Paul A. Lalor○, **Alan M. Stall**□, **Sharon Adams and Leonore A. Herzenberg**

Department of Genetics, Stanford University, Stanford

Permanent alteration of the murine Ly-1 B repertoire due to selective depletion of Ly-1 B cells in neonatal animals*

Studies presented here demonstrate that paternal allotype Ly-1 B cells are permanently depleted following neonatal treatment with antibodies to the paternal IgM allotype. Paternal allotype conventional B cells, in contrast, are temporarily depleted by treatment with either anti-IgM or anti-IgD allotype antibodies and return rapidly to normal frequencies once the antibody treatment disappears. These differences are explained by basic developmental differences between Ly-1 B and conventional lineage B cells. That is, the conventional B cell population is replenished from Ig^- precursors throughout life and, therefore, is only temporarily affected when depleted in neonates. The Ly-1 B cell population, in contrast, develops from Ig^- progenitors during the prenatal and neonatal life but survives because it is exclusively self-replenishing in adults. Therefore, elimination of a population of Ly-1 B cells from neonates is tantamount to removing it forever.

These findings suggest that while conventional B cells turn over rapidly and have an effectively unlimited repertoire, Ly-1 B cells express a repertoire whose composition is strongly influenced by neonatal conditions that favor or select against the retention of cells producing certain antibody molecules. Thus, Ly-1 B cells play a unique role in the immune system in that they retain indefinitely the history of the neonatal animal's immunological experience.

1 Introduction

Recent studies demonstrate the existence of two developmentally and functionally distinct B cell lineages in the mouse, referred to as the "conventional" and "Ly-1 B" lineages [1, 2]. Conventional lineage B cells predominate in spleen and lymph nodes and display most of the properties commonly assiciated with B cells in adult mammals [3, 4]. They derive from Ig^- progenitors that are represented in bone marrow and spleen throughout life [5] and readily reconstitute conventional B cell population(s) in irradiated recipients [4].

Ly-1 B lineage cells, in contrast, are rare in spleen and are not detectable in lymph nodes in normal animals [6]. They are mainly found in the peritoneum and usually account for more than half the B cells (> 20% of the lymphocytes) harvested in peritoneal washes [7]. Cells in this lineage seldom produce antibodies to exogenous antigens such as 2,4 dinitrophenyl-keyhole limpet hemocyanin; however, they routinely produce certain anti-carbohydrate antibodies [8], much of the serum Ig and many of the commonly studied autoantibodies in normal and autoimmune mice [9].

[I 7193]

* Supported in part by National Institutes of Health grant CA-42509.
○ Supported by Damon Runyon-Walter Winchell Foundation grant DRG-779. Current address: Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia.
□ Supported by National Institutes of Health grant AI-07290. AMS is a Special Fellow of the Leukemia Society of America.

Correspondence: Leonore A. Herzenberg, Department of Genetics, Stanford University, Stanford, CA 94305, USA

Abbreviations: **APC:** Allophycocyanin **FACS:** Fluorescence-activated cell sorter **6b:** Igh-6b **5b:** Igh-5b

Unlike conventional B cells, Ly-1 B are self-renewing cells that are only rarely reconstituted by transfers of Ig^- cells from adult bone marrow or spleen [1, 4]. Instead, they are reconstituted by transfers of either Ig^+ or Ig^- cells from neonatal spleen and liver [10] or by transfers of (self-replenishing) Ig^+ cells in the adult peritoneal Ly-1 B population itself [1, 4, 10]. Since the co-transfer of adult bone marrow does not block Ly-1 B development from either Ig^+ or Ig^- progenitors, the failure to reconstitute Ly-1 B cells by adult bone marrow transfers appears to be due to a specific deficit in Ly-1 B progenitors rather than to the presence of cells that inhibit Ly-1 B development from a common B cell progenitor [1, 4, 10].

Recent studies, together with data presented in the acompanying publication [11], distinguish two Ly-1 B subpopulations (or perhaps separate lineages). One, the predominant population in normal adult peritoneum, contains typical Ly-1 B cells that express the CD5 surface antigen. The second, a usually minor "sister" population, contains cells that do not express CD5 but otherwise appear identical to the predominant $CD5^+$ B cell population, *e.g.*, with respect to marker expression, function, organ localization and self-replenishing capability. Each of these Ig^+ Ly-1 B subpopulations/lineages replenishes itself, apparently exclusively, when sorted by a fluoresence-activated cell sorter (FACS) and transferred to irradiated recipients [1]. These findings are central to studies presented here examining the mechanisms responsible for the selective and permanent depletion of the Ly-1 B and related peritoneal B cells ("sister" subpopulation) in animals treated with Ig antibodies during neonatal life.

