Differential Activation of T Cells by Natural Antigen Peptide Analogues: Influence on Autoimmune and Alloimmune In Vivo T Cell Responses

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Differential Activation of T Cells by Natural Antigen Peptide Analogues: Influence on Autoimmune and Alloimmune In Vivo T Cell Responses

Luis R. B. Soares, Patricia L. Orr, Marvin R. Garovoy, and Gilles Benichou

Recent studies using synthetic altered peptide ligands (Analogue) have led to the fine dissection of TCR-mediated T cell functions elicited by Ag recognition. Certain Analogue behave as full agonists of the antigenic peptide while others are partial agonists in that they only trigger selected T cell functions. Additionally, peptide Analogue can behave as antagonists by inhibiting functions of T cell clones when coincubated with the wild-type peptide. In fetal thymic organ cultures, synthetic altered peptide ligands can impact T cell repertoire selection. However, the influence of naturally occurring peptide Analogue on T cell immunity in vivo remains hypothetical. We previously reported that, in B10.A mice, immunogenicity and tolerogenicity of the self-MHC class I peptide, L\textsuperscript{d} 61-80, were influenced by the presentation of a cross-reactive self-peptide, K\textsuperscript{k} 61-80. Here, we show that K\textsuperscript{k} 61-80 self-peptide represents a partial agonist of L\textsuperscript{d} 61-80 in that it induced the proliferation but not the lymphokine production of L\textsuperscript{d} 61-80-primed T cells. Next, we showed that presentation of K\textsuperscript{k} 61-80 Analogue peptide mediated T cell tolerance toward L\textsuperscript{d} 61-80 self-peptide. Alternatively, when L\textsuperscript{d} protein represented an alloantigen displayed on transplanted cells, immunization with K\textsuperscript{k} 61-80 Analogue sensitized recipient mice to L\textsuperscript{d} 61-80 peptide, thus inducing potent immune responses to donor cells. These results show that the presentation of natural Analogue peptides may represent an essential component of T cell responses involved in autoimmunity and transplant rejection. The Journal of Immunology, 1998, 160: 4768–4775.

Lymphocytes recognize protein Ags in the form of peptides bound to self-MHC molecules displayed at the surface of APCs (1, 2). Recent findings have demonstrated that peptide fragments processed from autologous proteins are continuously presented in a MHC-restricted fashion (3, 4) and constitute the vast majority of the peptides that have been eluted from MHC peptide-binding grooves (5–7). The presentation of self-peptides by thymic APCs has a crucial role in both positive and negative selection of immature T cells during ontogeny (8–10). Likewise, in adults, continuous presentation of self-peptides in the periphery presumably contributes to the maintenance of self-tolerance as well as the regulation of T cell responses to foreign Ags. Alternatively, altered presentation of self-peptides to autoreactive T cells can result in the breakdown of T cell tolerance and the initiation of autoimmune diseases.

The presentation of peptides is also an essential component of the response of recipient T cells to donor cells on transplanted tissues. During transplantation, self- and allopeptides are presented either by intact donor MHC (direct pathway) or by recipient MHC (indirect pathway) molecules, respectively (11, 12). These collective observations underscore the importance of the presentation of peptides to T cells in both autoimmune and alloimmune responses. Dissection of the different TCR-mediated functions elicited by peptide Ag stimulation is essential for understanding the mechanisms that govern T cell immunity. Historically, T cell activation was thought to be an “all or nothing” phenomenon. Recent studies using Analogue peptides displaying amino acid substitutions at key TCR contact positions of the Ag peptide have revealed, however, that TCR can interpret subtle modifications in its ligand, resulting in differential activation of T cell functions (13–15). Thus, apparently, the TCR can deliver selective transmembrane signals depending on the fine specificity of the Ag determinant recognized (16–19). Differential signaling through the TCR/CD3 complex by partial agonist peptides can lead to T cell activation or anergy (20, 21), selective induction of Th1 or Th2 CD4\textsuperscript{+} T cell subsets, or result in partial activation of T cell functions (cytolytic activity/proliferation/lymphokine release) (22–25). Additionally, coincubation studies have demonstrated that altered peptide ligands can also behave as antagonists of the wild-type peptide ligand and thereby inhibit certain signals delivered by the specific Ag to T cells (26–28). These observations suggest that peptide Analogue could be utilized to manipulate in vivo T cell responses in an Ag-specific fashion, a possibility that has been documented for autoimmune diseases (29–31).

