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Feedback regulation of murine Ly-1 B cell development*

Studies presented here, conducted with allotype homozygotes, demonstrate the existence of a feedback mechanism that regulates development of Ly-1 B cells from immature progenitors. In the preceding study (P. A. Lalor et al., *Eur. J. Immunol.* 1989. *19:* 501), conducted with allotype heterozygotes, we showed that treating neonates with monoclonal antibody to the paternal allotype IgM depletes roughly half of the neonatal B cell population (*i.e.* those expressing the paternal IgM allotype) and that paternal allotype Ly-1 B cells specificically remain depleted for the life of the animal. Here we show that treating allotype homozygotes with the same antibody depletes all (rather than half) of the B cells and that, under these conditions, relatively normal numbers of Ly-1 B cells reappear shortly after the treatment antibody disappears. This recovery, we also show, is prevented by restoring allotype-congenic Ly-1 B cells to the treated homozygotes, *i.e.* by reconstituting treated neonates with allotype-congenic peritoneal cells, sorted Ly-1 B cells or a monoclonal population of Ly-1 B "tumor" cells.

These findings in essence reveal a feedback mechanism through which mature Ly-1 B cells prevent further Ly-1 B cell development from Ig^- precursors. This feedback regulation is independent of Ig secretion by the mature Ly-1 B cells, since the monoclonal Ly-1 B "tumor" population that prevents endogenous Ly-1 B development does not secrete Ig. Furthermore, it appears to be independent of Ly-1 B surface Ig specificity, since a monoclonal population is sufficient to block all Ly-1 B cell development. This mechanism appears to operate normally to fix the composition of the Ly-1 B population, which survives through self-replenishment in adults, in accord with conditions that influence Ly-1 B development during neonatal life.

1 Introduction

Murine Ly-1 B cells expressing paternal Ig allotypes are depleted for life when allotype heterozygotes are treated neonatally with anti-IgM allotype antibodies that temporarily deplete the entire paternal allotype B cell population [1]. Thus, in principle, all Ly-1 B cells should be permanently eliminated when allotype homozygotes are treated with the same antibodies at dose levels that remove all B cells during the neonatal period. Nevertheless, as we will show, sizable numbers of Ly-1 B cells recover and persist indefinitely (along with conventional B cells) in allotype homozygotes treated with antibodies that completely deplete B cells in neonatal animals and keep these B cells depleted until well after weaning.

Studies presented here trace this apparently paradoxical difference in the potential for Ly-1 B recovery in similarly treated allotype homozygotes and heterozygotes to the operation of a developmental feedback mechanism that controls the development of Ly-1 B cells from endogenous Ig^- progenitors. This mechanism, which appears similar to one described some time ago for avian B cells [2-4], prevents further Ly-1 B development from immature progenitors once sufficient numbers of Ly-1 B cells have accumulated in the developing animal (usually around the time the animals are weaned).

Thus, as we will show, endogenous Ly-1 B development is not inhibited in adult animals that have no Ly-1 B cells, *i.e.*, in antibody-treated allotype homozygotes (once the treatment antibody disappears). However, this development is completely blocked by the neonatal introduction of allotype-congenic Ly-1 B cells, which grow and reconstitute a sizable Ly-1 B population (expressing the donor allotype) in the antibody-treated animals.

[I 7194]

* Supported in part by National Institutes of Health grant CA-42509.

○ Supported by Danom 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.

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Abbreviations: **FACS:** Fluorescence-activated cell sorter **PerC:** Peritoneal cells **6a:** Igh-6a **6b:** Igh-6b **5b:** Igh-5b

2 Material and methods

2.1 Materials

The antibodies, mice strains, immunofluoresence staining and fluorescence-activated cell sorter (FACS) analysis methods used here are described [1].

2.2 Treatment of neonatal $Igh^{b/b}$ homozygous mice with anti-Igh-6b (6b) antibody

Newborn C.B17 mice were injected within 1 day of birth with 0.1 mg of monoclonal anti-**6b** antibody (clone AF6-78.25) [5], followed by twice weekly injections of 0.2 mg for the first 4.5 weeks of life (a total of 2 mg). **6b** is the Igh^b allele of IgM. Greater than 10 μg/ml of the antibody is detectable in the serum of treated mice up to 6 weeks of age. Neonatal injection

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of 300 μg of antibody, the dose used for treatment of $Igh^{a/b}$ heterozygotes, depleted B cells from C.B17 mice for only 2 to 3 weeks.

