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Detection and Characterization of Hemopoietic Stem Cells in the Adult Human Small Intestine[1]

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The concept of lymphoid differentiation in the human gastrointestinal tract is controversial but is the focus of this study, which examined adult human small intestinal tissue for the presence of $CD34^+CD45^+$ hemopoietic stem cells (HSCs) and lymphoid progenitors. Flow cytometry demonstrated that over 5% of leukocytes ($CD45^+$ cells) isolated from human gut were HSCs coexpressing CD34, a significantly higher incidence than in matched peripheral blood or control bone marrow. HSCs were detected in cell preparations from both the epithelium and lamina propria of all samples tested and localized to the intestinal villous and crypt regions using immunofluorescence. A high proportion of gut HSCs expressed the activation marker CD45RA, and few expressed c-*kit*, indicating ongoing differentiation. The vast majority of intestinal HSCs coexpressed the T cell Ag, CD7 (92% in the epithelium, 80% in the lamina propria) whereas <10% coexpressed the myeloid Ag CD33, suggesting that gut HSCs are a relatively mature population committed to the lymphoid lineage. Interestingly, almost 50% of epithelial layer HSCs coexpressed CD56, the NK cell Ag, compared with only 10% of the lamina propria HSC population, suggesting that the epithelium may be a preferential site of NKR^+ lymphoid differentiation. In contrast, bone marrow HSCs displayed low coexpression of CD56 and CD7 but high coexpression of CD33. The phenotype of intestinal HSCs, which differs significantly from circulating or bone marrow HSCs, is consistent with a role in local lymphoid development. *The Journal of Immunology,* **2006, 176: 5199–5204.**

The human intestine, the largest mucosal surface of the body, is constantly exposed to a myriad of harmful and harmless Ags. It functions as the primary digestive organ, and due to colonization by commensal bacteria, the load of harmless foreign Ags requiring immunological tolerance in the gastrointestinal tract is larger than at any other site of regional immunity. At the same time, the gut must remain immunocompetent, as it is at high risk of pathogenic challenge and neoplastic transformation. This dual demand for immunoregulation in the gastrointestinal tract necessitates a specialized regional immune system. The lymphoid repertoire of the human small intestine is therefore complex. As well as classical $CD4^+$ and $CD8^+$ $\alpha\beta$ T cells, "unconventional" populations of T cells, that include $\gamma\delta$-TCR^+ cells (1) restricted by nonclassical MHC molecules such as CD1 and MHC class I polypeptide-related sequence A, and mucosal-associated invariant T cells (2) populate the gastrointestinal tract of humans and mice. Also included in this unconventional category are non-invariant NKR^+ T cells (3), as well as populations of NK cells (4). In addition, the gut harbors populations of lymphocytes that are unusual in terms of CD4 and CD8 expression and include CD4/CD8 double-positive and double-negative T cells as well as T cells that express the homodimeric form of the CD8 coreceptor ($CD8\alpha\alpha$) (5).

Several mechanisms are likely to influence the localization and complexity of the gastrointestinal lymphoid repertoire. In particular, the lymphocyte homing process directs lymphocyte subsets to specialized microenvironments, controls their differentiation, and regulates their survival and activity. The process is promoted by adhesion molecules, integrins, and proteoglycans. One of the first demonstrations of a tissue-specific homing molecule was the integrin α_4/β_7 (6, 7), which is expressed on gut homing T cells and binds, along with L-selectin, to mucosal addressin cell adhesion molecule-1 mediating T cell migration to the lamina propria (LP) of the gut. Most T cells that home to the epithelium of the gut express α_e/β_7, which binds E-cadherin expressed on epithelial cells (8). The gut mucosal environment plays a central role inducing trafficking and selecting intestinal lymphoid cell populations. The epithelial layer (EL) secretes chemotactic factors that activate integrins and attract lymphocytes thus directing localization of gut-specific populations. These include thymus-expressed chemokine, MIP-3α, MCP-3, and fractalkine, which are expressed by small intestinal epithelial cells in basal conditions (8, 9).

