Rejection of Skin Allografts by Indirect Allorecognition of Donor Class I Major Histocompatibility Complex Peptides

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Summary

LEW (RT1^l) rats were immunized with peptides corresponding to the α helical region of the $\alpha 1$ domain (peptide 1), the β sheet of the $\alpha 2$ domain (peptide 2), and the α helical region of the $\alpha 2$ domain (peptide 3) of the RT1-A^{av1} classical class I molecule of the DA (RT1^{av1}) strain. The immunizations were without carriers, and the objective was to prime to indirect allorecognition without influencing direct recognition of the RT1-A^{av1} molecule. The LEW rats mounted strong primary and secondary antibody responses to peptides 1 and 3, but only weak secondary responses to peptide 2. None of the antipeptide antibodies crossreacted with intact RT1-A^{av1} class I molecules. The immunizations also resulted in LEW antigen-presenting cell–dependent, CD4$^+$ T cell proliferative responses, which were very strong against peptide 1 and weakest against peptide 2. LEW rats immunized with peptides 1 or 3, but most effectively with both peptides 1 and 3 together, showed accelerated rejection of DA skin allografts. This effect was not observed in LEW rats immunized with peptide 2. In response to the DA skin allograft, the peptide-immunized LEW rats showed markedly accelerated kinetics of antibody production to the intact RT1-A^{av1} molecule. These data demonstrate that indirect allorecognition can play an important role in allograft rejection and have important implications for understanding allograft rejection and its regulation.

T he TCR normally recognizes foreign antigens as peptide fragments in association with self-class I and II MHC molecules. The major exception to this rule occurs in tissue transplantation and in in vitro models of alloreactivity. Here, the responding T cells are able to recognize the foreign MHC antigens as intact molecules on the surface of the allogeneic cells. The enigmas of direct recognition and alloaggression have been the subject of much research and speculation (1–5). However, it is clear that the precursor frequency for direct allorecognition is extremely high, with estimates of 1–10% of T cells in any individual being able to respond in proliferative responses to a foreign MHC (3). There is also little doubt that the direct pathway of allorecognition provides a strong stimulus both to proliferative responses for possible delayed-type hypersensitivity (DTH)[1] reactions, and for cytotoxic T cell responses to the foreign cells of the graft.

Recognition of the foreign MHC and other antigens of

the graft as conventional exogenous antigens, i.e., processed and presented as peptides in class II MHC molecules of recipient APC under the normal rules of MHC restriction (6), has received relatively little attention. This pathway is best termed "indirect allorecognition." Whether or not indirect allorecognition plays a significant role in allograft rejection is of fundamental importance in transplantation, since the requirements for activation and the mechanisms of regulation will be quite different from those for indirect recognition, as will be discussed later. Thus, the interpretation of transplantation phenomena and attempts at tolerance induction will be markedly influenced if both of these quite distinct pathways of T cell recognition play a significant role in rejection.

The possibility that processing of graft antigens by recipient APC might be important in allograft rejection was suggested a decade ago (7–9). However, the only experiments to specifically address this issue in transplantation have been those by Sherwood et al. (11). They were able to transfer specific sensitization for skin allograft rejection to naive mice by the transfer of T cell–depleted peritoneal and spleen cells from syngeneic mice injected 3 d previously with donor spleen

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[1] *Abbreviations used in this paper:* DTH, delayed-type hypersensitivity; NRS, normal rat serum; RAR, rabbit F(ab')$_2$ anti–rat F(ab')$_2$.

cells. Although these results were probably due to indirect recognition in the second hosts, Sherwood et al. (11) demonstrated undegraded donor class II MHC antigens in the transferred inoculum, so that priming to direct allorecognition in the secondary hosts could not be entirely excluded.

In a previous study (10), we immunized LEW rats with isolated, denatured RT1-A class I and RT1-Bα and RT1-Bβ class II MHC chains of the DA strain, with a view to priming to indirect allorecognition without influencing direct recognition. The use of synthetic peptides corresponding to short segments of the polymorphic regions of donor MHC antigens is a much better approach, since there is virtually no risk of priming to direct recognition, and the system lends itself more easily to further analysis. However, defining polymorphic MHC peptides able to prime allogeneic T cells is problematic. More importantly, MHC peptides able to influence allograft rejection have not previously been reported. Here we describe peptides from the RT1-A class I MHC molecule of the DA strain that can cause second-set rejection of DA skin grafts in LEW rats, and that can result in T helper or "carrier" type priming for antibody responses to the intact class I molecule.

Materials and Methods

Rats

Inbred DA (RTI^av1) and LEW (RT1^l) male rats were purchased from Harlan Olac (Bicester, Oxon, UK).

