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IL-2 receptor α chain (CD25,TAC) expression defines a crucial stage in pre-B cell development

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Abstract

The analysis of the expression of the α chain of the IL-2 receptor (CD25,TAC) on the surface of B lineage cells in mouse bone marrow reveals that it is a useful marker to distinguish pre-B-I from pre-B-II cells. CD25 is not expressed on CD45R(B220)$^+$ c-*kit*$^+$ CD43$^+$ TdT$^+$ λ_5^+ cμ^- sIg$^-$ IgH chain locus DJ_H-rearranged pre-B-I cells of mouse bone marrow. It is expressed on large cycling CD45R(B220)$^+$ c-*kit*$^-$ CD43$^+$ TdT$^-$ λ_5^+ cμ^+ sIg$^-$ and on small resting CD45R(B220)$^+$ c-*kit*$^-$ CD43$^-$ TdT$^-$ λ_5^- cμ^- sIg$^-$ IgH chain locus V_HDJ_H-rearranged pre-B-II cells. Therefore, the transition from pre-B-I to large pre-B-II cells is marked by the downregulation of c-*kit* and terminal deoxynucleotidyl transferase (TdT), and by the upregulation of CD25. SCID, RAG-2T, μMT and λ_5T mutant mice do have normal, if not elevated numbers of pre-B-I cells but lack all CD25$^+$ pre-B-II cells in their bone marrow. The expression of a transgenic H chain under control of the μH chain enhancer in RAG-2T bone marrow B lineage precursors allows the development of large and small CD25$^+$ pre-B-II cells. The results suggest that the differentiation of pre-B-I to pre-B-II cells in mouse bone marrow requires the expression of μH chains and surrogate L chains in membranes, probably on the surface of precursor B cells.

Introduction

B cell development in mouse bone marrow has been dissected into different stages of cycling, large and non-cycling, small cell populations which differentially express a variety of cell surface and intracellular markers. Furthermore these different cell populations either proliferate on stromal cells in the presence of IL-7, on IL-7 alone or not at all, and can or cannot populate B cell lineage compartments in severe combined immunodeficient (SCID) mice. Osmond (1) defined early, intermediate and late pro-B cells, and large and small pre-B cells by the differential expression of the common leukocyte antigen CD45R(B220) and IgM on the surface, and by the expression of terminal deoxynucleotidyl transferase (TdT) and μH chain in the cytoplasm (cμ). Measurements of the incidence, the size, proliferation capacity, turnover and production rates of these precursor B cell compartments complemented the analyses. Hardy *et al.* (2) distinguished fractions A – F by the differential expression of cell surface CD45R(B220), CD43, heat stable antigen, BP-1, IgM and IgD. These subpopulations were further characterized by their responsiveness to IL-7 and stromal cells *in vitro*, previously defined as differential properties of precursor B cells by Nishikawa *et al.* (3,4). Furthermore, the DNA of these subpopulations was analyzed by polymerase chain reactions (PCR) for the rearrangement status of H and L chain genes. These analyses were completed in a second publication (5) by reverse transcription polymerase chain reaction (RT-PCR) analyses of the expression levels of the genes encoding TdT, myosin-like light chain (6), λ_5 and $V_{pre\text{-}B}$ (7,8), as well as RAG-1 and RAG-2 (9,10).

We have previously distinguished stromal cell/IL-7-reactive, transplantable pre-B-I cells (11) which express c-*kit* (12) and surrogate L chain on the surface (13), from pre-B-II cells which have lost the capacity for long-term proliferation on stromal cells in the presence of IL-7 and which no longer express c-*kit* and surrogate L chain on the surface. Approximately 20% of all pre-B-II cells are cycling, while the remaining cells are resting and a very large proportion of them express cμ (14,15). A part of the cycling pre-B-II cells, but none of the resting pre-B-II cells, express surrogate L chain in the cytoplasm.

Mutant mice with defects in B lymphocyte development have

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been found [SCID/SCID (16)], or have been generated by targeted disruption of the RAG-1 or the RAG-2 gene (17,18), the transmembrane portion of the μH chain gene (19) or the λ_5 gene (20). Previous analyses have shown that all these mutant mice have normal, if not elevated numbers of pre-B-I cells but contain $<2.5\%$ of the normal number of pre-B-II cells in their bone marrow (18,19,21 – 24). As a consequence of all these analyses, two pathways of B cell development have been proposed (22,24). The decisive point of transition into the major pathway of development, defective in the mutant mice, is proposed to occur at the boundary of pre-B-I to pre-B-II cells.

