Both Rejection and Tolerance of Allografts Can Occur in the Absence of Secondary Lymphoid Tissues

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Both Rejection and Tolerance of Allografts Can Occur in the Absence of Secondary Lymphoid Tissues

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In this study, we showed that aly/aly mice, which are devoid of lymph nodes and Peyer’s patches, acutely rejected fully allogeneic skin and heart grafts. They mounted potent inflammatory direct alloresponses but failed to develop indirect alloreactivity after transplantation. Remarkably, skin allografts also were rejected acutely by splenectomized aly/aly (aly/aly-spl⁻) mice devoid of all secondary lymphoid organs. In these recipients, the rejection was mediated by allospecific CD8⁺ T cells presumably primed in the bone marrow. In contrast, cardiac transplants were not rejected by aly/aly-spl⁻ mice. Actually, aly/aly-spl⁻ mice that spontaneously accepted a heart allotransplant and displayed donor-specific tolerance also accepted skin grafts from the same, but not a third-party, donor via a mechanism involving CD4⁺ regulatory T cells producing IL-10 cytokine. Therefore, direct priming of allosecretory T cells, as well as rejection and regulatory tolerance of allogeneic transplants, can occur in recipient mice lacking secondary lymphoid organs. The Journal of Immunology, 2015, 194: 000–000.

Following allotransplantation, the immune response is initiated by T lymphocytes activated in either a direct or an indirect fashion (1). The direct alloreponse relies on the recognition by recipient T cells of intact donor MHC molecules displayed on donor APCs. This response is polyclonal, because it involves up to 10% of the entire T cell repertoire, owing to the high frequency of MHC determinants presented to T cells and the presentation of a multitude of peptides by allogeneic MHC molecules (2). In contrast, host T cells activated via indirect allorecognition interact with processed donor-derived peptides presented by self-MHC molecules on recipient APCs (3). The indirect alloreponse is oligoclonal in that it is mediated by a few T cell clones recognizing dominant determinants on alloantigens (4, 5). Although either of these pathways can lead to acute rejection of skin allografts (6), acute rejection of vascularized solid organ transplants is essentially mediated through the direct pathway. Alternatively, indirect alloreactivity is considered the driving force behind chronic allograft rejection (7–10), which is characterized by graft tissue fibrosis and blood vessel obstruction (11, 12).

Cellular trafficking is an essential element of the process associated with alloimmunity and transplant rejection. Following skin transplantation, donor dendritic cells emigrate via lymphatics to the recipient draining lymph nodes (13) where they activate naive recipient T cells, thereby initiating the direct alloreponse (14–17). Alternatively, in the case of primarily vascularized organs, such as hearts and kidneys, it is likely that donor APCs leaving the graft through the blood spread rapidly to various lymphoid organs where they can activate T cells. In addition, some studies suggest that vascularized allografts can be rapidly infiltrated by some host pre-existing memory T cells (TMEMs) (18). Indeed, unlike naive T cells whose homing is confined to lymphoid organs, TMEMs traffic regularly through peripheral tissues (19, 20). These TMEMs may be activated via direct recognition of MHC molecules on donor dendritic cells remaining in the graft and presumably endothelial cells expressing MHC class II and costimulatory molecules as a result of inflammation. This process could account for the direct activation of some alloreactive T cells following organ transplantation (21, 22). In contrast, it is still unknown where and how donor Ags are acquired, processed, and presented by recipient APCs to T cells for induction of the indirect alloreponse.

In this study, we investigated the role of secondary lymphoid organs in the T cell–mediated alloimmune responses, rejection, and tolerance of mice transplanted with fully allogeneic conventional skin grafts or primarily vascularized skin or cardiac transplants. We show that aly/aly mice devoid of lymph nodes and Peyer’s patches develop direct, but not indirect, alloresponses and acutely reject both skin and cardiac allografts. In contrast, splenectomized aly/aly (aly/aly-spl⁻) mice reject skin, but not cardiac, allografts. Remarkably, aly/aly-spl⁻ mice having spontaneously accepted heart transplants developed donor-specific tolerance mediated by CD4⁺ T cells. Therefore, both transplant rejection and tolerance can be induced in the absence of secondary lymphoid organs.

