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Immunology

The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity

(affinity maturation/anti-Jo-1 autoantibody/polymyositis/conformational epitopes)

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ABSTRACT **Patients with systemic autoimmune diseases make specific autoantibodies that are directed against self structures. According to one view, these autoantibodies arise as a result of an immune response to foreign antigens such as infectious agents that share, by molecular mimicry, common structures with host proteins. An alternative view is that the target autoantigen itself initiates, selects, and sustains autoantibody synthesis. We show here that anti-Jo-1 autoantibodies directed against histidyl-tRNA synthetase in the human autoimmune muscle disease polymyositis undergo, in addition to spectrotype broadening and class switching, the *sine qua non* of an immune response to the target antigen—affinity maturation to that antigen. We demonstrate further that these autoantibodies, unlike anti-synthetase antibodies induced in mice immunized with heterologous antigen, bind only nonlinear epitopes on the native human synthetase that remain exposed when the enzyme is complexed to $tRNA^{His}$. These data suggest that the native target autoantigen itself has played a direct role in selecting and sustaining the autoantibody response and sharply restrict the time and the way in which a molecular mimic might act to provoke autoantibodies.**

The origin and regulation of autoantibodies in the idiopathic inflammatory diseases characterized as autoimmune have provoked divergent theories, some of which include a role for the target autoantigen in initiating, selecting, and sustaining autoantibody synthesis and some of which do not (1–6). The findings that some autoantibodies bind more than one epitope per autoantigen or more than one protein per particle suggest that in some cases the identified target autoantigen itself sustains the autoimmune response (7). Even in these cases, however, what initiates and what selects the autoimmune response remain unknown.

In polymyositis, a rare human autoimmune muscle disease, there is a disease-specific family of autoantibodies that share certain remarkable characteristics. These autoantibodies are directed at phosphorylated cytoplasmic ribonucleoproteins involved in protein synthesis and tend to bind to conserved epitopes critical to the function of these proteins (1, 8–11). The most common of these, anti-Jo-1 autoantibodies (AJoA), bind to and inhibit activity of histidyl-tRNA synthetase (HRS) and are found in a genetically restricted and clinically distinct group of patients (11). We have previously shown (12) that AJoA are restricted mainly to the IgG1 isotype and that their levels are regulated independently of total immunoglobulins and vary in proportion to myositis disease activity. Although each patient appears to have a distinct polyclonal AJoA spectrotype, AJoA from all patients recognize the same proteolytic polypeptides of HRS (12). Here, in a study of a number of well-characterized patients, we explore the mechanisms by which AJoA arise and are sustained by analyzing serial changes in the classes, spectrotypes, and affinity of these autoantibodies and by defining the nonlinear nature of the AJoA epitopes.

MATERIALS AND METHODS

Immunoassays. All assays for AJoA used as antigen biochemically isolated HeLa HRS (13). AJoA serum levels were quantitated using class-, subclass-, or light chain-specific enzyme-linked immunosorbent assays (ELISAs) (12). Spectrotype analyses and Western blotting were performed as described (12). IgG was isolated from serum by using protein A-Sepharose and 5 μg was used in each HRS aminoacylation assay (1). For nucleic acid immunoprecipitation assays, $tRNA^{His}$ was immunologically isolated (14) and ^{32}P-labeled by standard kinase reactions (15); 10 ng (10 μCi, 370 kBq) of $tRNA^{His}$ was added to 100 μg of HeLa postribosomal supernatant and 5 μg of IgG and incubated for 30 min at 4°C before protein A-Sepharose-assisted immunoprecipitation, RNA extraction, and electrophoresis (1).

