Growth of Donor-derived Dendritic Cells from the Bone Marrow of Murine Liver Allograft Recipients in Response to Granulocyte/Macrophage Colony-stimulating Factor

By Lina Lu,* William A. Rudert,§ Shiguang Qian,* Delbert McCaslin,* Fumin Fu,* Abdul S. Rao,† Massimo Trucco,§ John J. Fung,* Thomas E. Starzl,* and Angus W. Thomson†

From the *Pittsburgh Transplantation Institute and Departments of Surgery, †Pathology, ‡Pediatrics, and §§Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Summary

Allografts of the liver, which has a comparatively heavy leukocyte content compared with other vascularized organs, are accepted permanently across major histocompatibility complex barriers in many murine strain combinations without immunosuppressive therapy. It has been postulated that this inherent tolerogenicity of the liver may be a consequence of the migration and perpetuation within host lymphoid tissues of potentially tolerogenic donor-derived ("chimeric") leukocytes, in particular, the precursors of chimeric dendritic cells (DC). In this study, we have used granulocyte/macrophage colony-stimulating factor to induce the propagation of progenitors that give rise to DC (CD45+, CD11c+, 33D1+, nonlymphoid dendritic cell 145+, major histocompatibility complex class II+, B7-1+) in liquid cultures of murine bone marrow cells. Using this technique, together with immunocytochemical and molecular methods, we show that, in addition to cells expressing female host (C3H) phenotype (H-2Kk+; I-E+; Y chromosome+), a minor population of male donor (B10)-derived cells (H-2Kb+; I-A+; Y chromosome+) can also be grown in 10-d DC cultures from the bone marrow of liver allograft recipients 14 d after transplant. Highly purified nonlymphoid dendritic cell 145+ DC sorted from these bone marrow-derived cell cultures were shown to comprise ~1-10% cells of donor origin (Y chromosome+ by polymerase chain reaction analysis. In addition, sorted DC stimulated naive, recipient strain T lymphocytes in primary mixed leukocyte cultures. Evidence was also obtained for the growth of donor-derived cells from the spleen but not the thymus. In contrast, donor cells could not be propagated from the bone marrow or other lymphoid tissues of nonimmunosuppressed C3H mice rejecting cardiac allografts from the same donor strain (B10). These findings provide a basis for the establishment and perpetuation of cell chimerism after organ transplantation.

A n unresolved enigma with the recent demonstration of persistent low-level donor leukocyte chimerism after organ transplantation (1-4) has been the means by which the chimeric cells can be perpetuated up to many years (2) postoperatively. Both in rodents (5-7) and in humans (8), dendritic cells (DC),1 which have well-recognized in vivo migratory ability (9-12), feature prominently in immunohistochemical analyses of this multilineage chimerism in long-term successful organ recipients. For some time, it has been recognized that DC in lymphoid and other organs arise from proliferating bone marrow-derived precursors (13-15). Only recently, however, have in vitro systems been developed for inducing the growth of these cells from precursors present in either the blood (16) or bone marrow (17). Similar methods have confirmed the presence of DC progenitors within the spleen (18) or liver (19). In each of these systems, liquid culture has been used to generate DC under the aegis of GM-CSF. This approach has permitted extensive characterization of the progeny DC, including their structural features, motility, immunophenotype, allostimulatory activity, and in vivo homing ability. With respect to murine liver-derived DC progenitors, it has been shown that their injection into nonimmunosuppressed allogeneic recipients gives rise to strongly MHC class II-positive cells that persist for months within T-dependent areas of secondary lymphoid tissue (20).

In mice, liver but not other organ allografts are accepted
permanently and with donor specificity between many strain combinations, without the requirement for immunosuppression (6). Here we present evidence that liver allografts export DC precursors that can be propagated in GM-CSF from the bone marrow or spleen of unmodified recipients. This novel finding, together with failure to propagate donor cells from the lymphoid tissues of nonimmunosuppressed mice rejecting cardiac allografts from the same donor strain, is congruent with the possibility that bidirectional leukocyte migration and donor cell chimerism play key roles in organ graft acceptance and acquired transplantation tolerance.

