DONOR HEMATOPOIETIC PROGENITOR CELLS IN NONMYELOABLATED RAT RECIPIENTS OF ALLOGENEIC BONE MARROW AND LIVER GRAFTS[1]

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Abstract

Background—Although the persistence of multilineage microchimerism in recipients of long-surviving organ transplants implies engraftment of migratory pluripotent donor stem cells, the ultimate localization in the recipient of these cells has not been determined in any species.

Methods—Progenitor cells were demonstrated in the bone marrow and nonparenchymal liver cells of naive rats and in Brown Norway (BN) recipients of Lewis (LEW) allografts by semiquantitative colony-forming unit in culture (CFU-C) assays. The LEW allografts of bone marrow cells (BMC) (2.5×10^8), orthotopic livers, or heterotopic hearts (abdominal site) were transplanted under a 2-week course of daily tacrolimus, with additional single doses on days 20 and 27. Donor CFU-C colonies were distinguished from recipient colonies in the allografts and recipient bone marrow with a donor-specific MHC class II monoclonal antibody. The proportions of donor and recipient colonies were estimated from a standard curve created by LEW and BN bone marrow mixtures of known concentrations.

Results—After the BMC infusions, 5–10% of the CFU-C in the bone marrow of BN recipients were of the LEW phenotype at 14, 30, and 60 days after transplantation. At 100 days, however, donor CFU-C could no longer be found at this site. The pattern of LEW CFU-C in the bone marrow of BN liver recipients up to 60 days was similar to that in recipients of 2.5×10^8 BMC, although the donor colonies were only 1/20 to 1/200 as numerous. This was expected, because the progenitor cells in the passenger leukocytes of a single liver are equivalent to those in $1–5 \times 10^6$ BMC. Using a liquid CFU-C assay, donor progenitor cells were demonstrated among the nonparenchymal cells of liver allografts up to 100 days. In contrast, after heart transplantation, donor CFU-C could not be identified in the recipient bone marrow, even at 14 days.

Conclusion—Under effective immunosuppression, allogeneic hematopoietic progenitors compete effectively with host cells for initial engraftment in the bone marrow of noncytoablated recipients, but disappear from this location between 60 and 100 days after transplantation, coincident with the shift of donor leukocyte chimerism from the lymphoid to the nonlymphoid compartment that we previously have observed in this model. It is possible that the syngeneic

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parenchymal environment of the liver allografts constitutes a privileged site for persistent progenitor donor cells.

The axiom that pluripotent hematolymphopoietic stem cells are confined to the bone marrow of mature animals and humans (1) has been challenged by evidence that pluripotent progenitor cells also reside in organs. It has been demonstrated that all hematopoietic lineages can be fully reconstituted by the transplantation of syngeneic livers or hearts to supralethally irradiated rodents (2, 3) or by infusing small numbers of allogeneic CD34+ stem cells purified from allogeneic livers (4). It was not clear from such experiments, however, where in the recipient these migratory stem cells of bone marrow origin take up residence, and specifically whether the transplanted organs themselves provide a particularly advantageous microenvironment for donor stem cell nesting and maintenance. We have examined this question by transplanting Lewis (LEW[*]) strain rat, liver, heart, and bone marrow cells (BMC) to transiently immunosuppressed Brown Norway (BN) recipients.

MATERIALS AND METHODS

Animals and surgical procedures

Male LEW (RT1^l) and BN (RT1^n) rats weighing 200–300 g (10–12 weeks old) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and maintained in a laminar-flow, specific pathogen-free atmosphere at the University of Pittsburgh. Heart and liver whole organ allografts, as well as BMC, were harvested from LEW donors. Orthotopic liver and heterotopic (intra-abdominal) heart transplantation was performed with previously described procedures (5, 6).

The LEW BMC were taken from the tibias and femurs, and processed in RPMI 1640 supplemented with 25 mM HEPES, 2 mM L-glutamine, and 50 μg/ml gentamicin (all from Life Technologies, Grand Island, NY). Trypan blue exclusion testing uniformly showed >95% viability of BMC. The LEW BMC (2.5×10^8) were injected into the penile vein of BN recipients. Based on the ability to reconstitute supralethally irradiated syngeneic rats, the number of pluripotent progenitor cells in this dose of leukocytes was estimated to be 20–200 times greater than that in a single liver (2).All BN recipients were treated with tacrolimus (FK506) (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), which was dissolved in HCO-60 and D mannitol and suspended in normal saline. Daily-intramuscular doses of 1.0 mg/kg were given on days 0 to 13 followed by single doses on days 20 and 27.

