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Pax5-Deficient Mice Exhibit Early Onset Osteopenia with Increased Osteoclast Progenitors

Mark C. Horowitz,‡* Yougen Xi,* David L. Pfugh,† David G. T. Hesslein,† David G. Schatz,¶§ Joseph A. Lorenzo,¶ and Alfred L. M. Bothwell†

Pax5 encodes BSAP, a member of the paired box domain transcription factors, whose expression is restricted to B lymphocyte lineage cells. Pax5−/− mice have a developmental arrest of the B cell lineage at the pre-B cell stage. We show here that Pax5−/− mice are severely osteopenic, missing 60% of their bone mass. The osteopenia can be accounted for by a >100% increase in the number of osteoclasts in bone measured histomorphometrically. This is not due to a lack of B cells, because other strains of B cell-deficient mice do not exhibit this phenotype. There was no difference in the number of osteoclasts produced in vitro by wild-type and Pax5−/− bone marrow cells. In contrast, spleen cells from Pax5−/− mice produce as much as five times the number of osteoclasts as control spleen cells. Culture of Pax5−/− spleen cells yields a population of adherent cells that grow spontaneously in culture without added growth factors for >4 wk. These cells have a monocyte phenotype, produce large numbers of osteoclasts when induced in vitro, and therefore are highly enriched in osteoclast precursors. These data demonstrate a previously unsuspected connection between B cell and osteoclast development and a key role for Pax5 in the control of osteoclast development. The Journal of Immunology, 2004, 173: 6583–6591.

osteoclasts (OC) are large, multinucleated, tartrate-resistant acid phosphatase (TRAP)-positive cells that are responsible for bone resorption. OC progenitors, like other hemopoietic cells found in bone marrow (BM) arise from hemopoietic stem cells and differentiate into the immediate precursor of functional OC. Activation of these cells with receptor activator of NF-κB ligand (RANKL) and M-CSF results in terminal differentiation to form mature multinucleated, bone-resorbing OC (1, 2).

Hemopoietic cell differentiation is marked by discrete stages that can be identified by the appearance of specific transcription factors and cell surface markers. Although many transcription factors have been identified as critical for the development of other hemopoietic lineages, only a small number are known to regulate OC differentiation.

Pax5 is a member of the multigene family that encodes the paired box transcription factors. This highly conserved motif was originally identified in Drosophila and presently nine paired box-containing genes (Pax1–Pax9) have been identified in mammals (3). The transcription factors encoded by the Pax genes recognize their target genes via the DNA binding function of the 128-aa paired domain (4). These genes are involved in regulation of pattern formation and morphogenesis and are expressed in distinct spatially and temporally restricted patterns during morphogenesis. For example, Pax1 is mutated in different forms of undulated, which results in skeletal changes in the vertebrae and Pax6 mutations result in Small eye, which fail to develop eyes and nose (5, 6). The Pax5 gene codes for the transcription factor B cell lineage-specific activation factor (BSAP), the mammalian homologue of the sea urchin protein TSAP (tissue-specific activation protein) (7). During embryogenesis Pax5 is expressed in the mesencephalon and spinal cord in a pattern different from other Pax genes (7). Later in development, expression moves to the fetal liver, where it correlated with the onset of B lymphopoiesis (7, 8). In the hemopoietic system, Pax5 is expressed exclusively in the B lymphocyte lineage extending from the B220− pro-B cells to mature B cells but not in terminally differentiated plasma cells (8). Testis is the only other tissue in the adult mouse known to express Pax5 (7).

When the Pax5 gene was deleted in mice, the most striking result, in addition to early postnatal lethality, was the loss of all B cell development beyond the early pro-B cell stage (8). B220− pro-B cells isolated from Pax5−/− BM can be grown in vitro on stromal/osteoblast (OB) feeder cells in the presence of IL-7, which maintains their undifferentiated phenotype (9). With removal of IL-7 and stimulation with the appropriate cytokines, these cells can differentiate into multiple hemopoietic lineages (9). Treatment of the Pax5−/− pro-B cells with M-CSF and RANKL induced the formation of TRAP+ multinucleated osteoclast-like cells (OCL), raising the possibility that Pax5 is involved in the regulation of OC development (9). These data and the severe running of the homozygous mutant mice prompted us to examine Pax5−/− mice in vivo to determine whether they had a bone phenotype.

