AN IMMUNOGLOBULIN LIGHT CHAIN FROM A LUPUS-PRONE MOUSE INDUCES AUTOANTIBODIES IN NORMAL MICE

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Identification of the immunogens responsible for the production of pathogenic autoantibodies is a major problem in the field of autoimmunity. In the principal diseases that are thought to involve autoimmunization, rheumatoid arthritis, type I diabetes, and multiple sclerosis are examples, the instigating stimulus is unknown. Another example of the gap in our knowledge of disease-causing immunogens can be found in SLE. The characteristic autoantibody of lupus binds to double-strand (ds) DNA yet this nucleic acid antigen is barely immunogenic, even in lupus-prone MRL-1pr/lpr mice (1). Certain bacterial DNAs can elicit anti-dsDNA antibodies in normal mice, but those antibodies and the spontaneously produced anti-DNA antibodies of SLE have different serological specificities (2). By contrast with the poor immunogenicity of mammalian dsDNA, other forms of DNA, such as Z-DNA (a double-strand molecule with a leftward spiraling helix instead of the right-handed helical structure of dsDNA), elicit brisk immune responses in MLR-1pr/lpr mice as well as in normal mice and rabbits (1, 3). The lack of immunogenicity of exogenous dsDNA does not disprove the participation of endogenous DNA in the origins of anti-DNA antibodies, however. Cell nuclei, for example, might release a form of dsDNA that is peculiarly immunogenic in patients with SLE.

In addition to nucleic acids, several polypeptides associated with uridine-rich small nuclear ribonucleoprotein (snRNP) particles are also important autoantigens in SLE. Patients with SLE and associated diseases (mixed connective tissue disease, scleroderma, rheumatoid arthritis, and Sjögren's syndrome) produce two categories of autoantibodies against these particles: anti-RNP antibodies, which bind the U1 snRNP-specific polypeptides 70 kD, A, and C, and anti-Sm antibodies, which bind to a shared epitope in the B', B, and D snRNP polypeptides (4). Anti-RNP autoantibodies, according to one hypothesis, originate from the release of snRNP antigens from the nuclei of dead or injured cells (5). The finding that normal mice respond to immuni-
zation with a U1 RNP polypeptide (6) supports the view that endogenous nuclear autoantigens can be immunogenic. However, this idea does not explain why the usual daily turnover of nucleated cells does not elicit anti-RNP autoantibodies in normal subjects. The observations that some mAbs can bind to both DNA and RNP (7), and that anti-DNA and anti-RNP antibodies can share the same idiotypic marker (8), suggest a link between anti-DNA and anti-RNP antibodies.

In this paper we report evidence that autoantibodies against U1 RNP, dsDNA, and ssDNA can be elicited in normal mice by a purified Ig light chain. The third hypervariable region of this light chain has two remarkable features: (a) it specifies an idiotype that is a component of a network of anti-DNA and anti-snRNP autoantibodies in MRL-lpr/lpr mice, and (b) of its 9 amino acids, a sequence of seven residues has strong homology to a sequence in the U1 RNP autoantigen. This model system demonstrates how autoantibodies can arise by autoimmunization with idiotypes that mimic autoantigens. It also identifies an amino acid sequence with the capacity of eliciting anti-DNA antibodies.

Materials and Methods

Preparation of Rabbit Anti-Id-28/12. A rabbit was immunized subcutaneously with 150 μg of affinity-purified mAb 28/12 in CFA and then boosted biweekly three times with 100 μg of the antibody in IFA. Sera that contained the highest anti 28/12 activity were pooled and made specific for the immunizing idiotype by repeated adsorptions on Sepharose 4B coupled to pooled mouse IgGs (TEPC 183, MOPC 104E, UPC 10, FLOPC, and MOPC 141, all purchased from Litton Bionetics, Inc., Charleston, SC). The purified rabbit anti-28/12 was used in a dilution of 1:2,000. With that dilution on the solid phase, 200 μl of biotin-labeled mAb 28/12 gave an OD405 of 0.600 within 20 min. The purified anti-28/12 did not bind to light chains F, G, and 105 (see below).

