Ribonucleoprotein complexes as autoantigens

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Many intracellular proteins and nucleic acids, that are involved in important biosynthetic pathways, are targeted by autoantibodies occurring spontaneously in the sera of patients with systemic autoimmune diseases. Frequently, the autoantigens are assembled into multicomponent complexes containing both nucleic acid(s) and proteins. Recently, progress has been made in the study of autoantigenic ribonucleoprotein complexes, the most important of which are spliceosomal ribonucleoproteins, nucleolar ribonucleoproteins, Ro/La ribonucleoproteins and complexes of aminoacyl-tRNA synthetase and tRNA. In addition to new structural and functional information, important results have been obtained on epitope spreading, as well as on a potential role for apoptosis during the development of an autoimmune response against these complexes.

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Introduction

In a number of rheumatic diseases, the presence of the so-called antinuclear antibodies is a dominant feature. These diseases include systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), polymyositis, scleroderma and Sjögren's syndrome. The autoantigens that are targets of such autoantibodies are often large cellular complexes that contain protein and nucleic acid components. Many autoantigenic complexes localize in the nucleus (reviewed in [1]), but autoantibodies directed to cytoplasmic organelles, such as mitochondria, centrioles and the Golgi system, have also been described. The list of interesting autoantigens targeted by antibodies found in patients with rheumatic diseases is steadily growing. Some autoantigens that have been cloned recently include one of the components of the Mi-2 autoantigen, a large dermatomyositis-specific antigenic complex [2], 56K/Annexin XI [3], PCM-1, a 228 kDa centrosome autoantigen [4], and CENP-A, a histone-like centromere autoantigen [5].

The types of autoantibody that occur most frequently, however, are directed to DNA–protein complexes (DNPs) or RNA–protein complexes, ribonucleoproteins (RNPs). Anti-DNP antibodies can be directed to double-stranded DNA, as well as to a large variety of proteins involved in DNA metabolism, and include targets such as histones, DNA topoisomerase I, proliferating cell nuclear antigen (PCNA), Ku and centromere proteins [1,6,7]. The anti-RNP antibodies can be directed to a variety of RNAs (28S rRNA, U1 snRNA or tRNA [8]), as well as to proteins associated with these RNAs (ribosomal proteins, U snRNP proteins and tRNA synthetases [6]).

These autoantibodies have two striking, and most interesting, characteristics. First, a large number of them are disease specific (reviewed in [7]); therefore, the presence of a certain 'marker' autoantibody in a patients' serum may help the clinician considerably in reaching a diagnosis. Second, some autoantibody specificities appear to be present very early in disease, long before a clinical diagnosis has been reached. In such cases, an antibody profile can be helpful in differentiating the future development of a certain type of disease (reviewed in [9]).

In this short review, we will discuss primarily recent findings concerning the RNP autoantigens in order to provide insight into the recent progress of this field. For additional information, the reader is referred to some complementary reviews on autoantigens [1,6,7].

Spliceosomal RNPs

Spliceosomes, large nuclear complexes involved in the processing of pre-mRNA, have been shown to contain a set of so-called small nuclear ribonucleoprotein particles (snRNPs), which contain both RNA and protein molecules, as well as non-snRNP proteins in addition

Abbreviations

DNP—DNA–protein complex; **hnRNP**—heterogeneous nuclear RNP; **MCTD**—mixed connective tissue disease; **MRP**—mitochondrial RNA processing; **PKR**—double-stranded RNA-activated protein kinase; **RA**—rheumatoid arthritis; **RNP**—ribonucleoprotein; **SLE**—systemic lupus erythematosus; **snoRNA**—small nucleolar RNA; **snRNA**—small nuclear RNA; **snRNP**—small nuclear ribonucleoprotein particle.

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to the pre-mRNA. Most of the snRNPs, and several additional proteins, have been shown to be targeted by autoantibodies in patients with rheumatic diseases [10]. Autoantibodies directed against snRNPs occur predominantly in sera of patients with SLE or MCTD. One particular prevalent antibody in MCTD is called anti-U1 snRNP, which is directed to one or more of the specific proteins, U1A, U1C and U1-70K, contained in the U1 snRNP complex. A related antibody is found predominantly in SLE patients, and is called anti-Sm after the code name of the patient serum used in the study that first described this antibody system. Anti-Sm antibodies are directed to the common (or Sm) proteins B′/B and D_{1-3} present in all major U snRNP complexes. The proteins B′/B and D_{1-3} share at least one Sm-antigenic epitope, as demonstrated by the cross-reaction of anti-B′/B antibodies with the D proteins, and *vice versa*. Sequence comparison has revealed that all the known Sm proteins share two evolutionarily conserved structural sequence motifs, which might explain their immunological cross-reactivity [11•,12•]. Although the most important autoimmunizing Sm epitopes are yet to be identified, the fact that both polyclonal and monoclonal anti-Sm antibodies cross-react with various core proteins suggests that they share common structural elements. It is thus possible that the Sm motifs comprise, at least in part, one or more Sm B-cell epitopes [12•].

