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CD4⁺ T cell-mediated rejection of major histocompatibility complex class I-disparate grafts: a role for alloantibody*

Experimental studies of the T cell requirement for rejection of class I major histocompatibility complex (MHC)-disparate grafts have generated controversy over both the autonomy of CD8⁺ T cells and the mechanism whereby CD4⁺ T cells are able to independently mediate rejection. In this study of rejection of RT1A^a class I MHC-disparate rat cardiac and skin allografts by high-responder PVG RT1^u recipients, we show that elimination of CD8⁺ T cells [by anti-CD8 monoclonal antibody (mAb) administration *in vivo*] fails to prolong graft survival, whereas partial depletion of CD4⁺ T cells (by anti-CD4 mAb treatment) markedly delays rejection of class I-disparate heart grafts, and marginally prolongs survival of skin grafts. Anti-CD4-treated PVG-RT1^u athymic nude rats reconstituted with CD8⁺ T cells failed to reject class I-disparate skin grafts for several weeks and eventual rejection correlated with re-emergence of a small number of donor derived CD4⁺ T cells. Conversely, anti-CD8-treated nude rats reconstituted with CD4⁺ T cells alone rapidly rejected class I-disparate skin grafts. Passive transfer of anti-class I immune serum to anti-CD4-treated euthymic recipients promptly restored their ability to specifically reject a class I-disparate heart graft. Similarly, passive transfer of immune serum to PVG-RT1^u nude rats bearing skin allografts caused destruction of class I-disparate but not third-party grafts. These results demonstrate that CD4⁺ T cells are both necessary and sufficient to cause rejection of class I-disparate heart and skin grafts in this model and that CD4⁺ T cell-dependent alloantibody plays a decisive role in effecting rejection.

1 Introduction

It is generally accepted that allograft rejection, in non-sensitized recipients, is mediated by cellular rather than antibody-dependent effector mechanisms [1–3]. Considerable progress has been made in defining the T cell populations responsible and the use of experimental models where graft and host differ at defined MHC subregions, most often for class I MHC, have proved invaluable in addressing this complex issue. The T cell populations involved have been studied most closely for the rejection of mouse skin grafts bearing either mutant or allelic class I MHC disparities [4–6]. In the case of mutant class I-incompatible skin grafts, CD8⁺ T cells, acting most likely as cytotoxic effector cells, play an essential role, whereas CD4⁺ T cells are neither necessary, nor by themselves sufficient, to mediate rejection, as shown by both adoptive transfer analysis and *in vivo* depletion studies with mAb [4–9]. Similarly, in allelic class I-disparate skin grafts CD8⁺ T cells are sufficient to cause rejection. However, CD4⁺ T cells also

participate in the rejection of certain non-mutant class I disparities, since depletion of CD8⁺ T cells by anti-CD8 mAb treatment does not prevent rejection in all strain combinations [6–8]. The cellular mechanisms whereby CD4⁺ T cells mediate rejection of class I MHC-disparate skin grafts, when conventional CD8⁺ T cells have been depleted, are not known with certainty and various non-classical effector T cell populations may be implicated [7, 10–12].

The T cell requirements for rejection of class I-incompatible organ allografts have been less clearly defined than those for skin graft rejection. We previously showed, in a comparison of rejecting and non-rejecting RT1A^a class I-disparate rat kidney grafts in recipients carrying the high (RT1^u) and low (RT1^c) responder alleles, respectively of the Ir gene for RT1A^a, that rejection did not correlate with the presence of cytotoxic T cells in the graft [13]. Moreover, high responder recipients treated with anti-CD8 mAb rejected class I-disparate kidney grafts at a normal tempo despite the absence of CD8⁺ cells within the rejecting grafts, thereby implying that CD4⁺ T cells may independently initiate organ allograft rejection in the rat.

