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The LY-1 B Cell Lineage

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INTRODUCTION

The murine Ly-1 lymphocyte surface glycoprotein was defined initially with conventional antisera in cytotoxic assays (Cantor & Boyse 1977). As such, it appeared to be expressed exclusively on helper T cells (Cantor & Boyse 1975). Later, however, Fluorescence Activated Cell Sorter (FACS) analyses and sorting studies with monoclonal antibody reagents showed that all T cells express Ly-1, regardless of functional subclass (Ledbetter et al. 1980). Furthermore, these studies (Lanier et al. 1981a, 1981b) showed that Ly-1 is expressed on several murine B cell tumors and introduced evidence suggesting that this glycoprotein may also expressed on a small proportion of normal murine splenic B cells (Manohar et al. 1982, Hayakawa et al. 1983).

Similar studies with human lymphocytes demonstrated the homologous (Leu-1) cell surface antigen on all normal T cells (Ledbetter et al. 1981), on some B cell tumors (particularly chronic lymphocytic leukemias) (Martin et al. 1981) and, as in the mouse, on a small proportion of apparently normal B cells (Calligaris-Cappio et al. 1982). Thus, a series of earlier findings foreshadowed contemporary evidence demonstrating Ly-1 and Leu-1, respectively, on subsets of murine and human B cells and showing further that Ly-1 marks functionally distinct B cells that play a major role in autoimmunity in the mouse.

In this paper, we summarize the physical and functional characteristics that distinguish Ly-1 B cells from the majority of splenic and lymph node (conventional) B cells. We focus on data from cell transfer and antibody treatment studies, which locate Ly-1 B cells in a separate developmental lineage that branches off from the conventional lymphocyte developmental lineage during prenatal or early neonatal life. We then consider various genetic defects that influence autoantibody production and Ly-1 B representation and, finally, we discuss potential homolog-

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ies between Ly-1 B and B cells in other species and the possible role(s) that Ly-1 B cells may play as producers of anti-idiotype and other autoantibodies found in normal animals. Thus we attempt to develop a fairly comprehensive view of the Ly-1 B lineage and thereby to create an overall context within which our studies and those of others presented in this volume can be understood.

METHODOLOGY

By and large, the serological and cell transfer studies that we have conducted rely on commonly used methods that have been perfected over the last 20 years in a variety of laboratories. The FACS studies, however, utilize certain unique methods and instrumentation developed quite recently in our laboratory, partly in response to needs generated by the B cell studies discussed here. In particular, we have relied heavily on the advanced capabilities provided by our six-parameter dual-laser FACS instrument (Parks et al. 1985) which is coupled to a VAX computer running FACS-DESK software written (in our laboratory) to enable rapid and efficient multiparameter FACS data collection, storage and processing (Moore & Kautz 1985). The availiability of this instrumentation and software allows us to routinely analyze and sort doubly- or triply-stained cells from large numbers of samples and to reliably detect minor subpopulations, even those containing only 0.5 to 1% of total live cells in the sample.

The FACS-DESK software provides novel types of contour maps that significantly improved the display of correlated multiparameter immunofluorescence staining data (Moore & Kautz 1985). In particular, we make intensive use of probability contour maps, which tend to emphasize subpopulations in proportion to their representation in the overall population. These contour maps, used to display virtually all correlated immunofluorescence data in our publications, tend to be more visually reliable than the maps (generated with commercial software) in standard use, in which the number of contours drawn around a peak is proportional to its height rather than to the number of cells it encompasses.

The smoothing method FACS-DESK uses when drawing the contour maps shown in our studies also improves the reliability and utility of the data display (Moore & Kautz 1985). In essence, use of this method minimizes the influence of statistical variation on the location of contours so that contour lines tend to have fewer random jagged edges. This substantially reduces distracting visual "noise" in the display and thus greatly facilitates the recognition and comparison of contour patterns that delineate subpopulations in the various analyses. The recognition and functional characterization of the minute Ly-1 B subpopulation in normal spleen (see Figure 3) attests to the validity of the smoothing and contouring algorithsm used in these studies.

Finally, the recent development of new, highly efficient fluorochromes that have suitable excitation and emission spectra for multiparameter analyses and can readily be coupled to monoclonal antibodies opened the way to effective three-color and four-color immunofluorescence studies (Parks et al. 1984, Hardy et al. 1984a). The availability of these plant-derived (phycobiliprotein) fluorochromes (Hardy 1986) and a multiparameter FACS system on which they could be utilized proved extremely important for our studies because it enabled the direct detection of cell surface phenotypes that could only be inferred from two-color analyses (Hardy et al. 1984a).

Definition of the Ly-1 B lineage

The extent of the differences between Ly-1 B cells and conventional B cells is sufficient in itself to suggest that the Ly-1 B belong to a separate developmental lineage. The Ly-1 B cells have a unique surface phenotype: in addition to being identifiable because they express Ly-1, they can be recognized because they express distinctive levels of several standard B cell surface markers, e.g., IgM, IgD, B220. Furthermore, they show a distinctly different localization pattern from conventional B cells in that they are present at quite high frequencies in the peritoneal cavity in normal animals but tend to be rare in spleen and undetectable in lymph nodes (Hayakawa et al. 1983, 1984, 1985, 1986a, 1986b, Hardy et al. 1984, 1986b, Stall et al. 1986, Sidman et al. 1985, Davidson et al. 1984).

B cell frequency differences in normal and immunologically defective mice also point to a basic distinction between Ly-1 B and conventional B cells (Hayakawa et al. 1983). By and large, individual normal and immunodeficient mouse strains show characteristic, genetically controlled Ly-1 B frequencies that vary independently of other B cell frequencies. In general, immunologically normal mouse strains have roughly the same number of Ly-1 B cells. Immunodefective strains, in contrast, tend to have either markedly elevated (e.g., NZB and motheaten viable mice) or markedly depressed Ly-1 B frequencies (e.g., CBA/N and other X-id strains) (Hayakawa et al. 1983, 1986; Sidman et al. 1986). Similar frequency abnormalities can be induced in normal mice by treating neonates with monoclonal anti-Ig antibodies. Such treatments result in the selective depletion or selective survival of the Ly-1 B cell population (depending on the specificity of the reagent), sometimes for the life time of the animal (manuscript in preparation).