Detection of Id-28/12. Polystyrene plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, VA) were coated with 50 μl rabbit anti-28/12 antiidiotype diluted 1:2,000 in 0.05 M borate buffer, pH 8.6, and blocked with 3% BSA in PBS. PBS containing 1% BSA and 0.05% Tween was used to dilute labeled and unlabeled antibodies. 25 μl of biotin-labeled mAb 28/12 (0.1 μg/ml) (9), at a concentration corresponding to 50% maximal binding to the antiidiotype reagent, was incubated on the plate together with the test antibody for 1 h at 37°C. After three washes in PBS/BSA/Tween, avidin-alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) was added and the bound enzymatic activity measured.

Antigen Binding. DNA and SmRNP binding by ELISA, Western blots, and liquid phase competition assays were conducted as described in detail elsewhere (9). The snRNP antigens were affinity purified from rabbit thymus extract on a mAb 28/12-Sepharose 4B column (9).

DNA Sequence of Light Chain G. Total RNA was isolated from hybridoma cells with guanidine isothiocyanate, and polyadenylated mRNA was prepared by passage over oligo(dt)-cellulose. cDNA, produced as previously described (10) with a 17-nucleotide 5' CS region primer (TGGATGGTGAGGAGATG) was ligated into M13 mpl8 and plaques were screened with a 35P-labeled 3 kb probe obtained from S. Lewis and D. Baltimore (II). Positive clones were sequenced by the dideoxy method (12).

Immunization of Mice. Light chain G was affinity purified on a goat anti-mouse IgG-Sepharose 4B chain and cross-linked to KLH with 0.25% glutaraldehyde. BALB/c mice (6 wk old) were injected three times intraperitoneally with 10 μg of light chain G conjugated to 40 μg KLH in RIBI adjuvant (RIBI Immunochemical Research, Hamilton, MT) at 15-d intervals. Control animals were given adjuvant alone or MOPC 104E in adjuvant in the same schedule.

Anti-peptide Antibodies. Purified synthetic peptides corresponding to the CDR3 of light chain G, to residues 117-123 plus flanking residues of U1 RNP and to the CDR3 of mAb R16.7 were a gift from Dr. Mai Chang Kuo (Immulogic Pharmaceutical Corp., Cambridge,
For detection of anti-peptide antibodies in serum, Immunolon II plates were coated overnight at 4°C with 5 μg/ml of peptide in PBS. The plates were blocked with PBS containing 3% BSA and 3% nonfat dry milk. Mouse serum diluted in PBS containing 2% nonfat dry milk was added to the peptide-coated plate and incubated for 2 h at 37°C. After washing, alkaline phosphatase goat anti-mouse IgG was added, the plates were incubated overnight at 4°C, and bound enzymatic activity was measured. For competitive assays, dilutions of serum corresponding to 50% maximal binding to peptide LCG G were incubated for 1 h at 37°C with increasing amounts of inhibitor peptide and transferred to a coated and blocked plate. The remainder of the assay was carried out as described above.

Results

**Definitions of Ab-1 and Ab-2.** The experiments reported here originated from studies of an idiotypic marker of MRL-lpr/lpr anti-DNA antibodies termed Id-H130. A substantial fraction of serum anti-DNA antibodies in that strain bear the H130 idiotypic, but the majority of Id-H130+ antibodies do not bind to DNA (13). To define the antigen-binding specificities of the latter, hybridomas derived from MRL-lpr/lpr mice were screened for Id-H130 and the Id-H130+ antibodies were then tested to exclude antibodies that bound to either ssDNA or dsDNA. Of the group of monoclonal antibodies identified in this manner, Id-H130+ mAb 28/12 was selected for further study because of its specificity for snRNP antigens (9). This mAb is of additional interest because it was found to be an anti-idiotype against 63/411 unselected MRL-lpr/lpr mAb (Koizumi, T., A. Puccetti, P. Migliorini, K. J. Barrett, R. S. Schwartz, manuscript in preparation). We will refer to mAb 28/12 as antibody-1 (Ab-1) and to the latter set of antibody-2 (Ab-2).

