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CD4 T Cell-Mediated Rejection of Cardiac Allografts in B Cell-Deficient Mice

Taiji Nozaki,†‡ Joshua M. Rosenblum,†‡ Daisuke Ishii,*† Kazunari Tanabe,‡ and Robert L. Fairchild²∗†§

CD4 T cell-dependent mechanisms promoting allograft rejection include expression of inflammatory functions within the graft and the provision of help for donor-reactive CD8 T cell and Ab responses. These studies tested CD4 T cell-mediated rejection of MHC-mismatched cardiac allografts in the absence of both CD8 T and B lymphocytes. Whereas wild-type C57BL/6 recipients depleted of CD8 T cells rejected A/J cardiac grafts within 10 days, allografts were not rejected in B cell-deficient B6.μMT−/− recipients depleted of CD8 T cells. Isolated wild-type C57BL/6 and B6.μMT−/− CD4 T cells had nearly equivalent in vivo alloreactive proliferative responses. CD4 T cell numbers in B6.μMT−/− spleens were 10% of that in wild-type mice but were only slightly decreased in peripheral lymph nodes. CD8 T cell depletion did not abrogate B6.μMT−/− mice rejection of A/J skin allografts and this rejection rendered these recipients able to reject A/J cardiac allografts. Redirection of the alloimmune response to the lymph nodes by splenectomy conferred the ability of B6.μMT−/− CD4 T cells to reject cardiac allografts. These results indicate that the low number of splenic CD4 T cells in B6.μMT−/− mice underlies the inability to reject cardiac allografts and this inability is overcome by diverting the CD4 T cell response to the peripheral lymph nodes. The Journal of Immunology, 2008, 181: 5257–5263.

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cute cellular rejection of allografts is characterized by the infiltration of donor Ag-primed T cells into the graft followed by their activation to express immune functions that mediate destruction of the graft tissue (1). During rejection of solid organ allografts CD8 T cells are the predominant infiltrating T cell population accompanied by a smaller number of CD4 T cells. In rodent models, CD4 T cells mediate rejection of skin and heart allografts when graft recipients are depleted of CD8 T cells by treatment with specific Ab or in recipients with a genetically targeted deficiency in CD8 T cells (2, 3). CD4 T cells not only promote graft tissue damage directly through production of cytokines that mediate delayed-type hypersensitivity-like inflammation but also through the provision of helper signals to B cells producing donor-specific Ab responses and donor Ag-specific CD8 T cell responses.

Graft- and recipient-derived dendritic cells are important APCs for priming donor Ag-reactive CD4 and CD8 T cells through the direct and indirect alloantigen presentation pathways, respectively (4, 5). Recipient B cells are another APC population capable of inducing CD4 T cell activation through the indirect alloantigen presentation pathway, and this activity is indicated in allograft recipients by the production of donor-reactive Ab (6–9). B cells and/or Ab are not required for allograft rejection in recipients with both CD4 and CD8 T cell compartments intact. Two recent studies have indicated the suboptimal activation and defective immune responses of CD4 T cells in mice in which a deficiency in the expression of class II MHC deficiency is restricted to the B cell compartment. This finding results in the inability of such mice to reject MHC-mismatched heart allografts (10, 11). These studies have been interpreted as indicating that B cell presentation of donor-Ag to reactive CD4 T cells through the indirect pathway, possibly with the participation of donor-reactive Ab production, is required to elicit rejection of heart allografts. However, the defect generated by the class II MHC deficiency in B cells must also affect the priming or function of the donor-reactive CD8 T cell response. Several studies have reported that B cell-deficient B6.μMT−/− recipients reject skin and heart allografts, although this rejection appears to be dependent on the donor strain tested because B10.A hearts are not rejected by these mice (12–14). The contribution of the individual donor-reactive CD4 and CD8 T cell compartments to this rejection has not been delineated and the ability of CD4 T cells in B6.μMT−/− recipients to reject skin and heart allografts independently of CD8 T cells has not been tested. The results from recipients with MHC class II-deficient B cells predict that μMT−/− mice should be unable to reject MHC-mismatched cardiac allografts in the absence of CD8 T cells. The current study was performed to directly test the CD4 T cell-mediated rejection of complete MHC-mismatched cardiac allografts in B6.μMT−/− recipients.

