

Rejection of H-Y Disparate Skin Grafts by Monospecific CD4⁺ Th1 and Th2 Cells: No Requirement for CD8⁺ T Cells or B Cells¹

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We wished to determine whether CD4⁺ T cells could reject a skin graft that was discordant for a single minor transplantation Ag in the absence of CD8⁺ T cells or Ab. Transgenic A1(M) mice were constructed that express the rearranged V β 8.2 and V α 10 TCR genes from a T cell clone that is specific for the male Ag (H-Y) in the context of H2-E^k. In addition, the RAG-1^{-/-} background was bred onto these mice to eliminate any endogenous TCR rearrangements. As expected, clonal deletion was found to be complete in the thymus of male A1(M)×RAG-1^{-/-} mice, while only CD4⁺ T cells were positively selected and found in the periphery of females. Female A1(M)×RAG-1^{-/-} mice were able to rapidly reject (in <14 days) male (but not female) skin grafts in a CD4-dependent fashion. After multiple grafts, it was confirmed that no CD8⁺ T cells or surface Ig⁺ B cells were present. An immunofluorescent analysis of spleen cells after grafting showed that the majority of T cells expressed activation markers (CD44, CD25, and intracytoplasmic IL-2) and a significant proportion were making IFN- γ and IL-4. Surprisingly, the transfer of either Th1 or Th2 CD4⁺ T cell lines from these mice into T cell-depleted recipients was sufficient to cause a specific rejection of male skin. *The Journal of Immunology*, 1998, 161: 1868–1874.

Despite recent increases in our understanding of T cell activation and differentiation in vitro and in vivo, the mechanisms of graft rejection remain obscure. It has previously been demonstrated that depleting or blocking CD4⁺ T cells with mAbs in vivo causes a delay or even an indefinite acceptance of multiple minor or multiple MHC-mismatched skin (1, 2) or vascularized heart grafts (3–5). In contrast, the depletion or blockade of CD8⁺ T cells frequently has very little influence on the rate of rejection (1, 6, 7). Although this would tend to support a direct role for CD4⁺ T cells in the rejection of grafts, it is generally thought that CD8⁺ cytotoxic cells are the critical effector cells. CD4 and CD8 subset depletion experiments have been interpreted in terms of the CD4 mAb, causing the elimination of the CD4⁺ help, that is presumed necessary for CD8⁺ effector T cells (that are somehow resistant to CD8 depletion) to elicit rejection (8).

One of the main functions of CD4⁺ T cells is to provide help for the development of both cytotoxic T cells and B cells. It has been

shown that the rejection of minor transplantation Ags is particularly dependent upon help; one example of the need for such help is that the participation of both CD4⁺ (anti-H-Y) and CD8⁺ (anti-Qa1) T cells is essential for rapid rejection in normal mice (9). Similarly, CD4⁺ T cells is generally required to elicit both graft rejection and the priming of CD8⁺ CTLs against both multiple (10) and “single” (11) minor transplantation Ag differences. Indeed, the so-called single minor transplantation Ags, such as the male Ag H-Y, are thought to be coded by small clusters of genes that provide multiple MHC class II and class I epitopes (for presentation mainly to CD4⁺ and CD8⁺ cells, respectively); in turn, these epitopes provide sufficient helper and effector activity, resulting in graft rejection (12–14). A dependence upon help is also seen when CD8⁺ T cells alone reject single (mutant) MHC class I differences, as this rejection is only observed when the frequency of IL-2-producing CD8⁺ cells is particularly high (15, 16).

The question arises as to whether CD4⁺ cells are only required to provide help or whether they can act to reject independently of CD8⁺ cells, as was originally suggested from the CD4 and CD8 depletion studies (1, 6) and experiments in which the adoptive transfer of purified CD4⁺ T cells into nude mice was sufficient to reject MHC class II-mismatched skin (17). This question has been addressed in CD8-depleted mice that can reject MHC class I allogeneic or mutant skin. This situation is in some ways analogous to a single peptide “epitope” of a minor transplantation Ag, because the MHC class I can be processed to a peptide that is indirectly presented to CD4⁺ T cells by the MHC class II of recipient APCs (18–20). However, it remains unclear whether Ab to the intact MHC class I molecules on the graft (20–22) or a direct (i.e., CD4-independent) recognition of MHC class I (23) is also involved in these experiments. Experiments that have used mice in which there is no MHC class I expression to promote the development of CD8⁺ cells have also failed to resolve the mechanisms of graft rejection; it now seems that MHC class I-restricted, cytotoxic cells can still be generated when mice are challenged with

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allogeneic skin or MHC class I-expressing tumor cells (24, 25). Similarly, although CD8^{-/-} mice normally reject MHC-incompatible skin and CD4^{-/-} mice have been found to accept such grafts indefinitely (26), this finding is confounded by the observation that mice that express neither CD4 nor CD8 can still reject allogeneic (but not minor-mismatched) skin effectively (27), although it has been shown that purified CD4⁺ cells from CD8^{-/-} mice can indeed reject either MHC class I or MHC class II disparate skin after transfer to nude recipients (28).

