Anti-P autoantibody production requires P1/P2 as immunogens but is not driven by exogenous self-antigen in MRL mice.

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ANTI-P AUTOANTIBODY PRODUCTION REQUIRES P1/P2 AS IMMUNOGENS BUT IS NOT DRIVEN BY EXOGENOUS SELF-ANTIGEN IN MRL MICE

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Considerable evidence supports the idea that autoantibody production in human and murine SLE is Ag driven. To determine whether Ag (the ribosomal P proteins) could initiate autoantibody production in lupus mice, 34 MRL/lpr mice were immunized with mouse ribosomal proteins in Freund's adjuvant. Neither intact ribosomes, denatured total mouse ribosomal proteins, nor the purified mouse ribosomal proteins, P1 and P2, induced the production of anti-P autoantibodies in the MRL/lpr mice. In contrast to these negative findings, MRL/lpr mice immunized with Artemia salina ribosomes produced anti-P antibodies as well as anti-P autoantibodies. Although the induced anti-P autoantibodies bound exclusively to the carboxyl terminus, these anti-P antibodies differed from spontaneously occurring anti-P autoantibodies in their predominant binding to mouse P0 on immunoblots and their preferential reactivity against A. salina synthetic peptides by ELISA. Induction of anti-P antibodies required the presence of P1 and P2 on the ribosome because ribosomal cores devoid of P1 and P2 dimers did not induce anti-P. Despite the presence of approximately 80 ribosomal proteins, autoantibodies to other mouse ribosomal proteins were rarely observed. Immunization of MRL/+ mice and a normal H-2-matched strain of mice, C3H, also resulted in anti-P antibodies reactive with the A. salina P proteins and mouse P0. Whereas anti-P levels gradually declined in C3H mice, anti-P levels either remained elevated (MRL/lpr) or showed a secondary rise (MRL/+ at the onset of autoimmunity. These observations indicate that: i) high levels of autologous Ag are not sufficient to drive antiribosomal autoantibody production in MRL mice, ii) multivalency of the P proteins may explain their potent immunogenicity and ability to break tolerance, and iii) immunized MRL mice show an abnormal persistence of high level anti-P production presumably reflecting T cell activation of presensitized B cells. MRL/lpr mice produce autoantibodies with similar specificities to patients with SLE. In addition to anti-DNA, anti-cardiolipin, and rheumatoid factors, the mice produce antibodies against intracellular proteins including histones (1), Sm (2), and the ribosomal P (3) and S10 (4) proteins. Approximately 10% of MRL/lpr mice spontaneously develop anti-P antibodies after the age of 3 mo (3). As in human SLE (5, 6), MRL/lpr anti-P antibodies selectively target the conserved C terminus3 that is shared by P0, P1, and P2 (3).

Although the stimulus for autoantibody production remains unknown, many recent studies in either SLE patients or MRL/lpr mice suggest that autoantibody production is Ag driven. The evidence supporting this hypothesis includes the limited number of self-proteins recognized as Ag (7–9), the absence of random polyclonal B cell activation (10), multiple epitopes on large autoantigens (reviewed in Reference 11), and the immunochromical (12, 13) and molecular genetic (14) properties of the autoantibodies as well as a role for T cells (15). Although a target autoantigen (thyroglobulin) has been shown to be necessary for autoantibody production in the Obese strain of chickens with autoimmune thyroiditis (16), there is little direct evidence indicating that self-Ag stimulates autoantibody production in MRL mice. In order to approach this question, we analyzed the humoral immune response of MRL mice to syngeneic ribosomes. To determine whether anti-P autoantibodies induced by xenogenic ribosomes were similar to anti-P antibodies produced spontaneously, we analyzed the fine specificity of anti-P by using a panel of synthetic peptides. Finally, because repetitive epitopes have been reported to be important for autoantibody production, we compared the ability of ribosomes with only one P protein and ribosomes with all five P proteins to induce anti-P autoantibodies.

MATERIALS AND METHODS

Mice. MRL/lpr (MRL/Mp-lpr/lpr), MRL/Mp-+/+ (MRL/+) and C3H (C3H/HeSnJ) mice were originally obtained from the Jackson Laboratory, Bar Harbor, ME. BALB/c mice were obtained from Charles River Laboratories, Wilmington, MA. Mice were bred in the small animal quarters at the Hospital for Special Surgery, Cornell University Medical Center, New York, NY.