Peptides

Three 22–24–amino acid peptides were chosen to correspond to the most variable regions of classical class I MHC molecules (12), and the amino acid sequence was that deduced from the cDNA sequence of the RT1-A^av1 classical class I gene of the rat (13). Peptides 1 and 3 correspond to the α helical regions of the first and second domains, respectively, and peptide 2 to the β sheet of the second domain, assuming a structure similar to HLA-A2 (14). The actual sequences, numbering from the beginning of the first domain, were: peptide 1 (57) Pro-Glu-Tyr-Trp-Glu-Gln-Gln-Thr-Arg-Ile-Ala-Lys-Glu-Trp-Glu-Gln-Ile-Tyr-Arg-Val-Asp-Leu-Arg-Thr; peptide 2 (94) Thr-Ile-Gln-Glu-Met-Tyr-Gly-Cys-Asp-Val-Gly-Ser-Asp-Gly-Ser-Leu-Leu-Arg-Gly-Tyr-Arg-Gln-Asp-Ala; peptide 3 (143) Thr-Arg-Asn-Lys-Trp-Glu-Arg-Ala-Arg-Tyr-Ala-Glu-Arg-Leu-Arg-Ala-Tyr-Leu-Glu-Gly-Thr-Cys.

An irrelevant 20–amino acid peptide corresponding to amino acids 60–79 of the α helical region of the RT1-Dβ^u class II MHC (15) molecule was used as a control. All peptides were purchased from Cambridge Research Biochemicals (Cambridge, UK) and were further purified by desalting on G10 columns (Pharmacia, Uppsala, Sweden), freeze drying, and reconstituting in 0.15 M NaCl at 1 mg/ml as stock solutions. Peptides were always used as free molecules, without any carriers.

Peptide Immunization

LEW rats received 50 μg of free peptide in CFA subcutaneously into each hind footpad (100 μg of peptide per rat) and were boosted 4 wk later with the same dose of peptide to the same site in IFA.

The only exception to this schedule was in rats being simultaneously immunized with peptides 1 and 3, in which case 100 μg of peptide 1 was given to the left footpad and 100 μg of peptide 3 to the right footpad.

mAbs

The BMAC-5 hybridoma cell line secreting mouse IgG1 antibody to rat macrophages has previously been described in detail (16). The cell lines secreting the W3/25 antibody to rat CD4 (17), and the MRC OX8 antibody to rat CD8 (18) and the MRC OX6 antibody to rat RT1-B class II MHC antigen (19), were kind gifts of Professor A.F. Williams (MRC Cellular Immunology Unit, Oxford, UK). These antibodies were used as immune ascites partially purified by ion exchange chromatography (20).

The MRC OX35 antibody (21) to a nonoverlapping determinant (with regard to W3/25) of rat CD4 and the MRC OX12 antibody (22) to the rat Ig κ light chain were purchased from Serotec (Bicester, Oxon, UK).

Conventional Antibodies

Immunoadsorbent purified rabbit F(ab')₂ anti–rat F(ab')₂ (RAR) was prepared as previously described in detail (23) and iodinated using the chloramine T method.

Cell Preparations

Unseparated Lymph Node Cells. Popliteal, cervical, para-aortic, and mesenteric lymph nodes were removed aseptically and dispersed into single cell suspensions in Hepes-buffered RPMI 1640 (Flow Laboratories, Irvine, Scotland) containing 1% heat-inactivated normal rat serum (NRS). The cell preparation was performed at 4°C, unless it was to be depleted of macrophages, when the procedure was carried out at room temperature. After washing, the cells were resuspended at 2 × 10⁶ cells/ml in RPMI 1640 supplemented with 2 mM glutamine, 5 × 10⁻⁵ M 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5% heat-inactivated NRS (Gibco, Paisley, Scotland), hereafter referred to as culture medium.

Depletion of Adherent Cells. Cell suspensions in Hepes-buffered RPMI 1640 with 1% NRS were applied to sterile Sephadex G-10 (Pharmacia, Milton Keynes, UK) columns previously equilibrated in this buffer at 37°C. After 30 min at 37°C, the cells were eluted with a one-column volume of Hepes buffer, washed once, and resuspended in culture medium at 2 × 10⁶ cells/ml.

Separation of CD4⁺ and CD8⁺ Cell Subsets. Cell suspensions were first depleted of adherent cells as described in the preceding section. All subsequent procedures were carried out at 4°C. For the "CD8⁺" preparation, the MRC OX12, MRC OX35, W3/25, MRC OX6, and BMAC-5 antibodies were added to coat the B cells, CD4⁺ cells, and any residual macrophages. For the "CD4⁺" preparation, the MRC OX12, MRC OX8, MRC OX6, and BMAC-5 antibodies were added to coat the B cells, CD8⁺ cells, and any residual macrophages. These reactions contained cells at 5 × 10⁷/ml and antibodies at saturating concentrations. The preparations were incubated on ice for 30 min and then washed twice by centrifugation. The cells were resuspended at 10⁸ cells/ml, and sheep anti–mouse IgG-coupled Dynabeads (Dynal [UK] Ltd., Wirral, Merseyside) were added to the cell preparation at a ratio of 10 beads per cell. This was incubated at 4°C for 60 min with frequent agitation, and the beads were then removed using a magnetic particle concentrator (Dynal [UK] Ltd.). The cells were washed once and resuspended in culture medium at 2 × 10⁶ cells/ml. Purity of the subsets was checked using flow cytometry with directly fluoresceinated MRC OX8 and W3/25 antibodies, and was always >98%.