In addition, there is the question of whether graft rejection is mediated by the specialization of CD4⁺ or CD8⁺ T cells expressing different patterns of cytokines, as is seen in the Th1 or Th2 subsets. It has been suggested for both graft rejection and some autoimmune models that the effector cells should have a Th1 (IFN- γ and IL-2) phenotype, while Th2 responses (IL-4 and IL-10) might be protective or regulatory (29, 30). However, there is recent data that suggest that, under some circumstances, CD4⁺ Th2 cells may also be able to induce autoimmune diseases (31, 32) or reject cardiac allografts (33–35).

We have taken the approach of generating mice that carry only a single transgenic TCR against the male minor transplantation Ag in the context of MHC class II (H-Y + H2-E^k). We show that these mice are able to rapidly reject male, but not female, skin grafts. The RAG-1^{-/-} background makes it possible to rule out any potential involvement of endogenous TCRs that might have allowed the recognition of Ag in the context of MHC class I and also any involvement of B cells or Ab. The female recipients also showed an absolute positive selection of transgenic TCR-positive cells into the CD4 compartment (and complete thymic deletion in the males); therefore, CD8⁺ T cells can be further ruled out. In addition, we generated Th1 and Th2 lines from these mice, and found that both were able to elicit male skin graft rejection after transfer into T cell-depleted recipients. To our knowledge, this provides the first direct evidence that all of the T cell functionality that is required for the skin graft rejection of a minor transplantation Ag can be provided by either Th1 or Th2 CD4⁺ cells.

Materials and Methods

Mice

CBA/Ca (Harlan/Olac, Bicester, U.K.) mice were bred under specific pathogen-free conditions, and all experimental mice were maintained in the animal facility of the Sir William Dunn School of Pathology (Oxford, U.K.) in a filtered cage system (Maximizer, Thorens Caging, Hazelton, PA). RAG-1^{-/-} mice that had been bred onto an H-2^k background were obtained from Dr. B. Stockinger (National Institute of Medical Research, London, U.K.).

Generation of A1(M) transgenic mice

To generate transgenic mice, we used the TCR α - and β -chain from the A1 CD4⁺ T cell clone that had been isolated from CBA/Ca mice (Ref. 36 and our unpublished observations). The A1 clone recognizes the minor histocompatibility Ag H-Y, which is present in male mice but absent in female mice, in the context of H2-E^k. TCR expression was identified using primers that were specific for the V α and V β gene families (37); the TCR genes were cloned and sequenced to check for productive rearrangement. The α -chain was found to be encoded by LV α 10-J α 30-C α , and the β -chain was encoded by LV β 5.1-V β 8.2-D β 2-J β 2.3-C β 2 (EMBL accession nos. AJ000157 and AJ000158, respectively). *EcoRI-EcoRI* fragments containing the productively rearranged α - or β -chains were generated by RT-PCR. The oligonucleotides used for the amplification of the α -chain were GCGAATCACAAGCACCATGAAGAG GCTG and GCGAATTCCAGACCTCAACTGGACCACAG. The oligonucleotides used for the amplification of the β -chain were GCGAATTCAGAG GAAGCATGTCTAACACT and GCGAATTCAGGATGCATAAAAAGTT TGCTCAGG. The full-length cDNAs were cloned into the human CD2 minigene (VA) in pBluescript (Dr. D. Kioussis, National Institute of Medical Research, London, U.K.). *Sall-XbaI* fragments were used for microinjections into CBA/Ca oocytes. Transgenic A1(M) founders were maintained on the CBA/Ca background and bred to homozygosity. Southern blot analysis indi-

cated that the A1(M) line carried a single copy per haplotype of each of the transgenic V α - and β -chains.

Skin grafting

Pieces of tail skin that were \sim 0.5 cm² were grafted onto the lateral thoracic wall of anesthetized recipient mice as described previously (1, 2). When two grafts were given simultaneously, they were placed side by side in the same prepared graft bed. Plaster casts were removed on day 7, and the grafts were observed daily; rejection was defined as the day when no viable graft tissue could be seen. Statistical significance was determined using the log rank method (38). All procedures were conducted in accordance with the U.K. Home Office Animals (Scientific Procedures) Act of 1986.

