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Stromal Cell-Derived Factor 1 (CXCL12) Induces Human Cell Migration into Human Lymph Nodes Transplanted into SCID Mice

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Stromal cell-derived factor 1 (SDF-1; CXCL12), a CXC chemokine, has a primary role in signaling the recruitment of hemopoietic stem cell precursors to the bone marrow during embryonic development. In postnatal life, SDF-1 is widely expressed and is induced in chronically inflamed tissues such as psoriatic skin and the rheumatoid synovium, but has also been implicated in the migration of lymphocytes to lymphoid organs. To investigate the role of SDF-1 in recirculation and homing in vivo, we have developed a model in which human peripheral lymph nodes (huPLN) are transplanted into SCID mice. We have shown that huPLN transplants are viable, vascularized by the murine circulation that forms functional anastomoses with transplant vessels. In addition, grafts retain some features of the pretransplantation tissue, such as lymphoid follicles, lymphatic and high endothelial venule markers. We also show that SDF-1 is capable of inducing the migration of a SDF-1-responsive cell line (U937) and human PBLs from the murine circulation into the grafts in a dose-dependent manner, inhibitable by CXCR4 blockade. The mechanism of action of SDF-1 in this model is independent from that of TNF-α and does not rely on the up-regulation of adhesion molecules (such as ICAM-1) on the graft vascular endothelium. This is the first description of huPLN transplantation into SCID mice and of the functional effects of SDF-1 in regard to the migration of human cells into huPLN in vivo. This model provides a powerful tool to investigate the pathways involved in cell migration into lymphoid organs and potentially to target them for therapeutic purposes. The Journal of Immunology, 2002, 168: 4308–4317.

Stromal cell-derived factor 1 (SDF-1)α was first identified as an 89-aa cytokine capable of supporting the proliferation of a stromal cell-dependent pre-B cell line (1). The amino acid sequence of the secreted protein classified SDF-1 as a member of the CXC chemokine (CK) family and has been recently renamed CXCL12 (2). In contrast to the majority of CK, SDF-1 interacts specifically and monogamously with a G protein-coupled serpine transmembrane receptor LESTR/FUSIN subsequently termed CXCR4 (3). Binding of SDF-1 to CXCR4 on the leukocyte cell surface results in intracellular calcium fluxes and morphologic changes associated with cell locomotion, such as the formation and retraction of lamellipodia and integrin activation (4).

Both SDF-1 and CXCR4 expression have been found to be essential for normal myelopoiesis and lymphopoiesis both in embryo and adult life. McGrath et al. (5) have shown that the expression of CXCR4 mRNA in embryonic ectodermal structures is complementary to the expression of SDF-1 by migrating endo- and mesodermal stem cells. Defects seen in SDF-1-deficient mice suggest an important role for this cytokine in the recruitment and retention of leukocyte precursor lineages to myelopoietic and lymphopoietic sites (6, 7). This is also suggested by the fact that SDF-1 has been shown to be instrumental in mobilizing hemopoietic precursors from the bone marrow into the circulation and from here into peripheral tissues (8, 9). In addition, indirect evidence for an important role of SDF-1 in regulating cell migration comes from the knowledge that SDF-1 associates with heparan sulfates on the endothelial surface (10) and stimulates integrin-mediated arrest of CD34+ cells on vascular endothelium under shear flow in vitro (11). Furthermore, SDF-1 preactivation of CD34+ cells in vitro up-regulates their migration in vivo following transfer into SCID animals (12).

In humans, on the basis of a wide expression of SDF-1 in psoriatic skin and rheumatoid synovium, it has been suggested that SDF-1 is important in facilitating cell migration to inflamed tissues (13–15). On the other hand, SDF-1 is also strongly expressed within splenic red pulp and lymph node medullary cords (16, 17), suggesting that SDF-1 is also involved in lymphocyte migration to secondary lymphoid organs. However, no human data are available to directly prove this point. For this reason, to investigate directly the functional role of SDF-1 in regulating human lymphocyte migration into human peripheral lymph nodes (huPLN), we have developed a new model (reported herein) in which huPLN are transplanted into SCID mice s.c.

In the work designed to validate this model, we demonstrate that 1) huPLN can be grafted onto SCID mice with a success rate greater than 90%; 2) huPLN transplants are vascularized by mouse

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subdermal vessels which form anastomoses with the human graft microvascular endothelium that expresses human ICAM-1; 3) mouse-human vascular anastomoses are patent and functional as shown by the capacity to deliver mAbs and human cells to the grafts via the mouse systemic circulation; 4) a proportion of graft venules retained high endothelial venule (HEV) morphology, MECA-79 and CD34 expression; and 5) markers of human lymphatic vessels were found within grafts.

