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Inhibition of an In Vitro CD4⁺ T Cell Alloresponse Using Altered Peptide Ligands

Claude Daniel, Arash Grakoui, and Paul M. Allen

In this study, we explore the potential of altered peptide ligands (APLs) to modulate the alloresponse of CD4⁺ T cells using elements of the murine hemoglobin (Hb) Ag model. We first demonstrated that the T cell 2.102, specific for the Hb(64-76)/I-Ek complex, was alloreactive against splenocytes of the H-2p haplotype. Using Ab-blocking and transfection experiments, we further showed that this alloreactivity was restricted to the class II molecule I-Ep. We tested a panel of APLs previously shown to antagonize the Hb response of 2.102 and found that these peptides could also effectively inhibit the alloresponse to I-Ep. Importantly, these peptides were able to antagonize the alloresponse of naive T cells derived from mice transgenic for the 2.102 TCR, as well as Th1 and Th2 cell lines. The antagonism required the presence of both I-Ep and I-Ek on the same APC. Our study demonstrates the effectiveness of APLs to antagonize the primary alloresponse of specific T cells and provides a basis for the development of immunotherapeutics for use in transplantation and immune-mediated diseases. The Journal of Immunology, 1998, 160: 3244–3250.

The recognition of allo-MHC Ag by T cells is the major barrier to successful transplantation. Although advances in immunosuppressive therapies have considerably improved graft survival for solid organ transplantation in the last decades (1), major obstacles still persist. For example, prolonged immunosuppressive therapy has been associated with opportunistic viral infections and development of carcinomas and kidney diseases (2, 3). However, the impact of these therapies on long-term graft acceptance still remains questionable (4, 5). Thus, the ultimate goal in transplant biology is to achieve long-lasting, Ag-specific unresponsiveness. Multiple potential targets exist for interference with T cell activation and tolerance induction. Peptides derived from conserved domains of MHC class I or class II molecules have been shown to be able to inhibit T cell activation in vitro and graft rejection in vivo (6). Inhibition of costimulatory pathways has also been shown to alter the immune response that leads to graft rejection. For example, prolonged cardiac graft survival was observed in a murine model after treatment with a combination of CTLA4Ig and Abs to CD40L (7).

The interaction of the TCR with its ligand and the subsequent activation of the T cell is another potential target for inhibition of detrimental immune responses. Variants of agonist ligands that induce partial T cell activation have been defined as APLs (8). They have been shown to induce a variety of biologic activities, such as cytokine production without proliferation (9), changes in profile of cytokines production (10, 11), and anergy induction (12). Moreover, APLs have been shown to inhibit IL-2 production or proliferation of CD4⁺ Th clones or hybridomas through TCR antagonism (13, 14) and to inhibit cytolysis mediated by CD8⁺ CTL (15). These observations have raised the possibility that APLs could be used in immunotherapy of autoimmune diseases mediated by populations of T cell with a defined specificity (16). Indeed, very promising results have already been obtained in rodent models of experimental autoimmune encephalomyelitis (17, 18).

The purpose of this study was to explore the potential of APLs to modulate the alloresponse of CD4⁺ T cells. To address these questions, we decided to take advantage of the Hb Ag system developed in our laboratory. This Ag model is based on the generation of T cell clones and hybridomas specific for the d allele of mouse hemoglobin β chain (Hbβd). These T cells recognize the immunodominant determinant Hb(64-76) and are restricted by the class II molecule I-Ek (19). The Hb(64-76) epitope has been extensively characterized in terms of TCR recognition and MHC binding (20). The crystal structure of the Hb(64-76)/I-Ek complex has been recently solved (21). Furthermore, TCR transgenic mice have also been generated in the laboratory for two of the T cells recognizing this complex (Kersh et al.; Grakoui et al.; manuscripts in preparation). Finally, and most importantly for this study, a large panel of APLs with a broad array of biologic functions has been defined in this Ag model (13, 22). In this report, we describe a new allore cognition model based on the reactivity of a Hb(64-76)/I-Ek-specific T cell toward the class II molecule I-Ep. Using a panel of APLs previously shown to antagonize the Hb response, we show that the alloresponse of naive Th1 and Th2 T cell populations bearing a specific TCR can also be inhibited by TCR antagonism.

