

Distinct Subsets of Dendritic Cells Regulate the Pattern of Acute Xenograft Rejection and Susceptibility to Cyclosporine Therapy¹

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We determined whether distinct subclasses of dendritic cells (DC) could polarize cytokine production and regulate the pattern of xenograft rejection. C57BL/6 recipients, transplanted with Lewis rat hearts, exhibited a predominantly CD11c⁺CD8 α ⁺ splenic DC population and an intragraft cytokine profile characteristic of a Th1-dominant response. In contrast, BALB/c recipients of Lewis rat heart xenografts displayed a predominantly CD11c⁺CD8 α ⁻ splenic DC population and IL-4 intragraft expression characteristic of a Th2 response. In addition, the CD11c⁺IL-12⁺ splenic DC population in C57BL/6 recipients was significantly higher than that in BALB/c recipients. Adoptive transfer of syngeneic CD8 α ⁻ bone marrow-derived DC shifted a Th1-dominant, slow cell-mediated rejection to a Th2-dominant, aggressive acute vascular rejection (AVR) in C57BL/6 mice. This was associated with a cytokine shift from Th1 to Th2 in these mice. In contrast, transfer of CD8 α ⁺ bone marrow-derived DC shifted AVR to cell-mediated rejection in BALB/c mice and significantly prolonged graft survival time from 6.0 \pm 0.6 days to 14.2 \pm 0.8 days. CD8 α ⁺ DC transfer rendered BALB/c mice susceptible to cyclosporine therapy, thereby facilitating long-term graft survival. Furthermore, CD8 α ⁺ DC transfer in IL-12-deficient mice reconstituted IL-12 expression, induced Th1 response, and attenuated AVR. Our data suggest that the pattern of acute xenogeneic rejection can be regulated by distinct DC subsets. *The Journal of Immunology*, 2006, 176: 3525–3535.

It has been known that the recipient genetic background plays an important role to transplant Ags. We previously reported that C57BL/6 (Th1-predisposition) and BALB/c (Th2-predisposition) mice had very different immune responses to Lewis rat heart xenografts (1). BALB/c mice rapidly rejected Lewis heart xenografts with aggressive acute vascular rejection (AVR),³ while C57BL/6 mice rejected the same xenografts with less aggressive cell-mediated rejection (CMR) (1). Furthermore, we found that cytokines play a key role in determining the pattern of xenograft rejection. Unlike allografts, Th1 cytokines such as IFN- γ and IL-12 are beneficial to xenograft survival because of their negative effect on AVR. In recent findings, we demonstrated that distinct cytokine profiles expressed by different mouse strains played an essential role in regulating the pattern of rejection and outcome of cyclosporin A (CsA)/rapamycin therapy in allotransplantation (2). These data suggest that the cytokine profile appears to be a key

factor in determining the pattern of rejection and susceptibility to immunosuppressive agents. The factors that regulate the polarization of cytokine profiles in these two murine strains following xenotransplantation are not clear. In this study, we used the same xenotransplantation model to extend our previous observations (1) and to determine the factors that direct distinct immune responses to a xenoantigen.

Dendritic cells (DC) are bone marrow (BM)-derived professional APC with the unique ability to both initiate and regulate immune responses (3). DC provide a critical link between the innate and adaptive immune responses. In the human, type 1 IFN-producing cells, also known as plasmacytoid DC differentiate into a unique type of mature DC, which directly regulate the function of T cells and thus link innate and adaptive immune responses when they are exposed to an Ag (4). In mice, DC can be broadly subdivided into at least three subsets in most lymphoid and non-lymphoid tissues: CD8 α ⁺ DC (5, 6), CD8 α ⁻ DC (5, 6), and B220 DC (7–9). Although CD8 α ⁺ DC and CD8 α ⁻ DC are reported to be efficient stimulators of T cell activation and proliferation, there is little evidence that B220 DC are important for the initiation of adaptive immunity. B220 DC are, however, the primary source of type 1 IFNs following exposure to viral Ags (7–10).

DC provide T cells with Ag-specific information that reflects the nature of the pathogen or irritant and affected tissue (signal 1). In addition, DC provide costimulatory signals (signal 2) that are necessary for efficient T cell priming. More recently, DC have been reported to provide a third signal that polarizes the Th response (11). CD8 α ⁺ DC have been shown to be the primary source of IL-12, sometimes referred to as the third signal necessary for T cell priming and a key determinant of type 1 T cell differentiation. Moreover, Ag-pulsed CD8 α ⁺ DC have been shown to sensitize naive T lymphocytes to induce a dominant Th1 response. In contrast,

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³ Abbreviations used in this paper: AVR, acute vascular rejection; BM, bone marrow; CMR, cell-mediated rejection; CsA, cyclosporin A; DC, dendritic cell; KO, knockout; POD, postoperative day; xAb, xenoreactive Ab.

immunization with CD8 α ⁻ DC that typically produce lower levels of IL-12 induce a predominant Th2 response (12, 13). In addition, selective expansion of either the CD8 α ⁺ or CD8 α ⁻ DC subset in vivo results in increased Ag-specific Ab titers with distinct isotype profiles (14). These data suggest that DC subsets initiate T cell responses and direct the type of adaptive immunity that ensues as a result of Th-polarizing cytokines. The nature of the Ag-presented mouse strain, maturation of DC, tissue of DC origin, and cytokines (12, 15) are all significant factors that impact the differentiation and polarization of Th cells. Because of the unique features of CD8 α ⁺ and CD8 α ⁻ DC in regulating T cell responses, we focused on evaluating these two subsets of DC in a mouse xenotransplantation model for this study.

Until now, the role of DC subsets in the manipulation of immune responses following xenotransplantation has not been investigated. As a result of the ubiquitous role of DC in controlling immunity, we hypothesized that DC subsets regulate the pattern of acute xenograft rejection, in part, through polarizing T cell cytokine profiles. In this study, we compared DC subsets, cytokine and Ig profiles, and graft rejection patterns in two well-defined mouse strains, BALB/c and C57BL/6, that had received Lewis rat cardiac xenografts. Adoptive transfer of syngeneic CD8 α ⁺ or CD8 α ⁻ DC to BALB/c or C57BL/6 mice was performed to determine whether transfer of a specific DC subset would change the pattern of xenograft rejection. We showed that transfer of CD8 α ⁺ BM-DC attenuated AVR and prolonged graft survival time in BALB/c mice. Furthermore, adoptive transfer of CD8 α ⁺ DC was found to act synergistically with CsA and to protect xenografts once rejection was shifted from AVR to CMR.

Materials and Methods

Animals and immunosuppressive drug

Two-week-old Lewis rats (RT1¹) (25–30 g) were used as heart donors. Male adult BALB/c (H-2^b) mice, wild-type C57BL/6 (H-2^b) mice, and IL-12p40 knockout (KO) C57BL/6 mice (C57BL/6-IL-12btm1jm) (16), weighing 25–30 g, were used as recipients (The Jackson Laboratory). There were eight mice in each group. In the groups receiving immunosuppression, the recipients were given daily s.c. injections of CsA (15 mg/kg) from day 0 until the study end point or graft rejection. Animals were housed under conventional conditions at the Animal Care Facility of the University of Western Ontario and were cared for in accordance with the guidelines established by the Canadian Council on Animal Care (17).

Heterotopic cardiac transplantation

Intra-abdominal heterotopic cardiac transplantation was performed as previously described by Corry et al. (18). Briefly, a median sternotomy was performed on the donor, and the heart graft was slowly perfused in situ with 1.0 ml of cold heparinized Ringer's lactate solution through the inferior vena cava and aorta before the superior vena cava and pulmonary veins were ligated and divided. The ascending aorta and pulmonary artery were transected and the graft was removed from the donor. The graft was then revascularized with end-to-side anastomoses between the donor's pulmonary artery and the recipient's inferior vena cava as well as the donor's aorta and the recipient's abdominal aorta using 11-0 nylon suture. The heartbeat of the graft was monitored daily by direct abdominal palpation. The degree of pulsation was scored as: A, beating strongly; B, noticeable decline in the intensity of pulsation; or C, complete cessation of pulsation. When the heartbeat was no longer palpable, the graft was removed for routine histology, immunohistochemistry, and RT-PCR. Serum samples were collected, and circulating anti-donor Abs were evaluated by flow cytometry.

Graft histology

Tissue samples were removed at necropsy and fixed in 10% buffered formaldehyde. Specimens were then embedded in paraffin, sectioned, and stained with either H&E or martius scarlet blue. Microscopic tissue sections were examined, in a blinded fashion for severity of rejection by a pathologist (B.G). Criteria for graft rejection included the presence of vasculitis, thrombosis, hemorrhage, and lymphocyte infiltration. These changes were scored as: 0, no change; 1, minimal change; 2, mild change; 3, moderate change; or 4, marked change.