In the work designed to investigate the functional role of SDF-1 in this model, we demonstrate that 1) SDF-1 has even a greater ability than TNF-α to induce human PBL (huPBL) and U937 cell migration into the grafts; 2) the SDF-1-induced migration was specifically inhibitable by blocking the SDF-1 receptor CXCR4; 3) contrary to TNF-α, the SDF-1-induced migration was not dependent on endothelial up-regulation of ICAM-1; and 4) SDF-1 and TNF-α effects are independent from each other.

To our knowledge this is the first time that huPBL have been successfully transplanted into SCID mice and that a functional effect of SDF-1 on the migration of huPBL into huPBL has been directly demonstrated. Since recirculation studies to secondary lymphoid organs in vivo in humans are impossible for obvious ethical considerations, this model offers a possible alternative approach for investigating the molecular pathways involved in regulating lymphocyte migration into lymphoid organs.

Materials and Methods

Tissue collection, preparation, storage, and transplantation

Para-aortic or cervical huPLN were obtained from patients requiring vascular surgery. huPLN were of normal size and macroscopic appearance. Samples of each node were processed for routine H&E histology before their use for transplantation studies and found to have a normal histological appearance. Procedures were performed after informed consent approved by the hospital Ethics Committee (LREC 9903/19). Samples were divided into two parts. One part was used for immunohistology and the second for transplantation. The part assigned for immunohistology was embedded in OCT compound (Miles, Torrence, CA), snap frozen in liquid nitrogen-cooled isopentane (BDH, Poole, U.K.), and stored at −70°C until analysis. The second part, assigned for transplantation, was cut into 0.5-μm pieces, frozen in 20% DMSO (Sigma-Aldrich, St. Louis, MO) in heat-inactivated FCS (PAA Labs, Linz, Austria), and stored in liquid nitrogen until engulfment as previously described (18).

Samples of huPLN were thawed from liquid nitrogen storage immediately before surgery, washed in saline, and kept in saline-moistened sterile gauze over ice until transplanted. Beige SCID C.B-17 (NOD/LttSz-scid/scid) mice, maintained under pathogen-free conditions in biological facilities of King’s College, were anesthetized by i.p. injection of 0.2 ml Dormitor (0.1 mg/ml GlaxoSmithKline, Uxbridge, U.K.) and 0.1 ml ketamine (0.1 mg/ml; SmithKline Beecham). A small incision was made in the dorsal skin behind the ear of each SCID mouse (4–6 wk of age) and the tissue was inserted s.c. The wound was closed with soluble suture material (Ethicon, Edinburgh, U.K.). Successful tissue transplantation was assessed before migration studies by immunohistology after 4–5 wk. This particular strain of mice was chosen to minimize this possibility that huPBL could be killed by mouse NK cells in their systemic circulation. NOD/LttSz-scid/scid mice are specifically bred not only to produce no T or B cells, but also to have no NK activity (although the animals retain nonfunctional NK cells).

Assessment of graft viability

Graft viability was assessed before immunohistochemical or morphometric analysis both macroscopically and by microscopy of HE-stained acetone-fixed cryostat sections. Grafts judged to be necrotic or those comprising tissues other than those transplanted (e.g., murine skin and muscle) were excluded from the study.

Assessment of human vasculature within grafts

To confirm the conservation of human vasculature-associated cell adhesion molecule (CAM) and to assess the modulation of CAM expression following cytokine stimulation of the grafts, we assessed the expression of human ICAM-1, VCAM-1, and E-selectin pre- and posttransplantation using species-specific mAb and standard immunohistochemical techniques (see below). The relative expression of CAM was quantified using an arbitrary scale of staining intensity from 0 to 4, where 0 indicated no staining and 4 indicated maximal staining. To determine whether the human transplant vasculature remained patent and connected with the murine vascular network in the grafts, transplanted mice were injected i.v. with either biotinylated anti-human ICAM-1 or a biotinylated isotype-matched control Ab (MOPC21). Mice were killed after 10 min and the transplants were embedded in OCT and snap frozen. Cryostat sections were then incubated with avidin-biotin alkaline phosphatase (AP) complex for 30 min followed by development using a Vector Red substrate kit. Sections were subsequently incubated with FITC-conjugated anti-human VWFVIII (Serotec, Oxford, U.K.) to identify human blood vessels and, therefore, determine the site of localization of the anti-ICAM-1 and control Abs. Sections were mounted in aqueous mountant (ImmunoMount; ICN, Basingstoke, U.K.) and examined by UV fluorescence microscopy.

