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EVIDENCE FOR THE PRESENCE OF MULTILINEAGE CHIMERISM AND PROGENITORS OF DONOR DENDRITIC CELLS IN THE PERIPHERAL BLOOD OF BONE MARROW-AUGMENTED ORGAN TRANSPLANT RECIPIENTS¹

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Abstract

We have postulated that the donor leukocyte microchimerism plays a seminal role in the acceptance of allografts by inducing and perpetuating variable degree of donor-specific nonreactivity in long-surviving organ recipients. Limited information is available, however, concerning the phenotype and function of these chimeric cells in humans. The unequivocal presence of donor dendritic cells (DCs), a prominent lineage in the microchimerism observed in rodents and clinical organ recipients, was difficult to demonstrate in bone marrow (BM)-augmented organ transplant recipients. This enigma was resolved by the recent description of a method for propagating circulating human DCs from their progenitors by culture in a medium enriched with granulocyte-macrophage colony-stimulating factor and interleukin 4, a condition known to inhibit outgrowth of monocytes, thus providing a selective growth advantage to committed progenitors of the myeloid lineage. Cells from BM-augmented organ recipients and normal control subjects harvested from 12- to 14-day cultures exhibited dendritic morphology and potent allostimulatory capacity. Using appropriate primers, the presence of donor DNA was verified by polymerase chain reaction within the lineage^{null}/class II^{bright} sorted DC. Phenotypic analysis of cultured DCs from BM-augmented patients, unlike that of controls, exhibited a marked down-regulation of B7-1 (CD80) while retaining normal levels of expression of B7-2 (CD86) cell surface molecules. The presence of donor DNA was also confirmed by polymerase chain reaction in individually sorted lineage⁺ (T, B, and NK) cells and macrophages, suggesting that the chimerism in BM-augmented patients is multilineage. The presence of progenitors of donor DCs in the peripheral blood of BM-augmented patients further substantiates the already convincing evidence of stem cell engraftment.

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The diffuse migration of passenger leukocytes from transplanted organs into the recipient has been proposed as the first step toward allograft acceptance and the induction of variable degrees of donor-specific tolerance (1–4). In this paradigm, acceptance of various organs hinges on the quantity and quality of tolerogenic resident migratory cells with a critical role for cells of the dendritic leukocyte lineage (5–11) first characterized by Steinman and Cohn (12,13). Although dendritic cells (DCs^{*}) and their progenitors have been isolated from various mouse (14–18) and rat (19) tissues, such studies were not possible in humans until Romani et al. (20) described a modified culture technique for propagation of DCs from their progenitors in human blood. We argue that demonstration of these progenitors in the presence of other lineages in peripheral blood mononuclear cells (PBMCs) would provide strong evidence for engraftment of stem cells in organ recipients given adjunct bone marrow (BM) infusion (21). Added to previous observations in rodents (22–26), such findings would explain the long-term perpetuation of multi-lineage donor cell chimerism in all successful organ allograft recipients decades after transplantation (3,4).

Here, we present evidence that chimerism in BM-augmented organ transplant human recipients is indeed multilineage. Culture of their peripheral blood in recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF)- and interleukin 4 (rhIL-4)-enriched medium resulted in propagation of progenitors of DCs, which were confirmed to be those of donor origin. The functional significance of this finding and its role in the induction of tolerance, however, remains to be clarified.

MATERIALS AND METHODS

Isolation of PBMCs

Peripheral blood (40 ml) was obtained from six patients who had previously received an ABO-compatible but HLA-mismatched organ (liver, n = 4; heart, n = 1; kidney, n = 1) transplant accompanied with perioperative augmentation of chimensm with $3\text{--}5 \times 10^8$ unmodified BM cells/kg body weight (21). The patients were not preconditioned with any cytoablative or cytoreductive regimens, and after transplantation they were maintained on routine daily immunosuppression with tacrolimus and steroids. Blood obtained from normal, healthy human volunteers served as controls for propagation of DCs in culture and for in vitro proliferative assays. PBMCs were isolated by centrifugation of peripheral blood on a ficoll gradient (density = 1.077) for 30 min at $160 \times g$ at room temperature. After lysis of erythrocytes with red cell lysing buffer (Sigma Chemical Co., St. Louis, MO), the cells were washed twice with RPMI and counted. Viability was determined by trypan blue dye exclusion technique.

