Dynamics and Localization of Early B-Lymphocyte Precursor Cells (Pro-B Cells) in the Bone Marrow of scid Mice

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Mice homozygous for the scid (severe combined immunodeficiency) mutation are generally unable to produce B lymphocytes, a condition attributed to defective rearrangement of immunoglobulin genes in precursor B cells. Some early B-lineage cells are present in the bone marrow (BM), however. In scid mice, we defined three subsets of early progenitor B cells lacking \( \mu \) heavy chains (pro-B cells) based on the expression of terminal deoxynucleotidyl transferase (TdT) and B220 glycoprotein: (a) early pro-B cells (TdT*B220\(^-\)), (b) intermediate pro-B cells (TdT*B220\(^+\)), and (c) late pro-B cells (TdT*B220\(^+\)). Double immunofluorescence labeling of BM cell suspensions has shown normal numbers of early and intermediate pro-B cells, substantially reduced numbers of late pro-B cells, and an absence of pre-B cells and B cells. Early and intermediate pro-B cells accumulated in metaphase in near-normal numbers after intraperitoneal (IP) vincristine administration. B220\(^-\) pro-B cells have been localized in BM sections by the binding of intravenously (IV) administered \(^125\)I mono-

clonal antibody (MoAb) 14.8, detected by light and electron microscope radioautography. Many B220\(^-\) cells were located peripherally in the bone-lining cell layers associated with stromal reticular cells. More centrally located B220\(^+\) cells were frequently associated with macrophages containing prominent cytoplasmic inclusions. Occasional B220\(^+\) cells were present in venous sinuses. These results demonstrate that many pro-B cells in scid mice occupy microenvironments in the BM near the surrounding bone. The pro-B cells maintain normal rates of production during stages of presumptive \( \mu \) heavy-chain gene rearrangement, apparently unaffected by the absence of a mature B cell pool. Nearly all defective cells then abort at the late pro-B cell stage and are deleted, apparently by macrophages. The findings contribute to models of in vivo differentiation, regulation, localization, and selection of early B-lineage cells in the BM.

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would include the stage of heavy-chain gene rearrangement. A measure of their proliferative activity has been provided by a metaphase arrest technique. Early B220+ precursor B cells have been proposed to occupy microenvironments near the surrounding bone, associated with stromal reticular cells, whereas resident macrophages in normal BM appear to play an active role in B-cell deletion. To examine localization of pro-B cells in the BM of scid mice, B220+ cells were labeled in situ by binding radiolabeled MoAb 14.8 administered IV and detected in radioautographs of tissue sections at the light and electron microscope levels.

MATERIALS AND METHODS

Animals. The scid mutation originated in the C.B-17/Icr (C.B-17) inbred mouse strain, an IgH-chain congenic partner strain of BALB/cAnIcr. Scid and normal C.B-17 control male mice (R.A. Phillips, Ontario Cancer Institute, Toronto, Ontario, Canada) were given sterilized food and water and housed in sterile microisolator units. The scid mice and control mice were used at age 8 to 15 weeks for immunofluorescence studies and at 5 weeks for in vivo labeling work.

Antibodies and radioiodination. Rat MoAb 14.8 was collected, affinity column-purified, and concentrated (2 mg/mL) from hybridoma cell supernatants (American Type Culture Collection, ATTC) and diluted 1:150 in phosphate-buffered saline (PBS), pH 7.2. FITC-conjugated goat anti-rat IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was diluted 1:15 in PBS and used as a second layer after cell surface binding of the 14.8 antibody. Rabbit anti-TdT (Supertechs, Bethesda, MD) was used at 1:20 dilution and detected with rhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG F(ab')2 (1:20 dilution, Jackson Immunoresearch Laboratories, Mississauga, Ontario). TRITC- and FITC-conjugated affinity-purified goat antibodies to mouse μ chains (Southern Biotechnology Associated, Birmingham, AL, and Kirkegaard & Perry Laboratories) were diluted 1:30 and 1:10, respectively, in PBS. All antibodies were ultracentrifuged for 30 minutes at 120,000g (Beckman Instruments, Palo Alto, CA) to remove aggregates just before use. MoAb 14.8 (130 to 140 μg) was coupled to carrier-free Na[^125]I (2 μCi) specific activity 1.5 × 10[^6] cpm/μg) by a modified chloramine-T method, yielding a final concentration of 50 μg/mL.