Preparation of APC. A single cell suspension was prepared at room temperature from the spleens of normal LEW rats, and contaminating RBC were removed by incubating in Tris-buffered NH_4Cl (24). The cells were exposed to 1,500 rad of irradiation from a ^{137}Cs source using a gamma cell 1000 apparatus (Isomedix Inc., Whippany, NJ) at 100% dose for 1.2 min. The cells were then washed once and resuspended at 2×10^6 cells/ml in culture medium.

Cell Proliferation Assays. 2×10^5 cells were incubated in round-bottomed, 96-well tissue culture plates (Sterilin, Feltham, England) in 200-μl volumes of culture medium. Incubations were at 37°C in 5% CO_2 in air. To some cultures, 10^5 irradiated APC were added. Cultures were stimulated with the RT1-A^{av1} peptides and irrelevant peptide, as well as Con A (Pharmacia) at 10 $\mu g/ml$ as a positive control. 24 h before harvesting, 1 μCi of [^3H]thymidine in 20 μl of culture medium was added to each well.

Cell-bound radioactivity was measured by placing dried filters in Optiscint Hisafe scintillation fluid (LKB, Milton Keynes, UK) and counting in a rackbeta 11 liquid scintillation counter (LKB, Bromma, Sweden).

Indirect Radioactive Binding Assays

Plate Assay for Alloantibodies to the Peptides. All procedures were at 4°C or on ice. 25 μl of peptide at 100 $\mu g/ml$ in 0.15 M NaCl was added in triplicate to wells in a 96-well polyvinyl chloride (PVC) microtiter plate (Dynatech Ltd., Billingshurst, Sussex). The plate was then washed three times in 0.1% BSA/PBS. 25 μl of serum diluted in 0.5% BSA/PBS was added and the plate incubated for 60 min. The serum was removed, and the plates were washed as above. 50 μl of ^{125}I-labeled RAR in 0.5% BSA/PBS ($\sim$300,000 cpm/well) was added and the plate was again incubated for 60 min. The iodinated antibody was removed, and the plates were washed three times as above. Individual wells were cut out, and the bound radioactivity was measured using an ultragamma counter (LKB).

Tube Binding Assay for Alloantibodies to Whole RT1-A^{av1} Class I Molecules. This was performed essentially as described by Morris and Williams (25). All procedures were at 4°C or on ice. Duplicate 25-μl aliquots of rat serum diluted in 0.5% BSA/PBS were transferred to LP3 tubes (Luckham Ltd., Burgess Hill, Sussex). 25 μl of DA strain RBC at 10^9/ml in 0.5% BSA/PBS was added as targets to each tube, and this was incubated for 1 h. The cells were then washed twice in 0.1% BSA/PBS, and 100 μl of ^{125}I-labeled RAR in 0.5% BSA/PBS (300,000 cpm/tube) was added to the pellet of the second wash. This was resuspended and incubated for 1 h, and the cells were washed twice as above. The pellet of the second wash was resuspended in 0.5 ml of PBS, transferred to fresh LP3 tubes, and the target cell–bound radioactivity was measured in the ultragamma counter (LKB).

Skin Transplantation

Full-thickness grafts of abdominal skin, $\sim$2 cm^2 in area, were placed on corresponding full-thickness defects over the dorsolateral thoracic wall of recipients. Corner sutures of 4-0 silk were used to secure the graft in place, and a dressing of vaseline gauze, dry gauze, and elastic bandage was applied. The dressings were removed at day 6 and the grafts allowed to dry for 4–6 h before assessment. Thereafter, they were assessed daily. The day of rejection was taken as the day on which >90% full-thickness loss had occurred.