Immunofluorescent analysis and Abs

The thymus, spleen, or lymph nodes were removed, and E were lysed by isotonic shock. Cells were labeled in PBS containing 0.1% (w/v) NaN₃, 1% (w/v) BSA, and 5% (v/v) heat-inactivated normal rabbit serum (to block FcRs) at 4°C. The Abs used were: CD3 ϵ (KT3-FITC), V β 8 (KJ16-FITC), V β 8.2 (F23.2-FITC), CD4-phycoerythrin (PE)⁵ (P2942; Sigma, St. Louis, MO), CD8 α -quantum red (QR) (R3762; Sigma), B220-QR (R4262; Sigma), CD25 (PC61-biotin), CD44-QR (R5638; Sigma), streptavidin-APC (13049A; PharMingen, San Diego, CA), and FITC goat anti-mouse IgG (F0257; Sigma). After labeling and washing, cells were fixed in 1% formalin and stored in the dark at 4°C. Four-color analysis was performed using a FACSort (Becton Dickinson, Oxford, U.K.) with dual laser (488 nm and 633 nm) excitation in combination with data acquisition and cross-beam color compensation using CellQuest 3.1 software. At least 50,000 events were stored in list mode for further analysis and gating on forward and side scatters.

Intracytoplasmic cytokine staining was performed using spleen cells that had been given a 4-h stimulation *in vitro* with 50 ng/ml PMA (P8139; Sigma) plus 500 ng/ml ionomycin (I0634; Sigma) in phenol red-free RPMI 1640 medium plus 10% FCS at 37°C with the addition of 10 μ g/ml brefeldin A (B7651; Sigma) added to the spleen cells for the second 2 h (39). After washing, cells were fixed in 2% v/v formaldehyde in PBS for 20 min at 4°C, washed, and permeabilized with PBS plus 0.5% saponin (S-2149; Sigma). The following Ab conjugates were added in saponin buffer for 30 min at 4°C: anti-IL-2 (S4B6-FITC) (18004A; PharMingen), anti-IL-4 (11B11-FITC) (18194A; PharMingen), anti-IFN- γ (XMG1.2-FITC) (18114A; PharMingen). The cells were extensively washed in saponin buffer followed by PBS plus 0.1% azide plus 1% BSA plus 5% heat-inactivated normal rabbit serum. Cells were finally labeled with Abs to surface CD4 and CD44, fixed in 1% formalin, and analyzed on a FACSort as described above. The conditions of stimulation, staining, and analysis were such that normal CBA/Ca CD4⁺ spleen cells were essentially negative for all cytokine stains.

Treatment with CD4 mAb

The nondepleting rat IgG2a anti-mouse CD4 mAb (YTS 177.9 (2)) was made by growing the hybridoma in a hollow fiber bioreactor and was purified under sterile and low endotoxin conditions by precipitation with 50% saturated ammonium sulfate (see the following Internet address: <http://www.molbiol.ox.ac.uk/pathology/tig/mprod.html>). Starting on the day of grafting, grafted A1(M) \times RAG-1^{-/-} mice were given 5 \times 1 mg of mAb *i.p.* over a 2-wk period.

Generation of Th1 and Th2 lines

Spleen cells were taken from an A1(M) \times RAG-1^{-/-} mouse that had been grafted 7 days earlier with male plus female tail skin, and 0.5 \times 10⁶ cells were cultured together with 5 \times 10⁶ mitomycin C-treated male CBA/Ca stimulators in 2 ml RPMI 1640 and 10% FCS plus either 50 U/ml of human rIL-2 (to generate the R2.2 Th1 line) or 200 U/ml of mouse rIL-4 (to generate the R2.4 Th2 line). These cell lines were maintained by restimulation with male cells in the appropriate cytokine at 2-wk intervals. Cytokine production and specificity were checked using [³H]thymidine incorporation, IFN- γ - and IL-4-specific ELISAs (PharMingen), and intracytoplasmic staining as described above. To determine whether these lines could reject male skin grafts, 10⁷ viable (histopaque 1083; Sigma) Th1 or Th2 cells were injected *i.v.* at 10 days after the last *in vitro* restimulation into adult thymectomized (ATX), T cell-depleted (with depleting CD4 plus CD8 mAbs (29)) female CBA/Ca mice that had been given a male plus a female skin graft in the same bed.

⁵ Abbreviations used in this paper: PE, phycoerythrin; QR, quantum red; ATX, thymectomized; MST, median survival time.

