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Indirect T Cell Allorecognition and Alloantibody-Mediated Rejection of MHC Class I-Disparate Heart Grafts

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Recent studies in the rat have identified a role for T cell-dependent alloantibody in rejection of MHC class I-disparate allografts. RT1Aa-disparate PVG.R8 heart grafts are rejected acutely in naive, and hyperacutely in sensitized, PVG.RT1u recipients by CD4 T cell-dependent alloantibody. Here, we examined the T cell Ag recognition pathways responsible and show that direct injection into skeletal muscle of plasmid DNA, encoding a water-soluble form of the RT1Aa MHC class I heavy chain (pcmu-tAa), stimulates T cell-dependent alloantibody. Here, we examined the T cell Ag recognition pathways responsible and show that direct injection into skeletal muscle of plasmid DNA, encoding a water-soluble form of the RT1Aa MHC class I heavy chain (pcmu-tAa), stimulates IgG2b cytotoxic alloantibody and markedly accelerates rejection of PVG.R8 heart grafts (median survival time 2 days). pcmu-tAa injection did not induce CTL to Aa, arguing against direct allorecognition of soluble Aa. Treatment with mAbs confirmed that the alloimmune response to pcmu-tAa injection depended on CD4, not CD8, T cells. Priming T cells for indirect allorecognition by injection of 15-mer peptides spanning the α1 and α2 domains of Aa failed to stimulate anti-Aa Ab but caused an accelerated Ab response to a PVG.R8 heart and a modest acceleration in graft rejection (median survival time 4 days). These results suggest that both soluble MHC class I and allopeptides prime CD4 T cells by the indirect pathway, but that soluble class I is a more effective immunogen for humoral alloimmunity because its tertiary protein structure provides B cell epitopes. We propose that priming humoral alloimmunity, like CTL priming, requires recognition of intact MHC on donor cells, but essential T cell help can be provided by CD4 T cells recognizing allogeneic class I exclusively by the indirect pathway. The Journal of Immunology, 1998, 161: 1292–1298.

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I heavy chain, stimulates a strong CD4 T cell-dependent anti-MHC class I Ab response and causes markedly accelerated rejection of MHC class I-disparate heart grafts. In contrast, immunization with synthetic 15-mer peptides, corresponding to the hypervariable regions of MHC class I, failed to stimulate anti-class I alloantibodies before heart transplantation but led to an accelerated Ab response following transplantation and a modest acceleration in graft rejection. These results highlight the importance of the indirect pathway of allorecognition in the rejection of MHC class I-disparate grafts, particularly where Ab-dependent effector mechanisms are involved. We propose that both soluble MHC class I and synthetic allopeptides are able to effectively prime CD4 T helper cells by the indirect pathway but that soluble MHC class I is a more effective immunogen than linear allopeptides for stimulating humoral alloimmunity because its tertiary protein structure provides the conformational B cell epitopes necessary for generation of pretransplant Abs directed against target cells expressing intact allogeneic MHC class I.

Materials and Methods

Animals

Congenic PVG.RT1u (A'B/D'C'), recombinant PVG.R8 (A'B'/D'C'), PVG (RT1u), and DA (RT1nu) rats were purchased from Harlan U.K. (Bicester, Oxon, U.K.). The derivation of the PVG.RT1u and PVG.R8 rat strains is cross-referenced elsewhere (5). All animals were maintained under standard conditions and used when 8 to 12 wk old.

DNA constructs

cDNAs encoding the full-length and a truncated, soluble form of the rat RT1Aα molecule in the pcev-1-neo plasmid, were kindly provided by Dr. Simon Powis (Wellcome Trust Building, University of Dundee, U.K.). The DNA insert in this vector is under the control of an SV40 early-late promoter, and the soluble form of the MHC class I Aα molecule differs from the original full-length sequence by the inclusion of a stop codon after the methionine residue at position 284 in the transmembrane region. In preliminary studies, plasmids encoding full-length or truncated Aα were used to transfect the rat myoblast cell line, L6 (Ref. 21, ECACC, Salisbury, U.K.) by liposome-mediated transfer using DOTAP (Boehringer Mannheim, Mannheim, Germany). Flow cytometric analysis of stably transfected L6 cells confirmed that only the full-length, and not the truncated, Aα molecule was expressed on the cell surface (Fig. 1). Conversely, soluble Aα, detected by ELISA using MN4-91-6 (anti-RT1Aα, Ref. 22) as the capture Ab and biotin-conjugated OX18 (anti-RT1A, Ref. 23) for detection, was present in the supernatant of cells transfected with the truncated, but not the full-length, Aα molecule.