Immunohistochemistry

Four-micrometer sections were cut from tissue samples embedded in Tissue-Tek OCT gel (Skura Finetek, Torrance, CA), mounted on gelatin-coated glass microscope slides, and stained by a standard indirect avidin-biotin immunoperoxidase staining method using an Elite Vectastain ABC kit (Vector Laboratories). Specimens were stained for CD4⁺ and CD8⁺ cells with biotinylated rat anti-mouse CD4 mAb (clone YTS 191.1.2; Cedarlane Laboratories) or anti-mouse CD8 mAb (clone 53-6; BD Biosciences), respectively. Mouse IgG and IgM deposition in grafts was detected using biotin-conjugated goat anti-mouse-IgG and goat anti-mouse-IgM (Cedarlane laboratories). Intra-graft IgG1 and IgG2a deposition was detected using a biotin-conjugated rat anti-mouse IgG1 mAb (clone A85-1; BD Biosciences) and rat anti-mouse IgG2a mAb (clone R19-15; BD Biosciences). Slides were washed with PBS between steps, and examined using light microscopy. Negative controls were performed by omitting the primary Abs. The sections of immunoperoxidase staining for intra-graft IgG, IgG1, IgG2a and IgM deposition were graded from 0 to 4+ according to the staining intensity: 0, negative; 1+, equivocal; 2+, weak staining; 3+, moderate staining; and 4+, very intensive staining. The sections of immunoperoxidase staining for intra-graft infiltration of CD4⁺ and CD8⁺ T cells were analyzed by counting the number of all positively stained cells in the whole section and dividing by the section area examined.

Generation and purification of BM-derived DC

Generation of DC from BM was performed as originally described by Inaba et al. (19) with modifications (20). Briefly, BM cells were flushed from the femurs and tibias of naive wild-type BALB/c and C57BL/6 mice, washed, and cultured at a concentration of 2×10^6 cells/well in 24-well plates (Corning) in 2 ml of RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FCS (Invitrogen Life Technologies), 100 U/ml penicillin, 100 μ g/ml streptomycin, 50 μ M 2-ME (Invitrogen Life Technologies), 10 ng/ml recombinant murine GM-CSF (PeproTech), and 10 ng/ml IL-4 (PeproTech). Nonadherent cells were removed after 48 h of culture, and fresh medium was added every 48 h. After 6 days of culture, the nonadherent cells (containing at least 90% DC, as assessed by morphology and specific staining, with anti-CD11c mAb N418) were labeled with anti-mouse CD8 α mAb-conjugated superparamagnetic microBeads (Miltenyi Biotec), and CD11c⁺CD8 α ⁺ and CD11c⁺CD8 α ⁻ cells were isolated and purified by passage through a magnetic column (21). Mouse BM has few CD8 α ⁺ DC when cultured as described. Thus, to obtain sufficient purified CD8 α ⁺ BM-DC for adoptive transfer, eight naive BALB/c mice were required to culture 6×10^6 CD8 α ⁺ DC and 126×10^6 CD8 α ⁻ DC. Similarly, eight C57BL/6 mice were required to generate 12×10^6 CD8 α ⁺ DC and 65×10^6 CD8 α ⁻ DC in vitro.

Adoptive DC transfer

The CD11c⁺CD8 α ⁺ and CD11c⁺CD8 α ⁻ DC purified from syngeneic wild-type BALB/c and C57BL/6 mice were adoptively transferred into wild-type BALB/c and wild-type or IL-12KO C57BL/6 recipients, respectively, by i.v. injection. Each recipient received 1×10^7 BM-DC. Eight animals were included in each group. One day after adoptive DC transfer, heart transplantation was performed in these recipient mice.

Flow cytometry

Phenotypic analysis of isolated or cultured DC was performed on a FACSCalibur flow cytometer (BD Biosciences). DC were labeled with FITC-conjugated anti-mouse CD11c mAb (BD Biosciences) and PE-conjugated anti-mouse CD8 α mAb (BD Biosciences). For detection of IL-12 expression, cells were permeabilized with a cell permeabilization kit (Caltag Laboratories) and labeled with PE-conjugated anti-mouse IL-12 mAb (BD Biosciences). All flow cytometric analyses were performed using isotype-matched, irrelevant Ig as negative controls (Cedarlane Laboratories).

The circulating anti-donor IgM and IgG (including IgG1 and IgG2a isotypes) were evaluated in the recipient serum by flow cytometry (22, 23). In brief, Lewis rat splenocytes were isolated and incubated at 37°C for 30 min with sera from all experimental groups. To stain for anti-donor IgG, IgG1, IgG2a, and IgM, the cells were washed and incubated with FITC-conjugated goat Ab specific for the Fc portion of mouse IgG or with PE-conjugated goat Ab specific for the μ -chain of mouse IgM (Jackson ImmunoResearch Laboratories), FITC-conjugated goat anti-mouse IgG1 (Caltag Laboratories), or with FITC-conjugated goat anti-mouse IgG2a (Caltag Laboratories). After 1 h of staining at 4°C, the cells were washed with PBS, resuspended at 5×10^6 /ml, and analyzed by flow cytometry. Data are expressed as mean channel fluorescence intensity (MFI), which represents the intensity of Ab binding. Naive sera from BALB/c, C57BL/6, or IL-12KO C57BL/6 mice were used as negative controls.

RT-PCR

To measure relative differences in cytokine transcript levels between cardiac transplants, we used a semiquantitative RT-PCR technique as previously described (24). Total RNA was obtained from grafted or normal tissue using TRIzol Reagent (Invitrogen Life Technologies) according to the manufacturer's specifications. Total RNA was reverse transcribed using a first-strand cDNA synthesis kit (Pharmacia). PCR amplification was conducted in 25- μ l reactions using PCR Supermix High Fidelity (10790-020; Invitrogen Life Technologies), which was prepared for multiple reactions. Each PCR consisted of 1 μ l of cDNA, 100 nM of each GAPDH primer, a reference gene, and 200 nM of each pair of specific primers (IFN- γ , sense AGC TCT GAG ACA ATG AAC GCT ACA C and antisense ACC TGT GGG TTG TTG ACC TCA AAC; IL-12, sense AAA CAG TGA ACC TCA CCT GTG ACA C and antisense TTC ATC TGC AAG TTC TTG GGC G; IL-2, sense ACA TTG ACA CTT GTG CTC CGT GTC and antisense TTG AGG GCT TGT TGA GAT GAT GCT; IL-4, sense AGC TAG TTG TCA TCC TGC TCT TC and antisense AGC ATG GTG GCT CAG TAC TACG; and IL-10, sense TGC TAT GCT GCC TGC TCT TAC TGA C and antisense AAT CAC TCT TCA CCT GCT CCA CTG). The PCR was amplified as follows: denaturation at 95°C for 3 min followed by 8 cycles at 95°C for 1 min, annealing of the primers at 60°C, at 72°C for 1 min, followed by 30 cycles at 95°C for 1 min, annealing of primers at 50°C for 1 min, at 72°C for 2 min, and a final extension at 72°C for 5 min. After PCR amplification, a standardized volume of each PCR product was subjected to electrophoresis using a 3% agarose gel. The PCR product size of 376 bp is for IFN- γ , 451 bp for IL-12, 417 bp for IL-2, 423 bp for IL-4, and 249 bp for GAPDH. The cytokine PCR product was compared with the GAPDH PCR product as an internal control for the same cDNA using the same master mixture prepared concomitantly. In addition, to differentially compare cytokine transcription levels between experimental groups, we used densitometry to measure the ratio of each cytokine RT-PCR product compared with standardized and titratable levels of GAPDH.

Statistical analysis

Data are generally reported as mean \pm SD, unless otherwise specified. Xenograft survival among experimental groups was compared using the rank log test. Histological and immunohistological findings of intragraft Ab deposition were analyzed using the ANOVA on rank. Immunohistological findings of intragraft cellular infiltration, flow cytometric data, and RT-PCR data were analyzed using one-way ANOVA. Differences with p values $<$ 0.05 were considered significant.