Ag. Ribosomes were isolated from rabbit reticulocytes, HeLa cells (a human cervical cell line), and MRL/lpr livers as described previously (4). In brief, the cells were broken and the mitochondria and nuclei removed by centrifugation. The ribosomes were then pelleted through a discontinuous sucrose gradient containing 500 mM KCl in a Sw 27 ultracentrifuge rotor. Total ribosomal proteins were obtained from MRL/lpr liver ribosomes by acetic acid extraction and acetone precipitation (17). These proteins are referred to as denatured rabbit reticulocyte, HeLa, and MRL/lpr ribosomes, respectively.

Abbreviations used in this paper: C terminus, carboxyl terminus; rHuP2, recombinant human P2 protein fused to β-galactosidase; A-C22, A. salina C22; Hc-C22, human C22; A.C11, A. salina C11.
tured ribosomal proteins. Ribosomes were isolated from Artemia salina cysts according to the method of Zasloff and Ochoa (18). The ribosomal pellets were then washed several times with buffer containing 500 mM KC1 to remove contaminating proteins (19). Intact ribosomes had A 260/280 ratios of 1.6 to 1.7. Total protein concentrations were measured by the method of Bradford (20) using OVA as a standard. Ribosomal proteins P1 and P2 were specifically extracted from intact ribosomes by 50% ethanol and 80 mM KC1 according to the method of McConnell and Kaplan (21). The proteins were pure as determined by SDS-PAGE and silver staining (22). A rhUPII was synthesized in Escherichia coli and isolated to >95% purity (23). Synthetic peptides corresponding to the carboxyl termini of the A. salina P12 (P2 homologue) (24) and human P2 (25) proteins were synthesized by stepwise phase methods as described previously (5, 6).

***RESULTS***

**Immunization with intact mouse ribosomes, denatured mouse ribosomal proteins, or purified P proteins.** To determine whether self-Ag could readily break tolerance and induce autoantibodies in an autoimmune strain of mice (MRL/lpr) known to produce anti-P (3, 27), 10 MRL/lpr and 10 BALB/c mice were immunized with ribosomes derived from MRL/lpr mouse livers. Age- and sex-matched control mice were immunized with BSA. None of the MRL/lpr or BALB/c mice immunized with mouse ribosomes developed antiribosomal antibodies on immunoblots or anti-P antibodies as determined by ELISA using the rhUPII as Ag. All of the BALB/c and 9/10 MRL/lpr mice immunized with BSA showed reactivity to BSA on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A). When mouse ribosomes were used as target Ag on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A). When mouse ribosomes were used as target Ag on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A). When mouse ribosomes were used as target Ag on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A). When mouse ribosomes were used as target Ag on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A). When mouse ribosomes were used as target Ag on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A). When mouse ribosomes were used as target Ag on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A). When mouse ribosomes were used as target Ag on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A). When mouse ribosomes were used as target Ag on immunoblots at bleed 1 and usually increased in intensity by bleed 3. Sera tested from the third bleed revealed that all of the MRL/lpr mice reacted against an A. salina protein of approximately the same m.w. as P0 and that 9/14 mice had an anti-P pattern of reactivity identical to the control anti-P-positive mouse (MRL/lpr mouse spontaneously producing autoantibodies reactive with P0, P1, and P2) (Table I and Fig. 1A).
TABLE I  Detection of anti-P antibodies by immunoblotting early (bleed 3) or late (bleed 5) after immunization with A. salina ribosomes

<table>
<thead>
<tr>
<th>bleed 3</th>
<th>bleed 5</th>
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<tbody>
<tr>
<td>A. salina</td>
<td>Mouse</td>
</tr>
<tr>
<td>No.</td>
<td>P0</td>
</tr>
<tr>
<td>MRL/+</td>
<td>14</td>
</tr>
<tr>
<td>C3H</td>
<td>12</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>11</td>
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<tr>
<td>C3H</td>
<td>6</td>
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Figure 1. Antiribosomal antibodies and anti-P autoantibodies in immunized mice. A total of 40 μg/lane of A. salina ribosomes (A) or MRL/lpr mouse liver ribosomes (B) were resolved by SDS-PAGE, blotted to nitrocellulose and probed with a 1/500 dilution of sera obtained from C3H, MRL/lpr, or MRL/++ mice as indicated above each figure. All of the sera except the negative control (open arrow) and positive control (black arrow) were from mice immunized three times with A. salina ribosomes according to the standard protocol. The results shown are those from third bleed. The positions of P0, P1, and P2 are indicated.