Culture medium

AIM-V serum-free lymphocyte medium (Life Technologies, Gaithersburg, MD), containing L-glutamine (2 mM), streptomycin sulfate ($50 \mu\text{g}/\text{ml}^{-1}$), and gentamicin sulfate ($10 \mu\text{g}/\text{ml}^{-1}$), was used in all cultures. For the selective propagation of progenitors of myeloid lineage, the culture medium was supplemented with rhGM-CSF ($10^3 \text{ U}/\text{ml}^{-1}$), and rhIL-4 ($10^3 \text{ U}/\text{ml}^{-1}$), which were generously provided by Schering-Plough (Kenilworth, NJ).

Culture of PBMCs in cytokine-rich medium

The method was similar to that of Romani et al. (20). PBMCs obtained from organ transplant recipients were resuspended in culture medium (without cytokine supplementation) and plated

* Abbreviations: BM, bone marrow; DC, dendritic cell; EM, electron microscopy; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; MLR, mixed leukocyte reaction; NK, natural killer; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PE, phycoerythrin; rhGM-CSF, recombinant human granulocyte-macrophage-colony stimulating factor; rhIL-4, recombinant human interleukin 4; SRY, sex-determining region of the Y chromosome.

at a concentration of 10^6 cells/well⁻¹ in 6-well plates (Coming, Inc., Coming, NY) for 2 hours at 37°C in 5% CO₂ in air. At the end of this incubation, the nonadherent cells were gently removed, and the wells (containing the adherent population) were washed twice with warm (37°C) medium; caution was exercised not to dislodge adherent and loosely adherent cells. Fresh cytokine-rich medium was then added to each well, and the cells were allowed to proliferate. Every 72–96 hr until harvest the plates were gently swirled, and one half of the culture medium was aspirated and replenished with an equivalent volume of fresh cytokine-supplemented medium. After 12–14 days of culture, the spontaneously released cells were harvested, and the DCs were further purified by flotation on metrizamide columns (Life Technologies) as described by Freudenthal and Steinman (27).

Sorting of DCs

To further enrich for DCs, we sorted the cells contained within the low-density fraction at 4°C. For sorting, 10^6 cells were aliquoted into each of 96 wells of a U-bottomed plate (Coming) and centrifuged at $160 \times g$ for 3 minutes. To block non-specific binding, cells were incubated with 10% heat-inactivated goat serum for 15 min and washed twice with washing solution (phosphate-buffered solution, 5% fetal calf serum, and 0.01% NaN₃). The cells were subsequently incubated for an additional 30 min with a cocktail of phycoerythrin (PE)-conjugated lineage-specific monoclonal antibodies (mAb; purchased from Becton Dickinson, Mountain View, CA) directed against T (CD3⁺), B (CD22⁺), and NK (CD56⁺) cells and macrophages (CD14⁺), and fluorescein isothiocyanate (FITC)-conjugated mAb against HLA-DR. Using an Epics Elite flow cytometer (Coulter Corp., Hialeah, FL) equipped with an air-cooled argon laser, lineage^{null}/HLA class II⁺ cells were sorted; both phenotypic and light scatter characteristics were used for the identification of various subpopulations. The sorted population (10^3 cells/sec⁻¹) was reanalyzed for the determination of their purity. Unstained cells and cells stained with appropriate fluorochrome-conjugated, isotypematched irrelevant mAb were used as negative controls. The presence of donor cells within the sorted population was subsequently examined by polymerase chain reaction (PCR) analysis. Additionally, purified sorted DCs were also processed for elaboration of their ultrastructure by electron microscopy (EM) and for phenotyping (see below).