In addition to intestine-induced homing from the periphery, extrathymic development of T cells in situ is also considered to be important in determining the specialized lymphoid repertoire in the intestine (10–14). Although the majority of classical intestinal T cells arise in the thymus (15) and home to the gut, there is evidence that specialized T cell subsets develop locally in the intestine (14, 16). If lymphoid development occurs in the intestine, precursors and factors required for their maturation should be present (14). A recent murine study suggests that hemopoietic progenitors can become committed to the T cell lineage outside of the thymus and that the intestine can support maturation of these cells (17). In addition, hemopoietic stem cells (HSCs)[4] and lymphoid precursors have been detected in the murine small intestine (12, 18, 19). There

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[4] Abbreviations used in this paper: HSC, hematopoietic stem cell; IEL, intraepithelial lymphocytes; LP, lamina propria; EL, epithelial layer.

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is some evidence to support the existence of a similar pathway of extrathymic T cell differentiation in human gut. Expression of RAG transcripts, the molecular machinery required for T cell development, has been detected in the human intestine (20, 21). The secretion of IL-7 by human intraepithelial lymphocytes (IELs) (22, 23), the only cytokine that has thus far been shown to be indispensable for T cell development (24) has also been demonstrated. Another cytokine thought be critical for NK/NKT cell development, IL-15 (25), is also expressed in the human intestine (26). In addition, T cell differentiation has been shown to occur within the neonatal human intestine (27). However, neither HSCs nor intestinal lymphoid progenitors have been demonstrated in adult humans, even though epithelial stem cells have been detected in the base of the intestinal crypts (28).

Hemopoietic stem cells are defined as clonogenic cells capable of self-renewal and multilineage differentiation (29). All mature circulating blood cells are derived from self-renewing pluripotent HSCs characterized by the expression of CD34 (30). Stem cells ($CD34^+$) of hemopoietic origin are identified by the coexpression of CD45, as expression of CD34 is not exclusive to HSCs and is also expressed by some nonhemopoietic cells (31). As pluripotent HSCs mature and begin to differentiate, CD34 is gradually down-regulated with the concomitant up-regulation of CD38 and CD45RA. Hemopoietic precursors destined for different cell lineages can be identified by the expression of lineage-specific markers. $CD34^+CD45^+$ cells committed to myeloid differentiation are identified by the coexpression of CD33, whereas immature lymphoid precursors are identified by the coexpression of CD56 (NK), CD7 (T cells), and CD19 (B cells). Expression of receptors for IL-7 and IL-15, cytokines required for T and NK cell development, indicates responsiveness to these cytokines (32).

It is well documented that the small intestine is a rich source of unique and unconventional T and NKR^+ cell populations. Although there is evidence to suggest that some of these populations may differentiate locally, lymphoid precursor populations have not to date been examined. In this study, using multiparameter flow cytometry and immunofluorescence, the presence and phenotype of hemopoietic progenitors, which may give rise to some of these complex lymphoid populations, were investigated in the normal adult human intestine.

Materials and Methods

Tissue specimens

Small intestinal biopsies (four per patient) were obtained from patients undergoing endoscopic examination for gastrointestinal symptoms (n = 17; mean age, 54 years; range 20–78 years, 3 males and 15 females). The biopsies were collected in ion-free (calcium and magnesium free) HBSS (Invitrogen Life Technologies) supplemented with antibiotics (100 U/ml penicillin, 100 mg/ml streptomycin) and 5% FCS (Invitrogen Life Technologies). In all cases, small intestinal disease was excluded by endoscopic observations and histological analysis. For nine cases, 5 ml of matched venous blood was collected in heparinized tubes. Nine normal bone marrow samples were used as controls. All samples were obtained with informed consent. The ethics committee at St. Vincent's University Hospital (Dublin) granted approval for this study.