Thymus

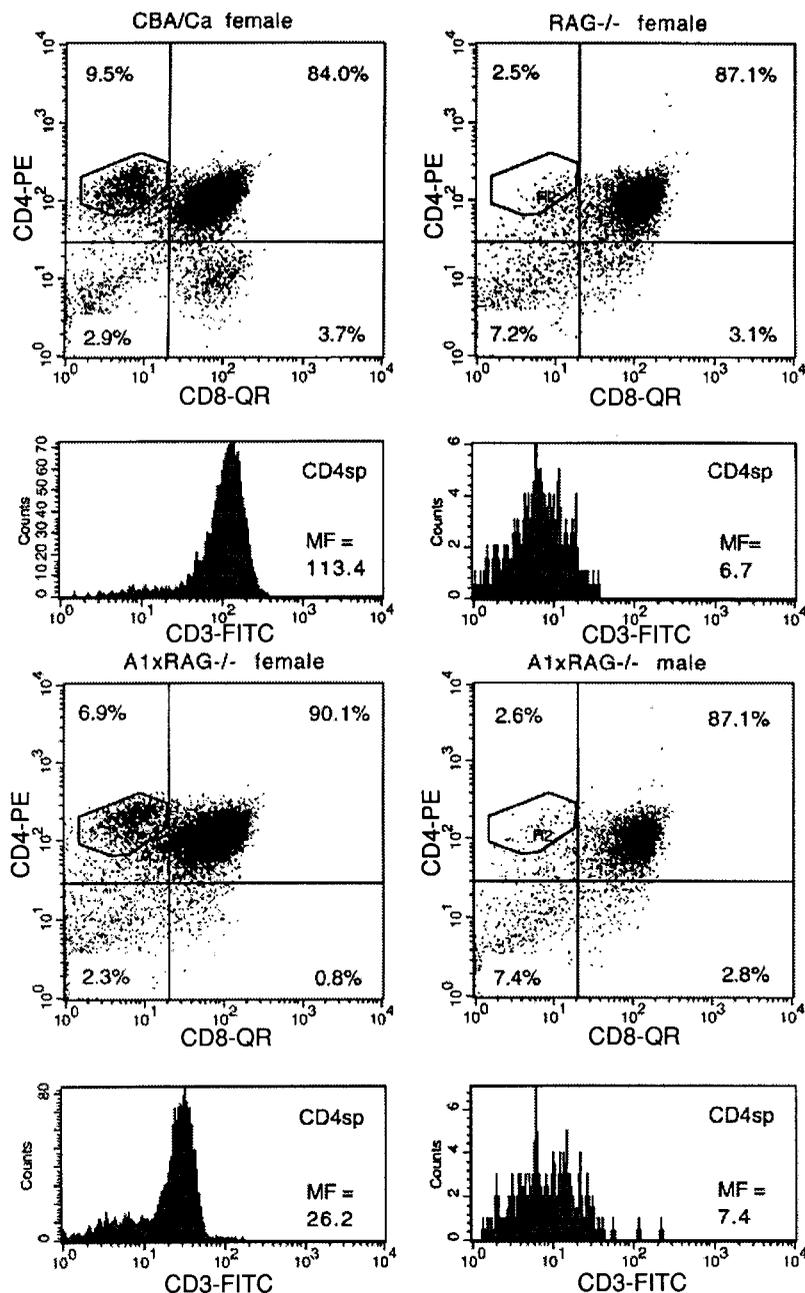


FIGURE 1. Positive selection in female, and clonal deletion in male, A1(M)×RAG-1^{-/-} thymi. Representative examples are shown of dot plot analyses of thymocytes from a normal CBA/Ca (top left), RAG-1^{-/-} (top right), A1(M)×RAG-1^{-/-} female (lower left), and A1(M)×RAG-1^{-/-} male (lower right) that were labeled with CD4-PE and CD8α-QR. Histograms of CD3-FITC staining gated on CD4 single-positive (SP) thymocytes are shown under each dot plot.

Results and Discussion

Analysis of A1(M) mice transgenic for TCR against H-Y + H2-E^k

The thymus, spleens, and lymph nodes from A1(M) mice were analyzed by three-color immunofluorescence to determine whether the expression of transgenic TCR would lead to the predicted functional modification of the T cell repertoire (data not shown). The thymi of female A1(M) mice were found to have a strong bias toward the generation of CD4⁺CD8⁻ rather than CD8⁺CD4⁻ T cells, as expected from an increased positive selection of the MHC class II-restricted anti-H-Y TCR. This bias led to a CD4/CD8 ratio in the peripheral lymphoid organs that was in excess of 10:1 and also to the expression of the Vβ8.2 transgenic receptor on >90% of CD3⁺ cells. In contrast, male A1(M) mice had smaller thymi ($0.75 \pm 0.14 \times 10^8$ total thymocytes compared with $1.9 \pm 0.23 \times$

10^8 at 7 wk of age in females), a mature CD4/CD8 ratio that was close to 1:1, and a similar expression of Vβ8.2 to nontransgenic CBA/Ca mice, suggesting the clonal deletion of anti-H-Y-transgenic T cells and the escape of endogenous TCR rearrangements. These A1(M) mice were then crossed onto a RAG-1^{-/-} background to eliminate all B cells and T cells expressing other TCR molecules encoded by endogenous TCR rearrangements, so that any ability of H-Y-specific CD4⁺ T cells to reject male skin grafts could be unambiguously identified.