Autoantibody Affinity Assays. AJoA affinity was determined by the ELISA binding-inhibition method of Friguet *et al.* (16). Extensive preliminary experimentation was performed to assure proper conditions for the ELISA (16). For determination of AJoA affinity, sera were diluted in BBSA (borate-buffered saline containing 5% bovine serum albumin) to a final concentration of 0.30 μg of total AJoA per ml. One hundred fifty microliters of diluted serum was mixed with 150 μl of BBSA containing serial dilutions of 2 μM to 0.2 pM HRS. After incubation at 4°C for 2 hr, 90 μl of the mixture was added in triplicate to wells of ELISA plates, previously coated with 0.5 μg of HRS and blocked with BBSA, and was incubated for 15 min at 4°C. The A_{410} was read after incubation with affinity-purified, alkaline phosphatase-labeled goat anti-human immunoglobulin antibody (Jackson ImmunoResearch) and development with *p*-nitrophenyl phosphate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) (12).

Induction and Analysis of Anti-HRS Antibodies. BALB/c mice were alternately immunized with 1 μg of biochemically isolated HeLa HRS (13) at 0 and 4 weeks and immunologically isolated HRS (17) at 3 and 7 weeks in Freund's adjuvant. Animals were bled by retroorbital plexus venipuncture and anti-HRS antibody levels were determined as before (12).

Autoantigen Epitope Studies. Hexapeptides representing all possible 508 overlapping hexapeptides of the linear sequence of human HRS (18) were synthesized in duplicate on solid-phase supports (Cambridge Research Biochemicals, Cambridge, England) as described (19). Peptides were screened for reactivity in a standard ELISA using protein A-Sepharose-isolated IgG (12). As a positive control, antisera from rabbits immunized with decapeptides HRS-(18–27), HRS-

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Abbreviations: HRS, histidyl-tRNA synthetase; AJoA, anti-Jo-1 autoantibody(ies); MHC, major histocompatibility complex.

(33–42), HRS-(411–420), and HRS-(436–445) each gave positive signals in the ELISA when tested against the appropriate hexapeptides on the solid-phase support. Background binding to each hexapeptide in the ELISA was determined by the reactivity of 0.8 μg of IgG from a normal BALB/c mouse and 2.0 μg of IgG obtained from patient 1 before he developed AJoA. This background was subtracted from the absorbance obtained after incubating 0.8 μg of IgG from an HRS-immunized mouse (1.5 ng of anti-HRS antibodies per well) and 2.0 μg of IgG from patient 1 (6.8 ng of AJoA per well), respectively. Significant binding was determined by net absorbance values greater than 3 times the standard deviation of the control values across the entire range of 508 hexapeptides.

RESULTS

The availability of serial serum specimens from a number of myositis patients, including specimens obtained from one patient before he developed disease (patient 1), allowed us to examine the spontaneous AJoA autoimmune response. IgM AJoA was first detected while patient 1 was being seen for dermatitis herpetiformis, an autoimmune skin disease. This was more than 5 months before he had any symptoms, physical findings, or laboratory evidence of muscle disease (Fig. 1*A*). The occurrence in the same patient of these two autoimmune diseases, both of which occur on the same genetic background, has been reported before (11, 20). In this patient, as in the others, the myositis was otherwise typical. It is also typical for myositis-specific autoantibodies, and indeed the majority of autoantibodies found in autoimmune diseases, to be present at the time the patient first seeks medical attention. The opportunity to study the early phases of such responses is, therefore, very rare.

Two months after the appearance of IgM AJoA, still before clinical myositis, and thereafter, IgG1 AJoA dominated the autoantibody response. The AJoA spectrotype profile increased in complexity from a narrow oligoclonal pattern to a broad pattern during this preclinical period and was fully established by the onset of the muscle pains, weakness, fevers, joint pains, and elevated serum levels of muscle-derived enzymes that characterized his clinical illness. Although the AJoA levels rose and fell in proportion to disease activity, in all but the earliest serum specimens in patient 1 the same broad AJoA spectrotype was present. AJoA immunoglobulin κ/λ light-chain ratios determined by ELISA (12) reflected those ratios seen in total immunoglobulins and remained relatively constant in this and several other patients, ranging from 2.3 to 3.1 over years of observation.

