LAH 323

Conventional and Ly-1 B-cell Lineages in Normal and μ Transgenic Mice

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Although Ly-1 B cells represent only a small percentage of the overall murine B-cell population, they produce much of the serum immunoglobulin M (IgM) and many of the commonly studied autoantibodies in autoimmune and normal animals (Hayakawa et al. 1984, 1986a; Herzenberg et al. 1986; Stall et al. 1988; Mercolino et al. 1988). These cells express typical B-cell surface markers and have much in common with conventional B cells. Nevertheless, when contrasted carefully with conventional B cells, they show key differences in cell-surface phenotype (Herzenberg et al. 1986), cell size and granularity (Herzenberg et al. 1986), tissue localization (Hayakawa et al. 1986a; Herzenberg et al. 1986), strain distribution (Hayakawa et al. 1983; Herzenberg et al. 1986; Sidman et al. 1986), and relative frequencies of expressed variable (V_H) genes (Förster et al. 1988; Pennell et al. 1988; Tarlington et al. 1988; Hardy et al. 1989). Furthermore, they tend to be substantially longer lived and to display a characteristic growth and generalization pattern when they become neoplastic (Stall et al. 1988). A series of studies (Hayakawa et al. 1985, 1986b; Herzenberg et al. 1986, 1987; Hardy et al. 1987; Lalor et al. 1987, 1988; Davidson et al. 1988; Hanecak et al. 1989; Palacios et al. 1989), culminating in the elegant experiments reported by J. Kearney and colleagues (this volume), support the placement of Ly-1 B cells in a separate developmental lineage. Collectively, these studies demonstrate the following:

1. Ly-1 B-cell progenitors are distinct from conventional B-cell progenitors (Hayakawa et al. 1985, 1986b; Herzenberg et al. 1986; Kearney et al., this volume) (e.g., progenitors for Ly-1 B cells are present in fetal omentum but are largely missing in adult bone marrow, whereas progenitors for conventional B cells are abundant in adult bone marrow but missing in fetal omentum).
2. Ly-1 B cells are more closely related to macrophages and monocytes than conventional B cells. That is, Ly-1 B cells tend to be adherent (A.M. Stall, unpubl.). They inhabit serosal cavities, such as the peritoneal (Hayakawa et al. 1986a) and pleural (F.M.G. Kroese, unpubl.) cavities and other sites frequented by macrophages. In addition, peritoneal (although not splenic) Ly-1 B cells express a classical macrophage surface marker, CD11/MAC-1 (Herzenberg et al. 1987; A.M. Stall, unpubl.) and look "macrophage-like" in electron micrographs (F.M.G. Kroese et al., in prep.).
3. The Ly-1 B-cell developmental pathway appears to contain pre-B cells that can differentiate either to Ly-1 B cells or to monocytes/macrophages (e.g., several pre-B cell lines have been recognized that develop in this way) (Davidson et al. 1988; Hanecak et al. 1989; Palacios et al. 1989).
4. Ly-1 B-cell developmental mechanisms differ from conventional B-cell mechanisms (e.g., conventional B cells arise later in ontogeny and are replenished from Ig^- progenitors throughout life, whereas Ly-1 B cells are derived from Ig^- progenitors only during the first few weeks of life and persist thereafter as self-replenishing B cells that readily reconstitute the Ly-1 B population in irradiated recipients (Hayakawa et al. 1986b; Lalor et al. 1987, 1988).

Figure 1 summarizes our concept of the lineage distinction between conventional B cells and Ly-1 B cells. We assign the Ly-1 B cells to an evolutionarily primitive, self-replenishing lineage whose development from Ig^- stem cells is regulated by a feedback mechanism that becomes effective shortly after weaning. The stem-cell population in this view differentiates further during early postnatal life and gives rise to the more highly evolved stem cells that replenish the conventional B-cell lineage throughout life.

We focus on B-cell development in Figure 1; however, the stem cells that we show are meant to be pluripotent stem cells that also give rise to lymphoid and myeloid cells. For example, if we were to extend this model to specify which T-cell and macrophage subsets derive from each of the stem cells, we would probably assign the $\gamma\delta$ T cells that pass through the thymus during fetal life to the primitive lineage and the $\alpha\beta$ T cells that emerge later in life to the conventional lineage.

