein antigens to be defined (Table I). Lerner and Steitz (8) showed that the Sm and nuclear RNP antigens were RNP particles that were complexes of small nuclear RNAs, called uridylate-rich (U) RNAs, and different proteins. These RNA-protein particles have been called small nuclear RNPs (snRNPs) and they comprise a large family, but of relevance to autoimmunity are the U1 to U6 snRNPs. The U RNA components of snRNPs are small RNAs and, with the exception of U6, have distinctive 5'-termini consisting of trimethyl guanosine cap structures (for review see reference 9). What were defined immunologically as the Sm antigens are four core proteins of snRNP particles with molecular masses of 29 (B'), 28 (B), 16 (D), and 13 (E) kD and perhaps two smaller proteins of 12 and 11 kD. These core proteins are intrinsic components of U1, U2, U4, U5, and U6 snRNPs and are the reason why antibody of the Sm specificity coprecipitates the associated U RNAs together with the core proteins. On the other hand, the antibody from lupus and MCTD, which had been designated antinuclear RNP, recognizes two proteins of 33 (A) and 22 (C) kD, which are distinctive components of U1 RNP and are not present in other U RNP species. Hence, antibody of nuclear RNP specificity only coprecipitates U1 RNA but not U2 or other RNAs. It is seen from Table I that anti–U1 RNP is also reactive with a 70-kD protein that has been shown to be associated with the nuclear matrix (10) and is therefore not an intrinsic protein of snRNPs.

It can be seen from this brief discussion that autoantibodies of anti-Sm and anti–nuclear RNP specificities have been instrumental in elucidating the molecular structure of snRNP particles, especially the core protein molecules of all particles and distinctive proteins associated with individual species. In addition, autoantibodies have been used as invaluable reagents to probe the function of snRNPs and these studies have led to important advances in the understanding of precursor RNA (pre mRNA) splicing. It was noticed that there was striking base pairing capability between the 5' end of U1 RNA and intron splice junctions, leading to the proposal that U1 snRNP could be playing a role in this process (11, 12). The stepwise

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1. *Abbreviations used in this paper:* CREST, calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia; MCTD, mixed connective tissue disease; PCNA, proliferating cell nuclear antigen; pre mRNA, precursor mRNA; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; snRNP, small nuclear RNP; U, uridylate-rich.

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Table I. Autoantibodies That Have Contributed to Knowledge of Cell Function

Autoantibody designation and disease association	Molecular structure of antigens	Function of antigens
Sm in SLE	Proteins 29-(B'), 28-(B), 16-(D), and 13-(E)kD core proteins of U1, U2, U4, U5, and U6 snRNPs	Splicing of precursor mRNA
U1-RNP in SLE and MCTD	Proteins 33(A), 22(C) kD-unique proteins of U1 snRNP; protein 70-kD associated with U1 snRNP	Splicing of precursor mRNA
SS-B/La in Sjogren's syndrome and SLE	Protein 48-kD	Associates with pol III transcription products; involved in termination of pol III transcription
PCNA in SLE	Protein 36-kD; auxiliary protein of DNA polymerase δ	DNA replication and repair
Centromere antigens in scleroderma	Proteins 140-, 80-, and 17-kD associated with centromeric DNA	Cell mitosis and spindle formation

involvement of different snRNP species in pre mRNA splicing has been the subject of intensive investigation and there are many excellent reviews of the subject (13–16). One interesting example of the utility of anti–Sm autoantibody was the demonstration of snRNA analogues in the yeast, *Saccharomyces cerevisiae* (17). The Sm antigens (core proteins) have been shown to bind to a conserved RNA motif that includes the sequence $A(U_{3-6})G$. Anti–Sm antibody is incapable of precipitating snRNAs in *S. cerevisiae* since Sm-like proteins appear to be absent in this organism. However, when labeled yeast snRNAs were microinjected into *Xenopus* eggs or oocytes, two snRNAs from *S. cerevisiae* became strongly immunoprecipitable with anti-Sm. These RNAs each contained the sequence $A(U_{5-6})G$ and were constituents of the spliceosome. It is known that *Xenopus* eggs or oocytes contain Sm-like proteins, and the microinjected yeast snRNAs carrying the conserved motif had complexed with *Xenopus* Sm and were rendered precipitable with autoantibody.

