Possible Mechanisms of Autoantibody Production and the Connection of Viral Infections in Human Autoimmune Diseases

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YAMAMOTO, K. Possible Mechanisms of Autoantibody Production and the Connection of Viral Infections in Human Autoimmune Diseases. Tohoku J. Exp. Med., 1994, 173 (1), 75-82 — The presence of autoantibodies is a characteristic phenomenon in an autoimmune disease. In order to investigate the mechanisms of the autoantibody production, we have performed epitope mappings of the autoantigens. It thus was found that there were multiple epitopes on an autoantigen molecule, and a serum from a patient with an autoimmune disease usually recognizes these multiple epitopes simultaneously, suggesting that autoantibodies are ultimately produced by an antigen-driven mechanism. However, other evidence suggests that tolerance to a self antigen is rather tightly maintained and a simple antigen-driven mechanism cannot easily take place. Based on the results of epitope mappings, it appears that there is a major or a usually recognized epitope on almost every autoantigen molecule. Some patients only recognize this epitope. It is unlikely that only a single epitope can be recognized if a large molecule is immunized to the host. Therefore, the recognition of this universal epitope may play some roles in the induction of the autoantibody production. In fact, some of these epitopes have sequences homologous to those of viral proteins. Thus, it is possible that an immune response to a certain virus might induce by molecular mimicry the recognition of an autoantigen. Possible mechanisms following this molecular mimicry that may induce antigen-driven autoantibody production are discussed. —— autoantibody; autoimmune disease; epitope mapping; molecular mimicry; viral infection

Although an autoantibody, the production of which is one of the major characteristic phenomena of autoimmune diseases, may not always induce tissue damages, a good correlation exists between the type of autoantibodies that are produced and the kind of symptoms or a diseases presented. Therefore, studies of the mechanisms of autoantibody production would appear to be important for analyzing autoimmune diseases (Tan 1989).

Recently, the techniques of molecular biology have been applied to the field of autoantibody research and in this regard, several cDNAs encoding targets of

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autoantibodies have been isolated (Wieben et al. 1985; Sturgess et al. 1988; Yamamoto et al. 1988) and have also been expressed as recombinant autoantigens. Therefore, by using these recombinant proteins, simple and reproducible methods to detect autoantibodies are now available. Further, by using these cDNAs, epitope mappings of the autoantigens can be performed, and information so obtained is helpful in understanding the mechanisms of autoantibody production.

On the other hand, with regard to the pathogenesis of autoimmune diseases, a viral infection has been speculated (Christian 1982). In fact, in mice an immune response to exogenous or endogenous viral antigens has been suggested to play a role in autoimmune diseases (Datta and Schwartz 1976). However, little evidence exists to support a viral infection as being responsible for an autoimmune disease in humans. Our study investigates the mechanisms of autoantibody production, focusing on the epitope mappings of target molecules of the anti-nuclear antibodies in systemic autoimmune diseases, and on the possible role of a viral infection in the induction of autoantibody production.

**Epitope mappings of autoantigen**

Several mechanisms responsible for autoantibody production have been speculated. These mechanisms include polyclonal B cell activation (Hang et al. 1983), antigen-driven response (Tan et al. 1988) and molecular mimicry (Oldstone 1987). To determine which of these mechanisms predominates in generation of each autoantibody, epitope mapping of the autoantigen is one of the most powerful methods.

Various methods have been employed for the epitope analyses of proteins, the chief methods being a synthetic oligopeptide analysis, the screening of an epitope library and the deletion mutant methods. Each approach has its merits and demerits. Synthetic oligopeptide analysis is easy to perform, although only limited linear epitopes can be detected. The use of an epitope library is an ideal method if a good library could be produced. However, only few serum samples were available for this screening (Habets et al. 1989). We thus mainly used the deletion mutant method, since surveying the sera of many patients is rather easy once the series of deletion mutants have been established (Kohsaka et al. 1990).