Materials and Methods

Mice and transplantsations

Six- to eight-week-old aly/aly mice used in this study were autosomal-recessive mutants of C57BL/6 (B6) mice displaying a point mutation in the gene encoding the NF-κB–inducing kinase (23). These mice lack lymph nodes and Peyer’s patches. In turn, heterozygous aly/+ mice displayed a normal immune system and secondary lymphoid organs. In some experiments, we used aly/aly-spl⁻ mice that are considered devoid of all secondary lymphoid organs. B6 (H-2b) mice, BALB/c (H-2d) mice, C3H (H-2k) mice, and B6 MHC class II–knockout mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were bred and maintained at Massachusetts General Hospital animal facilities under specific patho-

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Abbreviations used in this article: aly/aly-spl⁻, splenectomized aly/aly; B6, C57BL/6; CAV, cardiac allograft vasculopathy; MF, median survival time; TLO, tertiary lymphoid organ; TLS, tertiary lymphoid structure; TMEM, memory T cell; Treg, regulatory T cell; wt, wild-type.

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gen–free conditions. All animal care and handling were performed according to institutional guidelines. “Classical” nonvascularized full-thickness skin allografts (2 × 3 cm) were placed on the groin area of the recipients, according to the technique described by Billingham and Medawar (24). Vascularized skin grafts were performed using a technique described by our laboratory. Briefly, a 2 × 3-cm full-thickness flap was outlined in the groin and elevated. The epigastric vessels were dissected, the distal superficial and deep femoral vessels were ligated, and the femoral artery and vein were separated. The artery was flushed with 10% heparinized saline until the venous flow in the pedicle became clear. Then the femoral artery and vein were divided. For recipient mice, the same-sized defect was created in the groin area. The femoral artery and vein, right below the inguinal ligament, were separated and prepared for anastomosis. End-to-end anastomosis was performed for veins after the patency of the vessels was confirmed that the flap was sutured to the defect with interrupted sutures. Vascularized heterotopic cardiac transplantation was performed as described by Corry et al. (25). Transplanted hearts were monitored daily by palpation through the abdominal wall. Heart beat intensity was graded on a scale of 0 (no palpable impulse) to 4 (strong impulse). Rejection was described by Corry et al. (25). Transplanted hearts were monitored daily by palpation through the abdominal wall. Heart beat intensity was graded on a scale of 0 (no palpable impulse) to 4 (strong impulse). Rejection was defined by the loss of palpable cardiac contractions and verified by autopsy and pathological examination.

T cell and T cell subset isolation

T cells, as well as CD4+ and CD8+ T cell subsets, were isolated from the spleen and lymph nodes of transplanted and naive mice by negative selection using commercially available T cell purification columns, according to the manufacturers’ instructions (Accurate Chemical & Scientific, Westbury, NY; R&D Systems, Minneapolis, MN). Purified T cells were washed in HBSS and used in ELISPOT assays.

Preparation of sonicates

Stimulator spleen cells were suspended at 3 × 10^6 cells/ml in AIM-V containing 0.5% FCS and sonicated with 10 pulses of 1 s each. The resulting suspension was frozen in a dry ice/ethanol bath, thawed at room temperature, and centrifuged at 300 × g for 10 min to remove remaining intact cells (1).

ELISPOT assays

Direct and indirect alloresponses by T cells were measured as previously described (1). Briefly, 96-well ELISPOT plates (Polyfltronics, Rockland, MA) were coated with an anti-cytokine capture mAb in sterile PBS overnight. On the day of the experiment, the plates were washed twice with sterile PBS, blocked for 1.5 h with PBS containing 1% BSA, and washed three times with sterile PBS. Responder cells or purified T cells were added to wells previously filled with either intact donor cells (direct response) or syngeneic APCs, together with donor sonicates (indirect response), and cultured for 24 h at 37°C, 5% CO2. After washing, bio- tinylated anti-lymphokine–detection Abs were added overnight. The plates were developed using 800 μl AEC (Pierce, Rockford, IL; 10 mg dissolved in 1 ml dimethyl formamide) mixed in 24 ml 0.1 M sodium acetate (pH 5), plus 12 μl H2O2. The resulting spots were counted and analyzed on a computer-assisted ELISA spot image analyzer (C.T.L., Cleveland, OH).

mAb treatments

For leukocyte–costimulation blockade, recipient mice were injected i.p. with 0.25 mg anti-CD4, mAbs (MR1) at the time of transplantation and at days 2, 4, and 6 posttransplantation, as previously described (26). For Treg depletion, recipient mice were treated 1 d prior to transplantation with an anti-CD25 Ab (PC61; 1 mg given i.p) that was shown to deplete CD25high Foxp3+ Tregs in vivo (27). CD4+ or CD8+ T cells were depleted from recipient mice with anti-CD4 (GK1.5) or anti-CD8 (53.6.7.2) mAb (1 mg given i.p. at days −3 and −1 pretransplantation, respectively). NK cells were depleted using the mAb NK1.1 (PK136; 0.6 mg) given i.p on day −2 pretransplantation. The depletions of CD4+, CD8+, and NK cells were >95% (data not shown).

