Journal of Autoimmunity (1989) 2 (Supplement), 225–231

Are Ly-1 B Cells Important in Autoimmune Disease?

Leonore A. Herzenberg, Paul A. Lalor* and Alan M. Stall

Department of Genetics, Stanford University, Stanford, CA94305, USA

Although Ly-1 B cells produce autoantibodies and are found at elevated frequencies in certain autoimmune strains, very little is known about the role of these cells, if any, in autoimmune disease. In this publication, we summarize some recent findings relevant to Ly-1 B-cell development, clonal expansion and antibody production. We then consider the idea that Ly-1 B cells constitute the most primitive B-cell lineage in mammals and that the evolutionary niche occupied by these cells requires that they produce a basic set of autoantibodies that cross-react with common bacterial antigens.

Introduction

Ly-1 B cells clearly produce a significant portion of the IgM autoantibodies found in normal mice. However, the question of whether these cells produce autoantibodies or other products that contribute to active autoimmune disease has yet to be resolved [1–3].

The potential importance of Ly-1 B cells in autoimmunity and autoimmune disease first came to light early in our studies, when we found that spleens from NZB-related mice contain substantially more Ly-1 B cells than spleens from normal mice [1]. Since these elevated Ly-1 B frequencies occur in spleens from young mice, well before the onset of the characteristic NZB autoimmune disease, we decided (with some reservations) to use these spleens as a source of Ly-1 B cells for much of our initial work defining the basic physical and functional properties of Ly-1 B cells. However, when we discovered sometime later that relatively large numbers of Ly-1 B cells could be readily recovered from the peritoneal cavity in normal (and autoimmune) mice [4], we more or less abandoned the autoimmune animals and shifted the focus to characterizing Ly-1 B cells obtained from more normal environments.

*Current address: Walter and Eliza Hall Institute of Medical Research, Victoria, Australia.

0896–8411/89/03S225+07 $03.00/0

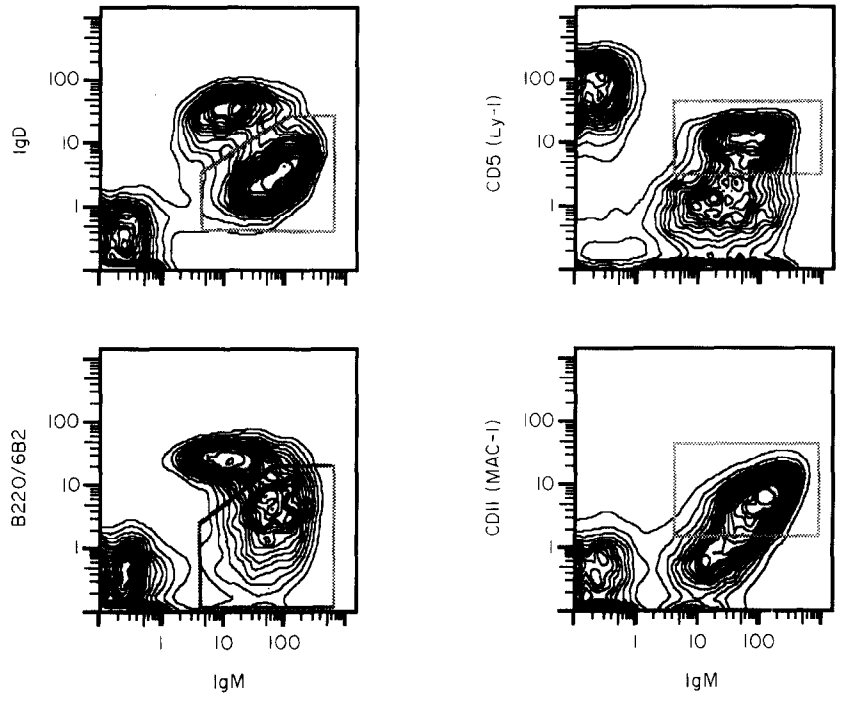

Figure 1. FACS phenotype for Ly-1 B cells in BALB/c peritoneum. A gray box surrounds the Ly-1 B cell population in each of the contour maps [5].