**Light Chains of Ab-2.** Three hybridomas (F, G, and 105) in the antibody-2 panel were found to produce only light chain dimers: the hybridoma products reacted with anti-k serum, but not with anti-μ or anti-γ chain antisera; only a 28-kD light chain band was present on SDS-PAGE of the Igs; and there was selective absence of Cμ and Cγ mRNA (data not shown). Biotin-labeled mAb 28/12 bound quantitatively to each of the three light chains (Fig. 1A); the possibility that the MOPC 21 light chain, produced in small amounts by the NS1 fusion partner, entered into those reactions is unlikely because biotin-labeled mAb 28/12 does not bind to MOPC 21 (9). Each of the three light chains inhibited the binding of biotin-labeled mAb 28/12 to a rabbit anti-28/12 idiotype in liquid phase competition assays (Fig. 1B). These
results indicate that the light chain contains a major component of the Ab-2 idiotypic recognized by mAb 28/12.

A remarkable feature of the Ab-2 panel recognized by mAb 28/12 is that almost one-third of its members are, like mAb 28/12 itself, anti-snRNP antibodies (Koizumi, T., et al., manuscript in preparation). This suggested the possibility that some of the Ab-2s we studied might contain a structure, perhaps in their light chains, that resembles the snRNP antigen to which mAb 28/12 binds. To test this hypothesis, we determined whether the light chains F, G, and 105 could compete against snRNP autoantigens for binding to mAb 28/12. Fig. 2 shows the result of this experiment: all three light chains inhibited the binding of mAb 28/12 to purified snRNP antigens in a liquid phase assay.

The Amino Acid Sequence of Light Chain G. To investigate the structural basis of idiotypic and autoantigen-mimicking properties of Ab-2 light chains, the nucleic acids encoding the variable region of light chain G were sequenced and compared with data banks of sequenced genes and proteins. Two of the related sequences were of substantial interest: the light chain of R16.7, a mouse anti-arsonate antibody, and the light chain of MOPC 173, a mouse myeloma protein (14, 15). They are shown, with the deduced amino acid sequence of light chain G, in Fig. 3. The VL regions of R16.7 and light chain G are identical except for two framework residues at positions 80 and 87, but their CDR3s differ considerably. By contrast, the CDR3s and framework regions of the VL regions of MOPC 173 and light chain G are identical, whereas their first two hypervariable regions differ. It is unlikely that the CDR1, CDR2, or frameworks specify the idiotype of light chain G since R16.7 does not bind to mAb 28/12. MOPC 173 does bind to 28/12, however (Fig. 4 A). Furthermore, MOPC 173 was found to compete in a liquid phase assay with purified RNP for binding to 28/12 (Fig. 4 B). This evidence suggests that the relevant idiotypic and immunochemical properties of light chain G are specified by its CDR3, either as a linear sequence or in a conformation made up of the CDR3 and some other part of the variable region of the light chain.

The interest in light chain G was heightened by the homology of a region of its CDR3 and an adjoining residue of its framework 4 with residues 117-123 within the RNA binding domain of the human U1 RNP polypeptide (16):