This model differs from earlier (current) concepts of B-cell development, which view B cells as deriving from self-replenishing hematopoetic stem cells that start populating the immune system early in life and provide a steady flow of cells thereafter. In essence, it states that successive stem cells give rise to progressively more advanced lymphocyte lineages whose characteristics recapitulate evolutionary advances in lymphoid and myeloid development. This idea is clearly novel; however, it is consistent with many of the known prop-

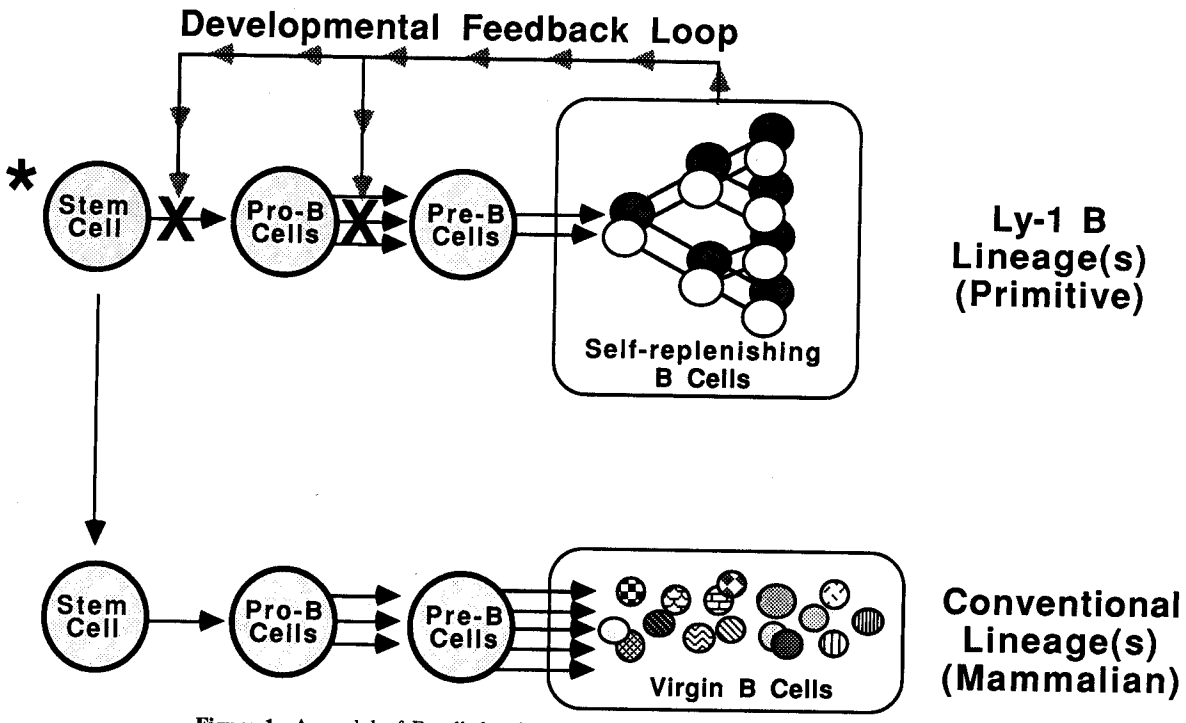

Figure 1. A model of B-cell development in the mouse. See text for explanation.

erties of the B-cell (lymphocyte) lineages as we have drawn them.

For example, the tendency for Ly-1 B cells to inhabit the peritoneal and pleural cavities and to participate extensively in mucosal immunity (Kroese et al. 1989) suggests that these cells may have a longer "biological history" than conventional B cells, which predominate in the more highly evolved lymphoid areas such as the B-cell follicles in spleen and lymph nodes, and tend to contribute more heavily to the immune responses that originate in these follicles. Similarly, the feedback regulation of Ly-1 B development and the maintenance of the Ly-1 B population by self-replenishment in adults approximates the more primitive B-cell development and maintenance mechanisms that operate in avian species (Pink et al. 1985; Ratcliff et al. 1986).