Although extensive studies using T cell clones have delineated the multiple effects of peptide agonism/antagonism on in vitro activation of T cell clones, little is known about the actual contribution of this phenomenon to bulk T cell responses in normal mice. Several lines of evidence accumulated in transgenic mouse models support the view that naturally processed Analogue peptides contribute to the process of T cell repertoire selection in the thymus (32–35).

We previously reported that the self-peptide L\textsuperscript{d} 61-80 elicited the proliferation of CD4\textsuperscript{+} MHC class II-restricted T cells in syngeneic B10.A mice (36). This self-peptide, despite its high binding affinity for self-MHC class II molecules, failed to induce elimination of autoreactive CD4\textsuperscript{+} T cells, presumably due to its incomplete processing and/or presentation in the B10.A’s developing
the core region of the determinant recognized by T cells in B10.A mice (residues shared by all immunogenic peptides).

One could extend this finding by determining whether these cross-reactive MHC-derived peptides represent natural Analogues and can behave as agonists or antagonists in autoimmunity and allogemnuce in vivo T cell responses. To address this question, we examined the different T cell functions elicited by the presentation of a series of cross-reactive MHC class I peptides during in vivo T cell responses. We discuss here the implications of our findings for determining the influence of self- and allopeptide Analogues on in vivo T cell responses involved in autoimmunity and transplantation.

Materials and Methods

Mice and immunizations

B10.A (K\textsuperscript{a},A\textsuperscript{e},E\textsuperscript{a},L\textsuperscript{d},D\textsuperscript{d}) and B10.A(2R) (K\textsuperscript{a},A\textsuperscript{e},E\textsuperscript{a},L\textsuperscript{d},D\textsuperscript{d}) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in germfree conditions at University of California at San Francisco animal facilities. Mice were used at 7 to 12 mo of age.

The mice were immunized in their hind footpads with 50 to 100 µg of the MHC class I peptide emulsified in CFA (Difco, Detroit, MI). In allogeneic stimulation experiments, spleen cell suspensions from donor mice (B10.A) were prepared, washed extensively in HBSS (ICN, Irvine, CA), and irradiated at 3000 R. Recipient B10.A(2R) mice were then injected in the hind footpads with 2 × 10\textsuperscript{6} allogeneic donor, MHC class I-mismatched, irradiated splenocytes along with 25 µl of CFA on the dorsal surface of the foot as described elsewhere (12).

Peptides

The peptides used in this study were synthesized at the Norris Cancer Center Microchemistry Laboratory, University of Southern California, with an Applied Biosystems (Foster City, CA) model 430A automated peptide synthesizer using modified Merrifield chemistry. They were cleaved from the resin and deprotected by using either hydrogen fluoride (Peninsular Laboratories) or trifluoromethane sulfonic acid. They were then fractionated on Sephadex G10 with 30% acetic acid and lyophilized. Each peptide was then purified by reverse phase HPLC at room temperature on a Brownlee 20 µM, 300 A, 25 × 1-cm Aquapore octyl prep 10 cartridge column, using 0.1% trifluoroacetic acid with a gradient of 80% aqueous acetonitrile containing 0.1% trifluoroacetic acid. Each peptide eluted essentially as a sharp single peak. All purified peptides were found to have the expected amino acid composition and sequence. Peptides were dissolved at a concentration of 1 mg/ml in PBS and further diluted to appropriate concentrations with assay medium. The amino acid sequences of the MHC class I-derived peptides used in this study are shown in Figure 1.

The complete alanine-substituted MHC class I peptides were synthesized using the pin synthesis technique. The procedure was modified as described in detail elsewhere (40) so that the peptides could be cleaved from the pins. The first amino acid residue added in each case was proline followed by Boc-lysine (F-Moc)-OH. The F-Moc-protecting group from the e amino residue was removed and additional F-moc-protected amino acids added in a stepwise fashion. The terminal amino group of each peptide was acetylated. After removal of all the protecting groups, cleavage from the pins was performed by exposure to neutral pH (PBS), under which conditions the carboxyl-terminal lysine-proline residues undergo diketopiperazine formation. This methodology allows peptides of any sequence to be cleaved. Peptide yield was estimated as described elsewhere (60).

T cell proliferation assay

Poplitical lymph node cells were collected 9 to 10 days after immunization and used in Ag-induced proliferation assays. Suspensions of 4 × 10\textsuperscript{4} lymph node cells/ml were prepared and washed in serum-free HL-1 medium (Ventrex, Portland, ME). CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells were then isolated from lymph node cell suspensions using CD4 and CD8 cell recovery columns, respectively (Accurate Chemical and Scientific, Westbury, NY). The cells were then cultured in 0.2 ml of HL-1 medium containing 2 mM glutamine alone, in the presence of serial concentrations of the MHC class I peptides, or with a control peptide in 96-well culture dishes for 4 days. Ag-induced proliferation was assessed by determining the incorporation of 1 µCi of [\textsuperscript{3}H]thymidine during the last 18 h of culture.

