The Self-Directed T Cell Repertoire Against Mouse Lysozyme Reflects the Influence of the Hierarchy of Its Own Determinants and Can Be Engaged by a Foreign Lysozyme

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The Self-Directed T Cell Repertoire Against Mouse Lysozyme Reflects the Influence of the Hierarchy of Its Own Determinants and Can Be Engaged by a Foreign Lysozyme

Kamal D. Moudgil, Scott Southwood, Akio Ametani, Kasey Kim, Alessandro Sette, and Eli E. Sercarz

The T cell repertoire is shaped by the processes of positive and negative selection. We have previously shown that mice are tolerant to a native self-Ag, mouse lysozyme (ML), but they respond vigorously when challenged with different ML peptides (“cryptic” self-determinants). In this study, we have addressed the issue of the physiological significance of both the hierarchy (dominance/crypticity) of self-determinants within ML and the anti-cryptic, self (ML)-directed T cell repertoire. Our results demonstrate that there are several ML peptides that bind well to MHC but are totally nonimmunogenic when tested for proliferative T cell response and cytokine secretion: a subset of these peptides presumably represent the originally dominant self-determinants of ML, which have rendered the T cells tolerant during thymic selection. Other ML peptides, which bind well to MHC and are immunogenic, correspond to the cryptic determinants of ML: T cells against cryptic ML determinants escape tolerance induction. Thus, the mature T cell repertoire against ML bears the direct imprint of the hierarchy of self (ML)-determinants. Interestingly, hen egg white lysozyme could prime T cells in vivo that were cross-reactive with certain cryptic ML determinants, and vice versa, without requiring any coimmunization with the foreign lysozyme and ML peptide(s). Moreover, repeated, deliberate priming and expansion of T cells by hen egg white lysozyme immunization concomitantly enhanced T cell response to such cross-reactive ML determinants. This reciprocal self-foreign determinant cross-reactivity may play a previously unrecognized, but crucial, role in the expansion and diversification of self-reactive clones in the autoimmune response.


In our earlier studies using either mice tolerized or transgenic for the foreign Ag, hen egg white lysozyme (HEL) or mouse lysozyme (ML) as a model self-Ag (9), we observed that mice are tolerant to the native neon/ML but that the T cells directed against cryptic self-determinants exist in the mature repertoire. Furthermore, we proposed that the anticryptic T cell repertoire might be involved in the induction of autoimmunity (10). In this study, we have examined the physiological significance of both the hierarchy (dominance/crypticity) of determinants within self-lysozyme, ML, and the T cell repertoire potentially directed against cryptic determinants of ML. We determined the MHC binding of ML peptides spanning the entire length of native lysozyme and evaluated the results in relation to the immunogenicity of these peptides. We also tested whether foreign (HEL) antigenic determinants make an impact on priming and expansion of the anticryptic self-T cell repertoire. We specifically addressed two crucial questions using the ML system: 1) are nonimmunogenic regions within ML capable of binding to the MHC, and thereby do they represent potentially dominant self-determinants, which can lead to tolerance induction in the thymus in vivo, or are they non-determinants, incapable of binding to the particular MHC of the mouse strains tested; and 2) can the foreign homologue of ML, HEL, prime and expand the T cell repertoire potentially directed against the cryptic determinants of self-lysozyme, ML, and vice versa?

Our results demonstrate that the mature T cell repertoire against ML bears a direct imprint of the hierarchy of self-determinants within ML. Of various ML peptides that bound well to MHC, a
subset of the nonimmunogenic ones correspond to putatively dominant determinants of ML, whereas the immunogenic peptides correspond to cryptic ML determinants. Furthermore, the T cells primed by HEL can also be engaged by peptides comprising cryptic self (ML)-determinants, and vice versa, without requiring any coimmunization with the foreign lysozyme and ML peptide(s). These results suggest that a reciprocal interaction between a pair of foreign and self-antigenic determinants through determinant mimicry (or molecular mimicry) might be of significance in induction as well as propagation of autoimmunity. This study provides a unique determinant-specific perspective on the shaping of the antigenic (self) T cell repertoire and its engagement by a related foreign Ag.