Positive selection in female and negative selection in male A1(M)×RAG-1^{-/-} mice

Immunofluorescent staining of A1(M)×RAG-1^{-/-} mice confirmed that the anti-H-Y TCR was functional, since positive selection and the generation CD3⁺CD4⁺CD8⁻ thymocytes was

Lymph-nodes

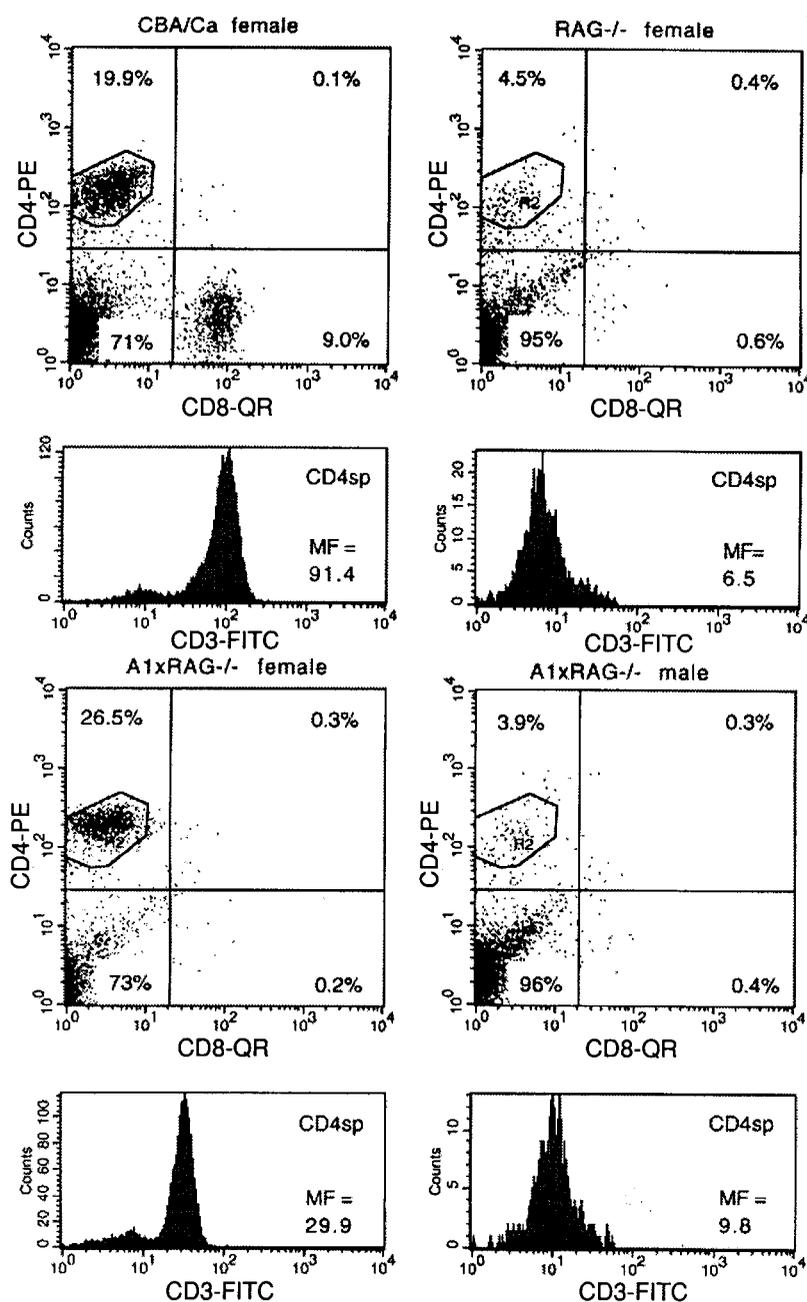


FIGURE 2. Immunofluorescent analysis of male and female A1(M)×RAG-1^{-/-} lymph nodes. Representative examples are shown of dot plot analyses of lymph nodes from normal CBA/Ca (top left), RAG-1^{-/-} (top right), A1(M)×RAG-1^{-/-} females (lower left), and A1(M)×RAG-1^{-/-} males (lower right) that were labeled with CD4-PE and CD8α-QR. Histograms of CD3-FITC staining gated on CD4 single-positive (SP) T cells are shown under each dot plot.

only observed in female thymi (Fig. 1); male thymi were much smaller, with very few CD4⁺CD8⁻ cells. When we looked in more detail at these few CD4⁺CD8⁻ cells, we found that they were present in similar numbers in both male A1(M)×RAG-1^{-/-} mice and RAG-1^{-/-} controls, and that none of them expressed CD3 but were mostly CD11c⁺ (data not shown), suggesting that they may be related to CD4⁺ immature dendritic cells (40) rather than to T cells that have somehow escaped deletion. The staining of lymph nodes (Fig. 2) confirmed that only CD4⁺ and not CD8⁺ T cells were present in female A1(M)×RAG-1^{-/-}, and that clonal deletion in the male reduced the number of CD4⁺ cells down to the level seen in RAG-1^{-/-} mice (again, these were CD3⁻CD11c⁺). The expression of the TCR in female A1(M)×RAG-1^{-/-}, as measured by CD3 (Fig. 2) or Vβ8.2 (data not shown) staining, was lower than that seen in a normal CBA/Ca mouse (~30% of the

median fluorescence level) but was similar to that of the A1(M) founders (data not shown), which may be a property of the CD2 expression system (G. Stockinger, unpublished observations).