This shift did not substantially alter the view of Ly-1 B function and phenotype defined from our studies with the NZB-related mice. We rapidly found that although Ly-1 B frequencies in spleen (and in peritoneum) are clearly lower in normal mice, the Ly-1 B cells present in these mice show the same phenotype and overall functional profile as the Ly-1 B cells in the autoimmune animals [5]. Thus, we were able to sever our studies of Ly-1 B function in autoimmunity from more general considerations of Ly-1 B functions in normal mice. (Current view of Ly-1 B phenotype and properties are shown in Figures 1 and 2).

During the next few years, much of our attention focused on reconstitution studies. We used allotype congenic mice to distinguish between the self-replenishing Ly-1 B-cell lineage found in neonatal spleen and adult peritoneum and the conventional, bone-marrow derived B-cell lineage that predominated in spleen and lymph nodes [6–8]. However, in a recent return to studies with NZB-related animals, we discovered a surprising developmental difference between Ly-1 B populations in normal and autoimmune mice that provides a basis for the Ly-1 B frequency differences in these two kinds of animals [9].

In essence, we found that individual Ly-1 B-cell clones expand and develop into CLL-like neoplasms (hyperplasias) that generalize from their initial development site in the peritoneum (see Figure 3) to normally alien locations in bone marrow and lymph nodes. This process takes over a year in normal mice; however, it occurs extremely rapidly in NZB-related animals and thereby raises Ly-1 B frequencies to

Phenotype:	$CD5^+$ $Mac1^+$ IgM^{hi} IgD^{lo} large
Location:	predominant in peritoneal and pleural cavities and lamina propria
Ontogeny:	arise early, self-replenishing
Progenitors:	present in neonate, missing in adult BM
Function:	produce serum Ig, autoantibodies, anti-bacterial antibodies

Figure 2. Ly-1 B cell characteristics. Summarized from [5].

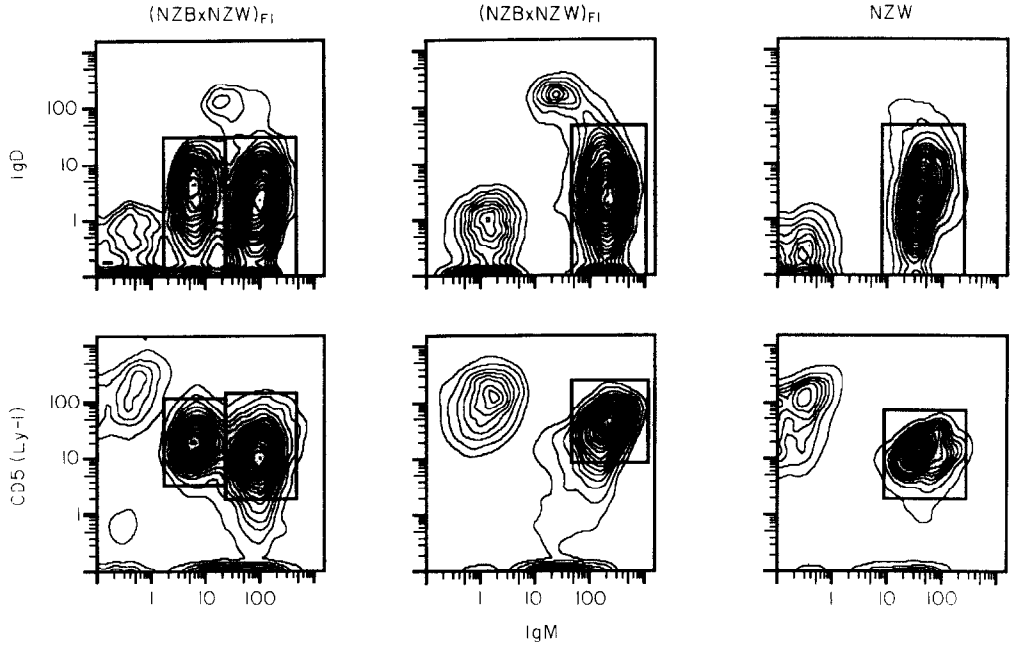

Figure 3. Expanded Ly-1 B-cell clones in peritoneum from NZB-related mice. Data shown are from 9–11-month-old mice. Similar (although usually smaller) clones are detectable in NZW and (NZB × NZW)F_1 mice as early as 2 months of age, and in BALB/c mice over 1 year of age [9]. Compare with normal peritoneal distribution in Figure 1.