Materials and Methods

Animals

Strains of A.CA/SnJ (H-2b), C57BL/6J (H-2b), B10.BR (H-2b), B6.AKR (H-2b), CBA/J (H-2k), DBA/II (H-2b), P/J (H-2b), PL/J (H-2b), RIILS/J (H-2r), SJL/J (H-2r), and SM/J (H-2r) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). BALB/cAnNCr (H-2b) mice were purchased from The National Cancer Institute (Bethesda, MD). The B10.P (H-2r) strain (23) was kindly provided by Dr. Ted Hansen (Washington University, St. Louis, MO) and bred in our animal facility. The 2.102
TCR-transgenic mouse (2.102tg) was generated and bred to RAG1-deficient mice as reported elsewhere (Grakoui et al., manuscript in preparation).

**Cells and mAbs**

The 2.102-G2 and 3.L2-12 hybridomas were derived from the Th2 clone 2.102 and the Th1 clone 3.L2, respectively, and are specific for the Hb(64-76) peptide/I-E\(^b\) complex as previously described (13, 24). The CH27 B cell lymphoma expressing the I-E\(^p\)/I-A\(^\lambda\) class II molecules was used as APC and maintained as described (22, 25). All cell lines were grown in RPMI 1640 (Life Technologies, Gaithersburg, MD) medium supplemented with 10% (v/v) heat-inactivated bovine calf serum (HyClone, Logan, UT), 2 mM glutamax (Life Technologies), and 2 \(\times 10^{-3}\) M 2-ME. The mAb EN (M. Olszowy and A. Shaw, unpublished data), which contains a geneticin-resistance gene, was obtained from the American Type Culture Collection (ATCC, Rockville, MD). To deliver one pulse of 270 V at 500 mAh (Bio-Rad, Hercules, CA) to deliver one pulse of 270 V at 500 mAh (Bio-Rad, Hercules, CA), cells were seeded into flat-bottom 96-well plates and stable transfectants selected for the I-E\(^p\)-chain, which was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The mAb EN-B6.8 (IgG2a), specific for the I-E\(^p\), was generated by fusion of the lymph node cells of A/J mice immunized with CH27-EN to the P3-X63-Ag8.653 myeloma according to the established procedure (26). The mAb LF1B9.16, specific for the hemoglobin peptide Hb(64-76), was used as an isotype control.

**Peptides**

Peptides were synthesized on a Rainin Symphony Multiplex synthesizer (Woburn, MA) using standard F-moc chemistry. Peptides were purified by HPLC on a C18 column, and their amino acid content and concentration determined by analysis on a Beckman amino acid analyzer, model 6300 (Beckman, Fullerton, CA). The identity of purified peptides with predicted molecular masses and composition was further confirmed by mass spectrometry analysis at the Washington University Mass Spectrometry Resource (St. Louis, MO). The peptides used in our study and their sequences in one letter amino acid codes are: Hb(64-76), GKVKI TAFNLEGK, S69, GKKVISAFAENGKL, N73, GKKVITAFNNGLGK, Q72, GKKVITAFQLEGK.

**Cloning of the I-E\(^p\) \(\alpha\) and \(\beta\)-chains**

Total RNA was isolated from P/J splenocytes using RNAzol B (Tel-Test, Friendswood, TX) according to the manufacturer’s protocol. I-E\(^p\) \(\alpha\) and \(\beta\)-chains were amplified into full-length cDNAs by RT-PCR using oligonucleotides selected from their 5' and 3' consensus sequences. These oligonucleotides were E\(^\alpha\)-GAATTC GAATTC ATGGCCACAATTG GAGCCCT-3' GAATTC GAATTC ATGGCCACAATTG GAGCCCT-3' and E\(^\beta\)-GAATTC GAATTC GAATTCTC

**Results**

The Hb(64-76)/I-E\(^p\)-specific T cell 2.102 is alloreactive against I-E\(^p\)**