**Materials and Methods**

**Animals.** 10-12-wk-old male C57BL/10J mice (B10; H-2b, I-A^k, I-E^k) and C3H/HeJ (C3H; H-2k, I-A^k, I-E^k) mice of both sexes were purchased from The Jackson Laboratory (Bar Harbor, ME). They were maintained in the specific pathogen-free facility of the University of Pittsburgh Medical Center (Pittsburgh, PA).

**Liver and Heart Transplantation.** Orthotopic liver transplantation (OLTx) was performed in a mouse strain combination (B10 to C3H; MHC class I, II and multiple non-MHC antigen disparities) with a high liver acceptance rate (6), using techniques described previously (21) with minor modifications. Briefly, after clamping the artery and portal vein, the donor liver was flushed thoroughly via the portal vein with cold lactated Ringer’s solution. A suprahepatic vena cava anastomosis was completed, with a 10-0 running suture. The cuff technique was used for anastomosis of both the intrahepatic vena cava and the portal vein. No attempt was made to reconstruct the graft arterial supply. The bile ducts were anastomosed by inserting a polyethylene tube into both. Blood lost during the operation was replaced with lactated Ringer’s solution. The method for heterotopic heart transplantation was adapted from the rat procedure of Ono and Lindsey (22). The heart was transplanted into the abdomen with end to side anastomosis of aorta to aorta and pulmonary artery to vena cava. Rejection was defined by the cessation of cardiac contraction after daily palpation through the abdominal wall, and it was confirmed by histological examination. No immunosuppressive therapy was used in either procedure, and no immunosuppressive therapy was used in either procedure, and no immunosuppressive therapy was used in either procedure, and no immunosuppressive therapy was used in either procedure.

**Isolation of Leukocytes from Tissues.** Before harvesting of organs from transplanted animals, whole body perfusion was performed to minimize blood contamination of tissues. 30 ml HBSS (GIBCO BRL, Gaithersburg, MD) was infused over a 3-min period via the inferior vena cava. Bone marrow cell suspensions and mononuclear cells from the spleen or thymus were prepared in RPMI 1640 (GIBCO BRL) using conventional methods. Liver nonparenchymal cells (NPC) (<5% hepatocyte contamination) were isolated as described in detail elsewhere (19). Briefly, the liver was perfused in situ via the portal vein with collagenase solution (type IV, 1 mg/ml in HBSS; Sigma Chemical Co., St. Louis, MO). It was then removed, diced, and further digested with collagenase in vitro. The washed cells were then centrifuged on Percoll (Sigma Chemical Co.) gradients, and the leukocyte-rich layer was harvested as described (19).

**Culture of Tissue-derived Cells with GM-CSF.** 1-2 × 10^6 cells obtained from the bone marrow, spleen, thymus, or liver were cultured per well in 24-well plates in 2 ml RPMI 1640, supplemented with 10% FCS and 0.4 ng/ml mouse rGM-CSF (R&D Systems, Minneapolis, MN). In some experiments, bone marrow-derived cells were cultured in GM-CSF (4 ng/ml) plus IL-4 (1,000 U/ml) (each cytokine was from Schering-Plough, Kenilworth, NJ) in an effort to maximize the development of their allostimulatory activity (23). The procedure used was similar to that described initially by Inaba et al. (17) for the propagation of large numbers of DC from mouse bone marrow. The medium containing GM-CSF (or GM-CSF plus IL-4) was refreshed every 2 d; after gentle swirling of the plates, half of the old medium was aspirated and an equivalent volume of fresh cytokine-supplemented medium added. This method was implemented to deplete nonadherent granulocytes without dislodging clusters of developing DC that attached loosely to a monolayer of firmly plastic-adherent macrophages. Morphological and phenotypic analyses of typical, single, nonadherent mononuclear cells released spontaneously from the clusters were performed after 10 d.