Brief mention will be made of other autoantibodies that have been used in elucidating important aspects of cell biology (Table I). SS-B/La is a ubiquitous nuclear protein of 48 kD which is an antigen recognized by autoantibodies in sera of patients with SLE and Sjogren's syndrome (18, 19). The antigen SS-B was shown to be transiently associated with RNA polymerase III transcripts and it was presumed to have a role in the maturation of certain precursor RNAs, including tRNA, 5S RNA, 4.5S RNA, and 7S RNA (20–22). Recently it was demonstrated that SS-B/La was required for termination of RNA polymerase III transcription (23). However, questions that remain to be elucidated include reasons for its close physical association with the SS-A/Ro antigen (see reference 1) and what role the latter contributes to the process.

Proliferating cell nuclear antigen (PCNA) is a nuclear protein of 36 kD that was first identified with autoantibody present in ~ 3% of lupus patients (24). A unique feature observed in the first study was that immunolocalization of the antigen in cell nuclei varied in intensity and configuration, and this was shown to be related to the proliferative state of the cell, with actively proliferating cells containing antigen detectable by immunofluorescence in contrast to weak or negative immunofluorescence in quiescent cells. There is a recent comprehensive review of this subject (25) that describes the sequence of studies showing the identity of PCNA with cyclin (26) and the auxiliary protein of DNA polymerase delta (27). Autoantibody to PCNA has been a key reagent in elucidating the mechanism of DNA replication regulated by DNA polymerase delta. In SV40 replication, PCNA is required for leading strand (continuous) but not for lagging strand (discontinuous) DNA synthesis (28). This effect is reported to be due to PCNA reversing an inhibitor that blocks the elongation reaction of SV40 DNA replication (29). When autoantibody was microinjected into eggs of *Xenopus laevis*, the relative contributions of DNA polymerases α and δ to replication of intrinsic egg chromosomes or injected plasmid pUC9 DNA could be analyzed (30). With the convergence of technologies and interests in the fields of immunology, biochemistry, and molecular and cell biology, it has been stated that the study of PCNA may serve as a paradigm for proteins that participate in replication or cell cycle events (25).

Biologists engaged in the study of cell events related to mitosis were highly interested in the identification of autoantibodies to centromeric proteins in the sera of patients with the CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) variant of scleroderma (31). These autoantibodies reacted with proteins of 17, 80, and 140 kD (32–34) that were tightly associated with centromeric DNA and were components of the kinetochore, a trilaminar structure demonstrable by electron microscopy and to which spindle fibers attach during daughter chromatid separation at mitosis. Kinetochore structures that had not been detected previously in the cell nucleus during interphase could now be easily identified with antibodies, and their existence in the interphase nucleus was established (31, 35). It was also demonstrated that duplication of kinetochores during mitosis could be pinpointed to G2, the cell-cycle phase after DNA replication (35). As in the other studies cited above, autoantibodies to centromere/kinetochore antigens have provided another dimension to research in cell biology and recently have been used to elucidate mechanisms associated with mitosis in unreplicated genomes (36).

Cloning of DNAs encoding autoantigens

Human autoantibodies have been used very effectively to clone DNAs encoding autoantigens. In the majority of instances, sera containing antibodies in high titer have been used to screen cDNA expression libraries. In other instances, affinity-purified protein antigens were isolated and sequenced, and synthetic oligonucleotides deduced from these sequences were used in hybridization screening of cDNA or genomic libraries. For the Sm-related antigens, cloned DNAs include those encoding the A (33 kD) (37), B' (28.5 kD) (38), C (22 kD) (39, 40), D (16 kD) (41), and E (13 kD) proteins (42). The 70-kD

protein which is associated with U1 RNP and detected by antibodies from patients with MCTD has been cloned by several groups (43–46). Full-length cDNA clones of the 60-kD protein of SS-A/Ro (47, 48) and the 48-kD protein of SS-B/La (49) as well as a genomic clone of the latter (50) have been reported recently.

