

Autoantibodies in Sjögren's syndrome

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Today, dysregulation of B cells and hypergammaglobulinemia are prominent recognised features of the syndrome described by Henrik Sjögren. The elevated levels of circulating immunoglobulins usually contain several specificities for self-molecules of both organ-specific and non-organ-specific targets, the most common autoantibodies being rheumatoid factor, anti-Ro/SS-A and anti-La/SS-B. Other described autoantibodies in Sjögren's syndrome (SS) are anti-vimentin, anti-alpha-fodrin, anti-histone, anti-erythrocyte, anti-thyroglobulin, anti-prostatic acid phosphatase and anti-salivary gland duct epithelium. These autoantibodies are of high affinity and show characteristics of an antigen-driven, T cell-dependent immune response, belonging primarily to the IgG1 and IgG3 subclasses of immunoglobulin, though recent reports have described both IgA and IgM specific for the Ro and La proteins (1).

As part of the histopathological development of Sjögren's syndrome, aggregates of lymphocytes form around the intralobular ducts and in the surrounding acinar epithelium. The majority of the lymphocytes are T cells, but 10-20% of the infiltrating lymphocytes are B cells, and an increase in the number of plasma cells producing antibodies of the IgG and IgM isotypes is seen. The relative number of IgA producing cells can be reduced. The B cells are most often found at the margins of the focal infiltrates, but are also seen as small conglomerates throughout the parenchyma. B and T cells can be segregated into separate areas similar to those seen in lymphoid tissues. The location of the B cells and the large number of plasma cells indicate that the B cells are driven to activation and autoantibody production with T cell help in the areas of inflammation (2).

The role of autoantibodies in the autoimmune process is not known, and it is also unknown whether the antibodies react with the intracellular targets *in vivo* to cause inflammation. However, the La antigen in salivary gland epithelial cells has been shown to re-localise from nucleoli to nucleoplasm, cytoplasm and to a lesser extent to the membranes of acinar cells in SS patients (3). Viral infection and apoptosis have also been described to cause translocation of autoantigens. This implies that the antigens stimulating the antibody producing cells are present and accessible within the salivary glands, and that the B cells can be stimulated by these antigens to produce autoantibodies which contribute to the salivary gland inflammation and destruction.

Autoantibodies to the Ro/SS-A and La/SS-B ribonucleoprotein antigens are found both in the sera of patients with Sjögren's syndrome (SS), systemic lupus erythematosus (SLE) and rheumatoid arthritis, or overlaps between these diseases. The prevalence of anti-Ro antibodies in patients with SS varies according to the method used to detect them and criteria used to define the disease between 50-90%. Similarly, anti-La antibodies are often found in the sera of patients with Sjögren's syndrome, around 35% by immunoprecipitation but up to 85% by ELISA. Presence of anti-Ro/SS-A or anti-La/SS-B autoantibodies is one of the items in the classification criteria suggested by the European Community Study Group on Diagnostic Criteria for SS (4).

The presence of these antibodies is associated with tissue damage. Anti-Ro and anti-La antibodies can be produced locally in inflamed salivary glands of Sjögren patients (5). Ro-antibodies have also been eluted from kidneys of patients with SLE (6), and are associated with photosensitive rashes. Antibodies to Ro and La also appear to predispose patients with SLE to the development of Sjögren's syndrome. These antibodies can cross the placenta and in about 1 of 20 cases induce a neonatal lupus syndrome, which is characterised by a transient skin rash and a cardiac conduction defect that may be permanent (7). Moreover, evidence has been presented for the presence of La antibodies at the surface of the myocardial fibres of a foetus with congenital heart block, and an electrophysiological study demonstrated that human anti-Ro and anti-La antibodies can bind to and

affect the transmembrane repolarisation of neonatal rabbit cardiac-conducting cells. Consequently, many investigators have explored the structure, origins and precise targets of these antibodies. In addition, recent advances in molecular and cell biology have contributed to our understanding of the cellular antigens recognised by autoantibodies in connective tissue diseases. These are often functionally important molecules in key cellular processes such as synthesis and transport of nucleic acids, and are very specific targets for autoimmunity in these disorders.