Detection of multilineage chimerism

The PBMCs of the recipients, stained with various lineage-specific (CD3, CD14, CD22, and CD56) mAbs (Becton Dickinson), were sorted and reanalyzed for purity using Epics Elite flow cytometer (Coulter). For the purpose of this sorting, an analysis gate was established using the forward and orthogonal light scatter profiles of unstained cells. Leukocyte subsets were determined both by the use of light scatter as well as Leucogate (CD45⁺/CD14⁺, Becton Dickinson). The variable degree of CD45 and CD14 staining also allowed for delineation of lymphocytes, monocytes, and granulocytes present in the peripheral blood of the recipients. The use of the latter technique provided an additional means for confirming the presence of cells of multilineage origin within the donor-specific class II⁺ population. Unstained cells and cells stained with appropriate fluorochrome-conjugated, isotype-matched irrelevant mAb were used as negative controls. For analysis, 5×10^4 events were acquired with wide gates, as well as with gates encompassing the lymphocyte or monocyte populations. As with DCs, the presence of donor cells within the sorted lineage⁺ cells was determined by PCR analysis.

Fluorocytometric analysis of cultured/sorted DCs

The cell surface phenotype of the cultured DC was determined by staining with FITC-labeled anti-HLA-DR and a cocktail of lineage (anti-CD31/CD14/CD22/CD56)-specific mAbs. Additionally, expression of CD1a (M-T102), CD80 (BB7.1; BB1), and CD86 (B7.2; IT2.2) was

also determined on cultured HLA-DR^{bright}/lineage^{null}-sorted cells using mAbs obtained from PharMingen (San Diego, CA).

PCR

The presence of donor DNA was detected in the unsorted and sorted cells of the recipients by a procedure described previously (3,21,28). Using oligonucleotide primers specific for the sex-determining region of the Y (SRY) chromosome (in male→female transplant recipients) or for the appropriate mismatched HLA class II allele, the DNA was amplified by PCR and resolved by electrophoresis on an agarose gel. After Southern blotting, the membranes were hybridized with an allele-specific radiolabeled probe, washed, exposed to an autoradiograph, and developed. This technique allowed for a detection sensitivity of one donor cell per 10⁶–10⁶ recipient cells. For detection of Y chromosome, PBMCs obtained from normal male and female individuals were used as positive and negative controls, respectively. Additionally, for the identification of heterotopic HLA-DR alleles, cells from normal matched and unmatched volunteers were used as positive and negative controls, respectively.

Mixed leukocyte reaction (MLR)

To ascertain the allostimulatory capacity of cultured DCs, primary MLR assays were performed. A constant number of autologous responders (10⁵ PBMCs or purified T cells per well⁻¹) and responders obtained from HLA mismatched healthy human volunteers were cultured with increasing concentrations of γ -irradiated stimulators (purified DCs, PBMCs, or splenocytes) in a 96-well U-bottomed microtiter plate (Corning) for 6 days in 5% CO₂. One day before harvesting, each well was pulsed with 1 μ -Ci of [³H]thymidine (NEN, Boston, MA), and the cells were harvested (Skatron, LKB, Wallac, Gaithersburg, MD) onto glass fiber filter mats (Wallac Oy, Turku, Finland), with incorporation of radioactivity determined by liquid scintillation counting (Betaplate 1205, Pharmacia LKB, Gaithersburg, MD). The results are expressed as the mean [³H]thymidine incorporation in triplicate cultures.

Electron microscopy (EM) of sorted DCs

To further confirm the identity of DCs, their distinct ultrastructure was determined with EM. For this purpose, cultured sorted DCs were centrifuged (160 $\times$ g) and fixed as a pellet in 2.5% glutaraldehyde (Sigma Chemical Co) in phosphate-buffered saline (pH 7.2). After fixation, the pellet was incised into small (1-mm) cubes and postfixed for 60 min in osmium tetroxide. At the end of this treatment, the cubes were dehydrated by passage through graded series of alcohols before being embedded in Epon (Energy Beam Sciences, Agawam, MA). Ultrathin (60-nm) sections obtained using Leica Ultracut S microtome (Leica, Dearborn, MI) were counterstained in 2% uranyl acetate for 7 min and 1% lead citrate for 2 min. Ultrastructure was examined using a JEOL 1210 (Peabody, MA) transmission electron microscope.

