T Cell Reactivity to MHC Class II-Bound Self Peptides in Systemic Lupus Erythematosus-Prone MRL/lpr Mice

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T Cell Reactivity to MHC Class II-Bound Self Peptides in Systemic Lupus Erythematosus-Prone MRL/lpr Mice

Chang-Hee Suh, John H. Freed, and Philip L. Cohen

The epitopes recognized by pathogenic T cells in systemic autoimmune disease remain poorly defined. Certain MHC class II-bound self peptides from autoimmune MRL/lpr mice are not found in eluates from class II molecules of MHC-identical C3H mice. Eleven of 16 such peptides elicited lymph node cell and spleen cell T cell proliferation in both MRL/lpr (stimulation index = 2.03–5.01) and C3H mice (stimulation index = 2.03–3.75). IL-2 and IFN-γ production were detected, but not IL-4. In contrast to what was seen after immunization, four self peptides induced spleen cell proliferation of T cells from naive MRL/lpr, but not from C3H and C57BL/6.H2k mice. These peptides were derived from RNA splicing factor SRp20, histone H2A, β2-microglobulin, and MHC class II 1-Aβ. The first three peptides were isolated from I-Ek molecules and the last peptide was bound to I-Ak. T cell responses, evident as early as 1 mo of age, depended on MHC class II binding motifs and were inhibited by anti-MHC class II Abs. Thus, although immunization can evoke peripheral self-reactive T cells in normal mice, the presence in MRL/lpr mice of spontaneous T cells reactive to certain MHC-bound self peptides suggests that these T cells actively participate in systemic autoimmunity. Peptides elicited from self MHC class II molecules may yield important clues to T cell epitopes in systemic autoimmunity. The Journal of Immunology, 2003, 170: 2229–2235.

Systemic lupus erythematosus (SLE) is characterized by the presence of a variety of autoantibodies against cell surface, nuclear, and cytoplasmic Ags. It has been shown that both B and T cells play a central role in this autoimmune disease (1). In contrast to other systemic autoimmune diseases, T lymphocytes in SLE do not appear to play a direct role in tissue damage. However, T cells are clearly involved in the development of autoantibody production (2, 3). In murine lupus in particular, disruption of T cell activation (4–6) or absence of T cells (7) abrogate autoantibody production and glomerulonephritis. The nature and specificity of the relevant autoreactive T cells have not been fully characterized. Some autoimmune T cells appear to be specific for nuclear Ags, such as histone (8, 9), small nuclear ribonucleoprotein (snRNP) (10, 11), heterogeneous nuclear (hnRNP) (12), Smith Ag (13), and DNA topoisomerase I (14). Although these studies have provided important information about T cell epitopes in autoimmune disease, the approach as a whole has permitted analysis of a limited number of epitopes and cannot confirm the actual epitopes present under physiological conditions. We reasoned that determination of the array of MHC class II-bound self peptides expressed by presumed APCs from autoimmune prone mice might reveal a broader spectrum of potential T cell epitopes.

We previously isolated and sequenced self peptides bound by MHC class II molecules from autoimmune MRL/lpr mice and identified a number of peptides that were not seen in similar preparations from nonautoimmune H-2-identical C3H/HeJ (C3H) mice (15). To test whether these peptides were T cell autoepitopes, T cell responses to them were evaluated both in normal and in MRL/lpr mice. The overall results indicate that self peptide responding T cells could be elicited even in normal mice after immunization, yet spontaneous T cells reactive to certain MHC-bound self peptides were present only in autoimmune prone mice. These T cells may represent expansions of self-reactive T cells which actively participate in systemic autoimmunity.

Materials and Methods

Mice

MRL/lpr, C3H, C57BL/6 (B6), and C57BL/6.H2k (B6.H2k) mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME). All animals were bred and maintained in the specific pathogen-free animal colony at the University of Pennsylvania (Philadelphia, PA).