Ontologically, Ly-1 B cells also present a substantially different picture (Hayakawa et al. 1983). They are among the earliest B cells detectable in spleen; however, their predominance recedes as conventional B cell populations develop and fill the spleen. Thus, by 6 wk of age, they represent less than 2% of total splenic B cells.

Finally, functional studies distinguish Ly-1 B from conventional B cells. Ly-1 B produce many of the commonly studied IgM autoantibodies in autoimmune mice. Furthermore, recent data suggest that they may be responsible for producing much of the autoantibody (Hayakawa et al. 1984) and anti-idiotype antibodies produced by normal animals (Vakil & Kearney 1986). Conventional B cells, in

contrast, tend to participate much less frequently in autoimmune responses and, instead, appear to be concerned principally with producing antibodies to exogenously introduced antigens (Hayakawa et al. 1984).

This extensive series of differences (discussed in more detail below) provides both the rationale and the tools for cell transfer studies that demonstrate directly that Ly-1 B cells belong to a distinct developmental lineage (Hayakawa et al. 1985). In essence, these studies identify the donor-derived B cell populations present in irradiated recipients reconstituted with progenitors from various sources. They show that progenitors for Ly-1 B cells are mainly found in the peritoneum and carry surface Ig, whereas progenitors that reconstitute conventional B cell populations typically lack surface Ig and are found in bone marrow rather than in peritoneum. We review this evidence, which dissociates Ly-1 B progenitors from the traditional B cell progenitors in bone marrow, in the sections that follow.

Ly-1 B cell characteristics

Ly-1 B cells express all of the usual B cell surface markers (in conjunction with Ly-1). Several of these markers, however, are expressed at levels that are substantially different from the levels found on most splenic and lymph node B cells (see Fig. 1). IgM, for example, tends to be 5- to 10-fold higher on Ly-1 B cells. IgD, in contrast, tends to be 10- to 20-fold lower. Ia, ThB and certain B220 determinants also show distinct (although less dramatic) level differences; and FACS light scatter levels similarly show small but clear differences (Fig. 2). None of these markers is individually sufficient to resolve Ly-1 B cells definitively; however, when used together in two-color and three-color FACS studies, they clearly delineate the Ly-1 B and other B cell populations for analysis and sorting.

Cytotoxic treatment with monoclonal anti-Ly-1 antibodies (particularly C3PO) have also been used to identify Ly-1 B cells (Bishop & Haughton 1985). In some studies, this method has been used to deplete Ly-1 B cells from splenic B cell populations in order to find out whether Ly-1 B participate in or influence a particular antibody response (Okumura et al. 1982, Sherr & Dorf 1984). In principle, direct analysis and sorting of Ly-1 B cells would be more satisfactory for these purposes; however, these studies clearly demonstrate that cytotoxic depletion analysis represents a viable alternative when the need for many cells or the lack of FACS facilities mitigates against FACS use.

Hemolytic plaque forming cell (PFC) assays that reveal cells producing autoantibodies to mouse erythrocytes treated with the enzyme bromelain (BrMRBC) have proven useful for detecting the presence of Ly-1 B cells, even when there are too few Ly-1 B cells present to reliably detect by FACS analyses. The failure to detect the anti-BrMRBC PFC does not necessarily mean that Ly-1 B are missing; however, in our experience, these PFC provide a reliable indicator for

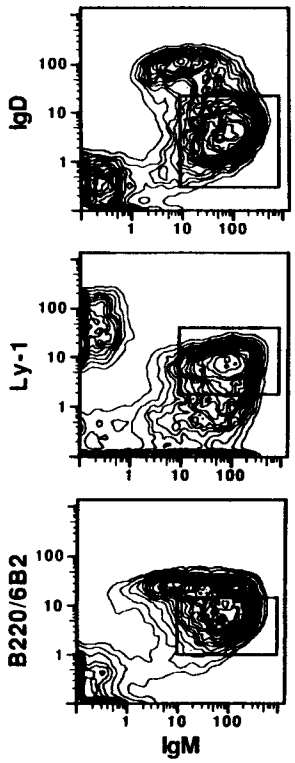

Figure 1. Cell surface phenotype of peritoneal Ly-1 B cells (revealed by three-color FACS analyses). Peritoneal cells from 3-month-old BALB/c mice were stained simultaneously with 3 monoclonal antibodies: anti-IgM, 331.12/FITC; anti-Ly-1, 53-7.6/allophycocyanin; anti-B220/6B2, RA3-6B2/biotin or an anti-IgD allotype antibody, anti-Igh-5a, AMS 15.1/biotin. Biotin-conjugated antibodies were revealed by Texas-Red/avidin. Dead cells were excluded by propidium iodide staining. All analyses were conducted as previously described (Hardy et al. 1984a, Parks 1986). The box in each panel delineates the Ly-1 B population.

Ly-1 B presence since 1) they are all contained within the Ly-1 B population (Hayakawa et al. 1984); 2) they are always present at reasonable frequencies in (LPS-stimulated) animals that have normal numbers of Ly-1 B cells; and, 3) they are always either undetectable or reduced in frequency in Ly-1 B-deficient animals (manuscript in preparation).

Ly-1 B location in vivo

Analysis of B cell frequencies in various lymphoid organs demonstrates that Ly-1 B cells localize quite differently from conventional B cells (Hayakawa et al.