The Ro/SS-A autoantigen is an RNP complex containing at least two proteins, Ro 60kd (8) and Ro 52kd (9), bound to one of four 83-112 base long RNAs termed human cytoplasmic RNAs (hY RNAs) (10). However, whether the Ro 52kd protein binds to the complex is controversial. The hY RNAs reside in the cytoplasm, while the Ro proteins are present in the nucleus as well as in the cytoplasm. The biological functions of the components of the Ro/SS-A complex are not known, but data suggesting the Ro 60kd protein to be involved in a discard pathway for defective 5S rRNA precursors have been presented. The La/SS-B autoantigen consists of a 48kd protein (11), which can transiently bind to the poly-U tail of RNA polymerase III transcripts, including the hY RNAs. A function for the La protein as a transcription termination factor for RNA polymerase III transcripts has been implied in mammalian cells, while the La yeast homologue was recently reported to be required for 3' cleavage of tRNA precursors.

By the use of synthetic peptides and recombinant antigens attempts have been made to define the important epitopes of these ribonucleoprotein antigens. When comparing results obtained by independent investigators, the most striking observation is that, although a number of important technical parameters such as type of assay, type of antigen probe and selection of sera differ between these studies, the position of the major Ro 60kd and Ro 52kd epitopes is consistent. For example, a major antigenic region in the middle of Ro 60kd (within residues 155-326) has been identified in six of seven studies published so far, and a major antigenic region present in the middle of Ro 52kd (within residues 136-292) has been identified in all eight published studies analysing this region (12). The major antigenic region of the Ro 60kd antigen represents the most hydrophilic, and presumably exposed part of the protein. In the Ro 52kd antigen, the most frequently identified antigenic domains contain a putative coiled-coil domain, and within this a leucine zipper motif.

The similarities in the autoimmune response to the Ro antigens can be interpreted as an indication that the immune response is stimulated by the endogenous proteins to produce autoantibodies. In mice, monoclonal antibodies have been raised against the Ro 60kd and Ro 52kd antigens by immunisation with recombinant antigen (13,14). Interestingly, several clones recognised the same domains of the proteins as do human autoimmune sera, showing that these domains are immunogenic. Also, immunisation of mice (15) and rabbits (16) with recombinant Ro antigen or synthetic Ro peptides has been shown to lead to intra and inter molecular spreading of immune-reactivity. This further supports the idea that autoantigens can act as immunogens, and that once an autoimmune response to one component has developed, it may spread to associated proteins. Antibodies to the Ro-associated RNA molecule hY5 RNA have been detected, and the epitopes of the hY5 RNA identified as non-protein binding and therefore probably exposed parts when the full Ro-complex is associated (17), further indicating the importance of the entire Ro particle in the autoimmune response. One should also consider the possibility that the initial immune reaction is to an exogenous antigen, and that the antibodies cross-react with some part of a Ro antigen, later leading to spreading of the immune response to include other epitomes of the Ro proteins.

In addition to the major epitomes, minor antigenic regions in other domains of the proteins which are less frequently recognised by patients' antibodies are identified in most of the studies for both Ro 60kd and Ro 52kd. The reactivity to these regions shows a more specific profile for different rheumatic diseases. For example, a majority of sera from patients with SS, as opposed to SLE sera, were found to react with the amino-terminal part of Ro 60kd (18, 19), while others (20) identified

reactivity to this region mainly in sera from patients with subacute cutaneous lupus. For the Ro 52kd antigen, the region spanning residues 69-216 was identified as specific for strong reactivity in SS sera, but not in SLE sera (21). This might be due to different mechanisms operating at onset of disease, or be caused by the different HLA associations in the diseases, which preferably presents slightly dissimilar peptides of the autoantigens to stimulate the immune response.

The accumulated knowledge from analysis of epitopes recognised by autoantibodies can be used for refined diagnostic tools, in assays with specific peptides or recombinant antigens containing major or disease associated epitopes, developed to facilitate autoantibody detection and setting of diagnosis and thereby prognosis. One might also consider the use of such peptides or antigens in immunomodulation of the autoimmune response. The challenge still present is to understand by what mechanisms this reaction pattern develops in autoimmune patients, the direct mechanisms of how the antibodies participate in tissue destruction and why intracellular complexes, often with central cellbiological functions, act as autoantigens.

References

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