Histology

Cardiac transplants were fixed in 10% buffered formalin, embedded in paraffin, coronally sectioned, and stained with H&E for evaluation of cellular infiltrates and myocyte damage (acute rejection) by light microscopy. For assessment of chronic rejection, cardiac grafts were stained with Verhoeff’s elastin (vessel arteriosclerosis scoring) or Mason’s trichrome (evaluation of fibrosis). Arteriosclerosis was assessed by light microscopy, and the percentages of luminal occlusion and intimal thickening were determined using a scoring system, as previously described (28). Only vessels that displayed a clear internal elastic lamina were included in the morphometric analysis (five to seven vessels/section). All arteries were scored by at least two examiners in a blinded fashion.

Statistics

All statistical analyses were performed using STATView software (Abacus Concepts, Berkeley, CA). The p values were calculated using the paired t test, and values < 0.05 were considered statistically significant.

Results

Rejection of allografts and alloresponses in the absence of secondary lymphoid organs

First, we investigated the rejection of fully allogeneic BALB/c (H-2b) skin and heart allografts in wild-type (wt) B6 mice (H-2b), B6 aly/aly mice (lacking lymph nodes and Peyer’s patches), and B6 aly/aly-spl−/− mice. As shown in Fig. 1A and 1B, control wt B6 mice acutely rejected skin and cardiac transplants within 10–12 d after placement. A slight prolongation of skin graft survival was observed in aly/aly mice (median survival time [MST] = 16 d) (Fig. 1A, 1B). In contrast, in aly/aly-spl−/− mice, whereas skin allografts also were acutely rejected (although in a delayed fashion, MST = 30 d), cardiac allografts survived indefinitely (Fig. 1A, 1B). Additionally, wt mice that had been splenectomized rejected skin and heart allografts at the same pace as did control wt B6 mice (Fig. 1A, 1B). Of note, histological examination of accepted hearts explanted from aly/aly-spl−/− mice revealed pathological features characteristics of mild chronic rejection detectable at day 50 posttransplantation (Fig. 1D). Therefore, rejection of cardiac allografts requires the presence of either the recipient’s lymph nodes or spleen, whereas skin allografts can be rejected in the absence of any secondary lymphoid organ.

Unlike conventional skin grafts, cardiac transplants are vascularized at the time of transplantation, a feature that contributes to their lower immunogenicity and greater susceptibility to tolerance induction (29). This prompted us to test whether primary vascularization of skin grafts would prolong their survival in aly/aly-spl−/− mice. As shown in Fig. 1C, aly/aly-spl−/− mice rejected vascularized skin allografts at the same pace as conventional skin allografts (Fig. 1A).

Direct, but not indirect, alloresponses are induced in transplanted aly/aly mice

Next, we measured T cell alloresponses in aly/aly mice transplanted with BALB/c skin allografts. Direct and indirect alloresponses by T cells collected from the recipient’s spleen were evaluated 10 d after transplantation using a previously described ELISPOT assay (1). As expected, transplanted wt B6 mice mounted potent direct and indirect alloresponses that were mediated primarily by proinflammatory T cells secreting IL-2 and IFN-γ and a few T cells producing type 2 IL-4 cytokine but not IL-10 (Fig. 2A). In contrast, in aly/aly mice, the direct alloresponse was dominated by T cells secreting IL-4 and IL-10 cytokines. In addition, aly/aly mice failed to mount an indirect alloresponse (Fig. 2B).

CD8+ T cells reject skin allografts in aly/aly-spl−/− mice

To identify the cells causing transplant rejection in recipients devoid of secondary lymphoid organs, aly/aly-spl−/− mice were injected with anti-CD4 (GK1.5)-depleting, anti-CD8 (56.8.72)-depleting, or NK1.1 (anti-NK cells)–depleting Abs, as described in Materials and Methods. As shown in Fig. 3A, although depletion of CD4+ and NK cells had no effect, treatment with anti-CD8 mAbs resulted in long-term survival of skin allografts. Therefore, CD8+ T cells mediate skin allograft rejection in aly/aly-spl−/− mice.