Other major antigenic components of spliceosomes are proteins contained in heterogeneous nuclear RNP (hnRNP) complexes. In particular, autoantibodies directed to the abundant hnRNP-A1 and hnRNP-A2 proteins can often be found in sera from patients with rheumatoid arthritis (RA), SLE and MCTD [13].

Nucleolar RNPs

The nucleolus contains a large number of autoantigens that are targeted by autoantibodies that are produced by patients with a connective tissue disease, mostly scleroderma [1,6,7]. A dynamic and expanding field of research is the study of the growing number of small nucleolar RNAs (snoRNAs), each of them presumably associated with common proteins (e.g. fibrillarin) and specific, as yet unidentified, proteins. Although some of these RNAs are coded for by independent genes transcribed by RNA polymerase II (e.g. snoRNAs U3, U8 and U13) or RNA polymerase III (7-2/MRP RNA), most are encoded within introns of mRNA coding genes (U14–U22, and more to come [14]). The major autoantigen in these snoRNP particles is fibrillarin, a common protein indirectly associated with most of these snoRNAs. Autoantibodies directed to fibrillarin are found in patients with scleroderma, but can also be induced in certain strains of mice by treatment with mercuric chloride ($HgCl_2$). The epitope regions in fibrillarin recognized by the spontaneous human and toxin-induced murine autoantibodies appear to be the same, and included amino- and carboxy-terminal regions [15•]. Given these striking similarities, it is to be expected that the murine $HgCl_2$ model may teach us what is actually happening in the patient when autoantibody production is initiated.

RNase P is an endoribonuclease that processes precursor tRNA transcripts to generate their mature 5′ termini. Many patients with an autoimmune disease produce antibodies against a 40 kDa protein (referred to as the Th40 antigen), which is one of the components of eukaryotic RNase P, as well as nucleolar 7-2 RNP, which is identical to the mitochondrial RNA processing (MRP) RNP. Reddy and coworkers [16] mapped the RNA-binding site of the Th antigen (presumed to be Th40) to nucleotides 20–75 near the 5′ end of human RNase P RNA. It was previously shown that the same antigen(s) bound to a non-homologous sequence at the 5′ end of 7-2/MRP RNA. Both sequences, however, are capable of assuming a similar secondary structure that corresponds to a 'cage' like structure, indicating that the major determinants for binding of Th40 are conformational and not contained in the primary structure of the RNAs.

Ro/La RNPs

Two of the main targets of autoantibodies from patients with SLE and Sjögren's syndrome are the La (SS-B) and Ro (SS-A) ribonucleoproteins (reviewed in [17]). Recently, interest in the biological and immunological properties of the La and Ro RNPs has grown markedly. The first indications of a biological function for a Ro protein have been obtained, and although La was previously only known to act in termination of RNA polymerase III transcription, evidence has also been obtained for additional function(s) of this protein in the cell.

The evolutionarily conserved 60 kDa Ro protein component (Ro60) was found complexed with certain variant 5S rRNAs in *Xenopus laevis* oocytes and was proposed to function in the quality control or discard pathway for 5S rRNA precursors [18•]. In agreement with such a function, part of Ro60 was shown to reside in the cell nucleus, in contrast to the Ro60 contained in RoRNP complexes, which is localized exclusively in the cytoplasm [19–21]. The recent identification of a Ro60 homolog in *Caenorhabditis elegans* [22•] will further aid to unravel the function of Ro60 via genetic approaches. The 52 kDa Ro protein (Ro52) was also found in both the nucleus and cytoplasm [19,21] but the majority of this protein is cytoplasmic, which is substantiated by the cytoplasmic accumulation of Ro52 observed in transfected cells that overexpress this protein [23]. The association of Ro52 with Ro RNPs remains a matter of controversy. Although Ro52 could not be detected in partially purified Ro RNPs [24,25•], the results of

Peek *et al.* [26•] indicate that only a restricted number of Ro52 epitopes is accessible in Ro RNPs, implying that the recognition of Ro RNPs by anti-Ro52 antibodies is highly dependent on the specificity of the antibodies. In any case, although present in the cytoplasm, the majority of Ro52 does not seem to be stably associated with Ro RNPs.