In this paper we first analyze the pre-B-I and pre-B-II cell populations of normal bone marrow for the expression of the markers used by Osmond, by Hardy *et al.* and by ourselves. We then employ a monoclonal antibody specific for the α chain of the mouse IL-2 receptor [CD25(TAC)] to distinguish pre-B-I cells [which do not express CD25(TAC)] from large and small pre-B-II cells (all of which express it). Finally, the analysis for CD25(TAC) expression of B lineage cells in bone marrow of the mutant mice shows that $<1\%$ of all precursors express CD25(TAC) and, thus, defines the defect in B cell development in the four types of mutants as an inability of precursors to undergo transition from the pre-B-I to the large, cycling pre-B-II compartment. The results are discussed in view of possible roles of surrogate L chains in association either with a complex or early proteins or with μH chains.

Methods

Mice

Female BALB/c, DBA/2J, C3H/HeJ, CBA/J and C57Bl/6J mice (4 – 5 weeks old) were purchased from Biological Research Laboratories (Füllinsdorf, Switzerland). CB17 SCID mice were originally obtained from M. Bosma (Institute for Cancer Research, Fox Chase, Philadelphia, PA), RAG-2T mice were originally obtained from F. Alt (The Children's Hospital, Boston, MA), μMT mice were originally obtained from K. Rajewsky (Institute für Genetik, Cologne, Germany) and λ_5 T mice were bred in our own facilities. Mice carrying the Sp6 μH transgene (25) were kindly provided by A. Iglesias (Max-Planck-Institüt für Psychiatrie, Martinsried, Germany). These transgenic mice were crossed with RAG-2T mice. Mice expressing the μH transgene were then backcrossed to RAG-2T mice to obtain homozygous RAG-2T mice expressing a μH transgene.

Antibodies

The FITC-, phycoerythrin (PE)- and allophycocyanin-conjugated mAb RA3 6B2 (anti-CD45R,B220), the FITC- and biotin-conjugated mAb 7D4 [anti-CD25(TAC)] and the FITC-conjugated mAb 6C3 (anti-BP-1) were all obtained from Pharmingen (San Diego, CA). The mAbs ACK_4 (anti-c-*kit*) (26), S7 (anti-CD43) (2) and LM34 (anti-λ_5) (13) were purified from culture supernatants on Protein G – Sepharose columns (Pharmacia, Uppsala, Sweden) as recommended by the supplier. Purified mAbs were conjugated with FITC, PE or biotin according to standard protocols. FITC-conjugated goat anti-mouse IgM was purchased from Southern Biotechnology Associates (Birmingham, AL) and FITC-conjugated goat anti-rabbit IgG was obtained from Jackson ImmunoResearch Laboratories (Milan Analytica AG, La Roche, Switzerland). The rabbit anti-mouse TdT antiserum was a kind gift of D. Mathis (CNRS, l'INSERM Institut de Chimie Biologique, Strasbourg, France). Streptavidin – PE was obtained from Southern Biotechnology Associates, streptavidin – Tri-Color was obtained from Caltag (San Francisco, CA) and streptavidin-RED 613 was obtained from Gibco BRL (Gaithersburg, MD).

Cell surface staining and flow cytometric analysis

Bone marrow cells were depleted of $sIgM^+$ B cells by the use of sheep anti-mouse Ig-conjugated Dynabeads (Dynal AS, Skøyen, Norway) as recommended by the supplier. Depletion was tested by flow cytometric analysis and revealed always $<1\%$ contamination of $sIgM^+$ B cells in the bone marrow cell preparations.

Cell surface staining was performed as described (23). Cell sorting was performed using the FACStar-plus (Becton-Dickinson, Mountain View, CA). Gates for sorting were set by forward and side scatter on either total, small or large nucleated bone marrow cells.

Intracellular staining

For testing cμ and cλ_5 protein expression cells were fixed for 10 min in 4% paraformaldehyde in PBS on ice and then permeabilized with 0.2% Tween-20 in PBS for 20 min at 37°C. For detecting cμ expression, cells were then incubated with FITC-conjugated goat anti-mouse IgM (Southern Biotechnology Associates) while cλ_5 expression was visualized by incubating the cells first with biotin-conjugated LM34 followed by streptavidin – PE or streptavidin – Tri-Color. Stained cells were analyzed by FACScan. For determining TdT expression cells were fixed for 5 min on ice with 1% paraformaldehyde and then permeabilized with 70% methanol on ice for 30 min. Cells were then incubated with rabbit anti-mouse TdT followed by incubation with FITC-conjugated goat anti-rabbit IgG. Stained cells were analyzed by FACScan.

Cell cycle analysis

Sorted bone marrow cells were fixed in 70% ethanol overnight at 4°C. Fixed cells were treated with 0.5 mg/ml RNase (Boehringer Mannheim, Mannheim, Germany) for 30 min at 37°C and then with 0.5 mg/ml Pepsin (Sigma, St Louis, MO) for 15 min at 37°C to prepare nuclei. Nuclei were subsequently incubated with 10 μg/ml ethidium bromide in 0.1 M Tris (pH 8.0) containing 0.25% (w/v) BSA for 15 min at room temperature. DNA content was analyzed using a FACScan instrument.