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Detection of alloresponses in the blood and bone marrow of transplanted aly/aly-spl− mice

Because aly/aly-spl− mice lacking all secondary lymphoid organs reject skin allografts through a process involving CD8⁺ T cells, we investigated whether an allospecific response could be detected in other lymphoid tissues. To test this, T cells from the peripheral blood and bone marrow of aly/aly-spl− mice were tested 10 d after BALB/c skin grafting for the presence of a direct alloresponse. As shown in Fig. 3B, direct alloresponses were detected with T cells isolated from bone marrow or peripheral blood. No response was detected in nontransplanted aly/aly-spl− mice (data not shown). These results demonstrate that transplanted aly/aly-spl− mice can mount an allo-specific T cell response, despite the absence of lymph nodes and spleen.

aly/aly-spl− mice mount memory alloresponses

Next, we investigated whether aly/aly-spl− mice can mount a donor-specific memory alloresponse following allotransplantation. To test this, aly/aly-spl− mice that rejected a BALB/c skin graft were retransplanted (20 d after rejection) with a skin allograft derived from the same or a third-party C3H (H-2k) donor. First, we observed an expansion of T cells expressing CD44 (mostly CD44⁺ CD62L⁻ effector TEMEs), which is characteristic of a memory phenotype (Fig. 4A). Consistent with this observation, these mice rejected a second BALB/c skin graft in an accelerated fashion (Fig. 4B). No accelerated rejection was observed after placement of control third-party C3H allografts (data not shown). Therefore, despite their lack of secondary lymphoid organs, aly/aly-spl− mice can mount a donor-specific memory alloimmune response resulting in a second graft rejection.

Differential rejection of heart and skin grafts does not depend on graft size

Minor Ag-mismatched (H-Y) heart transplants are less susceptible to rejection than are skin grafts due to their larger size,
presumably causing T cell exhaustion (30). However, this finding may only be relevant to transplants rejected via oligo-
clonal alloresponses, such as the anti–H-Y response. Never-
theless, given the overall paucity of lymphocytes in aly/aly-spl
mice, we reasoned that the graft size could influence the re-
jection process. To address this question, aly/aly-spl
mice were transplanted with skin allografts of increasing sizes: 2
(size of control grafts), 4, and 9 cm². Bigger-sized grafts were
rejected significantly faster than were the initial 2 cm²–sized
grafts (Fig. 4C). Therefore, larger skin grafts did not exhaust
T cells in aly/aly-spl− mice, and they were also more immu-
nogenic.

Finally, aly/aly-spl− mice, which had accepted a BALB/c heart for
50 d, were transplanted with a skin allograft from the same (BALB/c,
H-2b) or a third-party (C3H, H-2k) donor. Strikingly, BALB/c skin
allografts exhibited indefinite survival (>250 d), whereas C3H third-
party allografts were rejected within 20–25 d posttransplantation.
(Fig. 5). Therefore, despite the absence of secondary lymphoid organs, aly/aly-spl² mice developed donor-specific tolerance. Of note, heart transplants placed in aly/aly-spl² mice displayed histological features of cardiac allograft vasculopathy (CAV) that were detectable at day 50–75 posttransplantation (data not shown). Therefore, ongoing chronic rejection of cardiac allografts did not prevent tolerance of skin grafts.

Additional experiments were conducted to investigate the mechanisms underlying transplant tolerance in aly/aly-spl² mice. First, BALB/c skin allografts were placed on aly/aly-spl² mice either at the time of (d0) or 14 or 30 d (d14 and d30) after transplantation of BALB/c hearts. Skin grafts placed at day 0 or 14 were rejected acutely, whereas the majority of those transplanted at day 30 exhibited long-term survival (Fig. 6A). Therefore, tolerance requires 2–4 wk to become established in this model. Next, naive aly/aly-spl² mice were injected with 10⁶ CD4⁺ T cells isolated from the peripheral blood of tolerant or control aly/aly-spl² mice 3 d prior to placement of a BALB/c skin allograft. Adoptive transfer of CD4⁺ T cells from tolerant mice resulted in long-term survival of skin allografts in most recipients (Fig. 6B). Finally, aly/aly-spl² mice were injected with anti-CD4 Ab (GK1.5) or with anti-CD25 Ab (PC61), using a regimen known to deplete CD4⁺CD25highFoxp3⁺ Tregs (27) 3 d prior to and 20 and 47 d after BALB/c heart transplantation. All mice were transplanted with a BALB/c skin graft 50 d after cardiac transplantation. Recipients with anti-CD4 Abs accepted heart, but not skin, transplants (n = 4, MST = 32 d). In contrast, anti-CD25 mAb-treated mice accepted both transplants (n = 5, MST > 100 d). Altogether, these experiments show that, although transplant tolerance is dependent upon CD4⁺ T cells, it apparently does not depend on the presence of CD4⁺CD25highFoxp3⁺ Tregs.