Cytokine measurements

Lymph node-derived, purified CD4\textsuperscript{+} T cells were prepared and stimulated as described earlier. Culture supernatants were collected after 30, 60, and 90 h and kept frozen at −70°C before being assayed for the presence of different lymphokines. IFN-γ, IL-2, IL-4, and IL-5 concentrations were determined using a culture ELISA technique as described elsewhere (41, 42). mAbs R46A2 and XM1 1.2 for IFN-γ measurement, JES6-5H4/1A12 for IL-2, BVD6-D11/24G2 for IL-4, and TRKF-4/5 for IL-5 were purchased from PharMingen (San Diego, CA). Recombinant mouse IL-2 and IFN-γ were obtained from Genzyme (Cambridge, MA) and recombinant mouse IL-4 from Boehringer Mannheim (Indianapolis, IN).

T cell costimulation experiments

The requirement for T cell costimulation was tested using mAbs directed toward CD28 or in the presence of APCs expressing B7-1/B7-2 surface Ag (3–5 × 10\textsuperscript{6} cells/well). In anti-CD28 assays, CD4\textsuperscript{+} T cells from primed mice were treated with anti-CD28 mAb (37.51) or MR-1 control mAb (5 µg/ml), in the presence of the relevant MHC class I peptide Ags (5–10 µg/ml). In experiments using B7-1/B7-2-expressing APCs, syngeneic splenocytes were stimulated by LPS (from S. abortus, Sigma, St. Louis, MO) or rabbit F(ab\textsuperscript{’})\textsubscript{2}, anti-mouse IgG (Zymed, South San Francisco, CA) for 48 h as described previously (41). B cell-enriched populations were then prepared by eliminating T cells with a mixture of anti-Thy 1.2, anti-CD4, and anti-CD8 Abs in the presence of complement. B7-1 and B7-2 expression was confirmed by flow cytometry analysis using anti-B7-1 (1G10-FITC) and anti-B7-2 (GL1-FITC) mAbs, respectively. B7\textsuperscript{+} cell-enriched APCs were irradiated and cultured (3–5 × 10\textsuperscript{6} cells/well) with purified CD4\textsuperscript{+} T cells (1–2 × 10\textsuperscript{5} cells/well) in the presence of the relevant Ag. T cell proliferation and lymphokine release were then measured as described above.

In assays using peptide Analogues, purified CD4\textsuperscript{+} lymph node T cells from mice immunized with the wild-type peptide (10\textsuperscript{6} cells/ml) were incubated for 24 h in six-well dishes (Corning, Cornning, NY) in the presence of peptide Analogues alone or with peptide Analogues plus anti-CD28 mAb, along with irradiated, syngeneic (T cell-depleted) spleen cells as APCs (10\textsuperscript{6} cells/ml). Viable cells were then isolated by centrifugation on a Ficoll gradient and reincubated in 96-well dishes (10\textsuperscript{5} cells/well) along with irradiated spleen cells and serial concentrations of the immunizing peptide. After 48 h, Ag-induced proliferation was assessed by determining the incorporation of 1 µCi of [\textsuperscript{3}H]thymidine during the last 8 h of culture.

**FIGURE 1.** Sequence of the MHC class I-derived peptides used in this study. Amino acid sequences of the peptides corresponding to residues 61-80 of five different MHC class I proteins used in this study (L\textsuperscript{d} 61-80, D\textsuperscript{b} 61-80, K\textsuperscript{k} 61-80, K\textsuperscript{d} 61-80, and D\textsuperscript{d} 61-80) are shown. The shaded box represents the core region of the determinant recognized by T cells in B10.A mice (residues shared by all immunogenic peptides).
In addition, cell culture supernatants were collected after 24 and 48 h for cytokine determination as described above.

**MLR assays**

B10.A(2R) (K^k, A^k, E^k, L^b, D^b) mice were immunized with MHC class I disparate allogeneic B10.A (K^k, A^k, E^k, L^d, D^d) irradiated splenocytes as described above. Purified CD4^+ T cells from B10.A(2R)-grafted mice were used as responder cells in MLR assays. T cells were cultured for 72 h in 96-well plates in the presence of irradiated allogeneic (B10.A) or syngeneic (B10.A(2R)) stimulator spleen cells as indicated in the figure legends. In some experiments, MHC class I peptides were added (14 μM) to syngeneic or allogeneic lymphocyte cultures. Cell proliferation was assessed by determining the incorporation of 1 μCi of [3H]thymidine during the last 18 h of culture. Cell culture supernatants were collected after 24 and 48 h for cytokine determination as described above.