- Clones express Ly-1 (LEU 1) and MAC-1 (LEU-15)
- Development of clonal populations is characteristic of older animals
- Expansion of the clonal populations is relatively slow
- The clones eventually metastasize to tissues in which Ly-1 B cells are not normally resident (L.N.; B.M.)
- The clonal populations do not directly result in reduced lifespans of the mice

Figure 4. Clonal hyperplasia of Ly-1 B cells: a potential murine equivalent of human CLL. Summarized from [9].

well above normal. Thus, young NZB-related mice frequently have splenic clonal B-cell populations detectable in spleen by FACS analysis or by Southern gel analysis probing for monoclonal V_H gene rearrangements. Similar populations can be found in spleens from normal mice, but only much later in life [9].

The early development of these CLL-like clones (see Figure 4), like the development of autoimmune disease in NZB-related mice, appears to be under genetic control. The gene(s) that control early clonal development, however, are clearly able to operate independently of the genes that control the NZB autoimmune disease, since clonal Ly-1 B populations are detectable in young mice in the NZW strain, which shows virtually no autoimmune disease. Thus, the elevated Ly-1 B frequencies demonstrated in NZB-related mice may be more a reflection of a genetically-defined propensity for clonal expansion that a direct or indirect consequence of autoimmune disease [9].

The early studies with young NZB-related mice also demonstrated that their splenic Ly-1 B cells produce a variety of autoantibodies and are responsible for the well-known 'spontaneous' autoantibody production by cultured NZB spleen cells [1]. The pathologic consequences of the presence of these antibody-producing cells are not clear, since Ly-1 B cells from normal mice often produce similar autoantibodies, albeit after stimulation with LPS. Thus, Ly-1 B autoantibody production in autoimmune mice is probably more important at a quantitative than a qualitative level. Alternatively, however, this apparently excessive autoantibody production could be a consequence of, rather than a contributor to, the autoimmune disease and be basically irrelevant to the disease process [1, 10].

The posing of these alternatives (and the compromise hypotheses they engender) raises perhaps the key Ly-1 B cell question. Ly-1 B cells appear to have found a stable evolutionary niche and thus can be presumed to play some important role in the ecology of the normal animal. Since they are primarily adapted to producing antibodies, it seems likely that the antibodies they produce introduce a selective advantage that maintains these kinds of B cells in the evolving animal. Is this actually true? And if so, are the autoantibodies Ly-1 B cells produce important in the process? These questions are not easy to answer; however, some thoughts about the issue may be useful.

Ly-1 B cells can be viewed as the most evolutionarily primitive of the B lymphocytes present in the mammal (see Figures 5 and 6). Their developmental pattern is consistent with the idea that ontogeny recapitulates phylogeny. Thus they predominate in neonatal mice but rapidly become overshadowed when bone-marrow-derived conventional B cells begin to increase in frequency [5, 8]. Furthermore, they shun the well developed (evolutionarily mature) lymphoid sites, preferring peritoneal and pleural cavities to more organized lymphoid structures such as the spleen and lymph nodes [5].

The kinds of antibodies Ly-1 B cells produce and the characteristics of the antibody responses they mount are also consistent with these cells being relatively primitive B cells. That is, conventional B cells produce the typical well studied antibody responses to haptens such as NP and DNP, and to proteins such as keyhole limpet hemocyanin (KLH) (P.A.L. unpublished). Ly-1 B cells, in contrast, tend to produce antibodies to common determinants found on micro-organism cell surfaces, e.g. dextran [11], phosphoryl choline (A.M.S. unpublished), phosphotidyl choline [12], and pneumococcal coat polysaccharide (S-III) (in press).

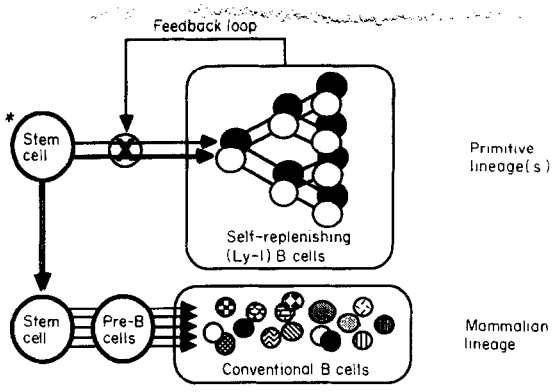

Figure 5. Proposed ontogeny pathway for B-cell lineages (and for other cells derived from hematopoietic stem cells). This model assumes that the self-replenishing neonatal hematopoietic stem cells that give rise to Ly-1 B cells are replaced (or replace themselves) in young animals with 'adult' self-replenishing stem cells that persist for the life of the animal and give rise to conventional B cells. *Stem cells also give rise to T cells, macrophages etc.