Quantification of human and murine vasculature

Cryostat sections of the transplants were immunostained for both human vascular markers (including VWFVIII, MECA-79, CD34, and CCL21) and mouse vessels using anti-murine CD31 (for details of Abs used, see Table I). Standard immunoenzymatic histochemistry methods (streptavidin-alkaline phosphatase; DAKO, Glostrup, Denmark) were used as described below. The volume fraction (Vv) of murine and human vessels within the transplanted tissues was determined morphometrically using a point counting technique (19). Briefly the number of point-sampled intersections overlaying the positively stained vessels was determined on serial cryostat sections using a light microscope equipped with a 5 × 5 eyepiece graticule. The ratio of positive hits to total possible hits was calculated to give the volume fraction of vessels per equivalent volume of transplant tissue. For each transplant, ~60 microscope fields from three cutting levels were examined.

Immu-nalloidal phosphatase stain

Indirect staining was performed using standard techniques as previously described (20). Briefly, 10-μm acetone-fixed cryostat sections of the grafts after incubating with appropriate nonimmune serum for 20 min at room temperature to block nonspecific binding were incubated with primary Ab for 1 h. Sections were washed (50 mM TBS, pH 7.6) and incubated with the secondary Ab for 30 min. If the secondary was conjugated to biotin, sections were washed and incubated with avidin-biotin-AP complex (DAKO) for 30 min. Sections were developed using the alkaline phosphatase substrate kit Vector Red containing 1 mM levamisole to inhibit endogenous alkaline phosphatase activity. Finally, sections were washed, counterstained with Meyer’s haematoxylin, dehydrated through graded ethanol, cleared in xylene, and mounted under DePeX (BDH) for examination by light microscopy.

Immunohistochemical analysis of SDF-1 and TNF-α in original lymph nodes and grafted tissue

SDF-1 tissue distribution was analyzed immunohistochemically using Abs specific for human SDF-1α and quantified using the same grading score described above. The tissue distribution of TNF-α was determined immunohistochemically using a modification of the method described by Ulfgren et al. (21). Briefly, sections were fixed for 20 min in TBS containing 2.5% paraformaldehyde (pH 7.6, 4°C) and washed in TBS plus 0.1% saponin (Sigma-Aldrich) for 20 min. All subsequent Ab incubations and washes were conducted in the presence of 0.1% saponin. A standard two-layer immunoalkaline phosphatase technique was used with a murine anti-TNF-α primary and goat anti-mouse AP-conjugated secondary Ab and vector red substrate for visualization (for details of Abs, see Table I). TNF-α-positive cells showed characteristic intracellular distribution, characteristically submembrane or a predominantly supranuclear cytoplasmatic localization as previously described (21). Sections of rheumatoid synovium were used as a positive control.

Cell preparation, culture, and labeling.

U937 cell culture and analysis of CXCR4 and LFA-1 expression by flow cytometry. The U937 human myelo-monocytic cell line was cultured in RPMI 1640 medium plus 10% FCS. Cells were subcultured and maintained at 0.5–1.0 × 10⁷ cells/ml. For FACS analysis, U937 cells were resuspended in PBS (0.5 × 10⁷ cells/ml), and 100-μl aliquots were added to a 96-well flat plate in triplicate for each treatment and incubated on ice. CXCR4 and LFA-1 expression was assessed by addition of specific mAbs (mAbs, see Table I) at their previously determined saturating concentration. Nonspecific binding was minimized by the addition of human IgG (6 mg/ml). Plates were incubated at 4°C for 1 h. Cells were washed and
incubated with P(ab\textsuperscript{2}) of goat anti-mouse IgG-FITC before further washing and analyzed by flow cytometry (BD Biosciences FACScan II analyzer; BD Biosciences, Mountain View, CA).

**huPBL.** HuPBL were isolated from peripheral blood using Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation as described previously (22). The isolated huPBL were suspended in tissue culture medium (10% heat-inactivated FCS (PAA) in RPMI 1640 (Life Technologies, Grand Island, NY)) and then incubated overnight in plastic flasks to deplete adherent monocytes. The remaining nonadherent cells, mostly lymphocytes as demonstrated by FACS analysis (see below), were then washed and resuspended in serum-free PBS ready for labeling.