RESULTS

Culture of DCs

After 3–4 days of culture in cytokine-supplemented medium, PBMCs from the BM-augmented recipients and from healthy human volunteers contained loosely adherent cells that exhibited typical dendritic morphology. Further incubation resulted in eventual proliferation of adherent aggregates, which over the next 7–10 days released typical “veiled” DCs.

Further purification of DCs

For determination of donor DNA (by PCR), phenotyping, and for EM analysis of the ultrastructure of the putative DC, cultured, metrizamide fractionated DCs were further purified by sorting. HLA-DR^{bright}/lineage^{null}(CD3/CD22/CD56/CD14) cells were gated (Fig. 1C) and

subjected to deliberate low-rate (10^3 cells/sec⁻¹) sorting. Subsequent to sorting, the cells were reanalyzed to confirm their purity (Fig. 1D). Only cells with 100% purity were used for subsequent analysis.

Phenotyping of cultured/sorted DCs

The low-density fractions of DCs purified on metrizamide columns were harvested and analyzed using immunofluorescent staining. Flow cytometric analysis of stained cells revealed marked HLA-DR but little or no expression of markers for T (CD3⁺), B (CD22⁺), NK (CD56⁺), cells and macrophages (CD14⁺; Fig. 2C). Within the cultured/sorted (lineage^{null}/class II^{bright}) DCs, evidence for the levels of expression of costimulatory (CD80, CD86) molecules and of those thought to be necessary for antigen presentation (CD1a) also was sought. The only discernible difference between the phenotype of DCs obtained from various PBMC donors was the remarkably down-regulated cell surface expression of costimulatory molecule B7-1 (CD80) in BM-augmented patients (Fig. 2E) compared with that of healthy controls. In contrast, high levels of expression of B7-2 (CD86; Fig. 2F) and that of CD1a (Fig. 2G) were comparable in the study and control groups.

Determination of allostimulatory capacity

The mature DCs present among the cells of spleens procured from the cadaveric donors of the BM-augmented organ recipients generated a moderate allostimulatory response when used as stimulators against naive allogeneic T cells and/or allogeneic PBMC responders (Fig. 3). Similar control studies using the PBMCs of the recipients as stimulators also showed modest allostimulation (Fig. 3), confirming the presence within this population of potent antigen-presenting cells. The most striking observation was made when the cultured DCs ultimately purified on metrizamide columns were used as stimulators. Although autologous PBMCs were nonresponsive, allogeneic T cells and PBMCs when used as responders exhibited two-to threefold higher proliferation than that after their exposure to spleen and/or PBMC stimulators (Fig. 3). The allostimulatory capacity of cultured DCs obtained from all BM-augmented patients, as well as healthy controls, was always at least 1–2 log higher than that of fresh splenocytes or PBMCs. A representative outcome in two BM-augmented recipients is shown in Figure 3.

EM analysis of ultrastructure of DCs

Highly purified sorted cells obtained from both study patients and normal healthy controls exhibited an ultrastructure characteristic of cells of dendritic leukocyte lineage (16,20). There was an abundance of slender “organelle-deficient” cytoplasmic processes (veils), sparse secretory granules, and scant rough endoplasmic reticulum (Fig. 4). Although the mitochondrial content varied between cells (Fig. 4A vs. 4B), the presence of characteristic “racket-shaped” Birbeck granules, known to be associated with endocytosis, could not be confirmed. The cytoplasmic development also was highly variable in the cells examined.