A variant Ro52 protein resulting from alternative splicing (exon skipping) has recently been identified ([27]; WJ van Venrooij, GJM Pruijn, unpublished data) but the physiological relevance of this variant, which appears to be ubiquitously expressed, still has to be established.

The role of the La protein in the termination of transcription by RNA polymerase III was further elaborated by Maraia *et al.* [28]: La was shown to mediate transcript release and to facilitate multiple rounds of transcription reinitiation by RNA polymerase III. Although La is believed to be localized mainly in the nucleus, several observations suggest that La may also be involved in some aspects of translation, which may be related to its stable association with the cytoplasmic Y RNAs and to a redistribution (cytoplasmic accumulation) under certain stress conditions, such as viral infection. The binding and unwinding of double-stranded RNA by La was shown to inhibit the double-stranded RNA-dependent activation of the protein kinase PKR *in vitro* [29]; PKR is known to be involved in the phosphorylation of the α subunit of protein synthesis initiation factor eIF-2. A more specialized role for La has been demonstrated in the translation of some viral mRNAs. La binds to the 5′ untranslated region of poliovirus mRNA and promotes (internal, cap-independent) initiation of translation at the correct AUG [30]. The La protein also binds to the HIV-1 leader RNA, the *trans*-activation response element (TAR), and alleviates translational repression by this element (cap-dependent initiation) [31•].

La proteins from *Drosophila melanogaster* and *Saccharomyces cerevisiae* have also recently been identified and characterized [32–34]. The gene encoding the yeast homolog of La appeared to be dispensable for viability, but at present, it can not be excluded that additional La homologs exist in yeast.

Many studies have addressed the characterization of the epitopes on the Ro and La proteins that are recognized by autoantibodies. In summary, multiple epitopes appear to be present on each of these proteins. Striking features of these epitopes are the discontinuity of the major Ro60 epitope(s), the apparent absence of important epitopes in the carboxy-terminal half of Ro52 [26•,35,36], and the presence of a conformational epitope in the RNA-binding domain (RNP motif) of La [37]. Maternal anti-La antibodies that cross-react with laminin have been proposed to contribute to the pathogenesis of congenital heart block [38]. Recently, novel antibodies that target deproteinized hY5 RNA have been identified in the sera of patients with anti-Ro antibodies [39].

Aminoacyl-tRNA synthetases

These enzymes perform an essential function in protein synthesis by catalyzing the esterification of an amino acid to its cognate tRNA. Histidyl-tRNA synthetase is the most frequent target of autoantibodies in patients with an idiopathic inflammatory myopathy (polymyositis or dermatomyositis), but autoantibodies directed to other members of this family (alanyl-, glycyl-, isoleucyl- and threonyl-tRNA synthetase) have occasionally also been found in myositis patients [1,6,7]. The autoantibodies directed to His-tRNA synthetase, also called anti-Jo 1 antibodies, precede the clinical illness, and the immune response bears the hallmarks of a typical secondary immune response. Why these particular proteins are selected as targets in myositis remains unclear. Plotz and coworkers [40] mapped the B-cell epitope region that is predominantly recognized by the anti-Jo 1 antibodies and found it to be contained in the amino-terminal 60 amino acids, a region of the protein with a high α helical content. This finding provides further support to the hypothesis that long, charge-rich α helices (coiled-coils) are found more frequently in (epitope regions of) autoantigens than in other proteins. Recently [41], the cDNA sequence of human glycyl-tRNA synthetase was published. As patient antibodies directed to this autoantigen were also able to inhibit Gly-tRNA synthetase activity, just like the anti-Jo 1 antibodies inhibit His-tRNA synthetase activity, it would be interesting to see whether the B-cell epitope of this autoantigen includes a coiled-coil region as well.

Why autoantibodies to RNP autoantigens?