Results

Preliminary Considerations. As only one classical class I MHC sequence is known in the rat (RT1-A^{av1}), and more
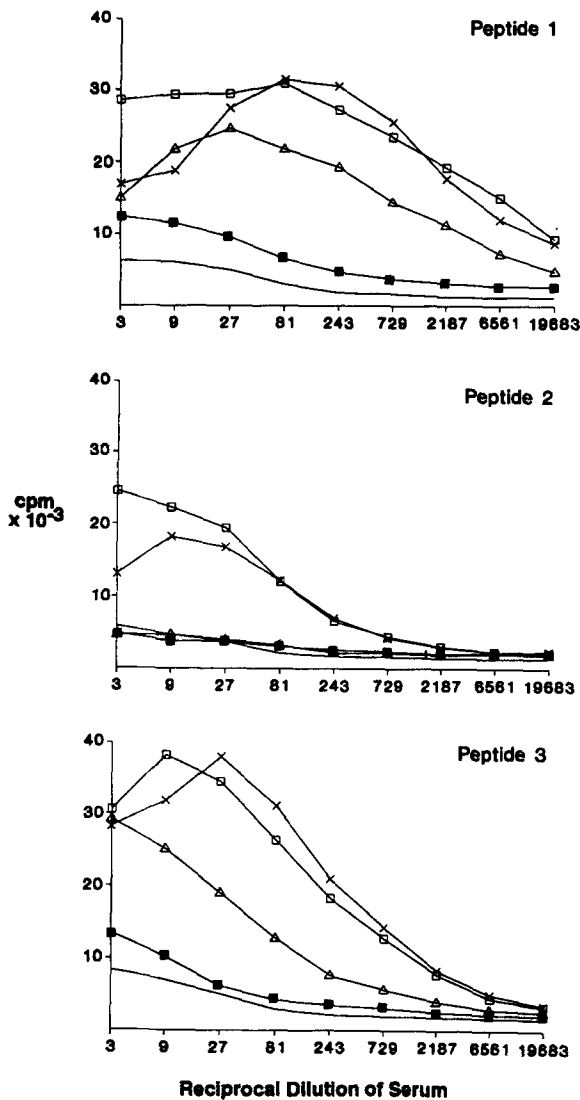
specifically since the RT1-A sequence of the LEW strain is not known, we synthesized relatively long peptides from the hypervariable regions of RT1-A^{av1} (12) to ensure that the peptides would encompass regions that were polymorphic between the DA and LEW strains. For our experimental design, peptide length was not of primary importance, as it was necessary only that direct recognition would not be triggered. Whether or not the peptides were further processed to smaller fragments was of no consequence from the point of view of our experimental objectives. Indeed, in view of recent data on optimal peptide length for class II binding (26), one would guess that further processing is likely to have occurred.

Alloantibody Responses to the Peptides. The antibody responses of LEW rats immunized with the RT1-A^{av1} peptides is given in Fig. 1. It can be seen that peptides 1 and 3 induced strong primary and secondary alloantibody responses, while peptide 2 induced no primary response and only weak secondary responses. These antibody responses were of interest because provision of T cell help for B cells requires recognition by the helper T cell receptor of antigenic peptides on the class II molecules of the B cells (27). The presence of an antibody response to free peptide therefore suggests that the peptide is able to provide helper determinants, i.e., is able to interact with LEW class II MHC molecules. An additional important point is that antibodies to the peptides might interact with the whole class I molecule, and thereby influence graft rejection either positively by contributing to graft damage (28), or negatively because of passive enhancement effects (29). In fact, antisera to the RT1-A^{av1} peptides did not react at all with whole RT1-A^{av1} class 1 molecules, as shown by their complete failure to react with DA erythrocytes in binding assays (Fig. 2).

The antibody responses also demonstrate that the regions of the RT1-A^{av1} molecule chosen for study were indeed polymorphic between the DA and LEW strains.
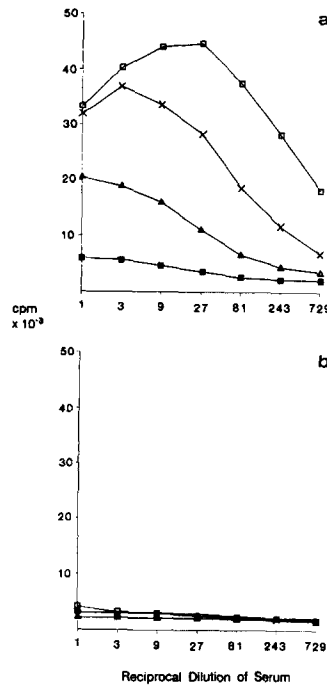
T Cell Reactivity to the Peptides. Confirmation of T cell reactivity to the peptides was obtained by testing the proliferative response of lymph node lymphocytes from peptide-immunized LEW rats. Fig. 3 *a* demonstrates that LEW rats immunized with peptide 1 showed strong proliferative responses to peptide 1, but not to peptides 2 and 3 or to the control peptide. Fig. 3, *b* and *c* show that this response is dependent on LEW APC and mediated entirely by CD4$^+$ T cells. LEW rats immunized with peptide 2 (Fig. 3, *d–f*) and peptide 3 (Fig. 3, *g–i*) showed similar but weaker responses. Peptide 2 consistently gave the weakest response. The absence of proliferation by CD8$^+$ T cells is consistent with the general rule that exogenous antigens are recognized by CD4$^+$ T cells (6).

The Influence of Peptide Immunization on Skin Graft Rejection. The preceding data demonstrate that indirect recognition of the three RT1-A^{av1} peptides does occur in the LEW strain. However, does it make a significant contribution to the effector mechanisms of rejection? This was tested by immunizing LEW rats with the RT1-A^{av1} peptide and seeing if this influenced the survival of skin allografts. The results are given in Table 1. In the first series of experiments, slight

Figure 2. Crossreactivity of anti-RT1-A^{avl} peptide sera on intact RT1-A^{avl} class I molecules. Sera taken from LEW rats 4 wk after the booster injection with peptide 1 ($\square$), peptide 2 ($\triangle$), or peptide 3 ($\times$), or from control LEW rats given Freunds adjuvant only ($\blacksquare$) were tested in binding assays on the immunizing peptide (*a*) or DA RBC (*b*). Antibody binding was detected using ^{125}I-labeled RAR. cpm refers to ^{125}I RAR bound per assay.

