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Epitope Mapping of the Indirect T Cell Response to Allogeneic Class I MHC: Sequences Shared by Donor and Recipient MHC May Prime T Cells That Provide Help for Alloantibody Production

Emma Lovegrove, Gavin J. Pettigrew, Eleanor M. Bolton, and J. Andrew Bradley

Indirect allorecognition occurs when T cells recognize donor MHC presented as peptide epitopes by recipient APC, but the precise nature of the epitopes involved remains unclear. Rejection of rat MHC class I-disparate PVG.R8 (RT1.Aa) grafts by PVG.RT1u (RT1.Au) recipients is mediated by indirectly restricted CD4 T cells that provide help for the generation of alloantibody. In this study, epitope mapping was performed using a functionally relevant readout (alloantibody production) to identify key peptides that prime an indirect alloimmune response, leading to graft rejection. PVG.RT1u rats were immunized with a series of overlapping 15-mer peptides (peptides 1–18) that spanned the α1 and α2 domains of the RT1.Au molecule. Several peptides were able to accelerate both the alloantibody response to the intact RT1.Au Ag and PVG.R8 heart graft rejection. An immunodominant epitope was identified within the hypervariable region of the α1 domain. Fine mapping of this region with a second series of peptides overlapping by single amino acids confirmed the presence of an eight-amino acid core determinant. Additional “subdominant” epitopes were identified, two of which were located within regions of amino acid homology between the RT1.Au and RT1.Aa molecules and not, as had been expected, within other hypervariable regions. The contribution of self-epitopes to indirect allorecognition was emphasized by the demonstration that i.v. administration of a 15-mer peptide encompassing one of the subdominant self-determinants diminished the recipient’s ability to mount an alloantibody response on challenge with intact Aa alloantigen. Our findings suggest that cryptic self-epitopes recognized by autoreactive T cells may contribute to allograft rejection and should be considered when designing novel strategies for inducing tolerance to alloantigen. The Journal of Immunology, 2001, 167: 4338–4344.

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MHC alloantigen. A series of overlapping peptides spanning the α1 and α2 domains of the RT1.Aa molecule were used to map the T cell epitopes responsible for generating help for alloantibody production in vivo. We reasoned that assaying the anti-Aa alloantibody response following priming with synthetic Aa peptides would, in contrast to relying on the use of in vitro T cell proliferation assays alone, provide a functionally relevant assessment of the indirect T cell response, since alloantibody is a known effector mechanism for graft rejection in this experimental model. Using this approach, an immunodominant epitope corresponding to the hypervariable region of the α1 domain was identified. Several subdominant epitopes were also identified and, unexpectedly, at least two of these were located in regions of amino acid identity in the Aa and Aa haplotypes. The implications of this finding and the potential contribution of autoreactive T cells to allograft rejection are discussed.

Materials and Methods

Animals

Congenic PVG.RT1a (Aa B/D C) and recombinant PVG.R8 (Aa B/D C) rats were purchased from Harlan U.K. (Bicester, Oxon, U.K.). The derivation of the PVG.RT1a and PVG.R8 rat strains is cross-referenced elsewhere (21). All animals were maintained under standard conditions and used when 8–12 wk old.

Allopeptides

Epitope mapping was undertaken using a series of eighteen 15-mer peptides (designated peptides 1–18), each overlapping by 5 aa and spanning the α1 and α2 domains of the RT1.Aa molecule (residue 1 (glycine) to residue 185 (proline) of the α1 domain) (22). Peptides were obtained from Immune Systems (Paignon, U.K.) and were synthesized by standard Fmoc chemistry, purified by HPLC, and assessed by mass spectrometry (peptide purity >80%). An additional 15-mer peptide (YAQWEIQRERQIT) was synthesized for use as an irrelevant control peptide. It comprised the same amino acids as those in peptide 7, but in random order.

Fine epitope mapping was performed using another series of 15-mer peptides overlapping by single amino acids and spanning the hypervariable region of the α1 domain of the RT1.Aa molecule (residue 57 (proline) to residue 85 (tyrosine) of the α1 domain, see Fig. 1).

Immunization with allopeptides

PVG.RT1a rats were immunized by s.c. injection into each hind footpad of 50 μg of peptide dissolved in 50 μl of distilled water and emulsified with a comparable volume of CFA (Sigma, Dorset, U.K.).