**Results**

L^d 61-80 self-peptide-reactive B10.A T cells proliferate but do not secrete lymphokines after in vitro challenge with the cross-reactive self-peptide, K^k 61-80.

We first compared the fine CD4^+ T cell responses directed to L^d 61-80 and its cross-reactive counterpart, self-K^k 61-80 peptide, in L^d 61-80 self-peptide-primed B10.A mice. Following in vivo priming with L^d 61-80 peptide, mouse lymph node CD4^+ T cells were challenged in vitro with either L^d 61-80 or K^k 61-80 peptide and tested for both proliferation and lymphokine production. In vitro restimulation of L^d 61-80-primed T cells with L^d 61-80 peptide induced a vigorous T cell proliferative response (Fig. 2A) as well as IFN-γ (Fig. 2B) and IL-2 production (data not shown), while no IL-4 or IL-5 were detected (data not shown). Alternatively, while the cross-reactive K^k 61-80 self-peptide also elicited T cell proliferation (Fig. 2A), it failed to stimulate the secretion by T cells of both IFN-γ (Fig. 2B) and IL-2 lymphokines (data not shown) when used at doses ranging from 0.01 to 100 μM. In contrast, T cells from K^k 61-80-immunized B10.A mice could be restimulated in vitro to both proliferate (Fig. 2C) and secrete IFN-γ (Fig. 2D) and IL-2 (data not shown) in the presence of K^k 61-80 but not L^d 61-80 peptide (Fig. 2, C and D). Collectively, these results show that 1) only immunization with L^d 61-80 self-peptide can reveal cross-reactivity between the two self-peptides, L^d 61-80 and K^k 61-80; and 2) K^k 61-80 self-peptide induces partial activation of L^d 61-80-primed T cells. We conclude that the cross-reactive self-peptide, K^k 61-80, represents a natural partial agonist of the self-peptide L^d 61-80 in B10.A mice.

Selective IFN-γ production of MHC class I peptide-specific T cells can be modulated by a single amino acid substitution within region L^d 61-80

The amino acid sequence of K^k peptide differs from that of L^d peptide (within the determinant recognized by T cells) by two residues at positions 70 and 73 (Gln^70 → Asp, Trp^73 → Ile) (Fig. 1). To determine the contribution of these amino acids to L^d 61-80 vs K^k-specific T cell responses, B10.A mice were immunized with the self-peptide L^d 61-80. After 9 days, lymph node CD4^+ T cells were collected and restimulated in vitro with a series of peptides displaying alanine substitutions at positions 70 and 73 in the L^d

![Image](http://www.jimmunol.org/Downloaded-from)
The results shown in position 69, which represents a well-conserved residue, was also tested. As shown in Figure 3, a phenomenon that is sometimes observed in MHC class I disparate peptides can result in the activation of selected TCR-mediated effector functions.

**Ld 61-80 autoreactive T cells are inactivated following preincubation with the cross-reactive Kk 61-80 self-peptide**

Our data suggest that concomitant T cell recognition of different endogenous self-peptides behaving as natural partial agonists could modulate autoimmune T cell responses in vivo. To address this possibility, Ld 61-80-primed CD4+ B10.A T cells were preincubated for 24 h in vitro either with the wild-type Ld 61-80 or the Analogue peptide, Kk 61-80. CD4+ T cells were then washed and tested for their proliferative responses and lymphokine release following restimulation with their specific Ag, the peptide Ld 61-80. We observed that Kk 61-80-pretreated T cells could no longer release IFN-γ (Fig. 5) and proliferate (data not shown) when challenged with Ld 61-80 peptide. Neither IL-4 nor IL-5 could be detected (data not shown). In contrast, T cells preincubated with either the wild-type Ld 61-80 or the control Dd 61-80 peptide secreted IFN-γ following challenge with Ld 61-80.

Next, we tested the influence of costimulation on Kk 61-80 peptide-mediated T cell unresponsiveness to Ld 61-80 peptide. Addition of anti-CD28 mAb to the preculture restored Ld 61-80-mediated IFN-γ release (Fig. 5) and proliferation (data not shown) of Kk 61-80 Analogue-preincubated T cells. This indicates that preincubation of Ld 61-80-reactive T cells with Kk 61-80 Analogue rendered these autoreactive T cells anergic.