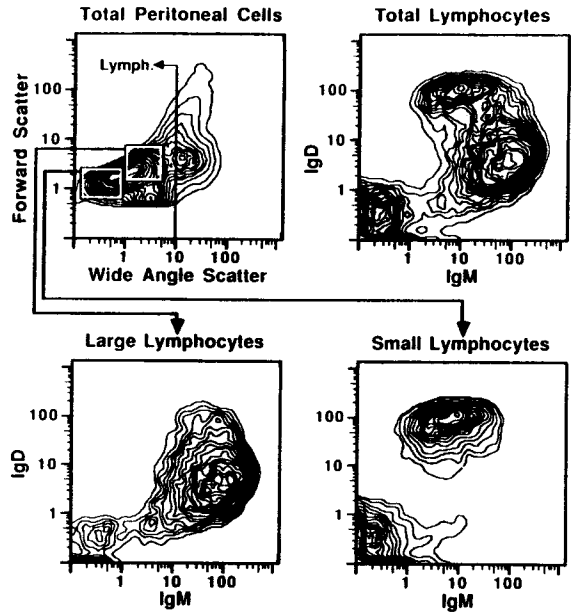

Figure 2. Forward angle and wide angle light scatter distinguish Ly-1 B cells. Peritoneal cells from a 3-month-old BALB/c mouse were stained for surface IgM (331.12/FITC) and IgD (AMS 15.1/biotin). The gated contour maps shown in the bottom two panels represent the IgM vs IgD distribution of the cells within the scatter gates depicted by the boxes shown in the upper left hand panel. All Ly-1 B cells in the analyzed peritoneal population are contained within the large lymphocyte population whose IgM vs IgD distribution is shown in the lower left panel. The Ly-1 B cells represent approximately 80% of this population. The remaining cells represent a related Ly-1⁻ population discussed later in the text.

1983, 1986a). In general, Ly-1 B are barely detectable in spleen (Fig. 3) and are almost never found in lymph nodes (except in animals with advanced autoimmune disease). Furthermore, they represent less than 1% of the total B cell population in a normal adult mouse. Nevertheless, they frequently comprise more than half the floating and loosely-attached B cells recoverable from the peritoneum (20–40% of total).

This concentration in the peritoneum appears to be due to the operation of a homing mechanism, since injecting irradiated animals intravenously with allotype congenic Ly-1 B cells results in the specific accumulation of donor-derived Ly-1 B cells in the peritoneum shortly after injection (Hayakawa, unpublished data). These cells gradually disappear and are no longer detectable a week to 10 days later. Their relationship to the reconstituted peritoneal Ly-1 B populations that become visible 3 to 4 wk after transfer has yet to be determined.

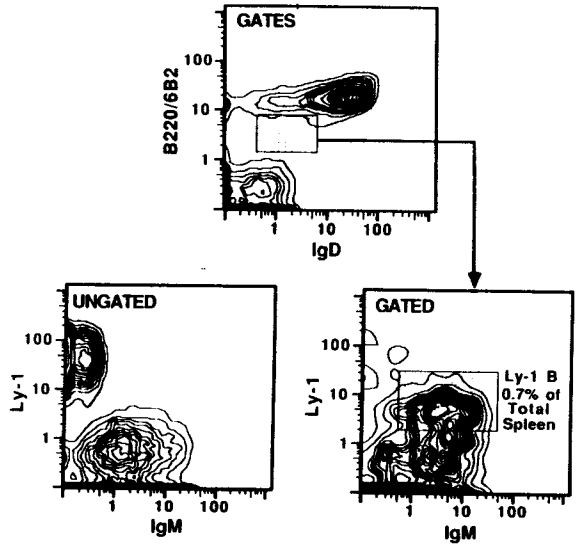

Figure 3. Cell surface phenotype of splenic Ly-1 B cells defined by four-color FACS analysis. Spleen cells from a 3-month-old BALB/c were simultaneously stained with 4 reagents: anti-IgM, 331.12/FITC); anti-Igh-5a (IgD), AMS-15.1/biotin; anti-Ly-1, 53-7.6/allophycocyanin; and anti-B220/6B2, RA3-6B2/phycoerythrin. The "ungated" contour map (lower left) shows the Ly-1 vs IgM distribution for all splenic lymphocytes. The "gated" contour map (lower right) shows the Ly-1 vs IgM profile for the cells that fall within the gates depicted by the shaded box in the B220 vs IgD contour map (upper panel).

The anatomical location of the small number of Ly-1 B cells found in normal spleen also remains to be determined; however, it is possible that these cells reside in or near the marginal zones that separate the white pulp and red pulp areas of the spleen. We make this suggestion based on the description, by McClennan, Gray, Bazin and co-workers, of two apparently distinct B cell lineages in the rat that exist in anatomically distinct locations in the spleen (Kumararatne et al. 1981, Bazin et al. 1982, MacLennan et al. 1982, Gray et al. 1984). The cells from one of these putative lineages, located mainly in the splenic marginal zone, appear quite similar to Ly-1 B with respect to Ig surface phenotype and several other properties (see Table I). Thus it is possible that the anatomical location of these cells (in the rat spleen) predicts the anatomical location of the mouse Ly-1 B population, i.e., in the marginal zone of the mouse spleen.

Potential Ly-1 B cell homologs in other species

In addition to the rat B cell population(s) discussed above, there is a human B cell population that appears to be homologous to the murine Ly-1 B lineage

TABLE I

Characteristics shared by murine LY-1 B cells and rat marginal zone B cells

Phenotype	Bright for IgM, low-to-negative for IgD. Slightly larger than bright IgD$^+$ follicular B cells
Organ Location	Present in spleen but not nodes
Ontogeny	Develop early in post-natal life
Precursors	Distinct from precursors for bright IgD$^+$ B cells that populate follicles in spleen and nodes
Antibody	Depleted by treatment of neonates with anti-IgM but persist at normal levels in anti-IgD-treated animals

(Calligaris-Cappio et al. 1982). This human population carries the Leu-1 antigen, which is structurally homologous to Ly-1 (S. Huang, manuscript in preparation). It has been associated with the production of a variety of autoantibodies (e.g., cold hemagglutinins, cytoskeletal antibodies, rheumatoid factor) (Bofill et al. 1985). Furthermore, it has been shown to be elevated in a proportion of patients with rheumatoid arthritis (Plater-Zyberk 1985; Hardy et al., personal communication; Taniguchi and Okumura, personal communication).

There is also reasonable evidence suggesting that Ly-1 B may be homologous to the predominant B cell population in chickens (see discussion of Pink et al. 1985 in section labelled B Cell Development Pathways below).