Cardiac transplantation

PVG.R8 heart grafts were transplanted into PVG.RT1a recipients according to the modified technique of Ono and Lindsey (23). The donor heart was transplanted heterotopically into the abdomen using standard microsurgical techniques with end-to-end anastomosis of the donor aorta and pulmonary artery to the recipient infrarenal aorta and vena cava, respectively. Cold ischemic times were <30 min. Grafts were assessed by daily palpation and rejection was defined as the complete cessation of myocardial contraction.

Skin transplantation

PVG.RT1a recipients were grafted on the flank with full-thickness PVG.R8 skin grafts as described elsewhere (24).

Blood transfusion

PVG.RT1a rats were injected with 1.5 ml of heparinized PVG.R8 blood i.v. into the dorsal penile vein.

Cytotoxic alloantibody determination

Lymphocytotoxic Abs in serum samples were detected by their ability to lyse 31Cr-labeled Con A-transformed splenic blasts in the presence of guinea pig complement, as described elsewhere (25). Percent specific 31Cr release was calculated by the formula: (experimental release – spontaneous release)/(maximum release – spontaneous release) × 100.

Anti-peptide Ab determination

Peripheral blood serum samples were tested for the presence of Abs to synthetic peptides using an indirect radioactive binding assay. Briefly, 96-well plates were coated with 50 μl/well peptide at 100 μg/ml in 0.15 M NaCl for 18 h at 4°C and then washed three times with PBS/0.1% BSA. Plates were blocked with 200 μl of Membryl III (Bionostics, Wyboston, Bedfordshire, U.K.) for 2 h at 37°C and washed before adding tripling dilutions of test serum diluted in PBS/0.5% BSA (50 μl/well). After a 2-h incubation at 37°C, plates were washed and bound anti-peptide Ab was detected with 50 μl/well 125I-labeled sheep F(ab)2 anti-rat Ig (Amersham International, Amersham, U.K.) diluted 100-fold in PBS/0.5% BSA. Following incubation for 1 h at 37°C, plates were washed and bound radioactivity was measured.

T cell proliferation assays

Lymph node cells (LNC)3 were prepared from pooled popliteal, cervical, and mesenteric lymph nodes and resuspended at a concentration of 4 × 106 cells/ml in RPMI 1640 enriched with 2% heat inactivated syngeneic normal rat serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine, and 50 μM 2-ME. LNC (100 μl) were added to 96-well U-bottom plates and peptide was added to a final concentration of 40 μg/ml in a final volume of 200 μl/well. Plates were incubated at 37°C in 5% CO2 for 72 h, pulsed with 1 μCi/well 3H-thymidine, and incubated for another 24 h before harvesting. Incorporated 3H-thymidine was determined using a liquid scintillation beta counter. Results are expressed as a stimulation index, calculated as experimental counts/control counts.

Statistical analysis

Differences between groups were compared by nonparametric analysis using the Mann-Whitney U test. All p (two tailed) <0.05 were considered to be significant.

Results

Naive PVG.RT1a rats reject Aa-disparate PVG.R8 allografts rapidly by CD4 T cell-dependent alloantibody-mediated effector mechanisms (20, 21). To map the T cell epitopes of RT1.Aa responsible for indirect CD4 Th cell activation in this model, a series of overlapping synthetic 15-mer peptides spanning the entire α1 and α2 domains of the Aa molecule were used. The initial set of peptides used were designated peptides 1–18. As shown in Fig. 1, peptides 7 and 8 (corresponding to the hypervariable region of the α1 domain) and peptides 15, 16, and 17 (corresponding to the hypervariable regions of α2 domain) contained the greatest number of amino acid disparities (4–9 residues). Eight of the 18 RT1.Aa-derived peptides used had identical sequences to the corresponding region of the RT1.Aa molecule.

Immunogenicity of allopeptides

Peptides encompassing T cell epitopes involved in the indirect allorecognition of Aa class I MHC would be expected to stimulate a peptide-specific in vitro T cell proliferative response and/or anti-peptide Ab response. We therefore sought to establish which of the 15-mer Aa peptides were immunogenic by immunizing PVG.RT1a rats s.c. with individual peptides emulsified in CFA. Twelve days later, LNC were assessed for their ability to proliferate in vitro to peptide and circulating serum was assayed for the presence of anti-peptide Ab by an indirect radioactive binding assay.