Results

Expression of c-kit, CD43 and CD25(TAC) by $CD45R(B220)^+$ $sIgM^-$ B lineage precursors of normal and mutant mouse bone marrow

Previously we have shown that pre-B-I cells which had DJ_H rearranged IgH chain loci and which possess the long-term capacity to proliferate on stromal cells in the presence of IL-7 express CD45R(B220) , c-*kit* and CD43, but not CD25 on their surface (27). Upon *in vitro* differentiation of these pre-B-I cells into more mature B cells by omission of IL-7 they lose c-*kit* and CD43 expression but gain the expression of CD25 on the surface. Therefore, CD25 might be a useful marker to distinguish DJ_H

rearranged pre-B-I cells from V_HDJ_H rearranged pre-B-II cells.

Here we analyzed the expression of c-*kit*, CD43 and CD25 on the surface of CD45R(B220)$^+$ sIgM$^-$ B lineage precursors of normal and mutant mouse bone marrow. In Fig. 1 the two-color FACS analysis of bone marrow depleted of sIgM$^+$ B cells of 4 – 5 week old BALB/c mice is shown. For the CD45R(B220)/c-*kit* and the CD45R(B220)/CD43 expression all nucleated bone marrow cells were analyzed, while CD45R(B220)/CD25 expression was analyzed on large cells (Fig. 1C, see inset) and on small cells (Fig. 1D, see inset). Table 1 summarizes the quantitation of these analyses in five normal inbred strains of mice and five mutant strains of mice for total bone marrow. In the bone marrow of 4 – 5 week old normal mice the percentages of CD45R(B220)$^+$/c-*kit*$^+$ and CD45R(B220)$^+$/CD43$^+$ range from 3 to 5 and from 5 to 8% respectively. Thus, the CD45R(B220)$^+$/CD43$^+$ population is 1.5 – 2 times larger than the CD45R(B220)$^+$/c-*kit*$^+$ population. Between 5 and 7% of the bone marrow cells of these mice are large CD45R (B220)$^+$/CD25$^+$ cells and between 15 and 20% are small CD45R(B220)$^+$/CD25$^+$ cells.

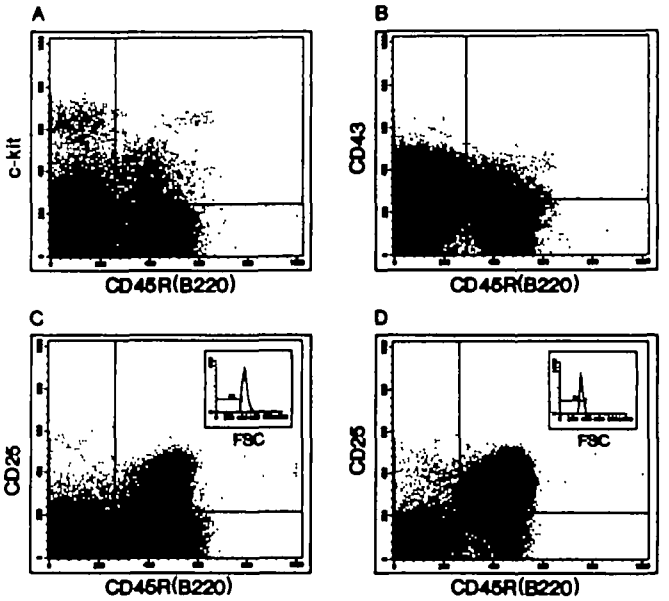

Fig. 1. Dual labeling analysis of sIgM depleted bone marrow cells of 4 week old BALB/c mice for CD45R(B220)/c-*kit* (A), CD45R(B220)/CD43 (B) and CD45R(B220)/CD25(TAC) (C and D). For the expression of CD45R(B220)/c-*kit* and CD45R(B220)/CD43, total nucleated bone marrow cells were analyzed while CD45R(B220)CD25(TAC) expression was analyzed by forward and side scatter gating (see insets) on large (C) and on small cells (D). Percentages of double positive cells are summarized in Table 1.