Feedback Regulation of Ly-1 B Development

The feedback mechanism that controls the development of Ly-1 B-lineage cells (see Fig. 1) was recently defined by P. Lalor in our laboratory. Lalor charted the recovery of conventional B cells and Ly-1 B cells in animals that had been treated during the first few weeks of life with anti-IgM allotype antibodies that deplete all B cells (in allotype homozygotes) or half of the B cells (in allotype heterozygotes). In essence, he found that conventional B cells recovered rapidly after the treatment antibody disappeared, regardless of whether the initial B-cell depletion was complete or partial. Ly-1 B-cell recovery, however, failed unless all B cells were removed initially. Thus, the neonatal depletion of paternal allotype Ly-1 B cells in allotype heterozygotes resulted in the lifelong depletion of these Ly-1 B cells with the consequent persistence of a Ly-1 B-cell population consisting only of B cells that express the maternal allotype. (These studies are summarized in Table 1.)

Lalor reproduced this selective failure of the depleted Ly-1 B cells to recover in the presence of Ly-1 B cells by depleting all of the B cells in allotype homozygotes and injecting Ly-1 B cells whose IgM allotype

Table 1. Feedback Inhibition of Ly-1 B Development

IgH allotype	Neonatal depletion of Ly-1 B[a]	Recovery of depleted Ly-1 B[b]
a/b	partial: paternal (b) allo in heterozygotes	no
b/b	complete: all B in homozygotes	yes
b/b (+ a/a)	complete: neonates restored with allotype-congenic Ly-1 B	no

Data summarized from Lalor et al. (1987, 1988).

[a]Mice were injected with 2 mg of anti-IgH-6b during the first 4 weeks of life. No IgH-6b$^+$ cells were detected for the first 6 weeks of age.

[b]Peritoneal cells were analyzed from mice up to 8 months of age. Conventional B cells are depleted and always completely recover.

did not react with the treatment antibody (like the maternal allotype Ly-1 B cells in the allotype heterozygotes). This procedure permanently established an allotype-congenic Ly-1 B-cell population derived from the injected cells and, by so doing, permanently prevented the recovery of the endogenous Ly-1 B population.

Without the injected (allotype-congenic) Ly-1 B cells, the endogenous Ly-1 B population recovered fully within a few weeks of the disappearance of the treatment antibody, no matter how long the antibody treatment was maintained after weaning. On the other hand, if the treatment antibody was allowed to fall below detectable levels during the neonatal period (3–5 weeks), the endogenous Ly-1 B population recovered despite the presence of a nondepletable (maternal allotype) Ly-1 B population. Thus, Ly-1 B cells differentiate de novo for the first few weeks of life without regard to the presence of other Ly-1 B cells; however, if sufficient numbers of Ly-1 B cells are present after this time, further de novo Ly-1 B-cell differentiation is blocked and the existent Ly-1 B population moves into its self-replenishing phase (which persists for the life of the animal).

The early termination of de novo Ly-1 B-cell development forces definition of the basic Ly-1 B repertoire during the first few weeks of life when newly emerging Ly-1 B cells committed to the production of particular antibody molecules are either accepted or depleted from the incoming pool. Once the feedback mechanism is triggered and de novo differentiation terminates, changes in the repertoire are limited to the expansion or depletion of existent clones. Thus, the neonatal repertoire continues to be expressed throughout life but tends to become progressively more narrow, particularly in older animals where dominant (faster-growing) clones and Ly-1 B tumors tend to take over the available "living space" (Stall et al. 1988).

This unique developmental pattern, coupled with the relatively large contribution Ly-1 B cells make to serum IgM and other serum immunoglobulin levels, means that factors that influence immunoglobulin V_H and V_L rearrangement and expression in Ly-1 B cells during neonatal life have a continuing effect on the antibodies produced by the animal. Similarly, antigenic stimulation, idiotypic network interactions, and environmental conditions that alter the Ly-1 B-cell repertoire in neonates or in young adults are reflected in the serum antibodies produced many months hence. In this sense, Ly-1 B cells provide a kind of primitive immunologic memory that keeps elements of the neonatal immunologic experience active throughout life.

B-cell Lineages, Antibody Responses, and Affinity Maturation

Although Ly-1 B cells play key role(s) in the mammalian immune system, they tend not to participate in high-affinity IgG antibody responses to haptens and proteins. These responses are mainly produced by conventional B cells which, according to our view, emerged later in evolutionary time and are more suited developmentally to producing such high-affinity responses (Herzenberg et al. 1986; P.A. Lalor, unpubl.).

Almost nothing is known about the mechanisms that determine which lineage responds to a given antigen. The neonatal mechanisms that control the antibody specificities present in the Ly-1 B repertoire probably contribute to these decisions. Physical characteristics of the antigen also appear to be important. Similarly, the anatomical habitat of the lineages may be a significant factor.