An ordinary immune response to an immunizing antigen is characterized by an initial rapid diversification of antibody-producing B-cell clones through somatic hypermutation re-

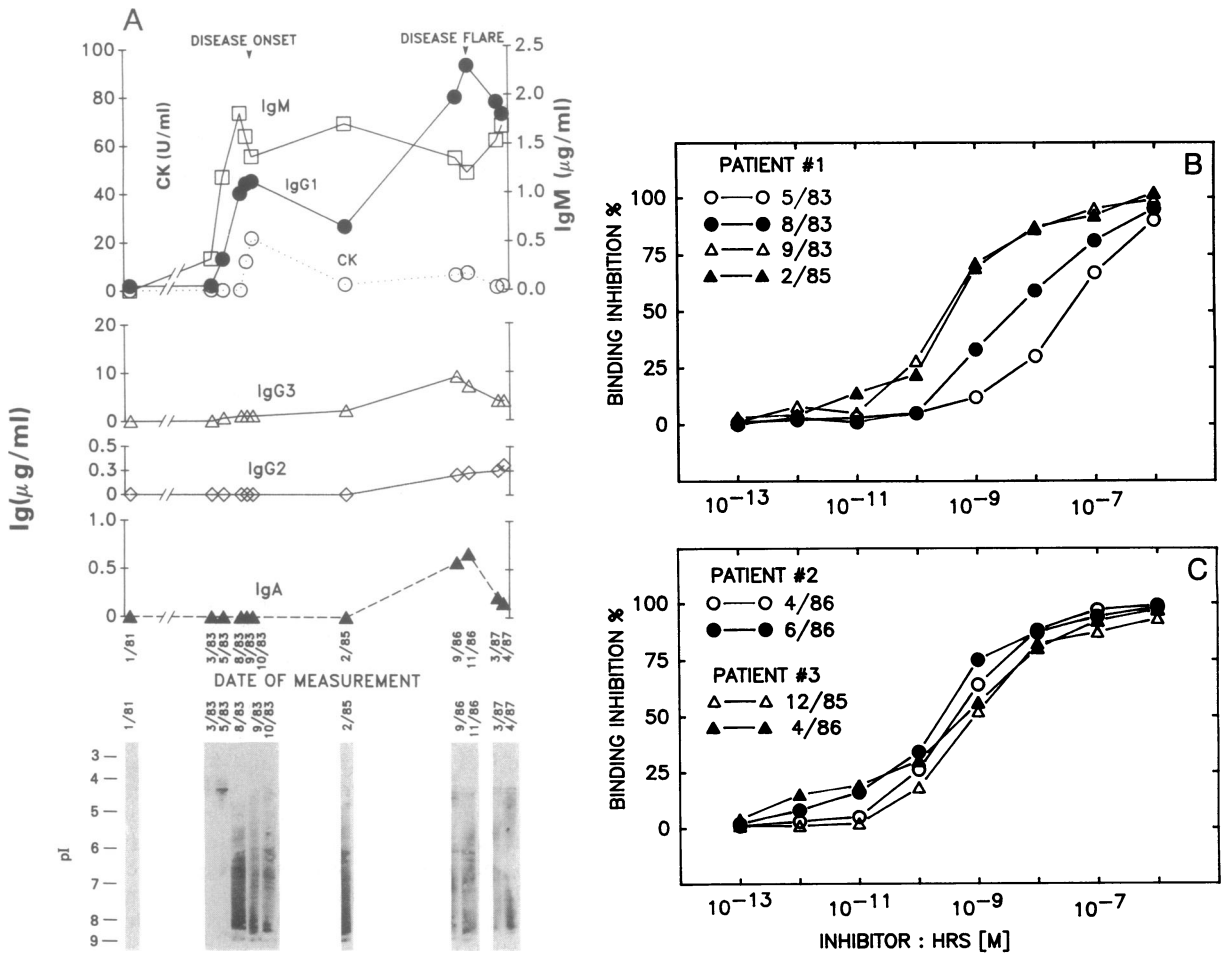

FIG. 1. Serial AJoA classes, subclasses, spectrotypes, and affinities suggest that the targeted autoantigen, HRS, selects for and maintains the autoantibody response. (*A*) AJoA immunoglobulin (Ig) class and subclass levels and isoelectric-focusing spectrotypes (below) as well as serum creatine kinase levels [CK, units (U)/ml] of patient 1 before, during, and after clinical disease onset in 9/83 (month/year). (*B*) Affinity maturation of the AJoA response of patient 1 to HRS, showing increasing affinity in the preclinical period and stable high affinity thereafter. The affinities can be read on the abscissa at the point of 50% binding inhibition. In this experiment, the amount of immunoglobulin was adjusted so that equal quantities of antibodies to HRS, as determined by a standard ELISA, were present in the specimens from the three different time points. (*C*) Stable high-affinity AJoA from two other polymyositis patients (nos. 2 and 3), studied several years after their disease onset.

sulting in increasing affinity of the antibodies for the antigen, followed by stable antibody polyclonality (21, 22) as long as antigen persists (23). The broadening of the AJoA spectrotype pattern in patient 1 was accompanied by a >50-fold rise in affinity of AJoA for human HRS and was followed by persistent high-affinity autoantibodies (Fig. 1*B*). Several other patients studied later in their disease showed similar persistent high-affinity AJoA (Fig. 1*C*) with stable broad AJoA spectrotypes, despite marked changes in AJoA levels associated with changing disease activity. Although it is possible that the earlier lower affinity reflects removal from the circulation of