Antigens

Based on the previously published amino acid sequences of the MHC class II-bound self peptides from MRL/lpr mice (15), 16 peptides (see Table I) were synthesized at the University of North Carolina (Chapel Hill, NC). These peptides represent the most abundant self peptides isolated from MRL/lpr MHC class II molecules. Eleven peptides were unique to MRL/lpr mice and were not detectable in parallel eluates from C3H mice. Five peptides (PCPHP, PCINK, PCRMV, PCYVR, and PCVVK) were found in both MRL/lpr and C3H mice. Synthetic peptides were analyzed for purity by HPLC and by mass spectroscopy. Two other peptides (PCDSCa and PCHLQa), where the anchor residue (P1, P4, P6 and P10, and P1, P6 and P9, respectively) MHC class II binding motif was replaced with alanine, were also produced. Most peptides were dissolved in PBS (pH 7.2). A few were insoluble in PBS and were dissolved in dH2O or DMSO/dH2O (1%) to produce 1 mg/ml stock solutions.

Immunization

Mice (2-mo-old) were immunized s.c. in the base of the tail with 50 μg of peptide Ag or saline, emulsified 1:1 in CFA. At least three mice were used in each Ag group. For analysis of T cell proliferation, mice were sacrificed after 2 wk, and regional draining lymph nodes (LNs; inguinal and periaortic) and spleens were collected aseptically and single cell suspensions were...
**Cytokine ELISA**

After a 72-h incubation, culture supernatants from each well were tested for the presence of IL-2, IL-4, or IFN-γ by sandwich ELISA using Abs from BD PharMingen (San Diego, CA). Standard curves performed with known concentrations of recombinant cytokines (BD PharMingen) were used for the test calibration. All steps were performed according to manufacturer's recommendations. 

**Table I. MHC class II-bound self peptides from autoimmune MRL/lpr mice**

<table>
<thead>
<tr>
<th>Name</th>
<th>MHC</th>
<th>Possible Donor</th>
<th>Amino Acid Sequence</th>
<th>Dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCDS C</td>
<td>I-Eβ</td>
<td>RNA splicing factor Srp20 (4–23)</td>
<td>DSCPRLDCCKVVGNLGNNGKNK</td>
<td>PBS</td>
</tr>
<tr>
<td>PCHLQ</td>
<td>I-Eβ</td>
<td>Histone H2A (84–103)</td>
<td>HPQLAINRDEELNLKLGGKVT</td>
<td>PBS</td>
</tr>
<tr>
<td>PCCHPα</td>
<td>I-Eβ</td>
<td>β2-Microglobulin (42–59)</td>
<td>HPHIEIEIQLNKGKIKP</td>
<td>PBS</td>
</tr>
<tr>
<td>PCINKα</td>
<td>I-Eβ</td>
<td>Clusterin (20-35)</td>
<td>INKEIQNVAVGKHIK</td>
<td>PBS</td>
</tr>
<tr>
<td>PCRMVα</td>
<td>I-Eβ</td>
<td>Heat shock cognate protein 70 (238–251)</td>
<td>RMVNHFIAEKRKH</td>
<td>PBS</td>
</tr>
<tr>
<td>PCAPQ</td>
<td>I-Eβ</td>
<td>Ferritin L chain (165–183)</td>
<td>APOQSLGELYELRLTLKHD</td>
<td>H₂O</td>
</tr>
<tr>
<td>PCPGV</td>
<td>I-Eβ</td>
<td>40S Ribosomal prot S19 (5–24)</td>
<td>PGVTNQWQDPFVRALAAF</td>
<td>DMSO/H₂O</td>
</tr>
<tr>
<td>PCVFR</td>
<td>I-Eβ</td>
<td>60S Ribosomal protein L14 (2–20)</td>
<td>VFRRFVEGVVAYIFSPH</td>
<td>DMSO/H₂O</td>
</tr>
<tr>
<td>PClFL</td>
<td>I-Aβ</td>
<td>26S Proteasome p112 (224–227)</td>
<td>IFLLDDFPASSVDE</td>
<td>PBS</td>
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<tr>
<td>PCLPD</td>
<td>I-Aβ</td>
<td>Saposin D (37–54)</td>
<td>LPDPQKQQDDDFVAYEYP</td>
<td>PBS</td>
</tr>
<tr>
<td>PCYVRα</td>
<td>I-Aβ</td>
<td>Aββ (37–49)</td>
<td>YVRFSDSVDGERYA</td>
<td>PBS</td>
</tr>
<tr>
<td>PCICN</td>
<td>I-Aβ</td>
<td>Aββ (110–129)</td>
<td>HNNTLVCSTVDTFPAXIKVR</td>
<td>PBS</td>
</tr>
<tr>
<td>PCSTQ</td>
<td>I-Aβ</td>
<td>Aββ (143–162)</td>
<td>STQILIRDWTPFQVULVLEM</td>
<td>DMSO/H₂O</td>
</tr>
<tr>
<td>PCVVα</td>
<td>I-Aβ</td>
<td>Transferrin (119–135)</td>
<td>VVKGKTDFLNLQLEGGK</td>
<td>DMSO/H₂O</td>
</tr>
<tr>
<td>PCQRV</td>
<td>I-Aβ</td>
<td>Nucleoporin NUP155 (120–134)</td>
<td>RQVRFDGVIELS LT</td>
<td>DMSO/H₂O</td>
</tr>
<tr>
<td>PCKTA</td>
<td>I-Aβ</td>
<td>14–3–3 Protein (95–107)</td>
<td>KTAFDREAIAELDT</td>
<td>PBS</td>
</tr>
</tbody>
</table>

