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Bone marrow cell trafficking following intravenous administration

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Summary. To address trafficking of transplanted marrow cells immediately after intravenous infusion, we examined the early fate of infused non-adherent, low-density donor bone marrow cells in a syngeneic mouse model. The presence of infused donor cells, marked with indium-111 oxine (^{111}In), with the fluorescent dye PKH26, or by a detectable transgene marker, was evaluated at 3–48 h in a variety of tissues, including peripheral blood. All three cell-marking methods indicated a rapid (< 4 h) influx of cells into the bone marrow, liver, spleen, muscle and other tissues. Moreover, these tissues remained positive for the 48 h observation period. Interestingly, analysis of PKH26-positive cells in non-myeloablated animals demonstrated that approximately 17% of infused donor marrow cells localized to the marrow space within 15 h, whereas a smaller proportion of donor cells (~ 1–2%) localized to the marrow in recipients preconditioned by irradiation. In an effort to enrich for cells that specifically home to the bone marrow, PKH26-labelled donor marrow cells were recovered from the first host and infused into a secondary recipient. Although this was a phenotypically undefined population of cells, no increase was observed in the relative fraction of PKH26-labelled cells returning or 'homing' to the marrow of the second recipient. Taken together, these data suggest both that marrow engraftment may be mediated by non-specific 'seeding' rather than a specific homing signal, and that efficient targeting of transplanted cells to the marrow is a complex multifaceted process.

Keywords: homing, bone marrow, haemopoiesis, cell migration, transplantation.

The remarkable capacity of the bone marrow to regenerate and reconstitute haemopoiesis provides the rationale for bone marrow transplantation (BMT). Within a period of several weeks, infused (donor) stem cells re-establish haemopoiesis at normal physiologic sites (i.e. the bone marrow). However, little is known about the early fate of haemopoietic stem cells following their intravenous administration until re-establishment of haemopoiesis within the marrow space. Evidence exists for the involvement of adhesion molecules such as VLA-4 (Williams *et al*, 1991; Rosemblatt *et al*, 1991; Miyake *et al*, 1991) and VLA-5 (Rosemblatt *et al*, 1991) and VCAM-1 (Simmons *et al*, 1992). Likewise, proteoglycans such as CD44, CD45 and Mac-1 also serve as ligands for specific adhesion receptors within the marrow microenvironment (Long, 1992; Coombe *et al*, 1994). However, these molecules are widely expressed, and their specific role in cellular localization is unclear.

It has been suggested that intravenously infused haemopoietic stem and progenitor cells return to the bone marrow through a specific 'homing' process (defined as the immediate, selective lodging of haemopoietic cells within the marrow space) that is mediated by the interaction of cell-surface receptors with corresponding glyco-conjugate ligands (Hardy & Tavassoli, 1988; Tavassoli & Hardy, 1990). Moreover, the partial inhibition of BMT efficiency by mannosyl- and galactosyl-neoglycoproteins further implies that the putative homing receptor contains critical galactose and mannose residues (Aizawa & Tavassoli, 1988).

In order to characterize the early events of marrow haemopoietic cell trafficking following BMT, we monitored intravenously infused donor bone marrow cells (albeit phenotypically undefined) by three different means: ^{111}Indium labelling, fluorescent labelling using the membrane-bound dye PKH26, or the use of a donor-cell-specific

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transgene marker detected by PCR. Early trafficking of infused donor cells was observed in the marrow of the recipient mice, as well as in all other tissues examined. Importantly, significant differences were observed in bone marrow cell trafficking for irradiated versus nonirradiated BMT recipients. Infusion of PKH26-labelled cells that had localized to the bone marrow of the primary donor into secondary recipients resulted in a similar tissue distribution pattern, with no increase in the percentage of cells returning to the marrow. These results suggest that bone marrow cells do not specifically home to the marrow in the period immediately following transplantation.

MATERIALS AND METHODS

Bone marrow transplantation. 8–12-week-old male C57BL/6J mice (Jackson Laboratories) were used for the ^{111}In labelling, and the PKH26-labelled bone marrow transplantation studies. Transgenic mice carrying a murine PAI-1 transgene under control of the cytomegalovirus (CMV) promoter were used for the transgene-marker PCR studies (Eitzman *et al*, 1996). Although the original transgenic founder mouse was SJL/J×C57BL/6J, all transgenic mice used in these studies were the product of more than five backcrosses to the C57BL/6J parental strain. All mice were maintained under specific pathogen-free (SPF) conditions.