Previous studies have shown that B cell development and Ig production are impaired when neonates are treated with antibodies to IgM and IgD determinants [12–14]. The introduction of these antibodies under conditions where most or all B cells are depleted usually produces only temporary defects in that B cell frequency and functionality return to normal shortly after injected or maternally transferred antibody disappears [15,

0014-2980/89/0303-0501$02.50/0

16]. The recovery of depleted B cell populations, however, takes substantially longer when neonates are treated with antibodies that deplete only a fixed proportion of B cells, *e.g.* when Ig heavy chain (Igh) allotype heterozygotes are treated as neonates with anti-allotype antibodies that react with only one parental allotype and hence deplete no more than half of the B cells expressing any given isotype [17–19].

We explore the mechanisms responsible for the differences in Ly-1 B recovery between allotype homo- and heterozygotes in the accompanying publication [11]. Here, we focus on the strikingly different effects on the composition of individual B cell lineages in allotype heterozygotes treated neonatally with anti-allotype antibodies. As we will show, neonatal depletion of the paternal allotype component of the conventional B cell population does not affect the long-term composition of this B cell population (since these B cells are continually replenished from uncommitted Ig^- stem cells). Depletion of the paternal allotype component of the (self-replenishing) Ly-1 B and related peritoneal B cell populations, in contrast, depletes these populations for the life of the animal and thus permanently alters the antibody repertoire that this population can produce.

2 Materials and methods

2.1 Animals

BALB/cN (Igh^a) and the Igh^b-congenic strains C.B17 and BAB/25, CBA/Ca (Igh^j) and the Igh^b-congenic CBA.Ig/bb, and SJL/J (Igh^b) mice were bred in the Stanford Genetics Department animal facility. CBA/Ca mice have the Igh^a alleles for IgM and IgD.

2.2 Antibodies

Mouse monoclonal anti-Igh-6b (**6b**) (IgM^b, AF6-78.25), anti-Igh-5b (**5b**) (IgD^b, AF6-122.2) and anti-Igh-5a (IgD^a, AMS 9.1) have been described [20]. Rat monoclonal antibodies, anti-IgM (331.12) [21], anti-CD5 and anti-Ly-2 (53-7.8 and 53-6.7) [22], anti-B220 (RA3-6B2) [23], anti-Mac-1 (M1/70) [24], and anti-BLA-1 and -BLA-2 (53-10.1 and 30-E2) [22, 25] were purified from supernatants obtained by culture in serum-free medium (HB 101; Hana Media, Inc., Berkeley, CA). All antibodies used in these studies were purified by ion-exchange chromatography on DEAE-Sephacel or QAE-Sephadex [26]. The conjugations of antibodies to fluorescein isothiocyanate (FITC) or to biotin, and of avidin (Vector Laboratories, Inc., Burlingame, CA) to Texas Red (TR; Molecular Probes, Inc., Junction City, OR), have been described previously [25, 26]. The preparations of allophycocyanin (APC) from the blue-green algae, *Spirulina platensis* and coupling of APC to monoclonal antibodies have also been described [25, 27]. Purified antibodies for injection into mice were dialyzed against phosphate-buffered saline (PBS), adjusted to a concentration of 2 mg/ml and filter sterilized.

2.3 Preparation of cells

Single-cell suspensions were prepared in chilled RPMI 1640 (Irvine Scientific, Santa Ana, CA; minus biotin and phenol red) supplemented with 3% newborn calf serum (NCS) by abrasing lymhoid organs between frosted microscope slides and filtering through 30 μm nylon mesh. Erythrocytes were lysed in spleen cell suspensions by a 3-min incubation in chilled 0.14 M NH_4Cl, 20 mM Tris, pH 7.4. Peritoneal washout cells were obtained by injecting 7 ml of chilled NCS-RPMI 1640 into the peritoneal cavity, followed by mild massage of the abdomen and collection of the fluid.

2.4 Cell staining and multiparameter FACS analyses

Three-color staining of cells has been described in detail elsewhere [25, 28]. In brief, 5×10^5 cells were incubated for 20 min on ice with predetermined saturating concentrations of each of the FITC-, biotin- and APC-conjugated antibodies in a final volume of 100 μl. After washing, the cells were incubated with TR-avidin for 15 min on ice. The cells were washed and resuspended in 100 μl of medium containing 1 μg/ml of propidium iodide. Cells were analyzed by use of a modified, dual laser FACS II (Becton Dickinson, Mountain View, CA) interfaced with a VAX 11/780 computer (Digital Equipment, Maynard, MA) [25, 27].