Results

BALB/c and C57BL/6 mice acquire distinct DC subsets after xenografting

We have previously demonstrated that Lewis rat hearts in BALB/c mice were rejected in 6.0 ± 0.6 days, while the same grafts lasted up to 20.6 ± 4.9 days in C57BL/6 mice (1). To investigate the role of DC in regulating the immune response to xenoantigens, we first studied DC subsets in BALB/c and C57BL/6 mice receiving Lewis rat cardiac transplants at a uniform time point, postoperative day (POD) 6. We found that C57BL/6 recipients transplanted with Lewis rat hearts displayed a predominant CD11c⁺CD8 α ⁺ splenic DC subset (Fig. 1A), as evidenced by a high ratio of CD8 α ⁺:CD8 α ⁻ DC on POD6 (Fig. 1B). Conversely, BALB/c recipients exhibited a predominant CD11c⁺CD8 α ⁻ splenic DC subset (Fig. 1A), evidenced by a low CD8 α ⁺:CD8 α ⁻ DC ratio on POD6 ($p <$ 0.01, Fig. 1B). As compared with naive animals, there was no change in the CD8 α ⁺:CD8 α ⁻ DC ratio in BALB/c mice after xenografting; however, a dramatic change in this ratio was observed in transplanted C57BL/6 recipients ($p <$ 0.01; Fig. 1B). We also found that the total number of CD11c⁺DC in spleen was $18.3 \pm 1.1 \times 10^6$ and $28.4 \pm 0.7 \times 10^6$ in BALB/c and C57BL/6 xenograft recipients, respectively. These DC contained $5.6 \pm 0.4 \times 10^6$ CD11c⁺CD8 α ⁺ cells and $12.7 \pm 1.0 \times 10^6$ CD11c⁺CD8 α ⁻ cells in BALB/c mice, as well as $20.6 \pm 0.9 \times 10^6$ CD11c⁺CD8 α ⁺ cells and $7.7 \pm 0.4 \times 10^6$ CD11c⁺CD8 α ⁻ cells in C57BL/6 mice. To note, the number of splenic CD8 α ⁺ DC in C57BL/6 mice was significantly higher than that in BALB/c mice after xenografting ($p <$ 0.05). In addition, the splenic

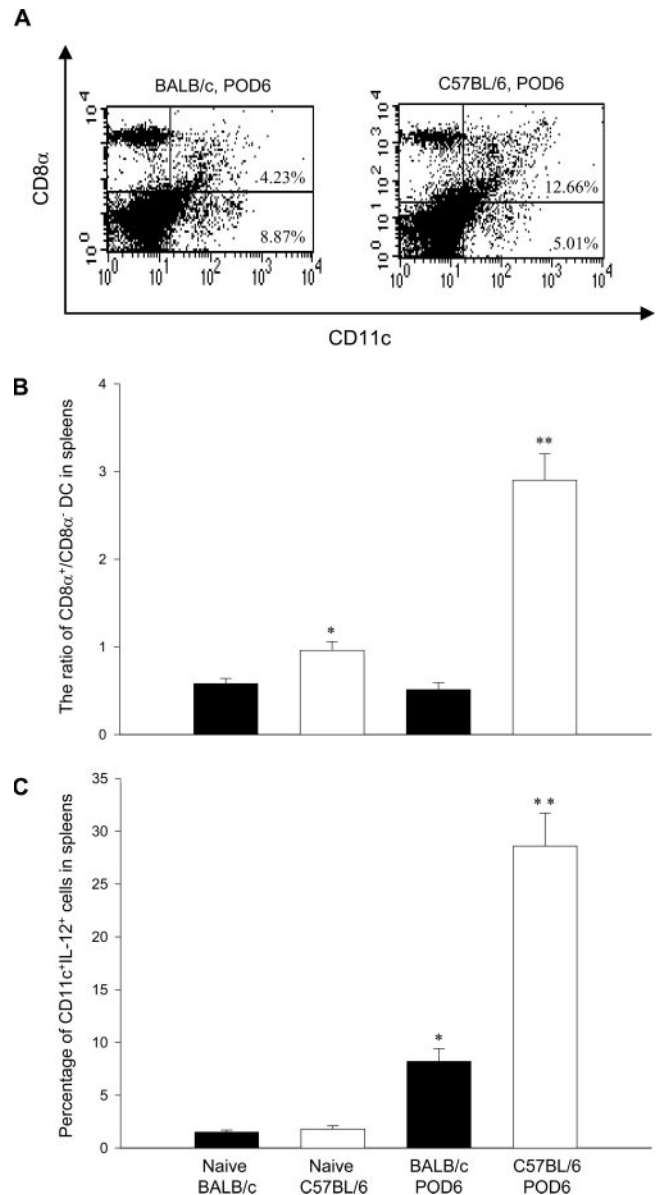


FIGURE 1. Comparison of distinct subsets of DC in BALB/c and C57BL/6 mice on POD6. Splenocytes were isolated from naive or transplanted BALB/c and C57BL/6 mice on POD6 as described in *Materials and Methods*. Phenotypic analysis of DC subtypes was performed using flow cytometry. DC were stained by FITC-conjugated anti-mouse CD11c mAb and PE-conjugated anti-mouse CD8 α mAb or PE-conjugated anti-mouse IL-12 mAb. The polarity of DC subsets in BALB/c and C57BL/6 recipients of xenografts is shown in a representative dot plot analysis (A). The ratio of splenic CD11c⁺CD8 α ⁺ cells:CD11c⁺CD8 α ⁻ cells (B) as well as the expression of intracellular IL-12 in splenic DC (C) were compared in these two strains of mice. Results are mean \pm SD of six independent experiments. B, Naive C57BL/6 mice vs naive or transplanted BALB/c mice: *, $p <$ 0.05; transplanted C57BL/6 recipients on POD6 vs naive BALB/c or C57BL/6 mice and transplanted BALB/c recipients at the same day: **, $p <$ 0.01. C, Transplanted BALB/c recipients vs naive BALB/c and C57BL/6 mice: *, $p <$ 0.01; transplanted C57BL/6 recipients on POD6 vs transplanted BALB/c recipients at the same day: **, $p <$ 0.01.

CD11c⁺IL-12⁺ cell population increased after transplantation in both C57BL/6 and BALB/c recipients. Importantly, CD11c⁺IL-12⁺ cells were significantly more abundant in C57BL/6 recipients compared with BALB/c mice on POD6 ($p <$ 0.01; Fig. 1C). Furthermore, to determine whether the polarity of DC subsets is also

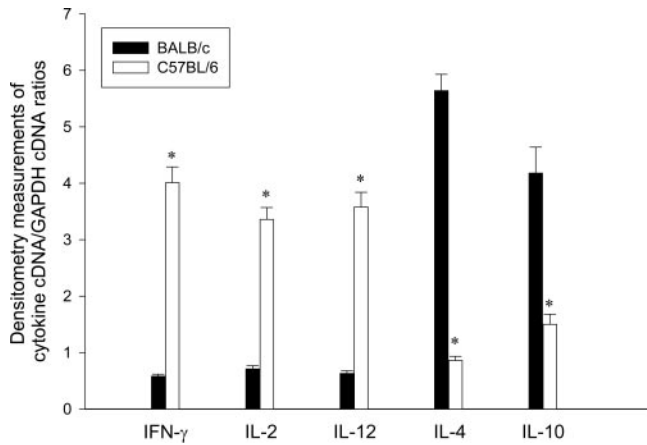


FIGURE 2. Intra-graft cytokine expression in BALB/c and C57BL/6 mouse recipients on POD6. Heart grafts were harvested from transplanted recipients on POD6. Semiquantitative RT-PCR was performed for intra-graft mRNA expression of IL-2, IFN- γ , IL-12, IL-4, and IL-10. GAPDH was used to ensure amplification of comparable quantities of cDNA. Data shown are mean densitometry measurements for cytokine cDNA:GAPDH cDNA ratios. Results are mean \pm SD of six independent experiments. C57BL/6 recipients on POD6 vs BALB/c recipients at the same day: *, $p < 0.01$.

found in lymph nodes, we measured the DC number in abdominal lymph nodes by flow cytometry. We found that the similar polarity of DC subsets was found in abdominal lymph nodes, even though the total number of CD11c⁺ DC in lymph nodes was significantly less than that in spleens. For example, abdominal lymph nodes in BALB/c and C57BL/6 xenograft recipients have total numbers of $(0.11 \pm 0.015) \times 10^6$ and $(0.16 \pm 0.009) \times 10^6$ CD11c⁺DC, respectively. These DC contained $(0.037 \pm 0.004) \times 10^6$ CD11c⁺CD8 α^+ cells and $(0.069 \pm 0.013) \times 10^6$ CD11c⁺CD8 α^- cells in BALB/c mice, as well as $(0.11 \pm 0.012) \times 10^6$ CD11c⁺CD8 α^+ cells and $(0.047 \pm 0.004) \times 10^6$ CD11c⁺CD8 α^- cells in C57BL/6 mice. Interestingly, the number of CD8 α^+ DC in the lymph nodes of C57BL/6 mice was significantly higher than that in BALB/c mice after xenografting ($p < 0.05$). These data indicate that these two mouse strains exhibit significant differences in both cell number and ratio of DC subsets after receiving the same type of xenograft.

Opposing cytokine profiles are present in BALB/c and C57BL/6 recipient mice following Lewis rat cardiac xenografting

To determine whether different DC populations would differentially affect the Th cytokine profiles following xenotransplantation,

we compared BALB/c and C57BL/6 intra-graft cytokine mRNA expression on day 6 after Lewis rat heart transplantation. We found that gene transcripts for Th1 cytokines such as IFN- γ , IL-2, and IL-12 were significantly higher in C57BL/6 recipients than those in BALB/c mice ($p < 0.01$; Fig. 2). In contrast, the Th2 cytokine transcripts, as evidenced by IL-4 and IL-10, were much higher in BALB/c mice than in C57BL/6 mice ($p < 0.01$; Fig. 2). These data suggest that following exposure to xenoantigen, different DC subsets may play important roles in regulating Th1/Th2 cytokine profiles.