Bone marrow and spleen cell suspensions. Mice were killed by cervical dislocation. Both femoral shafts were flushed with 1 mL cold Eagles’ minimal essential medium (MEM), pH 7.2, containing 10% (vol/vol) newborn calf serum (MEM/NCS, Gibco, Grand Island, NY) centrifuged (10 minutes, 250g, 4°C) to remove all aggregates and debris. After thorough resuspension, large particles were removed by sedimentation into 1 mL NCS for 5 minutes. The marrow cells were then centrifuged through 1 mL NCS (10 minutes, 250g, 10°C) and resuspended in 1 mL MEM/NCS. The nucleated cells recovered from two femurs were counted with an electronic particle counter (Model Z; Coulter Electronics, Hialeah, FL). Spleens were removed and teased through a fine-mesh screen with MEM/NCS, and the resulting suspended cells were counted.

Metaphase arrest. Mice were injected with vincristine sulfate (Sigma Chemical, St Louis, MO) intraperitoneally (IP) at a dose of 0.2 mL/20 g body weight to block cells in metaphase. Marrow cells were sampled after an interval of 2 hours 40 minutes, which represents the middle of the linear curve of increase of mitotic cells.

Double immunofluorescence labeling of MoAb 14.8 and μ chains. To detect surface membrane 14.8 antigen, 100 μL of BM cells (4 × 10^7 nucleated cells per milliliter were incubated for 30 minutes on ice with 100 μL of MoAb 14.8. The cells were washed twice by centrifugation (10°C, 10 minutes, 250g) through NCS, exposed to FITC-conjugated goat anti-rat IgG (30 minutes, 4°C), washed twice through NCS, and resuspended in 2.5 mL of 0.15 mol/L NaCl, with 2.7 mmol/L of disodium EDTA (Fisher Scientific, Fairlawn, NJ) and bovine serum albumin (BSA, Gibco) 5% (wt/vol). In a cytocentrifuge (Cytopsin, Shandon Southern Instrument, Sewickly, PA) samples of 4 × 10^5 cells were deposited (7,000 rpm, 5 minutes) on glass slides previously coated by centrifugation of 100 μL PBS/BSA 5% (wt/vol) (7 minutes, 9,000 rpm). The slides were quickly air-dried to preserve cell morphology, fixed in precooled (4°C) 5% (vol/vol) glacial acetic acid in absolute ethanol for 12 minutes on ice, and then washed four times in PBS. After excess PBS was removed, each cytospot was covered with 30 μL anti-μ-TRITC, incubated for 30 minutes in a humidified chamber, washed four times, and kept overnight in PBS at 4°C.

Surface and cytoplasmic μ chains. Bone marrow cell suspensions were incubated with anti-μ-FITC (30 minutes, 4°C) to label μs+, then washed, cytocentrifuged, and fixed as above. Each cytospot was exposed to 30 μL anti-μ-TRITC, thus labeling cytoplasmic μ chains.

MoAb 14.8 and TdT. After BM cells were surface labeled with MoAb 14.8, as described above, they were cytocentrifuged, fixed with absolute methanol for 20 minutes on ice, and gradually rehydrated by washing first with 50% (vol/vol) MeOH/PBS (3 to 5 minutes) and then with 100% PBS (3 to 5 minutes). After washing four more times at 10-minute intervals with PBS, each cytospot was exposed to 30 μL normal goat serum (NGS) for 30 minutes to block any nonspecific binding sites for the goat anti-rabbit antibody, incubated overnight with rabbit anti-TdT (12 hours at room temperature), and then exposed to TRITC-conjugated goat anti-rabbit IgG F(ab')2, for 30 minutes.

Slide preparations were mounted in 90% (vol/vol) glycerol (Fisher Scientific) in PBS, pH 8.0, containing 0.1% (wt/vol) p-phenylene diamine (Fisher Scientific) to reduce fading of the fluorochrome during microscopy. An epifluorescence microscope (Carl Zeiss of Canada, Don Mills, Ontario) equipped with a HBO 50 mercury lamp, phase-contrast optics, and a x×100 oil-immersion phase-contrast objective was used for analysis.