**Cell labeling.** U937 cells or huPBL were incubated with PKH26 dye (Sigma-Aldrich) at room temperature at a concentration of 100 μl of dye per 20 × 10\textsuperscript{6} cells in 5 ml of diluent for 2 min before stopping the reaction with the addition of heat-inactivated FCS. The cells were then washed twice to remove unbound dye and resuspended in sterile PBS (pH 7.6) at a cell concentration of 50 × 10\textsuperscript{6} cells/ml. Cell viability was determined via trypan blue exclusion and always found to be >95%. PKH26 labeling efficiency was confirmed before transplantation by examining a wet preparation of labeled cells under the UV microscope.

**In vivo cell migration assays**

PKH26-labeled U937 cells or U937 cells blocked with Ab anti-CXCR4 (5 × 10\textsuperscript{6} cells/animal), as described above, were injected i.v. into the tail vein of the transplanted SCID mice in a 100-μl dose volume. SDF-1α (PeproTech, Rocky Hill, NJ), TNF-α (Genzyme, Cambridge, MA), or saline was injected intragraft at the same time. In a separate experiment, PKH26-labeled huPBL were injected i.v. into the tail vein of transplanted SCID mice (5 × 10\textsuperscript{6} cells/animal in a 100-μl dose volume). As above, SDF-1α or saline was injected intragraft. The migration of U937 cells or huPBL into the grafts was assessed histologically by UV fluorescence microscopy (see below).

**Quantification of cell migration into grafts**

Samples of lymph nodes were stored at −70°C until analysis. Serial cryosections (10 μm) were mounted on Vectabond Reagent (Vector Laboratories, Burlingame, CA) coated slides and dried overnight at room temperature. Sections for immunohistochemical analysis were fixed in acetone at 4°C for 10 min, wrapped in aluminum foil, and stored at −70°C until further use. Sections assigned for analysis of PKH26-positive cells were washed for 10 min in PBS (pH 7.6) and mounted using aqueous mounting media (ImmunoFluor; ICN). Sections were analyzed using a fluorescence microscope (Olympus BX60; Olympus, Melville, NY). To obtain an accurate representation of the number of PKH26-positive cells present in the lymph node grafts, three different sections were taken from a different cutting level (i.e., from the top, bottom, and middle of the transplant) of each sample. The results were expressed as the average number of cells identified in each section per high-power field (×40 objective). On average, ~100 high-power fields were counted per transplant.

**EA.hy926 culture conditions and transmigration assay**

EA.hy926 cells were provided by Dr. C.-J. Edgell (Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, NC). EA.hy926 cells are a hybridoma between HUVECs and the epithelioma A549 and retain most of the features of HUVEC, including the expression of endothelial adhesion molecules and human factor VIII-related Ag (23). EA.hy926 cells were cultured in DMEM-F12 supplemented with 10% FCS and endothelial growth factors (cultured medium) and subcultured every 3 days. The transmigration assay was performed using a protocol modified from a previous study (24). The inserts of 8-μm Biocoat 24-well plates (Stratech Scientific, Oxford, U.K.) were coated with 0.5 ml of a human fibronectin solution (50 μg/ml in PBS to give a final concentration of 5 μg/cm\textsuperscript{2}) for 1 h at room temperature. After washes in PBS, EA.hy926 cells were added (5 × 10\textsuperscript{5} cells in 500 μl of cultured medium) and cultured for 48 h. U937 cells, previously demonstrated to be strongly positive for the CXCR4, were cultured as described below and added at 1.5 × 10\textsuperscript{7} cells on top of the inserts, while 0–50 ng/ml SDF-1 was added in the bottom compartment. After 18 h at 37°C in 5% air/5% CO\textsubscript{2}, cells that had migrated through the filters were retrieved from the lower compartment and counted using a Neubauer hemacytometer following staining in Turk’s solution. In some experiments, anti-CXCR4 mAb was preincubated with U937 cells for 30 min before cell addition into the Transwell. Data are reported as the mean number of cells ± SEM migrated per well. Three experiments were performed in triplicate.

**Statistical analysis**

In the in vitro transmigration assay, data are reported as the mean number of cells ± SEM migrated per well. Three experiments were performed in duplicate or triplicate analyzed by ANOVA, followed by the Bonferroni test for post hoc comparisons. A p value <0.05 was taken as significant. In the in vivo cell migration assays, results are expressed as mean ± SEM unless otherwise indicated. Nonparametric statistical analyses were performed using the PC analysis package SigmaStat 2.0 (Jandel, San Rafael, CA). Initially, either the Kruskal-Wallis nonparametric ANOVA or one-way ANOVA was performed. Post hoc significance testing was conducted using Dunn’s multiple comparison tests for nonparametric data or Dunnnett’s test for parametric data.