Rejection of male skin by female A1(M)×RAG-1^{-/-} mice

In initial experiments in two laboratories, a total of eight female A1(M)×RAG-1^{-/-} mice were given single male skin grafts, four of which were rapidly rejected (within 16 days); two additional grafts were eventually rejected in a chronic fashion. Subsequently, a group of five female A1(M)×RAG-1^{-/-} mice were simultaneously grafted with male and female CBA/Ca skin in the same graft bed. All of the male grafts were rapidly rejected (within 14 days), while the female grafts remained perfectly healthy (Fig. 3). A second group of five mice were grafted in an identical fashion but were also treated with saturating amounts of a mAb that blocks

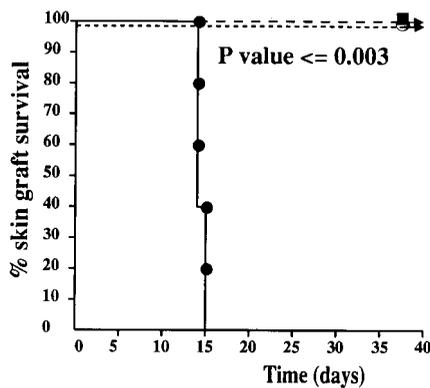


FIGURE 3. Female A1(M)×RAG-1^{-/-} mice show a CD4-dependent, specific rejection of male skin. Female A1(M)×RAG-1^{-/-} mice were grafted with male and female CBA/Ca skin in the same graft bed. Survival plots are shown for the male skin that rejected with a median survival time (MST) of 14 days (●; *n* = 5) compared with the accompanying female grafts that survived beyond day 30 (○; *n* = 5). The *p* value for statistical significance was <0.003 (log rank method). Also shown is the survival of male skin on similarly grafted A1(M)×RAG-1^{-/-} female mice that had been treated with 5 × 1 mg of nondepleting CD4 mAb (■; *n* = 5; MST of >30 days).

CD4 function *in vivo*. All these grafts were accepted, proving that the rejection was both CD4-dependent and male-specific. The experiment shown in Figure 3 has been repeated two additional times with similar results; it is not clear why the mice in the initial experiments rejected more slowly (although it might be relevant that these mice were still heterozygous for the A1(M) TCR at that time).

Mechanism of CD4-dependent graft rejection

The A1(M)×RAG-1^{-/-} mice should have no CD8⁺ T cells or Ab-producing B cells that might be able to act as effectors of graft rejection; the absence of these cells was checked by staining spleen cells from two female A1(M)×RAG-1^{-/-} mice that had been allowed to reject two sequential male grafts and had been grafted with a third male skin 7 days previously, such that if there was any hypothetical expansion of, for example, a novel CD8⁺ population during graft rejection, this expansion should become visible. However, it was confirmed that there was no CD3⁺CD8⁺ staining above background (Fig. 4), and that CD25 expression was limited to the CD3⁺CD4⁺ subset (data not shown). Similarly, there were no surface Ig⁺ B cells that might have been able to contribute an Ab response (although there is no convincing evidence that Ab responses are made to H-Y or to other minor transplantation Ags

FIGURE 4. Phenotypic and functional immunofluorescent analysis of rejecting A1(M)×RAG-1^{-/-} mice. Two female A1(M)×RAG-1^{-/-} mice that had rejected two sequential male skin grafts were given a third graft; their spleen cells were stained for a number of surface and intracytoplasmic Ags. Representative examples of four-color immunofluorescent analysis from one of the mice are shown. All samples were live-gated on forward and side scatters, and the dot plot in the *upper left panel* shows CD4-PE vs CD8-QR staining of the CD3-FITC-positive lymphocytes. The *upper right panel* shows that there were no B cells expressing surface Ig in the A1(M)×RAG-1^{-/-} (filled histogram) compared with an A1(M) control (broken-line histogram). The *middle left panel* shows the staining for CD44-QR of A1(M)×RAG-1^{-/-} lymphocytes that was used as the basis for gating the remaining anticytokine stains (rat IgG1 anti-IFN-γ, *middle right panel*; rat IgG1 anti-IL-2, *lower left panel*; rat IgG1 anti-IL-4, *lower right panel*), where the CD44⁺ cells are shown as filled histograms, the CD44⁻ cells are shown as open histograms, and the negative control histogram (based on the background staining of isotype-matched, rat IgG1 anti-IL-4-FITC in normal mice) is shown as a broken line.