**Staining of Cell Surface and Intracellular Antigens.** Cell surface staining was analyzed by cytofluorography using a FACSscan flow cytometer (Becton Dickinson & Co., Mountain View, CA). Staining with primary hamster or rat mAbs, including rat anti-B7-1 and -B7-2 (Pharmlingen, San Diego, CA) was followed by FITC-conjugated goat anti-hamster or mouse anti-rat IgGs as described (18, 19). Donor and recipient MHC class I- and class II-positive cells were identified using biotin-conjugated mouse anti-mouse mAbs (Pharmlingen, San Diego, CA) with FITC streptavidin (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) as the secondary reagent. After staining, the cells were fixed in 1% paraformaldehyde in saline before analysis. mAbs to MHC class II antigens and to the 2A1 DC-restricted granule antigen were tested on cytospin preparations as described (17, 18). The specimens were fixed in acetone and stained with biotinylated mouse anti-MHC class II or rat anti-2A1 mAbs, followed by streptavidin biotin peroxidase complex (ABC-P; Boehringer Mannheim Corp., Indianapolis, IN) or peroxidase-conjugated anti-rat IgG, respectively. The nuclei were counterstained with hematoxylin.

**Detection of Donor Male (Y) Chromosome in Cell Cultures by PCR Analysis.** DNA was prepared from freshly isolated and 10-d GM-CSF-stimulated cells from both normal male B10 and female C3H mice and from female C3H mice 14 or 8 d, respectively, after liver or heart transplantation from male B10 donors. The presence of male donor cells was determined as previously described for human analysis (24) by PCR amplification of the SRY region of the mouse Y chromosome. Primers (5'-CAAGGCTACACCCAGACF3' and 5'-CCAATCTCTGTTGACATT3') were chosen from the p4.2.2 sequence (25). 1-µg aliquots of DNA were amplified with 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s in buffer consisting of 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂, 0.2 mM deoxynucleotides, 0.1% gelatin, 0.2 µM primers, and 2.5 U of Taq polymerase (Perkin-Elmer Corp., Norwalk, CT). The products were separated in a 1% agarose gel, transferred to nylon membrane, and hybridized with an identical purified and radiolabeled 32P PCR product from male mouse DNA. Single-copy sensitivity and male specificity were verified with control samples consisting of serial dilutions of male mouse DNA into that of female mouse DNA.

**Sorting of DC.** Before staining with mAb, cultured bone marrow-derived cells were incubated with 10% goat serum (Vector Laboratories, Inc., Burlingame, CA) in HBSS (GIBCO BRL) at 4°C for 30 min to eliminate nonspecific binding. The cells were then washed once in HBSS containing 0.1% BSA (Sigma Chemical Co.) and resuspended to a concentration of 40 × 10^6/ml in the same buffer. A saturating concentration of nonlymphoid dendritic cells [NLDC] 145 mAb (a gift from Dr. R. M. Steinman, 380 Growth of Donor-derived Dendritic Cells from Allograft Recipients
The Rockefeller University, New York) or rat IgG2a isotype control (Sigma Chemical Co.) was added, and cells were incubated for 45 min at 4°C. After two washes in HBSS, the cells were stained with FITC-conjugated goat anti-rat IgG (Sigma Chemical Co.) under the same conditions. They were then washed and resuspended in complete RPMI 1640 medium supplemented with 2% FCS (GIBCO BRL) for cell sorting. NLDC 145* and NLDC 145+ cells were sorted into distinct cell populations based on forward scatter, side scatter, and FITC fluorescence intensity using a FACStar Plus® cell sorter (Becton Dickinson & Co.). Cells were excited with a 5-mW argon/HeNe laser (Coherent Inc.) with FITC-conjugated goat anti-rat antibody. The degree of thymidine incorporation was determined in cultures established from mouse liver or spleen previously (17). A similar technique was adopted to propagate the DC-restricted granule antigen 2Al (17, 18) using procedures modified after Inaba et al. (19). Similar results were obtained whether the cultures were established from normal bone marrow or from postulated small numbers of progenitor cells in freshly isolated bone marrow of unmodified liver allograft recipients. As shown in Fig. 1 (right), both cells expressing recipient (H-2k) and those expressing donor MHC class I (H-2Kb*) could be identified by flow cytometry in allografted mice. In several repeat experiments using pooled bone marrow cells from groups of three allograft recipients, the incidence of donor MHC class II+ cells in 10-d GM-CSF-stimulated cultures ranged from 5 to 17%.