**Results**

**Transplant viability, cytoarchitecture, and vascularization**

To establish whether huPLN could be successfully transplanted into SCID mice, 4 wk posttransplantation (optimal time determined for synovial tissues (18)) grafts’ viability was examined macroscopically and microscopically as described in Materials and Methods. As seen in the representative example shown in Fig. 1, huPLN were macroscopically healthy being fed by mice subdermal vessels with no macroscopic evidence of inflammation observed. By macroscopic assessment, transplantation was successful in >95% of cases. Microscopic analysis showed in some cases evidence of tissue necrosis but in ~90% of cases the transplant tissue appeared viable with no evidence of murine cell infiltration or signs of chronic inflammation. In subsequent experiments, one animal from each batch was sacrificed before migration studies to assess graft viability as above. No evidence of graft-versus-host or host-versus-graft disease was observed in any of the transplanted animals.

**Transplant cytoarchitecture.** The histological appearance of huPLN grafts 4 wk posttransplantation (shown in Fig. 2) was compared with pretransplant tissue on routine H&E sections and by immunohistochemistry using species-specific Abs to B cells (CD20), T cells (CD3), and follicular dendritic cells (FDC; CD21). In general, the cellularity and organization of the transplants was reduced compared with original lymph node. In four of six transplants, B cell aggregates (CD20+) were maintained (Fig. 2B), although smaller and reduced in cell number compared with the original tissue (Fig. 2A). In the remaining two transplants, a diffuse B cell distribution was observed (Fig. 2C). B cell aggregates were always found in close association with CD21+ cell clusters (Fig. 2E). In contrast, CD21+ cells were markedly depleted in those

![FIGURE 1. Macroscopic appearance of human lymph node, 4 wk posttransplantation. The transplant tissue (T) appears healthy and murine blood vessels are clearly visible feeding the graft (arrows).](http://www.jimmunol.org/DownloadedFrom/9050311.png)
transplants with a diffuse B cell distribution (Fig. 2F). CD3-positive cells were scattered throughout the transplants (Fig. 2, H and I) with no obvious organizational pattern compared with the original lymph node (Fig. 2G).

Transplant vascularization. To investigate the nature (human/murine) of the vasculature within the grafts, these were analyzed using species-specific blood vessel markers and double immunofluorescence. As can be seen in Fig. 3, A and B, both human and murine vessels were present and often the blood vessels of the two species appeared to be directly connected. To determine whether these connections were patent and functional, transplanted mice were injected i.v. in the tail vein with either anti human-specific ICAM-1 biotinylated or an isotype-matched control Ab biotinylated (MOPC21). As shown in Fig. 3C, immunohistochemical detection of anti-human ICAM-1 showed discrete staining of human vessels in the grafts that also stained positively for human VWF-VIII (Fig. 3D). This confirmed that Ab localization within the grafts occurred via anastomosis of the murine systemic circulation and the human graft vessels. Injection with control-biotinylated mAb produced no staining (Fig. 3E), although blood vessels could be clearly visualized when stained with anti-human VWFVIII-FITC (Fig. 3F). The functionality of the mouse-human vascular connection was further confirmed by the injection of PKH26-labeled huPBL i.v. into lymph node-transplanted SCID mice. As can be seen in Fig. 3G, PKH26-positive cells (red fluorescence) localize to the grafts via the human vasculature (counterstained with anti-human VWFVIII-FITC).

To investigate whether the microvascular endothelium of transplanted lymph nodes maintained the specific functional features of HEV, we examined by immunohistochemistry the distribution of peripheral node addressin (PNAd) using the mAb MECA-79 (25), CD34, and CCL21 in lymph nodes pre- and postimplantation. It can be seen that in the original huPLN the majority of VWFVIII-positive vessels (Fig. 4A) also express MECA-79 (Fig. 4C). However, the expression of MECA-79 in the grafts was significantly reduced in comparison to the original lymph nodes as shown by volume fraction (original lymph node = 10.6 ± 0.58; saline-injected transplant = 0.60 ± 0.03, p < 0.05), despite the presence of numerous VWFVIII-positive vessels (Fig. 4B). Interestingly, although VWFVIII-positive vessels were found both in areas of lymphocytic aggregation and diffuse cellularity, MECA-79-positive vessels were found only in areas of cellular aggregation (Fig. 4, B and D). There was no significant difference in the volume fraction (Vv) of MECA-79 staining between SDF-1/TNF-α stimulated transplants vs saline-injected controls (see later).