Ly-1 B cell reconstitution

Adult bone marrow transfers are commonly thought to reconstitute all B cell populations in lethally irradiated recipients. However, our studies show that although such transfers routinely reconstitute the predominant B cell populations in spleen and lymph node, they frequently fail to reconstitute even minimal numbers of Ly-1 B cells in transfer recipients (Fig. 4). This failure appears to be due to a deficit in Ly-1 B progenitors in the bone marrow, since co-transfer experiments combining allotype congenic progenitors from various sources fail to reveal any evidence suggesting that regulatory influences are responsible for the selectivity of these reconstitutions. In all cases, progenitors from a given source (adult PerC, adult BM, neonatal liver) quantitatively and qualitatively reconstitute the same B cell populations whether transferred alone or co-transferred with progenitors from a second (allotype congenic) source. Furthermore, the reconstituted populations persist in co-transfer recipients at essentially normal proportions for at least 6 months after transfer (Hayakawa et al. 1985).

Occasionally, we obtain an adult bone marrow suspension that either partially or fully reconstitutes the recipient Ly-1 B population. We have been unable to

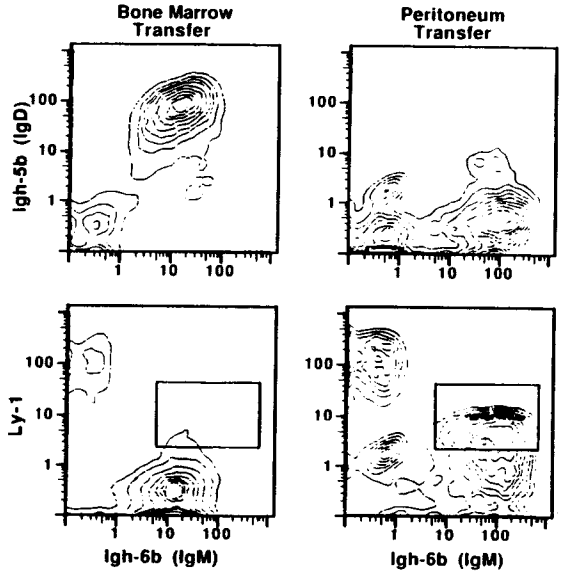

Figure 4. Peritoneal cells selectively reconstitute Ly-1 B cells. 10^7 bone marrow (BM) cells or 10^7 peritoneal cells (PerC) from 12-week-old BAB/14 (Igh^b) mice were transferred iv to 600R irradiated BALB/C (Igh^a) mice. One month after transfer, recipient PerC were analyzed for donor-derived (Igh^b) B cells (from Hayakawa et al. 1985).

identify the conditions required to reproducibly obtain such suspensions (or to reproducibly obtain suspensions that do not repopulate Ly-1 B). However, since bone marrow suspensions that successfully reconstitute Ly-1 B generally do so to the same extent in all recipients, we believe that the sporadic Ly-1 B reconstitution we have observed is due to variations in the frequency of functional Ly-1 B progenitors, either in the marrow cell suspensions or in the donor marrow itself (A. M. Stall, unpublished observations).

These difficulties in reconstituting Ly-1 B cells by transferring adult bone marrow cells to irradiated recipients contrast sharply with the complete and highly reproducible Ly-1 B reconstitution that occurs whenever recipients are given 3 million or more (allotype congenic) neonatal spleen cells, neonatal bone marrow cells (from animals less that 6 wk of age) or adult peritoneal cells. In all cases, Ly-1 B populations are reconstituted to normal levels with allotype-marked cells that identify the donor from which they came (see Fig. 4).

Host type Ly-1 B cells that survive the irradiation (600–850 R) and gradually reproduce as the animals age can sometimes be found in recipients, particularly 6 months or longer after transfer (A. M. Stall, unpublished observations). Simi-

larly, Ly-1 B cells derived from progenitors in the host-type bone marrow used to supplement the peritoneal cell transfers (to restore hemopoiesis) are sometimes detectable. However, the Ly-1 B progenitors present in the peritoneum (or in neonatal lymphoid organs) are clearly the most effective in reconstituting Ly-1 B cells.

Surface Ig on Ly-1 B progenitors

Recent FACS sorting and transfer studies show that the Ly-1 B progenitors present in adult peritoneum carry both Ly-1 and surface Ig and thus are contained within the Ly-1 B population itself (Hayakawa et al. 1985, 1986b). In addition, preliminary evidence indicates that progenitors that express lambda light chains mainly (or perhaps exclusively) reconstitute Ly-1 B populations that express lambda, and progenitors that express kappa light chains mainly reconstitute cells that express kappa. Studies with neonatal B cells similarly reveal the presence of Ig-bearing progenitors for Ly-1 B cells; however, neonatal spleen also contains some Ly-1 B progenitors that lack surface Ig (Hayakawa et al. 1986b)

These results, coupled with the relatively poor yield of Ly-1 B cells per progenitor transferred, raise some key questions about the nature of the reconstitution process. In general, the number of Ly-1 B cells in fully reconstituted recipients is no more than 2- to 4-fold greater than the number injected initially. Thus, if all of the Ly-1 B cells we inject were to survive and be detectable 6 months or longer after transfer, the reconstitution would require very little replication on the part of the progenitors.

This hypothesis is probably too extreme for several reasons. First, large numbers of transferred cells usually wind up trapped in the lungs and liver of the recipient. Thus the effective number of progenitors transferred is usually quite a bit lower than the actual number. Secondly, the transferred cells would have to temporarily reside in sites where they cannot be detected, since virtually no donor-derived Ly-1 B cells (or other B cells) are detectable in the recipient for a period of about 2 wk, i.e., between the time that the injected cells disappear from the peritoneum and the time that the permanently reconstituted Ly-1 B population begins to emerge. Thirdly, several Ly-1 B cell lines that growh indefinitely *in vitro* have been established, indicating that Ly-1 B cells are clearly not just end stage cells that cannot divide. And finally, certain donor/host strain combinations (e.g., CBA into CBA/N) tend to show more expansion of the Ly-1 B population than other combinations. Hopefully, current studies will resolve this question shortly.

A second peritoneal B cell lineage?