Detection of Cells Expressing Donor Phenotype in Freshly Isolated Bone Marrow from Liver Allograft Recipients. C3H mice accept livers from B10 donors spontaneously, with graft survival times consistently >100 d (6). To identify cells expressing donor phenotype in freshly isolated bone marrow cell suspensions obtained from nonimmunosuppressed C3H mice 14 d after OLTx from B10 donors, mAbs directed against donor MHC class I and II antigens were used. Typically, as shown in Fig. 1 (left), the incidence of cells expressing donor MHC class I determined by flow cytometry in allografted mice was very low (~1%), whereas that of recipient was, as expected, consistently very high (>84%).

Results

Propagation of DC from Normal Mouse Bone Marrow. In prior studies, aggregates of growing DC progenitors were described in cultures established from mouse liver or spleen cell suspensions (18, 19) using procedures modified after Inaba and co-workers (16, 17). Similar techniques were adopted to propagate DC progenitors from the bone marrow of B10 and C3H mice. At 2 and 4 d of culture, non- or loosely adherent granulocytes were removed, without dislodging clusters of developing DC that were attached to firmly plastic-adherent macrophages. After day 4, granulocytes were no longer significant contaminants, and the cultures were maintained routinely for 10 d. Numerous dendritic-shaped cells appeared to be released from the clusters. These floating cells exhibited typical dendritic projections and other structural features of DC (17, 19, 26) upon light microscopic examination.

Immunophenotypic Analysis of GM-CSF-stimulated Bone Marrow-derived Cells. To ascertain the surface phenotype of cells released from proliferating aggregates, flow cytometric analysis was performed after 10 d of culture in GM-CSF. Staining for cells of lymphoid lineage (CD3+, CD4+, CD8+, B220+) including NK cells (NK1.1+) was absent. The floating cells in 10-d bone marrow-derived cultures expressed surface antigens that are known to be associated with murine DC. These included CD45 (leukocyte common antigen), MHC class I, heat-stable antigen (J11D), CD54 (intercellular adhesion molecule-1), CD11b (membrane attack complex-1), and CD44 (nonpolymeric determinant of Fgg.l glycoprotein). In addition, staining of moderate intensity was observed for the murine DC-restricted markers NLDC 145 (interdigitating cells), 33D1, and CD11c (N418; β2-integrin); for the macrophage antigen F4/80; and for FcγRII, as described previously (17) for bone marrow–derived DC. The cells also expressed moderate levels of MHC class II and of the costimulatory molecule and CD28 ligand, B7-1. The cells were negative, however, for cell surface expression of the CD28 coligand B7-2. Expression of the low affinity IL-2 receptor (CD25) could also not be demonstrated (data not shown).

Cytospins were prepared to further characterize the released DC. By day 10, the majority of the cells were dendritic by Giemsa stain and expressed high levels of MHC class II. Granulocytes were rare. The majority of the cells strongly expressed the DC-restricted granule antigen 2Al (17, 18). Similar results were obtained when the cells were incubated from cells obtained from the bone marrow of either B10 or C3H mice.

Detection of Cells Expressing Donor Phenotype in Freshly Isolated Bone Marrow from Liver Allograft Recipients. C3H mice transplanted with male (B10) liver grafts killed 14 d after OLT. The cells also expressed donor MHC class I and II antigens were used. Typically, as shown in Fig. 1 (left), the incidence of cells expressing donor MHC class I determined by flow cytometry in allografted mice was very low (~1%), whereas that of recipient was, as expected, consistently very high (>84%).

Cells Expressing Donor Phenotype in Bone Marrow DC Cultures from Liver Allograft Recipients. We next determined whether DC expressing donor cell surface phenotype could be propagated using GM-CSF from postulated small numbers of progenitor cells in freshly isolated bone marrow of unmodified liver allograft recipients. As shown in Fig. 1 (right), both cells expressing recipient (H-2Kb*) and those expressing donor MHC class I (H-2Kb*) could be identified by cytfluorometry in 10-d cultures propagated using the same methods as for DC from normal bone marrow. Staining of cytopsins for recipient and donor MHC class II products confirmed the presence of small numbers of donor (I-A*) cells (Fig. 2). In several repeat experiments using pooled bone marrow cells from groups of three allograft recipients, the incidence of donor MHC class II+ cells in 10-d GM-CSF-stimulated cultures ranged from 5 to 17%.