Figure 1. Alloantibody responses to RT1-A^{avl} peptides in LEW rats. LEW rats were immunized with 100 μg of peptide from the RT1-A^{avl} class 1 molecule in CFA and boosted 4 wk later with the same dose of peptide in IFA. Peptides were used as free molecules, without any carriers. The rats were bled at 2 wk ($\blacksquare$), 4 wk ($\triangle$), 6 wk ($\times$), and 8 wk ($\square$) after the initial injection, and the sera assayed on PVC microtiter plates previously coated with immunizing peptide. Antibody to the peptide was detected using ^{125}I-labeled RAR. cpm bound refers to ^{125}I RAR bound to each well. Background binding to wells without peptide was similar for all sera and a representative serum is given (———). For each peptide, time course studies were performed on three rats, and all three within each group gave very similar results. Data from a representative rat from each group are given.

but significant shortening of skin graft survival was obtained in LEW rats immunized with peptides 1 and 3, but not peptide 2. In view of the weak effect observed, the entire experiment was repeated, and on this occasion a group of LEW rats given both peptides 1 and 3 was included. It can be seen that the effect observed with peptides 1 and 3, although small, was reproducible. Of particular significance was the more marked shortening of graft survival seen in the LEW rats

given both peptides 1 and 3. It is interesting that the weakest, and statistically insignificant, effect was seen with peptide 2, which induced the weakest antibody (Fig. 1) and T cell proliferative (Fig. 3) responses in LEW rats.

Possible Effector Mechanisms. Indirect allorecognition might influence graft rejection by two quite distinct mechanisms. The first involves a direct effector mechanism, mediated by a DTH-type reaction within the connective tissues of the graft, as previously suggested (30). The other involves the provision of help for conventional effector mechanisms requiring T cell help, i.e., CD8$^+$ cytotoxic T cell generation and alloantibody production. CD4$^+$ T cells, primed to donor MHC peptides, represent primed T helper cells and could amplify or accelerate CD8$^+$ cytotoxic T cell or antibody responses to the graft.

We were in a position to test the effect of priming LEW rats to the RT1-A^{avl} peptides on the antibody response to whole RT1-A^{avl} class I molecules on DA skin grafts. Since the B lymphocytes specific for the whole RT1-A^{avl} molecules would be virgin in the peptide-immunized rats, this would represent an unusual and interesting example of carrier-type priming in antibody responses (31). The results were similar for rats immunized with peptides 1, 2, or 3, and the results for peptide 3–immunized LEW rats are given in Fig. 4. Fig. 4 *a* gives the kinetics of the antibody to whole RT1-A^{avl} molecules in a typical control LEW rat after grafting with DA skin. There was no detectable response at day 7, a weak response at day 10, and a good response at day 14. Fig. 4 *b* gives the kinetics of the response in a typical peptide 3–immunized rat. It is worth noting (as previously demonstrated in Fig. 2) that at the time of grafting, i.e., at 4 wk after the booster injection with peptide, and at a time when