The eukaryotic expression plasmid, pcmu-IV (24), containing the full-length RT1Aα cDNA (25), was generously provided by Dr. Etienne Joly (Babraham Institute, Cambridge, U.K.). The RT1Aα sequence had been inserted into the pcmu-IV plasmid at the NotI and XhoI restriction sites (Fig. 2). For the DNA vaccination studies described in this study, an additional plasmid was constructed by excising the full-length RT1Aα sequence from the pcmu-IV plasmid and substituting it with the truncated RT1Aα cDNA (from the pcev-1-neo plasmid) at the NotI and XhoI restriction sites. Empty plasmid (pcmu-IV) for control studies was created by excising the class I MHC insert at the BamHI sites and re-ligating the ends of the parent plasmid. Plasmid DNA was purified using a Qiagen Plasmid Megakit (Qiagen, Crawley, U.K.) incorporating Endotoxin Removal Buffer, according to manufacturer’s instructions. Typical endotoxin levels in DNA purified using this procedure are <50 endotoxin units per mg DNA (26).

In vivo injection of plasmid DNA

Gene transfer of DNA encoding the truncated RT1Aα molecule into adult PVG.RT1u rats was achieved by direct injection of plasmid DNA into skeletal muscle (27). To induce regeneration of skeletal muscle fibers and thereby increase the efficiency of gene transfer (28, 29), 400 μl of 0.5% Bupivacaine (1-butyl-N[2, 6-dimethyl phenyl] 2-piperidine-carboxamide) were first injected into each tibialis anterior muscle using a 28-gauge needle. Three and eight days later, 200 μg of pcmu-IV encoding the truncated Aα molecule in 400 μl of saline were injected into each tibialis anterior muscle.

Monoclonal Abs

The following mouse mAbs were used for in vivo treatment: MRC OX8 (CD8, Ref. 30) and MRC OX38 (CD4, Ref. 31). Hybridoma cells secreting these Abs were injected i.p. into pristane-primed BALB/c mice to produce ascites, from which IgG was purified by protein A column chromatography (ProSep, Fisher Scientific, Loughborough, U.K.). The OX8 and OX38 mAb treatment regimens used to induce blockade of CD8 and CD4 T cell subsets, respectively, were based on our experience with these Abs in previous studies (5, 18). The mouse IgG2a mAb, ESH8, which is directed
Allopeptides and allopeptide immunization

A series of 18 overlapping (by 5 amino acids) 15-mer peptides that span the α1 and α2 domains of the RT1Aa molecule ( residue 28 (glycine) to 212 (phenylalanine) inclusive; Ref. 25) were obtained from Immune Systems (Paignton, U.K.). The allopeptides were synthesized by standard F-moc chemistry, purified by HPLC, and assessed by mass spectrometry (peptide purity >80%).

PVG.RT1u rats were immunized s.c. in each hind footpad with a single injection of 900 μg of peptide (comprising a mixture of 50 μg of each of the 18 individual allopeptides), dissolved in 50 μl of water and emulsified with a comparable volume of CFA (Sigma).

Cardiac transplantation

Heterotopic cardiac transplantation was performed by the modified technique of Ono and Lindsey (33), using standard microsurgical techniques with end-to-side anastomosis of the donor aorta and pulmonary artery to the recipient infrarenal aorta and vena cava, respectively. Cold ischemic times were less than 30 min. Grafts were assessed by daily palpation, and rejection was defined as the complete cessation of myocardial contraction.

Differences in graft survival were assessed by the Mann-Whitney U test. P values (two-tailed) of <0.05 were considered significant.

Skin transplantation

Recipients were grafted on the flank with full thickness skin grafts, as described elsewhere (34).

Cytotoxic alloantibody determination

Lymphocytotoxic Abs in serum samples were detected by their ability to lyse 51Cr-labeled, Con A-transformed splenic blasts in the presence of guinea pig complement, as described elsewhere (35). Percent specific 51Cr release was calculated by the formula: (experimental release - spontaneous release)/(maximum release - spontaneous release) × 100.

Class and subclass determination of anti-AaAb

The Ig class and isotype of serum Ab against RT1Aa class I MHC molecule is less effective at promoting accelerated rejection of Aa heart grafts (Table I). In a recent study (37) we showed that PVG.RT1u rats immunized s.c., with an emulsion of CFA and a single allopeptide corresponding to the α-1 allopeptides emulsified in CFA were injected s.c. into the hind footpads of recipient animals 12 days before heart transplantation. Control animals (group 3) received CFA alone.