Preparation of single-cell suspensions

Single-cell suspensions of EL and LP were prepared as previously described (33). In brief, the EL was removed by rotation at 37°C for 1 h in a mixture consisting of 1 mM solution of DTT (Sigma-Aldrich) and 1 mM EDTA (Sigma-Aldrich) in ion-free HBSS. The single-cell suspension was pelleted from the supernatant and washed once in RPMI 1640 medium (Invitrogen Life Technologies) supplemented with antibiotics and 10% FCS by centrifugation at 300 × g for 10 min. To release the mononuclear cells from the LP, the remaining tissue was disrupted with a scalpel and treated with collagenase (128 U/ml type 1A; Sigma-Aldrich) in RPMI 1640 medium for 3 h with continuous agitation at 37°C. Cells from the supernatant were washed once with RPMI 1640 medium by centrifugation at 300 × g for 10 min. Epithelial and LP cell pellets were resuspended in 1 ml of RPMI 1640 containing antibiotics and 10% FCS. Cell yields and viability were assessed by ethidium bromide/acridine orange staining. The cell suspension was adjusted to 1×10^6 cells/ml in RPMI 1640 medium containing antibiotics and 10% FCS. For preparation of bone marrow mononuclear cells, aspirated samples were drawn from the marrow with a 20-ml syringe, through a 14-gauge Salah needle, and were immediately transferred into lithium-heparin Vacutainers (BD Biosciences). Peripheral blood and bone marrow mononuclear cells were prepared by standard density gradient centrifugation over Lymphoprep (Nycomed) at 400 × g for 25 min. Cells were then washed twice with HBSS supplemented with HEPES buffer solution (Invitrogen Life Technologies) and antibiotics. Cell pellets were resuspended in 1 ml of RPMI 1640 medium, and cell yields and viability were assessed as before. The cell suspension was adjusted to 1×10^6 cells/ml in RPMI 1640 medium.

Cell surface staining of cells for flow cytometric analysis

Aliquots of 100 μl (1×10^5) of cells were labeled with mAbs directed against cell surface markers classically associated with differentiating hemopoietic progenitor cells. The appropriate mAbs (0.3 μg/ml final concentration) were added to cells, which were incubated in the dark at 4°C for 30 min. Cells were then washed twice with 1 ml of PBS-BSA-azide. Labeled cells were fixed in 0.5 ml of 1% paraformaldehyde (Sigma-Aldrich).

Monoclonal Abss used in this study

For the identification/detection of cells of hemopoietic origin, FITC-labeled anti-CD45 mAb (BD Biosciences) was included in all tubes. For the identification of stem/progenitor cells, PerCP-labeled anti-CD34 mAb (clone 8G12; BD Biosciences) was used. As well as anti-CD45-FITC and anti-CD34-PerCP, one of the following PE-labeled anti-differentiation/lineage marker mAbs was also used in each tube: anti-CD33 (myeloid), anti-CD117 (c-*kit*; early stem cell marker) anti-CD56 (NK cell), anti-CD127 (IL-7 receptor; all obtained from BD Biosciences), anti-CD7 (T cell), anti-CD45RA (activation, naive T cell marker, B cells and monocytes; both obtained from BD Pharmingen), anti-CD122 (IL-2/15 receptor β-chain; Immunotech). Appropriate isotype-matched fluorescent-labeled nonspecific Abs were used to correct for any background staining.

Flow cytometric analysis

Cells were analyzed using a FACScan flow cytometer and CellQuest software (BD Biosciences). For analysis of HSCs, lymphocytes were gated by their density and granularity using forward scatter and side scatter parameters. Leukocytes ($CD45^+$) were gated using side scatter and FL1 (FITC) parameters. All further analysis was performed on $CD45^+$ cells only. Thirty thousand $CD45^+$ events were acquired in each case. PE (variable markers) and PerCP (CD34) staining levels above that of appropriate isotype controls were analyzed for a low forward scatter-side scatter gate within the $CD45^+$ population, once it had been established that >98% of $CD34^+CD45^+$ cells were contained in the analysis region. Results were expressed as a percentage of the $CD45^+$ cells or as a percentage of $CD34^+CD45^+$ cells where appropriate.

Statistical analysis

Differences between groups were assessed using the Mann-Whitney U test for nonparametric data. A value of $p < 0.05$ was taken as significant.