2.5 Conditions for neonatal antibody treatments of allotype heterozygotes

Three kinds of $Igh^{a/b}$ allotype heterozygous mice were used for the studies presented here: $(BALB/c \times SJL/J)_{F1}$, $(BALB/c \times C.B17)_{F1}$ and $(CBA/Ca \times CBA.Ig^{b/b})_{F1}$. All express paternal Igh^b allotypes at about the same levels as maternal Igh^a allotypes and all yield essentially similar results with respect to neonatal antibody treatments (see below). The antibody dosage and injection schedules used for these studies were established in a series of preliminary experiments. Injection of neonatal $(BALB/c \times SJL/J)_{F1}$ mice with a total of 100 μg of monoclonal anti-(**6b**) (33 μg on days 1, 7 and 14 after birth) proved sufficient to induce short-term depletion of all $\mathbf{6b^+}$ B cells and chronic depletion of Igh^b Ly-1 B cells (data not shown). However, to provide a margin of safety, doses of 300 μg or more were administered to the neonates (usually 100 μg on days 1, 7 and 14) in all subsequent experiments. The dose of anti-**5b** injected into neonates for most of the studies presented here (300 μg) is roughly comparable to that used in previous studies [18]. At this dosage, the injected antibody remains detectable for roughly 6 weeks. To maintain continuous levels of the antibody into adulthood, some groups of neonatally injected mice were further injected at weekly intervals with 100 μg of anti-**5b**.

3 Results

3.1 Anti-5b and anti-6b treatments differentially affect B cell representation

Results from the antibody treatment studies presented here are summarized in Table 1. In essence, these studies show that neonatal treatment with anti-**6b** depletes all conventional Igh^b B cells for as long as the antibody is present and permanently and selectively depletes the Igh^b Ly-1 B . Treatment with anti-**5b**, in contrast, selectively depletes conventional B cells while the treatment antibody is present but does not affect Ly-1 B frequencies, which remain at near-normal levels throughout life.

Table 1. Summary: B cell populations are selectively depleted following neonatal injection of Ig allotype heterozygotes with monoclonal antibodies to IgD and IgM allotypes

Antibody injected[a]	Specificity	Age of mice (months)	Treatment antibody detectable	B Cell populations present[b] Igh^a Total[c]	Igh^b Conventional	Igh^b Ly-1 B
Anti-Igh-5b	IgD	1	Yes	+	–	+
Anti-Igh-5b	IgD	9	No	+	+	+
Anti-Igh-6b	IgM	1	Yes	+	–	–
Anti-Igh-6b	IgM	9	No	+	+	–

a) (SJL × BALB/c)F_1, (C, B17 × BALB/c)F_1 and (CBA.Ig$^{b/b}$ × CBA/Ca)F_1 (Igh$^{a/b}$ heterozygotes) were neonatally injected with 300 to 500 μg of antibody. The antibody was detectable in the mice until 5 to 7 weeks of age.
b) The presence of conventional B cells in spleen, lymph nodes and peritoneal cavity and of Ly-1 B cells in the peritoneal cavity was determined by FACS analysis.
c) Igh^a conventional and Igh^a Ly-1 B cells were detected in all animals tested.

3.2 Anti-6b treatment initially depletes all Igh^b B cells

All B cells expressing Igh^b allotypes are depleted in 6-week-old-allotype heterozygotes that were injected as neonates with monoclonal anti-**6b**. These mice, which still have detectable levels of the antibody in circulation, have normal (or somewhat elevated) numbers of cells expressing Igh^a allotype surface Ig but lack all detectable **5b**$^+$ or **6b**$^+$ cells (see Table 2 and Fig. 1).

Three lines of evidence rule out modulation of surface Ig as the explanation for the absence of the paternal Igh^b allotype cells in the treated animals. First, as indicated above, these mice do not have any cells that express **5b** (IgD of the b allotype). Second, the treated mice do not have elevated numbers of cells expressing B cell markers such as B220 and ThB in the absence of surface Ig (see Table 2). Finally, the treated mice do not have cells capable of rapidly expressing Igh^b allotypes in the absence of anti-**6b**. The rate of recovery of the (conventional) B cell population after the injected antibody decays would be substantially faster (see below) if this recovery were simply due to the reexpression of surface Ig receptors.

3.3 Anti-6b treatment permanently depletes Igh^b Ly-1 B cells

Ly-1 B cell populations differ substantially from conventional B cell populations in terms of their ability to recover following neonatal antibody treatment. Small numbers of conventional B cells expressing Igh^b allotypes begin to appear in spleen, lymph node, and peritoneum almost as soon as the injected antibody disappears (usually between 6 and 8 weeks of age with the dosage used here). The number of these cells then increases steadily over the next 3 months until the population stabilizes at its normal size, usually sometime around 6 months of age (see Fig. 2).

Ly-1 B cells and the sister population of B cells, in contrast, remain depleted for the life of the animal. In essence, treated heterozygotes lack all Igh^b Ly-1 B lineage cells in peritoneum, even when tested a year after disappearance of the injected antibody (see Figs. 3 and 4). In fact, the total population of B cells in peritoneum which expresses high levels of **6b** and low to negative levels of **5b** and includes both Ly-1 B cells and the related sister population is permanently depleted from treated mice (Figs. 3 and 4).