Adoptive transfer of CD8 α^- DC shifts CMR to AVR through manipulation of cytokine profiles in C57BL/6 recipients of Lewis rat hearts

To further determine whether a causal relationship exists between DC subsets and recipient immune responses to xenoantigen, we adoptively transferred syngeneic CD8 α^- or CD8 α^+ BM-DC (1×10^7 cells/mouse) wild-type, syngeneic C57BL/6 recipients. One day after BM-DC transfer, C57BL/6 recipients received Lewis rat heart transplants. To detect whether CD8 α^- DC transfer would change the polarity of DC subsets in C57BL/6 recipients, we sacrificed three recipients 3 days after DC transfer (e.g., 2 days after heart xenografting) and did find an $\sim 30\%$ increase in cell numbers of splenic CD11c⁺CD8 α^- cells in C57BL/6 recipients as compared with the same recipients without DC transfer (data not shown). After CD8 α^- DC transfer, graft survival time in C57BL/6 mice was significantly shortened from 20.6 ± 4.9 days to 9.5 ± 0.6 days ($p < 0.01$; Table I). In contrast, CD8 α^+ DC transfer did not accelerate graft rejection in C57BL/6 recipients. Moreover, there was no significant difference in xenograft survival between CD8 α^+ DC-transferred and non-DC-transferred animals (18.4 ± 1.3 days vs 20.6 ± 4.9 days, Table I). Based on these observations, we used POD9 as a uniform time point for comparison of immune changes in C57BL/6 recipients for all studies described hereafter.

At the end point of rejection (approximately POD9), transplanted heart grafts from C57BL/6 recipients infused with CD8 α^- DC exhibited a typical AVR profile with prominent interstitial hemorrhage, intravascular thrombosis (Fig. 3Ac), fibrin deposition (Fig. 3Af), and a few infiltrating CD4⁺ (Fig. 3, Ai and B) and CD8⁺ (Fig. 3, Al and B) cells. In contrast, at the same time point, grafts in C57BL/6 mice, with either no DC transfer or CD8 α^+ DC transfer, showed no evidence of AVR. Specifically, these mice had “clear” vessels (Fig. 3A, a, b, d, and e) and heavy CD4⁺ (Fig. 3A, g and h, and B) and CD8⁺ (Fig. 3A, j and k, and B) cell infiltration, a typical feature of CMR.

Table I. Survival time and histopathology of cardiac xenografts^a

Group (strain combinations)	Treatment	Mean Survival \pm SD (days)	Histopathology at End Point
1: Lewis rat \rightarrow C57BL/6	None	20.6 ± 4.9	AVR + CMR
2: Lewis rat \rightarrow C57BL/6	CD8 α^- DC transfer	9.5 ± 0.6^a	AVR
3: Lewis rat \rightarrow C57BL/6	CD8 α^+ DC transfer	18.4 ± 1.3	AVR + CMR
4: Lewis rat \rightarrow BALB/c	None	6.0 ± 0.6	AVR
5: Lewis rat \rightarrow BALB/c	CD8 α^+ DC transfer	14.2 ± 0.8^b	CMR
6: Lewis rat \rightarrow BALB/c	CD8 α^- DC transfer	5.8 ± 0.5	AVR
7: Lewis rat \rightarrow BALB/c	CsA	11.3 ± 1.2	AVR
8: Lewis rat \rightarrow BALB/c	CD8 α^+ DC transfer + CsA	80.6 ± 7.0^c	AVR + CMR (mild)
9: Lewis rat \rightarrow BALB/c	CD8 α^- DC transfer + CsA	12.8 ± 1.6	AVR
10: Lewis rat \rightarrow IL-12KO C57BL/6	None	6.2 ± 1.0	AVR
11: Lewis rat \rightarrow IL-12KO C57BL/6	CD8 α^+ DC transfer	16.4 ± 0.9^d	CMR
12: Lewis rat \rightarrow IL-12KO C57BL/6	CD8 α^- DC transfer	6.0 ± 0.8	AVR

^a Arrow, transplanted into: ^a, $p < 0.01$, compared with group 1; ^b, $p < 0.01$, compared with group 4; ^c, $p < 0.01$, compared with groups 5, 7, and 9; ^d, $p < 0.01$, compared with groups 10 and 12.

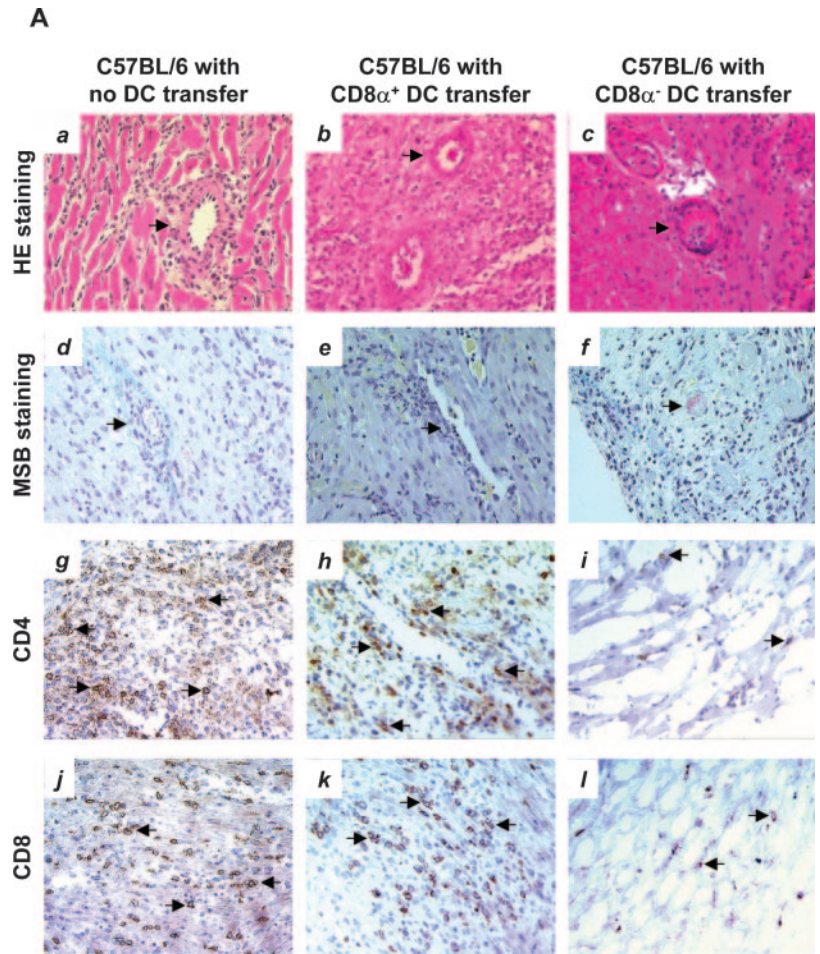
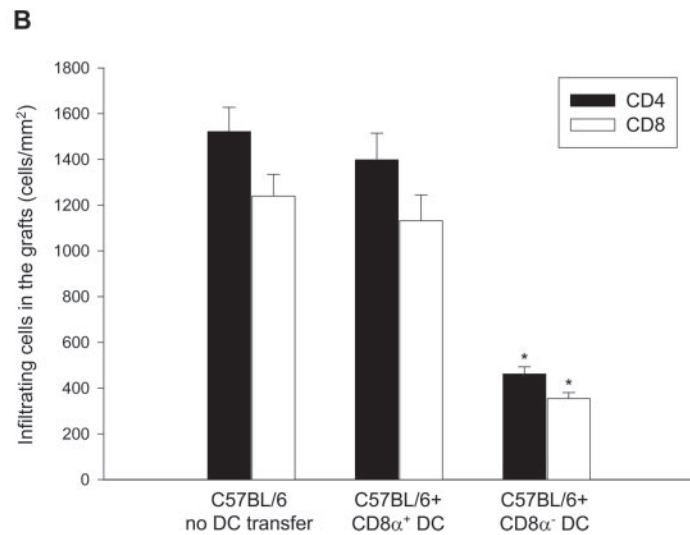


FIGURE 3. A, Histology and immunohistology for cellular infiltrates in cardiac xenografts of C57BL/6 mice with or without BM-DC transfer. Lewis rat heart grafts were harvested on POD9. H&E-stained or martius scarlet blue-stained paraffin sections of Lewis rat xenografts in C57BL/6 mice with no DC transfer (*a* and *d*), with CD8 α^+ DC transfer (*b* and *e*) or CD8 α^- DC transfer (*c* and *f*). Immunoperoxidase staining of frozen sections of CD4 $^+$ cells (*g*, *h*, and *i*) and CD8 $^+$ cells (*j*, *k*, and *l*) in C57BL/6 mice with or without BM-DC transfer. Arrows indicate positive staining. B, The number of infiltrating CD4 $^+$ and CD8 $^+$ cells were determined in the xenografts by counting all positively labeled cells in the section and dividing by the area assessed (cells/mm 2). Results are mean \pm SD of six experiments. C57BL/6 mice with CD8 α^- DC transfer vs C57BL/6 mice with no DC transfer or with CD8 α^+ DC transfer: *, $p < 0.01$.