We report here that Pax5−/− mice are severely osteopenic, missing ≥60% of their bone mass. This is the result of a 3- to 5-fold increase in the number of OC in bone while the number of...
OB in Pax5−/− mice is indistinguishable from wild-type (WT) controls. In vitro, spleen cells from Pax5−/− mice are enriched in OC precursors. A subpopulation of Pax5−/− spleen cells can be grown in vitro in the absence of added growth factors, have a monocyte/macrophage phenotype, and are highly enriched in OC precursors. The effects of Pax5 on bone turnover appear to be mediated by an indirect mechanism, as Pax5 is not expressed in bone cells. These data identify, for the first time, Pax5 as a transcription factor required for the normal regulation of OC development. In addition, Pax5−/− mice represent a novel system in which to study OC differentiation.

Materials and Methods
Mice
The Pax5−/− mice on the C57BL/6 background were obtained from Meinrad Busslinger (Research Institute of Molecular Pathology, Vienna, Austria). Pax5−/− mice were derived from matings of heterozygous mice as previously described (8). Heterozygous and homozygous WT age-matched littermates served as controls. Rag-1−/− mice (C57BL/6 background) were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred as homozygotes. WT mice using a modification of the technique of Robey and Termine (13). Calvarial cells (5×10⁴/well) were cultured in 96-well plates in 200 μl of medium for prescribed times. The CellTiter 96 Aqueous One Solution Proliferation Assay (Promega, Madison, WI) was used to determine cellular proliferation. In brief, this is a colorimetric method for determining the number of viable proliferating cells. A novel tetrazolium compound is bioreduced by cells into a colored formazan product that is soluble in tissue culture medium. Assays were performed by adding a small amount of the reagents directly to the culture wells, incubating for 4 h, and recording absorbance at 490 nm using a plate reader (16, 17).

Pit assay for bone resorption
Spleen cells (8×10⁴−10⁵) were overlayed on bovine cortical bone slices and M-CSF and RANKL were added to induce OC formation. Cells were cultured for 4–6 days, fixed with 2.5% glutaraldehyde, stained with TRAP. After counting, the cells were removed by sonication in 0.25 M NH₄OH. The slices were stained with 1% toluidine blue and the number of pits was counted using light microscopy (18, 19).

Flow cytometry
Cultured spleen cells were removed from dishes with trypsin/EDTA and washed with PBS containing 2% FCS. Staining was performed in PBS with 5% rat serum. Anti-cfms/M-CSFR/CD115 (AF598-1) was purified from hybridoma culture supernatants and biotinylated. Other Abs were purchased from BD Pharmingen (San Diego, CA).

ALP and osteocalcin assay
ALP and osteocalcin production was measured in OC cell lysates and conditioned medium, respectively. ALP activity was determined by substrate conversion in lysates prepared from preconfluent, confluent, and 1- and 2-wk postconfluent cultures. Using the same time points, osteocalcin levels in the CM were measured by a standard equilibrium radioimmunoassay using specific goat anti-mouse osteocalcin Ab (20).