LCG  Ser-Lys-Leu-Pro-Arg-Thr-Phe
RNP  Ser-Lys-Leu-Arg-Arg-Glu-Phe

FIGURE 2. Inhibition of binding of biotin-labeled 28/12 to snRNP by light chains. Biotin-labeled 28/12 was incubated for 1 h at 37°C with light chains and then transferred to Immulon II plates coated with affinity-purified nucleoprotein antigens (2.5 μg/ml). Inhibition was detected as described in A.
Immunogenic Properties of Light Chain G. To test the biological implications of these immunochemical and structural findings, we immunized 6-wk-old BALB/c mice with immunoaffinity-purified light chain G. If the autoantigen-mimicking idiootype of light chain G (Ab-2) is biologically relevant it should, in principle, evoke the production of antibodies with a resemblance to Ab-1, the anti-snRNP 28/12 mAb (16). Groups of control mice that were not injected, injected with adjuvant alone, or immunized with an adjuvant mixture containing 10 µg MOPC 104E, an IgM antidextran mAb that does not bind to any snRNP antigen or to DNA, were also tested. Fig. 5 A shows that anti-RNP antibodies developed in all BALB/c mice immunized with light chain G. The anti-RNP antibodies appeared in relatively high titers within 4 wk after immunization and remained high for at least 3 mo. None of the control mice produced such autoantibodies. Fig. 5 B shows Western blots of serum obtained from BALB/c mice before and after immunization with light chain G. Pre-immune serum contained no detectable anti-snRNP antibodies, whereas serum obtained after immunization reacted with a 70-kD RNA polypeptide, consistent with 70 kD U1 RNP, and an unidentified 52-kD band. Some human anti-RNP sera have also been noted.
FIGURE 5. (A) Production of anti-RNP antibodies by mice immunized with light chain G. The animals received three intraperitoneal injections of the light chain (10 μg conjugated to 40 μg of keyhole limpet hemocyanin) in RIBI adjuvant at 15-d intervals. Preimmunization antibody levels are shown at time 0; subsequent bleedings were obtained 4, 6, and 12 wk after the last injection. Bars represent the mean ± SE of serum antibody activity. All experimental mice (black bars) produced anti-RNP antibodies. That group consisted of three animals in which the initial observations were made plus five mice in which the results were reproduced. The injected controls (15 mice) were given either RIBI adjuvant alone or MOPC 104E plus adjuvant in the same schedule as the experimental groups. Results for all 15 control mice were pooled. ELISA was performed with a 1:200 dilution of serum and purified snRNP antigens. (B) Western blots of affinity-purified snRNP antigens probed with serum from mice injected with light chain G. (Lanes 1–3) Serum from three mice 4 wk after the last injection of the light chain. (Lanes 4–6) Serum from the same animals before immunization. The upper arrow points to a MADRNP polypeptide antigen; the lower arrow shows a 52-kD band, probably a degradation product of the 70-kD antigen. (C) Anti-ss DNA antibodies in serum of mice immunized with light chain G. The serum samples shown in A were tested for anti-DNA antibodies with a direct ELISA assay.
FIGURE 6. (A) Reaction of mAb 8B, produced by a hybridoma obtained from a BALB/c mouse immunized with light chain G, with snRNP antigens. (Inset) Speckled nuclear fluorescence (HEP-2 cells) typical of anti-RNP antibodies, produced by mAb 8B. (B) Reaction of mAb 21/6, also from a BALB/c mouse immunized with light chain G, to nucleic acid antigens. Note the reaction with dsDNA in this liquid phase competition assay. The strong binding to poly(dT) is typical of lupus anti-DNA mAbs. (C) Binding of serum antibodies from mice immunized with light chain G to synthetic peptides corresponding to the CDR3 of light chain G (open bars) and to residues 117-123 plus flanking residues of U1 RNP (hatched bars), but not to a control peptide, the CDR3 of anti-arsenate antibody R 16.7 (black bars). For each pair the left bar is the mean + SE of preimmunization serum and the right bar is the mean + SE for serum obtained 12 wk after the last injection of light chain G. The specificity of these results was confirmed in 6/7 sera by cross-competitive inhibition assays of the three synthetic peptides, a representative example of which is shown in Fig. 6 D. These findings demonstrate that the CDR3 of light chain G contains an immunogenic re-
region capable of stimulating normal mice to produce antibodies against an amino acid sequence in the NH$_2$-terminal region of the U1 RNP autoantigen. It is notable that the region in U1 RNP that is homologous to the CDR3 of light chain G occurs in a domain containing a major antigenic determinant for human anti-RNP antibodies (17).