Materials and Methods

Animals

C57BL/6 (H-2b) and A/J (H-2a) mice were obtained through C. Reeder (National Cancer Institute, Frederick, MD). C57BL/6-Igh-6tm1Cgn/B6.μMT−/− (B6.μMT−/−) and (C57BL/6J × A/J)F1 (B6AF1/J, H-2b/a) mice were purchased from The Jackson Laboratory. In all experiments, 6- to 12-wk-old males were used. All mice were housed and treated in accordance with Animal Care Guidelines established by the National Institutes of Health.
and the Cleveland Clinic. The use of mice in these experiments was approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic.

Ab analysis

The following Abs were used throughout this study: rat anti-mouse CD4 (GK-1.5) mAb and rat anti-mouse CD8α (53-6.7) mAb for immunohistochemistry and anti-mouse CD16/CD32 (2.4G2) mAb, PE-conjugated rat anti-mouse CD4 (GK-1.5) mAb, FITC-conjugated rat anti-mouse CD25 (7D4) mAb, and allophycocyanin-conjugated anti-mouse CD8α (53-6.7) mAb for flow cytometry (BD Pharmingen); rat anti-mouse macrophage (F4/80) mAb (Serotec); and, rat anti-mouse CD8α mAb TB105 and YTS169 and rat anti-mouse CD4 mAb GK1.2.5 and YTS191 (BioExpress). Rat anti-mouse CD25 mAb (PC61) and anti-mouse Gr-1 mAb (RB6-8C5) were purified from spent culture supernatant by protein G chromatography.

Surgical procedures

Heterotopic heart transplantation was performed using microsurgical methods detailed by Corry et al. (15). Briefly, the donor aorta and pulmonary artery were anastomosed to the recipient abdominal aorta and inferior vena cava in the peritoneal cavity. The strength of the heart graft was monitored by abdominal palpation each day until rejection, which was considered as total cessation of contraction and was confirmed visually by laparotomy.

Skin grafting was performed using a modified version of the Billingham and Medawar protocol (16). Briefly, full-thickness trunk skin was prepared from donor ventral skin and cut into 12-mm diameter circles using a punch. Graft beds were prepared by carefully excising 14-mm diameter circles of skin from the lateral dorsal thoracic wall of recipients and placed grafts were covered with Vaseline gauze and an adhesive bandage. On day 7 posttransplantation, the bandages and gauze were removed. Everyday thereafter, the grafts were visually monitored for rejection, when over 60% of the graft tissue was destroyed.

In vivo treatment with mAb

Wild-type C57BL/6 and B6,μMT-/- graft recipients were treated with a mix of rat anti-mouse CD8α mAb TB105 and YTS169 or with rat anti-mouse CD4 mAb GK1.5 and YTS191, 200 μg each/day i.p., on days −3, −2, −1, +4, +8, and every 4 days thereafter until graft rejection. Additional sentinel mice in each group were treated with Ab and the level of CD8 T cell depletion in the spleen and lymph nodes on days +7 and +14 was >99% when compared with control rat IgG-treated mice. Cardiac allograft recipients were treated with anti-CD25 mAb (PC61) by i.p. injection of 500 μg on day −1 and then 250 μg every other day on days 1–9 posttransplant as previously reported (17).

Flow cytometry analysis

Axillary and inguinal lymph nodes and spleens were obtained from C57BL/6 and B6,μMT-/- mice and 1 × 10⁶ cell aliquots were washed twice with staining buffer (Dulbecco’s PBS with 2% FCS) and anti-mouse CD16/CD32 (2.4G2) mAb was added to block FcγR binding. After 10 min on ice, the cells were stained with PE rat anti-mouse CD4 (GK-1.5) mAb, FITC rat anti-mouse CD8α (53-6.7) mAb, and allophycocyanin anti-mouse CD8α (53-6.7) mAb for 30 min on ice. The cells were washed three times, resuspended in staining buffer, and analyzed on a FACSCalibur and FlowJo software (Tree Star).