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Abbreviations: DAB: Dulbecco's A buffer MST: Median survival time

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The present study was undertaken to examine further the T cell requirements for the rejection of class I-disparate allografts in the rat. Because of possible differences between indirectly vascularized skin and directly vascularized organ grafts and to try and reconcile the mouse and rat data, we chose to define the T cell requirements for rejection of both heterotopic heart grafts and free skin grafts by high responder recipients. We show, in contrast to the mouse data, that CD8⁺ T cells alone are unable to mediate graft rejection. Conversely, CD4⁺ T cells are both

necessary and by themselves sufficient to initiate rejection of both types of graft, raising the intriguing question of the effector mechanism responsible. Here we provide evidence to show that CD4⁺ T cell-dependent alloantibody plays a decisive role in mediating first-set graft rejection in this class I-disparate rat strain combination.

2 Materials and methods

2.1 Animals

The following PVG congenic and recombinant rat strains, whose derivation is cross-referenced elsewhere [13], were used; PVG (RT1^c) (A^cB^cD^cC^c), PVG-RT1^u (A^uB^uD^uC^u) and PVG-R8 (A^aB^uD^uC^u). Male adult animals were obtained from Harlan Olac Ltd., (Bicester, Oxon, GB) and used when 8–12 weeks old.

The Rowett nude mutation (rnu) [14] carried by the PVG (RT1^c) strain [15] was bred into the high responder congenic PVG-RT1^u strain. PVG-rnu/rnu males were crossed with PVG-RT1^u females. The RT1^{u/c}-rnu/+F₁ offspring were backcrossed with PVG-RT1^u and the resulting offspring screened for RT1^{u/u} homozygosity by typing red blood cells for A^u and A^c (the analysis was kindly performed by Dr. Geoff Butcher, Babraham, Cambridge). RT1^{u/u} euthymic animals carrying the recessive nude gene (rnu/+) were identified by test mating with homozygous PVG nude males (RT1^{c/c}-rnu/rnu). Nude males from this cross (*i.e.* RT1^{u/c}-rnu/rnu) were paired with RT1^{u/u}-rnu/+ heterozygote females in a mother/son mating and the resulting offspring again typed to identify RT1^{u/u} homozygotes. We obtained three RT1^{u/u}-rnu/rnu males and four RT1^{u/u}-rnu/+ females which were selected to form the basis of the PVG-RT1^u nude colony. A companion PVG-RT1^u euthymic strain bearing the RT7^b allotype marker (a polymorphic variant of the leucocyte-common antigen) was also developed. The congenic PVG-RT7^b strain (developed by Dr. Simon Hunt, Oxford) was crossed with the PVG-RT1^u (RT7^a) strain. The resulting PVG-RT1^{u/c} RT7^{a/b} first generation F₁ hybrids were intercrossed. All the offspring were screened for A^c, A^u, RT7^a and RT7^b by FACS analysis of PBL using mAb YR5/12, MAC 161 (kindly supplied by Dr. G. Butcher), NDS58 [16] and 8G6.1 [17], respectively. Offspring homozygous for both A^u and RT7^b were selected and bred to establish the PVG-RT1^u-RT7^b congenic strain. Rats from both strains permanently accepted PVG-RT1^u (RT7^a) skin grafts. Breeding was maintained by brother/sister mating. Derivation of both of the above new strains was undertaken in the University of Manchester Animal Unit, under the direction of Mr. Neil Yates.

2.2 Cardiac transplantation

Heterotopic cardiac transplantation was performed by the modified technique described in detail by Ono and Lindsey [18]. Cardiac transplants were assessed by daily palpation and rejection, defined as the complete cessation of myocardial contraction, was confirmed by histopathological examination.

2.3 Skin grafting

Recipient rats were grafted on the flank as described previously [19]. Grafts were inspected daily and the end-point of rejection was defined as necrosis of 50 % of the grafted skin.

2.4 Antibodies

The following mouse mAb were used to treat rats *in vivo*: OX-8 labels the rat CD8 molecule [20], OX-35 and OX-38 bind to separate epitopes on the rat CD4 molecule [21] and OX-21 which recognises human C3b inactivator was used as a control [22]. All mAb were administered as ascites, diluted in PBS, by i.p. injection. Other mAb used in this study were: OX-1 (leucocyte common antigen) [23], OX-12 (rat Ig κ chains on B lymphocytes) [20], W3/25 (CD4) [20], R73 ($\alpha\beta$ TCR) [24] and ED1 (most tissue macrophages, monocytes and dendritic cells) [25] (all obtained from Serotec Ltd).

