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Heart, but Not Skin, Allografts from Donors Lacking Flt3 Ligand Exhibit Markedly Prolonged Survival Time

Zhiliang Wang,2* Antonino Castellaneta,2* An De Cres,*, William J. Shufesky,*, Adrian E. Morelli,* and Angus W. Thomson3*†

Fms-like tyrosine kinase 3 ligand (Flt3L) administration leads to dramatic increases in dendritic cells (DC) in lymphoid and nonlymphoid tissues. Conversely, mice lacking Flt3L (Flt3L−/−) show severe reductions in both myeloid (CD11c+CD8α+) and lymphoid-related DC (CD11c+CD8α+) in the thymus and secondary lymphoid organs. In this study marked reductions in CD11c+ interstitial cardiac DC and in dermal, but not epidermal, DC (Langerhans cells) were also observed. CD11c+ cells that migrated from Flt3L−/− skin explants expressed lower surface MHC class II and costimulatory molecules and naive T cell allostimulatory activity than migratory wild-type (wt) C57BL/6 (B6) CD11c+ cells. We examined the survival of Flt3L−/− heart or tail skin grafts (H2b) in allogeneic wt (BALB/c; H2b) recipients. The outcome of transplantation of BALB/c organs into Flt3L−/− recipients was also determined. Flt3L−/− mice rejected BALB/c heart or skin grafts with similar kinetics as B6 wt recipients. Trafficking of donor DC into host spleens or draining lymph nodes was markedly reduced after transplantation of Flt3L−/− heart, but not skin grafts, respectively. Compared with wt hearts, survival of Flt3L−/− hearts was markedly prolonged in BALB/c recipients (median survival time, 37 and 15 days, respectively; p < 0.001). Skin graft survival was unaffected. Rejection of Flt3L−/− hearts was precipitated by infusion of wt donor DC at the time of transplant. Thus, severe depletion of interstitial heart DC resulting from targeted gene disruption prolongs, but does not indefinitely extend, heart survival. Acute rejection of wt grafts in Flt3L−/− recipients reflects presumably an intact role of the direct pathway of allorecognition. The Journal of Immunology, 2004, 172: 5924–5930.

Dendritic cells (DC) are uniquely well-equipped APC that induce and regulate immune responses (1, 2). In transplantation, donor-derived and host DC play critical roles in the rejection response via the direct and indirect pathways of allorecognition, respectively. After organ transplantation, donor-derived DC (passenger leukocytes) leave the graft and migrate to secondary lymphoid organs (3) where they present MHC molecules to host T cells (direct allorecognition pathway). In the indirect pathway, recipient DC internalize and process donor Ags, then present alloantigen-derived peptides bound to self-MHC molecules to recipient T cells. Several observations support the view that direct allorecognition is principally involved in acute rejection (4). Thus, responder T cells are stimulated strongly in primary allogeneic mixed leukocyte cultures even after removal of responder APC (5). Furthermore, depletion of donor APC from tissue or organ grafts improves their survival in experimental models (6, 7). Although the indirect pathway has been thought to underlie events leading to chronic rejection, there is also evidence that it can play a role in acute rejection. Thus, prompt rejection of skin grafts from MHC class II-deficient mice by normal recipients indicates that indirect allorecognition can initiate rapid skin rejection (8, 9).

Fms-like tyrosine kinase 3 ligand (Flt3L) is a potent endogenous hematopoietic growth factor. Its in vivo administration in mice and humans leads to dramatic increases in various DC subsets in lymphoid and nonlymphoid organs (10–13). A crucial role of DC in modulating host responses to Ags has been shown using Flt3L-treated donor mice in transplantation models. Thus, liver allograft acceptance and induction of donor-specific tolerance are lost after Flt3L treatment of the donor due to marked increases in donor-derived DC that mature rapidly ex vivo into potent inducers of Th1 responses (11, 14). To further investigate the role of Flt3L in hematopoiesis, McKenna et al. (15) generated mice lacking Flt3L (Flt3L−/−), but with normal expression of its receptor (Flt3). These mice show several defects in different hematopoietic lineages. They exhibit severe reductions in both classic myeloid (CD11c+CD8α−; up to 11-fold reduction) and lymphoid-related DC (CD11c+CD8α−; up to 14-fold reduction) in spleen, lymph nodes, and thymus (15), and a major reduction in splenic plasmacytoid pre-DC (16). Flt3L−/− mice also display a decreased proportion of lymphocytes (in bone marrow, blood, spleen, and lymph nodes), an increase in neutrophils, and a marked reduction in splenic NK cells (15).