Materials and Methods

Mice

C57BL/6 (B6) (H-2b), BALB/c (H-2k), and CBA/J (H-2b) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in our animal facility. Female mice, 6–16 wk of age, were used in the experiments.

Hen egg white lysozyme

HEL, three times recrystallized, was purchased from Sigma (St. Louis, MO) and was further purified by chromatography on a weak cation-exchange column of Bio-Rex 70 (Bio-Rad Laboratories, Richmond, CA) as described (11).

Peptide synthesis and purification

ML (9, 12, 13) and HEL (14) (Fig. 1) peptides were obtained from the following sources. First, a panel of peptides was synthesized in our laboratory using the rapid simultaneous solid-phase multiple-peptide synthesis methodology, the so-called “teabag” method, which is based on Merrifield’s original solid-phase procedure (15, 16). Peptides were generated on a tert-butyloxycarbonyl-amino acid-Pam resin, and its amino acid composition was analyzed as previously described (17). Second, a set of peptides was synthesized in the University of California, Los Angeles Peptide Core Laboratory directed by Dr. Joseph R. Reeve, Jr. using a multiple peptide synthesizer (396 MPS, Advanced ChemTech, Louisville, KY) (16). The identity and purity of these peptides were determined by fast atom bombardment mass spectrometry at the Center for Molecular and Medical Sciences Mass Spectrometry (University of California, Los Angeles). Third, some peptides were obtained from Macromolecular Resources (Colorado State University, Fort Collins, CO) and from Chiron Mimotopes (San Diego, CA).

Lymph node proliferation assay

Mice were immunized with 7–14 nmol per mouse of native HEL or a ML peptide, each in PBS, in 1:1 (v/v) emulsion with CFA (Difco Laboratories, Detroit, MI), in a hind foot pad. After 9 days, the draining popliteal and inguinal lymph nodes were removed, and cell suspensions washed twice with HBSS (Life Technologies, Grand Island, NY) and then cultured in a flat-bottom 96-well plate at a concentration of 5 × 105 cells/well in X-Vivo-10 serum-free medium (BioWhittaker, Walkersville, MD) supplemented with 5 × 10−5 M 2-ME using different concentrations of the Ag (1.75–14 μM, final concentration) (16). Tuberculin purified protein derivative was used as a positive control. The cells were incubated with 1 μCi/ well of 3H]thymidine (International Chemical and Nuclear, Irvine, CA) for the last 18 h of a 5-day culture. The cells were then harvested using a micro cell harvester (Skatron Instruments, Lier, Norway), and the incorporation of radioactivity was assessed by liquid scintillation counting using the LKB 1205 Betaplate counter (LKB Instruments, Gaithersburg, MD). The results were expressed as mean cpm of duplicate or triplicate cultures. For presentation of data, background values of cpm (cpm of lymph node cells (LNC) cultured in medium without Ag) were subtracted from the cpm obtained with LNC plus Ag (Δ cpm). Alternatively, the value of cpm with Ag was divided by the cpm with medium alone, the stimulation index (SI). The final results from a group of animals immunized with the same peptide/Ag were expressed either as mean cpm ± SEM or mean SI ± SEM. Based on the value of average SI obtained with the optimal concentration of the peptide, the response to each peptide was arbitrarily graded as follows (18): SI of <3.0 = −; 3.0–3.9 = ±; 4–9.9 = +; 10–24.9 = 2 +; 25–49.9 = 3 +, and 50–100 = 4 +.

MHC binding assays

Binding to purified MHC class II molecules of ML peptides was tested in a competition assay using a reference peptide labeled either with biotin (for binding to the Aα molecule) or 125I (for binding to Aβ, Eα, Aβ, or Eβ molecules). The choice of these two different methods was not because of any special consideration for Aβ molecule over other MHC molecules, or for particular peptides of ML over others. In fact, we tested some of the ML peptides using these two different methods and obtained similar results.