The proliferative response of lymphocytes to the immunizing peptide was weak and only LNC obtained from animals immunized with either peptide 1 or peptide 7 showed a stimulation index of ≥2 (Fig. 2a). LNC from rats immunized with peptide 7 did not proliferate to peptide 1 in vitro and vice versa, suggesting the presence of distinct peptide 1- and peptide 7-specific T cells rather than T cells recognizing both peptides (data not shown). The 18

3 Abbreviations used in this paper: LNC, lymph node cell; MST, median survival time.
peptides tested also displayed limited ability to stimulate anti-peptide Abs. Only animals immunized with peptides 7 and 16 developed a detectable Ab response (Fig. 2b), suggesting that these two peptides encompass CD4 T cell determinants. It was not surprising that peptides 7 and 16 (which correspond to the hypervariable regions of the \( \alpha / H9251 \)1 and \( \alpha / H9251 \)2 domains of the RT1.A a Ag, respectively) were immunogenic. The ability of peptide 1 to stimulate a T cell proliferative response was not predicted from the amino acid sequence data, since the 15-mer sequence corresponded to a region of the \( \beta \)-pleated sheet of the \( \alpha 2 \) domain that is identical in the RT1.Aa and RT1.AA molecules. It is likely that peptide 1 failed to stimulate an anti-peptide Ab response because the linear peptide does not constitute a B cell determinant.

T cell proliferation to allopeptides after transplantation

To determine the T cell epitopes involved in the indirect allorecognition of the RT1.A a molecule, PVG.RT1u animals were immunized with a vascularized PVG.R8 cardiac allograft or a skin allograft and their LNC were tested for in vitro proliferation against individual peptides. LNC from PVG.RT1u rats primed with either a PVG.R8 heart graft or a PVG.R8 skin graft alone showed minimal proliferation to the panel of Aa peptides (data not shown). The lack of in vitro lymphocyte proliferation to allopeptide after transplantation with a single graft is consistent with the findings of Fangmann et al. (26), who noted that lymphocytes obtained from rats primed by one transplant alone did not proliferate effectively to donor allopeptides. PVG.RT1u rats were, therefore, primed with a full-thickness R8 skin graft followed 10 days later by a PVG.R8 cardiac allograft. LNC from such animals displayed a modest proliferative response to peptide 7 and a weaker response to peptide 8, suggesting the presence of a dominant T cell determinant within the hypervariable region of the \( \alpha \) domain (Fig. 3).

The ability of peptide immunization to accelerate the alloantibody responses to intact Aa MHC

PVG.RT1u rats develop a CD4 T cell-dependent anti-Aa lymphocytotoxic alloantibody response following exposure to intact Aa alloantigen in the form of a PVG.R8 blood transfusion (25). Blood transfusion, therefore, provides a straightforward and reproducible model in which to determine the ability of peptide immunization to influence the kinetics of this alloantibody response. Those peptides that accelerate the alloantibody response can be assumed to encompass a linear T cell epitope that is presented to CD4 T cells by Aa-specific B cells after internalization and processing of the intact RT1.Aa Ag. PVG.RT1u rats were immunized with individual 15-mer peptides in CFA and then 7 days later challenged with a PVG.R8 blood transfusion. Serum samples were obtained on days 4 and 7 after blood transfusion and the level of anti-Aa alloantibody was determined by assaying against PVG.R8 lymphoblast target cells. In this, as in our previous studies (18, 19), immunization with Aa peptides did not, in the absence of blood transfusion, stimulate the development of anti-peptide Abs cross-reactive with intact RT1.Aa on target cells (data not shown). By day 4 after blood transfusion, recipients that had been immunized with
Peptides 1, 7, 8, and 9 showed significantly higher levels of circulating cytotoxic anti-RT1.A<sup>a</sup> alloantibody than animals immunized with control peptide (Fig. 4). This experiment therefore confirmed the presence of a dominant T cell determinant(s) corresponding to the hypervariable region of the α1 domain. Additional T cell epitopes encompassed by peptides 1 and 9 were revealed and categorized as subdominant because they influenced the recall response to challenge with intact alloantigen. Peptide 9, like peptide 1, is consensual in the corresponding regions of the RT1.A<sup>a</sup> and RT1.A<sup>u</sup> molecules. None of the other consensual peptides (peptides 2, 3, 5, 12, 13, and 14) primed for RT1.A<sup>a</sup> alloantibody production. A possible explanation for their lack of immunogenicity is that they may encompass immunodominant self-epitopes, resulting in thymic deletion of peptide-specific T cells. Alternatively, some of the consensual peptides tested may lack immunogenicity because they do not bind with sufficient avidity to MHC class II to stimulate a T cell response. Peptide binding studies would help to distinguish these two possibilities.