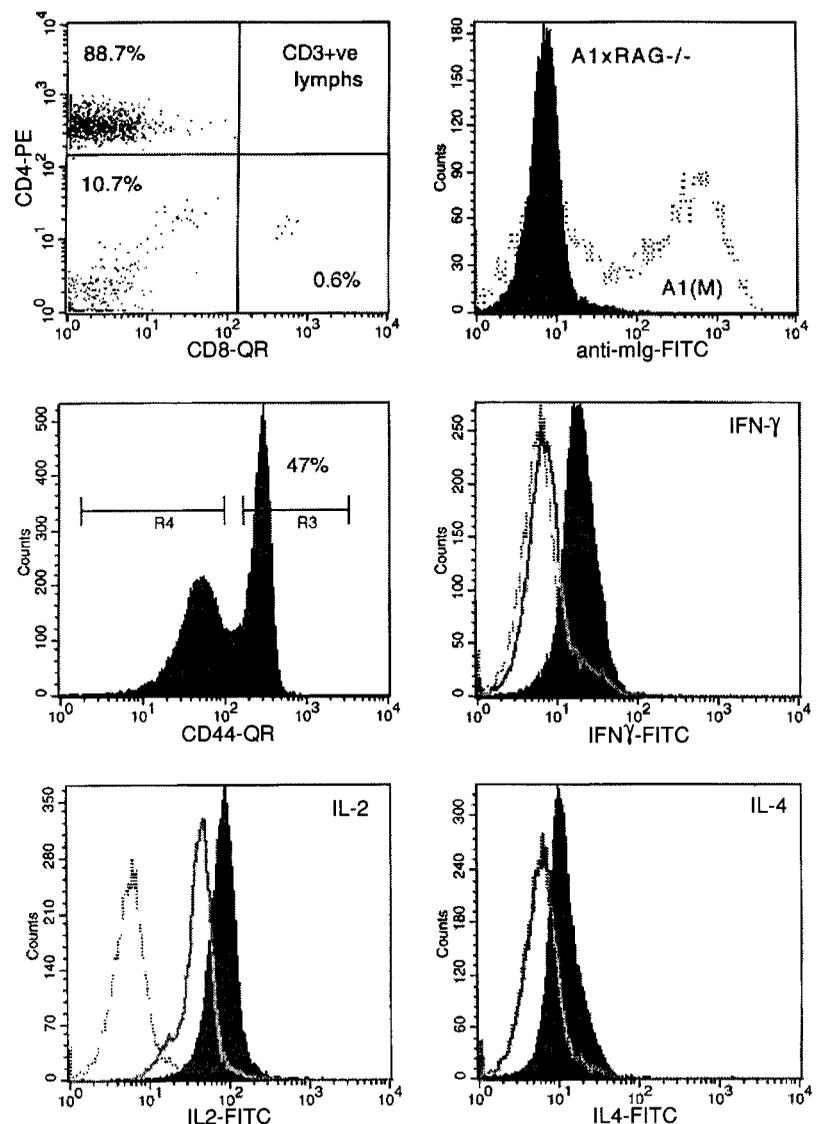


Table I. Specificity and cytokine production of Th1 and Th2 lines from A1(M)×RAG-1^{-/-} female mice

Line	No Stimulators	Dendritic Cells ^a		Assay
		Female	Male	
R2.2 (Th1)	99 ± 13	408 ± 102	34,201 ± 6,707	Proliferation ([³ H]thymidine: cpm)
	0	10	> 100	IFN-γ (ELISA: U/ml)
	0	0	< 1	IL-4 (ELISA: U/ml)
R2.4 (Th2)	100 ± 24	370 ± 178	23,958 ± 3,644	Proliferation ([³ H]thymidine: cpm)
	0	0	0	IFN-γ (ELISA: U/ml)
	0	0	12	IL-4 (ELISA: U/ml)

^a Male or female dendritic cells were generated by culturing bone marrow cells in RPMI 1640 plus 10% FCS containing recombinant human granulocyte-macrophage CSF (5 ng/ml) for 7 days; cells were treated with mitomycin C. A total of 5×10^4 of these cells were used to stimulate 2×10^4 R2.2 or R2.4 cells for 72 h at 37°C.

in normal mice (41)). There was clear evidence that the male graft was indeed being recognized by the transgenic anti-male TCR on CD4⁺ T cells, as the majority (~70%) of these could be categorized as memory or recently activated cells (expressing CD44) and produced both IFN-γ, and to a lesser extent, IL-4 (Fig. 4). All T cells (both CD44⁺ and CD44⁻) appeared to be expressing IL-2 by this method of intracytoplasmic staining.

