Immunogenicity and Tolerogenicity of Self-Major Histocompatibility Complex Peptides

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Summary

Mechanisms involved in self-antigen processing and presentation are crucial in understanding the induction of self-tolerance in the thymus. We examined the immunogenicity of determinants from major histocompatibility complex (MHC) molecules that are expressed in the thymus and have tested peptides derived from the polymorphic regions of class I and class II molecules. We found that two peptides corresponding to NH₂ termini of the class II α and β chains (A₂1-18 and A₃1-16) could bind to self-A₄ molecules with high affinity and, surprisingly, were immunogenic in that they could elicit strong proliferative T cell responses in B10.A mice (A₄,E₄). Neonatal injection of peptide A₃1-16 resulted in complete unresponsiveness to this peptide at 8 wk of age showing that these T cells were susceptible to tolerance induction. We have also tested certain class I MHC peptides and showed that some can interact efficiently with class II MHC peptides to induce an autoreactive T cell proliferative response. Among these class I peptides is one (D₄1-85) that has the capacity to bind to self-Ia without being immunogenic, and therefore represents an MHC determinant that had induced thymic self-tolerance. We conclude that some self-MHC molecules can be processed into peptides that can be presented in the context of intact class II molecules at the surface of antigen-presenting cells. Autoreactive T cells recognizing optimally processed self-peptide/MHC complexes are eliminated during development, whereas other potentially autoreactive T cells escape clonal inactivation or deletion. Incomplete tolerance to self-antigens enriches the T cell repertoire despite the fact that such T cells may eventually become involved in autoimmune disease.

Class I and Class II molecules encoded within the MHC have been shown to play critical roles in the immune system by acting as peptide binding molecules for presentation to cytotoxic and helper T lymphocytes (1-3). During an immune challenge, the peptides presented to T lymphocytes are derived from foreign antigens by ill-defined processing pathways. It has been hypothesized by Kourilsky et al. (4) that most self-cellular proteins are processed and presented as peptides in the context of I class I or class II MHC molecules for interaction with T lymphocytes. The self-peptides, which have access to and can bind efficiently to self-MHC molecules, should be continuously presented at the cell surface of the APC and may contribute to the acquisition of the T cell repertoire in ontogeny and to the regulation of the T cell response (5-7). Likewise, in the adult animal, it has been shown recently that self-molecules such as hemo-
globin are regularly processed and presented to induce T cell responses in the periphery (8). Mechanisms of self-antigen processing and presentation must also be important in the induction of self-tolerance in the thymus, where self-antigens presented to developing T cells cause deletion of autoreactive cells. One would therefore predict that examination of a panel of peptides derived from self-class I and -class II proteins from the MHC, expressed in the thymus, would reveal a subset of peptides that binds with high affinity to MHC molecules but is not immunogenic because of the deletion of the corresponding T cells. In this study, we have tested a number of peptides derived from the polymorphic regions of the α₁ and β₁ domains of the A₄ murine class II molecule and the α₁ and α₂ domains of the L₄ and D₄ class I MHC molecules. We found that certain MHC peptides bound to self-class II MHC molecules with high affinity and were immunogenic,
Materials and Methods

Peptide Synthetic and Purification. Peptides were synthesized in the Norris Cancer Center Microchemistry Laboratory, USC, with an automated peptide synthesizer (model 430A; Applied Biosystems, Foster City, CA) using modified Merrifield chemistry. They were cleaved from the resin and deprotected by using either hydrogen fluoride (Peninsula Laboratories, Inc., Belmont, CA), or trifluoroacetic acid with 80% aqueous acetonitrile containing 0.1% trifluoroacetic acid. Each peptide chromatographed essentially as a sharp single peak. All purified peptides were found to have the expected amino acid composition. Peptides were dissolved at a concentration of 1 mg/ml in PBS and further diluted to appropriate concentrations with assay medium.

T Cell Proliferation Assay. B10.A mice (H-2d) were obtained from The Jackson Laboratory, Bar Harbor, ME, and were bred at UCLA. Mice of either sex were used at 3-8 mo of age. Mice were immunized in both foot pads and at the base of the tail with 50 μg of MHC peptide emulsified in CFA (Difco Laboratories, Inc., Detroit, MI). Popliteal and inguinal LN cells were obtained 10 d later, and used in antigen-induced proliferation assays. A 5 x 10^6 LN cell suspension was prepared and washed in DMEM supplemented with 2 mM glutamine, 50 μM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS. Then the cells were cultured in 0.2 ml of HB-1 medium alone, containing 2 mM glutamine, or with 50 μg/ml (final concentration) of control lysozyme (HEL) or specific peptide (20 μg/ml of immunizing peptide) in 96-well culture dishes for 4 d (5 x 10^5 cells/well). Antigen-induced proliferation was assessed by the incorporation of 1 μCi [3H]thymidine during the last 18 h of culture.