In addition, our study revealed that on POD9, adoptive CD8 α^- DC transfer markedly increased intragraft IL-4 and IL-10 levels, but decreased IFN- γ , IL-2, and IL-12 levels in C57BL/6 recipients (Fig. 4). In contrast, CD8 α^+ DC transfer failed to change the intragraft expression of Th1 and Th2 cytokines in C57BL/6 recipients (Fig. 4). Combined, these results indicate that CD8 α^- DC transfer promotes a predominant Th2 response and facilitates AVR in xenotransplantation.

Change of Ab levels is associated with AVR after transfer of CD8 α^- DC

To determine whether the onset of AVR following adoptive transfer of CD8 α^- DC is associated with changes in xenoreactive Abs (xAbs), we measured serum levels of anti-donor Abs by flow cytometry on POD9. We found significantly higher levels of anti-donor xAbs, primarily IgG, were induced in C57BL/6 mice after

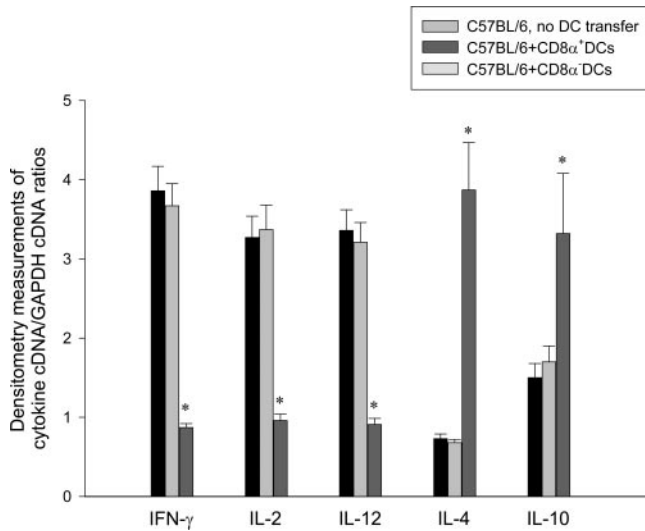


FIGURE 4. Intra-graft cytokine expression in wild-type C57BL/6 recipients with or without BM-DC transfer. Heart grafts were harvested from C57BL/6 recipients 9 days after xenografting. Semiquantitative RT-PCR was performed for intra-graft mRNA expression of IL-2, IFN- γ , IL-12, IL-4, and IL-10. GAPDH was used to ensure amplification of comparable quantities of cDNA. Data shown are mean densitometry measurements for cytokine cDNA:GAPDH cDNA ratios. Results are mean \pm SD of six experiments. C57BL/6 mice with CD8 α^- DC transfer vs C57BL/6 mice with no DC transfer or with CD8 α^+ DC transfer: *, $p < 0.01$.

CD8 α^- DC transfer when compared with C57BL/6 mice that had either CD8 α^+ DC transfer or no DC transfer ($p < 0.01$, Fig. 5A). In addition, serum levels of anti-donor IgM were slightly elevated in C57BL/6 recipients following transplantation as compared with naive animals ($p < 0.05$; Fig. 5A); however, there was no significant difference in IgM levels in these recipients with or without DC transfer (Fig. 5A).

Consistent with the cytokine profiles measured in the grafts, serum IgG isotypes switched to a Th2-predominant pattern, indicated by a high IgG1:IgG2a ratio after adoptive transfer of CD8 α^- DC in C57BL/6 mice ($p < 0.01$; Fig. 5B). Conversely, transfer of CD8 α^+ DC did not change IgG isoforms in these recipients. A low IgG1:IgG2a ratio, indicative of a Th1-predominant pattern, was shown in C57BL/6 recipients with either CD8 α^+ DC transfer or no DC transfer (Fig. 5B).

To further investigate the role of DC transfer in regulating xAb production, we investigated IgG, IgG1, IgG2a, and IgM deposition in grafts using immunostaining techniques. Table II shows that concordant with the circulating xAb levels, after CD8 α^- DC transfer, high levels of anti-donor IgG deposition, predominantly IgG1, was present in grafts on POD9. At the same time point, C57BL/6 mice with either CD8 α^+ DC transfer or no DC transfer were observed to have only mild xenograft deposition of anti-donor IgG with IgG2a-dominant Abs. Minimal IgM deposition was observed in all groups (Table II). These results indicate that Th2-promoting CD8 α^- DC can override a Th1 bias in C57BL/6 mice and cause IgG subclass switching from Th1 to Th2 dominant, thereby accelerating AVR.

Adoptive transfer of CD8 α^+ DC, but not CD8 α^- DC attenuates AVR and prolongs xenograft survival in BALB/c mice

To confirm the role of DC subsets in regulating the pattern of xenograft rejection, we adoptively transferred syngeneic CD8 α^+ DC or CD8 α^- DC (1×10^7 cells/mouse) into BALB/c recipients 1 day before Lewis heart transplantation. By sacrificing three re-

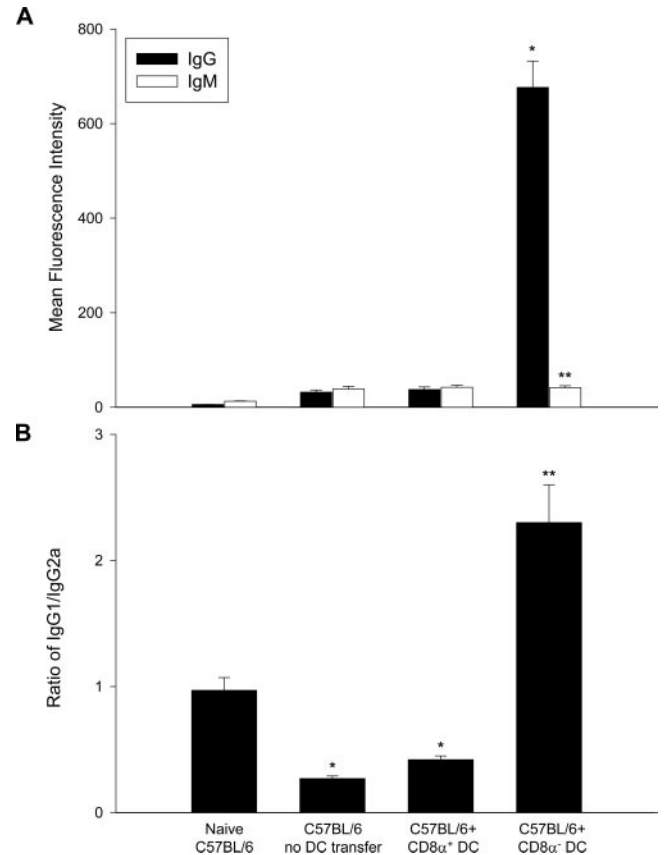


FIGURE 5. The levels of anti-donor Abs (A) and the ratio of IgG1:IgG2a (B) in the sera of C57BL/6 recipients with or without BM-DC transfer. Sera were collected from naive C57BL/6 mice and from C57BL/6 recipients 9 days after xenografting. Serum levels of anti-donor total IgG and IgM, as well as the ratio of IgG1:IgG2a, were determined by flow cytometry for comparison in different groups. Results are mean \pm SD of six independent experiments. A, C57BL/6 mice with CD8 α^- DC transfer vs naive C57BL/6 mice and C57BL/6 recipients with no DC transfer or with CD8 α^+ DC transfer: *, $p < 0.01$; C57BL/6 mice with CD8 α^- DC transfer vs naive C57BL/6 mice: **, $p < 0.05$. B, C57BL/6 recipients with no DC transfer or with CD8 α^+ DC transfer vs naive C57BL/6 mice: *, $p < 0.05$; C57BL/6 mice with CD8 α^- DC transfer vs naive C57BL/6 mice and C57BL/6 recipients with no DC transfer or with CD8 α^+ DC transfer: **, $p < 0.01$.

cipients 3 days after DC transfer, we found $\sim 30\%$ increase of splenic CD11c $^+$ CD8 α^+ cells in BALB/c recipients as compared to those without DC transfer (data not shown). With CD8 α^+ DC transfer to BALB/c mice, heart xenograft survival time was significantly prolonged from 6.0 ± 0.6 days to 14.2 ± 0.8 days (Table I). Accordingly, the graft rejection pattern had shifted from typical AVR (Fig. 6Aa) to predominantly CMR (Fig. 6Ac). In particular, after CD8 α^+ DC transfer, grafts had clear vessels (Fig. 6Ac) and massive CD4 $^+$ (Fig. 6B) and CD8 $^+$ (Fig. 6B) cell infiltration when compared with BALB/c recipients that had received either CD8 α^- DC or no DC ($p < 0.01$). Furthermore, adoptive CD8 α^- DC transfer neither prolonged graft survival nor changed the rejection pattern of BALB/c mice. These heart xenografts were rejected with predominant AVR (Fig. 6, Ae, and B) at 5.8 ± 0.5 days (Table I).