RNA extraction, Northern blot analysis, and PCR
RNA was isolated from cells using TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY). Northern blot analysis using 10 μg of total RNA was done as previously described (21). CDNA probes were labeled with 32PdATP and 32PdCTP by random hexamer extension. Total RNA was extracted from the cells with TRI-reagent (Molecular Research Center, Cincinnati, OH) (22). Total RNA was converted to CDNA by reverse transcriptase (Superscript II; Invitrogen Life Technologies) and random hexamer and aliquots of reverse transcriptase mixture were used for PCR. PCR amplification was done using gene-specific PCR primers and Taq polymerase (Ampliquest; PerkinElmer, Norwalk, CT). To verify that the amplification rate was in the linear range, PCR amplification was performed in eight different cycles with samples from bone marrow cell cultures (23). Specific amplifier sets were designed from published CDNA sequences for murine Pax5 (antisense, 5'-CATGTAGGCGCATGGAGCT 3', shared with 2.5% TGAATGAAGC and shared with 3'), sense, 5'-TGAAGGTGCGTTGACGTTTGCG-3') and murine GAPDH (23). The amplified products were run in a 1.5% agarose gel, stained with ethidium bromide, and photographed under UV illumination. Images were captured by a FOTO/Analyst Archiver Electronic Documentation System (Fotodyne; Hartland WI) and OD was determined using a digital image processing and analysis program (Scion Image; Scion, Frederick, MD). The identity of the amplified PCR products was confirmed by direct sequencing using an automatic DNA sequencer (Applied Biosystems, Norwalk, CT) (23).

Results

Mouse development, histology, and histomorphometry
At birth and until 7–9 days of age, the Pax5−/− pups are indistinguishable from their WT littersmates in physical appearance. From that point on, they lagged behind their control siblings in size and growth. At 15 days (Fig. 1A), the Pax5−/− mice were severely runted, being approximately two-thirds the length, while body weight was reduced by 50% relative to both heterozygous and WT controls. Few Pax5−/− mice survived past 18 days of age, although the cause of death remains unknown. The Pax5 mutant mice had normal tooth development and their eyes were normal, suggesting functional OC were present (M. C. Horowitz, unpublished data). Long bones from mutant mice, while smaller than...
FIGURE 1. Physical and histological assessment of mutant and WT long bones. A. Comparison of 15-day-old WT and Pax5<sup>-/-</sup> mice. Toluidine blue-stained proximal tibiae from 15-day-old Pax5<sup>-/-</sup> mice (C and E) and age-matched WT littermate control mice (B and D). Pax5<sup>-/-</sup> tibiae (C) show the striking loss of trabecular BV and thickness characteristic of Pax5 deficiency (original magnification, ×25); Pax5<sup>-/-</sup> bones show the large increase in the number of OC (arrows) on bone surfaces (original magnification, ×50 magnification); C57BL/6 littermate control (F), and Pax5<sup>-/-</sup> tibia (G) stained with safranin O show the delayed development of the secondary center of ossification in Pax5<sup>-/-</sup> mice characterized by the presence of chondrocytes with little bone. The articular cartilage is thinned and note the flattened appearance of the structure (original magnification, ×25).
those of WT controls, had appropriate structure and morphology. With the exception of size, no other visible skeletal changes were observed.

To evaluate bone remodeling, the femora and tibiae from 15-day-old Pax5−/− mice were processed for histomorphometric analysis and evaluated by light microscopy. Age- and sex-matched littermates (heterozygous and homozygous C57BL/6 WT) were used as controls. Histomorphometric measurements were performed on a fixed region just below the growth plate, corresponding to the primary spongiosa (11). In control animals, the primary spongiosa contained numerous spicules extending distally from the growth plate (Fig. 1B). These spicules formed the finger-like projections of the trabeculae and the resulting bone architecture was well organized. Individual trabeculae were covered with osteoid as would be anticipated for this period of highly active bone formation.

The bones of homozygous mutant mice exhibited profound differences. The most striking feature was the dramatic osteopenia (Fig. 1C). Overall, bone volume was reduced by 60% compared with WT controls (BV/TV) and osteoid volume was reduced by 55% (OV/TV) (Table I). Trabecular thickness was reduced by 17% (TbTh), the number of individual trabeculae was reduced by 51% (TbN), and the space between trabeculae, another indicator of bone resorption, was increased by 57% (TbSp) in Pax5−/− mice (Table I). Observed increases in bone resorption was likely due, at least in part, to the >100% increase in the number of OC (OcS/BS) in Pax5−/− bone (Table I). Numerous OC were observed attached to bone spicules (Fig. 1E, arrows) as compared with controls (Fig. 1D). In addition, the number of OC/bone perimeter was increased 88% in mutant vs control mice (data not shown). These data indicate not only a marked increase in the number of OC but also that the cells were functional.