2.5 Preparation and fractionation of lymphocytes

Lymphocytes for adoptive transfer were prepared from thoracic duct lymphocytes (TDL) of euthymic PVG-RT1^u-RT7^b rats and T cell subsets prepared by immunomagnetic separation [26].

PBL and single-cell suspensions of cervical and mesenteric lymph nodes (LN) were prepared and labeled for single-color fluorescence analysis as described previously [27, 28]. For two-color flow cytometry cells were stained with FITC-conjugated R73 ($\alpha\beta$ TCR) and either PE-conjugated W3/25 (CD4) or OX-8 (CD8) mAb (Serotec Ltd). Donor cells were identified with biotinylated 8G6.1 (anti-RT7^b) plus PE-streptavidin (Sera-Lab, Crawley Down, Sussex, UK) from PBL of reconstituted nude rats (RT7^a) by two-color fluorescence analysis (FITC-W3/25 or FITC-OX-8) [26]. Labeled cells were analysed on a FACScan Flow cytometer (Becton Dickinson, Oxford, GB).

2.6 Alloantibody determinations

Lymphocytotoxic antibodies were detected as described elsewhere [13]. Results were expressed as the highest dilution of serum giving > 20 % specific lysis. IgG and IgM serum alloantibodies were detected by a two-stage radio-labeled binding assay essentially as described elsewhere [29].

3 Results

3.1 Role of T cell subsets in rejection of class I-disparate heart grafts

In the following experiments, we studied the rejection by congenic PVG-RT1^u recipients (subsequently referred to as RT1^u) of class I MHC RT1A^a-disparate grafts from intra-MHC recombinant PVG-R8 (RT1A^aB^uD^uC^u) donors (subsequently referred to as R8). Rejection of allografts bearing

the RT1A^a class I antigen is under strict MHC-linked immune response gene control and the RT1^u rat strain is a high responder, rejecting rapidly class I-disparate R8 heart and skin grafts [30, 31].

To determine the role of CD8⁺ and CD4⁺ T cells in initiating and effecting rejection of RT1A^a-disparate heart grafts, recipients were treated *in vivo* with anti-CD8 and anti-CD4 mAb (Table 1). Administration of anti-CD8 mAb was highly effective at depleting CD8⁺ T cells (as shown here as well as in previous studies [13] by two-color FACS analysis of PBL and LN cells), but did not prolong the survival of R8 heart grafts in RT1^u recipients [median survival time (MST) 6.5 vs. 6.0 days in controls]. Immunohistological analysis in anti-CD8-treated recipients confirmed the complete absence of CD8⁺ cells from the graft infiltrate (data not shown). The histopathological appearances of rejecting heart grafts in anti-CD8-treated recipients were otherwise similar to those seen in unmodified recipients; rejecting hearts were heavily infiltrated by mononuclear cells (including numerous ED1⁺ macrophages and CD4⁺ T cells) and the heart microvasculature, a critical target during acute rejection [32, 33], showed swelling and disruption.

In contrast to anti-CD8 treatment, administration of anti-CD4 mAb markedly prolonged the survival of class I-disparate heart grafts and many recipients developed permanent tolerance towards their graft after cessation of antibody treatment (Table 1). Neither of the anti-CD4 mAb treatment protocols completely depleted CD4⁺ T cells from the blood or lymph nodes of RT1^u rats

(maximum depletion typically 60–80% depending on the anti-CD4 schedule used) although all residual CD4⁺ cells were coated with anti-CD4 mAb (detected using FITC-anti-mouse Ig). Immunohistological analysis of heart grafts in anti-CD4-treated recipients revealed a substantial day 5 cellular infiltrate which included numerous ED1⁺ macrophages and OX-8⁺ cells as well as W3/25⁺ cells.