While characterizing the Ly-1 B population and its (Ly-1 B) progenitors in the peritoneum, we often noted the presence of small numbers of peritoneal B cells

that had virtually the same phenotype as Ly-1 B but did not appear to express the Ly-1 surface molecue. In transfer experiments, these cells were reconstituted whenever Ly-1 B were reconstituted and usually failed to be reconstituted when Ly-1 B reconstitution failed (e.g., in bone marrow recipients). Thus, since Ly-1 staining with the reagents available at the time was dull even on the Ly-1 B cells that expressed the highest levels of Ly-1, we took a conservative view and treated these apparently Ly-1$^-$ cells as Ly-1 B cells that made up the dull end of the Ly-1 staining continuum.

Unfortunately, evidence from preliminary studies now indicates that this hypothesis is incorrect. One of us (C.S.) notes that although motheaten mice have large numbers of Ly-1 B cells, they lack virually all Ly-1$^-$ cells. Furthermore, the phycobiliprotein-conjugated anti-Ly-1 staining reagent that we presently use detects Ly-1 almost 10 times as well as our previous reagents and clearly resolves the Ly-1$^+$ B cell population from its Ly-1$^-$ "sisters" (which still fail to show even a trace of surface Ly-1).

Finally, preliminary evidence from a new series of transfer recipients given FACS-sorted Ly-1$^-$ or Ly-1$^+$ peritoneal B cells (IgM$^+$) indicates that each of these cell populations selectively reconstitutes itself (Fig. 5). Thus the peritoneum most likely contains two similar but relatively independent B cell populations that are reconstituted by what appear to be self-renewing progenitors that display the Ly-1 status of the population they will reconstitute (and from which they were drawn in the donor).

B CELL DEVELOPMENT PATHWAYS: A BROADER FORMULATION

The repopulation of lymphoid organs depleted by irradiation or drug treatment has been studied extensively for more than two decades. Not surprisingly, the consensus view of B cell development is based largely on the properties of BM-derived B cells (since these cells represent more than 99% of the total B cell population in normal adult mice). By and large, therefore, IgM-bearing lymphocytes are treated as relatively mature cells that have a limited capacity for self-renewal (except when triggered by antigen) and must continually be replenished from self-renewing Ig$^-$ progenitors present in spleen and bone marrow in adults. This formulation accurately reflects the developmental behavior of the conventional BM-derived B cell populations in mice; however, as we have shown here, rules for replenishing the murine Ly-1 B lineage are clearly different.

Reconstitution studies in birds also challenge the generality of the current B cell development paradigm. Pink and colleagues have recently shown that B cell progenitors in the bursae of young normal chickens carry surface IgM. These progenitors, which fully and permanently reconstitute B cells in cyclophosphamide-treated neonatal chickens, disappear from the bursa as the birds age, so that "... the sole source of B cells in adult birds (becomes) a pool of self-renewing,

IgM-bearing B cells with the capacity to proliferate in peripheral organs ..." (Pink et al. 1985). Thus the surprising reconstitution capability that we have demonstrated for Ly-1 B progenitors in mice may well reflect the isolated use in mammals of a self-renewal capability that is used generally to maintain B cell populations in birds (and perhaps other evolutionarily primitive species).

Ly-1 B frequencies in immunodefective mice

Our initial studies with Ly-1 B cells demonstrated that CBA/N and other mice affected by the X-linked immunodeficiency (X-id) lack Ly-1 B cells, while NZB and related autoimmune mice have excessive numbers of Ly-1 B cells in spleen and peritoneum (Hayakawa et al. 1983, 1986a). Extending these studies (partly in collaboration with Leonard Schultz, Jackson Laboratory, Bar Harbor) we have now shown the following:

1. Motheaten viable mice have large numbers of Ly-1 B cells and lack virtually all other (conventional) B cells (Sidman et al. 1985);
2. NZB-related autoimmune mice have large numbers of Ly-1 B cells but also have relatively normal numbers of conventional B cells (Hayakawa et al. 1983);
3. BALB/C and BALB/C-congenic mice tend to have somewhat elevated peritoneal Ly-1 B frequencies in comparison with most other normal mice (A. M. Stall, unpublished observation);
4. CBA, C3H and C57BL/10 have roughly similar Ly-1 B frequencies and, as such, can be taken as the normal phenotype (Hayakawa et al. 1983, 1986a);
5. SJL and SJL-congenic mice have substantially fewer than normal Ly-1 B cells as neonates and show progressively lower Ly-1 B frequencies with age (occasionally losing all detectable Ly-1 B in the peritoneum) (P. Lalor, unpublished observation);
6. BALB/C X SJL hybrids start life with relatively normal Ly-1 B frequencies but these frequencies drop dramatically with age and occasionally fall below detectablility (as in SJL) (P. Lalor, unpublished observation); and,
7. CBA/N and certain DBA/2Ha mice have no detectable Ly-1 B peritoneum or in spleen (Hayakawa et al. 1986a, Hayakawa, unpublished observation).

Ly-1 B deletion in anti-Ig-treated mice

Treating neonatal animals with antibodies to Ig heavy and/or light chain determinants has long been known deplete B cells, to reduce serum Ig levels, to interfere with antibody production and to induce chronic allotype suppression (in BALB/C X SJL mice) (Moller 1980, Black & Herzenberg 1979, Tokuhisa et al. 1981). Results from our current studies in this area, summarized in Figs. 6 and 7, demonstrate striking differences in the effects of treatment of neonatal mice with

monoclonal anti-IgM and anti-IgD antibodies. In particular, anti-IgM depletes all B cells, whereas anti-IgD depletes greater than 90% of the B cells in the animal but leaves the Ly-1 B population and some other minor B cell population(s) intact.