To further clarify the relative roles of donor interstitial DC and the direct vs the indirect pathways of allorecognition in graft rejection, we examined the survival of heart or skin allografts from Flt3L−/− donors (maintained on a C57BL/6 (B6; H2b) background) in wild-type (wt) BALB/c (H2b) recipients. Conversely, the outcome of wt BALB/c grafts in Flt3L−/− recipients was also studied. Severe depletion of graft interstitial DC resulted in significant prolongation of heart graft survival. By contrast, Flt3L−/− B6 skin allografts that lacked dermal DC, but exhibited
normal numbers of epidermal Langerhans cells (LC), were rejected with normal kinetics. Severe depletion of recipient lymphoid tissue DC did not affect acute rejection of wt grafts due, presumably, to an intact role of the direct pathway of allore cognition.

Materials and Methods

Animals

Ten- to 12-wk-old C57BL/6 (B6; H2Kb, IAa, IE+) and BALB/c (H2Kd, IAb, IE+) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, U.S.A.). Mice on a B6 background (Taconic Farms, Germantown, NY) were maintained in the specific pathogen-free central animal facility of University of Pittsburgh Medical Center (Pittsburgh, PA). They received Purina rodent chow (Ralston Purina, St. Louis, MO) and tap water ad libitum. Experiments were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals under an institutional animal care and use committee-approved protocol.

Immunofluorescence staining of tissue sections

Spleen, lymph nodes, heart, and tail skin were snap-frozen in OCT medium (Sakura Finetek USA, Torrance, CA). Eight-micron cryostat sections were fixed in 96% ethanol for 20 min at room temperature. After washing and blocking with 5% (v/v) normal goat serum in PBS for 20 min and avidin-streptavidin blocking kit (Vector Laboratories, Burlingame, CA), the slides were incubated overnight at 4°C with either anti-mouse CD11c mAb (HL3; BD PharMingen, San Diego, CA) in combination with biotin-conjugated anti-mouse IAa or IAb (AF6-120.1 and 39-10-8, respectively; both from BD PharMingen). The following day, after washing in PBS, the slides were incubated for 30 min at room temperature with streptavidin-cyamine 3 (Cy3) and cyanine 2 (Cy2)- conjugated anti-Armenian hamster IgG (both from Jackson ImmunoResearch Laboratories, West Grove, PA). Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (0.1 μg/ml; 1 min) and sections were fixed in 4% paraformaldehyde for 10 min.

Immunofluorescence staining of epidermal sheets

Ear pinna skin samples obtained from wt B6 and Flt3L−/− B6 mice were freed of fatty tissue and floated dermal side down in a petri dish containing EDTA (20 mM/ml; Sigma-Aldrich, St. Louis, MO) for 2 h at 37°C. Epidermal sheets were peeled from the underlying dermis and fixed in acetone for 15 min at 4°C. Subsequently, sheets were washed in PBS, then labeled with an FITC-conjugated anti-IAa mAb for 12 h at 4°C.

Migratory cells from skin explants

Migratory cells were obtained from separated ear pinna halves of wt B6 and Flt3L−/− B6 mice by floating the tissue dermal side down on 2 ml of RPMI 1640 complete medium in 24-well plates at 37°C. After 48 h culture at 37°C in 5% CO2 in air, the migratory cells were gently resuspended, washed, and counted in trypan blue (to assess viability) before further examination. For each experiment, migratory cells derived from the ear pinnae of pairs of mice were pooled.