T cells from 2.102tg mice were prepared by enrichment of spleen cells over a nylon wool column. For the preparation of naive 2.102tg T cells, splenocytes from 2.102tg/RAG1-deficient mice were stained (0.5 μg of Ab per 10^6 cells) with biotinylated anti-CD4 mAb (PharMingen, San Diego, CA) followed by fluorescein-conjugated streptavidin (Caltag, San Francisco, CA) and phycoerythrin-conjugated anti-CD4 mAb (H129.19, PharMingen). Naïve T cells (CD4\(^+\) and CD62L\(^hi\)) were isolated by cell sorting using a FACS-Vantage (Becton Dickinson, Mountain View, CA). Th1 and Th2 cell lines were established as described (27). Briefly, splenocytes (2.5 \(\times 10^6\)) from the 2.102tg/RAG1-deficient mice were stimulated in 2-ml cultures with 3 μM of Hb(64-76) and irradiated B6.AKR splenocytes (2000 rad, 5 \(\times 10^6\)) as APCs in the presence of 5 U/ml of IL-12 (Genetics Institute, Cambridge, MA) and 10 μg/ml of anti-IL-4 mAb (11B11) to promote Th1, or 200 U/ml of IL-4 (P815 mastocytoma cell culture supernatant) to promote Th2 phenotype development, respectively. The differentiated Th1 and Th2 cell lines were harvested on day 7. The phenotype of these lines was confirmed by evaluating IFN-\(\gamma\) or IL-4 production 48 h after restimulation with Hb(64-76) peptide and APCs.

**TCR antagonism**

TCR antagonism assays were performed as described (13). Briefly, CH27 cells were preincubated with 1 μM of Hb(64-76) peptide for 2 h at 37°C. The cells were then washed three times in HBSS and plated at 10^5 cells/well in flat-bottom 96-well plates. To analyze the antagonism of the alloresponse, CH27-EN were directly added to the 96-well plates at 10^4 cells/well. Serial dilutions of the peptides assayed were then added to these APCs. The activation of T cell hybridomas was evaluated by IL-2 production using the CTL-2 cell line as described above. In some antagonist experiments, irradiated splenocytes at 2.5 \(\times 10^5\) cells/well were used as APCs. Antagonism of primary T cell or Th1/Th2 line responses was evaluated directly by assaying proliferation after [H]HtD\(\alpha\) (0.4 μCi/well) incorporation over a 24-h period (28) using mitomycin-treated CH27 or CH27-EN cells as APCs.
ligands appeared to be similar. Thus, the reactivity of the 2.102 T cell with P/J splenocytes is not due to reactivity of its TCR with complexes made of Hb peptides presented by an H-2<sup>a</sup> MHC molecule.

We next sought to determine the restriction element recognized by 2.102-G2 in this alloresponse. Because this T cell is restricted by I-E<sup>b</sup> in the recognition of Hb(64-76) peptide, our initial inquiry focused on determining if the alloreactivity would be against the I-E<sup>p</sup> molecule. As shown in Figure 2B, the alloresponse of 2.102-G2 hybridoma to P/J splenocytes was completely inhibited by a mAb specific for the I-E <b>α</b>-chain (14-4-4s). No mAb specific for I-E<sup>p</sup> was available to directly involve the alloreactivity of the I-E<sup>p</sup> molecule in the 2.102 T cell alloresponse. We therefore generated such an Ab (2B6.B8) by fusion of lymph node cells from A/J mice immunized with CH27-E<sup>p</sup> cell line. Following analysis by cytofluorometry against the panel of splenocytes used in Figure 1, the mAb 2B6.B8 was shown to be highly specific for P/J splenocytes (data not shown). The alloresponse of 2.102-G2 to CH27-E<sup>p</sup> was also strongly inhibited by this I-E<sup>p</sup>-specific mAb (Fig. 2B). These results demonstrate that the 2.102 T cell alloresponse is restricted by the I-E<sup>p</sup> molecule.

The identification of I-E<sup>p</sup> as a second ligand for the 2.102-G2 hybridoma prompted us to characterize further this class II molecule and evaluate its capacity to reconstitute alloreactivity upon transfection in an APC. The I-E<sup>p</sup> <b>α</b>- and <b>β</b>-chains were amplified by RT-PCR from P/J splenocytes using oligonucleotides selected from conserved 5' and 3' nucleotide sequences of I-E <b>α</b>- or I-E <b>β</b>-chain genes. The cDNAs were then cloned and sequenced. The I-E<sup>p</sup> <b>α</b>-chain nucleotide and deduced amino acid sequences were identical to the I-E<sup>b</sup> <b>α</b>-chain. Wei et al. (29) reported the sequence of the I-E<sup>p</sup> <b>β</b>-chain in CH27 cells clearly demonstrated that the 2.102-G2 alloresponse is restricted by the I-E<sup>p</sup> molecule.