B cell populations in treated animals analyzed after the neonatally injected monoclonal antibody has disappeared from the circulation also demonstrate differences between anti-IgM and anti-IgD treatments. That is, anti-IgM (anti-Igh-6b) permanently depletes the Igh-6b$^+$ Ly-1 B cell population in Igh allotype heterozygotes (see Fig. 7) and selectively retards the recovery of this population

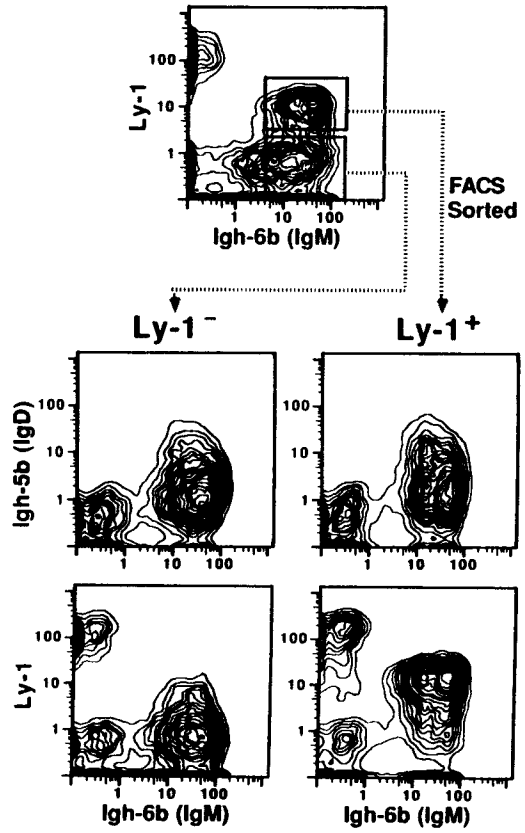

Figure 5. Ly-1 B and its Ly-1$^-$ sister population in the peritoneum selectively reconstitute themselves. CBA/bb (Igh^b) cells falling within the gates depicted in the upper panel were sorted and transferred to 850R irradiated CBA/N (Igh^b) recipients supplemented with CBA/N bone marrow cells. Recipients each received the yield from approximately 5×10^6 FACS-sorted peritoneal cells. The bottom four panels show the peritoneal lymphocyte populations in recipients analyzed 2 months after transfer.

in homozygotes. Anti-IgD, in contrast, shows no detectable effects that outlast its presence in the animal (P. Lalor, unpublished observation).

Autoantibody production by Ly-1 B cells

Our initial studies showed that Ly-1 B cells are responsible for producing most of the spontaneously secreted IgM and at least several of the IgM autoantibodies (NTA, ssDNA) produced by spleen cells from mice affected with the NZB autoimmune disease (Hayakawa et al. 1984). In addition, these studies showed that the Ly-1 B population contains virtually all of the IgM anti-BrMRBC PFC detectable in NZB-related mice and in normal (not autoimmune) animals but contains few (if any) cells that produce antibodies to typical exogenous antigens, e.g., DNP. These latter antibody producing cells are found in a different B cell population (low for IgM, negative for IgD) (Hayakawa et al. 1984).

This evidence is consistent with recent data from several laboratories (Kearney & Vakil 1986, Holmberg et al. 1986) demonstrating (collectively) that neonatal spleen and adult peritoneum are highly enriched for cells that fuse to make hydridomas producing antibodies that react with idiotypes and other self constituents. Since these hydridomas do not express detectable levels of surface Ly-1, their origin is still in doubt; however, Bona and co-workers have shown that spleens from motheaten mice, which are enriched for Ly-1 B cells, clearly provide an efficient source for generating hybridomas that produce autoantibodies. Thus,

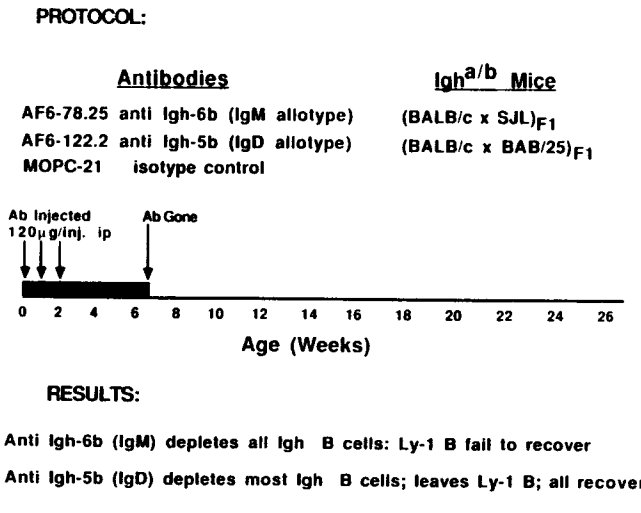

Figure 6. Neonatal treatment of Igh$^{a/b}$ allotype heterozygous mice with monoclonal antibodies to allotypic determinants on IgM (Igh-6b) and IgD (Igh-5b).

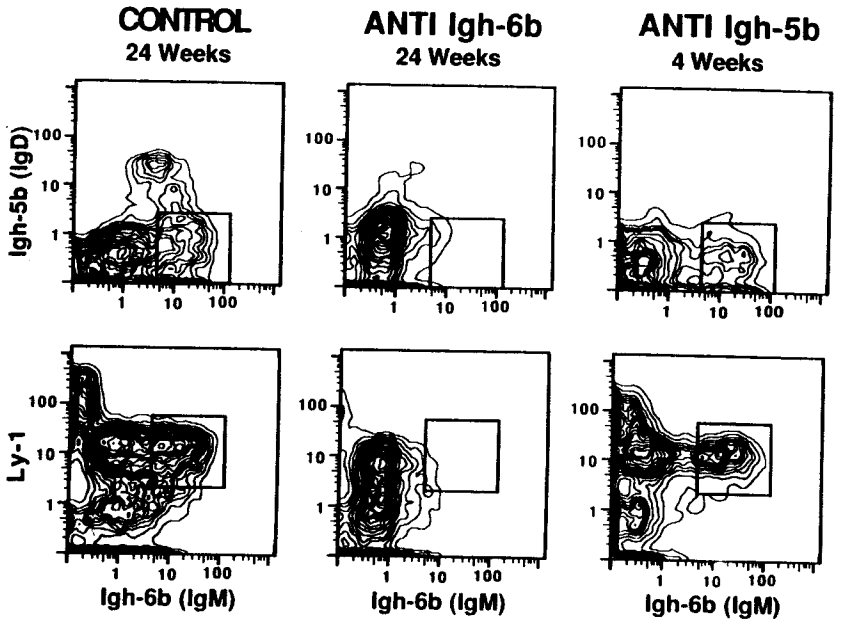

Figure 7. Differential effects of neonatal anti-Igh-6b (IgM) and anti-Igh-5b (IgD) treatments on the development of peritoneal Ly-1 B cells in (BALB/c × BAB/25)F1 allotype heterozygous mice. Mice were treated with antibodies to Igh^b allotypes according to the protocol shown in Figure 6. Peritoneal cells from treated animals were analyzed at the indicated times. The location of the Igh^b allotype Ly-1 B cells is shown by the boxes in the panels.

at a functional level, Ly-1 B cells emerge as the principal producers of several commonly studied autoantibodies and perhaps the major producers of natural antibodies that tend to recognize self consitutents and of "high-connectivity" antibodies that tend to recognize broadly expressed Ig idiotypes.