2.3 Isolation and maintenance of BCL-85, a clonal population of Ly-1 B cells

Clonal populations of Ly-1 B cells frequently develop in older mice of most mouse strains [6]. The clonal (Igh^a) Ly-1 B cell population (BCL-85) used here was isolated from an irradiated C.B17 mouse which had been reconstituted with C.B-17 bone marrow and 5-month-old BALB/c mouse peritoneal cells (PerC). The clone, designated BCL-85, were segregated from other transferred Igh^a Ly-1 B cells in the C.B17 recipient by subsequent passage of 10^4 PerC into several irradiated C.B17 mice. With this low number of transferred cells, the only Igh^a cells to establish in the recipients were the BCL-85 Ly-1 B cells, which grow slowly and do not kill the recipient for at least 18 months after transfer (P. Lalor, unpublished). The clonal origin of these cells was established by Southern gel analysis (D. Tarlinton and L. A. Herzenberg, unpublished observation).

3 Results

3.1 Neonatal treatment with monoclonal anti-IgM allotype antibody temporarily depletes all Ig^+ cells

Treatment of neonatal $Igh^{b/b}$ homozygous C.B17 mice with monoclonal antibodies to **6b** (IgM) allotype temporarily depletes all **5b**$^+$**6b**$^+$ (IgD^+ IgM^+) B cells from spleen, lymph nodes, peritoneum and bone marrow (Table 1). Injection of a total of 2 mg of antibody during the first 4 weeks of life is sufficient to deplete all Ig^+ cells until mice are approximately 6 to 7 weeks old. Neonatal injection of 300 μg of antibody, the dose used to deplete paternal **6b** B cells in heterozygotes (see accompanying papter), depletes B cells in the homozygotes only until mice are approximately 2 weeks old. Thus, conventional and Ly-1 B cells begin to appear in these mice shortly before they are weaned.

The absence of Ig^+ cells in 5-week-old animals that received 2 mg of antibody does not necessarily mean that all B cells are depleted in these animals. Small numbers of Ig^- cells that express the pan-B cell marker B220 are clearly detectable in spleen and lymph nodes in these mice. These cells, which could be pre-B cells or modulated B cells that have lost their surface Ig, comprise approximately 5% to 10% of cells in the spleen (compared to 1% to 3% in untreated controls) (see Table 1) and 1% to 2% in lymph nodes (controls have < 0.1%, data not shown). Gause et al. [7] have reported the presence of modulated (Ig^-, $B220^+$) B cells in spleens of Igh^b homozygous mice treated from birth with another anti-**6b** antibody.

Table 1. Neonatally injected anti-6b depletes all Ig^+ B cells in Igh^b homozygous mice

Treatment antibody	No. of spleen cells × 10^{-6} in 4-week-old mice[a]					
	Total cells	B lineage cells (B220$^+$)		T cells	Erythro-blasts	Others[b]
		IgM^+	IgM^-			
Anti-6b	110	< 1	9	30	52	16
None	130	57	3	44	4	20

a) C.B17 mice were injected with anti-6b antibody during the first 4 weeks of life sufficient to maintain detectable levels of antibody until 6 to 7 weeks of age.
b) Other cells include macrophages, null cells, etc.

3.2 Conventional and Ly-1 B cells in allotype homozygotes return after neonatally injected anti-IgM antibody disappears

Conventional B cells (dull IgM, bright IgD) begin accumulating in the spleen, lymph nodes and peritoneum immediately after the neonatally injected antibody (2 mg) disappears and reach normal levels in one location 4 to 6 weeks later. Ly-1 B lineage cells similarly begin accumulating (mainly in the peritoneum) once the treatment antibody disappears but take somewhat longer to reach stable levels (Figs. 2 and 3).