A rat cDNA clone encoding PCNA (51) was isolated by screening with synthetic oligonucleotides deduced from the published sequence of an amino-terminal fragment of human PCNA (52). Human cDNA clones for PCNA (53) have also been isolated, and more recently a human genomic DNA clone has been reported and a 5′ flanking sequence with promotor activity identified (54). Less than full-length cDNA clones for the 80-kD protein of CREST antigens (centromere protein B) were isolated, and the longest of these clones encoding for ~ 95% of the protein appears to contain most of the antigenic determinants recognized by autoantibodies (55). Antibodies in other autoimmune disease conditions have been used to clone cDNAs. They include the pyruvate dehydrogenase antigen of primary biliary cirrhosis (56, 57) and cytochrome P450 dbl (58), an antigen in a subset of autoimmune liver disease with anti–liver-kidney microsomal antibodies. All these cDNA and genomic clones are being scrutinized for several types of information, including sequences that might encode protein epitopes recognized by autoantibodies, nucleotide sequences that are known to occur in other DNA and RNA binding proteins, and regions of homology with other proteins, including those of viruses.

Contributions of molecular and cell biology to autoimmunity

In the early days, knowledge of the humoral response in autoimmune diseases consisted primarily of recognition of the autoantigens on the basis of their reaction with autoantibodies and their locations within the cell. This was somewhat like a team of players whose members were identified by names and addresses alone. The new data provide information on the physical characteristics (structure) of the players and the positions they are capable of playing (function). From this new information it might be possible to deduce certain hypotheses concerning what the different groups of autoantigens might be doing in the autoimmune process.

The immunogen is a subcellular particle

It is not possible to describe in detail here the great diversity of autoantibodies that have been identified and the corresponding antigens that have been characterized (see reference 1 for review), but certain distinctive features need to be emphasized. Certain autoantibodies are highly specific for a particular disease and they include anti-Sm and anti–native DNA for lupus, anti-tRNA^{His,Thr,Ala} synthetases for polymyositis, anti–Scl-70 for diffuse scleroderma, and anti–centromere/kinetochore for CREST. Antibodies of these specificities are rarely detected in other diseases. In addition to individual autoantibody specificity, there is group specificity which is manifested as autoantibodies that tend to be associated in unusual frequency with each other, and these antibody groupings can be recognized as distinct antibody profiles. In lupus, these profiles include the common association of anti-Sm with anti–U1 RNP, whereas in MCTD anti–U1 RNP is present without anti-Sm. In Sjögren's syndrome, anti–SS-A/Ro and anti–SS-B/La are present together without other recognizable autoantibodies, whereas these autoantibodies are also present in lupus but frequently in association with other antibodies such as anti-native DNA and anti-Sm. In scleroderma (Table II), the major antigen-antibody systems are anti–Scl-70 (DNA topoisomerase I) and anti–centromere antibodies. Several studies have shown that autoantibodies of these two specificities seldom occur simultaneously in the same patient, and anti–Scl-70 appears to be associated with the diffuse form of scleroderma, whereas anticentromere is associated with CREST (1). In addition, there are other defined autoantibodies that occur in lower frequency but appear to be highly specific for scleroderma.

The new information on the molecular identity of autoantigens suggests that in vivo immunogens might be subcellular particles and not individual proteins. The Sm immune response is directed against at least four proteins, the B′, B, D, and E core proteins of snRNP particles. Anti–U1 RNP immune response is directed against the A and C proteins of the U1 RNP particle and also the 70-kD protein, which is not in the family of snRNPs but as described earlier may be a nuclear matrix protein associated with U1 RNP. The autoantibody profile in scleroderma may be particularly revealing in that the antigens RNA pol I, fibrillarin, PM-Scl, To, and NOR-90 are all nucleolar in location or predominantly so and DNA topoisomerase I (Scl-70), which is a DNA supercoil relaxing enzyme, has been shown to be in the nucleolus associated with ribosomal DNA transcription centers (59) and in nucleolar organizer regions (60). The evidence that the immunogen in vivo might be subcellular particles or molecules coexisting in structurally distinct cellular compartments could account for the occurrence of autoantibodies directed against several different proteins, which is so characteristic of this group of diseases.