Testing the binding of ML peptides to Aβ/Eβ or Aβ/Eβ MHC molecules

Cells. The B cell lymphomas, A20-1.11 (A20) and CH27, were used as sources of I-Aβ/Eβ and I-Aβ/Eα, respectively (19, 20). Cell lines were maintained in vitro by culture in RPMI 1640 medium supplemented with 2 mM l-glutamine, 50 μM 2-ME, 10% heat-inactivated FCS, 100 μg/ml of streptomycin (Irvine Scientific, Santa Ana, CA), and 100 U/ml of penicillin (Life Technologies). Large numbers of cells were grown in spinner cultures. Cells were lysed at a concentration of 106 cells/ml in PBS containing 1% Nonidet P-40, (Fluka, Buchs, Switzerland), 1 mM PMSF (Sigma), 5 mM sodium orthovanadate (Sigma), and 25 mM iodoacetamide (Sigma). The lysates were cleared of debris and nuclei by centrifugation at 10,000 × g for 20 min.

Affinity purification of class II MHC molecules. Mouse class II molecules were purified as previously described (19, 20) using the mAb D6 (I-Aβ-specific), 10.3.6 (I-Aα-specific), and 14-4-4S (I-Eβ-specific), coupled to Sepharose-4B beads. Lysates were filtered through 0.8- and 0.4-μm filters and then passed over the appropriate Ab columns, which were then washed with 15-column volumes of 0.5% Nonidet P-40, 0.1% SDS and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, the MHC was eluted with 0.05 M diethylamine in 0.15 M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/20 volume of 1.0 M Tris, 1.5 M NaCl, pH 6.8, was added to the eluate to reduce the pH to about 7.5, and then the eluate was concentrated by centrifugation in Centricon 30 concentrators (Amicon, Beverly, MA).

Class II peptide-binding assays. Purified mouse class II molecules (5–500 nmol) were incubated with 1–10 nM 3H-labeled radiolabeled peptides for 48 h in PBS containing 5% DMSO (Sigma) in the presence of a protease inhibitor mixture. Purified peptides were iodinated using the chloramine-T method (21). Radiolabeled probes used were HEL Y,46–61 for I-Aβ, ROIV...
and ED molecules are presented in Table I. Also shown in the Table 4234 SHAPING OF THE SELF-DIRECTED T CELL REPERTOIRE AND AUTOIMMUNITY 7.0, but the other mouse class II alleles exhibited optimal binding at slightly acidic pH. I-A^d as well as I-E^a molecules were performed at pH 5.0. The final pH was adjusted as previously described (22). Peptide inhibitors were typically tested at concentrations ranging from 120 μM to 1.2 ng/ml. The data were then plotted and the dose yielding 50% inhibition (IC_{50}) was determined under appropriate stoichiometric conditions. The IC_{50} of an unlabeled test peptide compared to a prototype-labeled peptide is a reasonable approximation of the affinity of interaction with the purified MHC molecule (X). Peptides were tested in two to four completely independent experiments. Class II-peptide complexes were separated from free peptide by gel filtration on TSK2000 columns (model 16215; Tosohaas, Montgomeryville, PA), and the fraction of bound peptide was calculated as previously described (20). In preliminary experiments, each of the I-A and I-E preparations was titrated in the presence of fixed amounts of radiolabeled peptides to determine the concentration of class II molecules necessary to bind 10–20% of the total radioactivity. All subsequent inhibition and direct binding assays were then performed using these class II concentrations.