Donor cell phenotyping

As previously reported (6), LEW leukocytes can be identified in BN recipients by immunohistochemical staining with the L21–6 monoclonal antibody (mAb), which recognizes the MHC class II antigens of LEW, but not BN (7). The numbers and lineages of donor (LEW) hematolymphoid cells in the spleen and lymph nodes of these recipients 30 and 100 days after transplantation have been published elsewhere (6) and are summarized as follows:

[*]Abbreviations: BMC, bone marrow cells; BN, Brown Norway; CFU-C, colony-forming unit(s) in culture; FBS, fetal bovine serum; GM-CSF, granulocyte-macrophage colony-stimulating factor; IMDM, Iscove's modified Dulbecco's medium; LEW, Lewis; mAb, monoclonal antibody; 2-ME, mercaptoethanol; NPC, nonparenchymal cells; TCR, T cell receptor; PBS, phosphate-buffered saline; NMA, N^G-monomethyl-L-arginine.

Graft	Number of donor MHC class II^{+} cells[*]		Lineages
	30 days	100 days	
Heart	<5	—	Not tested
Bone marrow	5< <10	<5	Multilineage
Liver	10< <50	<5	T cell dominant
			Multilineage

[*] mAb L21–6^{+} cells in a whole mounted section of the spleen and lymph nodes.

Cell preparation

The BN recipients of the different kinds of LEW allografts were killed after 14, 30, 60, and 100 days. Liver allografts, and recipient tibias and femurs, were removed and examined for the presence of LEW hematopoietic progenitor cells by in vitro colony-forming unit in culture (CFU-C) assay and in vivo adoptive transfer into lethally irradiated recipients.

BMC—A crude fraction of BMC was obtained by flushing the tibial and femoral medulla with RPMI 1640 medium. After lysing the red blood cells by hypotonic solution, cells were resuspended in RPMI 1640, supplemented with 5% fetal bovine serum (FBS), 10 mM HEPES, 2 mM L-glutamine, 50 μg/ml gentamicin, and 5×10^{-5} M 2-mercaptoethanol (2-ME; Life Technologies).

Aliquots of $15–20 \times 10^6$ BMC were seeded into 10-cm culture dishes (Falcon. Lincoln Park, NJ). After an incubation for 2 hr at 37°C, the plastic dish-nonadherent BMC were collected and resuspended in complete Iscove's modified Dulbecco's medium (IMDM; Sigma, St. Louis, MO), supplemented with 10 mM HEPES. 8 mM L-glutamine, 50 μg/ml gentamicin, and 5×10^{-5} M 2-ME.

Liver nonparenchymal cells (NPC)—Nylon wool-nonadherent NPC were isolated from the liver by the collagenase digestion method of Berry and Friend (8) with some modifications. Briefly, the portal vein or the thoracic inferior vena cava was cannulated with a 16-gauge plastic catheter. The liver was perfused in situ at 30 ml/min initially with 120 ml of Hanks' balanced salt solution (Life Technologies), containing 5 mM EGTA (Sigma), 10 mM HEPES, and 50 μg/ml gentamicin, then with 300 ml of Leibovitz's L-15 medium (Life Technologies) containing 0.05% collagenase (type B; Boehringer Mannheim, Mannheim. Germany), 0.005% trypsin inhibitor (type II-S; Sigma), 2.5% FBS, 10 mM HEPES. and 50 μg/ml gentamicin. After perfusion, the liver was removed into a Petri dish, and NPC and parenchymal cells were liberated from the connective tissue by carefully raking the liver with scissors and shaking the liver gently.

The initial cell suspension was filtrated through a nylon mesh, and a crude fraction of NPC was separated from hepatocytes by low-speed centrifugation (five times at 70×g for 4 min) and washed by high-speed centrifugation (three times at 280×g for 8 min). The crude NPC suspension was subsequently overlaid on discontinuous Percoll density gradients and centrifugated at 750×g for 30 min. Purified NPC were obtained in an interface (1.051–1.084) by depleting debris, dead cells, contaminating hepatocytes, and red blood cells. The NPC suspension was then passed through a nylon wool column, and nylon wool-nonadherent NPC were finally obtained in complete IMDM.

CFU-C. Semisolid culture system—Our previously described CFU-C assay was performed, using a fibrin clot culture system with some modification (3, 9, 10). Plastic dish-nonadherent BMC and nylon wool-nonadherent hepatic NPC in complete IMDM were further mixed with 2% pokeweed mitogen (Sigma)-stimulated LEW spleen cell-conditioned medium, 20% FBS, 1% bovine serum albumin, 0.5 mM N^G-monomethyl-L-arginine-HOAc (NMA; Cyclo$_3$pss Biochemical Co., Slake Lake, UT), 0.5 mg/ml bovine fibrinogen solution (Sigma), and 0.5 U/ml bovine thrombin solution (Sigma) in final concentrations of $0.5–5 \times 10^5$ and 1×10^6/ml, respectively. A total suspension volume of 1.0 ml was plated in the middle of a 60-mm Parmanox dish (Nunc, Naperville, IL).