whole lymph node cells   **CD4⁺ T cells**   **CD8⁺ T cells**

cpm
x 10⁻³

Day of Culture
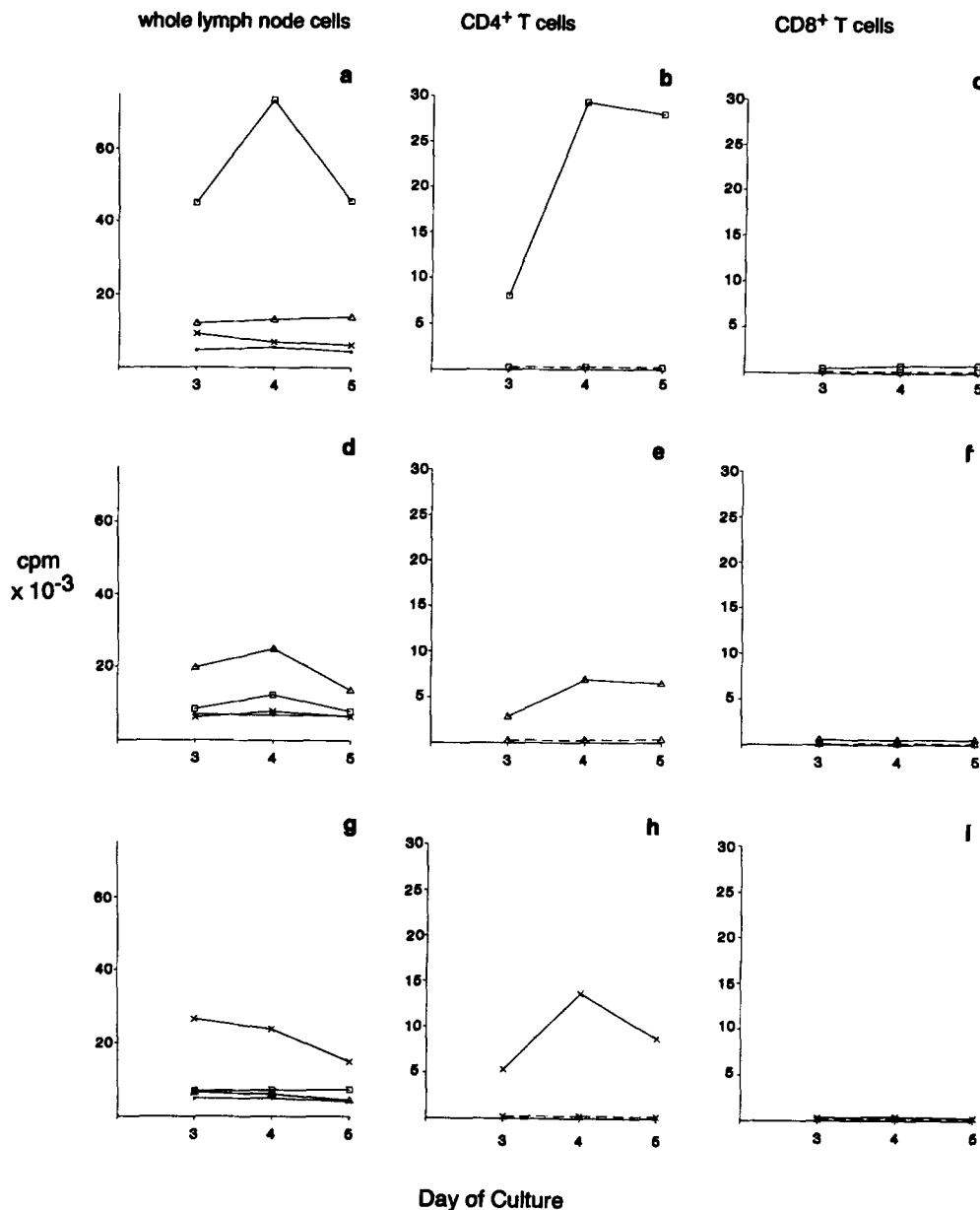
Figure 3. T cell proliferative responses to RT1-A^avl peptides in LEW rats. LEW rats were immunized with peptide 1 (*a–c*) peptide 2 (*d–f*), and peptide 3 (*g–i*) from the RT1-A^avl class I molecule. 10 d after the booster injection, whole lymph node cells (*a*, *d*, and *g*) purified CD4⁺ T cells (*b*, *e*, and *h*) or purified CD8⁺ T cells (*c*, *f*, and *i*) were incubated with 10 μg/ml of peptide 1 (□), peptide 2 (△), peptide 3 (×), or no peptide (■). The CD4⁺ and CD8⁺ T cell subsets were incubated in the presence (————) or absence (- - - -) of added APC. cpm refers to mean of triplicate counts of [³H]thymidine incorporated into the cells. The experiments with unseparated lymph node lymphocytes were performed on four occasions and the subset studies on two occasions, with similar results in all cases. A representative study is given.

the antipeptide response was very strong (refer to Fig. 1), there was absolutely no reaction against the whole RT1-A^avl molecule on DA RBC. At 7 d after skin grafting there was a small, but significant, level of antibody against whole RT1-A^avl class I molecules. This was greatly augmented at day 10, when the peptide-immunized rat had a very strong response to DA RBC, while the control rat had only a weak response. Fig. 4, *c* and *d* show the individual titrations against whole RT1-A^avl class I molecules at day 10 after DA skin grafting in five control LEW rats and all six peptide 3–immunized LEW rats of series 2 (Table 1). It can be seen that all peptide 3–immunized rats had greatly increased kinetics of the antibody response to whole RT1-A^avl class I molecules.

Discussion

The immune response to allogeneic MHC molecules is unique in offering two quite distinct pathways of T cell recognition. Since the activation requirements and the regulatory control of the direct and indirect pathways will be quite different, our demonstration that the indirect pathway can play an important role in allograft rejection is potentially of fundamental importance.