Where indicated, recipient mice were lethally irradiated with a total dose of 10·5 grays 24 h prior to transplantation using a ^{135}Cs irradiation source (Gammacell 40, Nordion International) fractionated into an initial dose of 7 grays followed by 3·5 grays at 4 h. For the ^{111}In and transgene marker studies, BMTs were performed by infusion via the tail vein of 10^7 nucleated marrow cells into each non-irradiated or irradiated primary recipient animal. In the case of secondary transplants (of PKH26-labelled cells, see below), a total of 5×10^5 pooled, marrow cells from the primary recipient (taken post-irradiation and transplant) were intravenously administered into irradiated secondary recipients. Donor bone marrow cells were obtained as previously described (Long & Shapiro, 1985), and red blood cells (RBC) were removed by incubation with RBC lysis buffer (145 mM NH_4Cl, 10 mM $KHCO_3$ and 0·1 mM EDTA), followed by washing with phosphate-buffered saline (PBS).

Labelling of donor bone marrow cells by ^{111}In, and RBC with technetium-99 (^{99}Tc). Bone marrow NALD cells were isolated from mouse femurs as described previously (Long & Shapiro, 1986) and labelled with ^{111}In (Medi-Physics Inc., Livonia, Mich.). For labelling, ^{111}In was added to the cell suspension and incubated at room temperature for 20 min (Thakur *et al*, 1977). Labelled cells were washed twice in Hanks' Balanced Salt Solution (HBSS, from Sigma Chemical Co., St Louis, Mo.), and resuspended in 1 ml HBSS. During each wash, cells were collected by centrifugation at 400 ***g*** for 7 min at 4°C. After two rinses, the unbound ^{111}In was <8%, and the labelling efficiency was 40%. Cell viability following labelling was >95%, as measured by trypan blue exclusion. Each of five recipient mice (per time point) received 5×10^5 bone marrow cells carrying approximately 592 kBq of ^{111}In. In order to determine the amount of ^{111}In label that was due to blood flow within a given organ (i.e. non-specific signal), RBCs were labelled with ^{99}Tc, using the Ultratag® kit (Mallinckrodt Medical, St Louis, Mo.). 1 ml of whole blood was collected from mice into a syringe containing approximately 15 units of heparin. The Ultratag kit was prepared according to the manufacturer's instructions, using the 1 ml of blood and 370 MBq of ^{99}Tc (Nuclear Pharmacy, University of Michigan Hospital, Ann Arbor, Mich.). The RBC labelling efficiency was determined to be 99%. Each recipient mouse was given 1·48 MBq of the ^{99}Tc-labelled RBC mixture, equivalent to 5 μl of donor blood, administered simultaneously with ^{111}In-labelled NALD cells.

The tissue-specific localization index of NALD cells was calculated by correcting the tissue specific ^{111}In signal for the degree of tissue non-specific localization due to the blood volume within a given organ. The tissue-specific ^{111}In signal was determined by dividing the decay-corrected ^{111}In counts per gram of a tissue by the ^{111}In counts found in circulating blood. The relative blood volume within a given tissue is determined by the ratio of ^{99}Tc counts/g of tissue to the ^{99}Tc counts/g of blood. The tissue-specific NALD cell localization index was then determined by dividing ^{111}In tissue/blood counts by ^{99}Tc tissue/blood counts. This calculation was performed on each tissue from each individual recipient animal in each group (irradiated and non-irradiated) and was analogous to the specific localization ratio for antibody targeting (Wahl *et al*, 1983). Statistical analysis was performed using Student's *t*-tests.

Labelling murine bone marrow cells with PKH26. Cell labelling with PKH26, a fluorescent membrane dye, was performed according to the manufacturer's instructions (Sigma, St Louis, Mo.). To define the optimal conditions for labelling murine bone marrow cells, PKH26 concentrations of 2, 5, 10 and 20 μM were tested. Dye concentrations of >2 μM were associated with significant fractional cell death as assayed by trypan blue exclusion. Optimal labelling was obtained following incubation of $1–5\times10^7$ marrow cells/ml in 2 μM PKH26 for 2 min at room temperature.