Double immunofluorescence analysis. Individual marrow and spleen cell preparations were scored for (1) single labeling with either FITC alone (14.8, sp) or TRITC alone (cp, TdT) or (2) double labeling with FITC plus TRITC (14.8+sp+, TdT+cp+). Flourescing cells were examined by phase-contrast to measure the cell diameter, using an eyepiece micrometer scale, and to detect cells in metaphase arrest. The incidence of TdT+14.8+, TdT+14.8-, and 14.8 + μ −pro-B cells, cp+sp−pre-B cells, and μ−B cells were derived by examining at least 3,000 nucleated cells. The counts included at least 100 cells of each phenotype. The proportion of the pro-B cell and pre-B-cell populations in metaphase (metaphase index, M1) was determined by counting 3,000 nucleated cells. To determine their size distribution, 70 TdT+ cells and 230 14.8− cells were measured by ocular micrometer. The absolute number of B-lineage cells in the BM was calculated from their incidence and the total BM cellularity.

In vivo labeling by MoAb 14.8. Under IP chloral hydrate anesthesia (1.6% in 0.9% sterile NaCl; 0.75 mL/25 g body weight), the external jugular vein of mice was exposed to allow a single IV injection of 100 μL ^125I-labeled MoAb 14.8 (5 μg; specific activity 1 × 10^6 cpm/μg). Three minutes after the injection, the whole-body arterial perfusion was established through the left ventricle (3 mL/min) with syringe pump (Model 355; Sage Instruments, White Plains, NY); the right atrium was opened to allow perfusate to
escape. Unbound $^{125}$I-MoAb 14.8 and blood were washed out by perfusing cold lactated Ringer’s solution at pH 7.4 for 10 minutes, followed by in situ fixation of body tissues by perfusion of a cacodylate-buffered solution of 2.5% glutaraldehyde and 2% acrolein (pH 7.4) for 10 minutes.

Tissue processing and radioautography. Perfusion-fixed femurs were removed, immersed in fixative (2.5% glutaraldehyde, 2.0% cacodylate-buffered solution of 2.5% glutaraldehyde and 2% acrolein (pH 7.4) at 4°C for 10 to 14 days, and washed in cacodylate buffer (pH 7.4). Femurs were postfixed in 1% potassium ferrocyanide-reduced osmium tetroxide for 3 hours at 4°C, dehydrated in acetone, infiltrated, and embedded in epon 812. For light-microscope preparations, 1-μm-thick transverse sections (mid-diaphysis) were cut, stained with iron alum/iron hematoxylin, and processed for radioautography as described. Electron microscopy radioautographic sections were cut at 70 to 84 nm, placed on celluloid-coated glass slides, dipped in Ilford L4 emulsion (Ilford Ltd, Ilford, Essex, England), and exposed for 42 to 105 days. After development, the sections were placed on copper grids, and the celluloid layer was removed with glacial acetic acid before staining with uranyl acetate and lead citrate.

Analysis of histologic radioautographs. Entire transverse sections of diaphyseal marrow of four sections from three mice were examined in successive square 2,025-μm² fields delineated by an ocular grid to quantitate radioautographic labeling indexes throughout the BM, as described elsewhere. In each field, the total number of nucleated cells (excluding megakaryocytes, endothelial cells, and adipocytes) and the grain count of each cell were recorded. Optimal grain densities over labeled cells with only minimal background grains over nonlymphoid cells were obtained after 7 days of radioautographic exposure. The percentage of cells labeled at various grain count thresholds was calculated in each field and plotted on maps of the BM sections. For EM radioautography, groups of five to seven sections from each femur were examined with a Philips 301 EM at three different exposure times.

