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EDITORIAL REVIEW

Autoantibodies to the RoRNP particles

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A number of themes have emerged from study of the heterogeneous autoimmune response in patients with systemic lupus erythematosus (SLE). These themes are typified by a current paper by Boulanger *et al.* on the detection of antibodies to HY^5 RoRNA [1]. In that paper, antibodies are described to HY^5 RNA which is part of a larger particle containing a number of autoantigens whose cognate autoantibodies frequently occur together.

At the simplest level of analysis such associated responses bespeak of an antigen-driven response in which the 'antigen' is not an isolated protein or pure nucleic acid, but a conjugate of the two, or at a more complex level a whole subcellular particle (e.g. a ribosome), some of whose components are antigenic. In the lupus spectrum of autoantibodies at least three other such complex systems are recognized. In one, antibodies to 28S ribosomal RNA are uniformly associated with antibodies to the ribosomal 'P' proteins [2]. In a second, encompassing most SLE patients, autoantibodies are recognized which may react with either DNA or histone and/or a DNA histone complex which are the components of chromatin [3]. In a third, autoantibodies to small nuclear ribonucleoprotein (SnRNP) particles or the spliceosomal complex are recognized which react with the U_1 specific polypeptides (70 kD, A and C) and the Sm-specific antigenic peptides BB′ and D as well as U_1 RNA [4]. Thus the major recognized antigenic targets for SLE sera are components of complex molecular particles or subcellular structures, and except for the RoRNP system whose cellular role is unknown have well defined biochemical functions.

A great deal has been learned about the autoimmune responses to the RoRNPs. The anti-Ro specificity was first recognized in gel diffusion [5] (also called SSA [6]) and was later identified as an RNA protein particle [7] in which a 60-kD protein [8,9] was associated with small RNAs termed HY RNAs [7]. Four small HY RNAs were recognized in human nucleated cells by immunoprecipitation which were designated HY^1, HY^3, HY^4, and HY^5. The picture became more complex when it was found that there was an erythrocyte form of the 60-kD protein which was distinctive but related to the 60-kD protein in nucleated cells [10]. At that time a 52-kD form of the Ro protein was described in nucleated cells, and was measurable only by Western blotting [11]. An analogous 54-kD Ro protein was characterized by Western blot in erythrocytes [10].

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In contrast to nucleated cells, erythrocytes contained only HY^1 and HY^4 bound to 60-kD Ro [10]. Later studies showed that human platelets contained only HY^3 and HY^4 Ro RNAs associated with the Ro proteins [12].

Biochemical studies revealed that the RoRNPs were heterogeneous and that the HY^5 RoRNP was unique among these particles [13]. First, patients were identified who produced autoantibodies specific for the HY^5 particle, but these specific anti-HY^5 RoRNP antibodies were also present in conventional anti-Ro sera which immunoprecipitated all four HY RoRNPs (HY^1, HY^3, HY^4 and HY^5). These HY^5 RoRNP-specific autoantibodies were also interesting in that no analogous RoRNP HY^5 had been found in rats, rabbits, or cows, but a RoRNP HY^5 particle has been identified in the guinea pig, revealed by reaction of guinea pig tissue extracts with HY^5-specific human sera [14]. Such studies suggest autoantibodies directed toward evolutionary non-conserved epitopes in RoRNP, an unusual finding among autoantibodies in which evolutionary conservation of epitope structure is the rule. Systematic studies of Ro antigenic reactivity with autoantibodies confirm that human anti-Ro is directed to epitopes that vary in evolution, such that the human antigen is the 'preferred' antigen [15–17]. Such findings also support the notion that the autoimmune response is antigen driven. Indeed, such targeting of non-conserved epitopes is the rule when animals are injected with heterologous proteins in conventional immunization schemes.

Further biochemical study has revealed that RoRNPs can be fractionated on ion exchange columns into several groups. HY^5 RoRNP contains a 60-kD Ro molecule, HY^5 RNA, and stably associated La protein. HY^4 RoRNP is similar, but replaces HY^5 RNA with HY^4 RNA. It is not clear if the La protein is stably associated with HY^1 and HY^3 RoRNP [18]. It is now thought that 52-kD Ro is not associated with HY RNA [19,20], or stably bound to either 60-kD Ro or La (also called SSB). The mechanism of its presence only in human sera with antibodies to native 60-kD Ro remains unresolved, but one study provides evidence that antibodies to denatured 52-kD Ro are a subset of autoantibodies to native 60-kD Ro [19].