Immunohistochemistry

Cross-sections of the center of cardiac grafts obtained at indicated time points posttransplant were frozen at −80°C in OCT compound (Sakura Finetek). Sections were cut at 7–8 μm, placed onto slides, and stained as previously detailed (17, 18).

ELISPOT assays

Allotigenic-specific T cell priming in heart graft recipients was assessed by enumerating donor-specific T cells producing IFN-γ using ELISPOT assays as previously described (17, 18). Spleen cell suspensions were prepared from graft recipients or from naive mice and the CD4⁺ T cells were separated by negative selection with Dynabeads (Invitrogen) and used as responder cells. Mitomycin C-treated donor and recipient spleen cell suspensions were added as stimulator cells in each assay in serum free HL-1 medium (Bio-Whittaker) with 1 mM l-glutamine for 24 h at 37°C in 5% CO₂. After development, the resulting spots were counted on an ImmunoSpot Series I analyzer (Cellular Technology).

FIGURE 1. Depletion of CD8 T cells abrogates heart allograft rejection in B6,μMT-/- recipients. a, Groups of wild-type C57BL/6 and B6,μMT-/- mice were treated with control Ig or with anti-CD8 mAb (200 μg/day on days −3, −2, −1, +4, +8, and every 4 days until rejection) and were transplanted with A/J heart grafts. b, Groups of the mice were treated with anti-CD4 mAb (200 μg/day on days −3, −2, −1, +4, +8, and every 4 days until rejection) and were transplanted with A/J heart grafts. Grafts were monitored daily by palpation and rejection was confirmed visually by laparotomy.

In vivo proliferation assays

Naive CD4⁺ T cells from spleens and lymph nodes of wild-type C57BL/6 and μMT-/- mice were purified from cell suspensions using a CD4⁺ T cell isolation kit (Invitrogen). For in vivo proliferation assays, CD4 T cells were labeled with 5 μM CFSE (Molecular Probes) and 2 × 10⁶ cells were transferred i.v. to wild-type C57BL/6 or (B6 × A/J)F1 recipients. After 48 h, recipient spleen cell suspensions were prepared and stained with PE anti-CD4 mAb and analyzed by flow cytometry. The CFSE profiles were determined for the CD4 gated cells.

Results

Allograft rejection in μMT-/- recipients with and without CD8 T cells

To test the ability of CD4 T cells to reject MHC-mismatched cardiac allografts in the absence of both CD8 T cells and B cells, rejection of A/J (H-2a) cardiac allografts was compared in wild-type C57BL/6 (H-2b) and B6,μMT-/- recipients treated with control IgG or CD8 T cell depleting mAb. Both wild-type and B6,μMT-/- recipients treated with control IgG rejected the allografts within 8–10 days (Fig. 1a). In wild-type C57BL/6 recipients, depletion of CD8 T cells extended cardiac allograft survival 4–7 days, whereas rejection was completely abrogated in CD8 T cell-depleted B6,μMT-/- recipients. CD4 T cells were required for rejection of the cardiac allografts in both wild-type and B6,μMT-/- recipients because deletion of CD4 T cells resulted in the survival of 75% of the grafts past day 50 posttransplant (Fig. 1b).
posttransplant (i.e., the time that CD8 T cell-depleted wild-type recipients rejected the allografts) had marked decreases in cellular infiltration. When random sections were counted, infiltration of CD4 T cells, neutrophils and macrophages into allografts from CD8 T cell-depleted B6.\textsuperscript{MT}/H11002/\textsuperscript{MT} recipients at day 14 posttransplant was \textasciitilde10% of the infiltrating cell populations observed in rejecting allografts in CD8 T cell-depleted wild-type recipients (Fig. 2).