**Flow cytometry analysis**

Unfractionated SC from 2-mo-old nonimmunized mice were plated in 24-well plates at a concentration of 1 × 10⁶ cells/ml and cultured for 18 h at 37°C with 100 μg of biotinylated peptide or unmodified peptide. The cells were washed in FACS staining medium containing HBSS/3% FCS/15 mM HEPES/0.1% sodium azide and were double-stained with FITC-conjugated mAb to mouse CD19 and PE-conjugated streptavidin for 20 min at 4°C. Cells were washed twice, resuspended in FACS buffer containing PBS with 0.1% sodium azide, fixed with 1% paraformaldehyde, and analyzed by flow cytometry using a BD Biosciences FACScan (San Jose, CA). Lymphocytes were gated according to their forward and side scatter characteristics. B cells (CD19-positive) and non-B cells (CD19-negative) were gated further based on PL2 staining. The samples were analyzed using FlowJo software (Tree Star, San Carlos, CA).

**MHC restriction**

To determine the MHC restriction elements involved in presenting peptides, proliferation assays of SC were conducted as described above in the presence of 100 μg/ml peptide and blocking mouse mAb to I-Aα (Y3P) or I-Eβ (M5114) at a dilution of 1:10. These mAbs were generous gifts from Dr. T. Lauffer (University of Pennsylvania). As a control, anti-IgM mAb (AF6-78.25) was used.

**Results**

Responses to the MHC class II-bound self peptides after immunization

We had previously characterized the principal MHC class II-bound self peptides from autoimmune MRL/lpr mice (15). There were a number of peptides that were not seen in similar preparations from nonautoimmune C3H mice. The peptide donors include histone, a protein component of the spliceosome, and ribosomal proteins. Potentially, some of these peptides represent T cell epitopes for Th cells for autoantibody production. To test the relevance of these peptides, a panel of 16 peptides was therefore synthesized and individual peptides were used as immunogens to assess their T cell stimulatory capacity (Table I). C3H mice were chosen as the normal strain for immunizations, given previous elution data and their haplotype compatibility with MRL/lpr mice (15). We immunized 2-mo-old mice with peptides in CFA or injected CFA without peptide as control. After 2 wk, draining LNs and spleen were removed and tested for ex vivo proliferation in response to the priming peptide. No proliferation or IL-2 secretion was observed when any of the self peptides were added to cultures containing T cells removed from control C3H mice injected with CFA alone. However, proliferative responses of varying magnitudes were reproducibly found with several peptides when they were added to cultures containing T cells removed from immunized mice (Fig. 1).
Optimal proliferative responses were observed at 10 or 100 μg/ml, depending on the peptides. In LNC, four peptides (PCPGV, PCVVK, PCRMV, and PCVFR) stimulated cells of MRL/lpr (stimulation index (SI) 2.21–2.64) and four (PCVVK, PCHHN, PCRMV and PCVFR) induced C3H (SI 2.26–2.98) responses. Three of these five peptides overlapped. In SC all five peptides stimulated cells in both strains, but their response (SI 2.2–5.62) was somewhat stronger than for LNC (SI 2.21–2.98). Another six peptides stimulated only SC from both MRL/lpr (SI 2.46–5.01) and C3H mice (SI 2.2–3.74). Taken together, 11 peptides induced SC proliferation in both mouse strains. IL-2, IL-4, and IFN-γ secretion were measured after 3 days of culture with the self peptides. Increased IL-2 and IFN-γ in the culture supernatant were detected in response to several peptides (Fig. 2). It was of interest that SC responses were stronger than those of LNC.