The high-turnover rate of the conventional B cells probably gives them a different type of advantage in high-affinity responses since the continual input of the newly rearranged cells into the "virgin" pool and the larger overall number of these cells in the pool in adult animals provides a wider range of antibody-combining site structures than can be provided in the Ly-1 B repertoire. Therefore, immunizing antigens are more likely to find a good (higher affinity) initial fit among combining sites expressed by conventional B cells and hence to trigger preferentially those B cells.

Somatic mutation, of course, plays a major role in increasing the affinity of the antibody produced by a B-cell clone once it is triggered. Conventional B cells are clearly capable of diversifying their combining sites by this mechanism (for review, see Rajewsky et al. 1987). Ly-1 B cells, in contrast, may not have a functional somatic mutation mechanism. Studies completed thus far favor this view since all of the Ly-1 B immunoglobulin V regions sequenced to date have germ-line encoded sequences (Förster et al. 1988; Pennell et al. 1988; Tarlington et al. 1988; Hardy et al. 1989). However, further work is required to determine whether these findings merely reflect the criteria used to select Ly-1 B immunoglobulins for sequencing or whether they are indicative of a more profound difference between the lineages, i.e., that the Ly-1 B lineage uses a simpler (more primitive?) mechanism for antibody diversification than the conventional lineage.

Developmental Changes in B-cell Lineages in μ Transgenic Mice

Given the major functional and developmental differences between the B-cell lineages outlined above, it is not surprising that these lineages are affected quite differently by the introduction of a μ (IgM) heavy-chain transgene. Conventional pre-B cells and B cells are often drastically depleted, particularly in older animals in certain strains (Herzenberg et al. 1987) (See Fig. 2 and Table 2). Furthermore, this defect in conventional B-cell development is accompanied by (or results in) a severe interference in the rearrangement and/or expression of endogenous immunoglobulin heavy (IgH) chains. In essence, those conventional B cells that manage to survive express the transgenic μ heavy chain (combined with a successfully rearranged

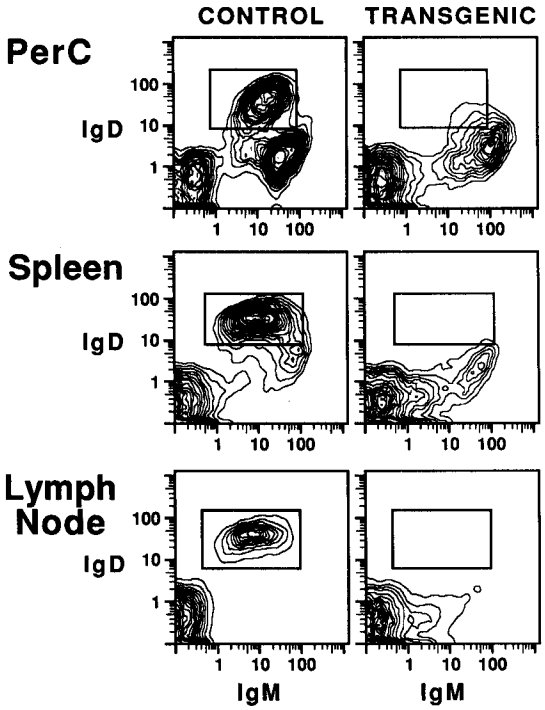

Figure 2. Development of conventional B cells is impaired in μ transgenic mice. Cells prepared from M54 peritoneum, spleen, and lymph nodes were stained with anti-IgM (331) and anti-IgD (AF6-122) and analyzed by a fluorescence-activated cell sorter (FACS) as described previously (Herzenberg et al. 1987). In each panel, the conventional B cells (dull IgM and bright IgD) are delineated by a box. (Data taken, with permission, from Herzenberg et al. 1987.)

light chain) and do not express detectable levels of endogenous μ (Stall et al. 1987; A.M. Stall, in prep.).