This permanent depletion is most easily seen in (BALB × C.B17)$_{F1}$ and (CBA/Ca × CBA.Ig$^{b/b}$)$_{F1}$ mice, since these

Table 2. Neonatally injected anti-Igh-5b and Igh-6b antibodies depletes target B cells from spleens of (BALB/c × SJL/J)F_1 mice

Treatment antibody	No. of splenic cells × 10^{-6} in 4 to 6-week-old mice[a] B lineage cells (B220$^+$) Total cells	Igh^b IgD$^+$	Igh^b IgD$^-$	Igh^a IgD$^+$	Igh^a IgM$^-$	T cells[b]	Erythroblasts[c]
Anti-6b	80	<1	<1	23	1.5	19	34
Anti-5b	70	<1	5	20	1.7	14	19
None	70	14	3	16	1.3	13	10

a) Mice were injected with antibodies as described in Table 1. Data are from at least four mice per group, analyzed between 4 and 6 weeks of age. The injected antibody was detectable in the mice at the time of analysis.
b) T cells defined as surface Ig$^-$, Ly-1$^+$ and either L3T4$^+$ or Ly-2$^+$.
c) Erythroblasts defined as BLA-1$^+$, B220$^-$ and Mac-1$^-$.

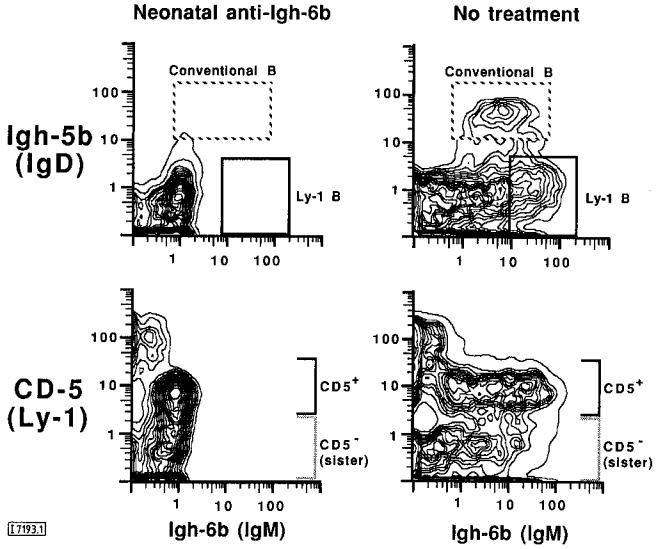

Figure 1. Neonatal anti-**6b** treatment depletes all Ig$^+$ B cells in Igh$^{a/b}$ heterozygous mice. (BALB/c × SJL/J)F_1 were injected with 360 μg of anti-6b in the first 2 weeks of life or left untreated. FACS analyses are of peritoneal cells from mice at 5 weeks of age when anti-6b antibody was still detectable in serum. In the IgD panels the position of the Igh^b Ly-1 lineage B cells is indicated by the black boxes and the position of Igh^b conventional B cells is indicated by the striped boxes. In the Ly-1 panels, the position of the Igh^b Ly-1 CD5$^+$ B and sister populations are indicated by the black and grey brackets, respectively.