Flow cytometric analysis of PKH26-labelled bone marrow samples. Bone marrow cells of recipient animals were harvested from both femurs at various time points following injection. After removal of RBCs by osmotic lysis, bone marrow samples were fixed with 1% paraformaldehyde in PBS for analysis using a Coulter Epics Elite Flow Cytometer (Coulter Corporation, Hialeah, Fla.) with an excitation wavelength of 514 nM and an emission wavelength of 575 nM. The following formula was used to calculate the seeding efficiencies (SE) of infused donor marrow cells: SE (%) = (PK×TM/TD) ×100, where PK = the percentage of PKH26-positive cells in the recipient marrow sample as determined by FACS analysis; TM = the number of nucleated marrow cells in the total marrow space of the recipient mouse; this number was estimated to be $1·7\times10^8$ for non-irradiated and $1·7\times10^6$ for irradiated hosts, based on average cell counts of both femurs from five animals, and assuming each femur to contain 6% of total marrow (Chervenick *et al*, 1968) and TD = total number of infused, PKH26-labelled donor cells.

DNA extraction and PCR amplification to detect donor cells. Femoral marrow cells and peripheral blood, along with sections of liver, lung, spleen and kidney, were collected from euthanized recipient mice, and total cellular DNA was prepared using standard methods (Sambrook *et al*, 1989). PCR primers (5′CCACAACTAGAATGCAGTGA3′ and 5′CCTACACAACGAAGAGGTCC3′) were designed to amplify a 290 bp segment of vector-specific sequence within the PAI-1 transgene. PCRs were performed (Kakuta *et al*, 1986) using 200 ng of each primer in a volume of 50 μl for 35 cycles. The PCR product was visualized by ethidium bromide staining after electrophoresis in 4% NuSieve agarose gels (FMC Bio Products, Rockland, Maine). Negative control reactions (no added DNA template) were routinely included. These control lanes were consistently negative for all PCR experiments.

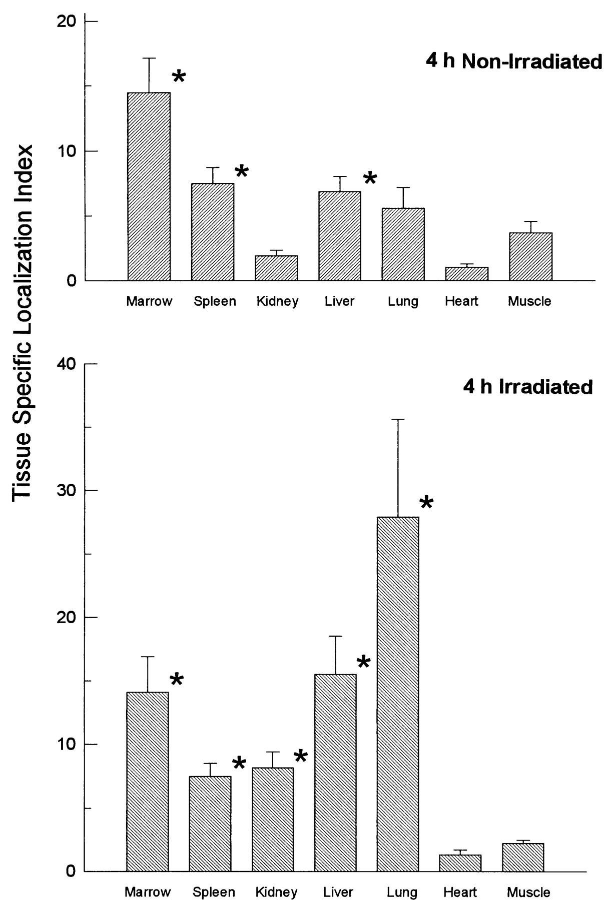

Fig 1. Tissue-specific localization of donor bone marrow cells 4 h following administration. Murine non-adherent low-density (NALD) bone marrow cells were collected from donor animals and labelled with ^{111}In, and donor red blood cells were labelled with ^{99}Tc as described in Methods. 4 h following simultaneous intravenous administration of both cell types into non-irradiated (upper panel) and irradiated (lower panel) hosts, tissue samples were obtained from euthanized animals, and the tissue-specific localization index calculated as described in Methods. $*P \leqslant 0{\cdot}05$ compared to the myocardium.