3.2 Role of T cell subsets in rejection of class I-disparate skin grafts

We next sought to determine the role of CD8⁺ and CD4⁺ T cell subsets in rejecting RT1A^a-disparate skin grafts, by *in vivo* treatment with anti-T cell mAb (Table 2). Like class I-disparate heart grafts, anti-CD8 treatment did not prolong the survival of R8 skin grafts, (MST 7.5 days in both OX-8-treated and unmodified recipients). However, the ability of anti-CD4 treatment to prolong survival of class I-disparate hearts did not extend readily to skin grafts. Using one regimen of anti-CD4 treatment (OX-35 + OX-38) skin grafts were rejected at a rate similar to that seen in untreated animals. Using high-dose mAb OX-38, which induced indefinite survival of class I-disparate heart grafts, skin graft survival was marginally, but not significantly, prolonged. Rats treated with anti-CD4 and anti-CD8 mAb simultaneously also rejected their grafts promptly (MST 8 days), suggesting that residual CD4⁺ T cells in such animals are able to reject class I-disparate skin grafts in the absence of CD8⁺ T effector cells.

Table 1. Ability of anti-T cell mAb to prolong survival of RT1A^a-disparate R8 cardiac allografts in RT1^u recipients

Group	mAb treatment ^{a)}	n	Graft survival (days) ^{b)}	MST (days) ^{c)}
1	None	8	6,6,6,6,6,7,7,8	6
2	Control mAb (OX-21)	3	6,6,7	6
3	Anti-CD8 (OX-8)	8	6,6,6,6,7,7,7,7	6.5
4	Anti-CD4 (OX-38 + OX-35)	7	14,34,57,73,> 100(×3)	73
5	Anti-CD4 (OX-38)	8	all > 100	> 100

a) Groups 2 and 3 received 3 mg mAb on day -1, 2 mg on day 0 (day of transplant) and 1 mg on days 1, 3, 6 and 9. Two anti-CD4 treatment protocols were used. Group 4 received 2.5 mg OX-38 + 0.75 mg OX-35 on day 0 and then twice weekly for 21 days. Group 5 received 5 mg OX-38 alone on days 0, 3, 6, 9 and 12.

b) Animals were examined daily and graft rejection was defined as complete cessation of myocardial contraction.

c) Median survival time.

Table 2. Ability of anti-T cell mAb to prolong survival of RT1A^a disparate R8 skin grafts in RT1^u recipients

Group	mAb treatment ^{a)}	n	Graft survival (days) ^{b)}	MST (days)
1	None	9	7,8,8,8,8,8,8,9	8
2	Anti-CD8 (OX-8)	6	7,7,7,8,8,8	7.5
3	Anti-CD4 (OX-38 + OX-35)	6	7,7,7,8,8,8	7.5
4	Anti-CD4 (OX-38)	7	7,7,8,8,14,14,16	8
5	Anti-CD4 (OX-35/38) + anti-CD8 (OX-8)	6	7,7,8,8,8,9	8

a) mAb treatment was given as described in legend for Table 1.

b) Rejection was defined as necrosis of > 50% of the grafted skin.

To clarify further the ability of CD4⁺ and CD8⁺ T cells to reject RT1A^a-disparate skin grafts, a series of adoptive transfer experiments was performed (Table 3). Congenitally athymic PVG-RT1^u (RT7^a) nude recipients were doubly grafted with allogeneic skin and then injected with highly purified T cell subpopulations obtained from allotype-marked histocompatible euthymic RT1^u RT7^b rats. In addition graft recipients were treated *in vivo* with mAb to eliminate any contaminating or host-derived lymphocytes of the unwanted subset.

RT1^u nude rats injected with $1 \times 10^7 - 2 \times 10^7$ Ig⁻ TDL rejected both class I-disparate R8 skin grafts and fully allogeneic DA RT1^a or PVG grafts at similar rates, and the tempo of rejection was not reduced by administration of anti-CD8 mAb. Adoptive transfer of 5×10^6 CD4⁺ T cells readily restored rejection of both R8 and fully allogeneic DA or PVG skin grafts, irrespective of whether recipients were treated with OX-8 or control mAb OX-21. In contrast, nude recipients given 2×10^7 CD8⁺ T cells from RT1^u-RT7^b donors and treated for 1 week with anti-CD4 mAb, failed to reject class I-disparate R8 or fully MHC-mismatched PVG grafts acutely. Donor-derived CD4⁺ T cells could not be detected in PBL during this period. However, rejection of both grafts was observed in four out of five recipients between days 47 and 78. Moreover, FACS analysis of PBLs from CD8⁺ T cell-restored recipients at day 70 revealed, in all five animals, a population of donor-derived CD4⁺ T cells (comprising 0.6–2.5% of circulating lymphocytes) arising as contaminants from the T cell inoculum (data not shown). Since this donor-derived CD4⁺ T cell population represents approximately $4 \times 10^6 - 15 \times 10^6$ recirculating CD4⁺ T cells, it seems likely that it was responsible for the late rejection of allogeneic skin grafts in the CD8⁺ T cell-reconstituted animals.