With regard to activation, the direct pathway of allorecognition is heavily dependent on allogeneic dendritic cells, at least in vitro (32). Whether or not interstitial dendritic cells (33) play the major role in stimulating direct allorecognition after organ transplantation is not certain, but the dramatic effects of their removal (9, 34) suggest that this is the case,

Table 1. *Survival of DA (RT1^{av1}) Skin Allografts on LEW (RT1^l) Recipients Immunized with RT1-A^{av1} Peptides*

Group*	Series 1		Series 2	
	Skin graft survival[‡]	p value[§]	Skin graft survival[‡]	p value[§]
	d		d	
Control	9, 9, 9, 9, 9, 9 10, 10, 10	–	9, 9, 9, 9, 9, 9 9, 10, 10	–
Peptide 1	7, 8, 8, 9, 9, 9	0.04	7, 8, 8, 8, 9	0.005
Peptide 2	8, 8, 9, 9	NS[‖]	8, 8, 9, 9, 9, 9	NS[‖]
Peptide 3	7, 7, 8, 8, 9, 9	0.01	8, 8, 8, 8, 9, 9	0.01
Peptide 1 plus Peptide 3	Not done		7, 7, 7, 8, 8, 8	<0.01

* LEW rats were immunized with peptides as described in Materials and Methods, and full thickness allografts of abdominal skin from DA rats were placed 4 weeks after the booster injection. Control rats received Freunds adjuvant only.

‡ For scoring, rats were presented in a random fashion, and the assessment was made without knowledge of the group to which the rat belonged. Results were not divulged until all rats in the series of experiments had rejected their grafts. The end-point of rejection was taken as >90% full-thickness graft loss.

§ Fisher's exact test, two tailed, in comparison with the control group.

‖ $p > 0.1$.

at least in come strain combinations in rodents. However, because donor antigens are processed and presented by recipient APC, the precise cellular origin of the donor antigens will be of no importance for the indirect pathway. Probably the only important factor will be quantity of donor antigen. In this regard, class I MHC antigens are likely to be of greater importance than class II MHC antigens, as class I antigens are much more abundant in the commonly transplanted organs (35), at least in the absence of overt rejection (36).

The situations in which direct and indirect allorecognition are important are likely to differ. T cells involved in direct recognition have a high precursor frequency and induce strong primary immune responses in vitro (3). By contrast, T cells involved in indirect recognition will have a low precursor frequency, as for any nominal antigen. Indirect recognition might therefore be important only some time after exposure to antigens, e.g., in the later stages of primary rejection responses, in chronic rejection, and after previous exposure to histocompatibility antigens (e.g., previous transplants, pregnancies, blood transfusions).

Direct recognition, however, might be of greatest importance in the early stages of the primary acute rejection response. This is not only because of the high initial T cell precursor frequency, but also because interstitial dendritic cells are migratory (33), being replaced by recipient interstitial dendritic cells within 2 wk of transplantation (37). If no other cells in the graft are able to efficiently stimulate direct recognition, this will leave the graft with little or no capacity to stimulate direct recognition. The major controversy concerns the capacity of class II–positive vascular endothelial cells, a permanent component of the graft, to stimulate direct T cell recognition (38–40). The relative importance of dendritic cells

and class II–positive vascular endothelial cells for stimulating direct recognition is unknown, but it is a crucial factor in clinical transplantation. For example, it will determine the long-term vulnerability of transplanted human organs to direct recognition and therefore the relative importance of direct and indirect recognition in chronic rejection, which is now the major immunological problem in clinical transplantation.

With regard to regulatory processes, recent advances in our understanding of the physiology of self-tolerance (e.g., reference 41), would suggest that thymic deletion of T cells with specificity for direct allorecognition of the graft will not occur in recipients of organ allografts. This follows from the fact that living donor cells, able to present intact donor MHC molecules, are not present in the thymus of these patients. By contrast, model studies in transgenic mice suggest that peripheral exposure to antigens such as OVA can lead to thymic deletion of specifically reactive T cells (42). It is therefore possible that the systemic release of graft antigens might result in thymic tolerances to indirect allorecognition (irrespective of its effect on peripheral T cells), as previously discussed in detail (43). Moreover, T cell anergy, a regulatory process consequent on T cell recognition of antigen in the absence of essential costimulatory signals (44), is unlikely to occur for indirect recognition, because, by definition, the presenting cell is a professional recipient APC.