Figure 2. Longitudinal study of anti-P levels in immunized mice. A. MRL/lpr and C3H mice were immunized with A. salina ribosomes and bled on days 21 (bleed 1), 35 (bleed 2), 49 (bleed 3), 70 (bleed 4), and 91 (bleed 5). Sera were diluted 1/200 and tested for reactivity against C22-A by ELISA. The mean and SE are shown for each bleed. Immunizations are indicated by V. The triangle (A) in A is the mean anti-P level obtained from five 4-month-old nonimmunized MRL/lpr mice that were negative for anti-P antibodies on immunoblots. B, the identical immunization protocol was performed on MRL/+ and C3H mice except that two additional bleeds were taken, 5 (bleed 6) and 6 (bleed 7) mo after the last boost. The asterisks indicate mean levels of anti-P antibodies statistically significantly higher in MRL mice compared with the C3H control mice (p < 0.05).

bleeds 1 to 5 appeared to parallel the C3H anti-P profile (Fig. 2B). By bleed 7, however, at the age of 9 mo, and 6 mo after the last Ag boost, the MRL/+ mice showed a second rise in anti-P antibodies (Fig. 2B). The anti-P levels in MRL/+ mice were significantly higher than those in the C3H mice at bleeds 6 and 7 (p < 0.05).

Fine specificity of anti-P antibodies induced by A. salina ribosomes. To verify that the antibodies induced in the immunized mice were true anti-P autoantibodies and to compare their fine specificities with anti-P antibodies produced spontaneously, further experiments were performed. Four sera (two MRL/lpr, one MRL/+, and one C3H) were analyzed by immunoblotting after this study could not be pursued because of the death of MRL/lpr mice at 5 to 6 mo of age (30). The immunization protocol was repeated in MRL/+ mice paired with a C3H control group. As shown in Figure 2B, C3H anti-P titers peaked at bleed 3 and then declined to background levels at bleeds 6 to 7 (5 to 6 mo after the last Ag boost). MRL/+ mice also produced higher anti-P levels than the C3H mice although the pattern of antibody production from
separation of the ribosomal proteins by two-dimensional gel electrophoresis. In each case, the presumed anti-P specificity was verified (not shown). Absorption of the same sera with concentrations of C22-A shown to completely inhibit binding to C22-A on ELISA plates revealed that although binding to A. salina P0, P1, and P2 was diminished on immunoblots, it could not be completely absorbed by C22-A. In contrast, anti-P autoantibodies were completely inhibited by C22-A when mouse ribosomal proteins were used as the target Ag on immunoblots (not shown). These observations indicate that the anti-P sera induced by immunization bind to multiple epitopes on the A. salina P proteins and bind exclusively to the conserved C terminus on mouse P proteins.

To evaluate the fine specificities of anti-P antibodies induced by immunization with A. salina ribosomes, sera obtained from the immunized mice were tested by ELISA against a panel of synthetic peptides conjugated to bovine thyroglobulin (6). The synthetic peptide Ag tested correspond to the C-terminal 22, 11, 7, 5 and 3 amino acid residues (C22, ... C3) of the A. salina proteins el.12' (P1) and el.12 (P2) (24) and to the carboxy-terminal 22 amino acids of human P2 protein (C22-H) predicted from the nucleotide sequence (25) (Fig. 3). Although the amino acid sequences of mouse P1 and P2 have not yet been published, the predicted sequences of the C-terminal 19 amino acids of human and mouse P0 (31) are identical (Fig. 3).

Figure 4A shows the fine specificity anti-P profile (bleed 2) from mice immunized with A. salina ribosomes. All strains reacted predominantly to the A-C22 and A-C1 peptides "immunization pattern". The binding to the Hu-C22 peptide was only slightly elevated compared with background binding to A-C3 and A-C5. In contrast, anti-P autoantibodies produced spontaneously by MRL/lpr and MRL/+ mice (Fig. 4B) showed higher binding to Hu-C22 than to A-C22 as well as elevated binding to A-C7 ("spontaneous pattern"). These findings indicate clear differences in the fine specificities of anti-P antibodies produced by immunization and those produced spontaneously.