3.3 Alloantibody levels in graft recipients treated with anti-T cell mAb

Anti-CD8-treated recipients bearing rejecting R8 heart grafts developed a strong cytotoxic antibody response apparent from day 3 and maximal by days 7–10 (Fig. 1c). This response was identical to that of untreated heart graft recipients. In contrast, anti-CD4 treated recipients showed only a weak and transient cytotoxic antibody response which was maximal at day 3 and declined thereafter. The pattern of cytotoxic antibody response in recipients of R8 skin grafts was essentially the same as that for heart grafts (data not shown). Serum IgG and IgM anti-RT1A^a responses in grafted animals were also determined by a two-stage binding assay using donor strain erythrocytes (Fig. 1a,b). Anti-CD8-treated recipients developed an IgG response which increased progressively from day 5 and an IgM response which peaked at day 7. In contrast, anti-CD4-treated recipients displayed a minimal IgG response and a much reduced IgM antibody response.

3.4 Role of antibody in mediating rejection of class I-disparate heart and skin grafts

We next examined whether passive transfer of immune serum restored the ability of anti-CD4-treated RT1^u recipients to reject R8 heart grafts. To mimic broadly the alloantibody response mounted by unmodified RT1^u recipients to R8 heart grafts, immune serum was obtained at day 10 after transplantation from unmodified recipients (when the circulating cytotoxic antibody response was maximal) and given *i.v.* to anti-CD4-treated RT1^u heart graft recipients on days 3, 4 and 5 after transplantation

Table 3. Ability of T cell subsets to reject skin allografts in PVG-RT1^u nude recipients

Group	T cells injected ^{a)}	mAb ^{b)}	n	grafted skin	Day of rejection ^{d)}	MST
1	$1 \times 10^7 - 2 \times 10^7$ Ig ⁻ TDL	OX-21	5	R8	18,12,25,13, 9	13
				DA	15, 9, 7	9
				PVG	13,15	14
2	$1 \times 10^7 - 2 \times 10^7$ Ig ⁻ TDL	OX-8	5	R8	13,25,8,13,11	13
				PVG	7,13,9,11,10	10
3	5×10^6 CD4 ⁺	OX-21	6	R8	15,13,45,19,11,11	14
				DA	12,12,12,10, 9,10	11
4	5×10^6 CD4 ⁺	OX-8	6	R8	20,13,38, 15,12,34	17.5
				DA	10, 9, (-) ^{c)} 10, 9,11	10
5	5×10^6 CD4 ⁺	OX-8	4	R8	21,16, 13,14	15
				PVG	17, (-), 13,13	13
6	2×10^7 CD8 ⁺	OX-38	5	R8	78,47,59,47,> 100	59
				PVG	49,78,47,51,> 100	51

- a) PVG RT1^u nude rats bearing two allogeneic skin grafts (engrafted 7–10 days previously) were injected *i.v.* with T cells or their subpopulations, prepared from TDL of euthymic RT1^u rats by negative selection (98% pure).
b) Recipients were treated *in vivo* with control mAb (OX-21), anti-CD8 (OX-8) (both 1 mg on days 0,2,5 and 7) or anti-CD4 (OX-38, 2.5 mg on days 0,2,5 and 7) after cell transfer.
c) Technical failure, graft not scored.
d) For each group, data for grafts on each individual animal are shown in vertical alignment.