Localization of hemopoietic stem cells in small intestinal tissue using immunofluorescence and confocal microscopy

Paraffin-embedded human small intestinal sections were obtained for three of the patients already involved in this study. These sections were stained for cells coexpressing $CD34^+CD45^+$. Before staining, all sections were baked at 60°C for 20 min to remove the wax layer. Sections were then deparaffinized and rehydrated. Heat-induced Ag retrieval was then performed in 10 mM citrate buffer (9 ml of 0.1 M citric acid, 41 ml of 0.1 M sodium citrate, 450 ml of distilled H_2O, pH 6.0). The sections were allowed to cool at room temperature before being washed in PBS. Tissues were blocked with normal goat serum (Vector Laboratories) for 30 min. Primary Abs, anti-CD34, (IgG1, 1/25 dilution with PBS solution, mouse monoclonal; Novocastra Laboratories), and mouse anti-human CD45-UNLB, (IgG2a, 1/50 dilution with PBS solution; Southern Biotechnology Associates) were added to the sections and incubated in a moist chamber in the dark at room temperature for 1 h. In addition, sections were stained with CD34 only and CD45 only to confirm specific staining with each Ab.

Slides were washed in PBS containing 0.1% Tween 20 for 1 h with constant agitation. Human adsorbed secondary Abs, goat anti-mouse IgG2a-FITC, and goat anti-mouse IgG1-Texas Red (both Southern Biotechnology Associates) were applied to the sections for 30 min. Sections were then washed in PBS containing 0.1% Tween 20 for 1 h with constant agitation. A DNA counterstain (TOTO-3 iodide, 1/200 dilution in PBS; Molecular Probes) was applied to sections at room temperature for 5 min, then washed gently in PBS. Finally, sections were dehydrated in alcohol and cleared in xylene and mounted in 90% glycerol containing diazabicyclo octane (fluorescent mounting medium; DakoCytomation) to retard fading and stored at −20°C in the dark. Digitized images of sections were obtained using a Zeiss Axiovert 100TV confocal microscope, with excitation at 488 nm for FITC, 543 nm for Texas Red, and 633 nm for TOTO-3 and analyzed using confocal assistance software (Bio-Rad).

Results

Cell yields and viability

Four duodenal biopsies were collected from each patient. The mean mononuclear cell yield obtained from the LP was 1.0×10^6 cells (range 0.3–1.5 $\times$ 10^6). From the EL, the mean mononuclear cell yield was 0.74×10^6 cells (range 0.3–1.6 $\times$ 10^6). Cell viability in all cases exceeded 80%.

Detection of small intestinal hemopoietic stem cells

Flow cytometric analysis was used to detect the presence and estimate proportions of intestinal HSCs ($CD34^+CD45^+$). HSCs were detected in both the EL (median 6.2% of total $CD45^+$ cells, range 3.0–14.0, $n = 14$) and the LP (7%, range 4.5–11, $n = 14$) of adult small intestinal tissue from all individuals studied, at significantly higher levels than in matched peripheral blood (0.10%, range 0–0.2, $n = 9$, $p = 0.0001$) or control bone marrow (3.91%, range 0.3–7.63, $n = 9$, $p < 0.01$; Fig. 1). The majority of intestinal HSCs expressed low levels of CD34 and high levels of CD45, indicative of a more differentiated phenotype than the primitive pluripotent $CD34^{high}$ HSCs found in the bone marrow (Fig. 2). Immunofluorescence and confocal analyses confirmed the presence of cells coexpressing CD34 and CD45 (HSCs) in sections of small intestine. Up to 20 villi from each of three individuals were examined. HSCs were found to be distributed in the villi, particularly at the tips in the epithelium and throughout the LP (Fig. 3*b*). They were also scattered throughout the crypt regions (Fig. 3, *a* and *c*). There were no HSCs found in the muscularis mucosae.

Early stem cell Ag expression

Three-color flow cytometric analysis was used to characterize the coexpression of cell surface Ags classically associated with immature, differentiating, and lineage restricted HSCs as well as expression of key lymphopoietic cytokine receptors on intestinal $CD34^+CD45^+$ HSCs. The early stem cell marker, c-*kit*, was found to be coexpressed on only a small proportion of intestinal HSCs, at approximately half the level detected in matched peripheral blood (Table I). This suggests that the HSC population in the small intestine is somewhat more mature than its equivalent circulating population. High levels of coexpression of the activation Ag CD45RA was detected on intestinal HSC populations. Similar coexpression of CD45RA was detected on HSCs from both layers of the gut; although, this was significantly reduced compared with matched peripheral blood (Table I).