**Preimmunization of B10.A mice with Kk 61-80-80 peptide elicits long-term in vivo tolerance to the self-peptide Ld 61-80**

B10.A mice were injected in the hind footpads with Kk 61-80 self-peptide emulsified in CFA. Three months later, these mice were immunized with Ld 61-80 peptide and tested after 9 days for their CD4+ T cell proliferative responses toward Ld 61-80 and Kk 61-80 peptides. Unexpectedly, vigorous T cell proliferation could be recorded only upon in vitro challenge with Kk 61-80 but not with Ld 61-80 peptide (Fig. 6). Control CFA-immunized mice and mice that only received the immunization with Ld 61-80 peptide responded equally well to both Ld 61-80 and its cross-reactive counterpart Kk 61-80 peptide (data not shown). Therefore, immunization of B10.A mice with the Analogue, Kk 61-80 peptide, resulted in complete and long-term in vivo T cell tolerance to the wild-type peptide Ld 61-80.

**Influence of MHC peptide Analogues on in vivo T cell responses to allogeneic grafted cells**

Next, we investigated whether T cell recognition of MHC peptide Analogues could influence in vivo T cell-mediated immune responses following allotransplantation. B10.A(2R) mice (Kk, Aa, Ea, Da, La) were injected with different peptides corresponding to regions 61 to 80 of the MHC class I molecule. B10.A(2R) mice were then grafted with 2 × 107 allogeneic, MHC class I disparate, irradiated B10.A splenocytes (Kk, Aa, Ea, Da, La). Nine days later, we examined the proliferation and lymphokine release of recipient B10.A(2R) CD4+ T cells in a MLR using irradiated donor B10.A spleen cells as stimulators. As shown in Table I, CD4+ T cells from recipient mice did not respond to allostimulator cells in vitro, a phenomenon that is sometimes observed in MHC class I disparate donorrecipient combinations. In contrast, both CD4+ T cell
proliferation and IL-2 and IFN-γ production were observed in MLR performed with T cells from Kk 61-80 peptide-preimmunized B10.A(2R) recipient mice (Table I). Apparently, pretransplant injection of B10.A(2R) mice with recipient-derived Kk 61-80 peptide sensitized recipient CD4+ T cells to allogeneic Ags on donor B10.A mouse cells. At the same time, preimmunization of B10.A(2R) recipient mice with control HEL 46-61 peptide or with two other MHC class I peptides, Ld 61-80 and Dd 61-80, did not sensitize T cell responses to donor B10.A cells (Table I).

We then investigated the mechanisms by which recipient B10.A(2R) CD4+ T cells became sensitized to donor (B10.A) MHC class I molecules following in vivo priming with Kk 61-80 peptide. To address this, CD4+ T cells from Kk 61-80-preimmunized B10.A(2R) mice were incubated with syngeneic (B10.A(2R)) irradiated cells as stimulators in the presence of various MHC class I peptides. As shown in Table II, T cells from Kk 61-80 peptide-presensitized B10.A(2R) recipient mice could be stimulated in vitro to produce IL-2 when incubated with syngeneic APCs and donor Ld 61-80 and Dd 61-80, did not sensitize T cell responses to donor B10.A cells (Table I).

We then investigated the mechanisms by which recipient B10.A(2R) CD4+ T cells became sensitized to donor (B10.A) MHC class I molecules following in vivo priming with Kk 61-80 peptide. To address this, CD4+ T cells from Kk 61-80-preimmunized B10.A(2R) mice were incubated with syngeneic (B10.A(2R)) irradiated cells as stimulators in the presence of various MHC class I peptides. As shown in Table II, T cells from Kk 61-80 peptide-presensitized B10.A(2R) recipient mice could be stimulated in vitro to produce IL-2 when incubated with syngeneic APCs and donor Ld 61-80 or Dd 61-80 MHC class I peptides. No T cell responses were obtained in the absence of donor, B10.A-derived MHC peptides or in the presence of the control HEL 46-61 peptide. Importantly, we observed that without grafting, T cells from Kk 61-80 peptide-immunized recipient mice did not respond to allogeneic B10.A-irradiated spleen APCs (Table III). This shows that in vivo sensitization of alloreactive CD4+ T cells required both grafting and the presentation of Kk 61-80 MHC class I peptide.