FIGURE 2. Histological analysis of cardiac allografts from CD8-depleted wild-type C57BL/6 recipients and \(\mu\text{MT}^{-/-}\) recipients at day 10 posttransplant. Wild-type C57BL/6 (a and c) and B6.\(\mu\text{MT}^{-/-}\) (b and d) mice were treated with anti-CD8 mAb and were transplanted with A/J heart grafts. On day 10 posttransplant the grafts were retrieved, and prepared sections were stained with H&E (a and b) or by immunohistochemistry with anti-CD4 mAb (c and d) as well as with RB6-8C5 to detect neutrophils and F4/80 to detect macrophages (data not shown). Magnification is at \(\times 200\). e, Positively stained cells were counted in four random fields from three different grafts retrieved from CD8 T cell-depleted wild-type (\(\square\)) or B6.\(\mu\text{MT}^{-/-}\) (\(\bullet\)) recipients. Data shown are expressed as mean number per field \pm SE. *, \(p < 0.05\).

Alloreactive T cell priming in wild-type and \(\mu\text{MT}^{-/-}\) recipients of cardiac allografts

One potential mechanism that could account for the inability of B6.\(\mu\text{MT}^{-/-}\) recipients to reject A/J allografts in the absence of CD8 T cells was a defect in alloreactive CD4 T cell priming. This possibility was first investigated by directly comparing the priming of alloreactive CD4 and CD8 T cells in the spleens of nontreated wild-type and B6.\(\mu\text{MT}^{-/-}\) cardiac allograft recipients. On day 8 posttransplant recipient spleen cell suspensions were prepared and tested by ELISPOT assay to enumerate donor-specific CD4 T cells producing IFN-\(\gamma\) in each recipient group. When unseparated spleen cells were tested equivalent numbers of donor-specific CD4 T cells producing IFN-\(\gamma\) were observed in wild-type and B cell-deficient recipients (Fig. 3a). When spleen cells from CD8\textsuperscript{+} T cell-depleted recipients were tested, however, an 85% reduction in the number of donor-specific CD4 T cells producing IFN-\(\gamma\) in the spleens of B6.\(\mu\text{MT}^{-/-}\) vs wild-type recipients was observed (Fig. 3b).

FIGURE 3. Low level priming of donor-reactive CD4 T cells in B6.\(\mu\text{MT}^{-/-}\) cardiac allograft recipients. Groups of wild-type C57BL/6 and B6.\(\mu\text{MT}^{-/-}\) mice were treated with control Ig (a) or with anti-CD8 mAb (b) and were transplanted with A/J heart grafts. ELISPOT assays were performed on days 8 (a) and 10 (b) posttransplant to enumerate donor-reactive IFN-\(\gamma\)-producing T cells in the spleens of the cardiac allograft recipients. Similar results were obtained in two individual experiments performed. *, \(p < 0.01\).
Previous studies have indicated that CD4+CD25+ T regulatory cells limit reactive CD4 T cell responses to single class II MHC disparate cardiac allografts and inhibit acute rejection of the allografts (17). Treatment of the recipients with anti-CD25 mAb removes this regulation leading to increased CD4 T cell priming and acute rejection of the class II MHC disparate allografts. The potential CD4+CD25+ T regulatory cell mediated restriction of donor-reactive CD4 T cell priming in CD8 T cell-depleted B6.MT−/− recipients of complete MHC-mismatched heart allografts was tested. Groups of wild-type and B6.MT−/− mice were first depleted of CD8 T cells and treated with or without anti-CD25 mAb and then received A/J cardiac transplants. On day 7 posttransplant, donor-specific CD4 T cells producing IFN-γ in the CD8 T cell-depleted spleens of the recipient groups were enumerated (Fig. 4). In wild-type recipients, treatment with anti-CD25 mAb resulted in a 2.5-fold increase in the number of donor-reactive CD4 T cells producing IFN-γ, whereas the treatment had little to no effect on donor-reactive CD4 T cell priming in the CD8 T cell-depleted B6.MT−/− recipients. Decreased CD4 T cell numbers in the spleens of B6.MT−/− mice

The decreased alloreactive CD4 T cell response in the spleens of B6.MT−/− recipients of cardiac allografts could be caused by an inherent defect in the ability of CD4 T cells from these mice either...
to respond to allogeneic stimulation or to a decrease in the number of CD4 T cells. These possibilities were first distinguished by directly counting the total number of mononuclear cells and, more specifically, CD4 T cells in the spleens and peripheral lymph nodes of wild-type vs B6.μMT−/− mice (Fig. 5). When compared with numbers in wild-type mice, the total number of mononuclear cells in peripheral lymph nodes of B6.μMT−/− mice was decreased ~40% and the number of CD4 T cells in the peripheral lymph nodes was decreased ~30%. In contrast, the number of mononuclear cells and CD4 T cells in the spleens of B6.μMT−/− mice were less than 10% of the number observed in the spleens of wild-type mice.