Initially a full length cDNA encoding for an autoantigen was subcloned into a plasmid vector and was expressed in *E. coli* as a recombinant antigen, usually in the form of a fusion protein. The cDNA was then enzymatically treated, after which several deletion mutant cDNAs were expressed in *E. coli*. The reactivities of these full length and truncated recombinant autoantigens were tested with serum samples taken from several patients by means of an immunoblot or enzyme-linked immunosorbent assay. Changes in the reactivities between these mutants indicated the cDNA segments responsible for shaping, at least in part, the epitopes on the autoantigen.

Representative results of our epitope mappings are shown in Fig. 1. SS-B/
La is a target molecule of the anti-SS-B/La autoantibody, a characteristic of Sjögren's syndrome. In the N terminus there is an epitope which is recognized by all the serum samples of anti-SS-B/La-positive patients. In the C terminus, there is a cluster of epitopes which sera from only a small number of patients recognize (Kohsaka et al. 1990). The snRNP-C protein is one of the target molecules of anti-nRNP antibodies, and in the C terminus, there is a cluster of epitopes (Misaki et al. 1993). Topoisomerase I is a target molecule of anti-Scl-70, a marker autoantibody of scleroderma. There are several epitopes on the molecules, and the sera of many patients react with these multiple epitopes simultaneously. However, there is a universally reactive epitope on the molecule and almost all sera from our patients recognized this epitope. Of interest, some sera were found to recognize only this epitope (Kato et al. 1993).

Poly (ADP-ribose) polymerase is a rare target molecule of autoimmunity, and after screening of several hundred sera from patients with rheumatic disorders, only seven sera were found to react with this molecule (Yamanaka et al. 1987). A serum having this autoantibody does not appear to possess other autoantibodies, indicating that polyclonal B cell activation might not take place. As the result of our epitope mapping, it was found that seven positive sera universally recognized one conformational epitope on the molecule. Three of these seven sera also

Fig. 1. Simplified summaries of epitope mappings of autoantigens.
recognized one of the minor epitopes (Miura et al. submitted for publication).

**Autoantibodies are ultimately produced by antigen-driven mechanisms**

Based on the results of our epitope mappings, we found that there are multiple epitopes on each autoantigen molecule. Each patient's serum usually recognized some of these multiple epitopes simultaneously with high-affinity IgG antibodies. These findings strongly indicate that the autoantibody is produced by an antigen-driven mechanism, and that there should exist antigen-specific helper T cells.

In fact, in an in vitro test using a soluble recombinant autoantigen, an antigen-specific T cell proliferation was detected (Okubo et al. 1993), and the cells that predominantly proliferated were CD4-positive T cells. The frequencies of these cells in the peripheral blood T cells were also calculated by the limiting dilution method, and these frequencies were found to be about one out of 4–8 thousand cells. Such frequencies are rather high, compared to frequencies of antigen-specific immune responses previously reported.

In this regard, however, several reports have already indicated that the tolerance to an autoantigen is rather tightly maintained, and that simple antigen-driven autoantibody production does not seem to easily take place. It is especially true for a target molecule of systemic autoimmune diseases. For example, Hines et al. (1991) have immunized MRL/lpr mice with purified mouse ribosomal proteins in both native or denatured forms. Although 10% of mice of this strain were already known to spontaneously produce autoantibodies to the ribosomal proteins, there were no increase in the incidence of the autoantibody production specific for this protein. Similarly, we have also tried to immunize rabbits or mice with purified recombinant autoantigens with strong immunogenic carriers and an adjuvant, but it was found to be extremely difficult to obtain good antibodies to the autoantigens. These results appear to indicate that simple immunization with an autoantigen does not break the immunological tolerance.

**Existence of a universal epitope on autoantigens**

During our epitope mappings, we also found that there is in general a universally recognized epitope on an autoantigen, and that almost all the positive sera recognized this epitope. In addition, the sera from some patients seem to react only with this epitope. For example, the serum samples obtained from a single patient, who was positive for anti-Scl-70 autoantibodies, were found to react only with this universal epitope on the topoisomerase I, and this restricted specificity sustained over a period of three years (Kato et al. 1993).