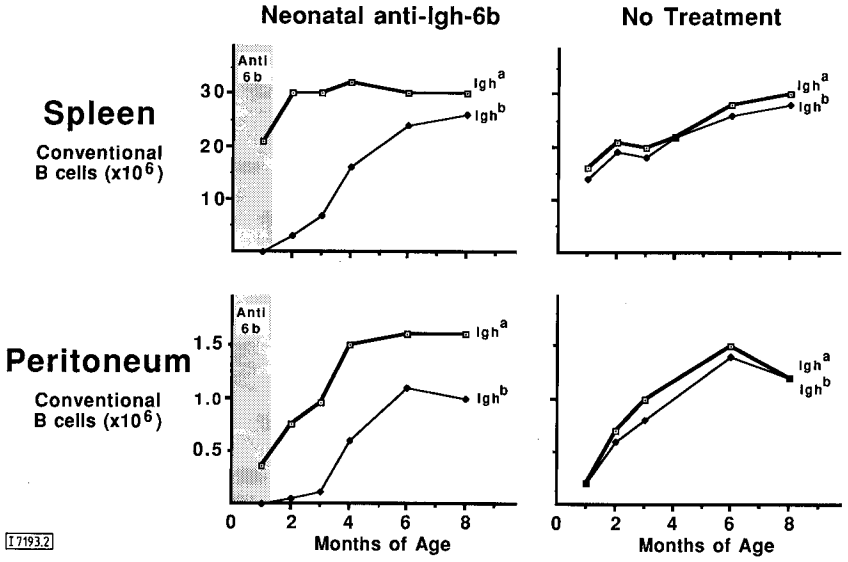

Figure 2. Igh^b conventional B cells recover following neonatal anti-**6b** treatment. (BALB/c × SJL/J)F_1 mice were treated with anti-6b antibody as described in Fig. 1. Antibody was detectable in serum for the first 5 weeks of life (shown by the shaded area). The number of cells in spleens from both treated and normal mice less than 3 to 4 months of age was between 7×10^7 and 10×10^7; from mice older than 4 months, the number of cells in spleen was generally between 1.5×10^7 to 2.5×10^7. The younger mice had approximately 3×10^6 to 5×10^6 peritoneal cells; older mice had between 5×10^6 to 8×10^6 cells.

heterozygotes have large numbers of peritoneal Ly-1 B cells that normally persist at high frequencies throughout life (see Fig. 4). The Ly-1 B recovery, however, also fails in anti-**6b**-treated (BALB × SJL)$_{F1}$ mice, although this failure is somewhat obscured by the natural decline in Ly-1 B frequencies that characteristically occurs with age in these mice (P. Lalor, unpublished).

To date, more than 30 neonatal allotype heterozygotes have been treated with anti-**6b** in our laboratory. Virtually all of these animals were permanently depleted for Igh^b allotype Ly-1 B cells, the only exceptions being those mice (in titration experiments) that were given so little antibody that their anti-**6b** levels fell below detectability by 3 weeks of age. thus, permanent depletion of the Ly-1 B cells and related "sister" population B cells requires injection of enough antibody to completely deplete these B cell populations until the treated animals reach roughly 5 to 6 weeks of age.

3.4 Neonatal injection with anti-5b selectively depletes 5b$^+$ conventional B cells

Neonatal injection of Igh$^{a/b}$ heterozygotes with anti-**5b** (which recognizes the paternal IgD allotypes) temporarily depletes all conventional B cells expressing the **5b** allotype (see Table 2 and Fig. 5). Igh^b allotype Ly-1 B cells, in contrast, are not depleted and remain at near-normal levels throughout the period of antibody treatment (see Fig. 5). Similarly, normal to elevated frequencies of conventional and Ly-1 B cells expressing Igh^a allotypes are continually maintained.

As with anti-**6b** treatment, the conventional B cells gradually reappear once the injected anti-**5b** antibody disappears. The depletion of these B cells can be maintained for at least 9 weeks (the last point tested) by injecting anti-**5b** at weekly intervals. During this time, no **5b**$^+$ cells are detectable. Furthermore, the frequency of **6b**$^+$ B cells lymphocytes in the

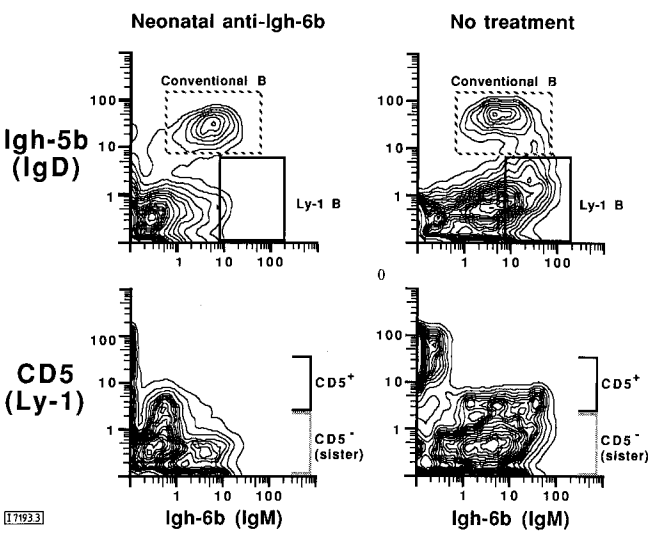

Figure 3. Neonatal anti-**6b** treatment permanently depletes Igh^b Ly-1 B cells in (BALB/c × SJL/J)F_1 Igh$^{a/b}$ heterozygous mice. (BALB/c × SJL/J)F_1 injected with anti-**6b** as described in Fig. 1. FACS analyses are of peritoneal cells of mice at 8 months of age. B cell populations are delineated as described in Fig. 1.