**Anti-DNA Antibodies in Mice Immunized with Light Chain G.** Besides its structural similarity to a region of the 70 kD U1 RNP autoantigen, the CDR3 of light chain G also contains an idiotype recognized by mAb 28/12 (Fig. 1). In theory (18), this idiotype has the potential to stimulate the production of antibodies with the idiotype of mAb 28/12, but with specificities other than anti-snRNP. Since mAb 28/12 shares an idiotypic marker with some anti-DNA antibodies (9), we sought anti-DNA antibodies in the animals immunized with light chain G. After receiving light chain G, all eight mice produced anti-ss DNA antibodies within 3 mo; two animals also had antibodies against dsDNA in their serum. The maximum response was observed about 2 mo after peak levels of anti-RNP antibodies were reached. Anti-DNA antibodies were not found in any of the control animals (Fig. 5 C).

**Binding Properties of mAbs from Mice Immunized with Light Chain G.** Direct validation of the specificities of the serum autoantibodies was sought by analysis of mAbs from animals that had produced antibodies against RNP, ssDNA, and dsDNA. Hybridomas were prepared from three such mice, and 127 mAbs were found to have the following specificities: anti-RNP (n = 16), anti-ss DNA (n = 31), anti-dsDNA (n = 4); anti-RNP + anti-ssDNA (n = 58). mAbs of each of those specificities were obtained from all three animals. Examples of the binding properties of two of these mAbs are given in Fig. 6 A for an anti-RNP mAb (mAb 8B) and in Fig. 6 B for a mAb with reactivity against both ssDNA and dsDNA (mAb 21/6). The inset of Fig. 6 A shows the typical speckled nuclear staining pattern of anti-RNP antibodies, produced by antibody 8B. Of the 127 mAbs, 18, including the antibody shown in Fig. 6 B, shared an idiotypic marker with the original 28/12 anti-snRNP antibody. A detailed idiotypic and structural study of this group of antibodies will be the subject of a separate report.

**Discussion**

Our finding that the CDR3 of an Ab-2 is structurally homologous to an autoantigen has several precedents. Fougereau's group (19) analyzed an idiotypic network evoked in mice by immunization with a random copolymer of glutamine, alanine and tyrosine (GAT): Ag $\rightarrow$ Ab-1 $\rightarrow$ Ab-2 $\rightarrow$ Ab-3. The Ab-1s and Ab-3s that expressed the dominant anti-GAT idiotype used closely related VH and Vx genes. The D region of the Ab-2s contained either a Tyr-Tyr-Glu or a Glu-Glu-Tyr sequence, which are characteristic of the major epitopes of GAT. This D region may therefore contain an internal image of the immunogen. Bona et al. (20) have described an antiidiotype that reacted with both an idiotype of a monoclonal IgG human rheumatoid factor and the Fc fragment of human IgG. They called this doubly reactive antiidiotype an *epibody*, an antibody that recognizes both an idiotype and an epitope on the original antigen. Subsequently, Chen et al. (21) found a second antibody example of an epibody: an antiserum against a synthetic peptide corresponding to the first hypervariable region of the $\lambda$ light chain of a human rheumatoid factor.
was found to bind pooled normal human IgG. This crossreaction involving the VL region of an Ig and the Fc domain of IgG was attributed to a Ser-Ser-Ser peptide in both the γ chains of IgG and the light chain of the rheumatoid factor. Bruck et al. (22) reported that an Ab-2 against an antireovirus hemagglutinin antibody contained an amino acid sequence that was also present in the viral antigen. Van Cleave et al. (23) described two mouse monoclonal Ab-2s that mimic the rabbit Ig al allotype. The CDR2s of the heavy chains of both antibodies contained sequences corresponding to the presumed mAb internal image, but in opposite orientation. Synthetic peptides corresponding to the internal image completely inhibited the binding of rabbit Ig to the anti-al antibody despite their opposite orientations.