Detection of Donor Y Chromosome in GM-CSF-stimulated Cultures from Liver Allograft Recipients. The apparent incidence of donor-derived DC in GM-CSF–stimulated bone marrow cell cultures was comparatively low. Therefore, to eliminate the possibility of false positive results due to minimal antibody cross-reactivity or to the uptake of donor MHC products by recipient APC, PCR analysis for donor Y chromosome was performed on cells cultured from female (C3H) recipients of male (B10) liver grafts killed 14 d after transplant. Detection of Y chromosome (Fig. 3) confirmed the
BONE MARROW: MHC CLASS 1

FRESHLY-ISOLATED CELLS

GM-CSF-STIMULATED CELLS (10-DAY CULTURE)

Figure 1. Flow cytometric analyses (MHC class I staining) of freshly isolated (left) and GM-CSF-stimulated bone marrow DC (right) from normal B10 (H-2b), normal C3H (H-2k), and unmodified B10 → C3H orthotopic liver allograft recipients 14 d after transplant. Donor strain (B10) and recipient strain cells (C3H) are identified by staining for H-2Kb and H-2Kk, respectively. Cultured cells were maintained for 10 d before analysis. Note the coexistence of cells expressing donor class I (H-2Kb line; arrowed) and recipient class I in the DC cultures propagated from transplanted mice (bottom right). The result is representative of three separate experiments.

Figure 2. Coexistence of cells expressing donor (H-2b; I-A+) and recipient strain (H-2k; I-Ek) MHC class II phenotype in 10-d cultures of GM-CSF-stimulated cells propagated from the bone marrow of liver-allografted [B10 (H-2b) → C3H (H-2k)] mice killed 14 d after transplant. Staining was performed using the appropriate biotinylated mouse anti-mouse mAb, followed by streptavidin biotin peroxidase complex, as described (19). (a) Ig isotype control; (b) evidence of occasional donor (I-A+) cells; as well as (c) DC expressing recipient phenotype (I-Ek+). Counterstained with hematoxylin. ×250.

growth of donor-derived cells in the 10-d bone marrow cell cultures and reinforced the immunocytochemical observations. Y chromosome signal was also detected in 10-d spleen cell cultures and was also increased in magnitude compared with freshly isolated cells. The signal, however, was substantially lower compared with bone marrow–derived cells (Fig. 3), indicating that comparatively few donor-derived, GM-CSF-responsive DC precursors had reached the spleen. A diminished signal for donor (male) cells in 10-d GM-CSF-stimulated liver-derived cell populations (Fig. 3) may reflect the deliberate physical removal and also the death of contaminating male hepatocytes (<5% at the initiation of the cultures). In addition, in situ replacement of donor (male) with host (female) GM-CSF–responsive cells within the liver by day 14 after transplant (5, 6), when the cells were freshly isolated, may contribute to the relative reduction in the donor signal from cultured liver-derived cells on day 10. In contrast to bone marrow and spleen, and despite histological findings (5) and evidence for chimerism in freshly isolated thymus cell suspensions, no signal for donor-derived cells could be detected in 10-d GM-CSF-stimulated cultures of thymocytes from liver allograft recipients. This suggested that, although a site in which chimeric cells are detected after OLTx, the thymus may not be a destination for adequate numbers of donor-derived immature DC progenitors.

Evidence that the Donor-derived Cells are DC. We next sought direct evidence that the donor-derived cells propagated from the bone marrow of liver allograft recipients were DC. Sorting
of donor-positive cells was considered, but the anticipated yield of cells was calculated to be too low for subsequent functional analysis. Instead, 10-d GM-CSF–stimulated bone marrow–derived cells were harvested, and NLDC 145⁺ cells were sorted (at least 90% purity by morphologic and FACScan® analysis; Fig. 4, a and b) and then investigated for the presence of donor Y chromosome. As shown in Fig. 4 c, the results of PCR analyses demonstrated convincingly that the highly purified DC population comprised ~1–10% Y chromosome–positive (donor-derived) cells. Further evidence for the presence of donor-derived DC was obtained by testing the allostimulatory activity of sorted, NLDC 145⁺ GM-CSF plus IL-4–stimulated, bone marrow–derived cells in primary MLR. As shown in Fig. 5 A, the purified NLDC 145⁺ population propagated from C3H recipients of B10 allografts strongly stimulated B10 responders but also stimulated a response in recipient strain T cells (Fig. 5 B). The extent of stimulation (P < 0.01 compared with negatively sorted cells or syngeneic DC) was similar to that achieved with "artificial mixtures" containing 1% donor strain DC.