It is unlikely, however, that a host would recognize only one epitope if larger molecules such as topoisomerase I or poly(ADP-ribose) polymerase were immunized to the host and antibodies were produced through an antigen-driven mechanism. In this regards, in a simulation study of antigen-driven antibody produc-
tion using β-galactosidase as an antigen and C3H mice as the host, it was found that from the first immunization the mice were able to produce the antibodies to multiple epitopes (unpublished observation).

If the recognition of the universal epitope on the autoantigen does not come from antigen-driven mechanisms, a cross-reactivity of the antibody might be the best explanation to account for this recognition.

Possible role of viral infection in autoantibody induction

On searching the amino acid sequences of the universal epitopes of the autoantigens, it was found that some epitopes have sequences homologous to viral proteins. For example, the universal epitope of the SS-B/La protein is homologous to a gag polyprotein of the feline sarcoma virus (Kohsaka et al. 1990). Further, the universal epitope of the U1 snRNP-C protein is homologous to ICP4 protein of herpes simplex virus (HSV). Using oligopeptides corresponding to the C protein and the HSV protein, it was found that the homologous sequences have immunologically cross-reactive antigenecities (Misaki et al. 1993).

Apart from our findings, several reports have indicated that epitopes on the autoantigens have sequences homologous to viral proteins (Query and Keene 1987; Maul et al. 1989; Guldner et al. 1990). Therefore, it is likely that an immune response to a virus could lead to the recognition of an autoantigen via a cross-reactivity. This mechanism is called molecular mimicry. In fact, molecular mimicry has been proposed as a possible mechanism of an autoimmune disease (Oldstone 1987). However, as has been described in this report, the ultimate phase of autoantibody production is antigen-driven responses. Multiple epitopes on an autoantigen are recognized by the host, and this could not be explained by molecular mimicry.

Molecular mimicry to an antigen-driven immune response: Progression of the reactivity

If a hypothesis were to be suggested, that is, a mechanism in which the progression of the reactivity from a universal epitope to other epitopes on an autoantigen occurs, in other words, the progression from molecular mimicry to an antigen-driven immune response, we could then naturally understand the reasons for the phenomena observed in our studies.

Lanzavecchia (1985) has proposed that antigen-specific B cells have powerful antigen presenting capacities to T cells specific for the same antigen. It thus becomes possible to speculate that these B cells play an important role in the tolerance breakdown. Based on this idea, we propose one possible scenario that is illustrated in Fig. 2.

If an exogenous immunogen such as a virus enters a host, the host immune system recognizes this antigen via the T cells that are specific to this immunogen. With their help, B cells reactive to the multiple epitopes on the molecule can be
activated and expanded. Even if there is a cross-reactive epitope between the immunogen and an autoantigen, the B cells that recognize such an epitope are usually anergic. However, with the specific help from the immunogen-reactive T cells, these B cells could also be activated and proliferate. As has been suggested by Lanzavecchia, these activated B cells could take and process the self antigen, and they could present the self peptide to the self antigen-reactive T cells. In turn, the self antigen-reactive T cells, which should be essentially anergic in the host, could thus be activated. If this process takes place, the tolerance to the autoantigen would no longer exist, and other epitopes on the autoantigen would then be recognized by autoantibodies through antigen-driven mechanisms.

This possible hypothesis was, to a certain extent, verified in a mouse model using cytochrome C (Lin et al. 1991). Further, if self antigen-specific T cells are
activated, it is also possible that self antigen-specific cytotoxic T cells (CTL) could be generated. These CTL might be restricted to a certain class I molecule, which is prominently expressed in some tissues. Tissue destruction in systemic autoimmune diseases could thus be explained based on the generation of these CTLs.

**Concluding remarks**

A possible mechanism of autoantibody production has been described and its relationship to a viral infection was discussed. It has been suggested that the existence of a virus or a bacteria dose not always cause tissue damage by itself, but that an immune response to the microorganism would cause the infectious disease, such as tuberculosis. Therefore, if microorganisms are truly capable of inducing some autoimmune diseases, then complicated immunological cascades, as has been described, would be required. It is conceivable, therefore, that the hypothesis we have described is far simpler than actual chain of events. In any case, pushing such studies will enable us to more clearly understand the precise mechanisms of autoimmune diseases, and provide the clues to overcome the diseases.

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