**Discussion**

Under appropriate circumstances, microbial peptides can elicit T cell responses to structurally or sequentially related cross-reactive self-Ags, thus initiating autoimmunity. This is a phenomenon known as Ag mimicry (44, 45). These microbial peptides represent an example of naturally occurring Analogues of our self-peptides. Recently, it has been hypothesized that presentation of naturally processed self-peptide Analogues may represent a driving force in T cell repertoire selection. Supporting this view, it has been shown that T cell repertoire selection in FTOC models can be influenced...
by addition of synthetic peptides that behave as Analogues of naturally processed self-peptides (32, 33). However, it is still unclear whether naturally occurring peptide Analogues are regularly processed self-peptides (32, 33). Recent elution of the peptides bound to MHC molecules has allowed the identification of some predominant naturally processed self-peptides. Recent elution of the peptides bound to MHC molecules has allowed the identification of some predominant naturally processed self-peptides (32, 33). However, the vast majority of MHC-bound peptides are not present in sufficient amounts.

by addition of synthetic peptides that behave as Analogues of naturally processed self-peptides (32, 33). However, it is still unclear whether naturally occurring peptide Analogues are regularly processed and whether their presentation impacts T cell immunity in normal mice.

Here, we have investigated the influence of different cross-reactive MHC-class I-derived peptides behaving as natural Analogues on in vivo mouse autoimmune and alloimmune T cell responses. First, we studied the relationships between different peptides derived from self-MHC class I proteins in B10.A mice. Although we previously showed that Ld 61-80 self-peptide is not continuously presented on APCs (cryptic) (36), its presentation can occur under defined conditions and lead to an autoimmune response (Ref. 43 and G. Benichou, unpublished observations). In this study, we observed that the cross-reactive Kk 61-80 peptide, despite its ability to stimulate the proliferation of Ld 61-80-primed B10.A autoreactive T cells, could not induce the production of IL-2 and IFN-γ by these T cells. This did not represent an intrinsic property of this peptide, as it elicited T cell secretion of these lymphokines in Kk 61-80-immunized mice. We concluded that Kk 61-80 self-peptide represented a natural partial agonist of Ld 61-80 peptide in B10.A mice. It is noteworthy that restimulation of Ld-specific T cells by Kk peptide did not elicit any significant release of IL-4 and IL-5, thus ruling out the possibility that the Analogue selectively activates Th2 cells. Two lines of evidence indicate that failure of the Kk peptide to mediate the full array of T cell functions of Ld peptide-primed T cells was due neither to inefficient presentation nor defective costimulation by APCs: 1) we previously showed that Ld and Kk peptides bind to B10.A MHC class II molecules with similar affinities (39), and 2) the provision of costimulatory signals by exogenous addition of anti-CD28 mAb or exposure to B7-expressing activated APCs did not restore lymphokine release by T cells (data not shown). This suggests that Kk 61-80 peptide delivered altered signals to TCR on Ld-specific T cells, a phenomenon that resulted in incomplete activation of T cell functions.

In another set of experiments, we observed that in vivo and in vitro exposure of Ld 61-80 peptide-specific T cells to the Kk peptide Analogue led to profound and long-term T cell unresponsiveness to their specific ligand, Ld 61-80. It is possible that partial or altered signals delivered by the Analogue peptide had rendered anergic the Ld 61-80-reactive T cells. Supporting this view, Ld 61-80 peptide-mediated T cell functions could be restored by addition of anti-CD28 mAb, a treatment that is known to overcome T cell anergy (46). It is noteworthy that Madrenas, Schwartz, and Germain have previously reported that the defect leading to anergy by partial agonists is related to IL-2 production (47). Work is in progress to address this possibility in our model.

Our data support the view that the presentation of Analogue self-peptides can influence autoreactive peripheral T cell responses. However, it remains to be determined whether Ag processing regularly generates a large variety of cross-reactive peptides. Recent elution of the peptides bound to MHC molecules has allowed the identification of some predominant naturally processed and presented self-peptides (5–7). However, the vast majority of MHC-bound peptides are not present in sufficient amounts.

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**Table I. Influence of pre- and coimmunization with Kk 61-80 peptide on T cellalloresponses in transplanted mice**

<table>
<thead>
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<th>Immunization</th>
<th>Peptide</th>
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<th>Exp. 3</th>
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<td>IL-2</td>
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* B10.A(2R) (Kk, Aβ, Eα, Lα, Dα) mice were transplanted with MHC class I disparate allogeneic B10.A (Kk, Aβ, Eα, Lα, Dα) irradiated splenocytes.Recipient mice were either preinjected (experiments 1 and 2) or coinjected (experiment 3) with different peptides corresponding to region 61-80 of MHC class I, or with the irrelevant control peptide, HEL 46-61. Purified CD4+ T cells from B10.A(2R) transplanted mice were then used as responder cells in vitro mixed lymphocyte reaction (MLR) assays along with irradiated donor B10.A splenocytes as stimulators. Results are expressed as cpm (T cell proliferation) and as pg/ml (IL-2 and IFN-γ measurements). The data are representative of two separate experiments, each including three mice tested individually. The data represent the mean of triplicate wells and the SD was less than 15%. ND, not determined. The values that are significantly over the background values ± SD are underlined.
The absence of B10.A allogeneic stimulator cells was also tested. Results are expressed as cpm (T cell proliferation) and as pg/ml (IL-2 and IFN-γ measurements). The data shown here are representative of three separate experiments each including three mice tested individually. The data represent the mean of triplicate wells and the SD was less than 15%. ND, not determined. The values that are significantly over the background values ± SD are underlined.