RESULTS

Precursor B-cell populations in BM of scid mice. Three populations of phenotypically distinct pro-B cells were detected in the BM of scid mice, using double immunofluorescence labeling techniques. TdT⁺ cells formed 2.8% ± 0.3% of all nucleated cells in the BM, falling into two categories, early pro-B cells (TdT⁺ B220⁻ μ⁺; 0.6% ± 0.2%) and intermediate pro-B cells (TdT⁺ B220⁻ μ⁺; 2.2% ± 0.2%). A group of B220⁻ μ⁺ cells accounted for 3.3% ± 0.9% BM cells, thus indicating by subtraction a low incidence of late pro-B cells (TdT⁻ B220⁺ μ⁻; 1.1% ± 0.6%). When calculated as the actual number of cells per femoral marrow and compared with age- and strain-matched controls, the early and intermediate pro-B cells showed normal or near-normal population sizes (Fig 1). B220⁻ μ⁻ cells, on the other hand, were considerably reduced to one fifth of control levels (Fig 1), and the population of late pro-B cells, derived by subtraction, was only one-tenth normal size. In an extensive series of cell counts, μ⁺ sμ⁺ pre-B cells and sμ⁻ B lymphocytes were completely absent, in contrast with the substantial populations of each cell type in control mice (Fig 1). Thus, B220 labeling, which in normal BM characterizes a large fraction of cells (intermediate and late pro-B cells, pre-B cells, and B lymphocytes) was limited in scid BM to pro-B cells; of these, two thirds were TdT⁺ immature pro-B cells, and the remainder were TdT⁻ late pro-B cells.

Measurements of cytocentrifuged TdT⁺ cells in scid BM showed them to be mostly medium-sized cells, 7 to 13 μm in diameter (mean 9.8 μm), similar to the control TdT⁺ populations. The B220⁺ μ⁻ cells in scid BM were also mainly medium-sized (mean 9.5 μm), however, in contrast to the usual predominance of large cells in this population (mean 11 μm).

Proliferative kinetics of pro-B cell subsets in BM of scid mice. The cells in each of the three populations of pro-B cells in scid mouse BM were in active mitotic cell cycle. The flow of cells through mitosis was indicated by the number of cells that had accumulated in metaphase at a standard interval of time (2 hours 40 minutes) after injection of vincristine sulfate to halt dividing cells in metaphase. TdT⁺ cells in metaphase showed TdT labeling dispersed throughout the cytoplasm. Whereas approximately normal numbers of TdT⁻ B220⁻ cells accumulated in metaphase, the number of TdT⁻ B220⁺ cells in metaphase was only half that of control values and mitotic B220⁺ μ⁻ cells were reduced considerably to about 15% of control numbers (Fig 2). The number of late pro-B cells entering metaphase, derived by subtraction of data shown in Fig 1, was only 5% of control values.

Cell populations in the spleen of scid mice. No sμ⁺ B cells, μ⁺ sμ⁻ pre-B cells were observed in scid mouse spleen. A low incidence of μ⁻ lymphoid cells bound MoAb 14.8 (3.8% ± 1.7%), as in control mice (3.5% ± 0.8%). The total number of nucleated cells in the spleen was greatly reduced (scid, 22.3 ± 7.4 × 10⁶; control, 132.6 ± 9.7 × 10⁶).

Localization of B-precursor cells in BM of scid mice. Young (5 weeks) scid mice were used to identify B220⁺ cells in situ by the binding of $^{125}$I-MoAb administered IV. In a preliminary immunofluorescence analysis, BM cell suspensions from a 5-week-old scid mouse showed the same general pattern of precursor B cells as that of scid mice aged 11 to 15 weeks, although TdT⁺ cells were less frequent. No pre-B cells or B lymphocytes were detected.
Fig 2. Number of B-lineage cells in metaphase in femoral BM of scid mice and control mice aged 8 to 15 weeks, sampled 2 hours 40 minutes after vincristine sulfate administration.

Radioautographic tissue sections viewed by light microscopy and EM after in vivo administration of 125I-MoAb 14.8 showed effective binding to B220+ cells (Figs 3 through 5). The locations of individual labeled B220+ cells were determined in four representative light microscope radioautographic sections from three scid mice, analyzed as detailed previously. 33,36,37

Sixty to 86 (mean 78) well-labeled B220+ cells (10 grains per cell) were detected in individual BM sections, each of which contained about 7,000 nucleated cells (incidence 1.1% ± 0.2%; mean ± SD). Histological mapping of labeled cells (Fig 4) showed that the B220+ cells were distributed widely in each BM section. Many of the B-lineage cells were located in a narrow peripheral zone of bone marrow, near the surrounding bone (Figs 3 and 4); one to three labeled B220+ cells were found in individual microscope fields (2,160 μm²). The single outermost fields lying immediately adjacent to the bone around a complete section of femoral marrow contained 44% of the total number of B220+ cells in the section, as compared with an expected value of 34% derived by distributing the same number of cells throughout the section randomly. Other B220+ cells were more centrally located, mainly as single cells widely separated from one another, but relatively few were located immediately around the central venous sinus (Fig 4). Some patchiness of distribution was apparent. The labeling was concentrated in particular areas; other areas were devoid of labeled cells (Fig 4). The labeling threshold restricted the observations to well-labeled B220+ cells: The minimum grain count per labeled cell was considerably greater than could be attributed to any background grains in the same radioautographic preparations.