The alloimmune reactivity of CD4 T cells from B6.μMT−/− vs wild-type mice was evaluated by testing an equivalent number of the CD4 T cells using an in vivo approach. Nearly equivalent proliferative responses were observed when CFSE-labeled CD4 T cells from the spleens of naïve B6.μMT−/− and C57BL/6 wild-type mice were injected into (B6 × A/J)F2 mice and the CFSE dilution of the labeled CD4 T cells was assessed in the spleens 48 h later (Fig. 6). In each of the three experiments, CD4 T cells from the B6.μMT−/− mice did exhibit a slightly lower rate of division when compared with CD4 T cells from the C57BL/6 wild-type mice.

CD4 T cell-mediated rejection of allogeneic skin grafts on B6.μMT−/− recipients

Because donor-reactive T cell priming to skin allografts occurs primarily in the peripheral lymph nodes draining the graft and the decrease in CD4 T cells in the lymph nodes of B6.μMT−/− mice was not as marked as the spleen, CD4 T cell-mediated rejection of MHC-mismatched skin grafts by B6.μMT−/− recipients was tested. Groups of wild-type C57BL/6 and B6.μMT−/− mice were treated with anti-CD8 mAb to deplete CD8 T cells and then received full-thickness trunk skin allografts from A/J donors. Wild-type recipients rejected the skin allografts between days 10 and 12 and the B6.μMT−/− recipients rejected the allografts between days 12 and 14 (Fig. 7a).

Based on these results the ability of skin allograft-mediated activation of donor-reactive CD4 T cells in the peripheral lymph nodes to provoke CD4 T cell-mediated rejection of cardiac allografts was tested. Groups of wild-type C57BL/6 and B6.μMT−/− mice were treated with anti-CD8 mAb to deplete CD8 T cells and then received A/J heart allografts in B6.μMT−/− recipients that had or had not been splenectomized. Groups of wild-type C57BL/6 and B6.μMT−/− mice were splenectomized or not as indicated and received A/J heart allografts. On day 8, posttransplant, CD4 T cells were purified from the spleens of the nonsplenectomized recipients as well as from the skin draining lymph nodes of nonsplenectomized and splenectomized recipients and ELISPOT assays were performed to enumerate donor-reactive IFN-γ-producing CD4 T cells.
producing IFN-γ. When purified CD4 T cells from the spleens of wild-type and B6,μMT−/− cardiac allograft recipients were tested, an equivalent number of donor-reactive cells producing IFN-γ were observed and these numbers were ~85% lower in the skin draining lymph nodes (Fig. 8b). Splenectomy resulted in a 2- to 3-fold increase in the numbers of donor-reactive CD4 T cells producing IFN-γ in the lymph nodes of both the wild-type and B6,μMT−/− cardiac allograft recipients.

Discussion

Allograft-infiltrating CD4 T cells produce IFN-γ and other proinflammatory cytokines that mediate tissue injury through delayed-type hypersensitivity-like immune responses (19, 20). During activation in secondary lymphoid tissues, CD4 T cells also promote rejection through provision of helper signals to donor Ag-presenting B cells to produce donor-reactive Ab that may synergize with graft-infiltrating T cells or directly mediate allograft rejection. The ability of CD4 T cells to reject solid organ allografts in the absence of both CD8 T cells and B cells remains unclear. The current study was directed to investigate the activation of CD4 T cells to MHC-mismatched heart allografts in the absence of B cells and CD8 T cells and to test the ability of the CD4 T cells to mediate rejection of the allografts. The model used in these studies was the B cell-deficient B6,μMT−/− mouse treated with specific Abs to deplete CD8 T cells. Previous studies had indicated the ability of B6,μMT−/− mice to reject skin as well as cardiac allografts indicating that neither donor-specific Ab nor B cell presentation of donor Ags were required to initiate a successful alloimmune response that ultimately resulted in rejection of the graft (12, 13). These studies did not distinguish, however, the contribution of the CD4 vs CD8 T cell compartment to cardiac allograft rejection in these recipients.