PCR detection of donor DNA in cultured/sorted DCs

Using primers specific for either heterotypic HLA alleles or else the SRY region of the Y-chromosome (in male→female recipients), the presence of donor DNA was detected by PCR in the cultured sorted DCs obtained from 4 of 5 (80%) BM-augmented organ recipients 279–755 days after transplantation (Table 1). Our inability to detect donor DNA in 1 of 5 patients evaluated probably reflected technical limitations inherent with extraction of an insufficient amount of DNA for PCR analyses from a very low ($<4-5 \times 10^2$) number of sorted cells. An alternative explication might be that donor-specific DNA was not present in the DCs population sorted from this patient.

Evidence for the presence of multilineage donor cell microchimerism

The potential engraftment of pluripotent stem cells contained within the infused BM in augmented organ transplant recipients and the subsequent establishment of multilineage chimerism in their peripheral blood was confirmed by two mutually corroborative techniques. Using orthogonal and side scatter flow cytometric profiles, the presence of reciprocal concentrations of recipient and donor cells was evidenced in open (Fig. 5, Ia, C, and D) as well as lymphocyte (Fig. 5, IIb, C, and D)- and monocyte (Fig. 5, IIIc, C, and D)-gated populations. This observation was further substantiated by the demonstration of the presence of donor DNA by PCR in the sorted T (CD3⁺; Fig. 6B), B (CD22⁺; Fig. 6C), and NK (CD56⁺; Fig. 6D) cells and monocytes/macrophages (CD14⁺; Fig. 6F) of the recipients. It is noteworthy that all evaluated patients exhibited evidence for the presence of T and B cells of donor origin within their peripheral blood, whereas the presence of NK cells and macrophages was highly variable (Table 2).

DISCUSSION

It was postulated after the discovery of microchimerism in the long-surviving human organ recipients that the donor-derived leukocytes were multilineage, that this outcome was no different in principle than what could be produced by a BM infusion to a noncytoablated recipient, and that persistence of the chimeric cells was dependent on the engraftment of pluripotent stem cells (1,3,4). These conclusions have been verified in rodents (7–10,22), but the constituency of the chimerism is not known to have been studied in humans except in a preliminary communication (29) and in a manuscript in press (30). In the studies reported herein, donor cells of various lineages (T, B, NK cells, macrophages, and DCs) were sorted from the peripheral blood of six BM-augmented organ allograft recipients who were at least 1 year after transplantation. The presence within the various sorted cells of DNA of donor origin was confirmed by PCR analysis.

Our most difficult objective, and the most crucial, was to establish the presence of circulating leukocytes of DC lineage in BM-augmented organ recipients. The role of DCs in initiating primary immune responses has been well documented (31). As immature cells, DCs display avid phagocytic capacity but as a result of a lack of expression of appropriate costimulatory molecules, they cannot induce optimal proliferation of naive T cells (31). Subsequent to antigen uptake, DCs undergo rapid transformation, which among other changes, involves differential up-regulation of costimulatory molecules, resulting in their acquisition of potent antigen-presenting capacity. There is considerable evidence that a majority of resident DCs within the nonlymphoid organs express the phenotype and function of immature cells (16,32,33), and that these cells mature and migrate to secondary lymphoid organs of the recipient after organ transplantation, initiating allograft rejection (34,35). The latter observation has prompted strategies designed to either deplete (36,37) or modify (38) the function of resident DCs before organ transplantation. This approach has resulted in moderate prolongation of allograft survival but has failed to induce donor-specific tolerance.

We have reported previously that livers transplanted orthotopically across many MHC-disparate mouse strain combinations were accepted spontaneously with contemporaneous establishment of stable microchimerism and the induction of donor-specific tolerance (9). There is now growing evidence that this outcome in mice hinges on the presence within the liver of an optimal number of “immature” DCs, many of which migrate and provide tolerogenic rather than stimulatory signals to the naive T cells of the host (9,16). This “diametrical” function of DCs has been implicated in the “veto” effect of donor leukocytes observed in a large animal model of transplantation tolerance (10), and was postulated to explain the significance of numerous donor cells of DC morphology found in tissues of humans bearing functional

allografts for up to 30 years after transplantation (1–4) and in experimental models of long-surviving organ recipients (6–8).