The differences observed at day 4 in alloantibody levels between animals primed with peptides 1, 7, 8, and 9 and the other members of the peptide panel were no longer apparent by day 7 after blood transfusion (data not shown). It was notable that peptide 16, which provoked a strong anti-peptide Ab response (Fig. 2b), did not lead to an accelerated anti-RT1.A<sup>a</sup> alloantibody response. Presumably, the T cell epitope encompassed by peptide 16 is not presented by A<sup>a</sup>-specific B cells that encounter intact A<sup>a</sup> alloantigen.

Peptides that had been shown to accelerate the alloantibody response to a PVG.R8 blood transfusion (peptides 1, 7, and 8), along with selected uninvolved peptides (peptides 4, 12, and irrelevant peptide), were tested further for their ability to influence the alloimmune response to a PVG.R8 heart graft. As shown in Table I (group 1), naive PVG.RT1<sup>a</sup> rats reject A<sup>a</sup>-disparate PVG.R8 heart grafts rapidly (mean survival time (MST), 7 days). Of the peptide-immunized animals, recipients immunized with peptide 7 (encompassing an immunodominant T cell determinant; Table I, group 4) rejected their grafts most rapidly and rejection was significantly faster than that of control animals (MST, 4 days vs 7 days, respectively, <i>p</i> < 0.05). Recipients immunized with peptides 1 and 8 (groups 2 and 5, respectively) also displayed accelerated allograft rejection (MST, 5 days, <i>p</i> < 0.05), whereas immunization with peptides 4 and 12 (groups 3 and 6, respectively) did not accelerate graft rejection (MST, 7 days). The effects of peptide priming were not synergistic; immunizing with all 18 peptides (group 8) resulted in a similar acceleration in PVG.R8 heart graft rejection as immunizing with individual peptides alone. The accelerated kinetics of heart graft rejection corresponded to a more rapid anti-A<sup>a</sup> alloantibody response; sera from PVG.RT1<sup>a</sup> animals primed with peptides 1, 7, and 8 contained higher levels of cytotoxic alloantibody at day 4 following heart grafting than peptides 4, 12, and the irrelevant peptide (Fig. 5).

**Identification of the core T cell determinant within the hypervariable region of the α1 domain of RT1.A<sup>a</sup>**

The above analysis revealed that peptides 7 and 8 both encompassed a Th cell determinant, but not whether this represented a shared, rather than two distinct, T cell determinant. Moreover, it was not clear whether the immunodominant T cell determinant identified was optimally represented by peptide 7 and did not, for example, extend to include adjacent C-terminal amino acids. A second set of fifteen 15-mer peptides (designated peptides A–O, Fig. 6), overlapping by single amino acid residues and spanning the hypervariable region of the α1 domain of RT1.A<sup>a</sup> (from residue 57 [proline] to residue 85 [tyrosine], see Fig. 1), were therefore used to map this region more precisely. Animals were primed by s.c. injection of individual peptides in CFA and the alloantibody response to challenge with a PVG.R8 blood transfusion determined. Peptides E through to L all caused accelerated alloantibody production (Fig. 7). This suggests the presence of a T cell determinant with a core amino acid octamer comprising KEWEQIYR that is located at the C-terminal end of peptide 7 (designated peptide E in this analysis), and contains four disparate amino acid.