After clot formation, 1.0 ml of complete IMDM with 20% FBS and 1% bovine serum albumin was added around the clot. The culture was kept for 6 days in a fully humidified atmosphere with 5% CO_2 in air at 37°C. On day 6, the outer medium layer around the clot was discarded, and the clot was slowly dried in the incubator for 5 hr. The flattened clot was then fixed either with absolute methanol for 30 min and washed with H_2O for Giemsa staining (Sigma), or fixed with 95% ethanol for 1 hr, air dried for another 1 hr, and washed with phosphate-buffered saline (PBS) for immunohistochemical staining. Clusters (10–50 cells/aggregate) and colonies (>50 cells/aggregate) were counted as CFU-C counts.

Liquid culture system—The assay previously described by Lu et al. (11) for the propagation of dendritic cells was performed with modifications, in parallel with the semisolid method to identify progenitor cells. Although the method of Lu et al. (11) was used, the cell population analyzed was different. In the original investigation of Lu et al. (11), the dendritic-shaped cells released from the clusters and floating in the medium were stained. In the present study, floating cells were removed from the culture and only adherent colonies were stained. Briefly, 2×10^6 liver NPC from the crude fraction or unfractional BMC were placed in a 24-well plate (Corning Inc., Corning, NY) in 2 ml of RPMI 1640, supplemented with 10% FBS, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 50 μg/ml gentamicin. 2×10^{-5} M 2-ME, 0.5 mM NMA, and 0.4 ng/ml recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF; R&D Systems Inc., Minneapolis, MN). The cultures were fed every other day with a fresh recombinant murine GM-CSF-supplemented medium after gently swirling the supernatant and discarding 50% of the medium in each well. Twenty-four hours before terminating the cultures, recombinant rat interferon-γ (100 U/ml: Genzyme, Cambridge, MA) was added to increase MHC class II expression of cultured cells.

Propagated cells in this system showed morphologic features of dendritic cells and macrophages. After culturing for more than 10 days, the number of propagated colonies in this system, in which floating cells were simultaneously generated, was much greater than that in the semisolid fibrin clot culture system. After removing nonadherent floating cells by gently aspirating the supernatants, loosely or firmly adhered colonies in wells were air dried for 2 hr, fixed with 95% ethanol for 1 hr, and air dried for another 1 hr for immunohistochemical staining.

Phenotyping of cultured cells

The CFU-C derived from LEW (donor) hematopoietic stem cells were identified by in situ immunochemical staining with mAb L21–6 of propagated colonies in the fibrin clot and liquid culture systems, using a standard three-step avidin-biotin complex method. Endogenous avidin-biotin activity and nonspecific bindings were blocked with avidin-biotin blocking kit (Vector, Burlingame, CA) and heat-inactivated normal BN serum (1:10), respectively. After incubation with mAb L21–6 for 2 hr at room temperature, samples were washed with PBS. placed in methanol containing 1% H_2O_2 for 10 min to deplete

endogenous peroxidase activity, washed in PBS for 20 min, and further incubated with normal rat serum (1:10; Sigma) for 20 min.

A biotinylated rat anti-mouse Fab_2 fragment (1:500: Jackson ImmunoResearch, West Grove, PA) was used as a secondary antibody for 45 min. After washing with PBS, samples were incubated with avidin-biotin complex (Vector) for 30 min, and color development was achieved with aminoethylcarbozole (ScyTek, Longan, UT). Cells were counterstained with hematoxylin. CFU-C in the fibrin clot culture and colonies in the liquid culture were considered to be derivative from LEW hematopoietic stem cells only when they were composed of L21–6–positive cells.

Established colonies were also analyzed for their surface lineage markers using the same in situ stain method as described above. mAb used were EDI (macrophages, 1:100), ED2 (macrophages, 1:100), and R7.3 (*αβ*-T cell receptor-[TCR], 1:25–100) (all from Serotec, Oxford, UK). All staining experiments were performed with controls including fibrin clot CFU-C and spleen sections obtained from normal LEW and BN rats. Nonimmune isotype antibody (mouse IgG1) was also used for negative control in each assay.

In vivo propagation of donor hematopoietic progenitor cells in lethally irradiated recipients

The same BMC used for CFU-C assay were infused into lethally irradiated (9.5 Gy delivered from a cesium source) BN recipients (1.5×10^8 unfractionated BMC per animal). Between 40 and 60 days alter bone marrow transplantation, the peripheral blood was taken and percentages of donor (LEW) cells were analyzed by flow cytometry using affinity-purified biotinylated mAbs 163 (rat IgG2b) and 42 (rat IgG2a), which are specific for the $RT1.A^2$ (MHC class I) antigen on LEW and $RT1.A^n$ antigen on BN, respectively (12). Subsequently, animals were challenged with LEW or ACI heart grafts to examine the development of LEW-specific graft acceptance.