Testing the binding of ML peptides to I-A^b molecules

I-A^b molecules. Murine MHC class II molecules were fractionated by affinity chromatography from murine splenocytes. Female C57BL/6 mice were obtained from Charles River Japan (Yokohama, Japan), and used for fractionation of A^b molecules. Splenocytes were teased, washed, and stored at −70°C until use. The cells were lysed and applied to anti-MHC class II Ab-bound beads as described above with the exception that protease inhibitors were excluded from the lysis buffer. Affinity chromatography was performed with a column of HiTrap N-hydroxysuccinimide-activated Sepharose-HP (Pharmacia) ligated with M5/114.15.2 (anti-A^b) Ab. Purity of the MHC molecules was confirmed by protein blotting using this Ab after SDS-PAGE in the absence of 2-ME.

MHC (I-A^b) binding assay. The binding assays of peptides to I-A^b molecules were performed essentially using the method described by Jensen (23). Bovine β-lactoglobulin 119-133 was biotinylated with sulfosuccinimidyl-6-(biotinamido) hexanctrone (Pierce, Rockford, IL). and used as the reporter peptide (this peptide was synthesized by F-moc chemistry with a 430A peptide synthesizer; Applied Biosystems, Foster City, CA). Fractionated A^b molecules at 20 nM were incubated with 0–400 μM competitor peptides and 2 μM of a biotinylated reporter peptide. The procedure for calculation of relative binding strength was as follows. First, binding curves were obtained by plotting concentration of competitor peptides vs absorbance. Then, the concentration of competitor peptide giving 50% inhibition of the binding to MHC of biotinylated reporter peptide was obtained. The geometric average of micromolar of test peptide inhibiting 50% binding of biotinylated reporter peptides to MHC molecules was derived from three to five repeat experiments. ML peptides were tested in one of these fraction ranges: either gel filtration on TSK2000, set II; or TSK2000, set II. According to the above data, binding strength to MHC of peptides was classified into different categories, (−) to 3 (with increasing binding strength) according to the most consistent pattern observed within all repeat experiments. The peptide either failed to show 50% inhibition at any of the concentrations tested [category (−); nonbinder] or showed it at peptide concentration of >100 μM (category 1; weak binder), 10–100 μM (or 40–400 μM) (category 2; intermediate binder), or 1–10 μM (or 4–40 μM) (category 3; strong binder).

Results

MHC binding of ML peptides to molecules of H-2^b, H-2^d, and H-2^k haplotypes

The binding of ML peptides spanning the entire sequence of ML (Fig. 1) to purified MHC molecules (e.g., A^b, A^d, E^b, A^d, or E^d) was measured in a competitive inhibition assay using labeled reporter peptides. The results of binding of ML peptides to A^b, A^d, and E^b molecules are presented in Table I. Also shown in the Table are the proliferative T cell responses to ML peptides (following immunization with the respective ML peptide) of C57BL/6 (H-2^b) and BALB/c (H-2^k) mice. It is evident that several ML peptides that were nonimmunogenic in C57BL/6 or BALB/c mice bound well to A^b or A^d/E^d molecules, respectively. For example, ML peptides 1-15, 22-36, 105-115, 105-119, 110-125, and 116-129 showed strong binding to the A^b molecule, whereas ML peptide 50-64 exhibited strong binding to the E^d molecule. Similarly, one of the ML peptides, 95-109, which is nonimmunogenic in C57BL/6 mice, was an intermediate binder to the E^d molecule (see Table I).

Upon further examination, we observed that ML peptides, nonimmunogenic in proliferation assays in a given mouse strain, also failed to induce IFN-γ, IL-4, or IL-5 (data not shown). The above MHC binding by ML peptides in the face of their nonimmunogenicity in vivo can be taken to represent potentially dominant self-determinants that are capable of tolerance induction in the thymus. The other ML peptides, which were neither immunogenic in vivo nor showed MHC binding, represent nondeterminants, which are incapable of inducing an immune response. On the contrary, the majority of MHC binding and immunogenic ML peptides comprise a set of cryptic determinants of ML. However, a few immunogenic peptides (e.g., ML peptides 37-50 and 40–53 in C57BL/6 mice) did not show detectable MHC binding within the range of assays employed (data not shown).