Initial experiments indicated the ability of B6,μMT−/− recipients to reject MHC-mismatched cardiac allografts and the inability to reject the allografts when these recipients were depleted of CD8 T cells. In contrast, B6,μMT−/− recipients rejected skin allografts whether CD8 T cells were participants or not. These initial observations suggested that CD4 T cells could not mediate rejection of cardiac allografts in the absence of both CD8 T and B cells. The underlying cause for this failure was a 90% decrease in the number of CD4 T cells in the spleens of B6,μMT−/− mice. This decrease in the number of CD4 T cells in the spleens of B6,μMT−/− mice had been previously noted during studies of CD4 and CD8 T cell responses to influenza virus in the B6,μMT−/− mice (21). These decreases are predicated from elegant studies indicating that B cells provide several different signals that are critical for T cell accumulation and organization into specific areas of the spleen (22). Thus, the location of CD4 T cells might not be properly organized in the spleen of B6,μMT−/− recipients to efficiently interact with allograft-derived APCs. However, the generation of optimal CD8 T cell responses to cardiac allografts is dependent on CD4 T cells (2, 3). The CD4 T cells in the spleens of B6,μMT−/− recipients clearly participate in the alloimmune response by providing help for the donor-reactive CD8 T cells that mediate rejection of the cardiac allografts at virtually the same time as wild-type recipients as depletion of CD4 T cells abrogated cardiac allograft rejection in B6,μMT−/− recipients. This indicates sufficient donor-reactive CD4 T cell localization in the spleen of the B6,μMT−/− recipients as well as a sufficient number to provide the help generating the CD8 T cell responses. The low number of CD4 T cells in the spleens of B6,μMT−/− recipients resulted in a marked decrease in primed CD4 T cell effectors induced in response to the heart allografts as well as infiltration into the graft. This result is consistent with the proposed need for threshold numbers of donor-reactive precursor T cells to achieve rejection of solid organ allografts (23, 24). However, the alloreactive potential of these CD4 T cells was equivalent to that of CD4 T cells from the spleens of wild-type mice when alloreactive proliferative responses were compared. Furthermore, direct comparison of purified CD4 T cells from the spleens of wild-type and B6,μMT−/− cardiac allograft recipients demonstrated an equivalent number of primed donor-reactive CD4 T cells producing IFN-γ again implying numerical rather than functional defects in the CD4 T cells in the B6,μMT−/− mice.

Two recent studies have demonstrated the poor activation of Ag-reactive CD4 T cells in mice having class II MHC-deficient B cells (10, 11). Furthermore, there was a marked extension in survival of MHC-mismatched cardiac allografts in recipients with B cells unable to express class II MHC and this extension was accompanied by poor alloreactive CD4 T cell responses when compared with responses in wild-type recipients. These results suggested a requirement for B cell presentation of alloantigen through the indirect pathway for optimal activation of CD4 T cells and to achieve rejection of the allografts. However, donor-reactive CD8 T cell responses that are a major component of the response to cardiac allografts were also obviously inhibited in these mice raising the alternative possibility that interaction of Ag-reactive CD4 T cells with class II MHC-deficient B cells results in the suppression of the donor-reactive CD4 and CD8 T cell repertoires. Such an unproductive interaction between CD4 B and T cells might be initiated by donor Ag-specific B cell stimulation following surface Ig receptor cross-linking with the Ag.