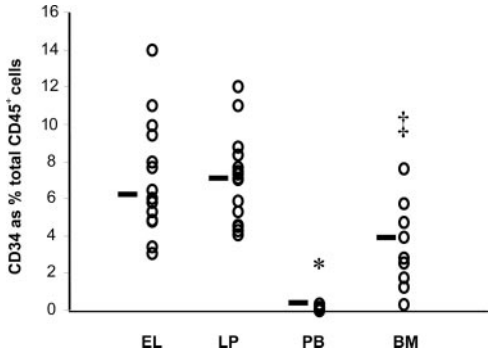

FIGURE 1. CD34 expression and levels of hemopoietic stem cells in the small intestine. $CD34^+CD45^+$ HSCs were detected by flow cytometry in both the EL ($n = 14$) and LP ($n = 14$) of normal adult human intestinal tissue at a significantly higher level than matched peripheral blood (PB; $n = 9$) or control bone marrow (BM; $n = 9$). Open circles represent individual samples and horizontal bars represent median levels. $p = 0.0001$; ‡, $p < 0.01$ (compared with matched EL and LP).

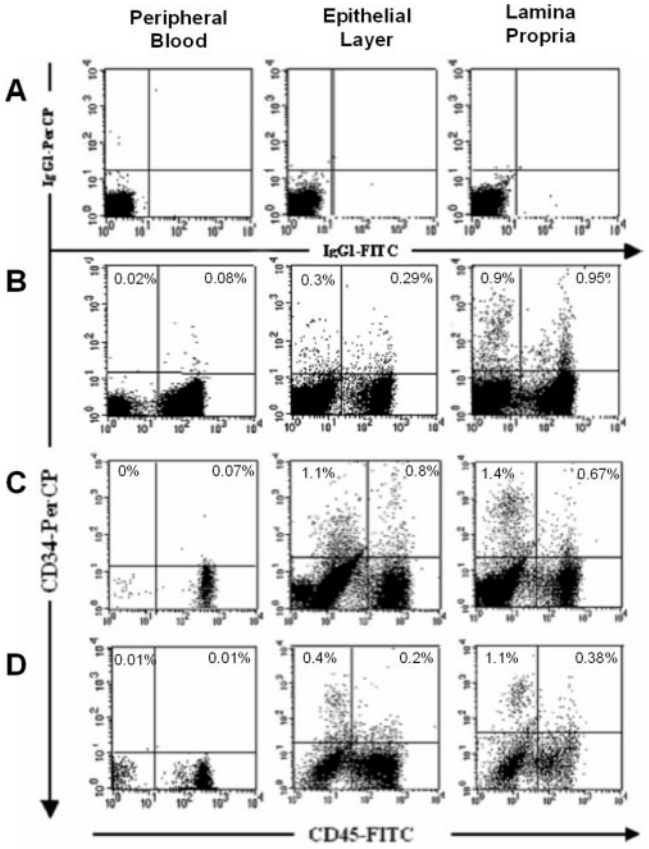

FIGURE 2. HSCs in the intestinal epithelium, LP, and matched blood. Flow cytometric dot plots of peripheral blood and matched intestinal HSCs from three patients. $CD34^+CD45^+$ HSCs fall in the *upper right quadrant*. Isotype-matched control staining is shown in the top panel (*A*). Three sample patients are shown in *B*, *C*, and *D*. The level of CD34 expression is lower on the vast majority of $CD45^+CD34^+$ cells (*upper right quadrant*) compared with $CD45^-CD34^+$ cells (*upper left quadrant*). Percentages of $CD34^+CD45^+$ and $CD34^+CD45^-$ cells are shown as a percentage of total lymphocytes for patients B, C, and D. Corresponding HSC ($CD34^+CD45^+$) levels as percentage of $CD45^+$ cells are 3.43, 7.7, and 9.9% (EL) and 8.77, 3.5, and 4.6% (LP) for patients B, C, and D, respectively.