In the bone marrow of age matched SCID, λ_5T, μMT and RAG-2, the percentages of CD45R(B220)$^+$/c-*kit*$^+$ and of CD45R(B220)$^+$/CD43$^+$ cells are slightly elevated when compared with the percentages found in the bone marrow of normal mice (see Table 1). In marked contrast, CD45R(B220)$^+$/CD25$^+$ large as well as small cells in the bone marrow of these mutant mice are below the FACS detection level. This indicates that both the λ_5 protein and a membrane-bound μ protein are required for the formation of a CD45R(B220)$^+$/CD25$^+$ precursor B cell population. To test this hypothesis RAG-2T mice which are rearrangement incompetent but express normal levels of λ_5 (13,18) were supplemented with a transgenic membrane-bound μ chain. Analysis of the bone marrow of these mice revealed a relatively normal number of CD45R(B220)$^+$/c-*kit*$^+$ and CD45R(B220)$^+$/CD43$^+$ cells when compared with normal inbred strains of mice, but a 2- to 3-fold lower number when compared with normal RAG-2T mice (Table 1). About 3 – 4% of the bone marrow cells of the RAG-2T mice expressing a transgenic membrane-bound μ chain co-express CD45R(B220) and CD25, and are large, and 13 – 14% co-express these markers and are small. Thus, the formation of a CD45R(B220)$^+$/c-*kit*$^+$/CD43$^+$ precursor B cell compartment in the bone marrow is independent of rearrangement and the expression of λ_5, while the formation of a CD45R(B220)$^+$/CD25$^+$ compartment is dependent on the expression of the λ_5 protein and a membrane-bound μ chain.

Expression of c-kit, CD43, CD25, BP-1, TdT, cμ and cλ_5 protein by CD45R(B220)$^+$/c-kit$^+$, CD45R(B220)$^+$/CD43$^+$ and CD45R(B220)$^+$/CD25$^+$ precursor B cells of normal mouse bone marrow

CD45R(B220)$^+$ sIgM$^-$ precursor B cells from bone marrow of 4 – 5 week old BALB/c mice were separated by preparative two-

Table 1. Phenotypic stages of development of progenitor and precursor B cells in normal and mutant mouse bone marrow[a]

Mice	Phenotypic markers (percentage of nucleated bone marrow cells)			
	CD45R(B220)$^+$ c-*kit*$^+$	CD45R(B220)$^+$ CD43$^+$	CD45R(B220)$^+$ CD25$^+$ (large)	CD45R(B220)$^+$ CD25$^+$ (small)
BALB/c	3.2	5.5	6.7	19.8
DBA/2	3.7	6.4	5.7	18.2
C3H/HeJ	3.7	5.9	5.9	14.8
CBA/J	5.5	8.5	5.4	15.0
C57Bl/6	3.3	6.3	5.0	22.0
SCID	4.5	8.5	0.1	0.6
λ_5T	6.2	9.5	0.5	0.7
μMT	5.0	8.0	0.2	0.2
RAG-2T	6.0	8.4	0.6	0.2
RAG-2T + tgμ	2.0	3.4	3.5	13.5

[a]*The data are values derived from studies on the bone marrow of a pool of three or four 4 – 5 week old mice.*

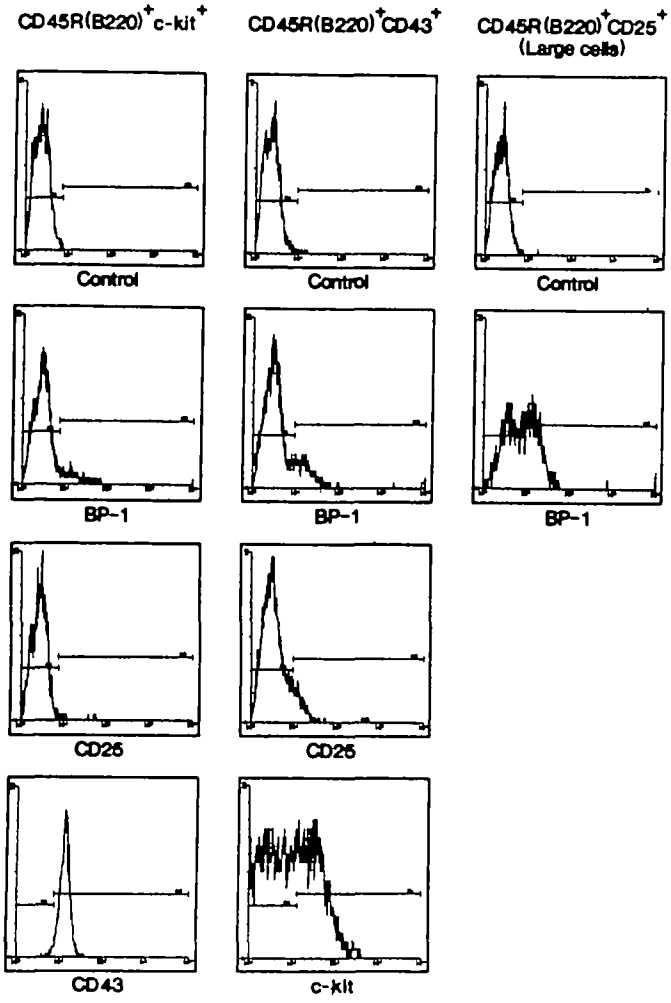

Fig. 2. FACS analysis of cell surface expression of BP-1, CD25 and CD43 on sorted CD45R(B220)$^+$/c-*kit*$^+$, of BP-1 CD25 and c-*kit* on sorted CD45R(B220)$^+$/CD43$^+$ and BP-1 on sorted large CD45R (B220)$^+$/CD25$^+$ bone marrow derived precursor B cells from 4 week old BALB/c mice. The percentages of positive cells are summarized in Table 2.