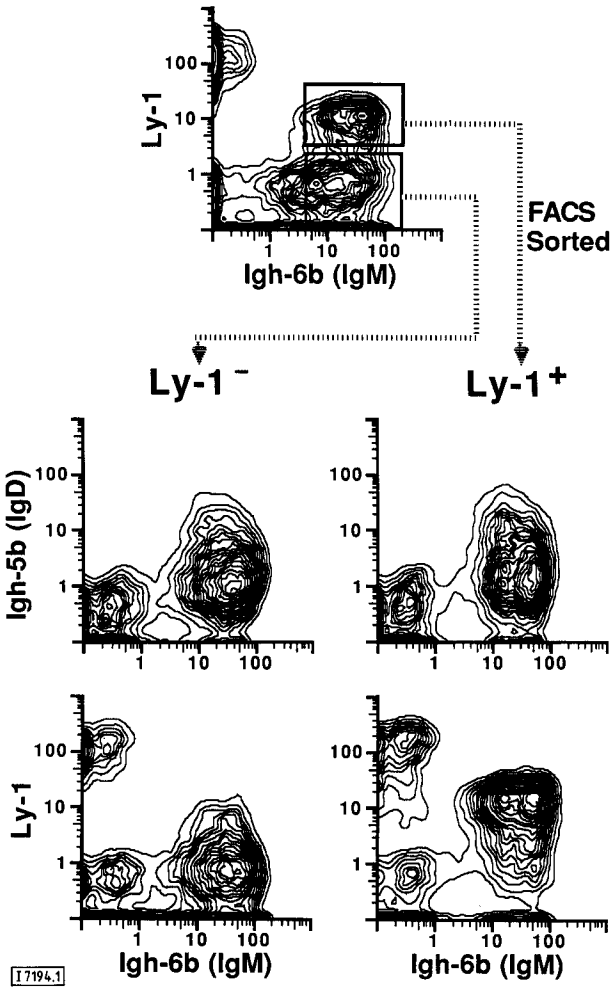

Figure 1. The adult Ly-1 B CD5 "sister" population is self-regenerated from IgM^+ progenitors. CBA/bb (Igh^b) cells falling within the gates depicted in the upper panel were sorted and transferred to 850R irradiated CBA/N (Igh^j) recipients supplemented with CBA/N bone marrow cells. Recipients each received the yield from approximately 5 × 10^6 FACS-sorted PerC. The bottom four panels show the peritoneal lymphocyte populations in recipients analyzed 2 months after transfer.

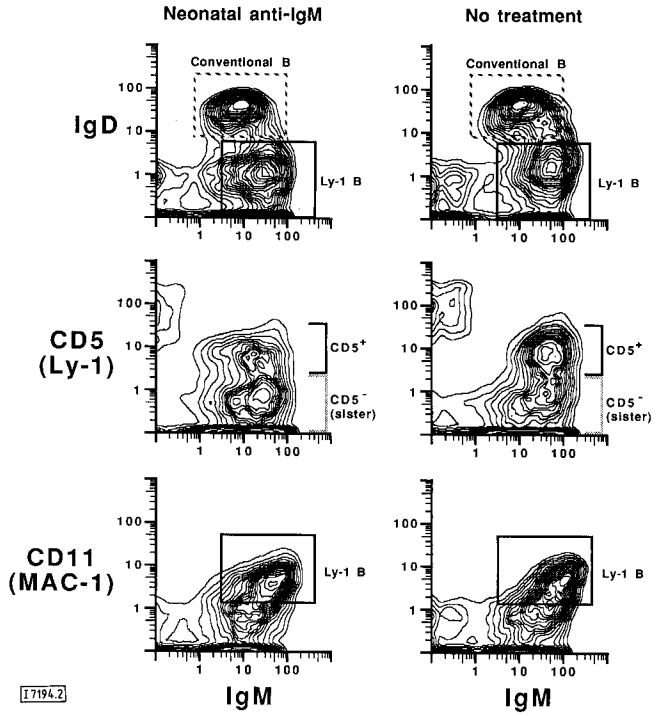

Figure 2. Conventional and Ly-1 B cells in allotype homozygotes return after neonatally injected anti-IgM (anti-**6b**) antibody disappears. C.B17 mice were treated with a total of 4 mg of anti-6b during the first 8 weeks of life. PerC were analyzed from mice at 8 months. In the IgD panels the position of the Ly-1 lineage B cells is indicated by the black boxes and the position of conventional B cells is indicated by the stripped boxes. In the Ly-1 panels, the position of the $CD5^+$ B and sister populations are indicated by the black and gray brackets, respectively.