Response of unprimed SC to the MHC II-bound self peptides

The responsiveness of both C3H and MRL/lpr mice to peptides upon immunization indicated that tolerance to these epitopes could be broken. We wished to know whether autoimmune MRL/lpr mice spontaneously generated T cells reactive to these peptides. SC of nonimmunized MRL/lpr mice (2-mo-old) were thus purified, and the proliferative responses and cytokine secretions were examined and compared with cells from C3H and B6.H2k mice. Unprimed SC proliferation was found only in MRL/lpr mice and was absent in control mice. Four peptides (PCDSC, PCHLQ, PCHPP, and PCHHN) significantly stimulated SC in MRL/lpr mice; these responses were generally smaller than immunized SC responses (Fig. 3). These peptides stimulated SC to secrete IL-2 and IFN-γ (data not shown). Three peptides were eluted from I-Ek molecules and one peptide from I-Ad. The donor proteins from which these peptides were derived were RNA splicing factor SRp20 (PCDSC), histone H2A (PCHLQ), β2-microglobulin (PCHPP), and MHC class II molecule I-Aβ (PCHHN). All of these molecules, except β2-microglobulin-derived peptide (PCHPP), were originally identified in MRL/lpr MHC class II, but not C3H. To evaluate at what age these peptide responses developed, unprimed SC of MRL/lpr mice were harvested from mice ranging from 1- to 5-mo-old. Interestingly, three peptides (PCDSC, PCHLQ, and PCHHN) induced SC proliferation in mice as young as 1-mo-old. These responses were mostly unchanged in older mice (Fig. 4). SC responses to PCHPP were observed at 2 mo. These results suggest that SC responding to these four peptides were already present in unprimed spleens from mice as young as 1-mo-old, well before the onset of autoimmune disease.
Self peptide-reactive cells are T lymphocytes

Because unfractionated SC were used to evaluate the self peptide response, we needed to verify that the cell population responsible for these responses was indeed T cells. Unprimed SC of 2-mo-old MRL/lpr mice were thus purified and depleted of T lymphocytes with anti-Thy1.2 Ab and complement. The resulting cells were evaluated for purity by FACS analysis. After gating on lymphocytes according to their forward and side scatter characteristics, this population consisted of >92% CD3-negative cells. These T cell-depleted SC showed no proliferation to peptides and the response to PHA was decreased by half (Fig. 5). The results of this study indicated that self peptide-responding cells in unprimed SC were T lymphocytes.

Proliferative responses of spleen cells are MHC II-restricted

The self peptides were derived from MHC class II molecules of the APCs in the LNs of sick MRL/lpr mice. To confirm that they could bind to MHC class II molecules, and to know which cells were important APCs, two peptides (PCDSC and PCHLQ) were biotinylated and evaluated for binding by two-color FACS analysis. These peptides were added to SC cultures, incubated overnight, and stained with FITC-conjugated anti-CD-19 mAb and PE-conjugated streptavidin. There was no peptide binding to CD19-positive cells (B cells). For PCDSC, derived from RNA splicing factor SRp20, a greater amount of peptide was bound to class II molecules from MRL/lpr mice than from B6.H-2k or B6 mice (Fig. 6). It was of interest that this peptide could bind to B6 mice (H-2b). There is a report that an I-Ak-derived peptide can bind to the I-Ak molecule (16). These observations raised the possibility that the processing and presentation of MHC molecules may play a role in the generation of alloantigenic determinants. For PCHLQ, derived from histone H2A, cells from MRL/lpr and B6.H-2k mice showed binding, yet B6 did not. To test the specificity of binding, two mutant peptides (PCDSCa and PCHLQa) were made by changing the MHC class II binding motif with alanine substitutions (see Table I). The proliferation of SC to these mutant peptides was determined and compared with the original peptides. Mutant peptides were not stimulatory; the cpm was the same as medium alone (Fig. 7). These results show that the MHC binding motif is important for binding to APCs for subsequent T cell stimulation.