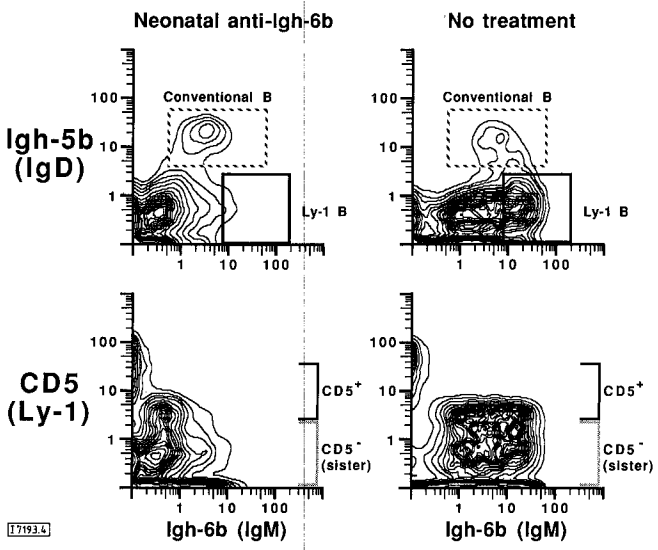

Figure 4. Neonatal anti-**6b** treatment permanently depletes Igh^b Ly-1 B cells in (BALB/c × C.B17)F_1 Igh$^{a/b}$ heterozygous mice. Untreated (BALB/c × C.B17)F_1 were injected with anti-**6b** as described in Fig. 1. Peritoneal cells from mice were analyzed at 10 months. B cell populations are delineated as described in Fig. 1.

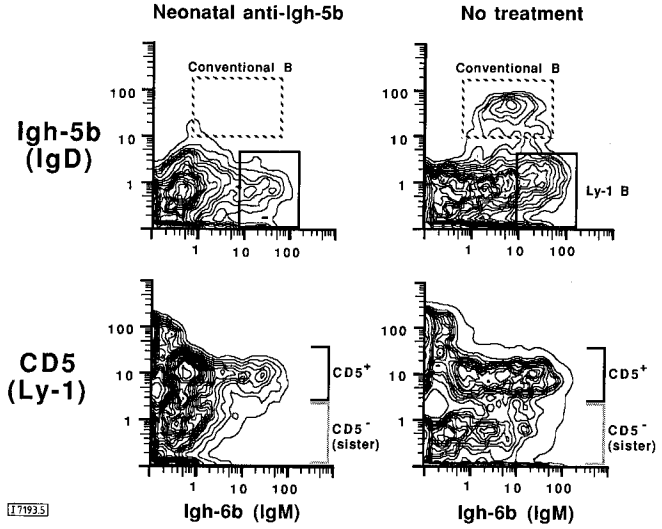

Figure 5. Neonatal injection with anti-**5b** selectively depletes **5b**$^+$ conventional B cells. (BALB/c × SJL/J)F_1 were injected with 400 μg of anti-5b in the first 3 weeks of life or left untreated. Analyses shown are of peritoneal cells from mice at 6 weeks of age when anti-5b antibody was still detectable in serum. B cell populations are delineated as described in Fig. 1.

spleen decreases from approximately 20% of cells at 3 weeks of age to 2% by 7 to 8 weeks of age.

A major proportion (approximately 30%–50%) of the Igh^b cells present in the spleens of long-term-treated (7-week-old) mice express CD5. The lineage origin of the CD5$^-$ proportion of the splenic **6b**$^+$, **5b**$^-$ B cells which persist in treated mice is still unclear. These curious B cells may belong to the conventional lineage; however, their phenotype is also consistent with them being part of the self-replenishing, Ly-1 B "sister" population [11].

When the injected anti-**5b** antibody disappears, conventional Igh^b B cells begin to appear in the spleen and peripheral lymph nodes (data not shown). The levels of Igh^b conventional B cells reach normal levels (*i.e.* levels comparable to the Igh^a conventional B cells) by approximately 6 months of age. The Igh^b Ly-1 B cells remain at frequencies comparable to those in control mice during this period. These findings confirm and extend previous studies from this laboratory, which showed that anti-5b treatment in heterozygotes depleted **5b**$^+$ B cells from spleen and lymph nodes, and that complete recovery of the depleted B cells took roughly 5 months [17].

3.5 Antibody treatment of neonatal mice induce high levels of nucleated erythrocyte precursors in spleen

Nucleated erythrocyte precursor cells, recognizable by light microscopy when FACS-sorted, are frequently present at elevated levels in the spleen of mice treated neonatally with anti-**6b** or 5b antibodies. These cells, which include both pro-erythroblasts and basophilic erythroblasts, do not have detectable levels of surface Ig, B220, CD5, Mac-1, L3T4, ThB or Thy-1; however, they express relatively large amounts of two other surface antigens, BLA-1 and BLA-2, found on some B cells as well as on macrophages and mature erythrocytes [2, 24].

These cells, which comprise 5% to 10% of spleen cells in control animals (see Table 2), often comprise up to 50% of the spleen cells obtained from 6-week anti-IgM-treated allotype heterozygotes. Their frequencies are also elevated (> 25% of spleen cells) in anti-IgM-treated allotype homozygotes [11], in neonatal mice and in several other types of immunologically "active" animals, *e.g.* in mice injected with high doses (50 μg) of bacterial lipopolysaccharide; in trypanosome-infected mice (S. Mahan and P. Lalor, unpublished data); and in mice of the highly autoimmune motheaten strain (C57BL/6 Me^v).