higher-affinity antibodies by binding to circulating antigen, both the extreme lability of HRS (24) and the much greater muscle damage with release of intracellular contents later in the disease course make this highly unlikely. These data, therefore, powerfully suggest that the identified target autoantigen has selected, by affinity maturation, and sustained the persistent AJoA responses in these patients.

Since some theories of molecular mimicry posit that similarities in linear amino acid sequences between autoantigens and infectious agents may account for autoimmunity (3, 4), we searched for linear AJoA epitopes of HRS for a clue to the origins of AJoA. IgG isolated from patient 1, as well as from three other AJoA-positive patients, did not specifically recognize any hexapeptides of human HRS (Fig. 2*B*). The nonlinear nature of autoantibody epitopes may be a general property of many human autoantigens. Patient autoantibodies that bind proliferating-cell nuclear antigen and those which bind the B'' protein from U2 small nuclear ribonucleoprotein do not bind to overlapping pentadecapeptides of proliferating-cell nuclear antigen (25) or to nonapeptides of the B'' protein (W. J. van Venrooij, personal communication), respectively. A peptide of 93 amino acids is required for full anti-mitochondrial autoantibody reactivity with the human pyruvate dehydrogenase-E2 autoantigen (26).

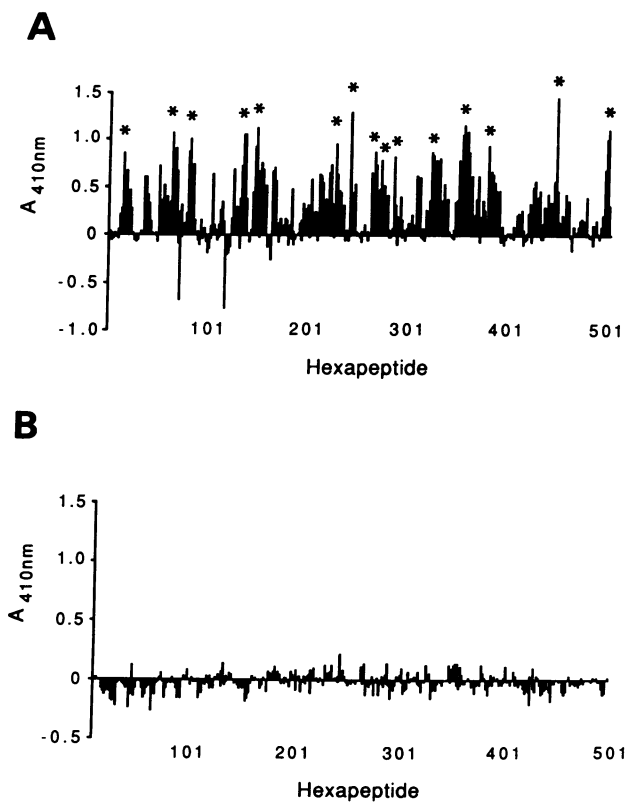

FIG. 2. AJoA, in contrast to mouse anti-HRS antibodies, do not recognize any linear hexapeptides of human HRS. Immunoglobulins from a mouse with high-titer anti-HRS antibodies (*A*) and from patient 1 after developing AJoA (*B*) were tested for reactivity with all 508 possible overlapping hexapeptides of human HRS (18). The asterisks above the peptides denote the area of significant binding, >3 SDs above the mean level of control antibody binding.