Flow cytometric analysis of cell suspensions

A low buoyant density fraction of cells obtained from draining lymph nodes (mesenteric and lumbar) of skin graft recipients or from spleens of heart recipients 1 day after transplantation were isolated using 16% (v/v) methoxyflurane inhalation anesthesia (Medical Development, Springvale, Australia). The heart was transplanted into the abdomen with end-to-side anastomosis of aorta to aorta and pulmonary artery to vena cava. To assess their impact on graft survival, 2 × 106 wt donor splenic bulk CD11c+ DC were adoptively transferred (i.v., via the lateral tail vein) at the time of transplant. Graft survival was assessed by daily transmural palpation. Rejection was defined as total cessation of cardiac contraction and was confirmed by histological examination. Skin grafting was conducted as previously described (19). Briefly, square, full-thickness skin grafts (0.5 cm2) were prepared from the base of the donor tail. Graft beds (0.5 cm2) were prepared on the backs of recipient mice. The skin was attached to the graft bed with eight interrupted sutures of 5-0 silk thread, then covered with protective tape.

Results

Flt3L−/− mice show differential reductions in tissue DC compared with wt mice

To investigate the relative roles of donor and recipient DC in inducing acute allograft rejection, we used Flt3L−/− B6 (H2Kb, IAa) and BALB/c (H2Kd, IAa) mice as donors and recipients alternatively in both heart and skin transplant models. We first compared, by immunofluorescence staining, heart and skin CD11c+ cell numbers in Flt3L−/− B6 and wt B6 (H2Kb, IAa) mice. Cryostat sections were stained for CD11c (green) together with anti-MHC class II mAb (red). As reported previously (16), Flt3L−/− B6 mice showed a profound reduction (>90%) in CD11c+ cells in both spleens and lymph nodes (data not shown) as well as in parenchymal organs. Thus, hearts from Flt3L−/− B6 mice exhibited pronounced depletion of interstitial CD11c+ MHC II+ cells compared with wt B6 mice (Fig. 1A, a and b). In contrast, no significant difference in LC numbers in the epidermal layer between Flt3L−/− B6 and wt B6 mice was observed in either stained sections (ear or tail skin; Fig. 1A, c and d) or epidermal sheets (ear skin; Fig. 1A, e and f; Flt3L−/−, 426 ± 41; wt, 483 ± 54 LC/mm2). However, Flt3L−/− B6 LC differed from wt LC in their morphology, as LC from Flt3L−/− B6 mice exhibited less extensive dendrites. In contrast to the normal LC density observed in the epidermis of Flt3L−/− B6 mice, these animals showed a marked reduction in CD11c+ cells in the underlying dermal layer compared with wt B6 controls (Fig. 1A, g and h).

Epidermal LC of Flt3L−/− mice exhibit normal ex vivo migration, but reduced functional maturation

To determine whether LC from Flt3L−/− B6 mice could migrate out of the skin, skin explants of Flt3L−/− B6 mice were used as an in vitro model. Skin explants of wt B6 mice served as positive controls. The ear skin of these mice was floated dermal side down
FIGURE 1. Flt3L−/− B6 mice show significant reductions in heart and skin DC compared with wt mice. A, Heart and tail skin cryostat sections from wt B6 (a, c, and g) and Flt3L−/− B6 mice (b, d, and h) were stained by immunofluorescence, as described in Materials and Methods, using primary anti-mouse CD11c mAb, followed by Cy2-conjugated goat anti-Armenian hamster IgG (green) and a biotin-conjugated anti-mouse IAβ developed with streptavidin-Cy3 (red). Epidermal sheets from ear skin of wt (e) and Flt3L−/− B6 (f) mice were stained for IAβ. Heart (b) and dermis (h) of Flt3L−/− B6 mice show a marked reduction in CD11c+ cells compared with wt mice (a and g). By contrast, no significant difference in the number of CD11c+ cells was found between the epidermal layers of Flt3L−/− (d and f) and wt mice (c and e). B, Leukocytes that migrated spontaneously from ear skin explants of wt and Flt3L−/− B6 mice were collected after 2 days of culture, as described in Materials and Methods, then double labeled with FITC-anti-CD11c and either PE-anti-IAβ or PE-anti-CD86. Flow cytometric analyses of ear skin migratory cells showed no significant difference in the frequency of CD11c+ cells between wt and Flt3L−/− B6 mice, but CD11c+ cells from wt mice expressed overall higher levels of CD86 and IAβ than those from Flt3L−/− B6 mice. C, Allostimulatory activity of migratory wt and Flt3L−/− skin DC for naive BALB/c T cells in 72-h MLR. Data are representative of results obtained from three separate experiments.