The induction of both CD4 and CD8 T cell responses in B6,μMT−/− mice has been characterized in many different viral and parasitic responses. B6,μMT−/− mice have been shown to have normal CD4 T cell responses to intranasal immunization with influenza virus, to i.p. injected allogeneic cells, and to s.c. injected neuropeptides (13, 21, 25). However, there is a clear defect in the development of CD4 T cell responses to viruses injected into the CNS and Ags i.v. injected (26–28). These results suggest that T cell responses to Ags delivered into peripheral tissues such as the skin and peritoneal cavity are relatively normal in B6,μMT−/− mice, whereas defective responses are observed to Ags introduced into the CNS and the vasculature. Dendritic cells in B6,μMT−/− mice have been reported to produce greater amounts of IL-12 than those from wild-type mice and this effect may skew Ag-specific T cell function to a type 1 cytokine-producing phenotype (29). An increase in IL-12 may compensate, at least partially, for the decreased number of CD4 T cells in the spleens of B6,μMT−/− mice during the development of CD4 and CD8 T cell responses. However, there was no noticeable increase in the number of donor-reactive T cells producing IFN-γ in the spleens of B6,μMT−/− vs wild-type cardiac recipients arguing against a role for increased IL-12 from the B6,μMT−/− recipients in the priming of the donor-reactive CD8 T cell response. Furthermore, wild-type and B6,μMT−/− recipients rejected skin allografts at similar times. Studies by Epstein and colleagues (13) have also indicated similar CD4 and CD8 T cell responses to a variety of Ags injected s.c. or i.p. into B6,μMT−/− vs wild-type mice. In addition, CD4 T cell responses to Pneumocystis infections in CD8 T cell depleted B6,μMT−/− mice are strongly skewed to a type 2 cytokine response that mediates overt pulmonary tissue pathology (30).

The initial activation of donor reactive T cells to heart allografts occurs in the recipient spleen, whereas in response to skin allografts this activation occurs in the peripheral lymph nodes draining the graft (31, 32). In contrast to the spleen, the decrease in CD4 T cell numbers in the peripheral lymph nodes of B6,μMT−/− mice was much less marked. Consistent with this decrease was the CD4
T cell-mediated rejection of skin allografts by B6.µMT−/− recipients that was virtually identical with the rejection observed in CD8 T cell-depleted wild-type recipients. Furthermore, rejection of skin allografts rendered the B6.µMT−/− recipients competent to reject subsequent heart allografts. These results suggest that expansion and mobilization of CD4 T cells by first encountering donor alloantigens in the peripheral lymph nodes allows CD4 T cell trafficking to the cardiac allograft. In support of this suggestion, splenectomy of CD8 T cell-depleted B6.µMT−/− recipients of cardiac allografts directed the primary donor-specific CD4 T cell activation to the lymph nodes where an increased number of donor-reactive CD4 effector T cells and rejection of the grafts were observed. Several recent studies have demonstrated a critical role of NK cells in the establishment of tolerance to allografts through killing donor APCs (33, 34). Thus, it is certainly possible that removal of splenic NK cells may also increase the longevity or killing donor APCs (33, 34). Thus, it is certainly possible that removal of splenic NK cells may also increase the longevity or killing donor APCs (33, 34). Thus, it is certainly possible that removal of splenic NK cells may also increase the longevity or killing donor APCs (33, 34). Thus, it is certainly possible that removal of splenic NK cells may also increase the longevity or killing donor APCs (33, 34). Thus, it is certainly possible that removal of splenic NK cells may also increase the longevity or killing donor APCs (33, 34).

The results of the current study clearly demonstrate that CD4 T cell-mediated rejection of MHC-mismatched cardiac allografts requires neither CD8 T cells nor B cells to reject cardiac allografts. When the recipients are depleted of CD8 T cells and the allogeneic response is directed to initiate in the spleen, the low number of CD4 T cells in the spleen is insufficient to expand the number needed to reject the graft. Removal of the spleen so that the initiation of the response occurs in the lymph nodes where the number of CD4 T cells is only slightly decreased when compared with wild-type C57BL/6 mice results in CD4 T cell-mediated rejection of the cardiac allografts. The implication of these studies is that indirect donor Ag presentation by B cells as well as Ab are under strict regulation by CD4+CD25+ T cells. J. Immunol. 174: 3741–3748.


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