Discussion

A single spontaneous autosomal mutation in the NIK gene on chromosome 11 results in lack of lymph nodes and Peyer’s patches in aly/aly mice (23). These mice display a disorganized thymic architecture and severe defects in the spleen associated with the absence of germinal centers, an atrophic white pulp, and no marginal zone (31). These multiple defects result in alymphoplasia and compromised cell-mediated and humoral immunity (31, 32). Despite such severe immunodeficiency, it is remarkable that aly/aly mice acutely rejected skin and cardiac allografts at the same pace as did normal mice. Our observations are in contrast with studies by Lakkis et al. (33) in the same aly/aly mouse model; they reported similar rejection of heart transplants but indefinite survival of skin allografts. Our results corroborate reports from two other laboratories showing the rejection of skin and lung allografts in aly/aly mice, as well as LTβR-knockout mice, which also lack lymph nodes (34–37).

Direct, but not indirect, T cell alloresponses were detected in skin-grafted aly/aly mice. This presumably reflects the fact that T cell indirect allosensitization occurs through traditional priming by peptides processed and presented by self-APCs in draining lymph nodes (38). In contrast, direct allorecognition is unconventional in that it involves TCR interaction with intact allogeneic MHC molecules present on foreign APCs and triggers a polyclonal response engaging up to 10% of the T cell repertoire (1, 39,
We surmise that, in skin-grafted aly/aly mice, donor APCs leaving the graft upon its revascularization (days 4–5 posttransplant) can initiate a direct alloresponse given the exceptionally high frequency of alloreactive T cells present in the recipient. In contrast, indirect allosensitization requires “classical” T cell priming in regional lymph nodes. This is supported by our recent report showing that primarily vascularized skin allografts, whose dendritic cells emigrate through blood vessels, elicit direct, but not indirect, alloresponses (29).

Unexpectedly, aly/aly-spl2 mice devoid of all secondary lymphoid organs acutely rejected skin allografts (Fig. 1). This process was mediated primarily by CD8+ T cells that were activated directly. Moreover, transplanted mice displayed a significant generation/expansion of effector TMEMs and rejected a second skin graft from the same donor in an accelerated fashion. Where do T cells from transplanted aly/aly-spl2 mice encounter allogens? There is accumulating evidence suggesting that naive alloreactive T cells can be primed either in secondary or tertiary lymphoid organs (TLOs) located outside of the graft or in tertiary lymphoid structures (TLSs) being formed within the graft itself during inflammation (41–44). However, it is unlikely that naive alloreactive T cells can be primed either in secondary or tertiary lymphoid organs (TLOs) located outside of the graft or in tertiary lymphoid structures (TLSs) being formed within the graft itself during inflammation. Instead, alloreactive T cells are likely to be activated directly by donor APCs that leave the graft upon revascularization (40).

Unusually, aly/aly-spl2 mice devoid of all secondary lymphoid organs acutely rejected skin allografts (Fig. 1). This process was mediated primarily by CD8+ T cells that were activated directly. Moreover, transplanted mice displayed a significant generation/expansion of effector TMEMs and rejected a second skin graft from the same donor in an accelerated fashion. Where do T cells from transplanted aly/aly-spl2 mice encounter allogens? There is accumulating evidence suggesting that naive alloreactive T cells can be primed either in secondary or tertiary lymphoid organs (TLOs) located outside of the graft or in tertiary lymphoid structures (TLSs) being formed within the graft itself during inflammation (41–44). However, it is unlikely that naive T cells of skin-grafted aly/aly-spl mice become sensitized in TLOs or TLSs, because aly/aly-spl2 mice are devoid of TLOs [with the exception of cryptopatches in the intestinal lamina propria (42)] and never form TLSs in the skin, even upon lymphotoxin-α transgenesis (45). Another possibility is that the direct alloresponse is mediated by “natural” alloreactive TMEMs already present in mice before transplantation and reactivated at the graft site (18). Unlike naive T cells, TMEMs recirculate through peripheral nonlymphoid tissues where they can be reactivated locally upon Ag presentation (19, 20). Indeed, our study shows the presence of 10–14% TMEMs in the blood of nontransplanted aly/aly-spl2 mice, presumably including some alloreactive cells. These pre-existing TMEMs are unlikely to mediate the alloresponse based upon previous studies showing their inability to trigger alloimmunity and allograft rejection upon adoptive transfer in aly/aly mice (18). Finally, because high frequencies of donor-specific activated inflammatory T cells were revealed in the bone marrow of transplanted aly/aly-spl2 mice, we surmise that this could represent the site of allosensitization. This hypothesis is supported by a recent study demonstrating that bone marrow donor APCs can initiate a direct alloresponse in aly/aly mice (29).