Lineage-specific Ag expression

To further characterize intestinal HSCs, coexpression of lineage-specific Ags, CD33 which identifies myeloid progenitors, CD56 which identifies NK cell progenitors, and CD7 which identifies T cell progenitors, was also investigated. A smaller proportion of HSCs coexpressing the myeloid Ag (CD33) was found in the intestine compared with matched blood and control bone marrow suggesting that, in contrast to bone marrow, production of myeloid

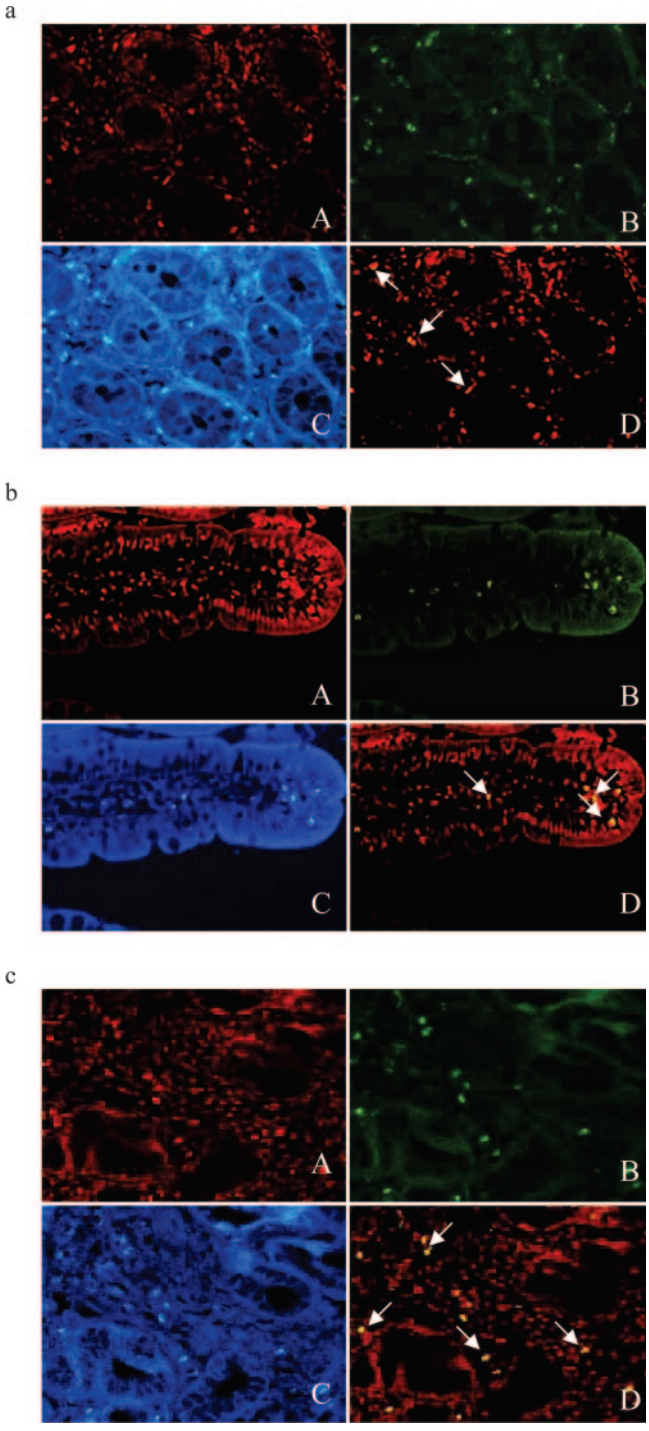

FIGURE 3. Localization of intestinal hemopoietic stem cells. CD34 Texas red staining (*A*) and CD45 FITC staining (*B*) are shown for the same field. The morphology of small intestinal sections was visualized using confocal microscopy at emission wavelength 640 nm at which none of the markers fluoresce (*C*). The location of double-positive $CD34^+CD45^+$ HSCs is highlighted by arrows in the composite image (*D*). HSCs are visualized scattered throughout the crypt regions (*a*) and (*c*) and epithelium at the villous tips and throughout the LP (*b*).

cells is not a primary function of intestinal HSCs. In contrast, lymphoid Ags CD56 and CD7 were coexpressed by a significantly higher proportion of intestinal HSCs compared with bone marrow suggesting a bias toward lymphopoiesis (Table I). Lymphopoietic cytokine receptors IL-7Rα and, to a lesser extent, IL-2/15Rβ were also detected on intestinal HSCs (Table I), suggesting that these cells are responsive to IL-7 and IL-15, cytokines with key roles in lymphoid differentiation.