Most labeled B220+ cells were located in the extravascular compartment of the bone marrow. Some in intermediate and central zones were apposed to the wall of venous sinusoids, including the central venous sinus. Two of the 311 labeled cells shown in Fig 4 were clearly located in the lumen of small venous sinusoids, however. Adipocytes were a prominent feature; about 30 voluminous fat-laden cells were noted in each section, mainly located peripherally and often adjacent to venous sinuses (Fig 3). Some heavily labeled cells were observed in the marginal zone and red pulp of the spleen (data not shown).

Cellular associations and ultrastructure of B-precursor cells in BM of scid mice. The precise location of B220+ cells and their relation with other cells were demonstrated by EM radioautography. B220+ cells were well labeled by 10 to 50 grains closely overlying the cell surface membrane, whereas background grains over other cells were virtually absent (Fig 5). Labeled B220+ cells were readily evident in subosteal locations near the bone interface (Fig 5a). The B220+ cells were often in direct contact with osteoblasts, identified as sheets of cells with cytoplasm packed with parallel profiles of rough endoplasmic reticulum and a well-developed Golgi apparatus (Fig 5a). These B220+ cells

Fig 3. Light microscope radioautograph of B220+ cell in femoral BM of scid mouse labeled by in vivo administration of 125I-MoAb 14.8. A well-labeled cell (>50 grains, arrowheads) is located near an adipocyte (A) and the endosteum-bone interface (arrows). Radioautographic exposure 15 days (original magnification ×1,250).
Fig 4. Distribution of labeled B220+ cells in transverse sections of femoral BM of scid mice after in vivo labeling with 125I-MoAb 14.8. The size of the circular symbols represents the number of labeled cells in successive rectangular microscopic fields throughout entire sections.

were of medium size and undifferentiated morphology. The nucleus had an open leptochromatic pattern with peripheral patchy chromatin condensation. The cytoplasm, small to moderate in volume, sometimes displayed prominent mitochondria and rough endoplasmic reticulum (Fig 5a).

Intimately surrounding many of the B220+ cells were complex processes of stromal reticular cells (Fig 5b) and, in some cases, a thick electron-dense extracellular matrix. The reticular cells were recognized as stellate cells with elongated nuclei surrounded by a small volume of cytoplasm containing small Golgi saccules and some rough endoplasmic reticulum and radiating numerous long tenuous extensions throughout the marrow parenchyma. In intermediate zones of the BM, further removed from the surrounding bone, B220+ cells located singly among cells of other lineages were associated closely with both stromal reticular cells and macrophages. The labeled cells in some cases displayed a wavy indented nuclear profile (Fig 5b) but otherwise were of undifferentiated morphology. The extensive electron-lucent processes of stromal reticular cells were in many cases in direct apposition to a considerable portion of the B220+ cell surface profile (Fig 5b). Large macrophages characterized by copious cytoplasm and conspicuous organelles were prominent in the BM of scid mice. Some labeled B220+ cells were closely associated with extensive cytoplasmic processes of the macrophages exhibiting inclusion bodies, that ranged widely in size, shape, and electron density (Fig 5c). The B220+ cells in some cases showed increased nuclear chromatin condensation and cytoplasmic electron density.

In the more central parts of the BM, some highly labeled B220+ cells were located immediately outside the tenuous wall of sinusoids, in contact with the thin layer of adventitial reticular cells and processes partially covering the endothelial lining. EM confirmed the occasional presence of a highly labeled B220+ cell in the lumen of a sinusoid (Fig 5d). The B220+ cells located in or adjacent to the sinusoids appeared to be smaller and to have a more condensed nuclear pattern and electron-dense cytoplasm with more numerous vacuoles than those located elsewhere. One B220+ cell in a sinusoidal lumen was noted to be attached to the sinusoid wall by processes that appeared to penetrate from the extravascular space.