Inhibition of self peptide responses with anti-MHC class II mAbs

To ask whether the T cell responses were functionally MHC-restricted, inhibition by anti-MHC class II mAbs to peptide-induced proliferation was evaluated. There was no inhibition of proliferation with a control Ab which bound to another cell surface protein (anti-IgM Ab, AF6-78.25). As expected, anti-I-Ek mAb (M5114) inhibited the proliferation of SC to all the peptides (PCDSC, PCHLQ, and PCHPP) purified from the I-Ek molecule (Fig. 8). The SC response to the single I-Ak bound peptide (PCHHN) was inhibited by anti-I-Ak mAb (Y3P). Surprisingly, it was also inhibited by anti-I-Ed mAb. After analysis of the sequence, the peptide PCHHN was found to have the three binding motif (P1, P4, and P9) for the I-Ek molecule. It was thus reasonable that this peptide could also bind to the I-Ek molecule, but the amount may have been the detection limit for eluted peptides. These results confirm that these peptides bind to the MHC class II molecule of B cells and stimulate T cells in autoimmune MRL/lpr mice.

FIGURE 3. Proliferative response of unprimed SC to the MHC class II-bound self peptides. SC of non-immunized 2-mo-old mice examined for proliferative responses to peptides in triplicate. Only four peptides produced significant proliferation of SC in MRL/lpr mice. No proliferation was seen in C3H and B6.H2k mice. A representative of at least six experiments is shown. The results are expressed as SIs. The background proliferation is 1211 ± 459 and 1653 ± 900 cpm in SC of MRL/lpr and C3H mice, respectively. SD within each experiment was <20%.

FIGURE 4. Proliferative responses of unprimed SC to the MHC class II-bound self peptides in MRL/lpr mice according to age. From MRL/lpr mice ranging from 1- to 5-mo-old, unprimed SC were harvested. Three peptides significantly induced SC proliferation as early as 1-mo-old and another peptide was stimulatory at 2-mo-old. A representative of at least six experiments is shown. The results are expressed as SIs. The background proliferation is 1079 ± 485 and 1660 ± 700 cpm in SC of MRL/lpr and C3H mice, respectively. All assays were performed in triplicate. SD within each experiment was <20%.
binding was non-specific. We also assessed binding to peptides by high concentrations of the respective peptide, indicating that the binding in many autoimmune sera, it could not be inhibited even in lpr mice and controls for binding. Although there was substantial expression of these peptides were apparently unique, in that they were absent from eluates from MRL/lpr mice, and that some of these peptides were naturally unique, in that they were absent in eluates from MHC-identical C3H mice.

The purpose of the present study was to define the immunological relevance of the self peptides bound to MHC in MRL/lpr mice.

Eluted peptides do not contain B cell epitopes

We coated ELISA plates with the peptides derived from histone, β2-microglobulin, and SRp20 and tested sera from 4-mo-old MRL/lpr mice and controls for binding. Although there was substantial binding in many autoimmune sera, it could not be inhibited even by high concentrations of the respective peptide, indicating that the binding was non-specific. We also assessed binding to peptides before and after immunization with peptide in Freund’s adjuvant, as detailed above. There was no consistent increase in binding at 2, 4, and 8 wk after immunization. These data suggest that these peptides do not contain important B cell epitopes for MRL/lpr mice.

Discussion

Th lymphocytes are required for autoantibody production in murine SLE, yet the nature of the principal peptides recognized by these T cells remains controversial. We have reasoned that identification of the most abundant peptides bound to MHC class II molecules from mice with ongoing autoimmunity might yield clues to the specificity of their pathogenic T cells. In a previous study, we demonstrated that self peptides with MHC class II binding motifs could be eluted from lymphoid cells from MRL/lpr mice, and that some of these peptides were apparently unique, in that they were absent in eluates from MHC-identical C3H mice.

The purpose of the present study was to define the immunological relevance of the self peptides bound to MHC in MRL/lpr mice.