We next examined the vascular distribution of the L-selectin ligand CD34 in original and posttransplantation lymph nodes. Like MECA-79, CD34 expression was also reduced posttransplantation, although to a lesser degree (Fig. 4, E and F). In addition, we analyzed the expression of CCL21. CCL21 was diffusely distributed in the T cell and subcapsular areas with an apparent association with lymphatic vessels but only modest staining of vascular structures (Fig. 4G). The tissue distribution was similar in the transplant tissue in comparison to original lymph nodes but with markedly reduced staining intensity (Fig. 4H).

Finally, we investigated whether human lymphatic vessels were retained within the grafts. We examined the expression of a cellular marker recognized by a new mouse anti human mAb (clone 3-155, see Table I) (26). Expression of 3-155 was clearly detectable in lymphatic vessels independently from VWFVIII-positive blood vessels both in the original huPLN (Fig. 4I) and huPLN grafts (Fig. 4J). The mAb 3-155 does not appear to cross-react with murine lymphatic vessels as we found no staining at the periphery of the transplants or in the surrounding subdermal mouse tissue.

SDF-1α induces U937 and PBL migration into huPLN transplanted into SCID mice

To investigate the capacity of SDF-1 to induce human cell migration into huPLN transplants, we injected SDF-1 intragraft and initially examined its ability to induce graft localization of U937 cells injected into the tail vein of the animal at the same time. This cell line was chosen because it expresses high levels of CXCR4 (Fig. 5A), the selective SDF-1 receptor, making it a useful tool to investigate the system. In addition, we also confirmed in vitro the capacity of U937 to migrate in response to SDF-1 using a Transwell model in which microporous filters were coated with the EA.hy926 endothelial hybridoma cell line (see Materials and Methods). The results, shown in Fig. 5B, demonstrate that SDF-1 caused a dose-dependant increase in U937 migration, which was...
significantly inhibited by preincubation of the cells with a CXCR4-specific mAb.

Similarly to these in vitro experiments, SDF-1 (0.3 μg/graft, optimal dose previously determined) induced U937 cell migration in vivo to huPLN grafts (Fig. 6A). These results were compared with the level of migration induced by TNF-α (200 ng/graft, optimal dose previously determined) as a positive control) and that of saline (negative control). It can be seen that SDF-1 was more effective than TNF-α at up-regulating U937 cell migration, causing ~3-fold increase in the number of migrating cells compared with saline-treated animals and a 2-fold increase over the TNF-α-injected group. Furthermore, SDF-1-dependent migration was completely inhibited by the anti-CXCR4-specific mAb.

We then tested the capacity of SDF-1 to induce migration of huPBL into huPLN grafts. The results shown in Fig. 6B indicate that the increase in migration of huPBL in response to SDF-1 vs saline baseline migration was of similar magnitude to that observed with SDF-1-induced U937 migration. However, huPBL showed a greater capacity than U937 to localize to the transplants both in unstimulated and stimulated conditions. This is indicated by the number of cells per high-power field detected (baseline migration: huPBL, 10.1 ± 2.03 vs U937, 3.49 ± 0.40; SDF-1-induced migration: huPBL, 29.3 ± 4.12 vs U937, 11.1 ± 1.02).

Mechanisms of action of SDF-1 in inducing U937 and PBL migration into huPLN transplanted into SCID mice

To investigate potential mechanisms of action of SDF-1 in relation to TNF-α, we concentrated on three aspects: first, we explored whether the effects of SDF-1 were mediated via the inductions of TNF-α and vice versa. Second, we examined the variation in ICAM-1 expression and the relationship of this to the degree of
migration of U937 cells and PBL into the grafts. Third, we analyzed the degree of human and mouse vascularity in treated and untreated transplants.

**SDF-1 and TNF-α do not induce reciprocal expression in hu-PLN grafts.** SDF-1 was diffusely distributed throughout the tissue of the SDF-1-injected grafts with staining of the vascular endothelium, transplant stroma, and interstitium. A similar pattern of distribution was seen in the TNF- and saline-injected grafts, but with greatly reduced staining intensity (saline, 1.68 ± 0.09; SDF-1, 2.26 ± 0.04*; TNF, 1.84 ± 0.12; mean ± SEM of four transplants per group examined). TNF-α immunoreactivity was detected in both the original preimplantation lymph node and in all treatment groups posttransplantation, with typically 5–10 positive cells per square centimeter of tissue. TNF-injected transplants showed a faint diffuse staining reaction in addition to the discrete cellular localization seen in the positive controls and other treatment groups. However, there was no significant difference in cellular TNF-α expression when comparing any of the treatment