To further investigate the ability of distinct DC subsets to regulate the cytokine profile and Ab production, we analyzed intra-graft cytokine expression and xAb production in BALB/c recipients transferred with DC subsets at the same time point, POD6. In comparison to BALB/c recipients receiving either CD8 α^- DC transfer or no DC transfer, adoptive CD8 α^+ DC transfer facilitated

Table II. Grades for immunoperoxidase staining of cardiac xenograft frozen sections^a

Group (strain combinations)	Treatment	Date for Sample Collection (POD)	IgG	IgG1	IgG2a	IgM
1: Lewis rat → C57BL/6	None	9	2+	0	1+	1+
2: Lewis rat → C57BL/6	CD8 α ⁻ DC	9	3+	2+	1+	1+
3: Lewis rat → C57BL/6	CD8 α ⁺ DC	9	2+	0	1+	1+
4: Lewis rat → BALB/c	None	6	4+	2+	1+	1+
5: Lewis rat → BALB/c	CD8 α ⁺ DC	6	2+	0	1+	1+
6: Lewis rat → BALB/c	CD8 α ⁻ DC	6	4+	2+	1+	1+

^a Grade: 0, negative; 1+, equivocal; 2+, weak staining; 3+, moderate staining; 4+, very intensive staining.

a Th1 response, as evidenced by increased intragraft IFN- γ , IL-2 and IL-12 expression, concomitant with suppressed IL-4 and IL-10 expression ($p < 0.01$; Fig. 7A). In contrast, transfer of CD8 α ⁻ DC did not change the cytokine pattern in BALB/c mice, in which predominant Th2 cytokine profiles were present (Fig. 7A). In addition, serum anti-donor IgG levels and the ratio of IgG1:IgG2a in BALB/c recipients was significantly down-regulated after CD8 α ⁺ DC transfer when compared with the same recipients with no DC transfer or transferred with CD8 α ⁻ DC ($p < 0.01$; Fig. 7, B and C). Circulating anti-donor IgM levels were elevated in BALB/c recipients following transplantation, but no significant difference was observed in these recipients with or without DC transfer (Fig. 7B). Concordant with serum xAb data, down-regulated deposition of anti-donor IgG with IgG2a-dominant Ab was observed in grafts of CD8 α ⁺ DC-transferred BALB/c recipients on POD6. Strong deposition of anti-donor IgG, with IgG1 dominant Ab, was found in the xenografts of BALB/c recipients which had received either CD8 α ⁻ DC transfer or no DC transfer at the same time point (Table II). Moreover, only minimal IgM deposition was observed in the grafts of BALB/c recipients with or without DC transfer (Table II). These data indicate that adoptive transfer of CD8 α ⁺ DC prolongs xenograft survival by up-regulating Th1 cytokines and shifting AVR to CMR.

Adoptive transfer of CD8 α ⁺ DC renders BALB/c mice sensitive to CsA, thereby achieving long-term graft survival after Lewis rat heart xenografting

We previously demonstrated that treatment with CsA only marginally prolonged Lewis rat heart graft survival in BALB/c mice due to the inability of CsA to prevent AVR in this model (25). Since adoptive transfer of CD8 α ⁺ DC had switched the pattern of rejection from AVR to CMR in BALB/c mice following xenografting, we hypothesized that CsA may be an effective treatment, as it is widely reported to successfully treat CMR (25). To test this hypothesis, we treated BALB/c recipients of Lewis rat hearts with daily injections of CsA at 15 mg/kg after CD8 α ⁺ DC transfer. We found that adoptive CD8 α ⁺ DC transfer rendered BALB/c recipients sensitive to CsA treatment, thereby increasing graft survival to 80.6 ± 7.0 days (Table I). Graft histology in BALB/c mice with CD8 α ⁺ DC transfer showed significant attenuation of both humoral and cellular rejection characterized by mild changes in vasculitis and cellular infiltration (Fig. 6, Ad, and B) at the end points. In contrast, CsA was unable to prevent AVR in BALB/c recipients with either no DC transfer (Fig. 6, Ab, and B) or CD8 α ⁻ DC transfer (Fig. 6, Af, and B). Heart grafts in these recipients were rejected at 11.3 ± 1.2 days or 12.8 ± 1.6 days, respectively (Table I). These data suggest that CD8 α ⁺ DC transfer rendered BALB/c recipients sensitive to CsA, facilitating long-term xenograft survival.

IL-12 plays an essential role in inhibition of AVR by transferred CD8 α ⁺ DC

It is known that IL-12 is a key molecule produced by CD8 α ⁺ DC (21, 26). We previously reported that disruption of the IL-12 gene accelerated AVR following xenotransplantation, suggesting that IL-12 was able to inhibit AVR (1). Therefore, we hypothesized that IL-12 played an essential role in AVR inhibition by the transferred CD8 α ⁺ DC. To test this hypothesis, we transferred CD8 α ⁺ DC into IL-12KO mice receiving Lewis rat heart grafts to determine whether such a transfer would inhibit AVR in IL-12KO mice. Similarly, an ~30% increase of splenic CD11c⁺CD8 α ⁺ cells was found in IL-12KO mice 3 days after DC transfer when compared with those without DC transfer (data not shown). Lewis rat heart grafts in IL-12KO mice, with either no DC transfer or CD8 α ⁻ DC transfer, were rapidly rejected by aggressive AVR (Fig. 8A, a and c), with a mean survival time of 6.2 ± 1.0 days and 6.0 ± 0.8 days, respectively (Table I). In contrast, after the adoptive transfer of CD8 α ⁺ DC from wild-type C57BL/6 mice 1 day before heart transplantation, Lewis heart graft survival was significantly prolonged from 6.2 ± 1.0 days to 16.4 ± 0.9 days (Table I). Accordingly, the graft rejection pattern shifted from typical AVR (Fig. 8Aa) to predominantly CMR (Fig. 8Ab). Furthermore, in comparison to IL-12KO recipients receiving no DC transfer or CD8 α ⁻ DC transfer, adoptive CD8 α ⁺ DC transfer reconstituted IL-12 expression, increased IFN- γ and IL-2 levels, and decreased IL-4 and IL-10 levels in the grafts of the IL-12KO recipients ($p < 0.01$; Fig. 8B). In addition, serum anti-donor IgG levels and the IgG1:IgG2a ratio was significantly down-regulated by CD8 α ⁺ DC transfer in IL-12KO recipients and the Ab changes were indistinguishable from those in CD8 α ⁺ DC-transferred BALB/c recipients (data not shown). These results indicate that IL-12 plays an essential role in the inhibition of AVR by transferred CD8 α ⁺ DC.

Discussion

In this study, we compared the effects of two (CD8 α ⁺ and CD8 α ⁻) DC subsets on cytokine profiles, IgG isotypes, and xenograft rejection patterns in two well-defined mouse strains, BALB/c and C57BL/6, after Lewis rat cardiac transplantation. We found that CD8 α ⁺ DC and CD8 α ⁻ DC were present in both C57BL/6 and BALB/c mice. Specifically, we found that these mouse strains exhibited distinct profiles of CD8 α ⁺ DC and CD8 α ⁻ DC and induced opposite cytokine profiles, leading to different patterns of rejection. BALB/c recipients displayed a predominant CD11c⁺CD8 α ⁻ splenic cell population and AVR when transplanted with Lewis cardiac grafts. In contrast, C57BL/6 recipients exhibited a predominant CD11c⁺CD8 α ⁺ splenic cell population and developed CMR. The similar polarity of DC subsets was also found in abdominal lymph nodes of these two strains of mice. Adoptive transfer of syngeneic CD8 α ⁻ BM-DC changed cytokine