RESULTS

Detection of donor cells in various tissues by ^{111}In labelling

The tissue-specific localization (TSL) of bone marrow derived non-adherent low-density (NALD) cells following transplantation of ^{111}In and ^{99}Tc-labelled cells is shown in Figs 1 and 2. A specific signal was detected in all tissues examined, but a number showed relatively low degrees of tissue localization. These include kidney, heart, skeletal muscle, fat and small intestine (latter two not shown). None of the localization signals in these five tissues were significantly different from each other ($P \leqslant 0{\cdot}05$). In contrast, tissues with a known predilection for accumulating labelled granulocytes (Thakur *et al*, 1977) showed increased cellular localization of (granulocyte-depleted) NALD cells. For the non-irradiated recipients, significant NALD cell localization (compared to the relatively low localization to the heart; so chosen as the heart had an intermediate absolute localization signal of the aforementioned 'low-localization' tissues) was seen in bone marrow, spleen and liver 4 h following the injection (Fig 1, upper panel). At 24 h, there was a continuing rise in NALD cell trafficking in most tissues (Fig 2, upper panel, note difference in *Y*-axis scale). In the irradiated recipients,

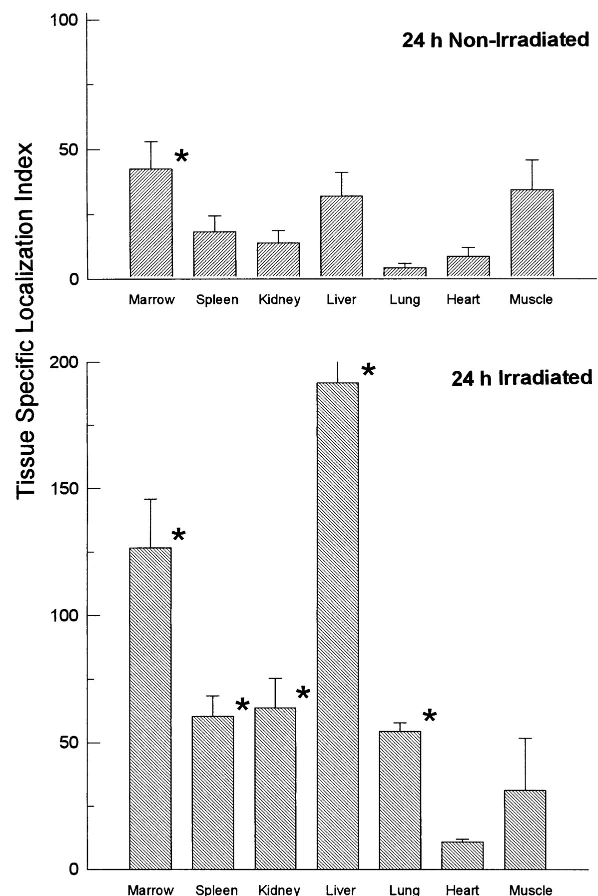

Fig 2. Tissue-specific localization of donor bone marrow cells 24 h following administration. Tissue samples were taken, and tissue-specific localization index determined, at 24 h following administration. Details as in Fig 1.

specific localization (in excess of that occurring in the heart) occurs within 4 h, and was seen in a variety of tissues: bone marrow, spleen, kidney, liver and lung (Fig 1, lower panel). These tissues also continued to show significant localization at 24 h, though the relative accumulation of labelled cells is markedly increased (Fig 2, lower panel, note change in *Y*-axis scale). Comparison of 4 h and 24 h data indicated that significant increases in tissue localization occurred in both 'haemopoietic' tissues (bone marrow, spleen and liver) and in non-haemopoietic tissues (kidney, lung, heart and muscle; compare Figs 1 and 2). However, the magnitude of the change in TSL was not greatly different between these two types of tissue, and was often equivalent or higher in non-haemopoietic tissues. Taken as a whole, the TSL data indicate that intravenously injected NALD cells showed no specificity for haemopoietic microenvironments (or micro-environments with haemopoietic potential) during the first 24 h following infusion.