T cells primed by HEL can be engaged by ML peptides representing cryptic self-determinants

To explore the physiological significance of the anticyryptic T cell repertoire directed against ML, we tested whether a foreign Ag (HEL) could prime in vivo T cells that were cross-reactive with one or more ML peptides comprising cryptic self-determinants. Mice (C57BL/6, BALB/c, and CBA/J) were immunized with HEL/CFA, and after 9 days, the draining LNC were tested with HEL or with a set of ML peptides. The results shown in Fig. 2 demonstrate that HEL-primed T cells are cross-reactive with some of the ML peptides despite a limited sequence homology between the corresponding determinants (Fig. 1), for example, p19-33 in C57BL/6 mice, p105-115/p105-119 in BALB/c mice, and p10-23, p19-33, and p99-113 in CBA/J mice. The remaining ML peptides tested (data not shown) did not elicit a significant recall response in these strains. In another set of experiments, we observed that the above three strains of mice immunized with OVA/CFA (an irrelevant Ag control) did not raise any proliferative response to any of the ML peptides tested (including those shown in Fig. 2) (data not shown). Thus, the positive proliferative responses shown in Table I and Fig. 2 represent ML peptide-specific responses induced in vivo and are not a result of in vitro priming of naive T cells by these peptides. In a different set of experiments aimed at testing the reverse cross-reactivity, we observed that T cells primed in vivo by certain ML peptides (e.g., p66-79 in C57BL/6 mice and both p19-33 and p66-79 in CBA/J mice) could indeed be restimulated in vitro by HEL and/or, in most cases, also by the corresponding HEL peptide(s) (data not shown).

Deliberate expansion of T cells directed against a foreign Ag (HEL) concomitantly leads to enhancement in response to a cryptic self (ML)-determinant

To further define the characteristics of the T cell repertoire shared between HEL and ML, we tested the effect of repeated antigenic challenge on expansion of the T cell repertoire. C57BL/6 mice (experimental group) were immunized with HEL (as described under Materials and Methods) first s.c. on the back twice (at an interval of 10 days), followed 10 days later by injection in the hind footpads. Another group of naive age- and sex-matched C57BL/6 mice (controls) were injected once only in the hind footpads. After 9 days of the footpad injection, the draining LNC were tested for a recall response in vitro to either the immunogen (HEL) or a peptide (p19-33) representing a cryptic ML determinant for this
Table I.  MHC class II binding characteristics and immunogenicity of ML peptides

<table>
<thead>
<tr>
<th>ML Peptide</th>
<th>Response&lt;sup&gt;a&lt;/sup&gt; in C57BL/6 (H-2&lt;sup&gt;b&lt;/sup&gt;) Mice</th>
<th>Binding&lt;sup&gt;b&lt;/sup&gt; to A&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Response in BALB/c (H-2&lt;sup&gt;k&lt;/sup&gt;) Mice</th>
<th>Binding&lt;sup&gt;c&lt;/sup&gt; to A&lt;sup&gt;k&lt;/sup&gt;</th>
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<td>3</td>
<td>–</td>
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<td>ND</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
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</table>

<sup>a</sup> The proliferative response to ML peptides is shown on a scale from (−) to +4. Mice were immunized with a ML peptide emulsified in CFA in a hind footpad. Nine days later, the recall response of draining LNC was tested in a proliferation assay using the same ML peptide. The results of the assay were graded on an arbitrary scale: SI of <3.0 = ; 3.0–3.9 = ; 4–9.9 = +; 10–24.9 = 2; 25–49.9 = 3; and 50–100 = 4. ML peptides found to be nonimmunogenic in proliferation assays also failed to induce IFN-γ, IL-4, or IL-5 (data not shown). For accurate comparison of the boundaries and sequences of peptides of ML and HEL we have numbered the extra glycine residue at position 48 in ML as “+” (Fig. 1).