Rejection of male skin grafts by Th1 and Th2 cells

The demonstration of staining for both IFN-γ and IL-4 in the spleen (Fig. 4) and draining lymph nodes (similar data not shown) of A1(M)×RAG-1^{-/-} mice rejecting a male skin graft raised the question of which of the CD4⁺ Th cell subsets (i.e., Th1 or Th2) were acting as effector cells. Skin graft rejection has generally been thought of as a Th1-mediated process, while Th2 responses have been considered as nonpathogenic or even protective (29, 30). To test which of these subsets was responsible for this rejection, we generated both Th1 and Th2 CD4⁺ T cell lines from a skin-grafted A1(M)×RAG-1^{-/-} mouse by repeated stimulation *in vitro* with male spleen cells in the presence of either IL-2 or IL-4 to promote Th1 or Th2 development, respectively. The Th1 and Th2 lines that were generated, which express the same transgenic A1 TCR (confirmed by Vβ8.2 staining, data not shown), were found to be specific for male, compared with female, syngeneic spleen cells in terms of proliferation and appropriate cytokine production (Table I); in addition, the lines were of a stable phenotype even if restimulated in the presence of the “opposite” cytokine (data not shown). Surprisingly, we found that both the Th1 and Th2 lines were able to cause a rapid and specific rejection of male skin after transfer into athymic, T cell-depleted recipient mice (Fig. 5), although the Th2 line rejected slightly slower with equivalent numbers of cells transferred ($p < 0.02$). It should be noted that male-specific skin graft rejection was also obtained in similar experiments using a CD4⁺ Th2 clone from A1(M) female mice (data not shown).

These results are reminiscent of recent data from two different autoimmune models; it had been thought that Th2 cells were protective, but instead they were found to transfer disease into T cell-depleted recipients (31, 32). In these examples the Th2-mediated disease was associated with granulocytic rather than the lymphocytic infiltrates, but we have not been able to observe any clear change in pathology between Th1- and Th2-mediated skin graft rejection, as granulocytes and macrophages seem to infiltrate in both cases. This observation is in agreement with the work of VanBuskirk et al. (33) who found that polyclonal Th2-like cells could cause a rejection of MHC-mismatched cardiac allografts with similar tempo and histology to Th1. There is the additional example of a Th2 clone that can cause rejection, in this case of a tumor cell graft (42), but this rejection was reportedly dependent upon CD8⁺ effector cells.

One limitation of the approach we have taken is that, while we observe that male skin grafts can be rejected by either Th1 or Th2 CD4⁺ T cells, this is in a situation in which the frequency of Ag-specific T cells is essentially 100%. In a normal mouse, the frequency of T cells that are specific for H-Y is significantly lower, and it may be important to determine the relative efficiency of rejection of different T cell subpopulations. This determination could be done by transferring various numbers of A1(M)×RAG-1^{-/-} T cells or clones into male skin-grafted normal female CBA/Ca (as these are “nonresponders” to H-Y (12)), and preliminary experiments suggest that 10^7 Th1 cells (R2.2) but not an equal number of Th2 cells (R2.4) can reject male skin in this situation; a similar situation is seen after the transfer of autoimmune Th2 cells into intact mice (31, 32). However, until we have clones from mice that are fully backcrossed (the mice used in our study are only four generations from 129 × A/J and are therefore allogeneic to CBA/Ca), we cannot reliably determine the minimum number that can cause rejection in such immunocompetent chimeras.

While it is therefore clear that the transgenic TCR⁺CD4⁺ T cells in A1(M)×RAG-1^{-/-} females are sufficient to reject male skin, it remains to be determined whether this rejection is due to CD4⁺ T cell-mediated cytotoxicity or to help for macrophages or another Ag-nonspecific effector cell. Recent data suggesting that

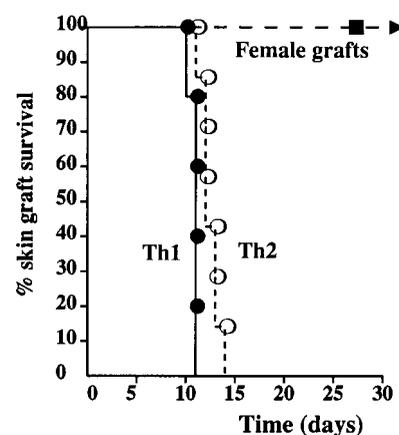


FIGURE 5. Rejection of male skin grafts by Th1 and Th2 CD4⁺ T cell lines. A total of 10^7 viable R2.2 (Th1) or R2.4 (Th2) cells were injected via the tail vein into female ATX T cell-depleted CBA/Ca mice (21) that were grafted the following day with both male and female syngeneic tail skin in the same graft bed. All the female grafts remained in perfect condition indefinitely (■), but the male skin was acutely rejected in those mice receiving either Th1 cells (●; MST = 11 days) or Th2 cells (○; MST = 12 days). Control ATX CBA/Ca mice receiving no cells did not reject male syngeneic skin, regardless of whether they had been T cell-depleted or not (data not shown).

neither Fas/FasL nor perforin (43) are required for the CD4-mediated rejection of MHC class I disparate skin would tend to favor the latter hypothesis. It also remains to be determined whether Th1 and Th2 CD4⁺ T cell subsets cause skin graft rejection by a common mechanism or whether they represent alternative routes to achieve the same end. Regardless of the mechanism, this result would seem to make it unlikely that deviating an immune response from Th1 to Th2 would be protective or therapeutic in the context of clinical transplantation.

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