PCR detection of infused bone marrow cells in multiple tissues

For these experiments, donor bone marrow cells were harvested from a transgenic mouse line carrying an inert PAI-1 transgene. Host animals were killed at varying times following transplantation, and tissue samples (from blood, marrow, liver, lung, spleen and kidney) were examined by PCR using oligonucleotide primers specific for transgene sequences. As with the ^{111}In/^{99}Tc studies, a transgene signal was obtained from bone marrow, peripheral blood, liver and spleen DNAs at all time points (Fig 3), with similar signals also observed in lung and kidney (data not shown). A constant signal was evident at later time points in bone marrow and spleen DNA, demonstrating that a persistent proportion of donor cells was localized in these two tissues (Fig 3), whereas the signal from liver and blood diminished somewhat after 20 h. The decreased signal from blood was consistent with a continued gradual migration of donor cells out of the peripheral circulation, and suggests that donor marrow cells rapidly leave the circulation following infusion, migrating into multiple organs and tissues.

PKH26 labelling of murine bone marrow cells

The PKH26 dye stably and irreversibly labelled the lipid component of the cell membrane for a broad range of cell types, without significant exchange between cells, and without apparent interference with cellular function or surface protein structure (Slezak & Horan, 1989; Samlowski *et al*, 1991; Ricketts *et al*, 1992). In addition, Hendrikx *et al* (1996) demonstrated that the presence of the PKH26 dye did not interfere with the formation of day 8 and day 12 spleen colonies in lethally irradiated mice.

Bone marrow from recipient mice was collected at 15, 25 and 40 h post transplantation, and analysed for the presence

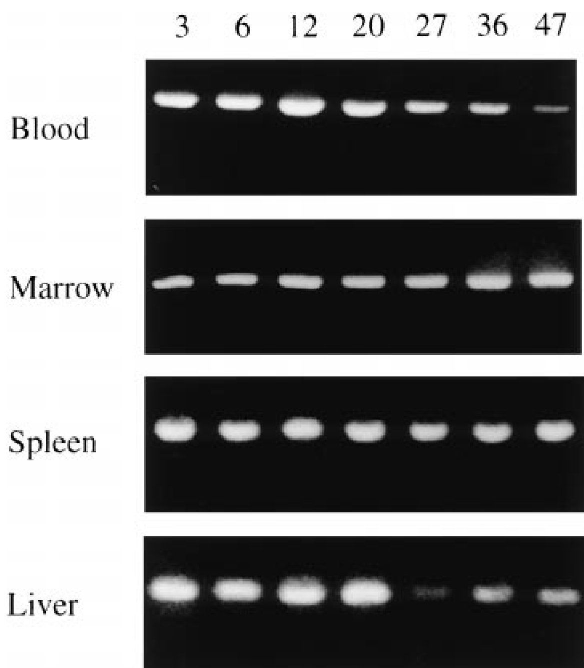

Fig 3. Detection of DNA from bone marrow cells in the recipient blood, bone marrow, spleen and liver by PCR. The 290 bp PCR product amplified from the PAI-1 transgene is a specific marker for donor cells. The number at the top of each lane shows the time (h) post-transplantation at which the samples were taken.