**FIGURE 4.** Representative micrographs of sections of lymph node pretransplantation (A, C, E, G, and I) and posttransplantation (B, D, F, H, and J) stained using immunokaline phosphatase methodology for the presence of VWFVIII (A and B), MECA-79 (C and D), CD34 (E and F) and CCL21 (G and H). Double immunofluorescence staining of pretransplantation (I) and posttransplantation (J) lymph node for lymphatic vessels using the mAb 3-155 (red staining) and VWFVIII-FITC (green staining). Arrows indicate MECA-79-positive vessels in lymph node grafts. Original magnification, ×20.
groups with either control (saline-injected) transplants, SDF-1-injected groups, or original preimplantation tissue. This strongly indicates that human SDF-1 and TNF-α expression is regulated in a nonreciprocally manner similarly to published data in mice (see Discussion) (27). In addition, this suggests that the mechanisms by which they increase cell migration into the grafts are independent from each other.

**SDF-1α-induced cell migration is independent of CAM up-regulation.** To investigate the mechanisms of action of SDF-1- vs TNF-α-induced cell migration, we examined the variation in endothelial CAM expression and the relationship of this to the degree of U937 and PBL migration into the grafts. Immunohistochemical analysis showed a significant increase of ICAM-1 expression in U937 and PBL migration to huPLN transplants does not correlate with the degree of human or murine vascular surface within the grafts

To determine whether the observed up-regulation in cell migration following intragraft injections of SDF-1 or TNF-α were simply due to an increase in the vascular beds feeding the graft, cryostat sections of the transplants were immunostained for human vascular endothelium (anti-VWFVIII) and mouse vasculature (anti-murine CD31). The volume fraction of murine and human vessels within the transplants was determined by point counting (see Materials and Methods). There was no significant difference between the treatment groups either in the total human endothelial surface (saline, 5.50 ± 1.82; SDF-1, 5.97 ± 0.91; TNF, 3.78 ± 0.43; mean ± SEM of four transplants per group examined) or mouse vascularity (saline, 10.78 ± 0.51; SDF-1, 11.12 ± 0.28; TNF, 11.01 ± 0.56; mean ± SEM of four transplants per group examined). In addition, scatter plots of Vv fraction vs PKH26 migration showed no discernible pattern of association of the two variables. Transplant vascularity showed no correlation with U937 or huPBL migration (Spearman’s rank correlation, p > 0.05 throughout) when either individual transplants or treatment group means were compared, indicating that the degree of migration is largely independent of the extent of graft vascularity.

**Discussion**

In this article, we report the development of a novel model in which huPLN are successfully transplanted into SCID mice. In addition, we demonstrated that SDF-1 is functional in this model as shown by its capacity to induce human cell migration into the huPLN grafts that is even greater than TNF-α.

Lymph node transplantation was successful in >90% of cases, with grafts remaining viable following revascularization by mouse subdermal vessels. This was confirmed both macroscopically and microscopically. The histological appearance of transplants, when compared with original preimplantation lymph nodes, showed a number of discernible morphological changes. There was a decrease in cellularity of the grafts, accompanied by a loss of organization primarily in the T cell areas but also apparent as a reduction in the number and size of B cell aggregates observed in the transplant tissue. This was mirrored by a reduction in the number of CD21+ FDC. However, where follicular aggregates of B cells were present, they were always associated with clusters of CD21+ FDC.
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chronic inflammation (25, 30, 31). MECA-79-positive vessels persisted following lymph node transplantation, although the volume fraction of the stained area was significantly reduced when compared with the original tissue. The distribution of MECA-79 in transplanted lymph node was mainly associated with lymphocytic aggregates but not with a diffuse cellular infiltrate underlining the cytoarchitecture and the actual presence of certain cell types and their products (e.g., lymphotoxin) in the maintenance of specialized lymphoid features (28). In addition, some of the graft vessels expressed CD34. Interestingly, despite the considerable cell loss within the grafts in comparison to the original tissue, presumably related to the lack of afferent lymph, some human lymphatic vessels appear to be preserved as assessed by the expression of the 3-155 marker. Future experiments will address the functional status of the graft lymphatic vessels (patency and potential anastomoses with mouse subdermal lymphatics) and their importance in the maintenance of specialized lymphoid features (28). This model may also allow investigating the factors involved in the maintenance of HEV morphology and lymphoid aggregates in human lymphoid organs.