A topic of considerable current interest is whether idiotypic networks can influence the process of autoimmunization. Such networks may modulate the production of autoantibodies via auto-antiidiotypes, or they might actually induce autoantibodies if the variable region of an Ab-2 imitates the structure or contact region of an autoantigen (the internal image effect, reference 24). The induction, by immunization of antiidiotypic antibodies that mimic self structures, has been reported by others (25–28). An example of this phenomenon is the development of myasthenia gravis in rabbits immunized with BisQ, a synthetic compound that binds to the acetylcholine receptor. Ab-2s against anti-BisQ antibodies (Ab-1) in the serum of the affected rabbits had properties of antiacetylcholine receptor antibodies (29). Another example was found in mice immunized with a human anti-DNA mAb. The animals produced antiidiotypic antibodies against the human Ig and also anti-antiidiotypes that had anti-DNA and other autoantibody activity (30). In this model the injected antibody probably triggered a cascade of idiotypes which culminated in a population of autoantibodies. Iribe et al. (31) have shown that injection of a rabbit antiidiotype against a murine anti-type II collagen mAb evoked the production of anti-type II collagen antibodies in DBA mice.

The Ig light chain we studied has two remarkable features: it contains a molecular mimic of a nuclear RNP autoantigen, and the very region of that mimicry functions as an idiotype. We propose that these dual properties can explain the ability of the light chain to elicit both anti-RNP and anti-DNA antibodies: the former probably arise from the structural similarities to U1 RNP, whereas the latter might originate from perturbation of idiotypic networks.

The autoantibodies produced by MRL-lpr/lpr mice have been attributed to polyclonal B cell activation, an important abnormality in that strain and in other lupus-prone mice (32). However, structural analysis has shown that IgG MRL-lpr/lpr anti-DNA antibodies arise from a limited number of B cell clones by a process of somatic mutation, a result consistent with a selective mechanism driven by antigen (33). Our data provide a basis for reconciling three puzzling aspects of the origins of anti-DNA antibodies: the lack of immunogenicity of DNA, the evidence for an antigen-driven mechanism, and the prominence of polyclonal B cell activation. Nonspecific expansion of the B cell compartment could increase the probability of the expression of autoantigen-mimicking idiotypes, which might in turn participate in the selection of autoantibody-producing clones. The activation of B cells by peptide fragments of V region (idiopeptides) is predicted by the “peptide self” model of Kourilsky et al. (34).
The finding that the region with sequence homology to an antigenic region of the U1 70-kD RNP in light chain G resides in its somatically generated CDR3 has another interesting implication. Autoantigen-mimicking idiotypes arising as a result of somatic rearrangements and insertion of N sequences in variable region genes might explain some of the perplexing, apparently random events in many autoimmune diseases. The production of anti-Sm antibodies in only ~30% of patients with lupus is an example (35). Anti-Sm antibodies develop in a similar minority of MRL-lpr/lpr mice even though they are an almost genetically uniform inbred strain. A thorough analysis has disclosed that stochastic events probably determine the production of anti-Sm autoantibodies in MRL-lpr/lpr mice; there was no evidence for genetic, environmental, gender, or parental influences on the tendency of individual animals to produce those antibodies (36). Whether somatically generated idiotypes could provide the stimulus that leads to pathogenic autoantibodies and tissue lesions is now under investigation.

Summary

Autoantibodies against the 70-kD U1 RNP nucleoprotein autoantigen and DNA were elicited in normal BALB/c mice with a purified Ig light chain. This light chain, derived from a lupus-prone MRL-lpr/lpr mouse, has two distinctive properties: it contains an idiotypic marker recognized by a monoclonal MRL-lpr/lpr anti-snRNP autoantibody, and the amino acid sequence of its third hypervariable region (CDR3) is homologous to a sequence in an antigenic region of the 70-kD U1 RNP polypeptide. The results demonstrate that an Ig idiotype that mimics an autoantigen can induce autoimmunization.

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References


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