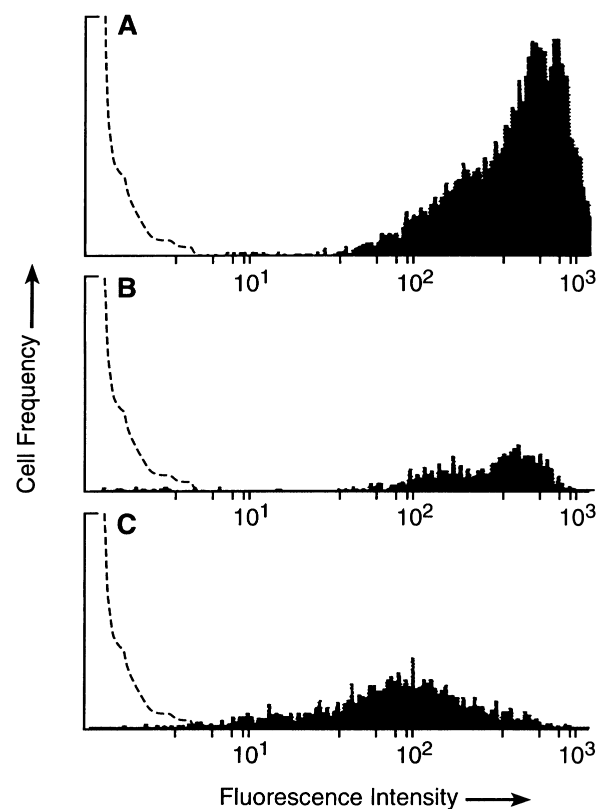

Fig 4. Detection of PKH26 labelled donor bone marrow cells by flow cytometric analysis in bone marrow samples obtained from recipient mice. Samples collected 15 h after marrow infusion. (A) Donor cells. (B) Non-irradiated recipient. (C) Irradiated recipient. Dashed line, autofluorescence (i.e. background fluorescence); solid profiles, PKH26 fluorescence.

Table I. Localization of PKH26-labelled cells to the bone marrow at 15 h.

Condition	% Labelled cells (PK)	Total marrow cellularity (TM)	Labelled cells infused (TD)	Seeding efficiency (%) (SE)
Primary recipient				
Non-irradiated	1·0	$1{\cdot}7\times10^8$	1×10^7	17·0
Irradiated	10·0	$1{\cdot}7\times10^6$	1×10^7	1·7%
Secondary recipient				
Irradiated	⩽0·1	$1{\cdot}7\times10^6$	5×10^4	⩽3·4

Seeding efficiency was calculated as described in Materials and Methods.

of PKH26-labelled cells by flow cytometry. A typical analysis of recipient mice is shown in Fig 4. The results of the analysis at the 15 h time point in non-irradiated recipients are shown in Fig 4B. Based on the numbers of infused donor cells and total host marrow cells, we calculated that approximately 17% of the infused donor cells (Table I) localized to the bone marrow of non-irradiated recipients within 15 h following injection. Though a larger fluorescence peak was seen at 15 h in irradiated mice (Fig 4C), compared to the similar experiments in non-irradiated hosts (Fig 4B), this was due to the marked decrease in the number of unlabelled host cells following irradiation. Calculations based on correction for numbers of unlabelled host cells indicated that approximately 3% of the infused donor cells localized to the marrow of irradiated recipients (Table I). For both groups of animals, maximal localization of infused cells to the marrow appeared to have been reached by the first time point (15 h), with no significant changes in the percentage of cells localizing to the marrow at up to 40 h (data not shown).

Within 15 h of transplantation, significant differences in donor-cell fluorescence intensity were seen in irradiated versus non-irradiated recipients (compare Figs 4B and 4C). In non-irradiated animals approximately 65% of the donor cells retain PKH26 staining equivalent to that of the input cells, whereas fluorescent intensity of the remaining 40% of cells was reduced by approximately one-half the original, which suggested that these cells had completed approximately one round of cell division. In contrast, the donor cells detected in irradiated recipients (Fig 4C) exhibited fluorescent intensities of one-quarter to one-half that of input cells, demonstrating a greater degree of donor cell proliferation in these animals.

Failure of bone marrow localization to enrich for a specific 'homing' subpopulation