	Ly-1 B	Conventional B
Adult population dynamics	Self-replenishing (no new entrants)	Constantly being renewed
Adult source for adoptive transfer	Mature Ly-1 B (Peritoneal)	Ig-progenitors (BM, spleen)
Developmental regulation	Feedback inhibition by mature Ly-1 B	unknown
Development blocked	Xid (CBA/N)	me^v (motheaten) μ transgenic
Ontogeny	Predominant in newborn	Predominant in adult
Main location	Serosal cavities	Lymphoid organs

Figure 6. Differential development of B-cell lineages. Summarized from [5–8, 15].

Neonates Treated with Anti Igh-6B (IgM allotype)*

Igh allotype	Status of Ly-1 B cells during treatment	Recovery of depleted Ly-1 B
a/b	Igh-6b (paternal) Ly-1 B depleted Igh-6a (maternal) Ly-1 B normal	No
b/b	All Ly-1 B depleted	Yes
b/b	All endogenous Ly-1 B depleted; Ly-1 B population restored with allotype-congenic (Igh-6a) Ly-1 B#	No

Figure 7. Feedback regulation of Ly-1 B development. All neonates were treated prior to weaning with a total of 2 mg of anti Igh-6b (spread over the first 3 weeks of life). Neonates that received (Igh-6a) Ly-1 B cells were injected at 2 days of age with peritoneal cells from Igha allotype congenic donors, or with an Igh-6a allotype congenic Ly-1 B clonal population (BCL-85) [7, 8].

The antibodies to phosphotidyl choline (PtC) may be particularly revealing with respect to the role of Ly-1 B cells and the evolutionary significance of the autoantibodies produced by these cells. In addition to being a common micro-organism coat antigen, PtC is present in the cell membranes of murine erythrocytes and is revealed as an antigenic structure when these erythrocytes are treated with the enzyme bromelain (BrMRBC). In this form, it becomes the well known autoantigen to which Ly-1 B cells produce IgM autoantibodies (detectable in a plaque-forming assay as 'bromelain PFC') [1, 12]. Thus one might reasonably wonder whether some or all of the other autoantibodies produced by Ly-1 B cells also recognize antigens associated with micro-organisms.

This hypothesis is reminiscent of earlier suggestions that autoantigens (self determinants) present in the neonate stimulate (or select) long-lived B cells that are committed to produce potentially protective antibodies that will cross-react with a variety of micro-organism coat antigens. Such ideas are particularly attractive within the context of the Ly-1 B development pattern, since these self-replenishing cells (or their progeny) apparently endure indefinitely. In addition, their repertoire appears to be selected and fixed during neonatal life, since the entry of newly rearranged cells essentially terminates shortly after the animals are weaned (see Figure 7) [7, 8].

The view of Ly-1 B cells as an antibody-producing population with a particular mission is consistent with evidence suggesting that Ly-1 B cells develop a more restricted functional antibody repertoire than conventional cells [13–15]. This could be due either to selection of Ly-1 B cells producing antibodies with relevant specificities or to an as yet unknown mechanism that favors rearrangement and expression of certain V genes in Ly-1 B cells. There is little evidence to indicate that Ly-1 B cells draw from a different pool of V_H and V_L genes than conventional B cells. However, as indicated above, the antibodies produced by the Ly-1 B population do appear to contain more V_H and V_L combinations that react with microbial antigens and autoantigens. Furthermore, certain germline V_H genes turn up repeatedly in the expanded Ly-1 B clones (neoplasms) mentioned above [13, 14].

All in all, it is still too early to decide whether the hypotheses we have advanced here are fanciful or fruitful. For the moment, we find clear value in considering the Ly-1 B-cell lineage as evolutionarily primitive and responsible for providing basic immune functions, perhaps including the production of broadly reactive antibacterial antibodies that also react with self determinants (autoantigens). The logic of this argument would thus suggest that if certain autoantigens prove to be important for triggering autoimmune disease, Ly-1 B cells will be found to play a key role in autoimmune dysfunction.