Anti-P antibodies at the onset of autoimmunity in MRL/+ mice. As shown above, anti-P levels in MRL mice immunized with A. salina ribosomes remain elevated or increase at the onset of autoimmunity (~3 mo for MRL/lpr and ~9 mo for MRL/+ mice (30)). To determine whether anti-P antibodies maintained their selectivity for A-C22 or switched to a self-reactive spontaneous pattern, the fine specificities of the six immunized MRL/+ mice were compared at bleed 2 and bleed 7. As shown in Figure 5, bleed 7, two mice (3-1 and 4-1) developed high levels of anti-Hu-C22 and/or anti-A-C7 antibodies similar to those observed in MRL mice with spontaneous anti-P antibodies (Fig. 4B). However, antibody binding to A-C22 and/or A-C11 remained elevated at bleed 7 in these mice, suggesting that the spontaneous pattern was superimposed upon the immunization pattern in mice 3-1 and 4-1. In the remaining four mice, the anti-P profile was similar in both bleeds 2 and 7 and maintained the immunization pattern (compare with Fig. 4A, above). Of 6 non-immunized MRL/+ littersmates, two (405-3 and 406-2 in Fig. 4B) also had elevated levels of both anti-A-C22 and anti-Hu-C22. The relative increases in anti-A-C22 and anti-Hu-C22 were also compared in six immunized MRL/lpr mice that survived for the sixth bleed (approximately 5 mo after the initial immunization). The median levels of anti-A-C22 increased twofold between bleeds 2 and 6 whereas the median levels of anti-Hu-C22 in-

**Figure 3.** C-terminal amino acid sequences (single letter code) of A. salina (A. sal), mouse (Mo), and human (Hu) P proteins. The human (25) and mouse (31) sequences are those predicted from the nucleotide sequences of the cDNA, whereas the A. salina sequences were obtained by direct microsequencing (24). The sequences of C22, C11, and C7 are shown. For C22, identity is shown in the line between the two sequences being compared and conservative changes are indicated by a colon.

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<td>AKVEAKKEESEEDDMGFGLFD</td>
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<tr>
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increased only 1.6-fold. Immunization of *A. salmon* ribosomes did not, therefore, induce typical anti-P autoantibodies with increased frequency in MRL mice.

To determine whether syngeneic ribosomes could drive autoantibody production once the antibodies were initiated, MRL/+ mice were immunized with syngeneic mouse liver ribosomes according to the standard protocol. MRL/+ mice positive for anti-P autoantibodies (Fig. 6, closed symbols) were immunized with ribosomes in Freund's adjuvant (mice 406-2, 405-3, and 3-1) or adjuvant alone (mouse 4-1). MRL/+ mice negative for anti-P autoantibodies (open symbols, mice 406-1, 405-1, and 405-2) were immunized with ribosomes in Freund's adjuvant. As shown in Figure 6, anti-P-positive mice immunized with ribosomes showed modest (less than twofold) elevations in anti-P titers after immunization and anti-P-negative mice did not respond at all. The mouse immunized with adjuvant alone (4-1) displayed a rapid fall followed by a rise in anti-P autoantibodies (Fig. 6). Overall, these findings suggest that exogenously administered Ag does not significantly influence anti-P autoantibody titers.