Ly-1 B cells, in contrast, are found in essentially normal numbers in μ transgenic animals and almost always rearrange and express endogenous μ chains (Herzenberg et al. 1987; Stall et al. 1987; A.M. Stall, in prep.). Surprisingly, however, many of these cells also express the μ transgene and can be triggered to secrete pentameric IgM molecules with "mixed" heavy chains (Herzenberg et al. 1987). Such "double-expressing" Ly-1 B cells predominate in certain transgenic strains, e.g., M54, whereas other strains mainly have Ly-1 B cells that express endogenous μ and do not express detectable levels of transgenic μ (see Fig. 3 and Table 2). Some transgene-only Ly-1 B cells can be found; however, they are relatively rare and appear to be restricted to a particular (sister) subset of the Ly-1 B lineage (A.M. Stall, unpubl.).

The characterization of the immunogloblin-expression pattern in the relatively few conventional B cells that survive in the transgenic mice was complicated by the presence of a normal-sized large population of Ly-1 B cells expressing both endogenous and transgenic μ in these mice. The conclusion (stated above) that the transgenic conventional B cells do not express endogenous μ required extension of our earlier studies to include the examination of these cells in lethally irradiated recipients reconstituted with transgenic bone marrow carrying a surface marker (Ly-5) that distinguishes donor-derived cells from surviving host cells. As expected (Hayakawa et al. 1985; Herzenberg et al. 1986), essentially all of the B cells in these reconstituted mice are conventional B cells that derive from progenitors in the donor (transgenic) bone marrow. Furthermore, as our earlier studies predicted (Herzenberg et al. 1987), essentially all of these donor-derived conventional B cells express the transgenic μ and virtually none express endogenous μ (see Fig. 4).

Taken together, the studies cited above demonstrate the following: (1) In Ly-1 B-lineage cells, the μ transgene is readily expressed but rarely interferes with endogenous IgH rearrangement and expression; and (2) in conventional lineage B cells, the μ transgene blocks expression (and most likely rearrangement) of endogenous μ and is the only surface immunoglobulin expressed on these B cells. These findings suggest a major difference between the two lineages in the way that they respond to the presence and activity of the μ transgene.

Transgenic μ, Endogenous μ, and Allelic Exclusion

B cells normally express IgH gene products encoded on only one of the two IgH chromosomes in the cell.

Table 2. B-cell Development and μ Gene Expression in μ Transgenic Mice

Mice	Transgene	Splenic B cells (% of sib control)	Peritoneal B cells detected: endogenous only	transgene only	double positive	Background
M54[a]	μ^a	<20	+	+	+	C57BL
M95[a]	μ^a	<20	+	+	+	C57BL
M52[a]	μ^a	<20	+	+	+	C57BL
M94[a]	μ^a	100[c]	+	−	−	C57BL
207-4[b]	μ^a, κ (αPC)	50–80	+	+	+	(C57BL × SJL)F_2 back-crossed to C57BL
243-4[b]	μ^a	>90	+	+	+	(C57BL × SJL)F_2
254-3[b]	μ^a, Δ mem	100[c]	+	−	−	(C57BL × SJL)F_2

[a]Lines developed by Baltimore and Costantini (Grosschedl et al. 1984).
[b]Lines developed by Storb and Brinster (Storb et al. 1986).
[c]No difference could be detected between transgene-positive mice and transgene-negative sibs.