Two principal findings emerged from the experiments. First, while T cells from normal mice were apparently tolerant to these self peptides, the state of tolerance could, for the most part, be broken by immunization with peptides in CFA. Of the 16 peptides tested, 11 stimulated T cells after immunization with CFA. T cells responding to these self peptides were thus in a state of apparent anergy, yet this tolerance to them could be broken by immunization in a strong inflammatory milieu in both autoimmune and control mice. The other five peptides did not produce proliferation, so these peptide-responding T cells might be deleted. The second noteworthy finding was that spontaneous T cell reactivity of unprimed autoimmune mice could be detected against four peptides. Such responses were not present in cells from nonautoimmune mice, and are of considerable interest as candidates for T cell targets for autoantibody-specific help.

We were surprised to observe that even after local immunization with self peptides, the SC response was much higher than that of regional LNC; in some such cases, peptide-induced proliferation was only found in SC. It was possible that many of the primed LNC might have migrated to the spleen at the time of evaluation of proliferative response (2 wk). In this regard, in experimental allergic encephalomyelitis, strong splenic proliferative responses were recalled by myelin basic protein at 40 days after immunization, yet no response was seen in LN (17). After 9 days of immunization, in contrast, the proliferative response was nearly comparable in spleen and LN. As it is known that ~70% of activated lymphocytes migrating through the body die within 24 h, reducing...
Anti-IgM b mAb (AF67825) was used. Anti-I-Eκ mAb inhibited the proliferation of SC to the peptides PCDSC, PCHILO, and PCHHPP, which were purified from I-Eκ. The SC response to I-Aκ bound peptide PCHHN was inhibited by anti-I-Aκ mAb and was also inhibited by anti-I-Eκ mAb. A representative of at least six experiments is shown. The results are expressed as relative proliferation compared with the peptide alone. All assays were performed in triplicate. SD within each experiment was <20%.

The spleen might also be a favored venue for these self-reactive T cells because the likely autoantigen-presenting cells (B cells) are more available than in LN.

Another explanation was that these proliferating cells to self peptides were already present in appreciable numbers in spleen. This is apparently the case for four key self peptides in MRL/lpr, but not in C3H, mice. These four peptides are derived from natural processing of their respective donor proteins: histone H2A, RNA splicing factor SRp20, β2-microglobulin, and the MHC class II I-Aκβ molecule.

Histones are the protein component of nucleosomes, which are believed to be primary immunogens that initiate the cognate interaction between the pathogenic T and B cells of SLE (19). Previous studies by Kahpayumeral (8) et al., performed in (SWR × NZB)F1 (SNF1) mice with 145 overlapping peptides covering the four core histones H2A, H2B, H3, and H4, revealed critical auto-epitopes for T cells encompassing residues 10–33 of H2B, 85–102 of H3, and 16–39 and 71–94 of H4. In their report, peptides 82–96 and 85–99 of H2A produced proliferation of splenic T cell from unmanipulated, 4-mo-old SNF1 mice, but not peptides 88–102 and 91–105 of H2A. In the present study using MRL/lpr mice, peptide 84–103 of H2A stimulated both naive and primed splenic T cells. These results suggest that this MHC class II-bound histone-derived peptide can serve as the T cell epitope for autoantibodies in autoimmune prone mice and suggest an overlapping H2A motif common to both autoimmune strains, despite their different MHC alleles.

RNA splicing factor SRp20 is a member of the highly conserved SR family of splicing regulators, which is one class of non-snRNP factors in a multicomponent protein-RNA complex termed the spliceosome (20). SR proteins are thought to be essential splicing factors important in constitutive and alternative splicing and also function in the regulation of splice site selection. SR proteins function at multiple steps during splicing, protein-protein interactions among SR proteins, and between SR proteins and other essential splicing components (e.g., U1 snRNP) mediate this function (21). There are several reports that some of the spliceosomal proteins can be the targets of autoimmune responses, but this is the first report of an autoimmune response to SR protein. Currently, reported self T cell epitopes of spliceosomal proteins in SLE are 26–40 and 56–70 of snRNP D (11), 50–70 of hnRNP B1 and 35–55 of hnRNP A2 (12), and 48–96 of the B2 polypeptide of snRNP (13). In addition, peptide 4–23 of RNA splicing factor SRp20 can provide determinants for autoreactive T cells.