Serum Ig production by Ly-1 B cells

Studies with mouse strains expressing various immunoregulatory defects (e.g., motheaten viable, X-id, NZB) reveal a loose correlation between serum IgM levels and the frequency of Ly-1 B cells. That is, strains that have high Ly-1 B frequencies generally have elevated serum IgM levels, while strains that have low Ly-1 B levels tend to have low serum IgM levels. This correlation suggests that Ly-1 B may play an important role in maintaining IgM serum levels in normal mice (Sidman et al. 1985, Stall et al. 1986).

Evidence from preliminary studies of serum Ig levels in peritoneal cell transfer

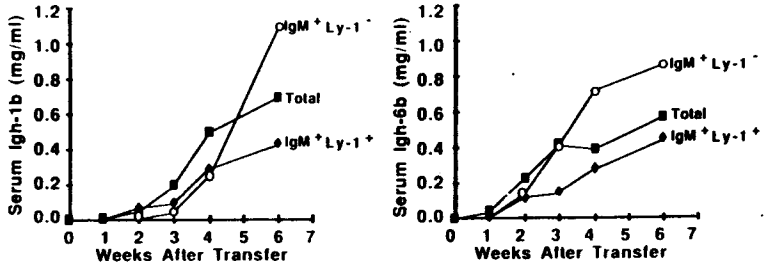

Figure 8. Serum Ig production by reconstituted Ly-1 B populations. Sera from irradiated CBA/N mice reconstituted with FACS-sorted CBA/bb cells (in the experiment shown in Figure 5) were pooled and analyzed at the indicated times for donor-derived IgM and IgG2a by RIA (Newby et al. 1986). Serum levels are shown for mice that recieved 5×10^6 unsorted cells, 0.5×10^6 IgM + Ly-1 + cells or 1.5×10^6 IgM$^+$Ly-1$^-$ cells.

recipients strongly supports this hypothesis (Fig. 8). In essence, these studies show that the peritoneal cells that reconstitute Ly-1 B populations in the allotype congenic recipients also reconstitute cells that produce significant amounts of IgM and IgG. Serum levels of Igh-6b, the IgM allotype produced by the Ly-1 B donor, rise rapidly after transfer and soon approximate the IgM levels maintained in normal adults. Serum levels of Igh-1b, the IgG2a allotype of the Ly-1 B donor, also tend to rise rapidly and to be maintained at approximately normal adult levels. Furthermore, other IgG isotypes are often produced in addition to (or instead of) IgG2a.

Transferring either FACS-sorted Ly-1 B cells or the FACS-sorted Ly-1$^-$ sister population from the peritoneum similarly results in the production of relatively large amounts of serum IgM and IgG marked with the peritoneal cell donor allotype. Furthermore, the level of serum Ig reached in recipients of the sorted cells is essentially equivalent to the level in recipients of the intact, unsorted peritoneal population which includes both IgM$^+$ and IgM$^-$ B cells (Fig. 8).

Significantly, however, IgG primary and secondary antibody responses to typical T-dependent antigens in reconstituted animals appear to be produced primarily by conventional B cells derived from BM donor. For example, in a recent study we found that, although both primary and secondary IgG responses to NP-KLH reached normal levels in animals reconsituted with bone marrow and allotype congenic peritoneal cells, less than 10% of the primary and virtually none of the secondary IgG response was produced by cells derived from the peritoneal donor (manuscript in preparation). Thus, Ly-1 B cells do not appear to participate in typical T-dependent primary and secondary antibody responses even though they appear to produce much of the serum Ig found in animals reconstituted with peritoneal B cells.

These findings (still incomplete), and a similar correlation between Ly-1 B presence and IgM production in animals treated neonatally with anti-IgM and anti-IgD (manuscript in preparation) suggest that *in situ* Ly-1 B cells and their Ly-1 sister population may play a far more important role in serum Ig production than their numbers would indicate. If so, then the old idea (Dresser 1978, Steele & Cunningham 1978) that serum Ig in healthy animals consists mainly of autoantibodies and anti-idiotype antibodies may not be so far off the mark (given the functional properties of Ly-1 B cells that we have charted so far). This would certainly recommend attention to the basic concepts of the Jerne network theory, which has sometimes been deemed untenable because of the large number of apparently useless antibodies that would have to be produced to maintain the network. Perhaps the commitment of an entire B cell lineage to the maintenance of what might be termed internal images is the price that has to be paid for the regulatory capabilities inherent in the mammalian immune system.

Ig light chain expression in Ly-1 B cells

The proportion of B cells expressing lambda light chains is significantly higher (10–20%) in the peritoneal Ly-1 B population and in its Ly-1$^-$ "sister" population than in the other B cell populations found in spleen or peritoneum (Hayakawa et al. 1986a). This is consistent with evidence demonstrating lambda (rather than kappa) light chain expression in a variety of Ly-1 B tumors (Davidson et al. 1984) and *in vitro* cell lines (Braun 1983). Curiously, in one of the tumors (NFS-5), kappa and lambda expression on the cell surface alternate as cells are grown alternately in the presence or absence of LPS (Hardy et al. 1986b). This switch in light chain expression does not occur frequently amongst the adult peritoneal Ly-1 B cells that repopulate irradiated recipients since FACS-sorted kappa$^+$ cells transferred to such recipients give rise almost exclusively to kappa$^+$ Ly-1 B cells and FACS-sorted kappa$^-$ cells give rise predominantly to lambda$^+$ Ly-1 B cells (Hayakawa et al. 1986).