**Requirement of P1 and/or P2 for immunogenicity.** To determine whether the multivalency of the P proteins on the ribosome (32) was related to their immunogenicity in general and their ability to induce autoantibodies, three groups of four age- and sex-matched MRL/lpr mice were immunized with the following Ag: group I, P1 and P2 eluted from *A. salmon* ribosomes, group II, intact *A. salmon* ribosomes, and group III, *A. salmon* ribosomes from which P1 and P2 had been removed ("ribosomal cores"). As shown in Figure 7A, silver staining of the immunogens revealed a high degree of purity of P1/P2 (lane 4) and no...
detectable difference in the composition of the intact ribosome (lane 2) and cores (lane 3). Moreover, gel filtration of the eluted P1/P2 labeled in vitro with casein kinase II\(^4\) and \([\gamma^32\text{P}]\) ATP on a Sephadex G-100 column confirmed that the proteins were dimeric (data not shown). Immunoblot analysis revealed that greater than 80% of P1 and P2, but little or no P0, had been removed from the ribosomal cores (Fig. 7B, lane I). When sera from these three groups of mice were tested for antiribosomal antibodies, sera obtained from mice immunized with intact ribosomes showed results identical to those described in Table I for other MRL/lpr mice, i.e., most sera recognized A. \textit{salina} P0, P1, and P2 and cross-reacted with mouse P0 (Fig. 7C, Rib.). Mice immunized with P1/P2 alone showed similar results (Fig. 7C, P1/P2). Interestingly, even though no P0 was detected in the P1/P2 immunogen (Fig. 7B, lane 3), the anti-P autoantibodies elicited by P1 and P2 reacted predominantly with P0 (Fig. 7C, mouse ribosomes). In striking contrast to these results, mice immunized with ribosomal cores did not make anti-P antibodies reactive against either A. \textit{salina} or mouse ribosomes (Fig. 7C, Cores), even though antibodies against other A. \textit{salina} ribosomal proteins were produced.

In order to exclude the possibility that the failure to produce anti-P antibodies was caused by inaccessibility or conformational change in P0, sera from mice immunized with intact A. \textit{salina} ribosomes were absorbed with either intact ribosomes or ribosomal cores at 4°C overnight. As shown in Figure 8, a serum preincubated with the same amount of intact ribosome (lane 3) or ribosomal cores (lane 4) showed virtual complete inhibition of binding to the P proteins as well as the other ribosomal protein Ag. These findings indicate that the C terminus of P0 is accessible on ribosomal cores.

Finally, MRL/lpr mice immunized with rHuP2 also failed to produce anti-P antibodies although antibodies against \(\beta\)-galactosidase were readily detected. Together with the experiments described above, these observations suggest that P1/P2, or multivalency is required for the induction of anti-P antibodies.

**DISCUSSION**

More than 30 years ago, Witebsky and Rose demonstrated that administration of a foreign protein Ag in Freund's adjuvant to a normal animal could induce antibodies to the immunogen and that these antibodies frequently cross-reacted with self-Ag (autoantibodies).

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\(^{4}\) Hasler, P. N., Brot, H., Weissbach, A. P., Parnassa, and K. B. Elkon. Ribosomal proteins P0, P1, and P2 are phosphorylated by casein kinase II at their conserved carboxyl termini. Submitted for publication.
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Figure 8. Accessibility of P0 on ribosomal cores. A high titer antiribosome serum obtained from a C3H mouse immunized with A. salina ribosomes was diluted 1/1000 and incubated with either buffer alone (lane 2), intact ribosomes (lane 3), or ribosomal cores (lane 4). The amount of ribosomes and cores used contained approximately 70 μg of each ribosomal protein. These sera and control anti-P positive (lane 1) and anti-P negative (lane 5) MRL/lpr sera were used to probe total A. salina ribosomal proteins by immunoblotting.

Since that time, numerous other examples of autoantibodies produced by this route have been published. Most recently, investigators have immunized normal animals with xenogenic lupus protein Ag. Although self-reactive antibodies have in some cases been induced, there is controversy as to whether the resulting autoantibodies had the same fine specificities as spontaneously occurring lupus autoimmunebodies (34–38). Some investigators have proposed that the ready ability to induce autoantibodies against certain lupus Ag by immunization suggests that the stimulus for antibody production in SLE is also exposure to high levels of endogenous Ag (35). In order to directly test this hypothesis in murine SLE, we immunized MRL mice with syngeneic mouse liver ribosomes before the onset of spontaneous anti-P production. A major finding in this study is that anti-P autoantibodies were not induced by immunization with mouse ribosomes. Neither the presence of RNA (which itself has been reported to have adjuvant properties (39, 40)) in intact ribosomes, nor protein denaturation—another potent enhancer of antibody production (28, 29)—was capable of breaking tolerance to ribosomal proteins in MRL mice. These findings are consistent with those of Madaio et al. who observed that MRL/+ mice responded poorly to nucleic acid autoantigens (41). These findings also indicate that, in addition to appropriate intrathymic deletion of Mls autoreactive T cells in MRL/lpr mice (42, 43), tolerance to soluble intracellular proteins is well preserved in young MRL mice.