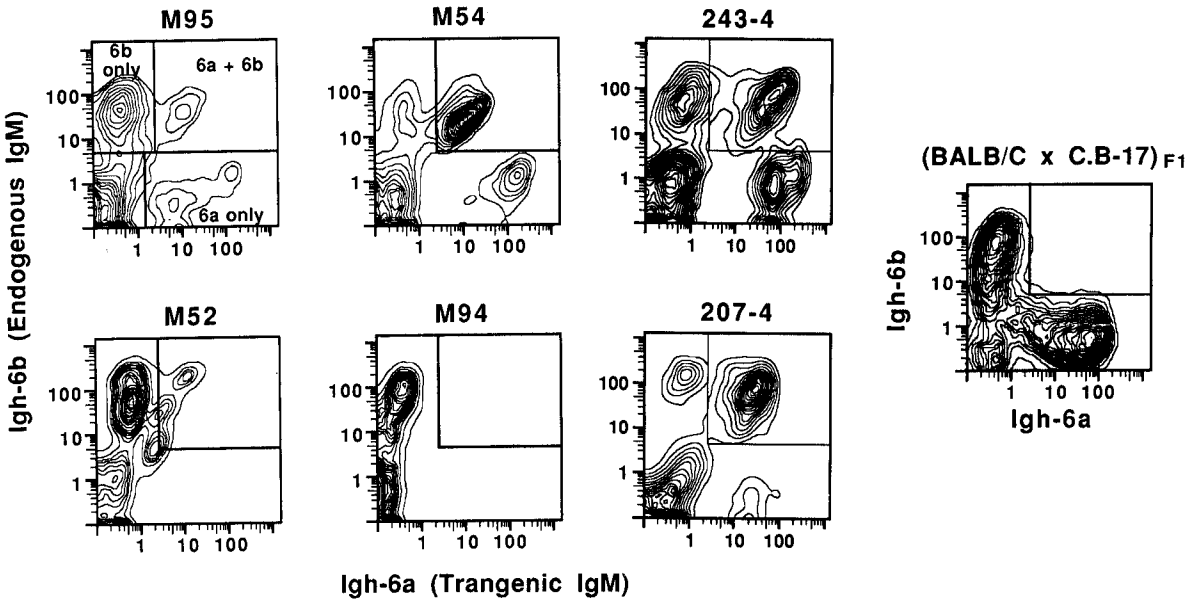

Figure 3. Independent transgenic lines have characteristic patterns of endogenous and transgenic μ expression. Peritoneal cells from the μ transgenic indicated were stained with anti-IgH-6a (DS-1) (transgenic μ) and anti-IgH-6b (AF6-78) (endogenous μ) and analyzed by FACS as described previously (Stall et al. 1987). In the M95 panel, cells expressing endogenous (6b)-only, transgene (6a)-only or double expressors (6a + 6b) are boxed. The stippled areas in each contour plot show the 6a + 6b "double-producer" region. The transgenic lines are more fully described in Table 2. An IgH-6a/IgH-6b contour plot of the allotypic heterozygous F_1 strain (BALB/c × C.B17)F_1 demonstrates allelic exclusion in nontransgenic mice.

The mechanism that mediates this allelic (IgH haplotype) exclusion appears to be triggered by the occurrence of a successful μ heavy-chain rearrangement and/or the production of a functional μ chain. Thus, the expression of a μ heavy-chain transgene prior to the successful rearrangement of an endogenous μ chain would be expected to block endogenous μ-chain production.

The failure to express endogenous μ in conventional B cells in M54 mice is consistent with this hypothesis, i.e., that the transgene product is mistaken for a successfully rearranged endogenous μ gene and that this "error" terminates IgH rearrangement before the endogenous genes can be rearranged. However, the unimpaired expression of endogenous μ in Ly-1 B cells in these animals challenges the generality (if not the very validity) of this commonly accepted hypothesis.

Can the Ly-1 B-cell data be reconciled with the current paradigm? We can dispense rapidly with the idea that the transgene product does not mediate the

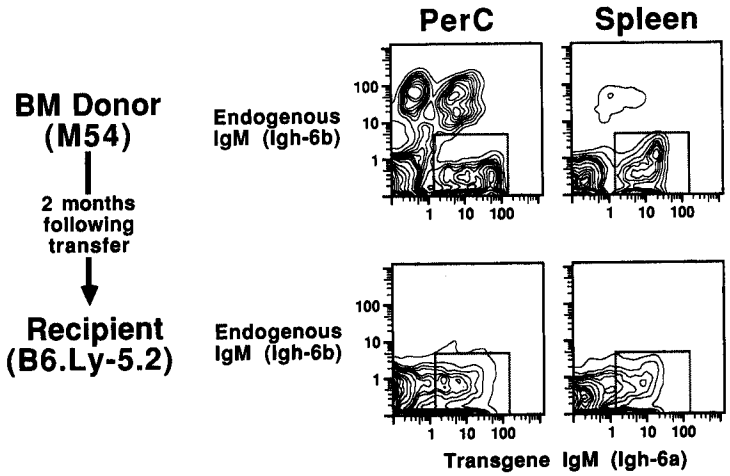

Figure 4. Conventional B cells reconstituted from M54 bone marrow do not express endogenous μ. Bone marrow cells from M54 mice were used to reconstitute a B6.Ly-5.1. These two strains are congenic, differing only with respect to their Ly-5 alleles and the μ transgene of the M54. After 2 months following reconstitution, the recipient mice were sacrificed and analyzed by FACS for donor-derived (Ly-5.2$^+$) B cells as described in Fig. 3. Only donor-derived (Ly-5.2$^+$) B cells are shown in the recipient plots. The donor-derived peritoneal B cells expressed no Ly-1 or MAC-1 antigens confirming that they are of the conventional lineage (not shown). Although the donor peritoneal B cells had a high frequency of endogenous μ-expressing cells, this population is missing in the recipient mice.