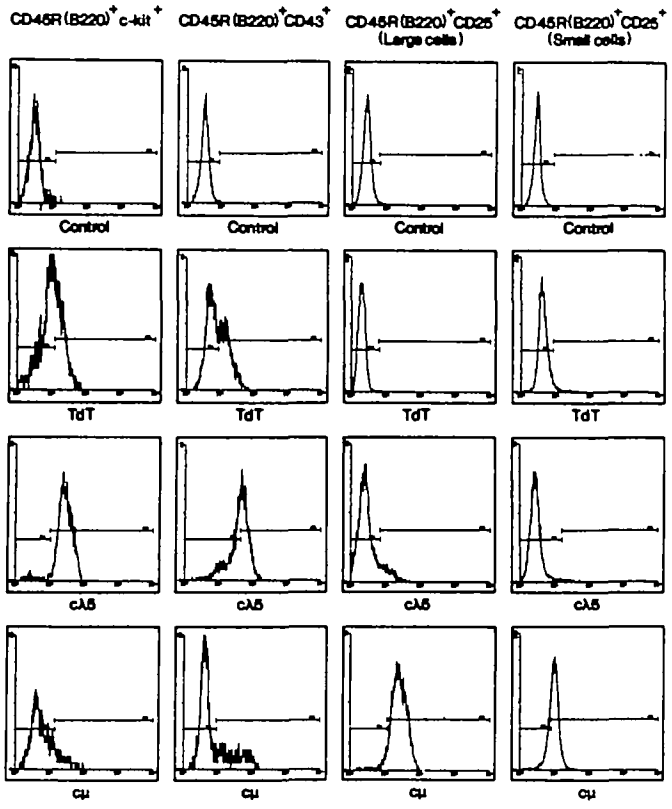

Fig. 3. FACS analysis of intracellular expression of TdT, λ_5 and μ in sorted CD45R(B220)$^+$/c-*kit*$^+$, CD45R(B220)$^+$/CD43$^+$, large CD45R (B220)$^+$/CD25$^+$ and small CD45R(B220)$^+$/CD25$^+$ precursor B cells derived from 4 week old BALB/c mice bone marrow. The percentages are summarized in Table 2.

color FACS into CD45R(B220)$^+$/c-*kit*$^+$, CD45R(B220)$^+$/CD43$^+$, CD45R(B220)$^+$/CD25$^+$ large and CD45R(B220)$^+$/CD25$^+$ small cells (Fig. 1). The separated cells were subsequently analyzed for the expression of BP-1, CD25, c-*kit* and CD43 on the surface (Fig. 2) as well as for the intracellular expression of TdT, λ_5 protein and μH chains (Fig. 3). Table 2 summarizes the quantitation of these analyses.

About half of the CD43$^+$ precursor B cells express c-*kit*, while none of the CD25$^+$ precursors do. Almost all of the c-*kit*$^+$ precursor B cells express CD43, while only 20% of the large and practically none of the small CD25$^+$ precursor B cells do. BP-1 expression in variable numbers was found on all subpopulations of bone marrow derived precursor B cells. One third of the CD45R(B220)$^+$/CD43$^+$ precursors do express CD25. TdT expression was observed in the vast majority of c-*kit*$^+$ precursors and in about half of the CD43$^+$ precursors. No TdT is detectable in CD25$^+$ precursor B cells. The cλ_5 protein is detectable in the vast majority of CD45R(B220)$^+$/c-*kit*$^+$ and of CD45R(B220)$^+$/CD43$^+$ cells. Only 25% of the CD25$^+$ large precursor cells express λ_5 protein in the cytoplasm, while none of the CD25$^+$ small precursors do. cμ expression is almost absent in CD45R (B220)$^+$/c-*kit*$^+$ precursors, whereas one third of CD43$^+$ precursors express cμ. The vast majority of CD25$^+$ B cell precursors express cμ. These analyses indicate that pre-B-I cells are almost all CD45R(B220)$^+$/c-*kit*$^+$/CD43$^+$/CD25$^-$/TdT$^+$/cλ_5^+/cμ^-. A part of them express BP-1.