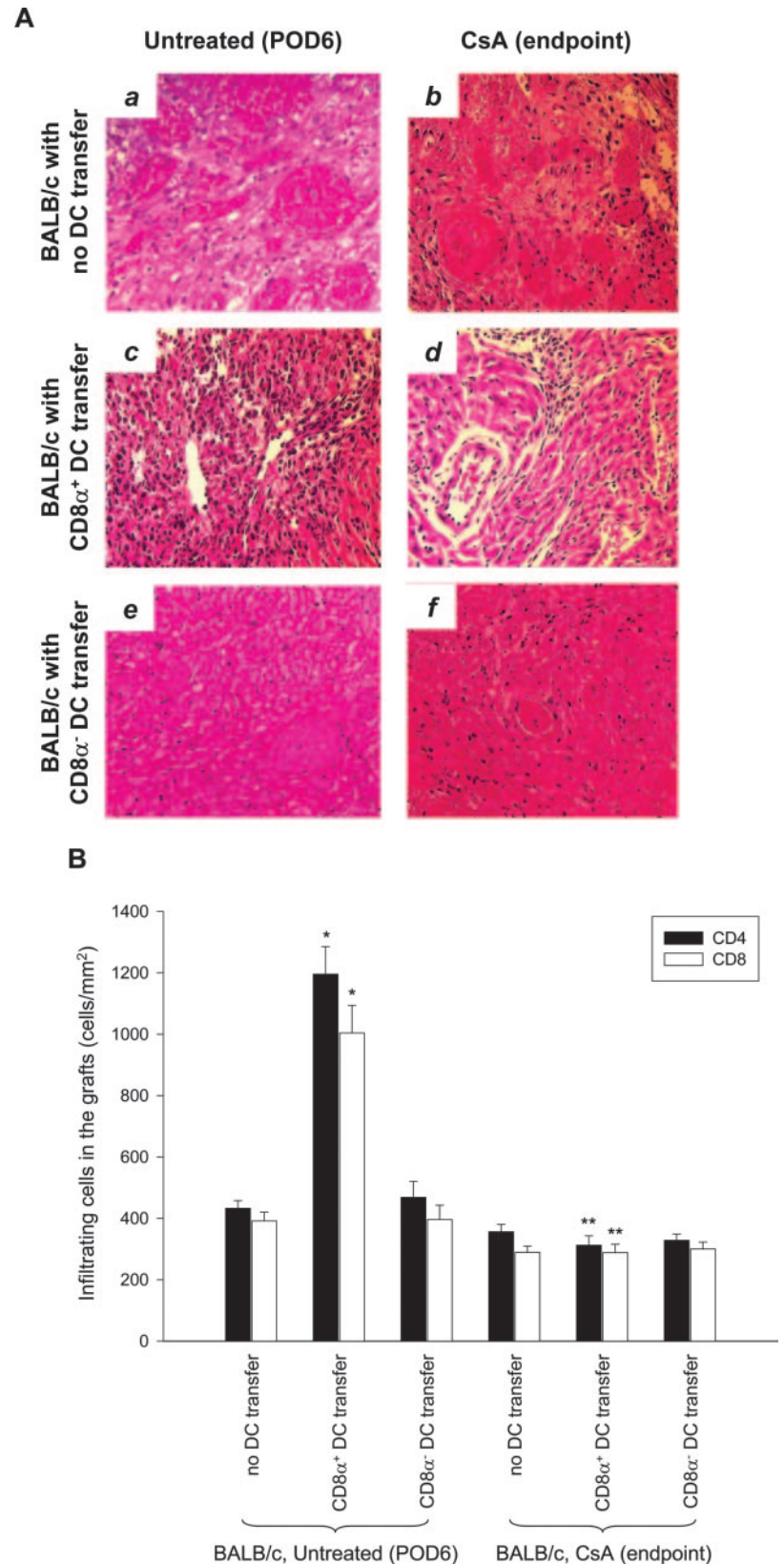


FIGURE 6. A, Histology of cardiac xenografts in untreated or CsA-treated BALB/c recipients with or without DC transfer. Heart grafts were harvested on POD6 in untreated recipients and at end point of rejection in CsA-treated recipients. H&E-stained paraffin sections of Lewis rat xenografts in untreated BALB/c mice without DC transfer (*a*), CD8 α^+ DC transfer (*c*), or CD8 α^- DC transfer (*e*), and in CsA-treated BALB/c recipients with or without DC transfer (*b*, *d*, and *f*). B, The infiltrating CD4 $^+$ and CD8 $^+$ cells were determined in the xenografts by counting all positively labeled cells in the section and dividing by the total area assessed (cells/mm²). Results are mean \pm SD of six independent experiments. Untreated BALB/c recipients with CD8 α^+ DC transfer vs untreated BALB/c recipients without DC transfer or with CD8 α^- DC transfer: *, $p < 0.01$; CD8 α^+ DC-transferred BALB/c mice with CsA treatment vs the same DC-transferred recipients without CsA therapy: **, $p < 0.01$.

profiles from Th1- to Th2-dominant and predominance of IgG isotype from IgG2a to IgG1, thereby accelerating AVR in C57BL/6 recipients, while transfer of CD8 α^+ BM-DC shifted the cytokine profile from Th2- to Th1-dominant and predominance of the IgG isotype from IgG1 to IgG2a, thereby attenuating AVR in BALB/c mice. Significantly, the CD8 α^+ DC transfer rendered BALB/c re-

cipients sensitive to CsA, which is effective in inhibiting CMR (3, 27–29), facilitating long-term xenograft survival. Finally, we demonstrated that IL-12 plays an essential role in inhibition of AVR by transfer of CD8 α^+ DC. These data indicate that DC subsets play an important role in regulating the cytokine and anti-donor Ig profiles and, as a result, the xenograft rejection patterns.

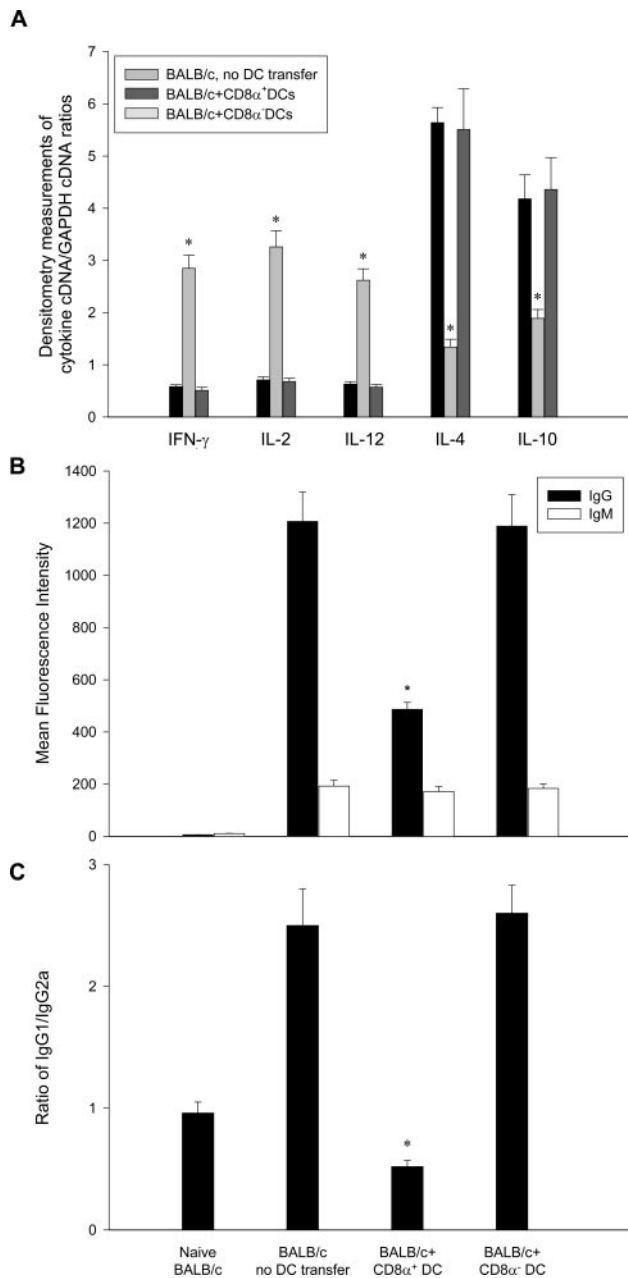


FIGURE 7. *A*, Intra-graft cytokine expression in wild-type BALB/c recipients with or without BM-DC transfer. Heart grafts were harvested from BALB/c recipients 6 days after xenografting. Semiquantitative RT-PCR was performed for intra-graft mRNA expression of IL-2, IFN- γ , IL-12, IL-4, and IL-10. GAPDH was used to ensure amplification of comparable quantities of cDNA. Data shown are mean densitometry measurements for cytokine cDNA:GAPDH cDNA ratios. Results are mean \pm SD of six independent experiments. BALB/c mice with CD8 α^+ DC transfer vs the same recipients without DC transfer or with CD8 α^- DC transfer: *, $p < 0.01$. *B* and *C*, The levels of anti-donor Abs (*B*) and the ratio of IgG1:IgG2a (*C*) in the sera of CD8 α^+ DC or CD8 α^- DC-transferred BALB/c mice. Mouse sera were harvested from naive and transplanted BALB/c mice on POD6. Serum levels of anti-donor IgG and IgM, as well as the ratio of IgG1:IgG2a, were determined by flow cytometry and were compared in different groups. Results are mean \pm SD of six independent experiments. BALB/c mice with CD8 α^+ DC transfer vs naive BALB/c mice and BALB/c recipients with no DC transfer or with CD8 α^- DC transfer: *, $p < 0.01$.