**Failure to Propagate Donor-derived Cells from Bone Marrow of Mice Rejecting Heart Allografts.** In contrast to liver allograft recipients, nonimmunosuppressed C3H mice reject heart allografts from the same donor strain (B10) within 8 d (6). PCR analysis for donor Y chromosome was performed on 10-d GM-CSF–stimulated cultures of bone marrow cells harvested from heterotopic cardiac allograft recipients 8 d after transplant. In contrast to the results obtained from liver graft recipients, no evidence was obtained for the propagation of donor-derived cells from the bone marrow of animals rejecting their cardiac grafts (Fig. 6), despite evidence of chimeric cells in freshly isolated bone marrow. It thus appears that these heart-derived cells, in contrast to those detected in fresh bone marrow of liver allograft recipients, do not comprise sufficient numbers of GM-CSF–responsive progenitors to allow growth of donor-derived cells (as opposed to amplification in the case of liver grafts) after 10 d of culture.

**Discussion**

In a previous search for myeloid progenitors in normal mouse liver using the same methods subsequently adopted in this study, cells were propagated with GM-CSF that exhibited many characteristics of DC (19). These included the expression of murine DC-restricted cell surface immunophenotypic markers (27). The cells did not, however, conform to the stereotypic features classically associated with ma-
These observations suggested, amongst other implications, a mechanism for liver DC precursor expansion and a possible basis for the establishment and perpetuation of donor-derived cell microchimerism after OLTx. The findings have now been extended to the context of whole organ transplantation. In the present study, an MHC class I- and II-disparate mouse strain combination (B10 → C3H) that accepts liver grafts spontaneously was used to determine whether liver-derived DC progenitors could be propagated from recipient lymphoid tissue. To confirm the presence of donor-derived cells, we first examined with flow cytometry the frequency of donor and recipient MHC class I+ leukocytes in freshly isolated cell suspensions from the bone marrow, spleen, thymus, and liver 14 d after OLTx. As anticipated, donor class I+ cells were rare (<1%) in the freshly isolated cell populations isolated from recipient primary and secondary lymphoid tissues. A higher incidence of donor class I+ NPC (5-9%) was detected in the liver allograft itself. When, however, cells were harvested from recipient bone marrow 14 d after OLTx and then cultured for 10 d in GM-CSF, a significant proportion of the developing DC exhibited donor phenotype. The same phenomenon was observed with cells propagated from the spleen, although the apparent growth of donor-derived DC was less striking. The expression of MHC class II on these cells could most accurately be studied after immunochemical staining of cytospin preparations. Similar to the class I results, donor class II+ cells were readily detected in the GM-CSF-stimulated cultures from bone marrow and spleen, both of which are hematopoietic tissues. In contrast, although donor class I+ and II+ cells were also detected in freshly isolated thymocyte preparations, it proved consistently difficult to demonstrate in vivo growth of DC progenitors from the thymuses of liver-allografted mice.

Definitive evidence for the growth of donor liver-derived
cells from the bone marrow (especially) and spleen was obtained by detection of the presence of the (donor) Y chromosome by PCR amplification and Southern blotting. These revealed a level of chimerism in the GM-CSF-stimulated cultures of ~1-10% (Fig. 3). Definitive evidence that donor-positive cells were DC was obtained by demonstration of the Y chromosome (Fig. 4) and allostimulatory activity for recipient strain naive T cells (Fig. 5 B) in highly purified NLDC 145+ cell populations. In marked contrast to these findings, donor-derived cells could not be propagated from the bone marrow, spleen, or thymus of unmodified cardiac allograft recipients rejecting their transplants at 8 d. Although donor-derived cells were detected in the freshly isolated bone marrow, these cells clearly did not comprise sufficient numbers of GM-CSF-responsive cells to allow perpetuation of donor signal for 10 d in culture. An implication of these observations is that acceptance of an organ allograft may be dependent on sustained chimerism comprising sufficient numbers of the appropriate donor-derived progenitor cell population(s).