Pre-B-II cells can be divided into large and small. All pre-B-II cells are c-*kit*$^-$/TdT$^-$/CD25$^+$/cμ^+. About 20% of the large pre-B-II cells are CD43$^+$, as well as cλ_5^+, the other 80% are CD43$^-$ and cλ_5^-. Half of them express BP-1. Within the small pre-B-II cells practically all cells are CD43$^-$/cλ_5^-. A part of them is BP-1$^+$. Thus, c-*kit* expression is restricted to pre-B-I cells, whereas CD25 expression is restricted to pre-B-II cells. Precursor B cells that express CD43 consist of at least a mixture of pre-B-I and pre-B-II cells.

Cell cycle analysis of CD45R(B220)$^+$/c-kit$^+$, CD45R(B220)$^+$/CD43$^+$ and CD45R(B220)$^+$/CD25$^+$ precursor B cells of normal mouse bone marrow

CD45R(B220)$^+$ sIgM$^-$ precursor B cells from bone marrow of 4 – 5 week old BALB/c mice were separated by preparative two-color FACS into CD45R(B220)$^+$/c-*kit*$^+$, CD45R(B220)$^+$/CD43$^+$, CD45R(B220)$^+$/CD25$^+$ large, and CD45R(B220)$^+$/CD25$^+$ small cells (Fig. 1). From the separated cells nuclei were prepared and their DNA content was analyzed as described in Methods. Of

Table 2. Phenotype marker analysis of sorted populations of progenitor and precursor B cells of normal mouse bone marrow

Phenotypic marker	Percentage of sorted			
	CD45R(B220)$^+$ *c-kit*$^+$ cells	CD45R(B220)$^+$ CD43$^+$ cells	CD45R(B220)$^+$ CD25$^+$ (large) cells	CD45R(B220)$^+$ CD25$^+$ (small) cells
c-kit	100	49	0.1[a]	0.1
CD43	96	100	20[a]	0.7
BP-1	14	25	60	30
CD25	4	30	100	100
TdT	80	40	0.1	0.2
$C\lambda_5$	95	90	25	0.5
$c\mu$	5	32	95	90

[a]These numbers are derived by calculation from staining done with CD45R(B220)$^+$/*c-kit*$^+$ and CD45R(B220)$^+$/CD43$^+$ sorting experiments (Fig. 2).

the CD45R(B220)$^+$/*c-kit*$^+$ precursors 45% are in S and G_2/M phases of the cell cycle (Fig. 4A). Of the CD45R(B220)$^+$/CD43$^+$ population ~20% is in S and G_2/M phase of the cell cycle (Fig. 4B), whereas ~70% and <5% of large (Fig. 4C) respectively small (Fig. 4D) cells of the CD45R(B220)$^+$/CD25$^+$ population are in the S and G_2/M phase of the cell cycle. Thus, CD45R (B220)$^+$/*c-kit*$^+$ pre-B-I cells and even more the CD45R(B220)$^+$/ CD25$^+$ large pre-B-II cells are actively cycling while small CD45R(B220)$^+$/CD25$^+$ pre-B-II cells do not cycle. The marker analyses described in the previous sections showed that CD45R(B220)$^+$/CD43$^+$ precursors consist of at least a mixture of pre-B-I cells and large pre-B-II cells. The finding that far less of the CD45R(B220)$^+$/CD43$^+$ cells are in S and G_2/M phases of the cell cycle than those of the pre-B-I compartment (20 versus 45%) and those of the large pre-B-II compartment (20 versus 70%) indirectly indicates that the CD45R(B220)$^+$/CD43$^+$ compartment must contain a third population of cells which does not cycle. The possible nature of this third population will be discussed below.

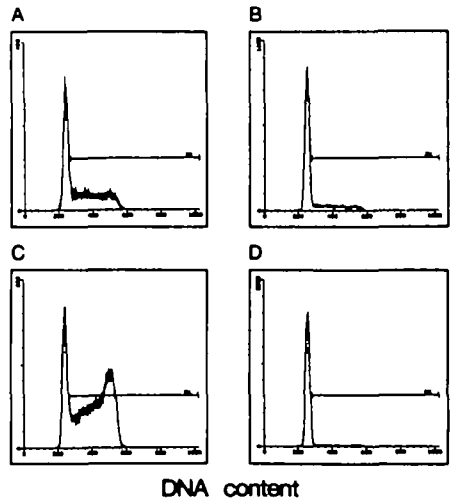

Fig. 4. DNA content analysis in nuclei prepared from sorted CD45R (B220)$^+$/*c-kit*$^+$ (A), CD45R(B220)$^+$/CD43$^+$ (B), large CD45R(B220)$^+$/ CD25$^+$ (C) and small CD45R(B220)$^+$/CD25$^+$ (D) precursor B cells derived from sIgM depleted bone marrow of 4 week old BALB/c mice. The marker indicates the percentage of cells in S and G_2/M phases of the cell cycle. These percentages of cells in S and G_2/M phases are A, 45%; B, 20%; C, 70%; D, 3%.