RESULTS

CFU-C in normal untransplanted animals

In BMC—The BMC of normal LEW and BN rats had a similar frequency of hematopoietic progenitor cells, as judged by the CFU-C in the fibrin clot culture system. The total CFU-C counts per 5×10^4 plastic dish-nonadherent BMC were 54±18 (SD) LEW (n=3) and 43±4 BN (n=3). By morphologic criteria after Giemsa staining, the majority of colonies were composed of macrophage lineage and dendritic-like cells. In situ immunohistochemical stain confirmed that the percentage of $ED1^{1+}$ and $ED2^{2+}$ cells (mature macrophages) were 60.9±17.4% and 40.9±11.2% in LEW BMC (n=3) and 55.8 ± 17.1% and 40.1±5.2% in BN BMC (n=3), respectively. With conventional cytoplasmic enzyme staining of the same samples, the majority of colonies were myeloid lineage (including weakly stained immature macrophages): 7.6±2.1% in LEW BMC (n=3) and 6.5 ± 1.7% in BN BMC (n=3) were peroxidase-positive granulocyte colonies, whereas 85.9% ± 2.1% in LEW (n=3) and 86.6 ± 1.9% in BN BMC (n = 3) were nonspecific esterase-positive macrophage colonies.

None of the CFU-C generated from BN BMC were stained with the mAb L21–6 (Fig. 1A). Interestingly, only 39±9% (SD) of CFU-C generated from the LEW BMC were stained in situ with mAb L21–6. The dispersed type of LEW CFU-C generally were composed of L21–6⁻ cells, whereas concentrated CFU-C tended to be L21–6⁻, suggesting that differences between L21–6-positive and -negative colonies depended on the maturation stages of macrophage and dendritic cell development from hematopoietic progenitors (Fig. IB).

To determine the sensitivity with which small numbers of LEW CFU-C could be detected, plastic dish-nonadherent BMC from naive animals were mixed at various LEW/BN ratios from 1:10 to 1:1000 and subjected to CFU-C assay. When the LEW BMC constituted 0.1, 1.0, and 10% of the 5×10^5 cells in the mixture, the numbers of L21–6-positive CFU-C were 0.2 ± 0.4, 2.3 ± 1.4, and 21.3 ± 5.7 (SD) (Fig. 2).

The majority of cells in the semisolid culture of LEW and BN BMC mixture were positive for EDI (>80%) and ED2 (>55%). There was no colony or individual cell stained with mAb specific for $\alpha\beta$TCR (R7.3), showing that detectable growth of activated lymphocytes does not occur in this culture system even after mixing allogeneic BMC. For the control, activated T cells obtained from standard mixed lymphocyte culture were stained with R7.3, and positive stain was confirmed.

In liver NPC—Using 10^6 nylon wool-nonadherent NPC obtained from naive LEW liver (rather than the 5×10^4 plastic dish-nonadherent cells used for the bone marrow studies), $7.9+3.9$ CFU-C were generated (n=3). Similar to those obtained from BMC in the same culture system, the majority of colony-forming cells were macrophages and/or dendritic-like cells; $35\pm6\%$ (SD) of the CFU-C stained with L21–6 compared with the $39\pm9\%$ in comparably prepared LEW BMC (see above).

CFU-C in bone marrow recipients

After infusion of 2.5×10^8 LEW BMC into BN recipients, LEW CFU-C were easily identified with the mAb L21–6 stain in the plastic dish-nonadherent recipient BMC (Fig. 3A). Mean donor CFU-C at 14, 30, and 60 days after transplantation were 5.4, 25.9, and 7.8, respectively (Table 1). Based on the results obtained from in vitro mixing of normal LEW and BN BMC (Fig. 2), the percentage of donor contribution to the total hematopoietic progenitor cell population in the recipient bone marrow was estimated to be 5–10% during the first 2 months after transplantation. By 100 days, however, donor CFU-C were no longer detectable with this assay.

CFU-C in liver recipients

In bone marrow—At 14 (n=4) and 30 days (n=2) after transplantation, all six recipient animals had donor CFU-C in their bone marrow: 1.0 and 0.3, respectively, at the two time periods (Table 1). The percentage contribution to the total hematopoietic progenitor cell population in recipient bone marrow was estimated to be 0.1–0.5%. This was only 1/10 to 1/100 that at comparable times after bone marrow infusion. This finding was consistent with the smaller number of progenitor cells thought to be contained in one liver compared with that in 2.5×10^8 BMC. No donor L21–6$^+$ colonies were found in later bone marrow samples with the semisolid culture system.

In the liver allografts—The invariable presence of multilineage microchimerism at 100 days in the LEW $\rightarrow$ BN liver transplantation model (6) as well as the persistence of a small population of donor leukocytes in the hepatic graft (13) caused us to suspect that the transplanted liver might be a nesting site for donor progenitor cells. However, when the nylon wool-nonadherent NPC from the grafted liver were cultured in the fibrin clot system, total CFU-C counts were too small even at 30 days after transplantation (6.1 ± 2.8 per 10^6 cells; n = 2) to study the frequency of L21–6$^-$ colonies. The possibility could not be ruled out. however, that the low progenitor cell yield was due in part to differences in the preliminary steps of cell separation (i.e.. more traumatic for liver NPC than for BMC).