Histomorphometric analysis also indicates that the number of OB in mutant and WT mice was similar (ObS/BS). Because of the age of the mice, the cortical bone had not yet compacted although it did appear to be thinned in the mutants. The BM from Pax5−/− mice had normal cellularity with no fibrosis. These data, taken as a whole, indicate markedly decreased trabecular bone due to the large increase in OC rather than a loss of OB.

In control animals, the growth plates were robust as would be expected during this early stage of life, with orderly columns of chondrocytes (Fig. 1F). In comparison, the columns of chondrocytes appear to be compressed in the Pax5−/− mice. Examination of the secondary center of ossification shows little bone formation with numerous chondrocytes in mutant mice (Fig. 1G). The Pax5−/− mice also lack a seam of bone separating the growth plate from the secondary center. The articular cartilage in the Pax5−/− mice is thinned and the entire structure is flattened. These data suggest a delay in development which, coupled with the increased OC number, would account for the runting.

### Table I. Histomorphometry measurements

<table>
<thead>
<tr>
<th>Mice</th>
<th>No. of Mice</th>
<th>BV/TV</th>
<th>OV/TV</th>
<th>ObS/BS</th>
<th>OcS/BS</th>
<th>TbTh</th>
<th>TbSp</th>
<th>TbN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pax5</td>
<td>6</td>
<td>5 ± 1b</td>
<td>1 ± 0.3</td>
<td>26 ± 7</td>
<td>9 ± 1b</td>
<td>14 ± 1</td>
<td>273 ± 38b</td>
<td>4 ± 1b</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>14 ± 1</td>
<td>2 ± 0.1</td>
<td>33 ± 1</td>
<td>4 ± 0.3</td>
<td>18 ± 1</td>
<td>118 ± 13</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Rag-1</td>
<td>3</td>
<td>18 ± 4</td>
<td>2 ± 1</td>
<td>25 ± 7</td>
<td>3 ± 1</td>
<td>29 ± 5</td>
<td>136 ± 12</td>
<td>6 ± 0.3</td>
</tr>
<tr>
<td>μMT</td>
<td>5</td>
<td>13 ± 1</td>
<td>1 ± 0.1</td>
<td>14 ± 3</td>
<td>4 ± 0.7</td>
<td>27 ± 2</td>
<td>177 ± 10</td>
<td>5 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>12 ± 1</td>
<td>1 ± 0.1</td>
<td>24 ± 4</td>
<td>3 ± 1</td>
<td>24 ± 2</td>
<td>155 ± 11</td>
<td>5 ± 0.3</td>
</tr>
</tbody>
</table>

a Results are presented as mean ± SEM.
b At least p > 0.05 compared to WT.

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**Pax5 expression**

Although Pax5 expression has been reported to be highly restricted to the B cell lineage, no data are available on its expression in bone cells. To determine whether Pax5 was expressed in bone cells, calvarial OB or long bone cells were grown to confluence, total RNA was collected, and Pax5 expression was determined by Northern blot analysis. RNA from a transformed pro-B cell line was used as a positive control (24). No Pax5 expression was observed in either cell population, while a characteristic 7.3-kb band was seen in the B cell control (Fig. 2). The failure of these cells to express Pax5 was confirmed by RT-PCR (data not shown).

OC were generated in vitro by culturing WT bone marrow cells with RANKL and M-CSF. After differential trypsinization to enrich for OC, total RNA was collected, and Pax5 expression was determined by RT-PCR. Pax5 was undetectable in authentic OC (data not shown). These data suggest that Pax5 is not expressed in mature OB, their immediate precursors, or in mature OC.

### OB function

Although the number of OB in Pax5 and control bone was similar, it was possible their functional activity was reduced, contributing to the decreased bone mass. To examine this possibility, the proliferative and ALP response was measured over time. Both the proliferative response (Fig. 3A) and the production of ALP (Fig. 3B) by calvarial OB from Pax5−/−, Pax5+/−, and WT mice were similar at all time points. These data suggest that Pax5−/− OB function normally.