Discussion

We have analyzed the precursor B cell compartments of normal and mutant mouse bone marrow. The results which are summarized in Table 1 are derived from analysis performed on 4 – 5 week old mice. Here it is important to note that with age, although the relative ratios between the different subpopulations stay constant, their absolute numbers decrease (23).

Previous work by Osmond *et al.* (1,28,29), Hardy *et al.* (2,5), Nishikawa *et al.* (4), Rajewsky *et al.* (19 – 21,30,31) and ourselves (14,24,32) has defined subpopulations of cells in the development of mature B cells from pluripotent stem cells and B-lineage-committed progenitors in mouse bone marrow. In this paper we add the analysis of the expression of the IL-2 receptor α chain (CD25,TAC) on the surface of defined stages of B cell development. It is a useful marker to distinguish pre-B-I from pre-B-II cells. Figure 5 shows a model of B cell development in mouse bone marrow based on our analyses, and Table 3 correlates this model and the corresponding nomenclature to those used by different laboratories. Generally, the different subpopulations can be correlated well with each other. Only a few discrepancies and uncertainties remain.

Osmond 's late pro-B cells and large pre-B cells, defined as $c\mu^-$ and $c\mu^+$ CD45R(B220)$^+$/ sIgM$^-$, TdT$^-$ respectively is one example of a discrepancy. Osmond's late pro-B cells are different from our large pre-B-II cells, since the latter are >85% $c\mu^+$ (Table 2). It might well be possible that Osmond's $c\mu^-$ late pro-B cells, detected *in situ* in bone marrow sections, are out-of-frame rearranged large pre-B-II cells (Fig. 5), which are on their way to die by apoptosis. By the time we have taken them out of bone marrow, stained them as single cell suspensions and analyzed them by FACS, they might already be dead. Alternatively, the level of expression of μ in the cytoplasm might differ in the two populations, so that by *in situ* fluorescence analyses under the microscope half of the cells might score as negative, while FACS detects them as positive.

Another area of uncertain correlations is the transition from Hardy's pro-B to pre-B cells, i.e. the transitions from fractions B to C, to C′, to D. Both Hardy's (5) and Rajewsky's (21) laboratories find cells with $V_HD_HJ_H$-rearranged H chain gene loci in fractions C, C′ and D, while practically all of our pre-B-I cells are DJ_H-rearranged and the vast majority of pre-B-II cells have undergone productive V_HDJ_H rearrangement. The transition from pre-B-I to large, and later small pre-B-II cells is most

Table 3. B lymphopoiesis in mouse bone marrow (correlation and comparison of nomenclature used in different laboratories)

Nomenclature						
This paper	pro-B	pre-B-I	large pre-B-II	small pre-B-II	immature B	mature B
Osmond *et al.*	early pro-B	intermediate pro-B	late pro-B large pre-B	small pre-B	immature B	mature B
Nishikawa *et al.*	B-pro-I PA6 only	B-pro-II PA6 – IL-7		CFU – IL-7 IL-7 only		
Hardy *et al.*	Fraction A pre/pro-B	fractions B, C → C' early pro-B late pro-B		D → pre-B	immature B	mature B

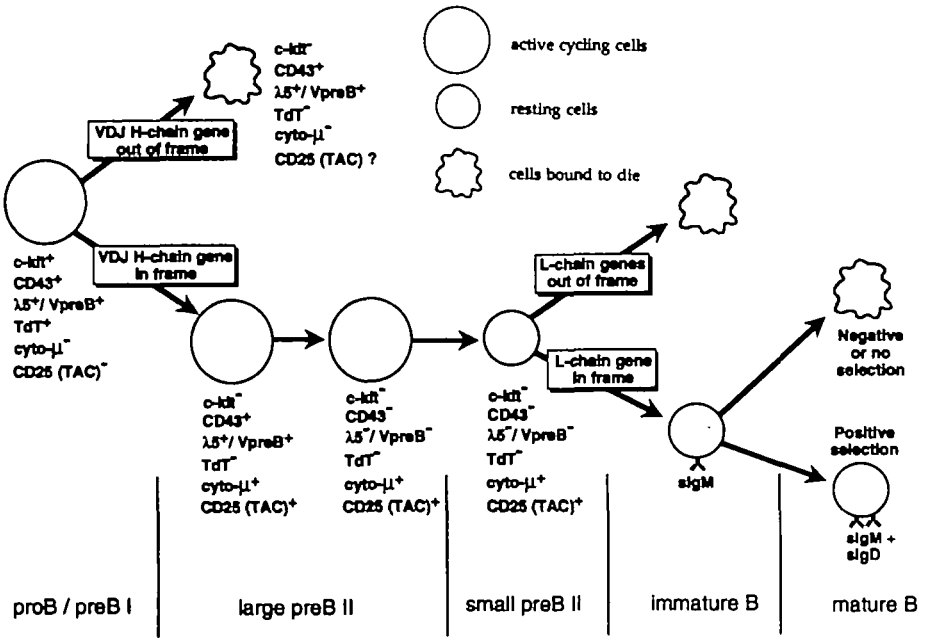

Fig. 5. A model of B cell development in normal mouse bone marrow based on the analysis described in this paper. This model describes the phenotypes of the vast majority of all B lineage cells in bone marrow. We expect minor populations to express other phenotypes in transition from one to another major compartment, and cannot rule out other, minor pathways of B cell development. Large circles, active cycling cells; small circles, resting cells; 'irregular' circles, cells bound to die.