Flt3L−/− donor DC are present in substantially reduced numbers compared with wt donor DC in secondary lymphoid organs of BALB/c recipients

To investigate the in vivo migration of donor leukocytes from Flt3L−/− B6 or wt B6 grafts to secondary lymphoid organs of BALB/c recipients, secondary lymphoid tissues (draining lymph nodes or spleens) of skin or heart graft recipients were harvested 1 day after transplantation, and examined by immunofluorescence microscopy. Sections were double stained for CD11c (green) and either donor (IAβ) or recipient (IAβ) MHC class II (red). Fig. 2 shows spleen and axillary lymph node sections of BALB/c mice given syngeneic, wt B6, or Flt3L−/− B6 grafts (heart or tail skin). The frequency of donor IAβ+ DC (yellow) in recipient secondary lymphoid organs was decreased markedly in recipients of both heart (10 ± 9 vs 35 ± 10%; p < 0.01) and skin grafts (11 ± 10 vs 24 ± 15%; p < 0.01) from Flt3L−/− donors. As expected, no double-positive donor cells were detected in the spleen or draining lymph nodes of syngeneic graft recipients (data not shown). We also determined the absolute numbers of donor-derived DC in the spleens of recipients of Flt3L−/− B6 or wt B6 hearts by flow cytometry. In a representative experiment of two performed, a 2-fold reduction in absolute numbers of IAβ+ CD11c+ H2Kd+ donor DC was found 1 day post-transplant in pooled splenocyte suspensions from Flt3L−/− heart graft recipients (96 × 10^3/spleen; n = 3 mice) compared with those from controls given wt grafts (144 × 10^3/ spleen; n = 3 mice).

Expression of costimulatory molecules on DC within secondary lymphoid organs of recipients of heart grafts from Flt3L−/− or wt donors does not differ significantly

After allogeneic organ transplantation, donor-derived DC migrate to secondary lymphoid tissues, where they localize in T cell areas. The interaction between these cell populations (predominantly) and also that between recipient DC, that take up and present donor Ag, and host T cells is thought to play an important role in both T cell sensitization against donor Ag and in modulation of anti-donor
reactivity. We next studied in the heart transplant model the expression of lineage markers (CD11b and B220) and costimulatory molecules (CD40, CD80, and CD86) on cell suspensions obtained from lymph nodes and spleens of BALB/c recipients 1 day after transplantation using Flt3L−/− B6 or wt B6 mice as transplant donors. Low buoyant density cells were isolated over 16% (v/v) metrizamide and analyzed for CD11b, B220, CD40, CD80, CD86, IAα, H2Kd, and IAα expression by dual color flow cytometric analysis after gating on CD11c+ cells. In the representative results shown in Fig. 3, no substantial differences in costimulatory molecule expression were detected between mice given Flt3L−/− B6 hearts and those given normal wt B6 hearts (CD40, 36.7 vs 34.8%; CD80, 53 vs 48%; CD86, 41.2 vs 56.5%, respectively). Moreover, in recipients of either Flt3L−/− B6 or wt B6 hearts, most of the CD11c+ cells coexpressed the myeloid marker CD11b (86.5 vs 87.9%, respectively), whereas a more modest number expressed B220, that is, associated with plasmacytoid pre-DC (23.7 vs 38.6%). The expression of CD8α and Ly6C (Gr-1) did not differ between the two groups. In addition, virtually all these cells coexpressed recipient MHC class I (99 vs 99%), emphasizing the very low level of DC chimerism even for recipients of wt B6 heart grafts.