To assess OB differentiation, the number of CFU-F, which is a measure of all mesenchymal cell precursors, and CFU-OB, which is a measure of OB precursor, was assessed. CFU-OB was determined as the number of ALP+ staining colonies while CFU-F was measured as the number of methyl violet-positive staining colonies at 7 and 10 days of culture. BM from Pax5−/− mice consistently contained 5–10 times the number of both colony types than did cultures of cells from littermate controls (Fig. 3C). In a typical experiment at 7 days of culture, control BM had 8 (average of 4 replicate wells) ALP+ colonies while Pax5−/− BM had 40 ALP+ colonies. At day 10, controls had 10 ALP+ colonies while Pax5−/− had confluent monolayers of ALP+ colonies.

To assess mineralization, BM cells were cultured for 14 or 28 days in mineralizing medium (ascorbate plus β-glycerol phosphate). Similar to the ALP data, Pax5−/− BM cells produced 5–10 times the amount of Von Kossa staining (specific for mineralized phosphate) than did cells from controls (data not shown). Taken as a whole, these data suggest that Pax5 deficiency results in increased numbers of OB precursors.
**FIGURE 2.** Lack of Pax5 expression in WT calvarial OB and long bone cells. An Abelson murine leukemia virus-transformed mouse pro-B cell line (63-12) derived from Rag-2-/- mice was used as a positive control. Northern blot analysis of total RNA (10 μg/lane) shows the characteristic 7.4-kb band for Pax5 (left panel) in the 63-12 cells with no expression in the OB or long bone cells. The panels below show the ethidium bromide loading control.

**FIGURE 3.** OB proliferation, ALP, and colony formation in Pax5-/- mice. Calvarial OB were grown in culture for different periods of time and their proliferative and ALP response was determined. Both the proliferative response (A, measured by MTT in triplicate) and the production of ALP (B) by calvarial cells were similar in Pax5-/-, +/-, and +/+ mice. C, BM from Pax5-/- mice produced as much as 10 times the number of ALP+ (CFU-OB) than WT BM.

**OC function**

One explanation for the histomorphometric data was that the Pax5-/- mice had increased numbers of OC precursors. To test this possibility, BM or spleen cells from Pax5-/- and WT controls were cultured with M-CSF and RANKL for 7–10 days, the cultures were then fixed and stained for TRAP, and the number of OC was counted. WT BM cells produced approximately equivalent amounts of OC as Pax5-/- BM cells. In contrast, mutant spleen cells generated as much as five times more OC than control spleen cells (Fig. 4A). These data suggest the number of OC precursors was dramatically increased in the spleens of Pax5-/- mice but not in the BM.

The Pax5-/- mouse spleens were substantially smaller than those of controls. The number of spleen cells in 7-day-old mice (19.3 × 10⁶, average of 8 mice) was reduced by 46% compared with those of WT controls (35.1 × 10⁶, average of 8 mice). Fifteen-day-old-mice spleen weights (0.017 g, average of 8 mice vs 0.36 g, average of 6 +/- mice) were reduced by 53% while spleen cell number (11 × 10⁶ vs 50 × 10⁶) was reduced by 78% as compared with WT controls. The low cell counts in the spleens of Pax5-/- mice were due in large part to the lack of B cells in these mice, which constitute ~60% of normal spleen cells.

Because the Pax5-/- spleen contains no mature B cells, this serves to concentrate the remaining cells within the spleen and could account for the observed increase in OC precursors. To examine this possibility directly, T and B cell cells were negatively gated (TCR αβ and CD45R/B220) from WT and Pax5-/- spleen cells and OC precursors (Mac-1 FcγR+) analyzed by flow cytometry. Data in Fig. 4B show that the number of OC precursors in Pax5-/- spleen is still higher by 21% than in WT. Although some of the increase in OC formation may be due to the concentration effect, this does not change the fact that the loss of Pax5 causes an absolute increase in OC precursors in the spleen.