**FIGURE 5.** aly/aly-spl2 mice that spontaneously accepted a BALB/c heart transplant developed donor-specific tolerance. aly/aly-spl2 mice that spontaneously accepted a BALB/c heart for 50 d were transplanted with a skin graft from the same (BALB/c) or a third-party (C3H) donor. The results are shown as percentage skin graft survival over time after skin transplantation (day 0). Graft survival was analyzed using the Kaplan–Meier method. Three to five mice were tested in each group. The inset shows a representative aly/aly-spl2 mouse that accepted a BALB/c skin allograft for 280 d.

**FIGURE 6.** Mechanisms involved in tolerance of skin allografts by aly/aly-spl2 mice. (A) aly/aly-spl2 mice were transplanted with a BALB/c heart and received a skin allograft from the same donor at the same time (d0) or fourteen (d14) or thirty (d30) days later. The results are shown as percentage skin graft survival over time after skin transplantation. Graft survival was analyzed using the Kaplan–Meier method. Three to five mice were tested in each group. (B) aly/aly-spl2 mice were injected with 10⁶ CD4+ T cells isolated from the peripheral blood of tolerant (mice having accepted a BALB/c heart for >100 d) or control naive aly/aly-spl2 mice 3 d prior to placement of a BALB/c skin graft. The results are shown as percentage skin graft survival over time after skin transplantation (day 0). Graft survival was analyzed using the Kaplan–Meier method. Three to six mice were tested in each group.
marrow can serve as a priming site for T cell responses to blood-borne Ag (46).

Why do aly/aly-spl mice acutely reject allogeneic skin transplants but not heart transplants? Skin allografts are notoriously more immunogenic and less susceptible to tolerogenesis than are heart transplants (29). We showed recently that this is due, in part, to the fact that cardiac allografts are immediately vascularized after their placement, whereas conventional skin allografts are not (29). However, our observation that primarily vascularized skin allografts are acutely rejected in aly/a/y mice (Fig. 1C) does not support the view that graft vascularization accounts for the longer survival of cardiac allografts in aly/a/y-spl mice. Alternatively, the presentation of some highly immunogenic skin-specific Ags described by Steinmüller et al. (47) could account for the rejection of skin, but not heart, allografts in aly/a/y-spl mice.

Although aly/a/y-spl mice did not acutely reject cardiac allotransplants, histological examination of these grafts revealed the presence of CAV typical of chronic rejection. No alloantibodies were detected in these mice (data not shown). It is conceivable that the few inflammatory T cells activated directly (Fig. 3B, 200–300 IFN-γ–producing cells/106 T cells) in these mice were insufficient to ensure acute rejection of heart transplants but elicited CAV, a phenomenon previously documented with adoptively transferred alloreactive T cell clones (30, 48). In addition, we surmise that chronic rejection may be associated with the high frequency of activated T cells secreting type 2 cytokines (IL-4 and IL-10) that was detected in these mice. The contribution of these T cells in chronic allograft rejection was documented in several studies (49–51). Likewise, we recently reported that donor-specific T cells secreting type 2 cytokines (IL-10) could prevent early acute rejection of skin allografts while causing CAV in an MHC class I–disparate mouse transplant model. We cannot rule out the possibility that allorecognition by some pre-existing TMEMs occurs in the graft. However, this hypothesis is not supported by the alloresponse kinetics or by previous observations in the same model. Alternatively, because we detected potent direct alloresponses in the bone marrow of transplanted aly/a/y-spl mice, and priming of naive T cells was documented previously in this tissue, it represents a plausible site for initiation of the alloresponse in transplanted mice lacking secondary lymphoid organs.

Disclosures
The authors have no financial conflicts of interest.

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