Table 4. Ability of passively transferred immune serum to initiate cardiac allograft rejection

Group	Donor/Recipient	mAb Treatment ^{a)}	Treatment ^{b)}	n	Graft survival	MST
1	R8/RT1 ^u	Anti-CD4	Normal serum	3	33,36,> 100	36
2	R8/RT1 ^u	Anti-CD4	Day 10 immune serum	5	5,6,6,7,7	6
3	PVG/RT1 ^u	Anti-CD4	Day 10 immune serum	4	29,50,> 100,> 100	75
4	PVG/RT1 ^u	None	None	4	6,6,6,7	6

- a) Anti-CD4 mAb treatment (OX-38 + OX-35) was given on the day of transplantation and twice weekly thereafter for 21 days (see Sect. 2.4).
- b) Immune serum was obtained from RT1^u recipients bearing rejecting R8 hearts 10 days after transplantation. After heat inactivation (56°C for 30 min) it was stored at -20°C and microfuged before use. One milliliter of immune serum (or normal RT1^u serum) was given to graft recipients by i.v. injection on days 3,4, and 5 after transplantation.

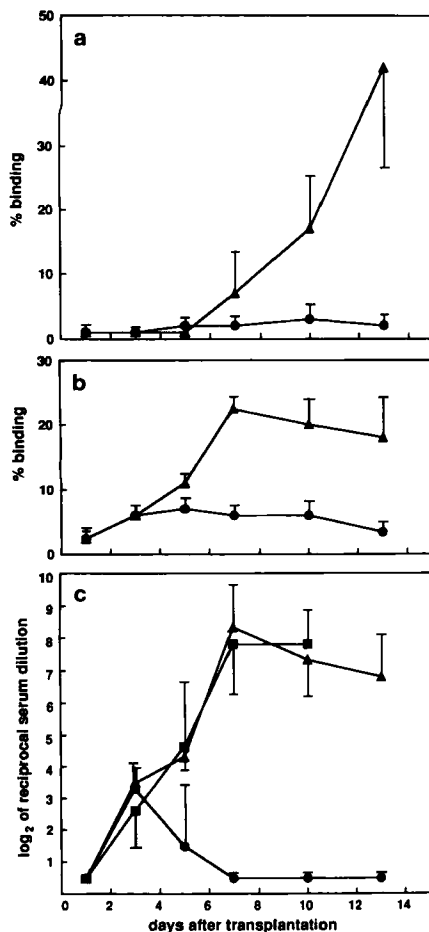


Figure 1. Circulating antibody response of RT1^u rats to RT1A^a-disparate R8 heart grafts. IgG (a) and IgM (b) antibodies were determined by assaying serum against R8 erythrocytes in a two-stage binding assay. Cytotoxic antibodies (c) were determined by assaying serum against ⁵¹Cr-labeled R8 Con A blasts in the presence of guinea pig complement. Graft recipients were treated *in vivo* with OX-8 (▲), OX-35 + OX-38 (●) or no mAb (■), according to the schedule given in Table 1. Values shown are mean and SD of four graft recipients. There was no binding to or lysis of third-party PVG (RT1^c) target cells (data not shown).

(Table 4). Passive transfer of anti-RT1A^a immune serum caused early rejection of R8 heart grafts (MST 6 days) and this effect was allospecific since third-party PVG heart

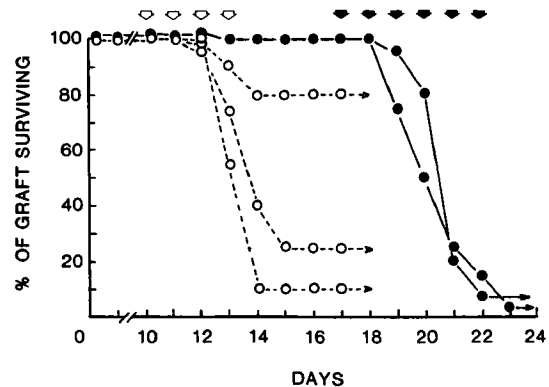


Figure 2. Ability of immune serum to destroy class I-disparate skin allografts. PVG-RT1^u nude rats ($n = 5$) were doubly grafted with R8 and third-party PVG (RT1^c) skin grafts and then 10 days ($n = 3$) or 17 days ($n = 2$) later (open or closed arrow, respectively), recipients received daily i.p. injections of 1 ml anti-RT1A^a immune serum prepared as described in the legend to Table 4. The R8 graft on each nude recipient was scored individually (open or closed circles) according to the area of grafted tissue (%) which remained intact and free of scabbing. (100% = graft fully intact; e.g. 5% = amount of non-rejected tissue remaining) All PVG skin grafts remained in perfect condition throughout.

grafts were not rejected acutely (MST 75 days). The histopathological features of tissue injury in rejected R8 hearts after passive transfer of immune serum were similar to those observed in acutely rejecting hearts from unmodified recipients.