The failure to induce anti-P antibodies in MRL mice by immunization with syngeneic ribosomes could be explained by an absence of autoreactive B or T cells, absence of an appropriate T helper signal, or failure to bypass normal suppressor circuits. Although concordance between splenic B cell precursor frequency and serum anti-Sm positivity has been observed (44), induction of anti-Sm antibodies in 100% of MRL/lpr mice by immunization with an anti-Sm mAb (45) suggests that all MRL/lpr mice are capable of producing specific autoantibodies given an appropriate stimulus. It seems likely, as discussed below, that the failure to induce anti-P antibodies is explained by the failure to recruit an autoreactive T cell population.

In contrast to the negative results obtained with mouse ribosomes, immunization of both autoimmune (MRL/lpr and MRL/+)+ and normal control (C3H) mice with ribosomes obtained from the brine shrimp, A. salina, proved to be a potent inducer of antibodies reactive against the immunogen. It is unclear why MRL mice (particularly the lpr substrain) produced antibodies to fewer A. salina ribosomal proteins compared with C3H mice. It remains possible that autoreactive B cells are already expanded in lpr mice, allowing less opportunity for exogenous Ag to engage the immune system ("antigenic competition"). Alternatively, non-MHC genes may influence the immune response to some ribosomal proteins. Although both autoimmune strains showed slightly higher anti-P (anti-A-C22) levels than C3H mice during and immediately after immunization (bleeds 1 to 3) suggesting possible B cell hyper-reactivity (30), statistically significant differences in anti-P levels between MRL/+ and C3H mice were only observed in later bleeds. The persistent elevation of anti-P in the lpr strain occurred during the autoimmune phase (3 to 5 mo of age (30)) and a second rise in anti-P levels occurred at the onset of autoimmunity in MRL/+ mice (9 months of age (30)). These findings could be explained by the spontaneous production of lymphokines released by activated T cells in MRL mice (46). However, longitudinal studies of the humoral immune response to phosphorylcholine-keyhole limpet hemocyanin revealed an age-related decline in anti-phosphorylcholine-keyhole limpet hemocyanin antibodies in both MRL/lpr and MRL/+ mice (47). The decline in antibodies against a foreign Ag but persistence or increase in anti-P autoantibodies suggest a possible role for self-Ag in the perpetuation of autoantibody production (see below).

Immunization with A. salina ribosomes induced anti-P autoantibodies because almost all MRL/lpr, MRL/+ and C3H sera contained IgG antibodies that bound to mouse P0 on immunoblots. As with spontaneous anti-P autoantibodies (3, 5), induced anti-P autoantibodies were absorbed by the A-C22 synthetic peptide indicating that the autoantibodies were exclusively directed against the C terminus. However, unlike spontaneous anti-P autoantibodies, the induced anti-P antibodies were only partially absorbed by A-C22 when A. salina P proteins were used as the test Ag. Similarly, the fine specificities of anti-P antibodies produced by immunization with A. salina ribosomes and that produced spontaneously in MRL mice were different when analyzed by a panel of synthetic peptides. The serologic preference of the immunized mice for A-C22 and A-C11 peptides demonstrate the exquisite specificity of the immune response since the Hu- and A-C22 peptides differ by only one nonconservative substitution 11 residues from the C terminus (C11, Fig. 3). The higher binding of the nonimmunized [spontaneous] MRL anti-P autoantibodies to the Hu-C22 and A-C7 peptides can be explained by the near identity of the predicted
human and mouse C-terminal amino acid sequences (Fig. 3) and the complete identity of the 7 C-terminal residues in human, mouse, and A. salina (Fig. 3) (assuming that the spontaneous anti-P antibodies are targeted to self-P proteins). These findings are similar to others in which autoantibodies induced by xenogeneic Ag in normal mouse strains were cross-reactive (36) or showed different fine specificities (48) compared with true autoantibodies. Furthermore, the anti-P autoantibodies induced in autoimmune MRL mice before the onset of autoimmunity were also cross-reactive, suggesting no inherent autoreactive bias in the B cell repertoire of the MRL mice.