Additional evidence for a B-cell epitope spreading mechanism has been published by a series of elegant papers from the laboratories of Mamula and Craft (see [42••]), McCluskey (see [43••]) and Harley (see [44••]). The latter paper especially points to the intriguing possibility that autoimmune diseases could be accompanied or induced by immune responses to relatively simple antigenic structures. In 1989, Habets *et al.* [45] showed that proline-rich sequences are cross-reacting targets of anti-Sm and anti-RNP autoantibodies. Harley and coworkers [44••] immunized rabbits with PPPGMRPP or PPPGIRGP (single-letter code for amino acids) and observed that the animals developed high titers not only against these peptides, but also to other parts of the molecules from which these peptides were derived (i.e. the Sm-B/B′ proteins) as well as against a variety of other spliceosomal components, including the U1-70K, U1C, U1A and Sm-D proteins.

The structural association of the La and Ro proteins in Ro RNP particles in the cell might also be related to the frequent co-occurrence of autoantibodies against these proteins in patient sera. In a recent study, Topfer *et al.* [43••] examined whether immunity to La and Ro60 autoantigens can be triggered by immunization with recombinant antigen. The data demonstrate the incomplete nature of T-cell and B-cell tolerance to these polypeptides in normal, healthy mice and reveal not only intramolecular spreading of the immune response, but also intermolecular spreading. Although the mechanism of intra- and intermolecular spreading is not known, it is tempting to speculate that endogenous La/Ro RNP complexes are involved. A potential role for microorganism infection in the etiopathogenesis of SLE and other related autoimmune diseases has been proposed in the past. Interestingly, immunization of rabbits with vesicular stomatitis virus N-protein, which had previously been shown to share sequences with peptide epitopes of Ro60, not only led to an immune response to the N-protein, but also to an anti-Ro60 autoimmune response, which is not restricted to the sequences shared with the N-protein [46].

One very interesting study published last year called attention to the possible association of apoptosis and autoantibody production. Casciola-Rosen *et al.* [47••] showed that UV irradiation of cultured human keratinocytes could induce changes consistent with apoptosis and that certain autoantigenic complexes (Ro RNPs, nucleosomes, snRNPs) accumulate in apoptotic blebs and apoptotic bodies in these dying cells. Even more interestingly, they showed in a subsequent study [48•] that one of the more important autoantigens, the U1-70K protein, is specifically cleaved in apoptotic cells. The possible implication of this work is that massive apoptosis in a genetically susceptible individual might be a mechanism via which appropriate MHC class II molecules might capture and present self-peptides that were previously cryptic. The immune response to this self-peptide may subsequently spread to other areas of the self-molecule to which the organism was previously tolerant. Indeed, such a sequence of events might explain why autoantibodies in a particular disease are directed at multiple antigens contained in the same RNP, DNP or protein–protein complex [48•].

Conclusions

Autoantibodies occurring in patients with rheumatic diseases can be directed to a large variety of cellular complexes, either in the nucleus or in the cytoplasm. Thanks to the availability of patient antibodies, we are now able to elucidate the structure and function of these complexes in the cell. The results of many studies have clearly established that most of these autoantibodies arise as a consequence of an antigen-driven response. Nevertheless, the autoimmune response may be triggered by the presence of a certain antibody that was originally raised against a foreign microorganismal invader but that displays some self reactivity. Spreading of the anti-self response may occur under certain conditions that are still undefined, but the finding that various antigenic RNP complexes, probably modified in some way [48•], seem to cluster in apoptotic structures like blebs near the membrane of apoptotic cells, which might enhance their availability to the immune system, certainly provides an attractive and testable explanation. The specific relation between the presence of a certain autoantibody specificity and a disease that is developing might thus be related to and dependent on at least three factors: first, the type of microorganism leading to cross-reactive anti-self antibody; second, the circumstances that lead to spreading of the anti-self response; and third, the fate of intracellular complexes during apoptosis.

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An important study suggesting that potentially immunogenic cellular components (Ro RNPs, snRNPs and nucleosomes) are clustered in two distinct populations of blebs at the surface of apoptotic cells. Massive apoptosis, together with chemical modifications occurring during this process (see [48•]), might be a mechanism to explain why certain antigenic complexes become autoantigens.

48. • Casciola-Rosen LA, Miller DK, Anhalt GJ, Rosen A: **Specific cleavage of the 70-kDa protein component of the U1 small nuclear ribonucleoprotein is a characteristic biochemical feature of apoptotic cell death.** *J Biol Chem* 1994, **269**:30757–30760.

This paper shows that an important autoantigen (i.e. U1-70K) is specifically cleaved during apoptosis. A possible implication could be that previously cryptic sites of the protein could become exposed to the immune system, possible leading to an autoimmune reaction.

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