Our observations appear to explain the persistence of low-level chimerism that does not require host immunosuppression in the B10 → C3H liver transplant model. They are also consistent with the adult liver containing comparatively large numbers of (potential migratory) hemopoietic cells (29), including DC progenitors with the capacity to propagate ex vivo in response to appropriate growth factors. The observation that organ transplantation permits the growth of donor as well as recipient progenitor DC is congruent with the paradigm of bidirectional immune reactivity, which, we have suggested, may explain whole-organ graft acceptance and acquired tolerance in a different way than previously envisioned (1, 5, 6, 8, 30). We postulate that the peripheral tissues and the graft itself become repositories of precursor cells of myeloid and probably other lineages, or perhaps even of pluripotent stem cells. The presence of these precursor cells can potentially be identified, as in this study, by their patterns of differentiation under controlled culture conditions.

The functional role of the precursors of chimeric DC is currently being investigated. Others have demonstrated a veto function for either subpopulations of murine DC (31) or putative immature DC in primate allogeneic bone marrow (32, 33). Moreover, there is strong evidence that the reduction of MHC class I-specific cytotoxic responses in mice injected with allogeneic donor lymphoid cells is a function of donor-deletional APC that inactivate class I-reactive CTL precursors that recognize them (34). The presence of donor-derived DC for up to 1 yr in rodents tolerant to their allografts (5, 6) and for many years after OLTx in humans (2) has been demonstrated. Moreover, using techniques described in the present study, we have preliminary evidence that donor-derived cells can be propagated from the bone marrow of unmodified murine liver allograft recipients up to 5 mo after transplant (our unpublished observations). The magnitude, tissue-specific site dependency, replicative capacity, and maturational stage of chimeric DC (including cell surface expression of key T cell-costimulatory molecules [35, 36]) that may be necessary to mediate postulated tolerizing effects of these cells have not been established.

Our failure to propagate chimeric DC progenitors from the thymus is of particular interest. Previous reports have shown that either intrathymic (37) or intravenous (38) donor bone marrow cell infusion in antilymphocyte serum/antilymphocyte globulin–treated mice results in only a comparatively low level of thymic chimerism that can no longer be detected after 3 wk. Indeed, permanent cardiac graft survival in mice is maintained despite the apparent absence of donor bone marrow cell–derived chimerism in the thymus (37). Moreover, the thymus is not required for the induction of liver-induced transplantation tolerance (39). Thus, chimerism in the periphery may be more important in maintaining a tolerant state (40–42). Conceivably, the recipient bone marrow may provide a repository of donor-derived DC progenitors with the potential to migrate to key peripheral sites. The unique observations in this study provide an opportunity for elucidation of the role of donor-derived cells in the induction and maintenance of donor-specific unresponsiveness after organ transplantation.

We thank Dr. A. J. Demetris for review of the manuscript, Dr. R. M. Steinman for constructive suggestions and the gift of mAbs, Dr. C. G. McAllister and Mr. R. Lakomy (Pittsburgh Cancer Institute) for expert assistance with cell sorting, Dr. Y. Li for immunocytochemical staining, Ms. Francy Chambers for preparation of figures, and Ms. S. L. Conklin for secretarial assistance.

This work was supported by National Institutes of Health grant DK 29961-14 and by the Competitive Medical Research Fund of the University of Pittsburgh Medical Center.

Address correspondence to Dr. A. W. Thomson, Department of Surgery, University of Pittsburgh Medical Center, W1544 Biomedical Science Tower, Terrace and Lothrop Streets, Pittsburgh, PA 15213.

Received for publication 3 October 1994 and in revised form 24 March 1995.
References


386 Growth of Donor-derived Dendritic Cells from Allograft Recipients