<sup>b</sup> The results of binding of ML peptides to the A<sup>b</sup> molecule were assigned categories (−) to 3. The peptide either failed to show 50% inhibition at any of the concentrations tested (category (−); non-binder) or showed it at peptide concentrations of >100 μM (or >400 μM) (category 1; weak binder), 10–100 μM (or 40–400 μM) (category 2; intermediate binder), or 1–10 μM (or 4–40 μM) (category 3; strong binder). The details of the binding assay are given in Materials and Methods.

<sup>c</sup> The data for BALB/c (H-2<sup>k</sup>) mice on binding of peptides to A<sup>k</sup>/E<sup>k</sup> molecules are presented as IC<sub>50</sub> (nM) with binding affinity of: ≤50 nM, good (strong) binders; 50–500 nM, intermediate binders; >500 nM, poor (weak) binders, and (−), nonbinders. The binding strength to the E<sup>k</sup> molecule (also presented as IC<sub>50</sub> (nM)) of 4 ML peptides that were nonimmunogenic in CBA/J (H-2<sup>k</sup>) mice was as follows: ML 31–45, (−); ML 34–47a, 9677; ML 95–109, 481; and ML 105–119, 1225. None of these four peptides showed detectable binding to the A<sup>k</sup> molecule (data not shown). For detailed procedure of MHC binding, see Materials and Methods.

Discussion
The results of MHC binding studies, and of assays measuring proliferative T cell responses and cytokine secretion, show that ML peptides tested belong to four distinct groups: 1) non-MHC binding and nonimmunogenic (nondeterminants); 2) MHC binding but nonimmunogenic (potentially dominant); 3) MHC binding and immunogenic (cryptic); and 4) a few non-MHC binding but immunogenic (cryptic). ML is a self-protein that is present in the blood, and the protein as well as its mRNA has been shown to be also present in the thymus and other tissues (12, 13). Taken together, our previous results showing that C57BL/6, BALB/c, and CBA/J mice are tolerant to native ML (9), and the new results showing the correlation between MHC binding and nonimmunogenicity/immunogenicity of ML peptides suggest that the shaping of the T cell repertoire potentially directed against ML is significantly influenced by the display hierarchy (dominance/crypticity) of its own determinants. ML peptides comprising dominant self-determinants are likely to have been rendered nonimmunogenic because of self-tolerance induced in the thymus by the display of the same determinants following processing of the native ML. Similarly, a hole in the repertoire might also be caused by a determinant within another self-Ag that might fortuitously cross-react with a particular determinant of ML (24). At this time, we cannot rule out the latter possibility. In contrast, crypticity of certain ML determinants allowed the potentially self-reactive T cells to escape tolerance induction (7–9, 25, 26); these T cells might have been positively selected on a distinct self-Ag or self-peptide (1, 3, 27, 28) and/or by the weakly MHC binding, poorly expressed (below the threshold for negative selection) ML determinant itself. The latter proposition is supported by the results of studies by us (H. Deng et al., manuscript in preparation) and others (29) showing that within a very narrow range of transgene expression, positive selection may be demonstrated toward a single self-peptide. Interestingly, in another situation but invoking a different mechanism, it has recently been proposed that altered thymic selection allowing high-affinity self-reactive T cells to escape into the periphery might explain the increased susceptibility of a particular mouse strain to an autoimmune disease as well as the association between MHC and autoimmunity (30). Finally, we observed that a few ML peptides did not show any detectable MHC binding but were immunogenic. A similar example but using peptide of staphylococcal nuclease has been reported earlier by other investigators (24). It is likely that such determinants might bind to MHC with a very fast off rate and therefore might not be detected in binding assays similar to that employed in our study.

Another important question addressed in this study relates to the specificity and physiological significance of the T cell repertoire directed against cryptic self (ML)-determinants. Our results show that T cells primed in vivo by the foreign lysozyme, HEL, can be

mouse strain. The results are given in Table II. Strikingly, there was a significant increase in the level of response not only to HEL but also to ML p19-33 in mice pretreated with HEL compared with control mice: based on average SI, there was a 6-fold increase in response to HEL and a 3-fold increase against ML p19-33. Thus, repeated priming and expansion of HEL-reactive T cells in vivo lead to a proportionate increase in response to a cryptic ML determinant.