Proving the presence of circulating DCs was a formidable undertaking. Until the report of Romani et al. (20), there was no way to reliably propagate DCs from their committed progenitors in humans. Using this new method, we were able to acquire from the PBMCs of healthy volunteers, as well as from BM-augmented organ allograft recipients, an adequate quantity of leukocytes that had typical characteristics of DCs (20,27) with which to undertake further investigations. Cultured DCs, when used as stimulators in primary MLR assays, triggered marked proliferation of naive allogeneic T cells. Additionally, cultured and sorted (class II^{bright}/lineage^{null}) cells had the distinctive morphologic features characteristic of mature DCs (veils, sparse secretory granules, and abundant mitochondria). With the phenotypic analysis, comparable levels of expression of CD1a and B7–2 (CD86) cell surface molecules were seen in the cultured/sorted DCs obtained from the peripheral blood of control and study patients. The expression, however, of B7–1 (CD80) was markedly down-regulated in the BM-augmented recipients as compared with that in the controls. The functional significance of this finding remains to be clarified.

Having confirmed that the propagated cells were indeed of dendritic leukocyte lineage, the next task was to determine their origin (donor vs. recipient). For this purpose, “culture/sorted” cells, the purity of which had been confirmed by reanalysis, were used. The final step was achieved by demonstrating by PCR within sorted (class II^{bright}/lineage^{null}) DCs, the presence of cells of donor origin.

Although *prima facie* evidence for engraftment of hematopoietic precursor and/or stem cells of donor origin was the detection of chimerism in successful long-term organ allograft recipients, this paper nevertheless provides strong evidence for the existence of progenitors of donor DCs in the peripheral blood of BM-augmented patients. It also establishes unequivocally the multilineage nature of donor cell chimerism in human organ allograft recipients. We anticipate to utilize the techniques reported herein as appropriate tools to initiate a systematic search for the cellular and molecular mechanisms involved in the induction of transplantation tolerance.

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Figure 1. Sorting for HLA-DR^{bright}/lineage^{null} cells using cultured metrizamide-fractionated putative DC generated using PBMCs obtained from a male→female kidney + BM recipient 345 days after transplantation. The sorting gates (C) were set for HLA-DR^{bright} but lineage^{null} population, and subsequent to low-speed sorting, the cells were reanalyzed for purity (D). Only cells exhibiting ~100% purity were used for EM studies and for phenotyping. The presence of donor DNA was confirmed by PCR analysis (D) using primers specific for SRY. For flow cytometric analysis, unstained and cells stained with fluorochrome-conjugated irrelevant isotype-matched mAbs were used as controls. PBMCs obtained from normal healthy males and females served as positive and negative controls, respectively, for PCR analysis. PGE, percentage gated events: SSC, side scatter channel: FSC, forward scatter channel.

Figure 2.

Determination of expression of costimulatory molecules on cultured/sorted DC generated from the PBMCs obtained from a liver + BM recipient 467 days after transplantation. Spontaneously released cells cultured in rhGM-CSF- and rhIL-4-enriched medium were purified further by flotation on metrizamide columns. The harvested low-density cells were stained with a cocktail of PE-conjugated lineage-specific (CD3/CD14/CD22/CD56) and FITC-conjugated anti-HLA-DR mAb. Lineage^{null}/HLA class II^{bright} cells were sorted (C) using an Epics Elite flow cytometer (Coulter) and reanalyzed (D) to determine their purity. Sorted cells were then subjected to staining with PE-conjugated mAbs against CD80 (B7.1; E), CD86 (B7.2; F), and CD1a (G). Unstained cells and cells stained with fluorochrome-conjugated irrelevant isotype-matched mAbs were used as controls. SSC, side scatter channel; FSC, forward scatter channel; PGE, percentage gated events.