Therefore, the liquid culture system developed by Lu et al. (11) was used, which allowed the generation of more colonies from the crude NPC preparation; in addition, the doses of

tacrolimus were increased to 1.5 mg/kg. After culturing these cells for 14 to 28 days, the propagated cells, which resembled macrophages and dendritic cells, were shown to be 97.8±1.0% (n=3) ED2 strong positive (Fig. 3B) and 84.1±2.6% (n=3) EDI weak positive. We were not able to detect any colony or individual cell stained with mAb specific for $\alpha\beta$TCR (Fig. 3C), confirming earlier observations by Lu et al. (11) using this long-term liquid culture enriched with GM-CSF.

The donor phenotype colonies were easily identified in the cultures of NPC obtained from grafted livers 30 days after transplantation m=3); the L21–6$^-$ colonies were estimated to range from 1 to 10% of all propagated colonies (Fig. 3D). Donor colonies also were seen in liver NPC 60 days after transplantation (n = 2), but at a <1% incidence. At 100 days after liver transplantation, donor L21–6$^+$ colonies were rare (<0.1%), but definitely existed among NPC obtained from the liver allograft (Fig. 3E).

Donor phenotype cells, but not colonies, were simultaneously detected in the bone marrow of these recipients using both semisolid and liquid culture systems. Interestingly, donor cells in the recipient bone marrow were mostly identified as an isolated single cell or aggregates of a few cells (Fig. 3F). These results suggested that some of the organ-based hematopoietic progenitor cells either persisted in, or else returned to, the grafted liver after transplantation.

CFU-C in heart recipients

The recipient bone marrow did not have detectable levels of CFU-C 14 days after heterotopic heart transplantation (Table 1). Samples were not obtained at later times.

LEW heart graft survival in secondary 9.5-Gy irradiated BN recipients reconstituted with transferred BMC

Controls—LEW heart grafts were rejected by naive BN animals with a median survival of 10 days (n=7). When lethally irradiated BN animals were reconstituted with BMC obtained from naive or tacrolimus-treated BN animals (without a LEW graft), LEW heart grafts were rejected within 16 days (group 1–4, Table 2). There was no significant difference in heart graft survivals, whether the BMC for reconstitution were obtained at 14 (group 2), 60 (group 3), or 100 days (group 4) after the initiation of tacrolimus treatment.

Adoptive transfer—When BMC were obtained 30 and 60 days after LEW bone marrow infusion, reconstituted animals accepted LEW heart grafts for 100 days without any immunosuppressive treatment (group 5 and 6). Flow cytometric analysis of the peripheral blood obtained from these animals before heart grafting revealed that 1.0 to 6.0% were LEW phenotype cells. In these animals, third-party ACI hearts were normally rejected in 7 days (n=3).

The adoptive transfer effect was not detectable in BN animals reconstituted with the BMC obtained from BN animals 100 days after LEW bone marrow infusion (group 7); LEW heart graft survival was reduced to 12 days. When lethally irradiated BN rats were reconstituted with BMC from BN animals that had been carrying LEW liver grafts for 14 (group 8), 30 (group 9), 60 (group 10), and 100 (group 11) days, animals rejected LEW heart grafts with median survivals between 7 and 13 days. Transfer of BMC obtained 30 days after LEW to BN heart transplantation had no effect on LEW heart graft survival (group 12).

DISCUSSION

It is well known that migratory organ "passenger leukocytes" of bone marrow origin, like infused BMC, home selectively at first to organized lymphoid collections (13–17). Our

specific purpose here was to obtain information about the destination of the subset of progenitor cells without which long-term perpetuation of posttransplantation chimerism would not be possible (18, 19). It was possible to make a precise distinction between donor and recipient hematopoietic cells with the mAb L21–6 that stains MHC class II$^+$ cells of LEW, but not of BN rats (7). This phenotyping tool in combination with the CFU-C technology made the search for progenitor cells feasible.

Because only 35–40% of the LEW colonies stained with the mAb L21–6, the number of donor CFU-C was considerably underestimated. Nevertheless, our experiments demonstrated the movement of donor progenitor cells into the recipient bone marrow. Although it has been a common assumption that recipient cytoablation is necessary to "create space" or "open niches" for infused leukocytes, many studies have suggested that syngeneic marrow cells can compete equally with host hematopoietic cells for long-term residence with an engraftment rate near theoretical maximum (20–23). A recent study by Ehl et al. (24) has shown the same thing in untreated mice despite a class I-restricted minor histocompatibility barrier.

The present study showing a 5–10% proportion of donor progenitor cells after infusion of 2.5×10^8 fully allogeneic BMC demonstrated the same thing, early after their infusion. The subsequent decline of these cells in our noncytoablated recipients occurred only after the cessation of immune suppression. Thus, it can be suggested that the effectiveness of cytoablation in the promotion of persistent allogeneic cell engraftment is more related to its weakening of the host immune response than to the provision of space.