To investigate the role of DC subsets in regulating the pattern of xenograft rejection, we generated DC for the adoptive cell transfer by culture of mouse BM with GM-CSF plus IL-4. Although the

capacity to generate CD8 α^+ DC in vitro has been contentious (30, 31), Basak et al. (32) recently demonstrated that small numbers of the CD8 α^+ DC subset can also be generated in vitro using GM-CSF and IL-4. For our adoptive transfer experiments, we collected BM from large numbers of naive mice (i.e., ~ 12 mice per 1×10^7 CD8 α^+ DC). The purity of DC subsets was confirmed by flow cytometry (data not shown). As described by Steinman et al. (33), immature DC are particularly good at Ag uptake and processing, but for a productive T cell response they must differentiate to fully mature DC, which express high levels of cell surface MHC-Ag peptide complexes and costimulatory molecules. When cultured in the presence of GM-CSF and IL-4, the precursor DC proliferated and developed into immature DC over a period of 3–5 days (34, 35) and can be further matured by a subsequent 2-day culture period using different stimuli. In addition, the observation of Pulendran et al. (36) showed that both subsets of CD8 α^+ and CD8 α^- DC subsets contained immature or partially mature DC that could be induced to undergo maturation after activation. In this study, DC used for adoptive transfer were generated from 6-day cultures of mouse BM cells, which contain a mixture of 60% immature CD11c $^+$ CD86 $^-$ cells and 40% mature CD11c $^+$ CD86 $^+$ cells (data not shown). We believe that these DC have the abilities not only in uptaking and processing xenoantigens, but also in driving the T cell responses.

The findings that transfer of specific subsets of DC results in the polarity of immune responses in two strains of mouse recipient after xenografting are quite interesting. It has been reported that injected DC can be identified in the spleen following i.v. administration (37). In this study, we found that 3 days after DC transfer there was significant increase in cell numbers of either CD11c $^+$ CD8 α^+ or CD11c $^+$ CD8 α^- cells in the recipients' spleens (data not shown). These data suggest that exogenous DC may directly play an important role in regulating the recipient's immune responses. Ultimately, distinct subsets of DC regulate cytokine profiles and, accordingly, the pattern of graft rejection following xenotransplantation. The novelty of this study is the potential for therapeutic intervention by transferring distinct DC subsets, which allows the manipulation of recipient cytokine profiles. The pattern of rejection is changed, which augments the efficacy of immunosuppressive drugs such as CsA, eventually facilitating long-term xenograft survival in this model.

The molecular basis of Th cytokine networks regulation by DC subsets still needs to be elucidated. In this study, we found that intracellular IL-12 expression by the CD11c $^+$ IL-12 $^+$ DC population in C57BL/6 recipients (CD8 α^+ DC dominant) was significantly higher than that of DC from BALB/c recipients (CD8 α^- DC-dominant). In support of our findings, it has been reported that CD8 α^+ DC are the major source of IL-12. Specifically, Pulendran et al. (26) reported that a DC subclass containing a majority of CD8 α^+ cells produced very high levels of IL-12 (26). Maldonado-Lopez et al. (21) also demonstrated that CD8 α^+ DC produced high levels of IL-12 heterodimer upon stimulation, whereas CD8 α^- DC secreted little IL-12. Since IL-12 is well accepted to be a key factor in determining and promoting Th1 differentiation (38, 39), we speculated that administration of IL-12-producing CD8 α^+ DC might directly mediate the changes in the observed immune responses. Consistent with this hypothesis, we found that adoptive transfer of CD8 α^+ DC reconstituted IL-12 in IL-12KO mice and shifted immune responses from Th2 to Th1, thereby inhibiting AVR. This data clearly demonstrate that IL-12 plays an essential role in inhibition of AVR by CD8 α^+ DC. However, whether administration of exogenous IL-12 would achieve the same goal as adoptive transfer of CD8 α^+ DC remains to be further investigated. In addition, it has been demonstrated that the main pathway to

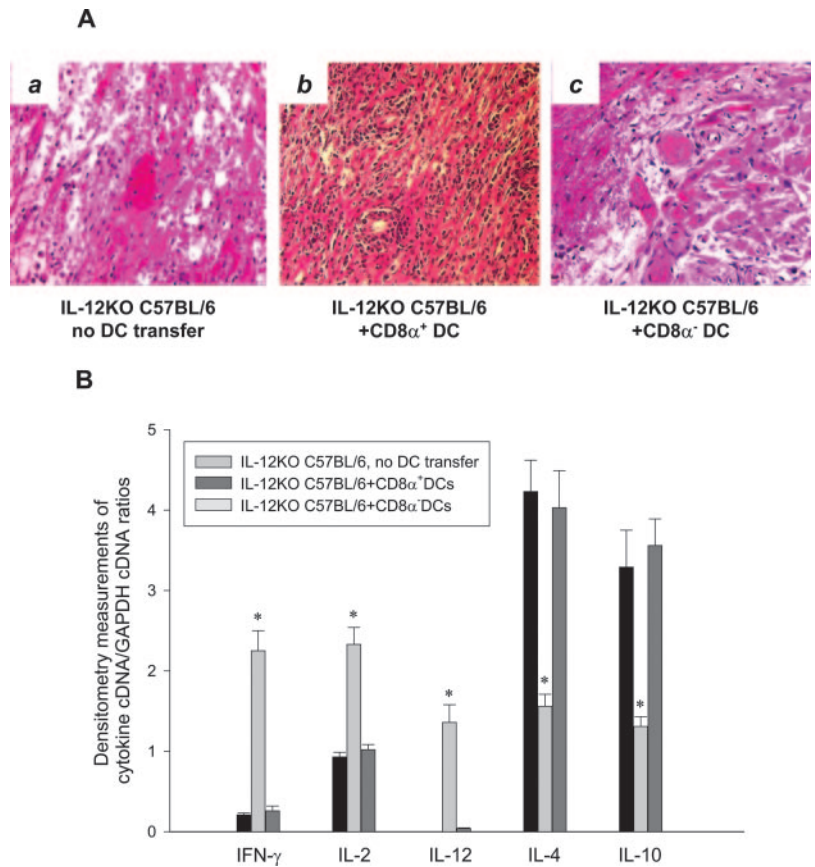


FIGURE 8. A, Histology of Lewis rat cardiac xenografts in IL-12KO C57BL/6 recipients with or without BM-DC transfer. Heart grafts were harvested on POD6. H&E-stained paraffin sections of xenografts in IL-12KO C57BL/6 mice with no DC transfer (a), or with CD8 α^+ DC transfer (b), or with CD8 α^- DC transfer (c). B, Intra-graft cytokine expression in IL-12KO C57BL/6 recipients with or without BM-DC transfer. Heart grafts were harvested on POD6. Semi-quantitative RT-PCR was performed for intra-graft expression of cDNA for IL-2, IFN- γ , IL-12, IL-4, and IL-10. GAPDH was used to ensure amplification of comparable quantities of cDNA. Data shown are mean densitometry measurements for cytokine cDNA: GAPDH cDNA ratios. Results are mean \pm SD of six independent experiments. IL-12KO C57BL/6 mice with CD8 α^+ DC transfer vs the same recipients with no DC transfer or with CD8 α^- DC transfer: *, $p < 0.01$.

induce IL-12 production by DC in acquired immunity is T cell dependent, and IL-12 level is up-regulated following DC interaction with T cells via CD40-CD40L signaling (40, 41). This may indicate that the changes in polarity of immune responses mediated by DC in this study require cellular interactions with T cells from either BALB/c or C57BL/6 mice.

DC subsets mediate different effects on T cells as a result of differences in the repertoire of costimulatory molecules expressed by the specific subset. After ligation of pattern recognition receptors, such as TLRs (42), CD86 and CD80 costimulatory molecules are critical for T cell activation (43, 44). Although controversial (45), it has been proposed that cellular interactions through CD86 promote a Th2-biased cytokine network, while interactions through CD80 promote Th1 cytokine production (46, 47). For example, in experimental allergic encephalomyelitis, pathology is associated with Th1-mediated autoreactivity. Administration of anti-CD80 Ab has been shown to reduce incidence and severity of this disease, while anti-CD86 Ab increased disease severity. This suggests that CD80 preferentially acts as a costimulator for a Th1-biased response, while CD86 induces a Th2 response (47). Recent publications using CD80/CD86-deficient mice support this notion (48, 49). Furthermore, we have recently demonstrated that the dependence of Th1/Th2 differentiation on CD80/CD86 also occurs in the context of xenotransplantation. By using either Ab against CD80/CD86- or CD80/CD86-deficient mice, we found that CD80 pathway drives Th1-dominant CMR and attenuates AVR, while the CD86 pathway drives Th2-dominant AVR. Most strikingly, indefinite rat heart xenograft survival can be achieved by channeling xenogeneic immune responses toward the CD80 pathway with the daily CsA therapy (50). Together with the findings from these studies, it might indicate that CD8 α^+ DC induce Th1 response and CMR through the CD80 pathway in C57BL/6 mice, while CD8 α^-

DC drive the Th2 response and induce AVR through the CD86 pathway in BALB/c mice following xenotransplantation.

In summary, we found that distinct subsets of DC regulate the pattern of acute xenograft rejection and susceptibility to CsA. These findings further explain the molecular and cellular mechanisms of genetic inherent difference to a xenotransplant Ag between BALB/c and C57BL/6 mice. Because of the heterogeneity of the human population, we speculate that genetically inherent response patterns to transplantation Ags also occur in humans. With the advance of genomics, it is important to evaluate the genetic predisposition on immune responses to transplant Ags and the susceptibility of the recipients to immunosuppressive drugs following transplantation.

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Disclosures

The authors have no financial conflict of interest.

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