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Our previous experiments have demonstrated inhibition of a T cell hybridoma alloresponse by TCR antagonism. To demonstrate a similar phenomenon with untransformed T cells, and to set the ground for further in vivo studies, we determined if the proliferative alloresponse of 2.102tg mouse (2.102tg) could also be antagonized. As shown in Figure 3, APLs that inhibited the response of 2.102-G2 to CH27-Ep complexes also antagonized the alloresponse to CH27-Ep. Interestingly, the range of effective concentrations for each peptide is similar for both ligands. These results demonstrate that the 2.102-G2 alloresponse can be antagonized, and that allo- and self-restricted responses do not appear to be intrinsically different in their sensitivity to TCR antagonism.

The alloresponse of naive 2.102 T cells and Th1/Th2 cell lines derived from TCR tg mice is antagonized by APLs

The alloresponse of naïve T cells and Th1/Th2 cell lines derived from TCR tg mice is also antagonized by APLs. CH27 cells pulsed with 1 μM of Hb(64-76) peptides (A) or CH27-Ep cells (B) were used to stimulate the 2.102-G2 hybridoma at the level indicated by the dashed line. Both APCs were used at 10^6 cells/well in a 96-well flat-bottom plate. The capacity of the variant peptides S69, N73, and Q72 to inhibit this response was evaluated. T cell activation was measured as described in Figure 1. The values represent the mean ± SD of triplicate wells. The results are representative of more than three experiments. 

The alloresponse of 2.102-G2 can be antagonized by APLs. CH27 cells pre-pulsed with 1 μM of Hb(64-76) peptides (A) or CH27-Ep cells (B) were used to stimulate the 2.102-G2 hybridoma at the level indicated by the dashed line. Both APCs were used at 10^6 cells/well in a 96-well flat-bottom plate. The capacity of the variant peptides S69, N73, and Q72 to inhibit this response was evaluated. T cell activation was measured as described in Figure 1. The values represent the mean ± SD of triplicate wells. The results are representative of more than three experiments.

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response, the purpose of the following experiment was to demonstrate antagonism of the alloresponse of phenotypically naïve T cells derived from 2.102tg mice. The 2.102tg mice used in these experiments were bred into a RAG1-deficient background, which allowed direct analysis of the transgenic T cell naïve phenotype without the need for a clonotypic Ab. This also eliminates the contribution of T cells with a TCR generated from pairing of the α-chains in the alloresponse observed. Naive T cells derived from 2.102tg/RAG1-deficient mice were purified by cell sorting, resulting in a population nearly 100% CD4<sup>+</sup> and CD62L<sup>high</sup> (data not shown). The observation that these transgenic T cells were alloreactive to CH27-Ep confirmed that the TCR made of TCRα4J48 and TCRβ1J2S4 chains was solely responsible for this alloreactivity. Importantly, the alloresponse of the naïve transgenic T cells was also antagonized by the S69 and N73 APLs (Fig. 4B). Furthermore, the alloresponse of 2.102tg/RAG1-deficient T cells was antagonized to a similar extent whether these T cells were purified by cell sorting or used after separation on a nylon wool column (90% CD4<sup>+</sup> and CD62L<sup>high</sup>) (data not shown).

Th1 and Th2 cellular responses and cytokines have been associated with allograft rejection and tolerance, respectively (30). To determine the sensitivity of these T cell populations to TCR antagonism, Th1 and Th2 cell lines were derived from 2.102tg/RAG1-deficient mice. We analyzed the effect of APLs on the alloresponse of these lines to CH27-Ep. Both Th1 and Th2 lines were determined if the proliferative alloresponse of 2.102tg mouse (2.102tg) could also be antagonized. As shown in Figure 3A, APLs that inhibited the response of 2.102-G2 to CH27 cells pre-pulsed with the agonist peptide Hb(64-76) (Fig. 3A), whereas the null peptide control, Q72, had no activity. This result recapitulated what has been previously described by Evavold et al. (13). To evaluate the effect of these peptides on the alloresponse of 2.102-G2, a similar assay was performed except that a concentration of CH27-Ep cells giving a submaximal stimulation was chosen, and the APLs were added directly to these cells. As shown in Figure 3B, APLs that inhibited the response of 2.102-G2 to Hb(64-76)/I-E<sup>k</sup> complexes also antagonized the alloresponse to CH27-Ep. Interestingly, the range of effective concentrations for each peptide is similar for both ligands. These results demonstrate that the 2.102-G2 alloresponse can be antagonized, and that allo- and self-restricted responses do not appear to be intrinsically different in their sensitivity to TCR antagonism.
could be antagonized by S69 and N73 peptides (Fig. 5). The effective dose range of APLs was also similar for both Th lines. Interestingly, even though the Th1 line alloresponse to CH27-Ep was weaker than the Th2 line in terms of proliferation, their sensitivity to antagonism was very similar. These experiments using the 2.102tg mouse showed that the alloresponse of naive T cells and Th1 or Th2 lines can be effectively inhibited by TCR antagonist peptides.