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Table I. Rejection of Aa class I MHC-disparate PVG.R8 heart grafts by PVG.RT1u recipients

<table>
<thead>
<tr>
<th>Group</th>
<th>Pretreatment</th>
<th>n</th>
<th>Heart Graft Survival (days)a</th>
<th>MST (days)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>none</td>
<td>4</td>
<td>6, 7, 7, 7</td>
<td>7</td>
</tr>
<tr>
<td>2a</td>
<td>PVG.R8 skin graft</td>
<td>4</td>
<td>1, 1, 1, 1</td>
<td>1</td>
</tr>
<tr>
<td>3a</td>
<td>CFA</td>
<td>4</td>
<td>6, 6, 7, 8</td>
<td>6.5</td>
</tr>
<tr>
<td>4a</td>
<td>CFA/peptide</td>
<td>5</td>
<td>4, 4, 5, 5</td>
<td>4</td>
</tr>
</tbody>
</table>

* Abbreviations used in this paper: LNC, pooled cervical and mesenteric lymph node cells; MST, median survival time; pcmu-IAa, pcmu-IV plasmid encoding truncated (soluble) RT1Aa class I MHC.

Compared with skin grafting, immunization of PVG.RT1u rats with synthetic allopeptides corresponding to the hypervariable regions of the Aα class I MHC molecule is less effective at promoting accelerated rejection of Aα heart grafts (Table I). In a recent study (37) we showed that PVG.RT1u rats immunized s.c., with an emulsion of CFA and a single allopeptide corresponding to the α-1 allopeptides emulsified in CFA were injected s.c. into the hind footpads of recipient animals 12 days before they received a PVG.R8 heart allograft. As shown in Table I (groups 3 and 4), recipients immunized with allopeptide rejected their heart grafts faster than control animals given CFA alone (MST 4 days vs 6 days, respectively, p < 0.02), but heart graft rejection in allopeptide-primed animals was still markedly slower than that observed in animals primed by a PVG.R8 skin graft (p < 0.02). The kinetics

![FIGURE 3. Circulating lymphocytotoxic RT1A Ab response of PVG.RT1u rats 12 days after application of a full-thickness PVG.R8 skin graft or immunization with Aα allopeptide. Allopeptide-immunized animals received 900 μg of pooled Aα peptides emulsified in CFA and given s.c. into each hind footpad. Control animals received adjuvant alone. Sera were assayed against 51Cr-labeled PVG. R8 Con A blasts in the presence of guinea-pig complement. Values shown are mean and SD of four animals per group.](http://www.jimmunol.org/By guest on June 8, 2017 http://www.jimmunol.org/Downloaded from http://www.jimmunol.org/By guest on June 8, 2017)

against human factor VIII (Scottish Antibody Production Unit, Law, Scotland, U.K.), was used as an isotype control Ab (32).
FIGURE 4. Cytotoxic RT1A\(^{a}\) Ab response of PVG. RT1\(^{u}\) rats 4 days after receiving a heterotopic PVG.R8 heart transplant. Recipients were pre-immunized with pooled A\(^{a}\) allopeptides emulsified in CFA (as detailed in the legend to Fig. 2), or with CFA alone, 12 days before heart transplantation. Results shown are mean and SD of four animals per group.

of heart graft rejection in animals immunized with CFA alone in this study were comparable to those observed in our previous study (37), when animals were immunized with CFA emulsified with an irrelevant control peptide (MST 6.5 and 6.0 days, respectively).

In this, as in our earlier study (37), immunization with A\(^{a}\) allopeptides did not stimulate the development of cytotoxic Ab recognizing intact RT1A\(^{a}\) on target cells, and sera obtained from peptide-primed animals on the day of heart grafting, i.e., 12 days after immunization, showed only background levels of PVG.R8 target cell lysis (Fig. 3). However, by day 4 after heart transplantation, recipients that had been immunized with allopeptides showed higher serum levels of cytotoxic alloantibodies than control animals immunized with CFA alone before heart transplantation (Fig. 4). Together, these observations are consistent with the idea that immunization with linear allopeptides is able to prime, via the indirect pathway, CD4 T cells that provide B cell help, but does not provide the relevant A\(^{a}\) conformational B cell epitopes for anti-A\(^{a}\) Ab production.