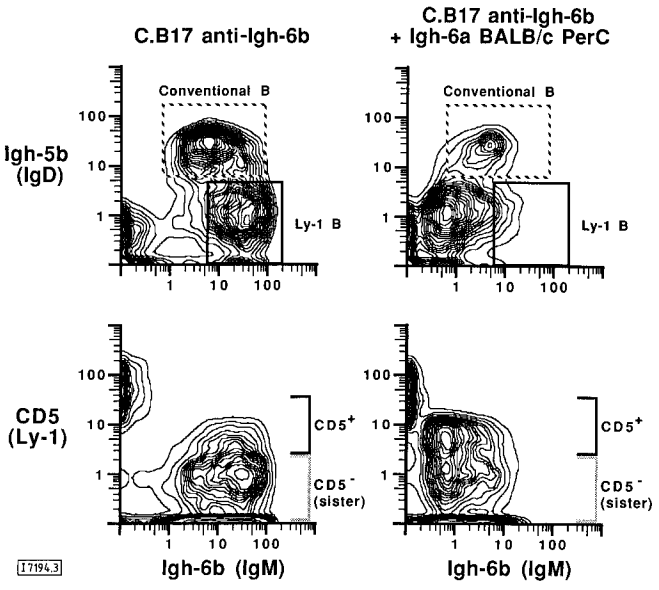

Figure 3. Transfer of allotype-congenic PerC into treated neonates impairs recovery of endogenous Ly-1 B cells. C.B17 mice were treated with 2.0 mg of anti-**6b** during the first 4 weeks of life. PerC from 6-week-old BALB/c mice were injected into treated mice on day 3 after birth. PerC from 6-month-old recipient mice were analyzed for B cell populations expressing endogenous Ig allotypes (**6b** and **5b**). The various B cell populations are indicated as described in Fig. 1.

Table 2. Ly-1 lineage B cells recovering following anti-treatment are predominately $CD5^-$ "sister" population

Animal[a]	Antibody treatment[b]	Total cells × 10^{-6}	PerC Conv. B lineage (%)	Ly-1 B lineage[c] $CD5^+$ (%)	Ly-1 B lineage[c] $CD5^-$ (%)
C.B-17	Anti-6b	4.0	23	17	30
C.B-17	None	9.5	16	16	34

a) C.B-17 mice were treated as described in Fig. 2.
b) PerC were analyzed from 8-month-old mice.
c) Ly-1 lineage B cells were identified as IgM^{bright}, IgD^{dull}, B220/6B2^{dull}, and Mac-1^{dull}.

Curiously, although typical Ly-1 B cells are clearly detectable in the antibody-treated C.B17 mice once B cell recovery begins, the majority of the Ly-1 B lineage cells that develop in these animals belong to the recently characterized Ly-1 B "sister" population [8]. Cells in this (sub)population lack detectable levels of surface CD5 but are otherwise indistinguishable from typical ($CD5^+$) Ly-1 B cells. They are self-replenishing; they show the typical Ly-1 B FACS phenotype (high IgM, low IgD, low 6B2/B220, low Mac-1) (Fig. 1); and insofar as they have been tested, they have the same functional properties as typical Ly-1 B^+ cells ([8] and A. M. Stall and P. A. Lalor, unpublished observations). Typically, the "sister" population cells constitute the minority of the overall Ly-1 B population in BALB/c and C.B17 mice; however, their representation increases dramatically in the Ly-1 B population that recovers in antibody-treated animals (Figs. 2 and 3; anti-Igh-6b and Table 2).

This increase in the frequency of "sister" population cells in Ly-1 B populations that develop in antibody-treated animals may be explained by recent evidence indicating that progenitors that reconstitute "sister" population cells tend to persist longer into adulthood than progenitors that reconstitute typical Ly-1 B cells. Data from the large series of bone marrow transfer experiments (> 100 recipients; > 50 donors) that we have performed over the last 3 years collectively demonstrate that in those experiments when Ly-1 B lineage cells are reconstituted (albeit usually at low levels) by transfers of adult bone marrow cells (> 2 months of age) the Ly-1 B lineage cells are predominately of the $CD5^-$ "sister" population (*e.g.* see Table 3).

Transfer of bone marrow from younger animals [9] or of Ig-pre-B cells from neonatal spleen [10], in contrast, tend to reconstitute both typical Ly-1 B and "sister" population cells although frequencies still tend to be somewhat skewed in favor of the "sister population" (see Table 3). Thus, the delayed development of the Ly-1 B population that occurs in antibody-treated mice appears to be responsible for the increased ratio of "sister" population cells to typical Ly-1 B cells in these animals, *i.e.*, because progenitors capable of reconstituting typical Ly-1 B cells are lost more rapidly with age than progenitors capable of reconstituting "sister" population cells.