Autoepitopes are active sites, catalytic centers, or functioning regions

An important effort in autoimmunity research has been directed at defining the autoepitopes on protein antigens. In

Table II. Cellular Antigens and Autoantibodies in Scleroderma

Antigen		Autoantibody frequency
Clinical designation	Molecular nature	
Scl-70	DNA topoisomerase I; native 100-kD protein and 70-kD degradation product	70% in diffuse scleroderma
Centromere antigens	Kinetochore proteins 17-, 80-, 140-kD	75–85% in CREST
RNA polymerase I	Complex of many subunit proteins 210–11-kD	4% in scleroderma
Fibrillarin	Nucleolar protein of 34-kD; component of U3-RNP	8% in scleroderma
PM-Scl	Nucleolar and nuclear complex of many subunit proteins 110–20-kD	3% in scleroderma
To	Nucleolar protein of 40-kD; complexed with 7-2 and 8-2 RNAs	Rare
NOR-90	Protein of 90-kD localized at nucleolar organizer region	Rare

studies involving PCNA (61), SS-B/La (62–64), centromere protein B (34), and the 70-kD protein of MCTD (43, 44, 46), more than one epitope has been identified for each antigen. Studies are continuing concerning the possible significance of these findings. Evidence pointing to the fact that autoantibodies inhibited function has been demonstrated for anti-Sm and anti-U1 RNP which inhibit splicing of adenoviral early RNA sequences and other forms of pre mRNA (65, 66). Autoantibodies to tRNA synthetases were identified by their ability to inhibit aminoacylation of the cognate tRNAs (67) and autoantibody to RNA polymerase I inhibited 28S and 18S RNA synthesis when microinjected into *Xenopus laevis* oocytes (68). The special significance of these observations was brought into focus when studies showed that autoantibodies, in contrast to experimentally induced heterologous immune sera, had the special capability to inhibit function, whereas most heterologous antisera did not have this property. Autoantibody to PCNA (from lupus patients) inhibited an in vitro assay simulating DNA replication (69), whereas experimentally-induced antisera consisting of two murine MAbs and a rabbit anti-peptide immune serum were without effect. Similarly, human autoantibody inhibited threonyl-tRNA synthetase activity, whereas rabbit polyclonal antibody to the purified protein did not (70). These studies suggested that certain autoantibodies were inhibitory in functional assays, perhaps by virtue of their reacting with functional or active sites of the antigens, whereas experimentally induced autoantibodies, although just as immunologically reactive with their respective antigens but recognizing different epitopes, were not inhibitory.

Perspectives on the role of autoantibodies in autoimmunity

When analyzing the significance of autoantibodies in the light of recently accumulated information on autoantigens, it is perhaps easier to say what they are not than what they are. The in vivo immunogens that drive the autoimmune response appear to be subcellular particles and not individual proteins. It is difficult to conceive that autoantibodies are pathogenic in the sense that they inhibit the function of their cognate antigens, because that would mean inhibition of such essential cell functions such as mRNA splicing (snRNPs), DNA replication (PCNA), transcription (RNA polymerase I, DNA topoisomerase I, SS-B/La), and translation (tRNA synthetases). However, autoantibodies might be pathogenic in special circumstances, such as when immune complexes of DNA antigen and antibody form in extracellular fluids, but these situations are not related to the function of their cognate antigens. The evidence that immunogens might be subcellular particles and that functioning sites of these particles appear to be stimulating immune responses raises the intriguing possibility that activated states of subcellular particles might be responsible. In such situations, the autoimmune response could be directed at several antigenic sites, one of which would be the functional region or catalytic center of the particle. In the final analysis, an important question in autoimmunity is the nature of the precipitating factor (or factors) initiating processes that cause intracellular particles to become immunogenic. Exogenous factors including chemicals and biological agents may play a role, and perhaps there are some clues to be learned from drug-induced lupus where procainamide is capable of inducing autoantibodies to histones (71) and from mercuric chloride–treated mice and rats where autoantibodies to the nucleolar antigen fibrillarin have been identified (72, 73).

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