Competition for MHC Binding. 10^6 AO4.H4.3T hybridomas cells specific for the HEL peptide, 20-35 in association with I-A^k, were cocultured for 24 h with CH27 (A^b, E^b) B lymphoma cells (10^5 cells) as APC, in the presence of the HEL peptide 20-35 (10 μM) and various concentrations of the known competitor (HEL 46-61) or of different MHC-derived peptides. The 96-well microplates were then centrifuged, and the culture supernatants (100 μl) were aspirated and transferred to a new microtiter tray which was frozen and thawed before assay for IL-2 production. IL-2 was assayed by [3H]thymidine incorporation of the IL-2-dependent cell line HT2. Briefly, 0.04 ml of culture supernatants was further incubated with 10^3 HT-2 for 24 h in total volume of 0.2 ml complete DMEM. Incorporation of 1 μCi [3H]thymidine was assayed during the last 4 h of culture.

Tolerance Induction. Tolerance was induced to the peptide A^k 1-16 by two intraperitoneal injections of 10 nmol of peptide in IFA, the first within 24 h after birth and the second between 72 and 96 h after birth. 8 wk later, tolerant animals were immunized with peptide in CFA for LN proliferation as described in the legend for Fig. 2.

Results

Immunogenicity of MHC Peptides. Immunization of naturally recombinant B10.A mice (K^k, A^k, E^b, D^b, L^d) with the class II-derived peptide A^k 1-18 or A^k 1-16 induces strong and specific T cell proliferative responses. Three other A^k-derived peptides from other polymorphic regions (whose sequences are indicated in Fig. 1) were found to be nonimmunogenic (Fig. 2). Immunization of B10.A mice with the class I peptide L^d 61-85, constituting the α1 helix in the native molecule, triggers specific LN cell proliferation that is class II-MHC restricted. T cell responses to these peptides were blocked by anti-CD4 but not anti-CD8 mAbs (data not shown). Two other class I peptides, D^d 61-85 and L^d 148-162 (at a corresponding portion of the α2 helix), were unable to induce T cell proliferation (Fig. 3). Thus, A^k 1-18, A^k 1-16, and L^d 61-85 are all self-MHC peptides that had not induced self-tolerance, but on the contrary, were highly immunogenic.

Binding to A^k MHC Molecules: Class II Peptides. To determine whether the class II-derived peptides were being presented to T cells in association with the self-molecule A^k, we showed that each of the immunogenic class II peptides was capable of inhibiting the in vitro IL-2 production of an A^k-restricted HEL 20-35-specific T cell hybridoma in response to the appropriate 20-35 peptide. As shown in Fig. 4, among the five Ia peptides tested, only A^k 1-18 and A^k 1-16 show a strong and dose-dependent inhibitory activity and their inhibition potency is comparable to that observed with the known HEL 46-61 competitor (9). The other three
Figure 2. Ak 1-18 and Ak 1-16 peptides induce T cell proliferative responses in B10.A mice (Ak, Ek). Results are expressed as stimulation indices ± SD; i.e., ratio of cpm obtained with cells stimulated in vitro with the relevant MHC peptide (■) or control antigen (HEL) (□) vs. cpm of cells treated with medium alone. The response to MHC-derived peptides of four individual mice in each group (starting from the left) averaged 30,291 ± 3,928; 1,959 ± 209; 2,150 ± 344; 32,396 ± 4,042; and 2,052 ± 263 cpm. The positive control response to purified protein derivative (PPD) ranged from 110,008 to 183,770 cpm. The background values (cells without antigens) ranged from 1,134 to 2,529 cpm.

peptides show no inhibition at all. Inhibition under these conditions can be directly attributed to competitive binding to the appropriate MHC restriction element by the extrinsic peptide (10). Lamont et al. (11) demonstrated a close correlation between binding of peptide to class II molecule and inhibition of activation of an unrelated hybridoma. In the case of Ak 1-16, no inhibition of an I-Ek-restricted HEL-17 response was observed (data not shown), confirming that competition is for the Ak molecule. We interpret these results as demonstrating class II-restricted T cell responses to self Ak peptides.