Significant differences were observed in the distribution of HSCs coexpressing lineage markers in the EL when compared with the LP. HSCs coexpressing the NK cell marker CD56, which were present only in low proportions in the bone marrow (3.73%), were almost twice as frequent in the gastrointestinal LP (7.56%). Surprisingly, over 40% of the HSCs in the LP coexpressed this NK Ag, significantly higher than in the LP ($p < 0.05$). In addition, a significantly higher frequency of CD7 coexpressing HSCs were detected in the EL (92%) compared with the LP (80%, $p < 0.05$, Table I).

Discussion

The concept of extrathymic T lymphocyte development predicts that T cell progenitors and all factors required for their maturation are present at extrathymic sites. In the murine intestine both hemopoietic stem cells (12, 18) and lymphoid progenitors (19) have been described. In addition to indirect molecular evidence, such as the expression of IL-7, which is critical for T cell development, direct evidence of local $\gamma\delta$ TCR^+ cell differentiation in the murine gut has been demonstrated (14, 16). A recent study has provided evidence of T cell differentiation in the human neonatal intestine (27); however, evidence for the existence of a similar intestinal T cell development pathway in adult humans is sparse. Indirect evidence, in particular the expression of RAG1 and RAG2 transcripts (20, 21) and IL-7 (22, 23), suggests that the normal adult human intestine may also support lymphopoiesis; however, hemopoietic stem cells and lymphoid progenitors have not previously been described. In this study, using flow cytometry, HSCs were detected in both the EL and LP of small intestinal tissue from all individuals studied. Indeed, the adult small intestine is enriched for HSCs when compared with matched blood and bone marrow. Disease had been excluded in all cases indicating that the presence of HSCs did not result from some underlying pathogenesis.

The presence of CD45RA, the sparse expression of c-*kit* and high levels of lineage-specific Ag expression on intestinal HSC populations suggests that gut HSCs are a relatively mature, lineage-committed population. The majority of $CD34^+CD45^+$ HSCs in the intestine coexpressed the lymphoid-associated Ags, CD7 and CD56, whereas <5% of EL and 10% of LP HSCs expressed the myeloid Ag (CD33). This is in contrast to normal bone marrow, where the majority of $CD34^+CD45^+$ cells coexpressed CD33 with T cell progenitors ($CD34^+CD45^+CD7^+$) comprising only a small proportion of HSCs. Intestinal HSCs also differed from matched peripheral blood with respect to myeloid commitment, 37% of which coexpressed CD33. These results suggest that in contrast to bone marrow HSCs, production of myeloid cells is not a primary function of intestinal HSCs. Most HSCs from both layers of the intestine express the T cell progenitor Ag, CD7, similar to matched blood but in stark contrast to control bone marrow where <10% coexpress CD7. The high level of CD7 expression on peripheral blood HSCs is consistent with circulation of T cell progenitors before their homing to the thymus and other organs that might support T cell differentiation. The equivalently high level of CD7 expression by gut HSCs suggests homing to and retention of T cell progenitors in the nondiseased adult human intestine.

Approximately 8% of HSCs in the LP coexpressed CD56, twice as many as found in the bone marrow. However, almost 50% of the HSCs in the EL have this phenotype suggesting that the epithelium may be a preferential site of NKR^+ lymphoid differentiation. Indeed, significant populations of small intestinal T cells coexpress NK cell receptors and classical NK cells have been shown to be

Table I. *Coexpression of lineage-associated Ags on CD34$^+$CD45$^+$ intestinal HSCs*[a]

Tissue	c-*kit*	CD45RA	CD33	CD56	CD7	IL-7-Rα	IL-15-Rβ
EL	3.76 (0–25)	54.2 (40–79)*	5.6 (0–11)*†	42 (20–85)†‡	92 (80–99)†‡	10.58 (4–36)†	1.61 (0–13.5)
LP	6.81 (0–16)	40.79 (22–55)*	3.51 (2.5–9)*†	7.56 (5–14.5)§*†	80 (44–92)†‡	9.1 (1–29.5)†	3.97 (0–12)
PB[b]	9.95 (7–60)	99 (98–99)	37.5 (32–95)	62 (39–80)	90 (70–90)	6.41 (5–10)	2.4 (2–13)
BM	NT	NT	54.3 (44–78.5)	3.73 (0.83–18)	9.97 (3–23)	3.12 (0–9)	1.45 (0–3.5)