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**Table I.** Rejection of MHC class I-disparate PVG.R8 cardiac allografts by peptide-immunized PVG.RT1<sup>a</sup> recipients

<table>
<thead>
<tr>
<th>Group</th>
<th>Pretreatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>n</th>
<th>Graft survival&lt;sup&gt;b&lt;/sup&gt; (days)</th>
<th>MST&lt;sup&gt;c&lt;/sup&gt; (days)</th>
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<td>7</td>
</tr>
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<td>P1</td>
<td>4</td>
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<td>5</td>
</tr>
<tr>
<td>3</td>
<td>P4</td>
<td>3</td>
<td>6, 7, 7</td>
<td>7</td>
</tr>
<tr>
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<td>P7</td>
<td>5</td>
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<td>P8</td>
<td>3</td>
<td>4, 5, 5</td>
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</tr>
<tr>
<td>6</td>
<td>P12</td>
<td>3</td>
<td>7, 7, 7</td>
<td>7</td>
</tr>
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<td>Irrelevant peptide</td>
<td>3</td>
<td>6, 7, 7</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>All 18 peptides&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4</td>
<td>4, 4, 4, 4</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Recipient RT1<sup>a</sup> animals were immunized s.c. with 100 μg of peptide emulsified in CFA 7 days before receiving an A<sup>a</sup>-disparate R8 cardiac allograft.

<sup>b</sup> Animals were assessed daily, and allograft rejection was defined as complete cessation of myocardial contraction.

<sup>c</sup> Group 1 vs groups 2, 4, 5, and 8, <i>p</i> < 0.05.

<sup>d</sup> Recipient animals were immunized with a mixture of all 18 peptides (100 μg of each peptide) emulsified in CFA 7 days before heart transplantation.

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**FIGURE 3.** T cell proliferation to peptide after transplantation. PVG.RT1<sup>a</sup> rats were grafted with a PVG.R8 skin graft followed after 10 days by a PVG.R8 heart graft. Ten days later, the proliferative response of LNC to individual peptides was determined. Results shown are the mean and SD of three animals.

**FIGURE 4.** Anti-A<sup>a</sup> alloantibody response to PVG.R8 blood transfusion after peptide immunization. PVG.RT1<sup>a</sup> rats were immunized with 100 μg of peptide in CFA and challenged 7 days later with a PVG.R8 blood transfusion. Serum was obtained 4 days after blood transfusion and assayed for cytotoxic alloantibody against <sup>51</sup>Cr-labeled PVG.R8 lymphoblast target cells. Results are expressed as mean and SD (three animals per group) of Ab titer (last dilution of serum that gave >20% cytotoxicity). *, <i>p</i> < 0.05 vs animals primed with control peptide.
residues. In addition peptides A, B, and O also accelerated alloantibody production (Fig. 7), suggesting the presence of further T cell epitopes flanking the central octamer and attributable to the presence of additional amino acid disparities within the hypervariable region of the α helix.

Down-regulation of the alloantibody response by administration of i.v. peptide

The identification of subdominant determinants within peptides 1 and 9 raised the question as to whether these self-determinants were of relevance as potential targets for manipulating the indirect helper T cell response to intact Aα alloantigen. In animal models of autoimmune disease, the i.v. administration of pathogenic peptide at a high dose may attenuate disease progression (27, 28). We therefore examined the ability of selected peptides (peptides 1, 7, and 15) when given i.v. to down-regulate the subsequent Ab response to Aα alloantigen. Peptides 1 and 7 both effectively down-regulated the cytotoxic alloantibody response to a PVG.R8 blood transfusion (Fig. 8). The functional relevance of our mapping studies was confirmed by the demonstration that the i.v. administration of peptide 15 (that corresponds to the hypervariable region of the α2 domain and contains 6 disparate amino acids) did not reduce the anti-Aα alloantibody response (Fig. 8). This experiment suggests that self-determinants may be legitimate targets for inhibiting T cell-dependent alloantibody production.

Discussion

In this paper, the T cell epitopes responsible for the provision of help for alloantibody production were defined in a class I-disparate model in which alloantibody is an important effector mechanism of graft rejection. Epitopes were identified by testing a series of 15-mer peptides spanning the α1 and α2 domains of RT1.Aα for their ability to prime CD4 T cells in PVG.RT1u rats and thereby accelerate the alloantibody response to subsequent challenge with intact Aα alloantigen. Two principal findings emerged. First, multiple T cell epitopes were identified within the Aα molecule, recognition of which provided T cell help for alloantibody production. Second, the T cell determinants identified were located not only in the

**FIGURE 5.** Anti-Aα alloantibody response to PVG.R8 heart graft after peptide immunization. PVG.RT1u rats were immunized with 100 μg of peptide in CFA and challenged 7 days later with a PVG.R8 heart graft. Serum was obtained 4 days later and assayed for cytotoxic alloantibody. Results are expressed as mean and SD (three animals per group) of Ab titer (last dilution of serum that gave >20% cytotoxicity). *p < 0.05 vs animals primed with irrelevant peptide.

