THE ubiquitous presence of donor cell chimerism in the tissues of long-term successful organ allograft recipients provided quintessential support to the argument that these cells play a seminal role in allograft acceptance and in the induction of donor-specific tolerance. A finding of commensurate significance was the observation that livers (unlike other organs) when transplanted orthotopically across most mouse	extsuperscript{4} and several rat-strain combinations	extsuperscript{5,6} and in few outbred pigs	extsuperscript{7,8} are spontaneously accepted without the necessity for exogenous immunosuppression. This ascertainment complemented by the novel search to establish the presence of progenitors of dendritic leukocyte lineage conceivably play an auspicious role. Given their functional significance, we initiated a systematic search to establish the presence of progenitors of dendritic cells (DC) in the peripheral blood of bone marrow (BM)-augmented organ transplant recipients. Additionally, the evidence for the multilineage donor cell chimerism was also sought; reported herein is the outcome of these investigations.

MATERIALS AND METHODS
Peripheral Blood Mononuclear Cells (PBMC)
PBMC were obtained from six BM-augmented organ transplant recipients who were at least 12 months' posttransplantation. For BM-augmentation, cells (6 \times 10^8/kg body weight) obtained from the vertebral bodies of the cadaveric donor were infused intravenously at the time of revascularization. PBMC from healthy normal volunteers served as standard controls for the propagation of DC (see below).

Culture/Sorting of DC
PBMC were cultured in AIM-V media supplemented with l-glutamine for 2 hours at 37°C in 5% CO\textsubscript{2} in air. Nonadherent cells were subsequently removed and the adherent population was replenished with rhGM-CSF and rhIL-4-enriched medium. The cells were then cultured for an additional 8 to 12 days, with fresh medium being exchanged every 3 to 4 days.

At the end of this incubation, the nonadherent cells were harvested and further purified by flotation over metrizamide columns. A portion of these cells were used in primary MLR assays to determine their allosstimulatory activity. The remaining cells were stained with a cocktail of PE-conjugated lineage (CD3/CD22/CD56/CD14)-specific and FITC-conjugated anti-HLA-DR MAH. Lineage\textsuperscript{null}/DR\textsuperscript{+} cells were sorted, and within them the presence of donor DNA was confirmed by PCR analysis.

Sorting of Lineage\textsuperscript{+} Cells
For the establishment of multilineage chimerism, PBMC obtained from BM-augmented recipients were stained with lineage-specific MAH and sorted. Within the sorted population, the presence of donor DNA was confirmed by PCR analysis.

RESULTS AND DISCUSSION
The cells harvested from proliferating cultures 8 to 12 days postincubation exhibited distinctive dendritic morphology. When tested in primary MLR, these cultured cells displayed very potent stimulatory capacity, confirming their origin as that of dendritic leukocyte lineage. In 4 of 5 evaluated patients, the presence of donor DNA was confirmed in sorted DC by PCR. Additionally, donor DNA was also found in sorted lineage\textsuperscript{+} cells, suggesting that chimerism is indeed multilineage. Although the presence of progenitors of donor DC in BM-augmented recipients years after transplantation provides unequivocal evidence for its engraftment, the functional significance of this finding, however, remains a subject for further investigation.

REFERENCES

From the Thomas E. Starzl Transplantation Institute and the Departments of Surgery and Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

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Address reprint requests to Abdul S. Rao, MD, Thomas E. Starzl Transplantation Institute, E1545 Biomedical Science Tower, 200 Lothrop St, Pittsburgh, PA 15251.