Heart, but not skin, allograft survival is markedly prolonged when Flt3L−/− mice are used as donors.

To evaluate the impact of donor DC deficiency on graft survival, Flt3L−/− or wt B6 mice were used as donors or recipients in different combinations with BALB/c mice: BALB/c hearts into B6 recipients, BALB/c hearts into Flt3L−/− B6, wt B6 hearts into BALB/c, and Flt3L−/− B6 hearts into BALB/c. As shown in Fig. 4A, Flt3L−/− mice, lacking secondary lymphoid tissue DC, rejected BALB/c heart grafts with kinetics similar to those observed in wt recipients (mean survival time, 16 ± 9 and 12 ± 2 days, respectively). By contrast, Flt3L−/− B6 heart graft survival was prolonged significantly in BALB/c recipients compared with that of wt B6 heart grafts (mean survival times, 37 ± 18 and 15 ± 2 days, respectively; p < 0.001). Histological examination of heart grafts on day 7 post-transplant confirmed the findings (Fig. 5), i.e., Flt3L−/− B6 and syngeneic BALB/c grafts showed a similar degree of mononuclear cell infiltration, that was markedly reduced compared with that observed in wt allogeneic B6 heart grafts. To

FIGURE 2. Flt3L−/− B6 donor DC (IAα) are present in substantially reduced numbers compared with wt B6 donor DC in secondary lymphoid organs of BALB/c recipients, 1 day after transplantation. Cryostat sections of spleens and axillary lymph nodes of BALB/c recipients given syngeneic, wt B6, or Flt3L−/− B6 grafts (heart and tail skin, respectively) were stained, as described in Materials and Methods, with primary anti-mouse CD11c mAb in combination with a biotin-conjugated anti-mouse IAα or IAα and then developed by Cy2-conjugated goat anti-Armenian hamster IgG (green) and streptavidin Cy3 (red), respectively. The frequency of double-labeled donor (IAα+) DC (yellow; arrows) in secondary lymphoid organs was decreased markedly in recipients of heart or skin grafts from Flt3L−/− donors. No double-positive donor cells were detected in the spleens or draining lymph nodes of recipients of syngeneic (IAα+) grafts. The counterstain was 4’,6-diamidino-2-phenylindole. Magnification, ×600.

FIGURE 3. Expression of costimulatory molecules on DC within secondary lymphoid organs of recipients of heart grafts from Flt3L−/− B6 or wt B6 donors does not differ significantly. Cells obtained from lymph nodes (mesenteric and inguinal) and spleens of wt and Flt3L−/− heart graft recipients 1 day after transplant were stained as described in Materials and Methods. Cells were labeled with FITC-anti-CD11c and one of the following PE-conjugated mAbs: anti-CD11b, anti-B220, anti-H2Kd, anti-CD40, anti-CD80, or anti-CD86. No statistically significant differences in costimulatory molecule expression were detected between the two groups. Most CD11c+ cells in both Flt3L−/− and wt B6 heart graft recipients coexpressed the myeloid marker CD11b, whereas a much more modest number expressed B220. Virtually all these cells (>99%) coexpressed recipient MHC class I (H2Kd). Data are representative of three separate experiments.
investigate whether fewer donor DC present in heart grafts from Flt3L−/− B6 mice led to the observed delay in graft rejection, we injected 2 × 10^6 wt B6 donor splenic DC i.v. at the time of transplant. As shown in Fig. 4A, cotransfer of wt donor DCs resulted in shorter graft survival times compared with those observed after normal wt B6 heart transplantation. These data suggest that reduced donor DC trafficking from the graft to the spleen and consequent suboptimal priming of recipient T cells accounts for the prolongation of heart graft survival. Using the same range of donor-recipient combinations, no significant differences were observed in skin graft survival time when either donors or recipients were Flt3L−/− B6 mice (Fig. 4B). These results seemed to conflict with the in vitro data, which showed that DC that migrated from Flt3L−/− B6 skin explants were less mature and exhibited diminished capacity to stimulate naive allogeneic T cells. Moreover, reduced numbers of Flt3L−/− B6 donor DC trafficked to secondary lymphoid organs after transplantation.