The presence of these cells effectively offsets the loss of splenic B cells in antibody-treated mice in terms of overall numbers of cells in spleen. Thus, frequencies of T cells or Igh^a B cells in spleens from these animals remain relatively normal despite the removal of a large proportion of B cells.

4 Discussion

Studies presented here demonstrate that conventional and Ly-1 B cell lineages differ strikingly with respect to their ability to recover from depletion in allotype heterozygous animals treated neonatally with monoclonal antibodies to paternal allotypes on cell surface Ig. In essence, conventional B cells, which are replenished throughout life from Ig$^-$ precursors [5], recover fully after neonatal depletion. In contrast, removal of self-replenishing Ly-1 B cells committed to production of paternal allotypes during the first 4 to 5 weeks of life depletes those cells from the lineage in heterozygotes for at least a year after disappearence of the antibody.

The functional repertoire of Ly-1 B cell lineage cells is clearly altered in the treated Igh heterozygous animals. Since these animals lack Ly-1 B cells capable of producing IgM molecules with heavy chains encoded by Igh^b allotype chromosome, they cannot produce Ly-1 B antibody responses utilizing V_H genes unique to the Igh^b chromosome. Thus, for those responses in which Ly-1 B antibody production predominates, certain idiotypes and/or antibody specificities produced by the Igh^b Ly-1 B cells in the normal allotype heterozygote will be lacking in the antibody-treated animals.

This alteration in the Ly-1 B repertoire is important in practical terms only insofar as responses by the Igh^a allotype Ly-1 B cells in the treated heterozygotes fail to compensate for the loss of the Igh^b encoded antibodies (*e.g.*, V_H-dependent idiotypes). However, the basic mechanism it demonstrates has far reaching consequences for the normal development of the immune system. In essence, it uses the generic depletion of Igh^b Ly-1 B cells to show that immunological experiences that influence Ly-1 B cells in neonatal animals condition the functional repertoire of the Ly-1 B population in adults. Thus, by extension, it indicates that neonatal encounters with antibodies and other agents (*e.g.*, antigens) capable of deleting or altering the frequency of individual neonatal Ly-1 B cells will also result in permanent alterations in the adult Ly-1 B repertoire.

These findings are reminiscent of many studies showing that neonatally introduced antigens [28–31] or anti-idiotype antibodies (injected or produced endogenously) [32–35] can modify the functional antibody repertoire in adults. The depletion or stimulation of Ly-1 B cells may play an important role in mediating such repertoire changes, either directly (in

responses that are mainly produced by Ly-1 B cells) or indirectly, through regulatory interactions mediated by Ly-1 B antibodies. In any event, the potential for early shaping of the Ly-1 B repertoire itself essentially provides a mechanism for permanently preventing production of unwanted antibodies and amplifying production of desirable antibodies by Ly-1 B cells throughout life.

The significance of this repertoire shaping is still not clear, since the Ly-1 B lineage appears to be evolutionarily more primitive and to have largely conceded its antibody-producing role to the newer and more broadly specific conventional B cells [11]. Nevertheless, Ly-1 B cells have been shown to produce many of the commonly studied autoantibodies and much of the serum Ig [7–9] in mice. Furthermore, these cells give rise to a major proportion of the IgA-producing plasma cells in the gut [36] and produce a large part of the antibody response to at least two bacterial cell wall antigens: $\alpha[1\rightarrow 3]$ dextran [8] and phosphorylcholine (A. Stall, unpublished observation). Thus, this lineage, whose repertoire is largely defined early in life, has retained key functions that apparently preserve its place as an important element in the mammalian immune system.

Not all neonatal antibody treatments can be expected to deplete Ly-1 B cells. As we have shown, the monoclonal anti-IgM allotype reagent used in these studies is quite effective for this purpose, while a monoclonal anti-IgD allotype reagent (of the same isotype) is ineffective. This could reflect the differences in the relative amounts of IgM and IgD expressed by Ly-1 B cells, or it could reflect differences in the way reactions with these surface Ig molecules affect the physiology of the cells. In any event, selective expansion of Ly-1 B cells clones in response to neonatal antibody treatments or antigenic exposures most likely occurs during the neonatal period (although such expansion has yet to be demonstrated directly).

Received September 12, 1988; in revised form October 24, 1988.