In an effort to enrich for a subpopulation of donor cells capable of specifically homing to the recipient marrow, cells localized to the bone marrow in the first transplant recipient were collected and infused into secondary recipient animals. For these experiments, PKH26-labelled cells were first injected intravenously into an irradiated primary host, and unfractionated bone marrow cells were collected from the femurs of recipient animals 15 h following the first transplant. These cells were then intravenously infused into irradiated secondary recipients. The secondary recipients were killed at 15 h, and femoral bone marrow cells were collected. Flow cytometric analysis of bone marrow cells from the secondary recipient detected only very small numbers of PKH26-labelled cells derived from the original donor animal (<0·1%). Calculations of seeding efficiency (Table I) indicated that ⩽ 3% labelled cells isolated from the marrow of the first recipient again localized to the marrow in the secondary recipient. This value did not represent a significant enrichment over the fraction of cells that localized to the primary recipient marrow. Thus, cell migration to the bone marrow of the primary transplant recipient failed to enrich for a subpopulation of cells capable of again specifically homing to the marrow of the secondary recipient. Analysis of experiments using non-irradiated primary and secondary recipients similarly failed to demonstrate significant enrichment of a specific homing subpopulation. To some degree, however, the high background of unlabelled cells could have obscured detection of marrow enrichment in these latter experiments. Also a formal possibility remains that, at 30 h post labelling, the PKH26-marked cells proliferated (in irradiated recipients) to a degree that their fluorescence-intensity was close to background and thus the cells were undetectable as originally labelled cells. We believe that this is unlikely, given the high fluorescence intensity of the PKH26-labelled cells (Fig 4A), and the data showing that the majority of cells undergo a single population doubling (Fig 4C). Thus, even three population doublings would yield a signal well above background (i.e. one-eighth that of original, within the second decade of the fluorescence histogram).

DISCUSSION

The localization of infused donor cells to the host marrow is a critical component of BMT engraftment, and the reconstitution of host haemopoiesis. A number of laboratories have presented data to support the existence of a specific homing receptor on the haemopoietic stem cell, with a corresponding ligand being present on the marrow endothelial cell and/or the marrow stroma. Our observations, demonstrating early localization of up to 17% of infused donor cells to the marrow of non-irradiated recipients, suggest that a broad range of phenotypically undefined bone marrow cells are

capable of initially localizing to the marrow, as well as other organs. The subsequent failure to enrich for a homing subpopulation in the secondary serial transplant was unexpected, and argues against the existence of a specific, highly efficient, 'homing' mechanism. Thus, the analysis of early NALD cell trafficking using both ^{111}In labelling and PCR detection of a marker transgene demonstrated that, within as little as 3 h, injected cells leave the circulation and migrate into a diverse number of organs and tissues in an apparently nonspecific pattern. Other PKH26-labelling studies, utilizing more purified subpopulations of haemopoietic cells, showed a similar temporal movement (i.e. within 3 h) of haemopoietic stem and/or progenitor cells into the bone marrow or spleen (Lanzkron *et al*, 1999; Szilvassy *et al*, 1999). However, these investigations did not monitor cell trafficking into other (non-haemopoietic) tissues. Interestingly, the NALD cell tissue distribution pattern observed in our study was very similar to that seen by the infusion of ^{111}In-labelled autologous peripheral blood leucocytes in humans, where the spleen, liver, lungs and marrow represent the site of greatest tracer (cell) accumulation (Thakur *et al*, 1977). This suggests that general mechanisms of vascular egress regulate the early events of phenotypically undefined marrow cell trafficking following intravenous infusion.

The increased uptake of ^{111}In-labelled NALD cells to a variety of tissues in irradiated mice relative to non-irradiated animals is probably due to the increase in general vascular permeability seen following total body irradiation of this magnitude (i.e. capillary leak) (Vriesendorp *et al*, 1994; Aizawa & Tavassoli, 1988). Although the subset of PKH26-labelled cells from the original donor detected in the secondary recipient by FACS analysis ($<0{\cdot}1\%$) was near the limit of sensitivity for this assay, the predicted significant increase in the percentage of specific homing cells was not observed. However, we cannot exclude the existence of a small subpopulation of cells (e.g. stem cells) which does efficiently home to the marrow, but was obscured by the nonspecific movement of the large background of other marrow cells.

Consistent with a recent report of utilizing PKH26 labelling (Hendrikx *et al*, 1996), we observed higher numbers of PKH26-labelled donor cells in the marrow of non-irradiated recipients than in irradiated animals. However, unlike that study, we observed a majority of cells in non-irradiated hosts that remained quiescent, whereas 40% underwent proliferation. The reason for the difference between these two studies is not clear, but may be related to the 5-fold differences in PKH26-labelling concentration, or other technical variations. Moreover, Hendrikx *et al* (1996) utilized an antigenically defined population of cells enriched in stem cell activity whereas our study employed non-adherent low-density cells. This latter cell population consists of $>99\%$ lymphoid cells and contains very few mature myeloid cells that would readily egress into tissues and represent a 'false positive' signal. This difference in cell populations undoubtedly accounts for the localization differences. However, it is interesting to note the similarities in trafficking: both populations performed better in non-myeloablated recipients, and both showed evidence of cell trafficking into organs other than the marrow.