The evidence that the AJoA response is initiated and sustained by human HRS suggested that the human autoantigen would be recognized preferentially compared with HRS from phylogenetically distant species. Relative to control immunoglobulins, immunoglobulins from a patient with AJoA inhibited HRS aminoacylation activity in standard assays (1) of human, reptile, amphibian, and fish extracts by 90%, 89%, 83%, and 52%, respectively, but did not inhibit yeast, euglena, amoeba, algal, or bacterial HRS. These data imply that the epitopes recognized by AJoA are relatively conserved among HRS molecules from representatives of the animal kingdom and are not present on HRS from species in the other four kingdoms. They also suggest that the autoantigenic epitopes are preferentially present on the human protein, as appears to be the case for other human autoantigens (27, 28), since HRS inhibition by AJoA decreases with increasing phylogenetic distance from humans.

Although the data above argued against it, we considered further the possibility that the response to the human enzyme had been elicited by a crossreacting HRS of another species by comparing the spontaneous human AJoA response to an antibody response to the same antigen induced in mice, where it would be seen as foreign. Immunization of four mice with HRS led to high-titer antibodies in each, which bound the antigen on Western blots (Fig. 3*A*) and depleted HRS enzyme activity from solution by immunoprecipitation (data not shown). Mouse anti-HRS antibodies also underwent changes indicative of an antigen-driven immune response: antibody class switching, from anti-HRS IgM to IgG to IgA, and an increasingly complex spectrotype. There was a gradual decline in antibody level several months after the last exposure to antigen (data not shown), and the mice did not develop inflammatory muscle disease. As expected from other experiments involving immunization with a foreign protein (29), IgG from mice possessing high-titer anti-HRS antibodies recognized many hexapeptides along the HRS protein sequence in a solid-phase assay (Fig. 2*A*).

A number of other lines of evidence suggest that the human and mouse antibodies recognize different epitopes on HRS. (*i*) All human sera with AJoA, including those from patient 1, inhibited HRS enzyme activity in proportion to the level of AJoA determined by ELISA (17). In contrast, none of the mouse sera containing anti-HRS antibodies inhibited HRS activity (Fig. 3*B*). (*ii*) All patient sera containing AJoA coprecipitated $tRNA^{His}$ along with the enzyme, although AJoA did not bind $tRNA^{His}$ alone. AJoA thus appeared to stabilize the enzyme–tRNA interaction and possibly inhibited HRS function by this mechanism. None of the mouse sera, however, coprecipitated $tRNA^{His}$ (Fig. 3*C*). (*iii*) AJoA from patients did not bind to heat-denatured HRS in a dot blot assay (30) whereas mouse anti-HRS antibodies did. (*iv*) Sera containing AJoA and anti-HRS antisera did not interfere with each other in binding HRS in ELISAs (12), even when the competing antibody was present in great excess (data not shown).

Together, the affinity maturation of AJoA to HRS, the preferential inhibition of human HRS by AJoA, and the differences between the antibody response of a rodent to a heterologous enzyme and the spontaneous human response, all support the view that the human response is directed against its own HRS.