Table II. Immunization of recipient mice with Kk 61-80 peptide sensitizes T cells that recognize donor-MHC peptides presented by recipient MHC during allotransplantation (indirect allore cognition)*

<table>
<thead>
<tr>
<th>Immunization</th>
<th>In Vitro Restimulation with Syngeneic Splenocytes Plus</th>
<th>T cell Response</th>
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<td>Dd 61-80</td>
<td>1716 &lt; 50 &lt; 50 ND</td>
<td>1716 &lt; 50 &lt; 50 ND 1716</td>
</tr>
<tr>
<td>HEL 46-61</td>
<td>988 &lt; 50 &lt; 50 ND</td>
<td>50 ND ND 50 ND 50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunization</th>
<th>No peptide</th>
<th>cpm</th>
<th>IL-2</th>
<th>IFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kk 61-80</td>
<td>Ld 61-80</td>
<td>50 ND</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Ld 61-80</td>
<td>Kk 61-80</td>
<td>3,320</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Dd 61-80</td>
<td>HEL 46-61</td>
<td>3,320</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Kk 61-80</td>
<td>allo spc</td>
<td>4,583</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Ld 61-80</td>
<td>allo spc</td>
<td>50 ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dd 61-80</td>
<td>allo spc</td>
<td>50 ND</td>
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* B10.A(2R) (Kk, Aa, Ea, Ld, Dd) mice were transplanted with MHC class I disparate allogeneic B10.A (Kk, Aa, Ea, Ld, Dd) irradiated splenocytes. Recipient mice were either preinjected with different peptides corresponding to region 61-80 of MHC class I, or with the irrelevant control peptide, HEL 46-61. Purified CD4+ T cells from recipient mice were then used as responder cells in vitro mixed lymphocyte reaction (MLR) assays. Irradiated syngeneic B10.A(2R) splenocytes either alone or in the presence of different donor-derived (Ld, Dd) or recipient-derived (Kk) MHC class I peptides were used as stimulators. Results are expressed as cpm (T cell proliferation) and as pg/ml (IL-2 and IFN-γ measurements). The data shown here are representative of three separate experiments each including three mice tested individually. The data represent the mean of triplicate wells and the SD was less than 15%. ND, not determined. The values that are significantly over the background values ± SD are underlined.

Table III. Immunization of recipient mice with Kk 61-80 peptide in the absence of transplantation is not sufficient to sensitize B10.A(2R) anti-B10.A alloimmune T cells in vivo.

<table>
<thead>
<tr>
<th>Restimulation with</th>
<th>IL-2</th>
<th>IFN</th>
</tr>
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<tbody>
<tr>
<td>Kk 61-80 + allo spc</td>
<td>36,480</td>
<td>888 4,583</td>
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<tr>
<td>Kk 61-80</td>
<td>41,200</td>
<td>774 7,392</td>
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<td>Ld 61-80 + allo spc</td>
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<tr>
<td>Ld 61-80</td>
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<td>Allo spc</td>
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<td>&lt; 50 104</td>
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<tr>
<td>Allo spc</td>
<td>1,612</td>
<td>&lt; 50 283</td>
</tr>
<tr>
<td>Dd 61-80 + allo spc</td>
<td>4,524</td>
<td>&lt; 50 629</td>
</tr>
<tr>
<td>Dd 61-80</td>
<td>3,334</td>
<td>&lt; 50 273</td>
</tr>
<tr>
<td>Allo spc</td>
<td>2,928</td>
<td>&lt; 50 451</td>
</tr>
<tr>
<td>HEl 46-61 + allo spc</td>
<td>3,320</td>
<td>&lt; 50 451</td>
</tr>
<tr>
<td>HEL 46-61</td>
<td>1,713</td>
<td>&lt; 50 471</td>
</tr>
<tr>
<td>Allo spc</td>
<td>2,170</td>
<td>&lt; 50 471</td>
</tr>
</tbody>
</table>