V_H gene expression in Ly-1 B cells

Although hybridomas, myelomas and normal splenic and lymph node B cells tend to express V_H genes from the J558 V_H family, several laboratories (Kearney, Holmberg, Bona, personal communication) have shown that hybridomas generated from sources enriched for Ly-1 B cells (neonatal spleen, adult peritoneum) frequently express V_H genes belonging to the 7183, Q52 and S-107 V_H gene families, which lie more proximal to the IgC_H region than the J558 family (Brodeur et al. 1984).

These proximal V_H genes also tend to be expressed frequently in hybridomas and Abelson pre-B cell tumors generated from prenatal and neonatal lymphoid sources (Yancopoulos et al. 1984, Perlmutter et al. 1985). Such tumors, which often express surface Ly-1, may be derived largely from cells in the Ly-1 B lineage.

since these cells apparently represent a fair proportion of the pre-B cells present during embryonic and early post-natal life. Thus, on average, Ly-1 B cells may tend to utilize a wider spectrum of V_H gene families but a more restricted spectrum of V_H genes than are utilized by the predominant B cell populations in adult spleen and lymph nodes.

Recent studies with Ly-1 B tumors have also revealed the existence of a mechanism that potentially would allow the replacement of V_H genes in cells that already possess a functional VDJ rearrangement and have initiated production of the Ig heavy chain specified by that rearrangement. In essence, these studies show that the existing, already rearranged, V_H gene on the active IgH chromosome can be replaced by a more distal V_H gene, which will then be expressed in the IgH chains produced by the cell (Kleinfield et al. 1986). At present, we and our co-workers have found 2 such V_H replacements on the active chromosome in the NFS-5 tumor cell line and another 5 such replacements on the inactive chromosome (D. Tarlinton, R. Kleinfield, unpublished observation).

There are currently no data that bear on the question of whether normal Ly-1 B cells (or other B cells) utilize this V_H gene replacement mechanism during development. If it were used in neonates, it might explain the puzzling entrance of progressively more distal V_H genes into the functionally rearranged V_H gene pool during the first few days of life (Yancopoulos et al. 1984); however, other (more traditional) mechanisms could also explain this observation.

COMMENTARY

Deadlines for reviews such as these invariably arrive just as the most interesting questions seem about to be resolved. Studies completed to date (discussed above) clearly establish the existence of two distinct B cell lineages, one that is consistently reconstituted by bone marrow transfers and a second that is consistently reconstituted by peritoneal cells and only sporadically reconstituted by bone marrow cells from adults. In addition, these studies show that the lineages differ with respect to cell size, cell surface phenotype, organ location, frequency elevations or deficits in immunodefective mice, differential sensitivity to neonatal anti-Ig treatments and the frequency of lambda light chain expression.

At a functional level, earlier studies revealed a "division of labor" between the lineages such that one (Ly-1 B) was found to be responsible mainly for producing IgM autoantibodies whereas the other (predomdinant in spleen and lymph nodes) was found to be responsible mainly for producing IgM responses to exogenous antigens. Preliminary results from ongoing cell sorting and transfer studies (outlined above) confirm and extend this formulation. These results indicate 1) that Ly-1 B and it Ly-1 sister population produce IgG in addition to IgM; 2) that these cells produce a large proportion of the serum IgM and IgG in transf recipients; but, 3) that despite their apparent ability to produce relatively lare amounts of IgG, they participate minimally in primary responses to NP-KLHn transfer recipients and do not detectably participate in secondary responses o this typical T-dependent antigen.

Evidence suggesting that the spectrum of V_H genes used by Ly-1 B cells ad their sister population may be different from the spectrum used by conventiol B cells further distinguishes the these lineage(s). In addition, evidence from L-1 B tumor studies demonstrating shifts in kappa/lambda expression and pr-ductive V^H gene replacements introduce molecular mechanisms which, if thy occur in normal cells, may be unique to the Ly-1 B lineage(s). Thus there is a extensive series of recent findings, many from studies that have yet to be comple-ed, that offer key insights into the distinctions amongst B cell lineages.

At present, taking all of the evidence (firm and suggestive) into accour, we have developed the following speculative view. The peritoneally-derived 3 cell lineage(s) are likely to be more primitive evolutionarily than the conventiol mammalian B cell lineage(s) that predominate in spleen and lymph nodes a adults. Both lineages must derive from a common progenitor; however, tl branching point that separates the lineages could occur either early or late i ontogony. If it occurs quite early in prenatal development, then the primitive an the more advanced lineage(s) might be expected each to have their own comp lement of T cells and macrophages; however, there is no evidence at presen bearing on this point.

At a functional level, the Ly-1 B and their sister population can be see as having evolved to continually produce autoantibodies useful for regulator purposes and perhaps additionally to produce certain antibodies that offer firs line protection against common pathogens in the environment. They appear t initiate antibody production without dividing (Pages & Bussard 1975), withou becoming permanently committed to antibody production and without losin their self-renewal capability, i.e., their future capacity to divide and reconstitut a portion of the lineage. Some cells in the lineage (perhaps the Ly-1 componen may require a T cell signal to initiate antibody production. Other cells probabl function independently of T cells.

The more advanced B cell lineage(s), in contrast, apparently demonstrate th classical B cell response characteristics. They appear to be reconstituted solel from Ig^- progenitors; they are responsible for most T-dependent and T-indepen-dent responses to exogenous antigens; they usually must be triggered to divide before they differentiate to antibody production; and, once differentiated, they become end-stage cells that apparently produce antibody until they die.

Thus, taken together, considerations of the properties of Ly-1 B cells and their differences from conventional B cells introduce a substantially new perspective on B cell functions and the mechanisms required to regulate the

production of antibodies, autoantibodies and serum Ig. Hopefully these thoughts provide a more up-to-date context for considering evidence from studies (discussed here and in other articles in this volume) concerned with defining functional differences among B cell lineages and the role(s) that these lineages play in normal and immunodefective immune systems.

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