To determine whether specific antibodies were involved in the rejection of class I-disparate skin grafts, we double-grafted 5 RT1^u nude rats with R8 and third-party PVG skin grafts. After 10 days, to ensure re-vascularization, three of the recipients were injected daily for 4 days with 1 ml of anti-A^a alloantiserum (raised in RT1^u rats against R8 hearts, thereby avoiding possible skin specific antigens). One day following antiserum injection, the R8 skin (but not the PVG graft) became reddened and 2 days later, the entire R8 graft bed was acutely inflamed and a proportion of the grafted tissue was destroyed. Starting on day 17, the remaining two nude recipients were injected daily for 6 days with 1 ml of anti-class I immune serum. Again, the R8 grafts alone underwent progressive destruction of > 90% of their surface area. Interestingly, the non-rejected R8 graft component, in some cases merely a tiny triangle of skin, subsequently recovered and was identified months later as a tuft of normal hair on an otherwise nude rat.

4 Discussion

The results reported here provide further support for the role of CD4⁺ T cells in the rejection of grafts differing from their host for class I MHC antigens alone [6–9, 11, 13, 34]. Rat CD4⁺ T cells were able to mediate rejection of class I-disparate heart and skin grafts, in the absence of CD8⁺ effector cells. RT1^u recipients treated *in vivo* with anti-CD8 mAb showed no impairment in their ability to reject RT1A^a class I-disparate R8 heart or skin grafts, as reported herein, or RT1A^a-disparate kidney allografts as reported in our previous studies [13], despite the effectiveness of anti-CD8 treatment in selectively depleting CD8⁺ cytotoxic T cells. Moreover, adoptive transfer experiments in congenitally athymic RT1^u nude rats revealed that the ability of these animals to reject an RT1A^a class I-disparate skin graft was readily restored by the transfer of purified CD4⁺ T cells alone. Nude rats have appreciable numbers of extrathymically derived CD8⁺ cells [28, 35], but these were not a necessary component of the rejection response in CD4⁺ T cell-restored recipients since their elimination by anti-CD8 treatment did not impair rejection of class I-disparate skin grafts. The nature of the class I alloantigen recognized by CD4⁺ T cells was not studied here but we have argued elsewhere [36], that CD4⁺ T cells are most likely to recognise graft class I alloantigens which have been processed and presented as class I peptide fragments in the antigen-binding cleft of self MHC class II molecules, *i.e.* via the indirect pathway of allorecognition [37, 38].

A further issue addressed by this study concerns the autonomy of CD8⁺ T cells in rejection of class I-disparate grafts. Mouse CD8⁺ T cells are able to mediate rejection of class I-disparate skin grafts, in the absence of CD4⁺ T cell help [3–6]. Information in the rat is more limited, but a recent study showed that congenitally athymic PVG nude rats can only be induced to reject class I-disparate skin grafts by adoptive transfer of both CD4⁺ and CD8⁺ T cell subsets [27]. However, PVG nude animals carry the low-responder RT1^c haplotype and their euthymic counterparts reject RT1A^a disparate skin grafts with a reduced tempo, raising the possibility that the lack of autonomy of CD8⁺ T cells in this strain combination may simply reflect an Ir gene effect. The results from the present study in the high-responder RT1^u rat strain do not support this view. We found no evidence, from either *in vivo* T cell depletion studies or adoptive transfer analysis, that CD8⁺ T cells are able to mediate rejection of class I-disparate grafts effectively in the absence of CD4⁺ T cell help. Anti-CD4 treatment alone markedly prolonged the survival of class I disparate heart grafts and although the non-depleting anti-CD4 schedule used here did not significantly extend the survival of class I disparate skin grafts, we attribute rejection in such animals to residual CD4⁺ T cell function, rather than CD8⁺ T cell autonomy for the following reasons. First, the number of functional CD4⁺ T cells required to induce skin allograft rejection in the rat is much less than that necessary to initiate organ allograft rejection [19, 28, 39]. Second, RT1^u recipients treated simultaneously with both anti-CD4 and anti-CD8 mAb also rejected R8 skin grafts rapidly in the absence of CD8⁺ effector cells. Third, RT1^u nude rats reconstituted with purified CD8⁺ T cells were unable to reject R8 skin grafts in the absence of CD4⁺ T cells. There is, therefore, no evidence from this