DISCUSSION

The findings described here lead us to conclude that AJoA are driven by the target antigen, are induced months before the onset of myositis, and react only with nonlinear epitopes

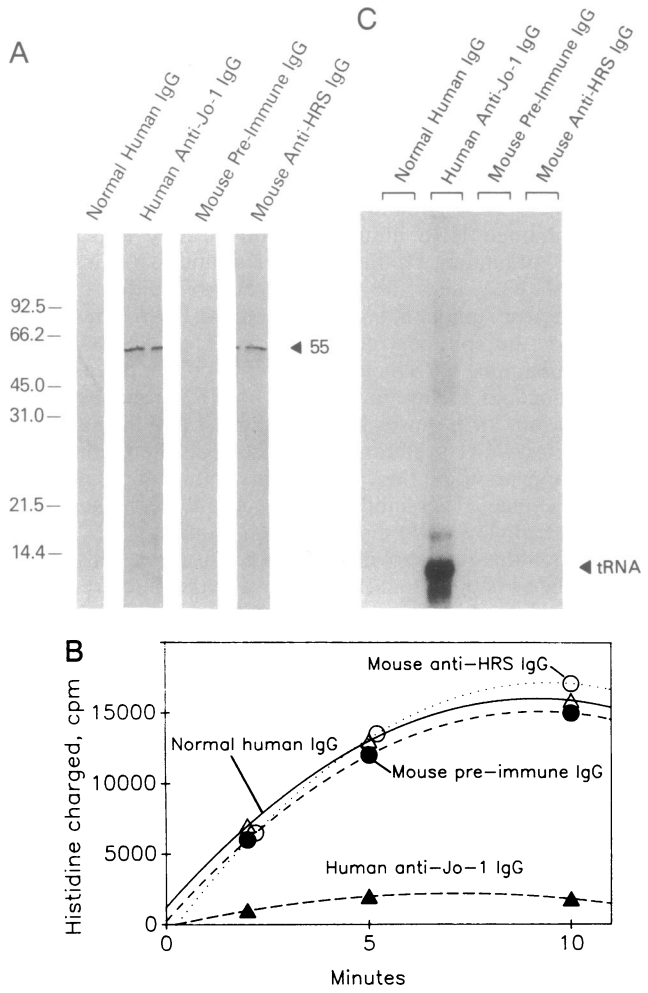

FIG. 3. Human AJoA, but not mouse anti-HRS antibodies, target conformational epitopes on native HRS that stabilize the enzyme–$tRNA^{His}$ interaction and inhibit enzyme function. (*A*) Western blot demonstrating the binding of AJoA and anti-HRS antibodies to the 55-kDa antigen; the migration positions of molecular mass (kDa) markers are shown at left. (*B*) Aminoacylation assays showing HRS enzyme inhibition by AJoA but not by mouse anti-HRS or control antibodies. (*C*) Autoradiograph of urea/polyacrylamide gel electrophoresis of ^{32}P-labeled $tRNA^{His}$ after immunoprecipitation.

preferentially found on the native human target autoantigen, which remain exposed when HRS is complexed to $tRNA^{His}$. These data are consistent with studies of other autoantibodies in patients and mice with autoimmune diseases that in carefully analyzed cases, closely resemble ordinary immune responses inasmuch as they exhibit class switching, somatic mutation, private idiotypes, and polyclonal spectrotypes (7, 12, 31–34). We also describe here a rising affinity over time of the interaction between human HRS and AJoA, which strongly supports the role of the autologous target autoantigen in selecting for as well as sustaining the autoantibody response.

Although polyclonal activation (5), direct (3) and indirect (2) molecular mimicry, increased expression of major histocompatibility complex (MHC) class II antigen (6), and altered autoantigen presentation (1), among other theories, have been proposed as autoimmune mechanisms, none adequately accounts for all the findings in autoimmune diseases. Indeed, multiple mechanisms may be at work simultaneously or in temporal succession in a single autoimmune disorder, and different mechanisms may operate in different diseases. The combined findings of an autoantigen-selecting immune response and the nonlinear nature of the AJoA epitopes powerfully argue against molecular mimicry on the basis of shared linear amino acid sequences as proposed (35) or random polyclonal activation as sufficient explanations for autoimmunity in myositis. They are consistent, however, with models that require a role for autoantigen-induced B-cell clonal expansion, selection, and continued stimulation by the persistence of the autoantigen in its native state. Of most importance, these findings constrain still further the time and the way in which molecular mimicry might play a part in autoimmunity: a molecular mimic could conceivably initiate the differentiation of B cells, which the autoantigen then selects, or the tertiary structure of the mimic must sufficiently resemble the autoantigen so that affinity maturation to the autoantigen occurs. A foreign protein that shares only a short stretch of amino acids identical to the autoantigen, embedded in a nonhomologous polypeptide, is extremely unlikely to fold so as to yield an identical continuous tertiary epitope allowing affinity maturation to the autoantigen (36). In fact, we have been unable to demonstrate binding to native HRS of high-titer rabbit antibodies directed at four decapeptides of the HRS sequence predicted to be antigenic by computer algorithms (29).