These findings suggest that the two kinds of Ly-1 B cells develop from distinct Ig^- progenitors in bone marrow and, hence, that they may belong to separate, albeit closely related, lineages. However, until the progenitors for $CD5^+$ B and its

Table 3. Sources of progenitors for the CD5⁻ Ly-1 B "sister" population

Cells transferred		Donor-derived B cell in recipient PerC[a]		
		Conventional	Ly-1 B lineage[b]	
Source	Population	B CD5⁻	CD5⁻ (%)	CD5⁺ (%)
Adult BM[c]	Unsorted	34, 32, 31, 33	3, 4, 6, 13[f]	1, 1, 2, 1
Adult BM	IgM⁻, B220⁻	37, 24, 27, 16	2, 3, 4, 17	1, 1, 2, 1
Neonatal spleen[d]	Unsorted	28, 28	16, 25	12, 14
Neonatal spleen	IgM⁻	26, 22	16, 14	8, 9
Neonatal spleen	IgM⁺	8, 6	2, 3	7, 6
Adult PerC[d]	Unsorted[e]	3, 1, 2, 2	15, 11, 14, 16	28, 25, 28, 33
None (control PerC)	–	12, 18, 16, 28	15, 13, 12, 8	31, 27, 24, 23

a) Donor-derived B cells were identified by their IgM and IgD allotype markers.
b) Ly-1 lineage B cells were identified as IgM^{bright}, IgD^{dull}, $B220/6B2^{dull}$, and $MAC\text{-}1^{dull}$.
c) BALB/c adult bone marrow (BM) and FACS-sorted BM were transferred to lethally irradiated allotype-congenic (C.B-17) mice supplemented with syngenic (C.B-17) BM. Recipients were analyzed 6 weeks after transfer. Between 2×10^6 to 5×10^6 total PerC were recovered from the mice. No donor-derived B cells were recovered in a third transfer group that received FACS-sorted IgM^-, $B220^+$ cells (plus syngeneic BM).
d) BAB/25 neonatal speen cells, FACS-sorted populations and adult PerC were transferred to lethally irradiated allotype-congenic (BALB/c) mice supplemented with syngenic (BALB/c) BM. Recipients were analyzed 3 months after transfer. Between 10^6 to 15×10^6 total PerC were recovered from the mice.
e) Previous studies [10] indicate that all Ly-1 B lineage cells in recipients are derived from Ig^+ cells in donor PerC.
f) "Sister" population frequencies as high as these generally occur in less than 15% of BM recipients.

"sister" population are more clearly distinguished from one another, prudence dictates that these two putative lineages be treated as subpopulations of a single (Ly-1 B) lineage. Thus, in this publication, we use the terms Ly-1 B lineage and Ly-1 B cells inclusively to mean both $CD5^+$ and "sister" population cells, except where findings merit separate reference for each subpopulation/lineage.

3.3 Transfer of allotype-congenic PerC into treated neonates impairs recovery of endogenous Ly-1 B cells

The recovery of endogenous Ly-1 B cells in Igh^b allotype homozygotes treated with anit-**6b** antibody fails almost completely when BALB/c congenic PerC containing Igh^a Ly-1 B cells are transferred into the treated animals during the neonatal period. Conventional B cells, in contrast, recover normally under these conditions (Fig. 3, Table 4). Furthermore, the injected BALB/c (Igh^a) Ly-1 B cells, which are unaffected by the treatment antibody, survive and permanently reconstitute the Ly-1 B lineage in the treated animals (Fig. 4; anti-6b C.B17 + BALB/c PerC). Thus, this treatment and reconstitution protocol produces chimeric animals that have endogenously derived Igh^b allotype conventional B cells and donor-derived Igh^a allotype Ly-1 B (Table 4, Fig. 3 and 4).

The effective replacement of the endogenous Ly-1 B population in allotype homozygotes requires both neonatal antibody treatment and PerC transfer. Littermates (of the treated and reconstituted animals) that were injected with antibody but not with cells had normal numbers of endogenously derived Ly-1 B cells that developed (recovered) after the injected antibody disappeared (Fig. 2 and 3). Furthermore, littermates injected with PerC but not with antibody had essentially normal numbers of endogenously derived Ly-1 B cells plus a small number (< 5%) of donor-derived Ly-1 B (Fig. 4; untreated

Table 4. Neonatal injection of BALB/c Ly-1 B cells into anti-**6b**-treated C.B17 mice permanently prevents recovery of endogenous Ly-1 B cells