With the LEW-BN strain combination used in our experiments, long-term organ engraftment is routine despite the variable development of low-grade chronic rejection (6). In this model, Terakura et al. (5) have demonstrated the previously unrecognized secondary migration LEW leukocytes of heart allografts and of BMC from BN recipient lymphoid organs and blood to nonlymphoid sites (e.g., skin and native heart) between 56 and 100 days after transplantation. Thus, it is conceivable that donor progenitor cells may be found in unexpected nonlymphoid locations.

One of the candidate sites for survival of allogeneic progenitor cells consistent with observations in the present study, is the microenvironment of the transplanted organ, which is syngenic to the surviving donor leukocytes. In a well-tolerated organ allograft, most of the departed passenger leukocytes are replaced by functionally nonreactive host cells of the same lineages (18, 25–27), a change that is nearly complete within 14 days in the rat (26). However, an estimated 5% of the leukocytes in an "accepted" organ allograft remain of donor phenotype (13). It is not known whether this residual population has failed to migrate or whether it is the consequence of recirculation. Despite technical difficulties some of these donor leukocytes within the liver grafts were shown in the present study to be progenitor cells.

It remains to be determined whether an organ allograft, syngeneic to its peripheralized passenger leukocytes, provides an especially advantageous microenvironment for the nesting of donor stem cells. If so, this would be consistent with evidence (summarized in 6 and 28) that microchimerism is easier to maintain in a recipient who also has an organ. Such chimerism or even macrochimerism often is lost in controlled experimental models when the organ allograft is removed.

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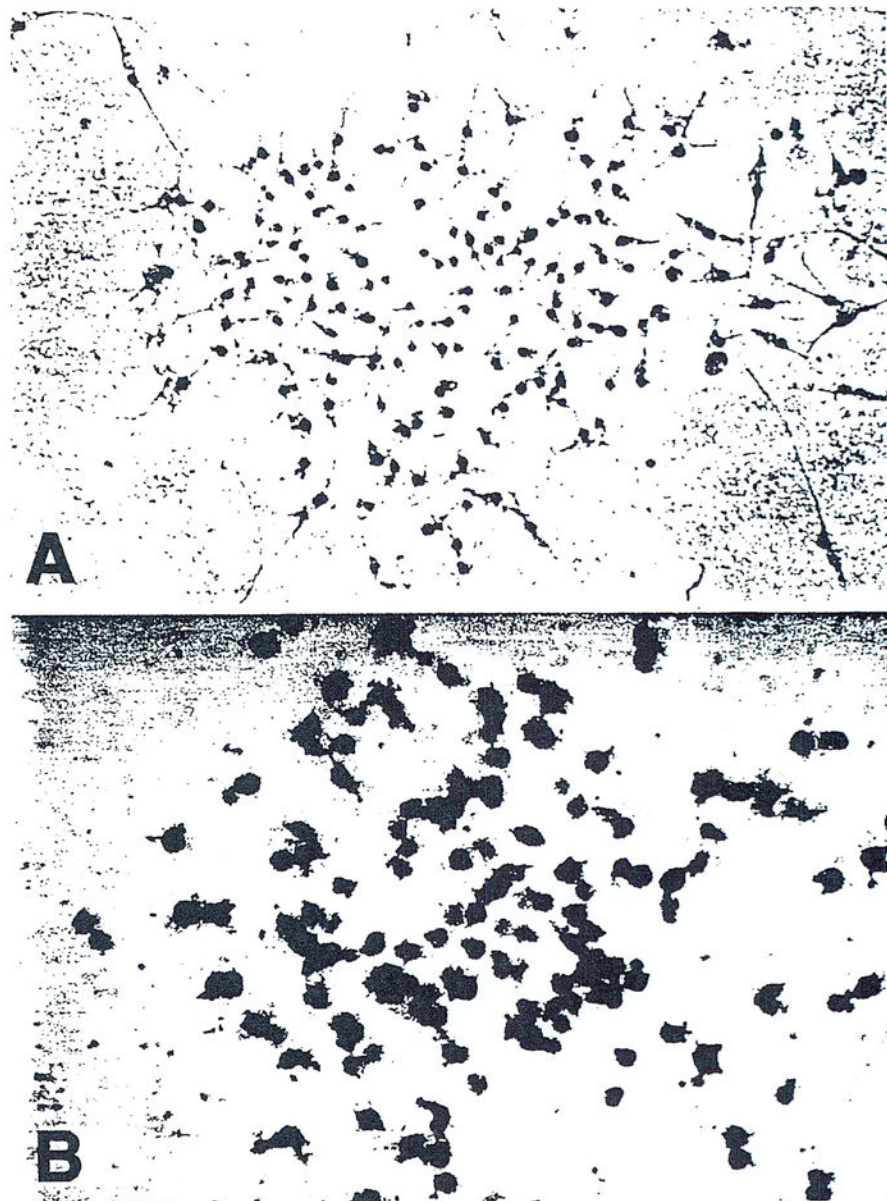
Figure 1.
L21–6-positive CFU-C in normal BMC. Plastic dish-nonadherent LEW or BN BMC
(5×10^4) were cultured in a fibrin clot culture system for 6 days and generated CFU-C that
were exposed to mAb L21–6. (A) CFU-C in BN BMC were not stained with L21–6. (B) The
positively stained dispersed-type CFU-C in LEW BMC were composed of macrophages and
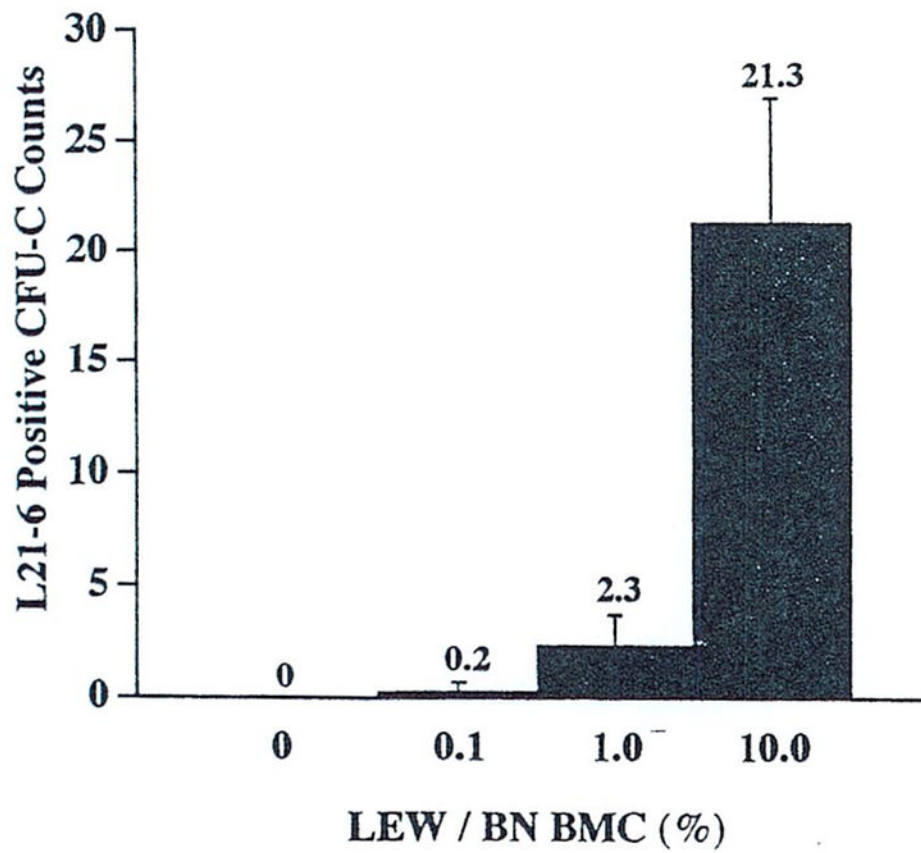dendritic like cells (without counterstain) (Original magnification; $\times$ 100).