An attractive parsimonious hypothesis to account for these and other findings is that autoimmunity resembles standard immunity in the mechanisms by which autoantibodies are selected and are sustained. Although spontaneous autoantibodies and antibodies induced in animals by immunization with human autoantigens both appear to be antigen-driven (7), they often differ in the epitopes they recognize (30, 37–39). In contrast to antibodies produced by immunization, spontaneous autoantibodies preferentially target conformational epitopes exposed after ribonucleoprotein complex formation and often inhibit the function of the autoantigen. These differences in the epitopes targeted in human autoimmunity from those seen in animals immunized with the human autoantigen may occur because of differences, both genetic and environmental, that determine how B cells are exposed to the antigen. Susceptibility to autoimmune diseases is strongly linked to certain MHC class II amino acid sequences located in regions considered to be important for antigen binding (40) and shown to govern immune responses in animals (41). The immune attack on nonlinear epitopes of autologous autoantigens may result from interactions of those autoantigens with specific environmental agents in the context of the disease-associated MHC class II sequences. Such a scenario may occur in polymyositis, where the necessary interaction of translational proteins with nucleic acids from infecting positive-stranded RNA viruses, which have been implicated in the etiology of myositis by many other lines of evidence (11), may be a mechanism by which conformational epitopes on these cytoplasmic proteins become exposed and targeted for immune attack in genetically susceptible individuals.

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1. Mathews, M. B. & Bernstein, R. M. (1983) *Nature (London)* **304**, 177–179.
2. Plotz, P. H. (1983) *Lancet* **ii**, 824–826.
3. Fujinami, R. S. & Oldstone, M. B. (1989) *Immunol. Res.* **8**, 3–15.
4. Query, C. C. & Keene, J. D. (1987) *Cell* **51**, 211–220.
5. Klinman, D. M. & Steinberg, A. D. (1987) *J. Exp. Med.* **165**, 1755–1760.
6. Bottazzo, G. F., Todd, I., Mirakian, R., Belfiore, A. & Pujol-Borrell, R. (1986) *Immunol. Rev.* **94**, 137–169.
7. Plotz, P. H. (1990) in *The Role of Microorganisms in Non-*