Anti-**6b**[b] (mg)	BALB/c cells injected[c] Source	No. × 10^{-6}	Total[d] cells recovered × 10^{-6}	PerC from C.B17 recipients[a] Conventional B cells (host)[d] × 10^{-6}	Ly-1 B Host cells × 10^{-6}	donor cells × 10^{-6}	Serum IgM[e] host (μg/ml)	Donor (μg/ml)
1.6	Unsorted PerC	3	6	1.2	0.1	2.1	nt	nt
1.6	Sorted Ly-1⁺ B	1	6	0.8	0.1	2.5	200	600
2.0	BCL-85	2	10	0.5	< 0.1	6.5	20	< 1
1.6	Sorted Ly-1⁻ B	1.5	7	0.9	1.9	0.4[f]	nt	nt
2.0	None	–	7	1.6	2.6	–	600	–
None	None	–	8	1.5	3.0	–	600	–

a) Data shown are averaged from three mice per group analyzed at 4–5 months of age. nt = not tested.
b) C.B17 mice were injected with anti-**6b** antibody as described in Table 1.
c) On day 2 or 3 after birth, treated mice were injected with sorted or unsorted BALB/c PerC or BCL-85 cells (a clonal population of BALB/c Ly-1 B cells maintained in irradiated C.B17 mice). Injected $Ly\text{-}1^+$ and $Ly\text{-}1^-$ B cells were sorted from 3×10^6 PerC from 6-week BALB/c donors. The $Ly\text{-}1^-$ B cells contained 90% conventional B cells and 10% (approximately 1×10^5–2×10^5) Ly-1 B sister population.
d) No donor (**6b**) conventional B cells were detectable in any mice.
e) IgM levels were measured in nonimmune serum from 5-month-old mice.
f) The Igh^a cells present in the recipient C.B17 are Ly-1 B sister population as determined by expression of **6a**, **5a** and Mac-1, and represent progeny of the transferred BALB/c Ly-1 B sister cells.

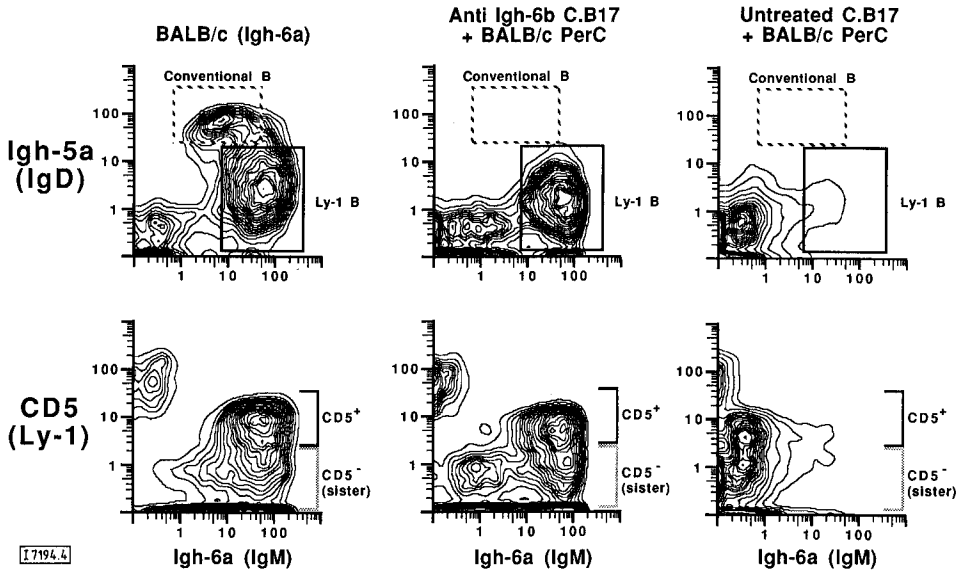

Figure 4. The effective replacement of the endogenous Ly-1 B population in allotype homozygotes requires both antibody treatment and PerC transfer. Neonatal C.B17 mice were injected with anti-**6b** and BALB/c PerC as described in Fig. 2. BALB/c PerC were also injected into normal untreated neonatal C.B17. PerC from 6-month-old treated and control mice were analyzed for B cells expressing the donor Igh^a allotypes. The various B cell populations are indicated as described in Fig. 1.

C.B17 + BALB/c PerC). Thus, antibody injected alone delays but does not block endogenous Ly-1 B development and (allotype-congenic) PerC injected alone persist at low levels but do not markedly hamper development of the endogenous Ly-1 B population.

Studies by others [11] have demonstrated the development of sizable Ly-1 B populations following transfer of PerC to untreated neonates; however, we have had little success with this protocol. In our hands, Ly-1 B cells transplant far less efficiently when transferred to untreated animals than when transferred to animals in which the endogenously derived population is disrupted either by antibody treatment (as shown here) or by irradiation [9, 10]. The reasons for these differences are unclear at present.