Figure 2.
The number of L21–6-positive (LEW) CFU-C per 5×10^5 cells in three mixtures of naive LEW and BN BMC. Culture and assay conditions as in Figure 1. Data indicate average ± SD from three experiments.
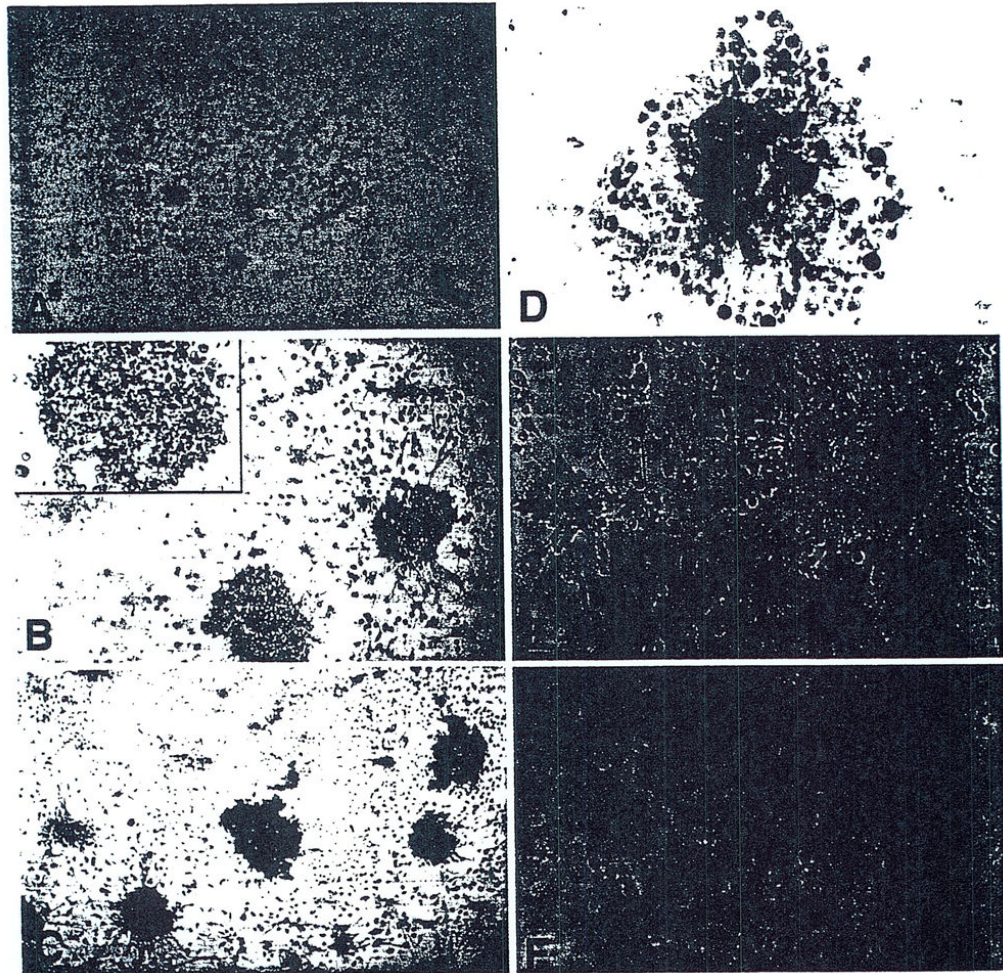
Figure 3.
(A) L21–6-positive donor (LEW) CFU-C in BN recipient bone marrow after LEW bone marrow infusion. CFU-C in BN recipient BMC obtained 30 days after LEW bone marrow infusion and studied with the same assay as in Figure 1. Three individual L21–6-positive (LEW) CFU-C are identified in this field (Original magnification; ×40). Colonies developed from liver NPC (30 days after transplantation) in a liquid culture system were mostly (B) ED2-positive and (C) αβTCR-negative. L21–6-positive donor colony in liver NPC (D) 30 days and (E) 100 days after transplantation into BN recipient. A crude fraction of NPC was obtained from liver allografts and cultured in a liquid culture system with recombinant murine GM-CSF. After 18 days of culturing, loosely or firmly adherent colonies in the wells were fixed and in situ stained with mAb. ED2, R7.3, and L21–6. A colony is shown that contains both weak and strong (L21–6) positive macrophage lineage and dendritic like cells (original magnification; B, ×40; C, ×40; D, ×200; E, × 100). (F) Singly identified donor phenotype cells in the liquid culture of BMC 100 days after liver transplantation (tacrolimus 1.5 mg/kg/day on days 0–13, 20, 27; Original magnification; ×100 without counterstain).