**DISCUSSION**

We used scid mice to examine properties of early B-lymphocyte precursor cells in BM. Our results demonstrate that early and intermediate pro-B cell populations expressing TdT persist in scid mice and continue to proliferate actively, although with no compensatory hyperplasia. Many of these early precursor B cells are located among layers of cells that line the surrounding bone, associated with stromal reticular cells and adipocytes, often immersed in a prominent extracellular matrix. The developing B-lineage cells abort early in the late pro-B cell stage, a process that in the intact BM appears to be mediated by resident macrophages.

Precursor B cells in mouse BM pass through a sequence of phenotypically identifiable differentiation stages from the rearrangement of heavy chains on. Our findings indicate that precursor B cells in scid mice can develop through the stages of Vn B gene rearrangement and for a short time thereafter. The absence of cυ- pre-B cells and sy- B lymphocytes in the BM of scid mice has been a universal finding. The incidence of B220+ cells, at first undetected, has been variously estimated by flow cytometry to range from less than 1% to 4% of BM cells. The present work confirms the higher value; it also shows the presence of TdT+ cells and demonstrates that most of the B220+ cells are actually the earliest B220+ cells that coexpress TdT.

Pro-B cells are normally in a state of proliferative cell cycle, undergoing at least one mitosis at each of the three stages. We showed that these populations are in a proliferative state in scid mice, passing into mitosis with time. Thus, the cells still appear to be moving along their differentiation pathway rather than being in a static state of developmental arrest. This is consistent with reports that few B-cell clones can emerge with age, resulting possibly from low-efficiency VDJ recombination activity or a reversion of the scid mutation. The data suggest that after attempting to undergo VDJ recombination while dividing as TdT+ cells, the developing pro-B cells generally survive no more than one further mitotic cell cycle. This interpretation is supported by cell size distribution profiles. The profiles of the TdT+ and intermediate pro-B cells in scid mice resemble those of normal mice. In contrast, the B220+ cells present in scid mice are unusual in that they include many medium-sized cells instead of the normal predominance of large cycling cells. With rare exceptions, the developing B cells in scid mice thus appear to abort soon after they enter the late pro-B cell stage.

The mechanisms leading to pro-B cell abortion in scid mice appear to be related to the DNA lesions and chromosome breaks resulting from failure to recombine chromo-
some ends bearing V, D, or J coding elements.\textsuperscript{19-24} Introduction of a rearranged $\mu$ gene into the genome of scid mice (\(\mu\)-transgenic scid mice) enables pro-B cells to synthesize $\mu$ and to develop successfully into pre-B cells.\textsuperscript{42} Cell loss in normal B-cell genesis, estimated to be 75% in mice\textsuperscript{1,13,15} and 60% in rats,\textsuperscript{12} although it occurs mainly at later stages of differentiation than in the scid mouse, has also been attributed in part to nonproductive Ig gene rearrangements.\textsuperscript{44} That such genetic errors alone are always sufficient to be lethal to the cells is not clear, however, BM of scid mice can establish in vitro cultures in which lymphoid cells proliferate despite having defective Ig gene rearrangements and being unable to express $\mu$ chains.\textsuperscript{19-24} Additional mechanisms in the BM microenvironment in vivo must normally delete the aberrant cells.

The present work implicates macrophages in the deletion process. Many B220\textsuperscript{+} cells in scid mouse BM are intimately associated with macrophages. The B220\textsuperscript{+} cells in some cases show nuclear condensation and cytoplasmic electron density characteristic of cells undergoing programmed cell death (apoptosis). The macrophages contain inclusion bodies, consistent with being phagolysosomes derived from multiple ingested cells. Similar though less marked appearances have been noted in normal BM,\textsuperscript{37} suggesting that differentiating B-precursor cells encounter the processes of resident macrophages that can recognize and rapidly ingest defective cells. This hypothesis has been supported by evidence of enhanced macrophage activity in anti-IgM-treated mice in which all B cells are deleted by ligation of surface IgM on newly formed B cells, a model of clonal deletion.\textsuperscript{43} The scid mouse provides a system for further studies of the mechanisms by which aberrant or undesirable cells are culled from the B lineage, safeguarding against entry of potentially dysregulated cells into the peripheral B-cell pool.