5 References

1 Herzenberg, L. A., Stall, A. M., Lalor, P. A., Sidman, C., Moore, W. A., Parks, D. R. and Herzenberg, L. A., *Immunol. Rev.* 1986. *93:* 81.
2 Hardy, R. R. and Hayakawa, K., *Immunol. Rev.* 1986. *93:* 53.
3 Hardy, R. R., Hayakawa, K., Haaijman, J. and Herzenberg, L. A., *Nature* 1982. *297:* 589.
4 Hayakawa, K., Hardy, R. R., Herzenberg, L. A. and Herzenberg, L. A., *J. Exp. Med.* 1985. *161:* 1554.
5 Osmond, D. G., *Immunol. Rev.* 1986. *93:* 103.
6 Hayakawa, K., Hardy, R. R., Parks, D. R. and Herzenberg, L. A., *J. Exp. Med.* 1983. *157:* 202.
7 Hayakawa, K., Hardy, R. R. and Herzenberg, L. A., *Eur. J. Immunol.* 1986. *16:* 450.
8 Forster, I. and Rajewsky, K., *Eur. J. Immunol.* 1987. *17:* 521.
9 Hayakawa, K., Hardy, R. R., Honda, M., Herzenberg, L. A., Steinberg, A. D. and Herzenberg, L. A., *Proc. Natl. Acad. Sci. USA* 1984. *81:* 2494.
10 Hayakawa, K., Hardy, R. R., Stall, A. M., Herzenberg, L. A. and Herzenberg, L. A., *Eur. J. Immunol.* 1986. *16:* 1313.
11 Lalor, P. A., Herzenberg, L. A., Adams, S. and Stall, A. M., *Eur. J. Immunol.* 1989. *19:* 507.
12 Cooper, M. D., Kearney, J. F., Gathings, W. E. and Lawton, A. R., *Immunol. Rev.* 1980. *52:* 29.
13 Layton, J. E., Johnson, G. R., Scott, G. W. and Nossal, G. J. V., *Eur. J. Immunol.* 1978. *8:* 325.
14 Skelly, R. R., Baine, Y., Ahmed, A., Xue, B. and Thorbecke, G. J., *J. Immunol.* 1983. *130:* 15.
15 Lawton, A. R. and Cooper, M. D., *Contemp. Top. Immunol.* 1974. *3:* 193.
16 Manning, D. D., *J. Immunol.* 1974. *113:* 455.
17 Black, S. J. and Herzenberg, L. A., *J. Exp. Med.* 1979. *150:* 174.
18 Tokuhisa, T., Gadus, F. T., Herzenberg, L. A. and Herzenberg, L. A., *J. Exp. Med.* 1981. *154:* 921.
19 Jacobson, E. B., Baine, Y., Chen, Y. W., Flotte, T., O'Neill, M. J., Pernis, B., Siskind, G. W., Thorbecke, G. J. and Tonda, P., *J. Exp. Med.* 1981. *154:* 318.
20 Stall, A. M. and Loken, M. R., *J. Immunol.* 1984. *132:* 787.
21 Kincade, P. W., Lee, G. W., Sun, L. and Watanabe, T., *J. Immunol. Methods* 1981. *42:* 17.
22 Ledbetter, J. A. and Herzenberg, L. A., *Immunol. Rev.* 1979. *47:* 63.
23 Coffman, R. L. and Weissman, I. L., *Nature* 1981. *289:* 681.
24 Springer, T., Galfré, G., Secher, D. S. and Milstein, C., *Eur. J. Immunol.* 1979. *9:* 301.
25 Hardy, R. R., Hayakawa, K., Parks, D. R., Herzenberg, L. A. and Herzenberg, L. A., *J. Exp. Med.* 1984. *159:* 1169.
26 Hardy, R. R., in Weir, D. M., Herzenberg, L. A., Blackwell, C. C., Herzenberg, L. A. (Eds.), *The Handbook of Experimental Immunology, 4th edn.*, Blackwell Scientific Pub., Edinburgh 1986, chapter 31.1.
27 Parks, D. R., Hardy, R. R. and Herzenberg, L. A., *Cytometry* 1984. *5:* 159.
28 Freitas, A. A., Rocha, B. and Coutinho, A., *Immunol. Rev.* 1986. *91:* 5.
29 Freitas, A. A., Rocha, B., Forni, L. and Coutinho, A., *J. Immunol.* 1982. *128:* 54.
30 Sprent, J. and Basten, A., *Cell. Immunol.* 1973. *7:* 40.
31 McLennan, I. C. M. and Gray, D., *Immunol. Rev.* 1986. *91:* 61.
32 Vakil, M., Sauter, H., Paige, C. and Kearney, J. F., *Eur. J. Immunol.* 1986. *16:* 1159.
33 Takemori, T. and Rajewsky, K., *Immunol. Rev.* 1984. *79:* 103.
34 Nossal, G. J. V., *Annu. Rev. Immunol.* 1983. *1:* 33.
35 Kearney, J. F. and Vakil, M., *Immunol. Rev.* 1986. *94:* 39.
36 Kroese, F. G. M., Butcher, E. C., Stall, A. M. and Herzenberg, L. A., *Immunol. Invest.*, in press.