Table 1

Donor MHC class II$^+$ CFU-C in the bone marrow of BN recipients after liver, heart, or bone marrow transplantation

Graft/cells	Days after transplantation	n	Donor MHC class II$^+$ CFU-C counts (per 5×10^5 BMC)	Mean
Bone marrow	14	3	4.1, 5.3, 6.8	5.4
	30	2	21.5, 30.2	25.9
	60	2	6.3, 9.3	7.8
	100	4	0, 0, 0, 0	0
Liver	14	4	0.3, 0.6, 1.5, 1.5	1.0
	30	2	0.1, 0.5	0.3
	60	2	0,0	0
	100	2	0,0	0
Heart	14	2	0,0	0

Plastic dish-nonadherent BMC from BN recipients after LEW bone marrow (2.5×10^8), liver, or heart transplantation were cultured in the fibrin clot culture system (5×10^5 cells/plate, 10 plates/each experiment). After culturing for 6 days, established CFU-C were in situ stained with mAb L21–6 (LEW MHC class II), and the number of CFU-C composed of strongly stained cells was counted.

Table 2

LEW heart graft survival in secondary 9.5-Gy irradiated BN recipients reconstituted with transferred BMC

| Group | Bone marrow donor | | n | LEW heart graft survival[a] (days) | Median (days) |
	Original LEW graft/cells	Days after original transplantation			
1	None	NA[c]	3	8, 8, 13	8.0
2		14	5	11, 11, 12, 12, 12	12.0
3		60	4	8, 11, 11, 11	11.0
4		100	10	9, 11, 11, 11, 12, 13, 13, 13, 16, 16	12.5
5[b]	Bone marrow	30	5	>100×5	>100
6[b]		60	10	10, >100×9	>100
7		100	10	8, 10, 11, 11, 11, 13, 13, 14, 14, 15	12.0
8	Liver	14	5	6, 6, 7, 7, 8	7.0
9		30	4	6, 7, 8, 8	7.5
10		60	5	9, 10, 11, 12, 13	11.0
11		100	8	10, 11, 11, 13, 13, 14, 14, 15	13.0
12	Heart	30	5	10, 12, 13, 13, 13	13.0

[a]LEW heart graft survival in naive BN recipients was 8, 8, 9, 10, 10, 11, and 11 days.

[b]Third-party (ACI) heart graft survival in these animals was 7 days (n=3).

[c]NA, not applicable.