Figure 3.

Allostimulatory capacity of γ -irradiated cultured DC was determined using a primary MLR assay. For the method used to generate DC, refer to legend of Figure 1. PBMCs were obtained from two liver + BM recipients 781 days (A) and 467 days (B) after transplantation. A constant number (10^5 cells/well) of autologous ($\square$) and allogeneic ($\circ$) PBMCs and allogeneic purified naive T cells ($\blacksquare$) were used as responders. In addition to cultured DC (1), splenocytes obtained from the cadaveric donor (2) and the PBMCs of recipients (3) were also used as stimulators. The results are expressed as the mean [^3H]thymidine incorporation in triplicate cultures.

Figure 4.

Analysis of the ultrastructure of the cultured/sorted (lineage^{null}/HLA class II^{bright}) DC obtained from the PBMCs of a BM-augmented liver allograft recipient 390 days after transplantation. All cells examined exhibited typical “organelle-deficient” cytoplasmic veils (arrow) with sparse secretory granules. The mitochondrial content varied between cells; some had low (A) but others very high (B) numbers. As is evident, the cytoplasmic development was also highly variable in the cells examined. Ultrastructure was determined using a JEOL 1210 (Peabody, MA) transmission electron microscope (magnification, $\times 4500$).

Figure 5.

Establishment of the multilineage nature of chimerism in the peripheral blood of a BM-augmented lung allograft recipient 475 days after transplantation. The orthogonal and side scatter profiles were used to establish analysis gates for lymphocyte (II) and monocyte (III) subpopulations. Within this population, the presence of cells staining with either donor (D)- or recipient (C)-specific mAbs was determined. Unstained and cells stained with fluorochrome-conjugated, irrelevant, isotype-matched mAbs (B) were used as controls. SSC, side scatter channel; FSC, forward scatter channel; PGE, percentage gated events.

Figure 6.

The detection of donor DNA by PCR in lineage-positive cells sorted from PBMCs obtained from a male→female liver + BM organ recipient 402 days after transplantation. Scatter profile was used to establish gates specific for the lymphoid (A) and myeloid (E) populations. Subsequently, the purity of sorted T (B), B (C), and NK (D) cells and monocyte/macrophages (F) was determined by reanalysis. Within the sorted population, the presence of donor DNA was confirmed by PCR analysis using primers specific for the SRY. For flow cytometry, unstained and cells stained with fluorochrome-conjugated, irrelevant, isotype-matched mAbs were used as controls. PBMCs obtained from normal males and females served as positive and negative controls, respectively, for PCR analysis. SSC, side scatter channel; FSC, forward scatter channel; PGE, percentage gated events.

Table 1

Detection of donor DNA within the sorted (lineage^{null}/class II^{bright}) putative DC obtained from rhIL-4 and rhGM-CSF-enriched cultures of recipient PBMC

Transplanted organs	PCR probe	POD ^a	Donor DNA within lineage ^{null} /class II ^{bright} cells
Liver + BM	DR14	755	+
Liver + BM	SRY	348	+
Liver + BM	SRY	390	+
Kidney + pancreas + BM	DR2	279	—
Kidney + BM	SRY	345	—

^aPostoperative day on which blood sample was recovered.

Table 2

Detection of donor DNA within lineage-sorted cells obtained from bone marrow-augmented organ transplant recipients^a

Transplanted organs	PCR probe	PCR detection of donor DNA within:			
		CD3 ⁺	CD14 ⁺	CD22 ⁺	CD56 ⁺
Kidney + BM	SRY	ND ^b	+	+	-
Heart + BM	DR2	+	-	+	-
Liver + BM	DR14	+	-	+	-
Liver + BM	SRY	+	+	+	+
Liver + BM	DR53	+	-	+	-
Liver + BM	SRY	ND	ND	+	ND

^aPeripheral blood was obtained from patients who were at least 1 year post-transplantation.

^bND, not done; insufficient cell yield.