References

1. Hayakawa, K., R. R. Hardy, M. Honda, L. A. Herzenberg, A. D. Steinberg, and Leonore A. Herzenberg. 1984. Ly-1 B cells: Functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc. Natl. Acad. Sci. USA* **81:** 2494–2498
2. Sidman, C. L., L. D. Shultz, R. R. Hardy, K. Hayakawa, and Leonore A. Herzenberg. 1986. Production of immunoglobulin isotypes by Ly-1(+) B cells in viable motheaten and normal mice. *Science* **232:** 1423–1425
3. Stall, A. M., P. A. Lalor, L. A. Herzenberg, and Leonore A. Herzenberg. 1986. Ly-1 B cells and autoantibodies. *Annales d'Immunologie del'Institut Pasteur* **137D:** 173–175

4. Hayakawa, K., R. R. Hardy, and Leonore A. Herzenberg. 1986. Peritoneal Ly-1 B cells: genetic control, autoantibody production, increased lambda light chain expression. *Eur. J. Immunol.* **16:** 450–456
5. Herzenberg, Leonore A., A. M. Stall, P. A. Lalor, C. Sidman, W. A. Moore, D. R. Parks, and L. A. Herzenberg. 1986. The Ly-1 B cell lineage. *Immunol. Rev.* **93:** 81–102
6. Hayakawa, K., R. R. Hardy, Leonard, A. Herzenberg, and Leonore A. Herzenberg. 1985. Progenitors for Ly-1 B cells are distinct from progenitors from other B cells. *J. Exp. Med.* **161:** 1554–1568
7. Lalor, P. A., A. M. Stall, S. Adams, Leonard A. Herzenberg, and Leonore A. Herzenberg. 1989. Permanent alteration of the murine Ly-1 B repertoire due to selective depletion of Ly-1 B cells in neonatal animals. *Eur. J. Immunol.* (In press)
8. Lalor, P. A., Leonore, A. Herzenberg, S. Adams, Leonard A. Herzenberg, and A. M. Stall. 1989. Feedback regulation of murine Ly-1 B cell development. *Eur. J. Immunol.* (In press)
9. Stall, A. M., M. C. Farinas, D. M. Tarlinton, P. A. Lalor, Leonard A. Herzenberg, S. Strober, and Leonore A. Herzenberg. 1988. Ly-1 B-cell clones similar to human chronic lymphocytic leukemias routinely develop in older normal mice and young autoimmune (New Zealand Black-related) animals. *Proc. Natl. Acad. Sci. USA* **85:** 7312–7316
10. Farinas, M. C., A. M. Stall, J. J. Solovera, D. M. Tarlinton, Leonore A. Herzenberg, and S. Strober. 1989. Ly-1 B cells and disease activity in NZB/NZW_{F1} mice: Effect of total lymphoid irradiation. (Submitted)
11. Forster, I. and K. Rajewsky. 1987. Expansion and functional activity of Ly-1^+ B cells upon transfer of peritoneal cells into allotype-congenic newborn mice. *Eur. J. Immunol.* **17:** 521–528
12. Mercolino, T. J., L. W. Arnold, L. A. Hawkins, and G. Haughton. 1988. Normal mouse peritoneum contains a large population of Ly-1^+ (CD5) B cells which recognize phosphatidyl choline: Relationship to cells which secrete hemolytic antibody specific for autologous erythrocytes. *J. Exp. Med.* **168:** 687–698
13. Pennell, C. A., L. W. Arnold, G. Haughton, and S. H., Clarke. 1988. Restricted immunoglobulin variable region gene expression among Ly-1 B cell lymphomas. *J. Immunol.* (In press)
14. Tarlinton, D., A. M. Stall, and Leonard A. Herzenberg. 1988. Repetitive usage of immunoglobulin V_H and D gene segments in $CD5^+$ Ly-1 B clones of (NZB × NZW)F1 mice. *EMBO J.* **7:** 3705–3710
15. Herzenberg, Leonore A., A. M. Stall, J. Braun, D. Weaver, D. Baltimore, L. A. Herzenberg and R. Grosschedl. 1987. Depletion of the predominant B-cell population in immunoglobulin mu heavy chain transgenic mice. *Nature* **329:** 71–73
16. Painter, C., M. Monestier, B. Bonin, and C. A. Bona. 1986. Functional and molecular studies of V genes expressed in autoantibodies. *Immunol. Rev.* **94:** 75–98