B10.A(2R) Kk, Aa, Ea, Ld, Dd) mice were injected s.c. with Kk 61-80 peptide along with CFA. Purified CD4+ T cells from these mice were then used as responder cells in vitro mixed lymphocyte reaction (MLR) assays. Irradiated allogeneic B10.A splenocytes either alone or in the presence of different donor-derived (Ld, Dd) or recipient-derived (Kk) MHC class I peptides, or the control HEL 46-61 peptide, were used as stimulators. Restimulation with each of the different peptides in the absence of B10.A allogeneic stimulator cells was also tested. Results are expressed as cpm (T cell proliferation) and as pg/ml (IL-2 and IFN-γ measurements). The data shown here are representative of three separate experiments each including three mice tested individually. The values that are significantly over the background values ± SD are underlined.

To be isolated and sequenced, it is noteworthy that most characterized peptides eluted from MHC class II molecules have been found in the form of a nested set of protein fragments with variable N and C residues (5). These self-peptides could encompass different cross-reactive T cell determinants behaving as natural TCR agonists or antagonists. During thymic selection, degenerate self-Ag processing and/or T cell recognition of self-proteins may have evolved to enrich the diversity of the T cell repertoire. In this scenario, clonal T cell tolerance may be the result of multiple and simultaneous interactions with various self-peptides displaying partial agonistic or antagonistic properties. Additionally, continuous presentation of cross-reactive self-peptides behaving as agonists and antagonists could represent a mechanism to regulate T cell responses to autoantigens and prevent autoimmunity in adult individuals. In turn, any combination of events that alters the presentation of these self-peptides may disrupt this balance and initiate an autoimmune process (43, 48, 49).

Next, we investigated the effects of MHC peptide Analogues on alloimmune CD4+ T cell responses in grafted mice. We showed that 1) immunization of B10.A(2R) recipient mice with Kk MHC class I peptide sensitized T cells to allogeneic B10.A donor target cells, and 2) the alloreactive T cell response was mediated by allospecific T cells that presumably recognize Ld and Dd 61-80 cross-reactive peptides presented on recipient APCs. Therefore, in contrast to its tolerogenic effect on Ld 61-80-specific autoreactive T cells, the Kk 61-80 Analogue sensitized T cells to the Ld 61-80 peptide when the Ld molecule was present as an allogeneic protein on B10.A-grafted cells. Importantly, in the absence of grafting, immunization of recipient B10.A(2R) mice with Kk peptide was not sufficient to sensitize alloreactive T cells in vivo. This showed that concomitant presence of Kk peptide and donor MHC class I-bearing cells was necessary to trigger T cell allosresponse. Immunization of Kk 61-80 may have sensitized some low affinity CD4+ T cells by lowering the threshold necessary to respond to the cross-reactive allopeptide Ld 61-80 on grafted cells. Alternatively, costimulation provided by the alloresponse along with Kk 61-80 cross-reactive peptide presentation may have elicited the activation of anti-Ld 61-80-reactive T cells, as suggested by previous studies by Mitchison and O’Malley (50). It is at first glance surprising that the presentation of a self-peptide can contribute to the sensitization of alloreactive T cells. Interestingly, a recent study in our laboratory by Fedoseyeva et al. has demonstrated that immune response to alloantigens during transplant rejection is associated with the breakdown of T cell tolerance to cross-reactive determinants on self-proteins (51). Further supporting this view, the present finding shows that the presentation of a self-peptide, such as Kk 61-80, can prime alloreactive T cells specific for a cross-reactive peptide on donor transplantation Ags, a phenomenon that results in the sensitization of host cells to allogeneic Ags on donor cells. Work is in progress to establish the contribution of this phenomenon to the process of organ transplant rejection.

This study shows the diverse effects of an MHC class I-derived natural peptide Analogue on in vivo autoimmune and alloimmune T cell responses in mice. We observed that the same Analogue peptide could behave as a partial agonist and could tolerate autoimmune or sensitize alloimmune T cell responses. These observations further underscore the complexity of the relationship between Ag recognition by bulk T cells and the subsequent in vivo T cell responses in mice. It has become clear that utilization of Ag peptide Analogues represents a promising strategy to selectively inhibit deleterious T cell responses or, alternatively, to induce or strengthen other immune responses for the design of new vaccines. Our study suggests, however, that peptide Analogue-based immunotherapies must be carefully designed so as to avoid undesirable effects on the multiple compartments of the immune system.
Acknowledgments

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References

30. E. Fedoseyeva, R. C. Tam, and J. M. Kanellopoulos for helpful discussions and critical review of this manuscript.