3.4 The Ly-1 B population reconstituted in antibody-treated neonates functions normally

The levels of serum IgM of the donor and endogenous allotypes (**6a** and **6b**, respectively) in these various mice at 5–8 months of age largely reflect the composition of the peritoneal Ly-1 B population (see Table 4). Thus, **6b** serum levels are high in antibody-treated mice that did not receive BALB/c PerC and are low in treated mice reconstituted with the PerC [which have high (**6a**) serum levels]. PerC recipients that were not treated with antibody had normal endogenous serum IgM (**6b**) levels and not detectable donor-derived **6b**, even though they had a few donor-derived Ly-1 B (data not shown). Thus, Ly-1 B function (i.e., IgM production) is largely normal in these animals, regardless of whether the Ly-1 B derive from the donor or from the treated host.

3.5 FACS-sorted Ly-1 B cells injected into antibody-treated mice impair development of endogenous Ly-1 B cells

FACS sorting studies demonstrate that the interference with endogenous Ly-1 B cell development following PerC injection into antibody-treated neonates is caused by the Ly-1 B cells in the PerC innoculum (see Table 4). Transfer of 10^6 FACS-sorted BALB/c Ly-1 B cells into neonatal C.B17 mice treated with anti-**6b** antibody reconstitutes the Ly-1 B population and completely prevents recovery of endogenous Ly-1 B cells. Transfer of the same number of FACS-sorted $CD5^-$, Ig^+ PerC, in contrast, does not detectably interfere with the development of the endogenous Ly-1 B population. Thus, the neonatal introduction of Ly-1 B cells that reconstitute a typical Ly-1 B cell population is sufficient to prevent endogenous Ly-1 B from developing in antibody-treated animals.

Further studies are required to determine how many and/or which Ly-1 B lineage cells are required to block endogenous Ly-1 B development. Although studies cited above show that endogenous Ly-1 B cells recover to normal levels in recipients of FACS-sorted $CD5^-$, Ig^+ B cells, these recipients in fact have small numbers of Ly-1 B "sister" population cells, apparently derived from the small numbers (roughly 10%) of "sister" population cells present in the sorted population. Thus, Ly-1 B "sister" cells either are incapable of preventing endogenous Ly-1 B development in antibody-treated neonates or are present at too low a frequency to be effective in these recipients.

3.6 Introduction of a monoclonal population of Ly-1 B cells prevents endogenous Ly-1 B development in antibody-treated neonates

Blocking endogenous Ly-1 B development does not require the introduction of polyclonal Ly-1 B cells. Endogenous development is also blocked by the introduction of a slow-growing, transferrable BALB/c Ly-1 B tumor, BCL-85, that we have recently isolated. This relatively benign, monoclonal tumor can grow for at least 15 months in syngeneic or congenic recipients without causing evident illness or stress. Like normal Ly-1 B cells, it tends to be free living and to localize mainly in the peritoneal cavity. It is present initially at very low levels in the spleen and only begins accumulating at higher, abnormal levels in peripheral lymphoid sites (spleen, lymph nodes, blood) after long-term growth (> 4 months).

Transfer of 10^6 BCL-85 cells into C.B17 neonates treated with a total of 1.8 mg of anti-**6b** antibody permanently establishes the BCL-85 cells in the recipients at a frequency level that is

comparable to the overall frequency of Ly-1 B cells in normal animals, *i.e.*, the tumor population represents roughly 40% of PerC in 4-month-old recipients. The presence of these cells does not impair recovery of conventional B cells in the spleen or lymph node of antibody-treated animals; however, endogenous Ly-1 B cell recovery is completely prevented. Thus, BCL-85 cells are the only Ly-1 B cells detectable in PerC in the antibody-treated animals (see Table 4).

3.7 Impaired development of endogenous Ly-1 B cells is not dependent on Ig secretion by transferred Ly-1 B cells

Ly-1 B PerC secrete high levels of serum IgM antibody upon transfer into antibody-treated allotype-congenic recipients (Table 4). The clonal Ly-1 B cells, BCL-85, in contrast, do not secrete detectable amounts of IgM (Table 4) or any other Ig isotypes (data not shown) following passage into Igh-congenic mice. Therefore, suppression of development of endogenous Ly-1 B cells following transfer of BCL-85 cells into anti-**6b**-treated neonatal C.B17 mice is not mediated by secreted Ig. Thus, the presence of mature Ly-1 B cells themselves, or of non Ig product that they secrete, is required to trigger the feedback mechanism that prevents endogenous B cell development in antibody-treated animals.