β2-Microglobulin, a low m.w. protein constituent of cell membrane, is secreted by lymphocytes and other cells, and is normally present in low concentration in serum. Amino acid sequence analysis of β2-microglobulin indicates a close homology with constant region domains of Ig polypeptide chains (22). Although the exact function of β2-microglobulin is unknown, it is thought to play a role in immunologic reactions because it is part of the class I MHC Ag complex on the lymphocyte cell surface (23). The peptide derived from β2-microglobulin was found in both MRL/lpr and C3H mice (15), but it stimulated only splenic T cells from naive MRL/lpr mice. The tolerance to this peptide was broken at 2 mo of age in MRL/lpr. β2-Microglobulin may thus represent a target for autoantibody. In this regard, it is of interest that rheumatoid factors reactive to β2-microglobulin are present in a large number of rheumatoid arthritis sera. This specificity has also been described in SLE and may contribute to functional effects of anti-lymphocyte Abs (24–26).

It was not surprising that self-MHC class II molecule-derived peptides were found in eluates. Endogenous MHC class II molecules were processed and presented by MHC class II on the same cell (16, 27). It has been shown that peptides from both I-Aκ and I-Aκ were immunogenic in syngeneic mice (28–30). Benichou et al. (28) examined peptides derived from the polymorphic regions of the MHC class II molecule and found that peptide 1–18 of I-Aκα and 1–16 of I-Aκβ could elicit strong proliferative T cell responses in B10.A mice after immunization. In this report, we have demonstrated that peptide 110–129 of I-Aκβ stimulated splenic T cells. The T cell response was ablated after changing the MHC class II-binding motif with an alanine or by inhibition with anti-I-Aκ Ab, indicating that this peptide-induced T cell response is MHC-restricted. Unexpected was that this response was also inhibited with anti-I-Eκ mAb. We examined the amino acid sequence of this peptide and the binding motif I-Eκ molecule. For all MHC class II molecules examined, there appear to be a three to four peptide-MHC binding motif (31, 32). The binding motif for the I-Eκ molecule is known based on the solved crystal structure (33). P1 is an amino acid with a hydrophobic side chain (I, L, or V, but also F, Y, or W); P4 is also hydrophobic (I, L, V, F) or S; P6 is usually polar (Q, N, E) or A; and P9 (or P10) is R or usually K. The P1 and P9 positions appear to exert a greater role in controlling binding specificity than do P4 and P6. This peptide had the three binding motif (P1, P4, and P9), so it could bind to the I-Eκ molecule. It is possible that this peptide was bound to the I-Eκ molecule in vivo, but that the amount was below our detection limit. Analysis of self peptides with current technology allows identification of only a handful of the estimated 2000 peptides bound by a single MHC allele (34). A limitation of our approach is that only the most abundant peptides are detected. These may not be the most relevant T cell epitopes. An advantage of this approach, in contrast, is that it imposes no bias on the detection of T cell epitopes. This study demonstrates the value of this opened approach to define the T cell epitope.

In summary, tolerance to MHC class II-bound self peptides can be broken by immunization with adjuvant in both MRL/lpr and C3H mice. Spontaneous self peptide-reactive T cells were present only in MRL/lpr mice and as early as 1-mo-old. These T cells likely participate in a spontaneous breakdown of self tolerance leading to systemic autoimmunity.
A recent study of naturally processed epitopes isolated from eluates of class II MHC molecules of chromatin-fed I-A\(^{b}\)-expressing B lymphoma cells is of special interest to this work (35). These investigators observed several dominant autoepitopes which elicited T cell reactivity even in young (SWR \(\times\) NZB)F\(_1\) mice. In this regard, the finding is consistent with our observation of spontaneous T cell reactivity to eluted peptides in preautoimmune MRL mice. It is not surprising that the epitopes recognized are different for our mice, which have a different class II allele, nor that the pattern of eluted peptides from ex vivo-isolated MHC class II molecules is more complex than that observed for Ag-fed monoclonal APC. Unlike Kaliyaperumal et al. (35), we did not observe serological reactivity with the eluted peptides, nor did immunization with peptides augment autoantibody production.

The analysis of MHC II-eluted peptides is potentially a powerful one in deducing T cell reactivity in systemic autoimmune disease. We anticipate future insights from identifying autoantigen-reactive T cells, their products, and their function from this approach.

Acknowledgments
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