The other important aspect that was addressed in this article was the question of the actual functional capacity of SDF-1 of inducing lymphocyte migration to lymphoid organs in vivo in humans. The literature is rich in indirect evidence in support of this hypothesis: first, SDF-1 is expressed on the luminal surface of HEV in peripheral lymph nodes (32). Second, SDF-1 is thought to be important in mobilizing hemopoietic precursors from the bone marrow into the circulation and from there into peripheral tissues (8, 9). Third,
SDF-1 associates with heparan sulfates on the vascular surface (10) and stimulates integrin-mediated arrest of CD34+ cells on vascular endothelium under shear flow in vitro (11) and in animal models (12). However, direct in vivo evidence in humans was still missing. The main reason for this is that human recirculation studies would be very difficult and unethical. The model described herein overcomes these problems and provides the first direct demonstration in vivo of the functional capacity of SDF-1 to induce human cell migration into huPLN grafts. The SDF-1-induced migration was specifically mediated by CXCR4 as demonstrated by the ability of a blocking mAb to inhibit migration of U937 cells to the grafts. Although in this article we have not formally proved this for human PBL, it is known from the published literature that the majority of circulating monocytes, B cells, and naive (CD45RA+L-selectin+) T cells are CXCR4+ and SDF-1 responsive (33). This, taken together with the recently published work of Kollet et al. (34), who demonstrated the functional capacity of SDF-1 to mediate the migration of CD34+CD38lowCXCR4+ human hematopoietic stem/progenitor cells to the bone marrow of SCID mice and that this effect was inhabitable by preincubation of cells with anti-CXCR4 mAbs, leads us to believe that the same would be true of our experimental model.

Finally, we demonstrated that SDF-1 was more effective than TNF-α causing approximately a 2-fold increase in migration over the TNF-α-injected group. Although the experiments described herein do not allow us to determine the proportion of injected cells which ultimately localize to the grafts, we have made such an estimate in our previously published work on cell migration to synovial transplants in SCID mice using radiolabeled and fluorescently labeled cells (18). A comparison between the numbers of cells per high-power field in the lymph node grafts vs those found in synovial grafts of previous experiments indicates that the proportion of migrated cells is comparable. Therefore, considering that in both models the number of human PBL injected i.v. was the same (5 × 10^6/animal), it can be deduced that the percentage of cells localizing in the lymph node grafts is similar to the one that localizes into synovial grafts (3–7%).

To investigate the mechanisms of action of SDF-1, we first considered the level of expression of endothelial adhesion molecules. As previously reported (19), TNF-α strongly up-regulated ICAM-1 expression; however, no up-regulation was detected in the SDF-1-injected grafts. This suggested that the SDF-1 effects were mediated independently of an increased transcription/mobilization of endothelial surface ICAM-1. Therefore, the likely explanation for our observation is that SDF-1 may be acting in the huPLN-SCID chimera model via the classical CK mechanism, namely, the activation of surface integrins (4, 12) of the human cells circulating within the grafts. Activated integrins would then be able to bind more avidly to endothelial ICAM-1 expressed at basal level. Indirect evidence for this comes from our in vitro experiments that confirmed that SDF-1 could induce transmigration of U937 even when using nonstimulated endothelial cells that express ICAM-1 at basal level (35).

To make sure that some of the effects of SDF-1 were not mediated by the induction of TNF-α, we examined their tissue distribution in all experimental conditions. As expected, SDF-1 injection into the grafts caused an increase in detectable SDF-1-specific immunoreactivity compared with the saline controls but not of TNF-α. Reciprocal results were obtained when the grafts injected with TNF-α were analyzed. This suggests that the mechanisms by which SDF-1 and TNF-α increase cell migration into the grafts are independent from each other. In addition, these data indicate that TNF-α and SDF1 are independently regulated in line with the information from gene-targeted animals, where TNF-α or lymphotixin-β deletion leads to BLC, SLC, ELC deficiency but has no effect on SDF-1 production (27).

Finally, to exclude the possibility that a variable degree of graft vascularization could have influenced the level of cell migration into the grafts, we analyzed the total endothelial surface and the number of human and murine blood vessels. We found that there was no significant difference between SDF-1 and TNF-α of saline-treated groups. In addition, there was no correlation between the number of cells infiltrating the grafts and the level of human or mouse vasculature. Taken together, these observations indicate that the level of cell localization to the transplant relates directly to the effects of SDF-1 or TNF-α.

In summary, we have demonstrated for the first time that SDF-1-injected intragrafts can induce the migration in vivo of human cells into huPLN grafted in SCID mice. In addition, we have described in detail a new model that may be of great help in dissecting the specific functions of distinct molecules involved in regulating the migration of human cells into human lymphoid organs. Also, it may allow the investigation of factors involved in the maintenance of lymphoid architecture in humans.