Both antagonist stimuli and agonist stimuli are required on the same APC for effective antagonism

The antagonism results shown above were obtained using the CH27-Ep cell line as APCs, which express both I-Ek and I-Ep. We wanted to ascertain if the alloligand and the antagonist ligand were required to be presented on the same cell. To address this question, we performed a set of experiments using splenocytes from B10.P (H-2p), B6.AKR (H-2k), or (B10.P 3 B10.BR)F1 (H-2p/k) mice as APCs. The antagonist peptides could not inhibit 2.102-G2 activation by B10.P splenocytes, ruling out the possibility that APLs antagonize the alloresponse through a direct interaction with I-Ep (Fig. 6A). The activation of 2.102-G2 was inhibited when both I-Ep and I-Ek were present on the same APC, as in the (B10.P 3 B10.BR)F1 splenocytes (Fig. 6B), suggesting that APLs were presented by I-Ek. In contrast, we could not observe any inhibition of 2.102-G2 when B10.P splenocytes where mixed with B6.AKR splenocytes in the antagonist assay (Fig. 6C). Thus, the presence of both agonist and antagonist ligands on the same APC was absolutely required to observe effective antagonism.

Discussion

The discovery of TCR antagonist peptides has generated hopes for their use as specific therapeutics in autoimmune diseases and graft rejection. To further explore this issue, we have established a new model to study MHC class II-restricted alloreactivity based on the allorecognition of I-Ep by the Hb(64-76)/I-Ek-specific 2.102 T cell. We then showed that antagonist peptides could effectively inhibit in vitro the alloresponse of various T cell populations bearing the 2.102 TCR. Hence, our data corroborate the study of Racioppi et al. (31), which showed that a Th1 clone alloresponse to a mutant I-Ek molecule could be antagonized, and extend these findings to demonstrate that Th2 and, more importantly, naive T cell alloresponses are also sensitive to TCR antagonism.

The alloreactivity of the 2.102-G2 hybridoma was assayed by stimulation with splenocytes from mice of different haplotypes without the addition of exogenous peptides. Only splenocytes from P/J mice could stimulate this hybridoma. However, we cannot rule out reactivity against some class II molecules in A.Ca (H-2f), DBA/1 (H-2q), SJL (H-2s) strains because these mice do not express I-E class II molecules (32). Nevertheless, 2.102-G2 was not stimulated by (B6.AKR 3 C57Bl/6)F1 splenocytes, demonstrating that it is not alloreactive against I-Ep (data not shown). Thus, our
results suggest that the 2.102-G2 hybridoma is specific in its recognition of the alloligand I-E\éta. The molecular nature of alloreactivity remains subject to debate and investigation (33, 34). Peptide-independent alloractive responses have been suggested by various studies, mostly by using cell lines defective on Ag processing as APCs (35, 36). Numerous studies have also demonstrated the role of peptides in allorecognition of class I and class II molecules (37–41). It has been postulated that the molecular basis for the recognition of non-self MHC molecules by self-MHC-restricted TCRs would be molecular mimicry between nominal and alloge- menace Ags. However, this interpretation was recently questioned by the work of Brock et al. (42), which analyzed using biologic assays and molecular modeling the reactivity of the 2C T cell clone against self-K\éta-peptide and allo-L\éta-peptide ligands. Their study suggested that cross-reactivity of 2C with these ligands was based on adaptation of the TCR to different ligand structures rather than molecular mimicry. Furthermore, it has been also suggested that TCR affinities for allo-restricted ligands tend to be higher than for self-restricted ligands (43). Thus the interaction between a TCR and self- or allo-MHC ligands could be different in numerous aspects. However, our results demonstrate that T cell activation that follows recognition of these two ligands can be inhibited by TCR antagonism with similar efficacies. The recent elucidation of the 3-D structure of the Hb(64–76)/I-E\éta complex (21) provides a unique opportunity, following further characterization of the allo-ligand recognized by the 2.102 TCR, to study the molecular nature of MHC class II-restricted alloractivity.