Another possible explanation for the increase in splenic OC precursors is that B cells negatively regulate osteoclastogenesis. To examine this possibility, two animal models of B cell deficiency were examined. B cell development in Rag-1- and Pax5-deficient mice are arrested at a similar stage of differentiation (8, 25). To determine whether Rag-1-/- mice had a bone phenotype that was similar to the Pax5-/- mice, tibia from Rag-1-/- mice were analyzed histomorphometrically. The data (Table I) indicate that Rag-1-/- mice are not osteopenic as compared with age- and sex-matched WT mice. One caveat to these data was that Rag-1-/- mice also lack T cells. Therefore, to further examine whether the loss of bone in Pax5-/- was not due to a loss of mature B cells, we analyzed the bones of μMT H chain mutant mice. These mice lack membrane-bound IgM and therefore are devoid of mature B cells.
The effect appears to be a direct result of lack of B cells does not in itself potentiate OC development; rather, similar to that seen in the

We noticed that culture of

This revealed numerous resorption pits, indicating that OC derived

wells were stained for TRAP while the cells on the chips were

removed by sonication and the chips examined for resorption pits.

wells containing bovine bone chips and cultured with M-CSF and

growth factors of any kind. To determine whether the SCL could

be induced to form OC, cells were treated with M-CSF and

RANKL. The cells were subsequently stained for TRAP and the

number of OC was counted. As can be seen in Fig. 5

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FIGURE 5. Analysis of the Pax5−/− SCL. A, Photomicrograph of SCL after passage 1. Note the thinned, fibroblastic appearance of the majority of cells (original magnification, ×25). Pax5−/− SCL (B) and control cells (C) cultured with RANKL and M-CSF, fixed, and stained for TRAP. Pax5−/− SCL produced many more TRAP+, multinucleated, OCL as compared with controls. The Pax5−/− OCL were much larger in size than control OCL: D, Pax5−/− SCL were cultured (with RANKL and M-CSF) on bovine bone chips for 7 days. Numerous resorption pits can be observed in the bone. E, SCL were cultured with RANKL and M-CSF in the absence or presence of IL-4 (30 μg/ml), IL-10 (30 μg/ml), or Ct (10−11 M). IL-4 completely blocked OC formation while IL-10 and Ct reduced formation by 66 and 90%, respectively. F, SCL were analyzed for expression of cell surface determinants by FACS. All cells were FcγR− Mac-1−, 31% c-fms− and B220−. Mac-1 (y-axis) was the same in all panels.
The Pax5-deficient mouse model is another example of the growing body of evidence that a relationship exists between B cell and OC development. Although both B cells and OC are hematopoietic in origin, B cells arise from the common lymphoid progenitor while OC are believed to arise from the common myeloid progenitor. PU.1 is one of the first in a series of transcription factors, which include E2A, early B cell factor, and Pax5, that function in a specific sequence that is time dependent and are required for B cell development (30). PU.1+/− mice, in addition to having no B cells, also fail to develop OC and macrophages (30). It has not been reported whether early B cell factor-deficient mice have a bone phenotype although they are runted (Ref. 31; D. G. T. Hesslein and M. C. Horowitz, unpublished data).

RANKL-deficient mice have severe osteopetrosis with no OC but normal numbers of macrophages in contrast to osteopetrotic (op/op) mice, which are deficient in both macrophages and OC (32, 33). Interestingly, RANKL−/− mice also have defects in B cell lymphopoiesis with markedly reduced numbers of pre-B cells in their marrow (32). During normal osteoclastogenesis, an early OC progenitor (Mac-1+c-fms+) can be induced to express B220 following culture on a stromal/OB cell line plus IL-7 (34). IL-7 is the major growth factor for B cells and is secreted by stromal cells. Pax5−/− pro-B cells were grown on a similar stromal/OB cell line with IL-7, which was required to maintain the progenitor status of the cells. Only when the IL-7 was removed were the Pax5−/− pro-B cells able to differentiate into other lineages (9). It is likely that the stromal/OB cells provided both M-CSF and RANKL, aiding in the differentiation to OC. IL-7 has different effects on OC development depending on whether it is administered systemically or locally. Systemic treatment of normal mice with IL-7 stimulates B220+ pro-B cell proliferation and osteoclastogenesis (35). Similarly, IL-7-overexpressing mice exhibit expanded numbers of pre-B cells and increased bone resorption (36). Conversely, IL-7R-deficient mice have suppressed B cell development and increased bone mineral density (35). In contrast, IL-7 has inhibitory effects on osteoclastogenesis when given in vitro to BM cultures (37).