DNA transfer studies

To define further the nature of A\(^{a}\) class I MHC alloantigen necessary to initiate alloantibody production and accelerated rejection of A\(^{a}\)-disparate heart grafts, PVG.RT1\(^{u}\) rats were immunized by direct injection into skeletal muscle of pcmu-IV plasmid encoding a truncated form of the A\(^{a}\) class I MHC molecule (pcmu-tA\(^{a}\)). Because the truncated A\(^{a}\) protein lacks cytoplasmic and transmembrane regions and is not, therefore, expressed on the cell surface (see Materials and Methods and Fig. 1), we reasoned that immunization with pcmu-tA\(^{a}\) would not be effective in priming alloreactive CD4 T cells by the direct pathway but that soluble A\(^{a}\) protein resulting from gene transfer might provide a ready source of class I MHC alloantigen for recognition by CD4 T cells through the indirect pathway as well as relevant conformational B cell epitopes for stimulating B cells with specificity for the intact A\(^{a}\) molecule. These predictions were supported by the observation that i.m. injection of pcmu-tA\(^{a}\) on two occasions before heart transplantation was very effective at priming PVG.RT1\(^{u}\) rats to A\(^{a}\) alloantigen and led to a marked acceleration in the rejection of PVG.R8, but not third-party PVG.RT1\(^{c}\), heart grafts (MST 2 days, p < 0.02, and 6 days, NS, respectively, Table II, groups 2 and 3). Control recipients injected with “empty” pcmu-IV plasmid rejected PVG.R8 heart grafts at the same rate as naive PVG.RT1\(^{u}\) recipients (MST 7 days, NS, Table II, group 1).

In contrast to allopeptide immunization, injection of rats with pcmu-tA\(^{a}\) led to high levels of circulating cytotoxic anti-RT1A\(^{a}\) Ab before heart transplantation (Fig. 5). The class and subclass of the resulting anti-A\(^{a}\) Ab was characterized by flow cytometric analysis using PVG.R8 target cells and FITC-conjugated anti-Ig mAb. As shown in Figure 6, immunization with pcmu-tA\(^{a}\) stimulated a strong IgG alloantibody response that was predominantly of the IgG2b subclass; in the rat, this is the most effective IgG subclass for mediating complement-dependent cell lysis (38).

Table II. Accelerated rejection of R8 heart grafts by PVG.RT1\(^{u}\) recipients after vaccination with DNA encoding water soluble A\(^{a}\) class I MHC

<table>
<thead>
<tr>
<th>Group*</th>
<th>DNA Vaccination*</th>
<th>mAb Treatment</th>
<th>Heart Donor</th>
<th>n</th>
<th>Heart Graft Survival (days)</th>
<th>MST (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pcmu (control)</td>
<td>–</td>
<td>PVG.R8</td>
<td>4</td>
<td>6, 7, 7</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>pcmu-tA(^{a})</td>
<td>–</td>
<td>PVG.R8</td>
<td>5</td>
<td>1, 2, 2, 2, 2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>pcmu-tA(^{a})</td>
<td>–</td>
<td>PVG.RT1(^{c})</td>
<td>3</td>
<td>5, 6, 7</td>
<td>6*</td>
</tr>
<tr>
<td>4</td>
<td>pcmu-tA(^{a})</td>
<td>–</td>
<td>PVG.R8</td>
<td>4</td>
<td>1, 1, 1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>pcmu-tA(^{a})</td>
<td>anti-CD8(^{d})</td>
<td>PVG.R8</td>
<td>3</td>
<td>1, 1, 1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>pcmu-tA(^{a})</td>
<td>anti-CD4(^{d})</td>
<td>PVG.R8</td>
<td>4</td>
<td>8, 12, 14, 17</td>
<td>13</td>
</tr>
</tbody>
</table>

* Group 1 vs group 2, p < 0.02. Group 1 vs group 3, NS.

* PVG.RT1\(^{u}\) rats were immunized with pcmu-IV plasmid encoding a truncated A\(^{a}\) class I MHC (pcmu-tA\(^{a}\)), or empty plasmid as control, as described in Materials and Methods. Twelve days after the first DNA injection, rats were given a heterotopic PVG.R8 (or PVG.RT1\(^{c}\)) cardiac allograft.

* Unmodified PVG.RT1\(^{u}\) rats reject PVG.RT1\(^{c}\) heart grafts on days 6-8.

* Animals in group 4 received 2 mg anti-CD8 mAb (OX8) on days 1, 0, and 1 relative to heart transplantation.