Elucidating the precise mechanisms whereby indirect recognition contributes to the effector mechanisms of rejection was not the major objective of this paper. However, it is interesting to speculate on the possible mechanisms. There are two broad ways in which indirect allorecognition might be important. A direct role would involve a DTH-type reaction
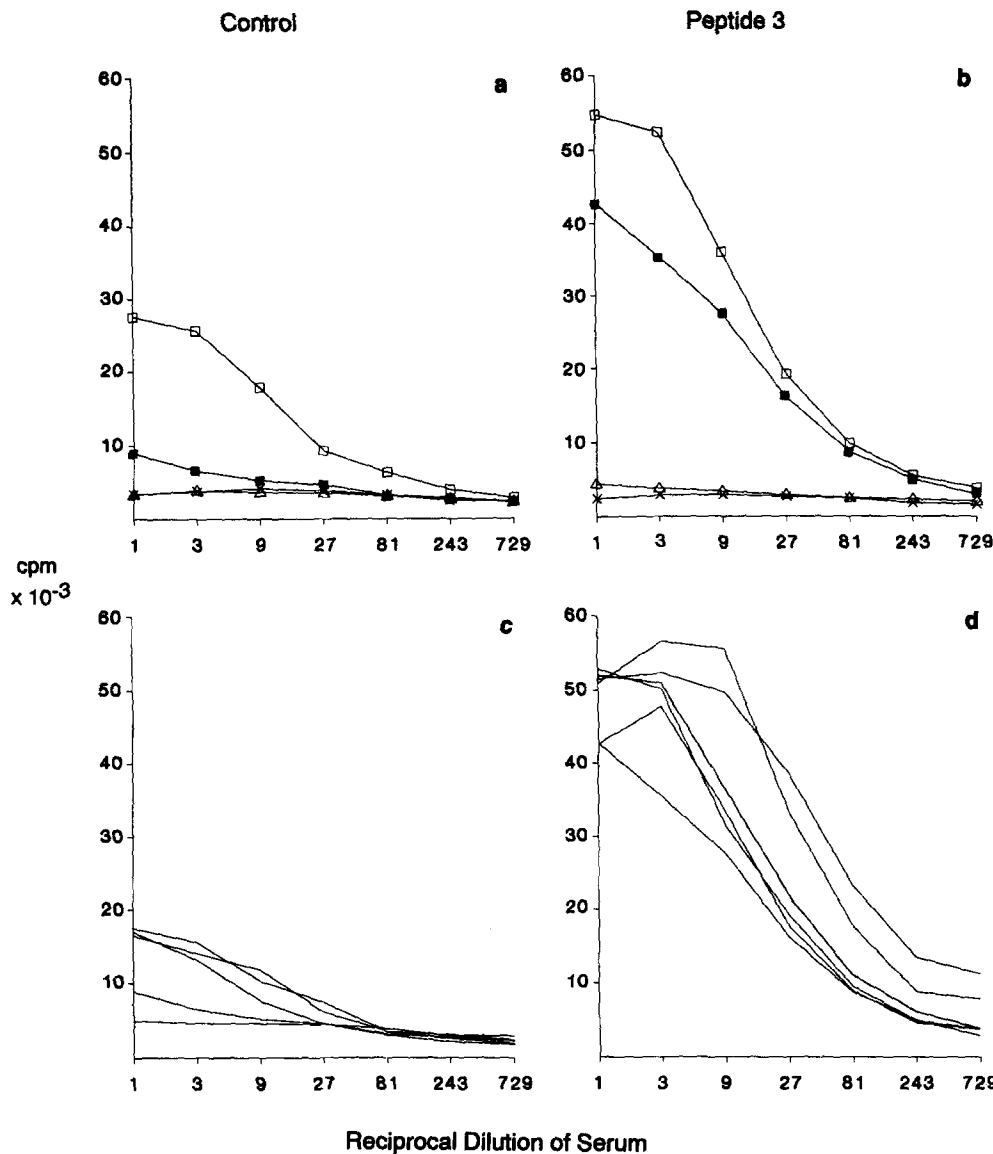
Control

Peptide 3

Figure 4. Alloantibody response to the intact RT1-A^avl class 1 molecule after DA skin grafting in LEW rats. LEW rats were immunized with peptide 3 of the RT1-A^avl class 1 molecule (*b* and *d*) or with Freunds adjuvant only (control) (*a* and *c*). 4 wk after the booster injection, they received full-thickness DA skin allografts and were bled at the time of grafting (×) and at 7 (△), 10 (■), and 14 (□) d after grafting. The sera were assayed for alloantibodies to whole RT1-A^avl class 1 MHC molecules by using DA RBC targets and ^125I-labeled RAR in binding assays. *a* and *b* show typical time course of antibody development in one control and one peptide-immunized rat, respectively. *c* and *d* show, respectively, the individual day 10 titrations of sera from five control rats and the six peptide 3–immunized rats, of series 2 in Table 1. cpm refer to ^125I RAR.

within the connective tissues of the graft, which would obviously require adequate numbers of recipient APC to migrate into the graft. This does not present any problems since macrophages represent a major component of the cellular infiltrates of acute and chronic rejection (45) and macrophages can function as APC (46). Moreover, as mentioned in a preceding section, recipient interstitial dendritic cells colonize the graft within 2 wk of transplantation (37).

Indirect recognition might also influence rejection by providing help for effector mechanisms involving direct recognition, i.e., T cell cytotoxicity and alloantibody formation. Our studies demonstrate that priming for indirect allorecognition can markedly accelerate the kinetics of alloantibody formation against the graft. Whether or not this was a con-

tributory factor to the accelerated graft destruction in our experiments is uncertain, but we would think not. In rodent systems, although alloantibodies to donor MHC antigens have occasionally been known to be harmful (e.g., references 28, 47, and 48), these are exceptional cases, and in most instances such antibodies are protective by the process of passive enhancement (e.g., references 28, 29, and 47). However, antibodies to the donor can be harmful in humans, and this effect on antibody production might therefore be of substantial importance in the clinical setting. With regard to the generation of cytotoxic T cells, indirect recognition, i.e., T cell help provided by responder APC, has been shown to be effective in vitro (49–51), but whether or not this will occur in vivo is not known.

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