Binding to Ak MHC Molecules: Class I Peptides. It was of interest to determine whether the self-peptide Dd 61-85 could bind to class II molecules of the k haplotype despite the absence of a response after immunization. We tested this class I peptide for its capacity to competitively inhibit an Ak-restricted HEL-specific T cell response. In fact, Dd 61-85 completely blocked the in vitro IL-2 production of an Ak-restricted T cell hybridoma in response to the relevant HEL peptide, to a comparable extent as the prototype high-binding HEL peptide p46-61 (Fig. 5). Likewise, immunogenic Ld 61-85 bound, while the nonimmunogenic, truncated Ld 61-72 failed to inhibit. We conclude therefore that Dd 61-85 is capable of binding to the Ak molecule, but is incapable of triggering a T cell response in B10.A mice displaying the Ak restriction element. It is noteworthy that the same class I peptide, Dd 61-85, when seen as an allopeptide in the context of the same Ia molecule (Ak) in CBA/J and B10.BR mice (H-2k), induces a T cell proliferative response (Table 1). This confirms that this peptide can be efficiently presented by the Ak molecule and can induce an in vivo T cell response in mice whose T cells, specific for this class I peptide/Ak determinant, have not been rendered tolerant.

Tolerance Can Be Induced to Immunogenic Class II Peptides. To determine whether autoreactive T cells against immunogenic MHC peptides were capable of being inactivated during on-
Figure 5. D^d 61-85 competitively inhibits the A^k-restricted response of a T cell hybridoma to p20-35 from HEL. Results are expressed as % inhibition; i.e., 100 \times \text{cpm obtained with HEL 20-35 + MHC peptide/cpm obtained with HEL 20-35. The following peptides were tested in this inhibition assay: D^d 61-85 (\textbullet{}), L^k 61-72 (\Delta{}), L^k 61-85 (\textsquare{}), and HEL p46-61 (\textcircled{O}) (used as positive control). The responses to p20-35 ranged from 89,134 to 101,443 cpm.

togeny, neonatal B10.A mice were injected with peptide A^k 1-16 and tested 2 mo later for their capacity to develop a T cell response against this peptide. As shown in Fig. 6, neonatal injection of peptide A^k 1-16 resulted in complete unresponsiveness to this peptide at 8 wk of age, showing that there is no barrier to tolerance induction by this (or presumably other) MHC peptide(s). The self-reactive T cells we have detected, therefore, have managed to escape deletion or inactivation.

Discussion

It is generally accepted that autoreactive T lymphocytes recognizing self-antigen/MHC complexes are inactivated during ontogeny (12–14). Therefore, it would be expected that all potential self-determinants, self-peptides that bind MHC with high affinity, would not be immunogenic owing to the clonal deletion of inactivation of corresponding T cells. The data presented in this article clearly show that this is not the case. Certain class I- and class II-derived peptides can bind with high affinity to self-class II molecules and also induce potent T cell proliferative responses. Class I peptides have previously been shown to interact with allogeneic class I molecules in CTL recognition (15–17), but this is the first instance of class I peptide presentation by class II molecules to self-reactive T cells. Several groups have recently provided direct evidence that endogenous peptides can interact with class II MHC molecules within the Golgi compartment and subsequently be presented at the cell surface of APC in the context of class II MHC (18, 19). But was this also true for peptides from self-MHC molecules themselves? The feasibility of such functional interactions was suggested by Saskia de Koster et al. (20), who obtained T cell clones from human peripheral blood that recognize an HLA-DR3 peptide, using another self-class II MHC molecule as restriction element, DPw3. In related work by Murphy et al. (21), an antibody was raised that seems to recognize a fragment from a class II E molecule in place within the binding groove of class II A molecules. Most pertinently, they reported that such complexes with this one peptide represent >10% of the total surface A molecules. It appears, therefore, that abundant opportunity exists for the binding and presentation of self-class II peptides.

Some self-MHC peptide-reactive T cells may escape tolerance because the potentially immunogenic determinants they recognize fail to make an impact on the T cell repertoire.

Table 1. D^d 61-85 Induces T Cell Proliferative Responses in B10.BR and CBA Mice But Not in B10.A Mice

| Mice     | Haplotype | K | A_a | A_b | E_a | E_b | D          | T cell response to D^d 61-85 |
|----------|-----------|---|-----|-----|-----|-----|------------|-----------------------------|
| B10.A    | H-2^k     | k | k   | k   | k   | k   | d          | 1,600 ± 660                  |
| B10.BR   | H-2^k     | k | k   | k   | k   | k   | k          | 18,200 ± 1,500               |
| CBA/J    | H-2^k     | k | k   | k   | k   | k   | k          | 28,350 ± 2,120               |

Results are expressed as cpm obtained with cells stimulated in vitro with D^d 61-85. Each group represents six mice tested individually. The positive control responses to PPD ranged from 130,000 to 158,480 cpm. The background values (cells + medium alone) ranged from 1,500 to 2,860 cpm.