BM-derived B220+ pro-B cells express RANKL and supported OC formation better than B220− cells (38). In fact, B220+ cells isolated from normal BM produce authentic OC when cultured with M-CSF and RANKL (39). Ovariectomy caused a 200% increase in the number of OC from the B220+ cells while having no effect on the B220− population (34). These data, taken as a whole, strongly support the idea that a regulatory relationship exists between early B cell and OC development; the apparent plasticity of early B cell and OC progenitors supports the idea that these cells may have a common ancestor, and this relationship appears to be physiologically relevant.

We have been able to identify an adherent monocyte/macrophage-like cell in the spleens of Pax5−/− mice that grows for long periods of time in culture without added growth factors and is highly enriched in OC precursors. This is similar to Pax5−/− pro-B cells, which can also be propagated for long periods of time in vitro, although these cells require stromal cell support and added growth factors (9). One explanation for the unusual growth factor independence of the SCL may be the increased expression of growth factor receptors. This is not unusual in Pax5 deficiency because Pax5−/− pro-B cells express c-fms, which is not seen in Pax5-replete cells (9). Therefore, loss of Pax5 may result in the expression of new growth factor receptors or the increased expression of existing receptors on the SCL. In addition, the SCL may secrete cytokines that are recognized by the up-regulated receptors on the SCL themselves or other OC precursors in a positive feedback mechanism, resulting in the increased number of OC and the associated bone loss. It is important to remember that the regulatory activity of Pax5 must be exercised on bone cells by an unknown indirect mechanism because its expression is restricted to the B cell lineage and cannot be detected in either OB or OC lineages. This suggests that the loss of Pax5 causes the loss of an inhibitory factor/cytokine resulting in the increased expression of growth factor receptors. Alternatively, a direct cell–cell interaction to support cell viability could also account for the cell growth. Conversely, loss of Pax5 may blunt or delay apoptosis.

That the SCL was enriched for OC precursors was consistent with data showing that Pax5−/− splenocytes contain more Mac1+FcyR+ cells. This was in contrast to the BM where the number of OC inducible in vitro was similar in Pax5−/− and WT mice. These data imply that although the BM pro-B cell population may contribute to the overall number of OC, they were not directly responsible for the large increase in bone resorption and OC on the bones of Pax5−/− mice. One explanation for the data is that the Pax5−/− pro-B cells, which are known to be progenitors of multiple lineages including OC, migrate to the spleen where they differentiate into OC precursors, develop the SCL phenotype, and produce the large increase in OC precursors found in this organ. This expanded population may then migrate back to the bone, but not to the BM and account for the increased number of OC found in the bones of Pax5−/− mice. It is also possible that there may be increased OC precursors in BM from direct differentiation of local precursors. However, our finding of no increase in OC formation in BM cultures from Pax5−/− cells compared with controls argues that this hypothesis is incorrect. Pax5−/− pro-B cells could express on their cell surface or secrete a factor(s) (e.g., TNF or IL-1) that induces increased RANKL expression on stromal/OB. This in turn could also induce more OC formation in the bone.

Alternatively, the pro-B cells and the SCL could be unrelated developmentally and arise independently due to the Pax5 deficiency. SCL cells could still migrate from the spleen to the bone and once in the bone microenvironment differentiate to mature OC and possibly induce the differentiation of resident OC precursors to bone-resorbing OC.

It has been shown that, with the exception of the B cell lineage, other hematopoietic lineages develop normally in Pax5-deficient mice (9). Our data demonstrate that the development of the OC lineage is dramatically increased in these mice, in distinction to the B cell lineage. Pax5−/− mice are a novel system to study both OC lineage development and the relationship between B cells and OC. Our results identify Pax5 expression as a prerequisite for normal OC development and consequently bone homeostasis.

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References