The mechanism of antagonism has not been clearly established. Recent studies have suggested that TCR antagonism would also be related to early tyrosine phosphorylation events in signaling pathways (44, 45), as was previously shown for partial agonists (46, 47). Our study clearly shows that antagonism requires that both agonist and antagonist ligands are presented on the surface of the same APC. Similar observations have been previously reported by Ruppert et al. (48) and have also been performed in our laboratory (K. Vidal, unpublished data). This strongly supports the notion that TCR antagonism occurs through the TCR and interferes with an early event of T cell activation rather than the induction of a general state of T cell unresponsiveness. Improper TCR oligomerization has been proposed as a mechanism for antagonism (16). In the context of this model, it is interesting to observe that heterogeneous MHC class II molecules can participate in the same oligomerization process; complexes made of antagonist peptides presented by I-E\éta could prevent oligomerization of I-E\éta/TCR complexes. It is also noteworthy that the interaction of the 2.102 TCR with its nominal Ag and the alloantigen are both antagonized in the same range of peptide concentrations, suggesting that there are no important differences in the efficacy to form E\éta homo-oligomers or E\éta/E\éta hetero-oligomers.

The model reported in this study could also be very useful to further explore the therapeutic usage of variant peptides. Several mechanisms have been described by which APLs or variants of agonist peptides can modulate harmful immune responses in vivo. TCR antagonism was suggested in addition to MHC blocking in the inhibition of adjuvant arthritis using variant peptides of the mycobacterial 65-kDa protein (49). A single TCR antagonist peptide was also shown to inhibit the development of disease in the experimental allergic encephalomyelitis (EAE) model (50). Furthermore, a pool of APLs was superior to MHC blockers or to a single APL in inhibiting induction of EAE (51). Interestingly, multiple antagonist peptides (S69 and N73, this study; A74, our unpublished data) have been defined in our model of allorecognition using the 2.102 TCR. A pool of these APLs could also be more efficient to inhibit graft rejection mediated by T cells bearing this TCR. Peptides that antagonize T cell clones with different fine specificities can be defined. Indeed, a high proportion of T cell clones generated against the Hb(64–76)/I-E\éta complex recognize peptides substituted at position 73 (i.e., N73) as APLs (20). Crystals of antigens have revealed that residue E73 of the Hb peptide is an MHC anchor residue (P6) adjacent to the primary TCR contact residue N72 (P5) (21). It remains to be determined if the N73 APL could also antagonize I-E\éta alloreactive T cells that have a specificity similar to the 2.102 TCR. Another mechanism shown to mediate the in vivo effect of variant peptides is modulation of cytokine production. In their study of EAE with myelin proteolipid protein peptide 139–151, Nicholson et al. (52) have shown that disease induction could be prevented when mice were preimmunized or immunized with peptides substituted at the primary TCR contact residue. These authors proposed that immune deviation of T cell differentiation, from the disease-inducing Th1 to a less aggressive Th0 or Th2 phenotype, was involved. Similar observations have been made in EAE induced by myelin basic protein peptides (18). Brocke et al. (53) have directly shown that protective variant peptides could have a trans-effect on T cell clones with different fine specificities. Interestingly, variant peptides that affect differentiation of 2.102 T2 cells toward Th1 or Th2-like phenotype have been identified in our laboratory (Grakoui et al., manuscript in preparation). These peptides could also be tested in vivo to assess their capacity to alter graft rejection mediated by 2.102 T cells.

Our cumulative data show that different T cell populations bearing the same TCR can be antagonized. A recent study by Dittel et al. (54) showed that antagonist peptides could inhibit the proliferation and the cytokine production of Th1, Th2, and Th0 clones derived from TCR transgenic mice. Interestingly, our study shows that antagonism of Hb(64–76)/I-E\éta and alloresponses were observed at similar peptide concentrations for the T cell hybridoma, Th1/Th2 cell lines, and naive T cells studied, suggesting a similar mechanism of antagonism for all three cell types. Furthermore, this study constitutes to our knowledge the first demonstration that phenotypically naive T cells can be antagonized by APLs. The inhibition of naive T cell activation is particularly relevant for transplantation because direct alloractivity is thought to be mediated by the activation naive T cell populations. Overall, these studies highlight the potential usefulness of APLs to control alloresponses.

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References

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