* Animals in groups 5 and 6 were treated with anti-CD8 (2 mg OX8 on days 1, 0, and 1) or anti-CD4 (5 mg OX38 on days 3, 0, 3, 6, and 9) relative to plasmid injection.
was dependent on CD4 or on CD8 T cells, recipients were treated in vivo with mAbs directed against T cell subsets following DNA injection. As shown in Table II, the ability of PVG.RT1u animals injected with pcmu-tAa to reject PVG.R8 grafts very rapidly was not prevented by treatment with the anti-CD8 mAb, MRC OX8 (MST 1 day, groups 4 and 5). We have confirmed, previously, that treatment with OX8 mAb is highly effective at depleting T cells expressing CD8 from both the blood and lymphoid tissue of PVG.RT1u rats (5) and, in the present study, we showed that this phenotypic depletion was accompanied by functional depletion of CD8 CTL precursors. LNC obtained from unmodified PVG.RT1u rats and cultured in vitro for 5 days with fully allogeneic PCMUTAA-irradiated splenic stimulators (as described in legend to Fig. 8) developed high levels of CTL activity against PVG.R8 lymphoblasts (>50% cytotoxicity at E:T ratio of 100:1); but CTL activity could not be generated from LNC obtained from PVG.RT1u animals during the first week after in vivo treatment with OX8 mAb (<10% cytotoxicity at E:T ratio of 100:1).

In contrast to anti-CD8 mAb treatment, in vivo treatment of pcmu-tAa-immunized PVG.RT1u rats with the anti-CD4 mAb MRC OX38, which produces depletion of approximately 50% of peripheral CD4 T cells (18), not only prevented accelerated heart graft rejection but extended graft survival well beyond that observed in naive PVG.RT1u recipients (MST 13 days, Table II, group 6).

The effects of in vivo treatment with mAbs to T cell subsets on the cytotoxic RT1Aa Ab titer following immunization with pcmu-tAa and heart grafting are shown in Figure 9. It can be seen that administration of anti-CD4 mAb completely abrogated the early cytotoxic alloantibody response, whereas cytotoxic alloantibody responses in anti-CD8−treated recipients were similar to those observed in control animals.

Discussion

Rejection of MHC class I-disparate allografts has, historically, been attributed to CD8 cytotoxic effector cells with specificity for direct recognition of allogeneic MHC class I expressed by target cells within the graft (1, 2). However, several recent studies have shown that CD4 T cells may be sufficient to initiate rejection of class I-disparate allografts and, at least in the rat, CD8 T cells are not essential for effecting graft rejection (2, 5, 17, 18). Since CD4 T cells participating in rejection of class I-disparate grafts most

CTL responses

To determine whether immunization with DNA-encoding soluble Aa induced a CTL response, spleen cells were obtained from PVG.RTiu rats that had been immunized with pcmu-tAa or with a PVG.R8 skin graft and assayed against 51Cr-labeled PVG.R8 Con A blasts. Lymphoid cells from PVG.RTiu recipients primed with PVG.R8 allografts do not generally display very high levels of in vitro cytotoxic T cell activity (5). However, as shown in Figure 7, spleen cells from animals grafted with PVG.R8 skin showed significant levels of CTL activity whereas cells from animals immunized with pcmu-tAa displayed minimal cytotoxicity. In additional experiments, LNC obtained from PVG.RTiu rats 12 days after immunization with pcmu-tAa were stimulated in vitro for 5 days with irradiated PVG.R8 spleen cells, and CTL activity was then determined. After in vitro stimulation, the level of CTL activity observed in LNC from animals primed with pcmu-tAa was comparable to those seen in T cells from control animals immunized with empty pcmu-IV plasmid (Fig. 7).

In vivo T cell depletion studies

Finally, to determine whether the ability of immunization with pcmu-tAa to promote accelerated rejection of PVG.R8 heart grafts

FIGURE 6. Ig class and subclass of serum anti-RT1Aa Ab response after immunization with pcmu-tAa. PVG.RTiu rats were immunized with pcmu-tAa, as described in the legend to Figure 5, and 12 days later serum was assayed for Aa alloantibody by flow cytometric analysis using PVG.R8 LNC as targets and FITC-conjugated mouse anti-rat Ig subclass-specific mAb. Results (as mean channel fluorescence) are shown as mean and SD. There was minimal lysis of third-party Lewis (RT1l) lymphoblast target cells.