allelic exclusion mechanism to terminate IgH rearrangement in Ly-1 B cells because the allelic exclusion mechanism does not function in these cells. Data from multiple FACS analyses taken over the last several years demonstrate clearly that, like conventional B cells, Ly-1 B cells in allotype heterozygotes always express either one parental μ heavy chain or the other, not both (Stall et al. 1987; Palacios et al. 1989). Thus, these cells definitely have a functional allelic exclusion mechanism.

Can the long-term survival of Ly-1 B cells, and hence their susceptibility to selection, account for their endogenous immunoglobulin expression? Perhaps. Ly-1 B cells develop early and persist thereafter by self-replenishment. Conventional B cells, in contrast, turn over rapidly throughout life. Therefore, a few successful rearrangements of endogenous genes in conventional B cells might tend to be lost rapidly, whereas the same number of successful rearrangements in the Ly-1 B population might tend to be "trapped" and expanded into a normal-sized population expressing the endogenous μ with or without transgene accompaniment. In essence, this hypothesis views the transgene as having the same effect in Ly-1 B cells and conventional B cells, and it relegates the observed difference factors that affect the survival rather than the development of cells expressing endogenous μ.

Differential Selection of Ly-1 B and Conventional B Cells

A selection-based hypothesis is particularly attractive since selective forces that operate on the Ly-1 B population in neonates would certainly favor the expansion of those cells that rearrange endogenous μ and enter the population at this time. Nevertheless, evidence from current studies suggests that such differential survival does not account for the lineage differences (A.M. Stall et al., unpubl.). For example, if the success rate for IgH rearrangements were as low in Ly-1 B cells as it is in conventional B cells, the development of the Ly-1 B population should be retarded in neonates (since the input rate to the population should be greatly reduced). Preliminary frequency estimates of Ly-1 B cells in transgenic and normal neonates, however, suggest that the Ly-1 population increases at the same rate in both kinds of animals.

Similarly, if the Ly-1 B population is being generated from a smaller number of successfully rearranged cells, its repertoire should tend to be more restricted and clonal populations of chronic-lymphocytic-leukemia-like tumors should develop earlier than in normal animals. However, such a dramatic change in the frequency or age of onset of clonal populations does not occur in the transgenic animals. Furthermore, preliminary studies indicate that the Ly-1 B repertoire in these animals is relatively normal, at least with respect to the specificities tested so far (A.M. Stall, in prep.).

Finally, a differential survival model would predict the presence of many more Ly-1 B cells that express the transgene in combination with a light chain that makes the cells desirable from a selective standpoint. The occurrence of such cells should easily be as frequent as the occurrence of cells in which a successfully rearranged endogenous μ gene product is coupled with an appropriate light chain to create a desirable (and hence selectable) antibody specificity. However, as we have indicated, transgene-only cells are very rare in the Ly-1 B population. Thus, although the evidence is still not conclusive, we feel that differential survival of successfully rearranged Ly-1 B cells is not likely to account for the observed differences between conventional and Ly-1 B cells in μ transgenic mice.

If we rule out the selection hypothesis, then the lineage differences must be explained by intrinsic developmental differences that allow endogenous rearrangement to proceed successfully in Ly-1 B cells but prevent it in conventional B cells. A variety of such differences can be proposed, e.g., the transgene might be expressed at lower levels or too late in development to be effective in Ly-1 B cells; the Ly-1 B-lineage allelic exclusion mechanism might be qualitatively different and be insensitive to the presence of the transgene or its product; or, the basic assumption that the transgene controls endogenous rearrangement via the allelic exclusion route might be incorrect and the differences between the lineages may result from the operation of entirely unsuspected mechanisms.

These kinds of hypotheses are difficult to evaluate and even more difficult to test; however, that does not necessarily deny their validity. Given the striking series of physical, functional, and developmental differences that have already been recognized between the Ly-1 B-cell and the conventional B-cell lineages (see above), there are good reasons for suspecting that intrinsic developmental differences lie at the heart of the differences in immunoglobulin expression in these lineages in μ transgenic mice. Future studies hopefully will resolve these questions.

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