The RoRNPs are therefore targets for a rich variety of autoantibodies, a large proportion of which are directed to the native 60-kD Ro structure. The antibodies specific for HY^5 RoRNP are particularly interesting because their reactivity depends on both protein and RNA and they are therefore conformational. Some sera only react with native 60-kD Ro and all sera react predominantly with native 60-kD Ro [21]. Essentially all anti-La sera also contain large amounts of

autoantibodies to anti-60-kD Ro. Sera apparently monospecific for La when studied with bovine tissue extracts invariably contain large amounts of anti-Ro exclusively directed to the human protein [16]. In this issue of *Clinical and Experimental Immunology*, Boulanger and colleagues provide evidence that two of 50 anti-Ro-containing sera also have antibodies highly specific for HY^5 RNA, and map the binding site to the stem region formed by hydrogen bonding of 5′ and 3′ ends near or in the Ro protein binding region. The clinical and/or biological significance of this response thus far seen in only 4% of anti-Ro sera remains to be seen.

The clinical significance of antibodies to components of the RoRNPs has been recently reviewed [4]. Antibodies to RoRNPs invariably occur in mothers who bear children with complete congenital heart block (CCHB), and while antibodies to 60-kD Ro are invariably present, anti-La is present in 80–90% of cases [4]. In a recent report, antibodies to native 60-kD Ro were found to be enriched in eluates of a child's heart who died at birth with CCHB [22]. While antibodies to 52-kD Ro were also enriched in the eluates, more than 90% of the eluate antibody activity was directed to the 60-kD Ro protein. SLE patients with anti-Ro precipitins alone have a much higher prevalence of nephritis than those with both anti-Ro and anti-La precipitins [23–25]. Patients with SLE and homozygous C_2 deficiency uniformly have anti-Ro precipitins and only rarely have accompanying anti-La precipitins [24,26]. Finally, SLE in the elderly tends to be enriched for patients with both anti-Ro accompanied by anti-La. The disease tends to be milder with more sicca, and like younger SLE patients with anti-La, a lower prevalence of nephritis [27–29]. The mechanisms of the associations of such antibodies with subsets could be easily understood if anti-Ro antibodies were the immunopathogenic basis for clinical findings such as the heart disease in CCHB and the skin disease in lupus dermatitis. Evidence from tissue eluate studies for the pathogenic potential of anti-Ro antibodies has been collected for parotitis [30], nephritis [31], and CCHB [22], and indirect evidence from tight clinical associations is strong. The role of anti-La in 'protecting' against the development of renal disease is still obscure, but it is associated with a low prevalence of antibodies to DNA [23–25]. A recent report describes the potential role of anti-La/SSB as an anti-idiotype to anti-DNA in SLE patients producing anti-La/SSB [32]. Clearly in the next decade one can expect the mechanism of these relationships to be revealed by combined immunochemical and clinical studies.

Finally, what is the 'immunogen' amongst the various Ro proteins, and what is the mechanism of autoimmunization? While the answers to these two questions are unknown, one can make some reasonable speculations. One central observation makes the native 60-kD Ro protein the leading candidate for the 'primary immunogen', since all sera with anti-Ro precipitins have antibodies that bind native 60-kD Ro [21,33]. Two patients described by Boire and Craft did not have anti-Ro precipitins, but had antibodies which only bound HY^5 RoRNP, and this specificity required both HY^5 RNA and protein for reactivity [13]. It would also be of great interest to know if this very restricted immune response persisted in these patients. However, such anti-HY^5 RoRNP antibodies are not present in every serum with anti-Ro precipitins [13]. Similarly, antibodies to 52-kD Ro are absent from all SLE sera with anti-Ro and anti-U_1RNP and/or anti-Sm precipitins (about 15–20% of all such anti-Ro sera) and anti-La is absent in more than half of all sera with anti-Ro precipitins. These facts make these antigens unlikely candidates for the primary immunogen.

In the '60-kD Ro as immunogen' hypothesis, the native 60-kD RoRNP (perhaps HY^5 Ro RNP plays a special role) confronts antibody-forming cells accompanied by antigen-presenting cells with the correct MHC class II molecules (DQ_1/DQ_2 heterozygotes for high responders [24,25,34]) and induces autoantibody production. Ro-specific T helper cells are probably involved, but their functional reality has yet to be demonstrated. Once, the anti-60-kD Ro response is initiated, it 'spreads' to other components of the RoRNP particle depending on the genetics of the host and other as yet unidentified factors. A recent report shows that such spreading occurs in mice immunized with either La or Ro. Tolerance to RoRNPs is either weak or easily broken in normal mice, since immunization with recombinant mouse La induces in normal mice production of autoantibodies to 60-kD Ro, and *vice versa*, immunization with 60-kD Ro leads to autoantibodies against first the 60-kD Ro protein and then the La protein. Viruses or other infectious agents as molecular mimics could be possible cofactors for immunization, as could sunlight. As yet unidentified genes which promote this specific autoimmune response are being sought in several laboratories. Given the pace of research in this field, it would not be surprising to observe the surfacing of new fundamental insights into the nature of autoimmunity to the RoRNPs.

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