T cells from mice given Flt3L−/− skin grafts show normal anti-donor proliferative response

We therefore compared the anti-donor proliferative responses of T cells from wt B6 or BALB/c mice and from BALB/c recipients of skin grafts from allogeneic wt B6 or Flt3L−/− B6 donors 7 days previously. Fresh wt B6 bulk splenocytes were gamma-irradiated and used as stimulators. As shown in Fig. 6, lymph node T cells from Flt3L−/− B6 skin graft recipients showed anti-donor T cell responses similar to those of mice given wt B6 grafts. These results correspond with the graft rejection data and suggest that recipient skin DC could, via the indirect pathway of allore cognition, play a major role in acute skin graft rejection. As expected, anti-donor responsiveness was reduced markedly in recipients of Flt3L−/− B6.
hearts compared with normal BALB/c mice or those given syngeneic grafts. The comparatively low level of anti-donor T cell proliferation detected in spleens of wt heart graft recipients may reflect different kinetics or redistribution of effector T cells to the allograft site at this time post-transplant (data not shown).

Discussion
These studies show, for the first time, that MHC-mismatched cardiac allografts from mice genetically deficient in the hemopoietic growth factor Flt3L undergo markedly extended, but not indefinite, survival in unmodified (nonimmunosuppressed) wt recipients. The findings contrast with the accelerated rejection of heart allografts from mice treated with Flt3L, in which donor interstitial DC are augmented (11). Prolongation of heart graft survival from Flt3L−/− B6 donors can be ascribed to the pronounced reductions observed in potential immunostimulatory interstitial CD11c+ MHC II+ DC, the crucial component of the migratory passenger leukocyte population that initiates the acute rejection response (3, 20). In contrast, the normal rejection kinetics of skin allografts from Flt3L−/− donors could reflect an apparently normal constitutency of epidermal MHC II+ LC despite marked reductions in dermal DC, implicating and confirming LC as the principal instigators of skin rejection (21–23). However, our ex vivo results indicate that DC migrated from the skin of Flt3L−/− mice were less mature and less potent in stimulating allogeneic T cell responses than migratory wt skin DC. In addition, fewer donor DC migrated to the draining lymph nodes after skin transplantation compared with donor DC that migrated from wt skin grafts. Thus, our data also implicate a possible role for the indirect pathway mediated by recipient DC during acute skin rejection.

Normal numbers of epidermal LC detected in situ in Flt3L−/− B6 mice (not described in an earlier characterization of DC populations within these animals) (15) suggest that LC are not dependent on Flt3L for their generation. In contrast, the skin of TGF-β1 null mice is devoid of epidermal LC (24), implying a critical role of TGFβ-1 in the development of this DC subset. The DC that migrated readily into culture medium within 2 days from combined epidermal and dermal layers of ear pinnae of Flt3L−/− B6 mice displayed lower surface levels of MHC II and costimulatory molecules (CD86). Moreover, migratory Flt3L−/− B6 DC, in contrast to DC that migrated from wt B6 mouse ear skin, were less potent APC after interaction with allogeneic T cells. In addition, two-color immunohistchemistry revealed a clear reduction in the overall number of migrating donor MHC II+ cells (DC) that reached host secondary lymphoid tissue from Flt3L−/− B6 skin grafts. However, the survival of these transplants was not prolonged, indicating that the fewer, less efficient allostimulatory donor APC (presumably LC) that did migrate were still sufficient to elicit an effective anti-donor T cell response. Alternatively/additionally, indirect presentation of donor alloantigens to recipient T cells via host DC (indirect allore cognition) may have contributed to the acute skin rejection response, as elicitation of anti-donor T cell proliferative responses ex vivo revealed that priming was as effective as in animals that received wt grafts.