Although both local and systemic factors can influence proliferation of B-precursor cells,\textsuperscript{40,45} it is not clear how the homeostatic level of B-lymphocyte production is maintained in vivo. Renewal of some other cell lineages, notably erythropoiesis, can be regulated by humoral intermediaries by negative feedback controls from the end cell pool. In contrast, deletion of B lymphocytes in anti-IgM-treated mice produces no compensatory increase in either lymphocyte production\textsuperscript{45} or pre-B cell proliferation\textsuperscript{46} in the BM. The findings in scid mice now extend these observations to control of pro-B cells which, despite the absence of B cells and pre-B cells, show no increase in proliferation above control levels. Thus, in vivo proliferation of early B precursors appears to be remarkably independent of the size of the peripheral B-cell pool or the level of circulating immunoglobulins, emphasizing the importance of intrinsic and microenvironmental controls.

The scid mouse provides a model to study the microenvironmental organization of early precursor B cells by the binding of radiolabeled MoAb 14.8 in vivo. In scid mice, MoAb 14.8-binding shows a narrow "window" of B-lineage development restricted mainly to B220\textsuperscript{+} pro-B cells that coexpress TdT. Many such pro-B cells of undifferentiated morphology in scid BM are located close to the surrounding bone among the layers of endosteal cells. In normal BM, proliferating B220\textsuperscript{+} precursor cells tend to be concentrated in the outer areas.\textsuperscript{35,37} The B220\textsuperscript{+} cells that first appear during postirradiation regeneration are also intimately associated with bone-lining cells.\textsuperscript{36} Curettage of bone-lining cells yields enriched populations of early B220\textsuperscript{+} and TdT\textsuperscript{+} cells.\textsuperscript{38} In rat BM, immunofluorescence labeling of frozen sections has shown numerous TdT\textsuperscript{+} cells in the outer zones.\textsuperscript{51} The present observations strengthen the view that early B-cell differentiation occurs near the marrow–bone interface, a region reported to contain other hematopoietic progenitor cells.\textsuperscript{32}

Although the properties of the subosteal microenvironment are largely unknown, our findings show two of its distinctive features also observed in both normal\textsuperscript{39} and regenerating\textsuperscript{40} BM, ie, close associations between B220\textsuperscript{+} cells and stromal reticular cells and a prominent electron-dense extracellular matrix. The extensive membrane contact noted between pro-B cells and reticular cells demonstrates that the adherence of B lineage cells to certain stromal cells in BM cultures\textsuperscript{51} has a counterpart in intact BM. Sustained B cell genesis in vitro requires close interaction with stromal cells and mediation of stromal cell-derived soluble growth factors.\textsuperscript{46,50} Pro-B cells in vivo thus may also be subject to control by short-range growth factors produced by subosteal reticular cells. Many cloned BM stromal cells capable of supporting B-cell genesis in cultures are preadipocytes which develop into mature fat cells in vivo.\textsuperscript{46,53} Well-developed adipocytes are unusually prominent in peripheral regions of the BM in scid mice. The frequent close proximity of labeled pro-B cells to adipocytes raises the possibility of a functional relation between them in intact BM. The heterogeneity of subosteal stromal cells and the nature of local growth factors, receptors, and cell adhesion molecules require further in vivo study.

The present findings raise the possibility that some early B-lineage cells in scid mice may be able to leave the BM and migrate to the spleen. A few MoAb 14.8-binding cells are located in the lumen of BM venous sinusoids, and others are apparently about to traverse the sinusoidal wall from the extravascular space into the bloodstream. The spleen is...
devoid of μ-bearing pre-B cells and B cells but contains some MoAb 14.8-binding cells lacking μ chains. In normal mouse spleen, MoAb 14.8-binding μ-negative cells have been identified as a small subset of T cells. In scid mice, lacking both B and T cells, some MoAb 14.8-binding μ-negative cells in the spleen also may represent non-B, non-T lymphoid cells derived from the BM. Such “null” cells, which could include regulatory and natural killer cells, may not be impaired by the scid defect.

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