clearly defined by the change of expression of c-*kit* from positive to negative, of TdT from positive to negative and of CD25 from negative to positive. It underlines the usefulness of CD25 since it is so far the only surface marker which is upregulated during this transition.

Approximately twice as many CD45R(B220)$^+$ cells express CD43 than do c-*kit* (Table 1). Practically all the c-*kit*$^+$ cells appear to co-express CD43 (Table 2) and most of them are cycling. Some of the remaining CD43$^+$, c-*kit*$^-$ cells are large, cycling cells and express $c\mu$, $c\lambda_5$ and CD25, but not TdT. They are expected to be a subpopulation of large pre-B-II cells in transit from pre-B-I to small pre-B-II cells. However, the relationship of pre-B-I to large pre-B-II cells, which are either CD43$^+$ or CD43$^-$, to small pre-B-II cells has to be investigated in future experiments. CD43 appears to be down-regulated later than c-*kit* and TdT, and also later than CD25 is up-regulated, and maybe at the same time as the expression of surrogate L chain is down-regulated.

Another part of the CD43$^+$, c-*kit*$^-$ population appears to be less actively cycling and is $c\mu^-$. They might be pre-B-II cells with non-productively V_HDJ_H rearranged H chain gene loci on their way to apoptosis (Fig. 5). Alternatively, they could be pro-B cells before the expression of c-*kit* and TdT, maybe related to fraction A of Hardy *et al.* (2,5). In the latter case, they should have all their H chain gene loci in germline cofiguration. Future experiments are aimed at testing this.

While CD25 on the surface of pre-B-II cells is a useful marker to distinguish them from pre-B-I cells and from immature B cells, its function in B cell development at these early stages remains unknown. IL-2 has no effect on either proliferation or differentiation of pre-B cells in tissue culture (unpublished observations). Moreover, mice with a targeted deletion of the IL-2 gene have no obvious B cell deficiency (33). Therefore it remains to be investigated whether the other, i.e. the β and γ chains, of the IL-2 receptor are expressed at this early stage or whether

the α chain is connected to other polypeptides, possibly with other functions.

B lineage committed, CD45R(B220)$^+$ precursor B cells in RAG-2T, μMT, λ_5T and SCID mice lack CD25$^+$ cells almost completely, while they are present in almost normal numbers in RAG-2T mice expressing a transgenic μH chain gene under the control of the heavy chain enhancer (Eμ) (Table 1). This indicates that the expression of the μH chain and surrogate L chain in membranes (and probably on the surface) of precursor B cells allows their entry into the pre-B-II compartments and their proliferative expansion. Since the composition of cells at the transition point from pre-B-I to large pre-B-II cells is indistinguishable in RAG-2T, SCID, μMT and λ_5T mice, and since no large CD25$^+$ cells can be found in the bone marrow of any of these mutant mice the block in the differentiation appears to be at the same stage of B cell development for all these mutants.

Pre-B-I cells express surrogate L chain together with a complex of polypeptides (p130, p65) on their surface (13), while large, cycling c-*kit*$^-$, CD43$^+$ pre-B-II cells co-express the μH chain and surrogate L chain, and are likely to assemble them in an Ig-like complex, in membranes, probably on the surface. The role of the complex of p130/p65 with the surrogate L chain is unclear. One possible role could be to signal pre-B-I cells to enter the large pre-B-II compartment, while the complex of μH chain with surrogate L chain in or on the large pre-B-II cells might signal the cells to expand by proliferation. Such a scenario would have predicted that SCID, RAG-2T and μMT mice should have some CD25$^+$ large pre-B-II cells (since they are potentially capable of expressing the p150/p65 surrogate L chain complex) while λ_5T mice would not (since they cannot express this early complex of proteins on the surface). This, however, appears not to be the case, since bone marrow of all these mutant mice lack practically all of the CD25$^+$ large pre-B-II cells. Consequently, the only role of surrogate L chain in B cell development identified so far is to expand by proliferation those pre-B-II cells which express membrane-bound μH chains.

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Abbreviations

cλ_5	cytoplasmic λ_5
cμ	cytoplasmic μH chain
PE	phycoerythrin
TdT	terminal deoxynucleotidyl transferase

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