FIGURE 7. Analysis of CTL response to RT1Aa. PVG.RTiu rats were grafted with PVG.R8 skin or immunized with pcmu-tAa or with pcmu-IV (empty vector). After 12 days, spleen cells were assayed against 51Cr-labeled R8 Con A blast target cells in a 6-h cytotoxicity assay. Values shown are mean and SD of three animals per group. This experiment was repeated on two occasions with the same result.

FIGURE 8. In vitro generation of CTL to Aa alloantigen. LNC were obtained from PVG.RTi u rats 12 days after immunization with pcmu-tAa (or pcmu-IV control plasmid) and used as responders (4 × 107 in 20 ml) in a bulk MLR. Stimulators were irradiated (20 Gy), fully allogeneic (RT1i) spleen cells (2 × 107). After 5 days of in vitro stimulation, responders (from three animals per group) were tested for their ability to lyse 51Cr-labeled PVG.R8 Con A blast targets in a 6-h cytotoxicity assay. Values shown are mean and SD. There was minimal lysis of third-party Lewis (RT1i) lymphoblast target cells.
likely recognize allogeneic class I MHC by the indirect pathway, the effector mechanisms by which T cells of this allospecificity destroy target cells expressing intact MHC class I have been the subject of considerable speculation. Our own recent studies of MHC class I-disparate graft rejection in the rat have identified a hitherto neglected role for T cell-dependent alloantibody-mediated effector mechanisms in graft rejection (5, 17, 18).

In the present study, the approach of in vivo gene transfer was used to provide insight into the Ag recognition pathways of allo- geneic MHC class I. Direct injection of naked plasmid DNA into skeletal muscle has been shown, for a variety of protein Ags, to be an effective means for inducing a strong humoral and cellular immune response to the Ag encoded by the plasmid DNA (39, 40). We found that i.m. injection of DNA encoding a truncated, water-soluble form of rat MHC class I heavy chain resulting from gene transfer is released in monomeric form of multimeric forms of MHC class I to cause cross-linking of the TCR on CD8 T cells. Interestingly, Wang et al. (48) observed that s.c. immunization of PVG.RT1u rats with soluble Aa class I MHC heavy chain protein, produced in a baculovirus expression system, also primed for heart allograft rejection. Heart allograft rejection was not as rapid as in the present study, and the effect of Aa immunization on the humoral immune response was not determined.

In contrast to soluble MHC class I, priming with synthetic al- lopeptides did not, in this or in our previous experiments (37), stimulate a cytotoxic Aa alloantibody response, nor was it as effective in accelerating heart graft rejection. PVG.RT1u rats, immunized by s.c. injection of allopeptides corresponding to the α1 and α2 domains of Aa MHC class I, rejected Aa-disparate heart grafts more quickly than control animals (mean graft survival 4 vs 6.5 days), but graft rejection was less rapid than after immunization with pcmu-tAa injection (MST 2 days). Shirwan et al. also found, in the PVG.RT1u rat strain, that immunization with Aa allopeptides led to only a marginal decrease in survival of PVG.R8 heart allografts when compared with control animals (mean graft survival 5 vs 6 days, Ref. 49), and these results for class I-disparate grafts are typical of those reported in other rat strain combinations, where immunization with synthetic MHC class I allopeptides has been shown to shorten the survival of fully allogeneic skin and heart grafts by, at most, 2 days (11, 50).

In contrast to immunization with allopeptides, sensitization ofPVG.RT1u rats with intact MHC class I on the surface of donor APCs by application of an Aa-disparate PVG.R8 skin allograft, as in the present study, or by s.c. injection of irradiated PVG.R8 spleen cells, as in the study of Shirwan (49), led to prompt rejection of Aa-disparate heart grafts within 1 to 2 days of transplantation. Shirwan suggested that immunization with donor cells expressing intact RT1.Aa was more effective than allopeptide because it primed T effector cells recognizing RT1.Aa directly
(49). The results of the present study suggest that the immunogeneity of donor cells may also reside in their ability to generate a humoral alloimmune response that is contingent on the presence of conformational B cell epitopes displayed on intact allogeneic MHC class I. Priming of humoral effector mechanisms is, therefore, analogous to priming of CD8 CTL (51), in that there is a requirement for recognition of intact allogeneic MHC class I expressed by and, in the case of Ab, derived from donor cells, but essential T cell help for the generation of these effector mechanisms can be provided by CD4 T cells recognizing allogeneic MHC class I exclusively by the indirect pathway.

References