The present findings are consistent with results obtained when thyroid, pancreatic islet, skin, or kidney grafts have been purged of donor leukocytes by means including tissue culture (6, 25, 26), residence of the graft in an intermediate allogeneic host (20), or treatment with specific anti-DC mAb (7, 26). In humans, perfusion of kidneys with anti-CD45 mAb before grafting has been reported to reduce the number of rejection episodes (27). More recently, it has been shown that the role of the direct vs indirect pathway of allore cognition depends on the type of tissue/organ graft, the experimental model, and the state of rejection (acute vs chronic) (28).

Moreover, difficulties inherent in assessing the indirect pathway in vitro and in vivo may explain in part the conflicting results regarding the relative contributions of direct vs indirect allore cognition in acute rejection. In a mouse model of vascularized heart transplantation similar to that used in this study, immunodeficient SCID and Rag1−/− recipients reconstituted with syngeneic CD4+ T cells rejected allogeneic MHC class II-expressing transplants, but accepted MHC class II-deficient grafts unable to induce direct allore cognition (29). Moreover, MHC II-deficient Rag1−/− recipients rejected allogeneic hearts when reconstituted with syngeneic CD4+ T cells (29). These results together with those of the present study suggest that the direct pathway is the mechanism that triggers acute heart rejection in mice. By contrast, using the same experimental model, but different strain combinations, the absence of costimulatory molecules on APC from cardiac grafts of CD80−/− CD86−/− double KO mice, unable to stimulate via the direct pathway, did not prolong graft survival. However, the expression of these costimulatory molecules by recipient DC (indirect pathway) elicited acute rejection (30). Benichou et al. (4) have shown that during acute rejection of allogeneic skin in mice, >90% of the recipients’ T cells recognize donor MHC Ags (direct pathway), whereas <10% of T cells respond to donor-derived MHC peptides presented via the indirect pathway.

Classic experiments have revealed that the indirect pathway by itself is sufficient to trigger acute rejection of rodent skin grafts (8). MHC class I-deficient mice (that lack CD8+ T cells) acutely rejected allogeneic skin grafts from MHC class II-deficient donors. In this model, CD4+ T cells only recognized alloantigens via the indirect pathway through presentation by recipient MHC II+ APC (8). The controversial results on the relative contributions of indirect vs direct pathways of allore cognition may reflect the participation of both mechanisms in the early phases of acute rejection. Another explanation is that in those models in which the direct pathway plays a dominant role in acute rejection, its absence or blockade may allow the indirect pathway to play a more prominent role in activation of allo-specific T cells. In fact, in the case of direct pathway deficiency (as with heart grafts from Flt3L−/− donors), kidney grafts depleted of DC are rejected significantly later (by the indirect pathway) than nondepleted grafts (20). Our results in the skin rejection model also suggest that if the direct pathway of allore cognition is defective or less potent, recipient DC may act as instigators of acute rejection.

In conclusion, the outcome of transplantation of hearts from donors with genetic deficiency in interstitial DC supports the importance of the direct pathway of allore cognition in acute vascularized organ rejection. This view is reinforced by the normal kinetics of acute rejection of wt grafts by Flt3L−/− B6 recipients. Severe deficiency of donor APC was not sufficient to prevent the ultimate rejection of the grafts. Retention of development, but deficiency in maturation, of epidermal LC in Flt3L−/− B6 mice in the virtual absence of dermal DC development confirm the comparative importance of the former APC in acute skin graft rejection and/or suggest a role for the indirect pathway of allore cognition during acute skin graft rejection in the absence of a fully competent direct pathway of alloantigen presentation and recognition.

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