Discussion
The results of MHC binding studies, and of assays measuring proliferative T cell responses and cytokine secretion, show that ML peptides tested belong to four distinct groups: 1) non-MHC binding and nonimmunogenic (nondeterminants); 2) MHC binding but nonimmunogenic (potentially dominant); 3) MHC binding and immunogenic (cryptic); and 4) a few non-MHC binding but immunogenic (cryptic). ML is a self-protein that is present in the blood, and the protein as well as its mRNA has been shown to be also present in the thymus and other tissues (12, 13). Taken together, our previous results showing that C57BL/6, BALB/c, and CBA/J mice are tolerant to native ML (9), and the new results showing the correlation between MHC binding and nonimmunogenicity/immunogenicity of ML peptides suggest that the shaping of the T cell repertoire potentially directed against ML is significantly influenced by the display hierarchy (dominance/crypticity) of its own determinants. ML peptides comprising dominant self-determinants are likely to have been rendered nonimmunogenic because of self-tolerance induced in the thymus by the display of the same determinants following processing of the native ML. Similarly, a hole in the repertoire might also be caused by a determinant within another self-Ag that might fortuitously cross-react with a particular determinant of ML (24). At this time, we cannot rule out the latter possibility. In contrast, crypticity of certain ML determinants allowed the potentially self-reactive T cells to escape tolerance induction (7–9, 25, 26); these T cells might have been positively selected on a distinct self-Ag or self-peptide (1, 3, 27, 28) and/or by the weakly MHC binding, poorly expressed (below the threshold for negative selection) ML determinant itself. The latter proposition is supported by the results of studies by us (H. Deng et al., manuscript in preparation) and others (29) showing that within a very narrow range of transgene expression, positive selection may be demonstrated toward a single self-peptide. Interestingly, in another situation but invoking a different mechanism, it has recently been proposed that altered thymic selection allowing high-affinity self-reactive T cells to escape into the periphery might explain the increased susceptibility of a particular mouse strain to an autoimmune disease as well as the association between MHC and autoimmunity (30). Finally, we observed that a few ML peptides did not show any detectable MHC binding but were immunogenic. A similar example but using peptide of staphylococcal nuclease has been reported earlier by other investigators (24). It is likely that such determinants might bind to MHC with a very fast off rate and therefore might not be detected in binding assays similar to that employed in our study.

Another important question addressed in this study relates to the specificity and physiological significance of the T cell repertoire directed against cryptic self (ML)-determinants. Our results show that T cells primed in vivo by the foreign lysozyme, HEL, can be
restimulated by ML peptide(s) comprising certain cryptic ML determinant(s), and vice versa, despite a limited sequence homology between the corresponding regions of the two proteins. Furthermore, the results of our deliberate, repeated priming experiments provide convincing evidence that activation of the HEL-reactive T cell repertoire concomitantly expands the T cells directed against a cryptic self-determinant of ML. In this experimental scheme designed to represent induction of autoimmunity by a foreign Ag, whole HEL is equivalent to the native Ag of a pathogen, whereas ML peptide represents a cryptic self-determinant of the host Ag, whose display can be up-regulated under inflammatory or other immune-stimulating conditions (10, 31, 32). Using the cytochrome system, Mamula et al. (33) have shown that T cell tolerance to self-cytochrome c (Cyt c) can be broken either by coimmunization with foreign Cyt c and self-Cyt c or by first priming with foreign Cyt c and then boosting with self-Cyt c. In contrast to these results, we could induce cross-reactive T cell responses to self (ML)-peptides by a single challenge with the foreign Ag, HEL, and vice versa, without any coimmunization with foreign lysozyme (HEL) and self-lysozyme (ML)/ML peptide(s).