Figure 6. Neonatal injections of A^k 1-16 result in complete unresponsiveness to this peptide in adult mice. The proliferative responses of three tolerized (\textbullet{}) and three control (\textcircled{O}) mice tested individually ± SD are indicated. The open squares represent the cpm ± SD obtained with normal mice in response to MEM. The response to HEL of three adult mice neonatally tolerized with A^k 1-16 was 116,232 ± 18,423 cpm.
It has been shown recently that T cells specific for minor determinants on a foreign antigen can escape tolerance induction (22). This evasion apparently is based upon inefficient processing in vivo, and a resulting failure to assemble enough complexes of the minor or cryptic determinant and the MHC molecule. T cells specific for such cryptic determinants are not activated during immunization with the native antigen, although the determinant in the form of the peptide may bind strongly to the MHC and induce excellent T cell responses. It could have been proposed that the self-MHC peptides used in this work were not capable of tolerance induction during ontogeny because of competition by other self-peptides that bound to MHC with higher affinity (23). However, such a competitive blocking mechanism would now seem unlikely, given that these MHC peptides bind with high affinity compared with HEL p46-61 (Figs. 4 and 5). Apparently, the block in tolerance induction is rather the result of incomplete antigen processing. Accordingly, there is a similar dominance hierarchy among determinants constituting the MHC antigen as for any other antigen: some determinants will be made readily available, via processing, for presentation to T cells, while others never become available and remain cryptic. Therefore, it would have been predicted that those class II peptides that are not cryptic determinants would have had the capacity to induce neonatal tolerance, as we show by experimental manipulation in Fig. 6. With respect to the five polymorphic Ak peptides examined in this work, however, we were unable to find any binding peptides that had induced self-tolerance. Only the two NH2-terminal peptides Aa 1-18 and Ak 1-16 bound to self-MHC and they had not induced neonatal tolerance.

Since it has been demonstrated that some endogenously produced MHC peptides have continuous access to the class II binding sites, we must assume that Aa 1-18 and Ak 1-16, found to be strongly immunogenic, are different and presumably poorly processed. This failure of these MHC determinants to influence the developing repertoire may be attributed to their position in the molecule. The three-dimensional model of class II MHC proposed by Brown et al. (24) shows that the NH2 termini are part of the b pleated sheets, which may be protected in the native molecule from proteolysis by the a-helices that reside on top of them. A similar failure to induce tolerance by a self-protein has been recently described by Stockinger and Lin (25). In this case, presentation of the pro-C5 self-peptide in the context of class II MHC also did not appear efficient enough in vivo to ensure induction of tolerance to this self-component. Another possible alternative is that pro-C5 as well as class II peptides resulting from self-processing appear in a cell compartment where they might preferentially interact with class I MHC, leaving a suboptimal concentration of determinants for the class II presentation pathway.

In contrast to these cases of presumed difficulties in processing and presentation, the example of the class I-derived peptide from Dd, amino acids 61-85, demonstrates that processing of some portions of MHC molecules occurs regularly. Furthermore, processing of this self-MHC molecule results in the induction of tolerance. In the B10.A mouse, Dd peptide 61-85, which we show binds to Ak (Fig. 5), cannot induce an immune response because presumably the Dd 61-85 peptide/Ak complex induced tolerance during development. An analogously positioned peptide from the a2 helix, Dd 148-162, induces neither a response nor tolerance because it does not bind to class II molecules. Class I MHC molecules, as was shown previously for class II, are also processed into peptides that can interact efficiently with class II MHC to activate T helper lymphocytes for the induction of cytotoxic T cells. Our data suggest that Dd 61-85 represents an available determinant on class I MHC molecules in that it induces complete tolerance when present as a self-component in the thymus. It is noteworthy that its counterpart on the Ld molecule (Ld 61-85) was found to be clearly immunogenic in a self-context. This difference in neonatal tolerance induction may be attributed to the fact that the Ld molecule is displayed at much lower concentrations than the Dd molecule (26).

It is now generally accepted that the T cell repertoire is shaped by both positive and negative selection resulting from recognition of self-molecules associated with self-MHC. Although no direct evidence is currently available, it is possible that self-MHC molecules include a set of determinants of profound importance in the acquisition of the repertoire and its subsequent regulation, as has been proposed by Kourilsky et al. (4). Our data strongly suggest that both class I and class II self-MHC molecules are processed into fragments that can be presented in the context of intact class II molecules at the surface of APC. Autoreactive T cells capable of recognizing the "immune-self" complexes (which include portions of Ig, TCR, and MHC molecules) presented during development are deleted or inactivated within the thymus. However, some self-MHC peptide-reactive T cells escape elimination because certain complexes are present at subtolerogenic concentrations, at least in the compartment in which the MHC becomes loaded with peptides. This incomplete tolerance to self-components should normally be no cause for concern since minor determinants are generally not presented, whether they are derived from foreign or self-molecules. However, under conditions of upregulation of MHC display, tissue damage, or increased lymphokine secretion (or any combination of circumstances), potentially autoreactive T cells may become involved in autoimmune disease. Despite this potential, the system has evolved to not eliminate all self-reactive T cells, because to do so presumably would have led to excessive purging of the T cell repertoire.

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