study that rat CD8⁺ T cells are able to effectively mediate allograft rejection autonomously.

The final issue addressed in this report concerns the nature of the effector mechanism whereby CD4⁺ T cells destroy class I MHC-disparate allografts. Evidence is presented that CD4⁺ T cell-dependent alloantibody is responsible for rejection of class I-disparate R8 grafts by RT1^u recipients. Passive transfer of immune serum to anti-CD4-treated recipients readily restored their ability to reject class I-disparate heart grafts and this finding is consistent with the earlier observation that passive transfer of immune serum restores the ability of cyclosporin-treated RT1^u rats to reject R8 kidney allografts [13]. Because of potentially important differences in the nature of the rejection response between directly vascularized organ grafts and secondarily vascularized skin grafts, it is interesting that passive transfer of alloantibody also caused rejection of class I-disparate skin grafts in nude rats which are genetically devoid of functioning CD4⁺ T cells [40]. There was no evidence, therefore, to suggest fundamentally different mechanisms of rejection between organ and skin grafts to a class I disparity.

The role of alloantibody in mediating acute rejection has, to a large extent, been neglected and the present results are at variance with the findings from many early animal studies, where passive transfer of immune serum not only failed to cause rejection of skin or tumor allografts [41, 42] but led, in some rodent models of organ transplantation, to a paradoxical improvement in graft survival [43, 44]. To reconcile our findings with those from earlier studies, it is notable that the immune serum used in earlier studies was often raised by immunization protocols or in rodent strain combinations which may have produced a predominance of anti-class II antibody (which potentially enhances survival, reviewed in [45]), rather than anti-class I antibodies. Moreover, the influence of alloantibody on graft survival is critically dependent on the nature of the experimental model, the strain combination and differences in complement regulation [13, 46, 47]. For example, transfer of anti-RT1A^a serum into anti-CD4-treated or nude RT1^u recipients produces graft rejection, as shown here, but transfer of identical antiserum into PVG RT1^c recipients bearing an RT1A^a-disparate kidney allograft leaves the graft intact [13].

What is the nature of the cellular targets for antibody-mediated rejection of class I-disparate heart and skin grafts? In the case of heart grafts, the microvascular endothelium, which is strongly class I positive, is probably the principal target of the rejection response [32, 33]. Here, rejecting heart grafts following passive transfer of immune serum, like those in unmodified rejection, showed progressive destruction of the microvasculature followed by interstitial hemorrhage and myocardial cell necrosis. The mechanism whereby alloantibody mediates vascular endothelial cell injury remains to be determined but *in vitro* studies suggest a role for complement activation since anti-RT1A^a immune serum readily lyses monolayers of R8 heart microvascular endothelial cells by complement-dependent cytotoxicity [48]. Analysis of the target cell in skin grafts is complicated by incomplete knowledge as to the origin of the vascular bed of the grafted skin. A study of xenogeneic human skin grafted onto nude mice showed

the initial capillary bed to be human derived, but with time it was replaced by endothelial cells of mouse origin [49]. The vascular origin of allografted skin (*i.e.* host or donor), in the nude rat model has not, to our knowledge, been established and further analysis is, therefore, limited.

Finally, recent information from clinical transplantation studies also points to an important role for anti-class I alloantibody in some episodes of severe rejection [50]. Overall, the role of antibody in acute rejection has probably been underestimated and it will be important to define in more detail the mechanisms underlying antibody-mediated rejection in experimental models such as those described here.

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