infectious Diseases, eds. De Vries, R., Cohen, I. & Van Rood, J. (Springer, Heidelberg), in press.
8. Bunn, C. C. & Mathews, M. B. (1987) *Science* **238**, 1116–1119.
9. Targoff, I. N., Arnett, F. C., Berman, L., O'Brien, C. & Reichlin, M. (1989) *J. Clin. Invest.* **84**, 162–172.
10. Targoff, I. N., Arnett, F. C. & Reichlin, M. (1988) *Arthritis Rheum.* **31**, 515–524.
11. Plotz, P. H., Dalakas, M., Leff, R. L., Love, L. A., Miller, F. W. & Cronin, M. E. (1989) *Ann. Intern. Med.* **111**, 143–157.
12. Miller, F. W., Twitty, S. A., Biswas, T. & Plotz, P. H. (1990) *J. Clin. Invest.* **85**, 468–475.
13. Biswas, T., Miller, F. W., Twitty, S. A. & Plotz, P. H. (1987) *J. Immunol. Methods* **98**, 235–241.
14. Rosa, M. D., Hendrick, J. P., Jr., Lerner, M. R., Steitz, J. A. & Reichlin, M. (1983) *Nucleic Acids Res.* **11**, 853–870.
15. Silberklang, M., Gillum, A. M. & RajBhandary, U. L. (1977) *Nucleic Acids Res.* **4**, 4091–4108.
16. Friguet, B., Chaffotte, A. F., Djavadi-Ohaniance, L. & Goldberg, M. E. (1985) *J. Immunol. Methods* **77**, 305–319.
17. Targoff, I. N. & Reichlin, M. (1987) *J. Immunol.* **138**, 2874–2882.
18. Tsui, F. W. & Siminovitch, L. (1987) *Nucleic Acids Res.* **15**, 3349–3367.
19. Getzoff, E. D., Geysen, H. M., Rodda, S. J., Alexander, H., Tainer, J. A. & Lerner, R. A. (1987) *Science* **235**, 1191–1196.
20. Kalovidouris, A. E., Miller, F. W. & Lawley, T. J. (1989) *Arthritis Rheum.* **32**, 1179–1181.
21. Askonas, B. A., Williamson, A. R. & Wright, B. E. G. (1970) *Proc. Natl. Acad. Sci. USA* **67**, 1398–1403.
22. Siekevitz, M., Kocks, C., Rajewsky, K. & Dildrop, R. (1987) *Cell* **48**, 757–770.
23. Gray, D. & Skarvall, H. (1988) *Nature (London)* **336**, 70–73.
24. Biswas, T., Miller, F. W. & Plotz, P. H. (1988) *FEBS Lett.* **229**, 203–205.
25. Huff, J. P., Roos, G., Peebles, C. L., Houghten, R., Sullivan, K. F. & Tan, E. M. (1990) *J. Exp. Med.* **172**, 419–429.
26. Surh, C. D., Coppel, R. & Gershwin, M. E. (1990) *J. Immunol.* **144**, 3367–3374.
27. Francoeur, A. M., Peebles, C. L., Gompper, P. T. & Tan, E. M. (1986) *J. Immunol.* **136**, 1648–1653.
28. Reichlin, M., Rader, M. & Harley, J. B. (1989) *Clin. Exp. Immunol.* **76**, 373–377.
29. Geysen, H. M., Tainer, J. A., Rodda, S. J., Mason, T. J., Alexander, H., Getzoff, E. D. & Lerner, R. A. (1987) *Science* **235**, 1184–1190.
30. Dang, C. V., Tan, E. M. & Traugh, J. A. (1988) *FASEB J.* **2**, 2376–2379.
31. Shlomchik, M. J., Aucoin, A. H., Pisetsky, D. S. & Weigert, M. G. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9150–9154.
32. Shlomchik, M., Marshak-Rothstein, A., Wolfowicz, C., Rothstein, T. & Weigert, M. (1987) *Nature (London)* **328**, 805–811.
33. Eisenberg, R. A., Dyer, K., Craven, S. Y., Fuller, C. R. & Yount, W. J. (1985) *J. Clin. Invest.* **75**, 1270–1277.
34. Delves, P. J. & Roitt, I. M. (1988) *Clin. Exp. Immunol.* **71**, 459–463.
35. Walker, E. J. & Jeffrey, P. D. (1988) *Med. Hypotheses* **25**, 21–25.
36. Barlow, D. J., Edwards, M. S. & Thornton, J. M. (1986) *Nature (London)* **322**, 747–748.
37. Chan, E. K. & Tan, E. M. (1987) *J. Exp. Med.* **166**, 1627–1640.
38. Portanova, J. P., Cheronis, J. C., Blodgett, J. K. & Kotzin, B. L. (1990) *J. Immunol.* **144**, 4633–4640.
39. Mimori, T., Hardin, J. A. & Steitz, J. A. (1986) *J. Biol. Chem.* **261**, 2274–2278.
40. Todd, J. A., Acha-Orbea, H., Bell, J. I., Chao, N., Fronek, Z., Jacob, C. O., McDermott, M., Sinha, A. A., Timmerman, L., Steinman, L. & McDevitt, H. O. (1988) *Science* **240**, 1003–1009.
41. Hochman, P. S. & Huber, B. T. (1984) *J. Exp. Med.* **160**, 1925–1930.