**FIGURE 6.** Amino acid sequence of the 15-mer peptides A–O used to map precisely the location of the dominant epitope in the hypervariable region of the α1 domain of RT1.Aα. Peptide E and peptide O correspond to peptides 7 and 8, respectively, of the initial peptide set. The shaded box encloses the amino acids that comprise the core octamer (see text for details).

**FIGURE 7.** Identification of the core T cell determinant within the hypervariable region of the α1 domain of RT1.Aα. PVG.RT1u rats were immunized with 100 μg of a single peptide (peptides A–O) in CFA and challenged 7 days later with a PVG.R8 blood transfusion. Serum was obtained 4 days later and assayed for cytotoxic alloantibody against 51Cr-labeled PVG.R8 lymphoblast target cells. Results are expressed as mean and SD (three animals per group) of Ab titer (last dilution of serum that gave >20% cytotoxicity).

**FIGURE 8.** Inhibition of cytotoxic alloantibody response to PVG.R8 blood transfusion by pretreatment with i.v. peptide. PVG.RT1u rats received 300 μg of peptide i.v. 12 days before challenge with a PVG.R8 blood transfusion. Serum was obtained 4 and 7 days after blood transfusion and assayed for cytotoxic Aα alloantibody. Results are expressed as the mean and SD of three to four animals per group. *p < 0.05 vs control animals pretreated with i.v. saline.
hypervariable region of RT1.A<sup>a</sup> but also in regions of the A<sup>a</sup> molecule where there was sequence identity with RT1.A<sup>u</sup>.

Comprehensive mapping of the T cell epitopes involved in indirect allorecognition by analysis of their ability to prime the response to challenge with intact alloantigen has not previously been undertaken. Earlier studies have assessed the ability of T cells from recipients primed with intact alloantigen to respond in vitro to challenge with allopeptides (8, 16, 17, 29). The most detailed study of this type was performed by Benichou et al. (14). In each of three different mouse strain combinations tested, T cells from animals immunized with allogeneic MHC proliferated in vitro to a single immunodominant epitope that was located in the hypervariable region of the donor class II MHC. When, in the present study, the in vitro proliferative response of lymphocytes from recipients primed by sequential class I MHC-disparate allografts was examined, an immunodominant T cell epitope (encompassed by peptide 7) corresponding to the hypervariable region of the α1 domain of RT1.A<sup>a</sup> was identified. The location of an immunodominant epitope within the hypervariable region of the RT1.A<sup>a</sup> molecule is consistent with previous less detailed mapping studies (16, 30). There are several amino acid disparities between the α1 domains of RT1.A<sup>a</sup> and RT1.A<sup>u</sup>, and presumably the four disparate amino acid residues within the core octameric epitope identified in this study provide a recognition motif for alloreactive T cells.

The additional T cell epitopes identified in this study were not apparent from measurement of either the proliferative response or the Ab response to linear peptide. They were instead identified through an in vivo functional readout that reflected priming of T cells providing help for alloantibody production. Previous studies have also found that the nature of the T cell epitopes differs according to whether an in vitro or in vivo approach was adopted (31, 32). In the present study, failure of in vitro proliferation assays to reveal all of the immunogenic peptides can be explained on the basis that lymphocyte proliferation correlates poorly with T cell cytokine production. We chose, for the reasons already outlined, to map T cell epitopes through their ability to promote T cell help for B cells, but it would also be of interest to examine whether alternative in vivo assays of T cell cytokine production, such as a delayed-type hypersensitivity assay, reveal the same or a different set of immunogenic epitopes.