4 Discussion

In this publication, we have shown that the presence of mature Ly-1 B cells in young mice triggers a feedback mechanism that terminates the development of additional Ly-1 B cells from immature (Ig^-) progenitors. We revealed this feedback mechanisms by injecting (allotype-congenic) Ly-1 B cells into neonates whose endogenous Ly-1 B population was completely depleted by treatment with antibodies to the endogenous IgM allotype. In the absence of these exogenous Ly-1 B cells, endogenously derived Ly-1 B cells would have begun to develop almost as soon as the treatment antibody disappeared and would have reconstituted a normal-sized Ly-1 B population shortly thereafter. However, in the presence of the exogenous Ly-1 B, endogenous Ly-1 B development was blocked for the life of the animal and the only detectable Ly-1 B cells were those derived from the injected, allotype congenic cells.

In normal animals, the mechanism that mediates this experimentally induced block in Ly-1 B cell development apparently serves to terminate Ly-1 B differentiation sometimes around weaning and thus functions to restrict the composition of the (self-replenishing) Ly-1 B population in adults to progeny of those Ly-1 B cells that develop and survive through the neonatal period. This hypothesis is supported by evidence presented in the preceding publication [1], which shows that neonatal depletion of a subpopulation of Ly-1 B cells (*i.e.*, those expressing the paternal allotype in an allotype heterozygote) results in the specific depletion of that subpopulation thoughout life.

Precisely how Ly-1 B cells trigger this feedback mechanism and how Ly-1 B development is actually blocked has yet to be determined. Evidence presented here demonstrating that the introduction of a monoclonal Ly-1 B population is sufficient to block endogenous Ly-1 B development argues against the participation of multiple idiotype-anti-idiotype regulatory interactions in this process. Sensitivity to increased serum IgM levels is similarly unlikely, since the monoclonal Ly-1 B population does not secrete detectable amounts of IgM. Thus, it is possible that Ly-1 B cells produce a lymphokine that signals the end of the Ly-1 B lineage differentiation period. Alternatively, the Ly-1 B cells might physically block the sites at which Ly-1 B differentiate or might otherwise alter the local differentiative environment so as to prevent further Ly-1 B development.

The evaluation of these hypotheses are complicated by preliminary findings which suggest that the presence of Ly-1 B cells is necessary but not sufficient to block Ly-1 B development. In several experiments in which animals were treated with suboptimal doses of the anti-IgM allotype antibody, endogenous Ly-1 B cells recovered prior to, or shortly after, weaning and persisted thereafter despite the presence of sufficient numbers of exogenous (allotype-congenic) Ly-1 B cells to completely block endogenous Ly-1 B development in animals that received higher antibody doses. These findings suggest that the feedback mechanism that blocks endogenous Ly-1 B development cannot be triggered prior to weaning or perhaps prior to the onset of puberty and thus that a hormonal signal may be required to trigger the feedback mechanism in addition to the presence of sufficient numbers of mature Ly-1 B cells.

To our knowledge, feedback regulation has not been recognized in mammalian B lymphocyte development; however, this kind of developmental control has been clearly shown for avian B cells [2–4, 12, 13]. In a series of studies analogous to those presented here, Ratcliffe, Pink and co-workers [12, 13] have shown that the peripheral B lymphocyte pool, which develops initially from the bursa, is maintained in adult chickens by self-replenishing IgM^+ (post-bursal) B cells. Depletion of a subpopulation of these B cells (in allotype-heterozygous hatchlings treated with anti-allotype antibody) results in the permanent depletion of the subpopulation; however, complete depletion of the B cells (in allotype-homozygous hatchlings treated with the same antibody) permits recovery of the depleted population [1]. Again, as in the mouse, this recovery is prevented by transfer of allotype-congenic B cells to the treated hatchlings [2, 3].

The similarity between these findings in birds and our findings with Ly-1 B cells in mice suggest that Ly-1 B cells represent an evolutionarily conserved, primitive B cell lineage analogous to bursally derived B cells in birds. This hypothesis is consistent with evidence from recent studies demonstrating a bursa-like organ in sheep [14, 15] and possibly in human [16] that operates during fetal and juvenile life as a developmental site for B cells whose properties are reminiscent of murine Ly-1 B. Thus, the highly evolved immune system in mammals appears to be layered over a more primitive system that predominates and fixes its repertoire [1] in young animals but is present and retains certain key functions throughout life [8, 14, 17].

We thank Drs. D. R. Parks and D. M. Tarlinton for helpful discussions, J. Jackson (DNAX Corporation) and S. P. Klinken for identification of nucleated erythrocyte precursors and subsequent discussions; Ms S. Philips for expert assistance with animal experimentation; Mr. W. Moore for discussions on computer analyses of FACS data; and Ms L. Lloyd for preparation of these manuscripts.

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

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