[a] Numbers represent the percentage of total CD45$^+$ cells (range).
[b] PB, peripheral blood; BM, bone marrow; NT, not tested.
*, $p < 0.05$ compared to matched PB.
†, $p < 0.05$ compared to control BM.
‡, $p < 0.05$ EL vs. LP.

present and functional in the human small intestine (4). It is possible that some of these differentiate from intestinal CD56$^+$ HSCs. The only other place in the body where large numbers of CD56$^+$ HSCs have been detected is the liver (34, 35), another regional immune organ with significant populations of NKR$^+$ T cells (36). Hepatic HSCs are similar to intestinal HSCs with respect to the coexpression of CD45RA, CD33, and CD56. However, the HSC population in the adult human liver differs from their intestinal counterparts with respect to T cell Ag expression patterns. The vast majority (>80%) of intestinal HSCs coexpress CD7, whereas only 40% of the hepatic HSC population are T cell progenitors. Taken together with the relatively low coexpression of CD33, this suggests a T lymphoid bias in the human intestinal compartment.

Murine thymic-independent IEL subsets display differential dependence on both IL-7 and IL-15 with IL-7 being essential for TCR-$\gamma\delta$ (14) and IL-15 for TCR-$\alpha\beta$CD8$\alpha\alpha$ IEL (37) and NKT cell development (25). Small proportions of intestinal HSCs coexpress the IL-2/15 receptor β-chain (~3%); however, a significantly higher proportion coexpress the IL-7 receptor (~10%). The presence of these lymphopoietic cytokine receptors on the intestinal HSC population is further evidence of intestinal lymphoid progenitors that can respond to IL-7 and IL-15, both of which are expressed locally (22, 26).

Immunofluorescent double staining of intestinal tissue revealed HSC populations scattered throughout the crypt region. They were also found in the villi, particularly in the tips, where they are interspersed between the epithelial cells and also throughout the LP. The intestinal villous region is the site of many T cells; however, it is not known exactly from where they have arisen. It is widely accepted that the majority of intestinal lymphocytes have homed to the gut from the periphery. However, it may also be possible that some have differentiated from intestinal HSC populations as the vast majority of the intestinal HSCs express the early T cell marker CD7.

Although all hemopoietic stem and progenitor cells express CD34 (30), not all CD34$^+$ cells are HSCs, as CD34 is expressed on cells that are not of hemopoietic origin (31). In this study, we used coexpression of CD45 on intestinal CD34$^+$ cells to distinguish HSCs. As seen in Fig. 2, less than half the CD34$^+$ population detected in both the EL and LP of the human intestine were of hemopoietic origin (coexpressed CD45). This is similar to what has been shown previously in the adult human liver (35). As our interest lay in hemopoietic progenitor cells, the CD34$^+$CD45$^-$ population was not analyzed further. However, it is interesting to speculate as to what these cells may be. It is unlikely that all nonhemopoietic CD34$^+$ cells are mature endothelial cells, because the gating technique used would have excluded the majority of endothelial cells on the basis of size. It is possible that this population contains noncycling HSCs that have yet to up-regulate CD45. The presence of epithelial stem cells in the intestine is established (28), and therefore, it is possible that the nonhemopoietic CD34$^+$ cells represent an epithelial progenitor population. The CD34$^+$CD45$^-$ intestinal cell population may therefore include small mature endothelial cells, quiescent HSCs, and/or epithelial progenitor cells; however, further studies would be required to define this population.

The results of this study show for the first time that the normal adult human intestine harbors lineage-committed hemopoietic progenitors, which differ significantly from their circulating, bone marrow, and hepatic counterparts. The vast majority of these progenitors expresses lymphoid-associated Ags and are thus likely to have a role in the emergence of lymphoid repertoires important to local immunoregulation and function.

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Disclosures

The authors have no financial conflict of interest.

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