Epitopes identified through the ability of peptide immunization to influence the recall response to intact protein Ag can be designated subdominant (12). The location of subdominant T cell epitopes within the RT1A<sup>a</sup> molecule could not have been predicted on the basis of amino acid disparity alone. It is notable that immunization with peptides corresponding to the hypervariable region of the α2 domain, where there are several amino acid disparities between RT1A<sup>a</sup> and RT1A<sup>u</sup>, did not prime for accelerated alloantibody production. Conversely, immunization with peptides 1 and 9 (derived from the β-pleated sheets of the α1 and α2 domains, respectively) resulted in an accelerated alloantibody response and this correlated with accelerated heart graft rejection. Both peptides correspond to regions of RT1A<sup>a</sup> where there is amino acid identity with RT1A<sup>a</sup>. They therefore encompass cryptic self-epitopes (33) recognized by potentially autoreactive T cells, and immunization with these peptides in the presence of adjuvant is evidently sufficient to overcome the peripheral mechanisms that normally prevent the development of autoimmunity. Benichou and colleagues (34) have recently reported that immunization of mice with allogeneic splenocytes led to an autoimmune T cell response directed against a self class I MHC peptide. A subsequent study by the same group demonstrated that cardiac transplantation in the mouse triggered a T cell autoimmune response directed against cardiac myosin and that sensitization of recipient mice with cardiac myosin accelerated heart graft rejection (35). The results of the present study and those of Benichou and colleagues (33–35) highlight the potential contribution of T cells directed against cryptic self-epitopes to allograft rejection.

The identification of subdominant epitopes through their ability to prime helper T cells for B cell help may be of particular relevance to the investigation of chronic allograft rejection. In the early phase of the alloimmune response, the direct pathway of allorecognition may be dominant due to the large precursor frequency of recipient T cells that recognize intact donor MHC on the surface of donor APC (36). Because donor APC within a graft are soon replaced by APC from the recipient (37), T cells that recognize donor MHC indirectly as peptide fragments are likely to play an increasingly important role and may be responsible for chronic rejection (1). A role for indirect allorecognition in chronic rejection is supported by the recent observation that allograft vasculopathy is accelerated in animals primed with donor MHC class I-derived peptides (38). Moreover, chronic allograft vasculopathy is still observed in experimental models in which the graft lacks donor strain APC (39). Evidence that indirect allorecognition contributes to chronic allograft rejection in humans is provided by the demonstration that T cells obtained from patients with chronic rejection display specificity for donor MHC-derived peptides (40–43).

As the alloimmune response develops, indirect T cell specificity spreads to include additional epitopes other than the immunodominant epitopes that were the focus of the initial acute response (41). Similar shifts in T cell reactivity have been noted in experimental models of autoimmunity (13) and rather than simply representing a marker of ongoing immune activation, they appear integral to the progression of disease (44). B cells may play a pivotal role in epitope spreading because their unique efficacy for capturing Ag-Ab complexes confers them with the potential to present additional donor epitopes at levels above the threshold required for T cell activation (45, 46). In the present study, the T cell epitopes identified reflect not only the presence of an appropriate T cell repertoire, but also the ability of the B cell to process the intact class I alloantigen and present a relevant T cell epitope. We hypothesize that, in the context of chronic rejection, presentation of subdominant epitopes by B cells results in the activation of additional helper T cell clones and that this spreading of T cell reactivity contributes to the development of chronic rejection.

The effector mechanisms by which T cells with indirect specificity for alloantigen effect chronic rejection remain unclear. One of the likely mechanisms is through provision of B cell help for alloantibody production (11, 47), and several recent studies have emphasized the role of anti-MHC class I alloantibody in the development of chronic allograft vasculopathy (48–50). In addition, helper T cells with indirect specificity for donor alloantigen may effect chronic graft rejection by initiating a delayed-type hypersensitivity-like response (10), or by providing help for CD8<sup>T</sup> cell effector functions (51).

The results from the present study clearly demonstrate the ability of cryptic self-epitopes to provide help for alloantibody production. It is interesting to speculate that during chronic allograft rejection, T cell-B cell determinant spreading results in the activation of autoreactive T cells recognizing cryptic self-epitopes. This may promote alloantibody production and augment chronic rejection. The observation in the present study that the i.v. administration of a self-peptide reduced the subsequent alloantibody response suggests that peptide-based strategies for inducing Ag-specific tolerance may be more effective if cryptic self-epitopes are additionally targeted.
In conclusion, our results highlight the complexity of the indirect T cell response to alloantigen. They suggest that cryptic self-epitopes recognized by autoreactive T cells may contribute to the alloimmune response and might be an important consideration in the design of novel strategies for inducing tolerance to alloantigen.

References