Our intent was to examine the trafficking of bone marrow NALD cells to determine if localization specificity or transplant-mediated enrichment could be observed at this level of 'resolution', and this was not the case. Hendrikx *et al* (1996) also noted a (calculated) specific localization index for cells localizing to the marrow, that was higher than that of cells localizing to the spleen, suggesting that specific 'homing' had occurred, especially for the enriched population of cells used in that study. However, their study also observed cells in the liver, demonstrating that cells also localized to this tissue. Given that the sensitive PCR and indium-labelling studies reported here showed a rapid egress to all tissues, and that the secondary bone marrow transplant of labelled cells failed to specifically enrich a population of donor cells, we believe that non-specific tissue migration plays a previously unappreciated role in early haemopoietic cell trafficking.

Our data are consistent with a number of investigations suggesting that no preferential selection of haemopoietic cells occurs within the bone marrow. Papayannopoulou *et al* (1995) demonstrated that selective modulation of bone marrow cell trafficking (at 3 h) was mediated by the VLA4/VCAM-1 integrin system, but that other cytoadhesion molecules also played a role in bone marrow localization. Their observations, in turn, were in agreement with earlier studies that reported the presence of haemopoietic progenitor cells in many tissues (lung, liver, kidney, spleen, blood and bone marrow) shortly following cell infusion into irradiated recipients (Vos *et al*, 1972; Kretchmar & Conover, 1969; Lahiri & Van Putten, 1969). Our data confirm and extend these observations indicating that donor bone marrow cells localize to most tissues, for periods of up to 48 h following infusion. Taken together, these investigations argue against the preferential uptake of haemopoietic cells by the bone marrow. None of these studies, however, distinguish between the preferential retention of haemopoietic progenitor cells in haemopoietic tissues (after 48 h), or a preferential survival and proliferation within a haemopoietic microenvironment.

Myeloablation is assumed to be a necessary prerequisite for bone marrow transplantation that ensures removal of host cells and the creation of appropriate niches for engrafting donor cells (Schofield, 1978; Takada *et al*, 1971; Micklem *et al*, 1968). However, several studies have shown that donor cells are capable of stable engraftment, even without prior chemotherapy, or radiation (Takada *et al*, 1971; Brecher *et al*, 1979; Micklem *et al*, 1968; Quesenberry *et al*, 1994), suggesting that marrow spaces ('niches') do not have to be created to ensure engraftment. Our PKH26-labelling data and that of Hendrikx *et al* (1996) suggest that immediate trafficking of infused bone marrow cells to the marrow space is inhibited by prior radiation, possibly due to damage to the marrow sinusoidal endothelial cells, or damage to other components of the haemopoietic microenviroment. The ^{111}In-labelling studies confirmed this concept, showing increased tissue specific localization in a number of tissues that was similar to cell-distribution patterns observed when ^{111}In-labelled granulocytes were

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infused (Thakur *et al*, 1977). We conclude that an early adverse affect of irradiation might not be appreciated in the standard stem cell transplant protocols, as successful engraftment generally is not assessed until a much later time point.

Based on the above data, we hypothesize that the early migration of infused marrow cells may represent nonspecific 'seeding' rather than a specific homing process. Infused marrow cells, at many stages of differentiation, may first adhere to vascular endothelial cells, and exit the circulation nonspecifically into many tissues. In this model, infused marrow cells would be distributed to all tissues randomly, but only those within the bone marrow would encounter the appropriate environment for proliferation and differentiation. Though this model is consistent with our observations of phenotypically undefined marrow cell trafficking at early time points, we cannot exclude a more complex specific homing process requiring prior cell processing in other tissues, or later changes in the cell surface, to selectively localize cells to the marrow. We conclude that bone marrow cell trafficking is a complex multifaceted process, and that the efficient targeting of transplanted cells to the marrow cannot be explained by a simple single receptor/ligand interaction.

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