Because young MRL mice immunized with A. salina ribosomes developed anti-P antibodies that cross-reacted with self-Ag and increased in titer after the onset of autoimmunity, we were interested to know whether the fine specificity of anti P-antibodies switched to a self-reactive pattern suggesting that mouse P proteins were driving antibody production. Although 2 of 6 MRL/+ mice did show a spontaneous pattern superimposed upon the immunization pattern at the age of 9 mo, the frequency was no higher than that occurring spontaneously in the MRL/+ littermates. Similarly, the relative increase in anti-A-C22 levels was higher than the increase in anti-Hu-C22 levels in MRL/lpr mice at bleed 5. Together, these findings suggest that the increase in anti-A-C22 levels is most likely explained by activation of B cells presensitized to A. salina P proteins. Deliberate immunization of the 4 MRL/+ mice producing anti-P autoantibodies with either adjuvant alone or adjuvant plus mouse ribosomes did not result in consistent increases in anti-Hu-C22 levels. Although the relatively small numbers studied do not allow definitive conclusions to be made, failure of the majority of MRL mice to switch anti-P specificity to self-Ag and the limited response to exogenously administered mouse ribosomes are consistent with the view that high levels of self-Ag are not sufficient to drive anti-P levels in MRL mice even if the B cells are already producing anti-P autoantibodies.

The P proteins were 3 of approximately 6 [MRL] to 10 (C3H) A. salina ribosomal proteins recognized as Ag by mice immunized with intact A. salina ribosomes. Although the anti-P antibodies bound to multiple epitopes on the A. salina P proteins, the binding of induced anti-P autoantibodies, like spontaneous anti-P autoantibodies (3, 5), was completely inhibited by the A-C22 synthetic peptide. Mouse mAb reactive against the C terminus of all of the 3 P proteins have also been produced by immunization of BALB/c (H-2d) and NZB/W F1 (H-2d/z) mice with xenogeneic ribosomes (49, 50). In addition, humans with chronic Chagas's heart disease have antibodies that bind to the conserved C terminus of a Trypanosoma cruzi ribosomal P protein (51). Induction of autoantibodies to the C terminus is surprising in view of the striking conservation of C22 from yeast (52) to humans (25). The potent immunogenicity of the P proteins in several mouse strains and in humans with Chagas's heart disease (51) may be related to the exposed location of the P proteins on the ribosome (49, 53) and the hydrophilicity of the C termini (6). Surface exposure alone, however, cannot account for the ready ability of the P proteins to induce autoantibodies because most ribosomal proteins are accessible to antibodies (54). One property unique to the P proteins is their multivalency on the ribosome. Cross-linking experiments suggest that P1 and P2 both exist as homodimers bound to P0 because ribosomal cores absorbed most anti-P activity. In addition, the rHuP2 protein did not induce anti-P antibodies in MRL mice. Although these observations strongly suggest that the multivalent ligand is necessary for the induction of anti-P antibodies and anti-P auto-

![Figure 9](http://www.jimmunol.org/)
antibodies, we cannot at present exclude the possibility that A. salina P1 and/or P2 is uniquely immunogenic.

As described above, considerable evidence supports the idea that autoantibodies occurring in human and murine lupus are Ag driven. The observations in the present study do not contradict this hypothesis but indicate that, unlike thyroiditis in the Obese strain of chickens (16), high levels of exogenously administered autoantigen are not sufficient to drive autoantibody production in the pre- or autoimmune phases of the disease. This, in turn, suggests that "spontaneous" autoantibodies in MRL mice are initiated by modified self-Ag different from the immunogen prepared from MRL livers and that: or low levels of endogenous self-Ag are sufficient to trigger the humoral immune response in the presence of a T cell stimulus. T cells are necessary for autoantibody production in MRL mice (15, 58, 59) but loss of suppression or excessive helper function cannot explain the selectivity of autoantibodies produced (7-10). Because multivalency may be necessary for the production of anti-P autoantibodies in the presence of a presumed T cell stimulus (foreign P proteins), we propose a two-signal model for autoantibody production similar to that suggested for T-B collaboration in the normal immune response (60, 61) and also in the graft-vs-host model of lupus (57) (Fig. 9). The reasons for T cell activation and for the activation rather than tolerization of B cells by multivalent Ag (65, 66) remain important questions for further studies.

REFERENCES


RIBOSOMES AS IMMUNOGENS IN MRL MICE