The above findings of cross-reactivity between determinants within HEL and ML, and enhancement of spontaneous self (ML)-reactivity by repeated priming and expansion underlie a key mechanism for initiation and propagation of autoreactivity. For example, in the case of a healthy individual, repeated exposure to a foreign Ag (e.g., bacteria, virus, etc.) might lead to induction of a T cell response to its dominant determinant(s). During the course of such inflammation and/or infection, the local milieu might favor up-regulation of processing and presentation of previously cryptic determinants of the corresponding self-Ag via different mechanisms discussed elsewhere (31, 32, 34–36). These newly displayed self-determinants could restimulate and expand T cells primed by the foreign Ag, besides inducing self-reactive T cells with new specificities. Thus, as individuals become exposed throughout their lives to repeated bacterial and/or viral infections, inadvertent expansion of a primed self-reactive T cell repertoire is to some extent inevitable;

Table II. Priming and expansion of HEL-reactive T cells leads to a concomitant enhancement in spontaneous response to a self (ML)-determinant

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Recall Response (mean SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HEL</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>2</td>
<td>19.6</td>
</tr>
<tr>
<td>3</td>
<td>6.5</td>
</tr>
<tr>
<td>Average SI ± SEM</td>
<td>11.7 ± 4.0</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>30.9</td>
</tr>
<tr>
<td>5</td>
<td>93.5</td>
</tr>
<tr>
<td>6</td>
<td>93.2</td>
</tr>
<tr>
<td>Average SI ± SEM</td>
<td>72.5 ± 20.8</td>
</tr>
</tbody>
</table>

C57BL/6 mice were immunized with HEL as described under Materials and Methods. The experimental group of mice (Nos. 4–6) were pretreated with antigen twice (with a 10-day interval) s.c. on the back prior to injection of the same Ag in the hind footpads 10 days later. Another group of age- and sex-matched naive C57BL/6 (control; Nos. 1–3) were immunized once only in the hind footpads. After 9 days of the footpad injection, the draining popliteal and inguinal LNC were tested in a proliferation assay using native HEL or ML peptide 19–33 as the recall Ags. The results were expressed as SI. The ratio of average SI for HEL of experimental and control mice was 6.2 in comparison to 3.0 for ML p19-33. Similar results were obtained in a repeat experiment (data not shown).

FIGURE 2. Response to self (ML)-peptides of mice immunized with the foreign lysozyme, HEL. Mice were immunized with HEL/CFA in both hind footpads, and after 9 days the draining LNC were tested for in vitro proliferative recall response to HEL or different ML peptides; only the latter are plotted. A, B, and C correspond to C57BL/6 (H-2b), BALB/c (H-2d), and CBA/J (H-2k) mice, respectively. The results (mean cpm ± SEM) from four mice of each strain are shown. Med, Medium control. Responses to HEL of these mice were as follows: C57BL/6 = 57,307 ± 47,800; BALB/c = 128,838 ± 33,452; and CBA/J = 109,029 ± 58,765.

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this repertoire can be tapped at an appropriate time by a cross-reactive foreign antigenic determinant (9, 10). Furthermore, we suggest that the self-foreign relationship may not necessarily be restricted to homologous proteins. Rather, any foreign Ag that harbors an epitope fortuitously cross-reactive with self (37–40), can also exploit such a situation. It is becoming increasingly recognized that the TCR has degenerate specificity and can bind to other apparently unrelated MHC-peptide complexes (38, 41–43). The novel feature of this study is to demonstrate that the self-directed T cell repertoire to ML is shaped by the dominance/crypticity of its own determinants, and that the anticryptic (ML) T cells can be primed and expanded by the related native foreign Ag, HEL, and vice versa, without requiring any coimmunization with the foreign Ag and self-peptide. Furthermore, our observations suggest that an unknown but chronic history of cross-stimulation of the T cell repertoire shared between a pair of self- and foreign Ag may underlie the onset of some human autoimmune diseases. Although the autoimmune episode may be traced to a recent infection or other antigenic exposure (37, 39, 44), in fact its origins may have a longer historical record.

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