Ly-1 B: A Functionally Distinct B-Cell Subpopulation*

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I. Introduction

T-Cell differentiation antigens such as Ly-1 and Ly-2 (and the recently defined L3T4a) have been generally accepted as murine T-cell markers. However, in addition to being present on T cells (Thy-1$^+$ cells), the Ly-1 antigen is also expressed on a small proportion of IgM-bearing B cells (Ly-1 B) in spleen from all mouse strains (Hayakawa *et al.*, 1983). These cells are not detectable in lymph nodes. They generally represent about 1% of spleen cells and, curiously, about 10% of

*This work was supported in part by National Institutes of Health grants GM-17367, HD-01287, and CA-04681. Dr. Hardy is a Fellow of the American Cancer Society.

ISBN 0-12-768240-6

peritoneal exudate cells. The frequency of Ly-1 B in the peritoneum is influenced by gene(s) within or closely linked to the MHC. The CBA/N X-linked immunodeficiency also strongly affects this frequency.

Ly-1 B secrete IgM autoantibodies, even in normal mice (BALB/c). Furthermore, these cells are present at much higher frequencies in autoimmune mouse strains such as NZB and (NZB × NZW)F_1. Some murine B lymphomas have been shown to carry Ly-1, and chronic lymphocyte leukemia cells in the human frequently express surface Ig and Leu-1 (the human homolog to mouse Ly-1). These findings suggest that Ly-1 B may constitute a distinct set of cells arising in the course of lymphocyte differentiation.

II. Distribution of Ly-1 B

We have defined the Ly-1 B subpopulation as cells that simultaneously express Ly-1 and IgM on their surfaces. The amount of Ly-1 on Ly-1 B is very low in comparison with the amount of Ly-1 on T cells, and the amount of IgM expressed is quite high (in comparison with other splenic B cells). Ly-1 B express other B lineage cell surface antigens, IgD (low), ThB, BLA-2, and Ia; however, they do not express Thy-1, Ly-2, or L3T4a. Although the frequency of Ly-1 B is low in spleen (usually 1–2%), we are able to visualize and enumerate this cell population by a careful two-color analysis using the fluorescence-activated cell sorter (FACS). Our first studies of these cells show (1) Ly-1 B reside in spleen but not (at detectable limits) in lymph nodes or bone marrow; (2) most mouse strains, including nude mice, have Ly-1 B in spleen; (3) Ly-1 B constitutes a significant fraction of B cell in newborn (3–5 days) mice but decreases to a minor population with age; and (4) Ly-1 B frequency is considerably increased in NZB mice (5–10% of spleen, see Fig. 1).

III. Ly-1 B Produce Autoantibody

In addition to being increased in frequency, the Ly-1 B in NZB mice are unique with respect to IgM secretion. *In vitro*-incubation of Ly-1 B isolated from NZB results in secretion of large amounts of IgM. This secretion is spontaneous in that it occurs in the absence of exogenously

V. Ly-1 B Are Enriched in Peritoneal Exudate Cells

In addition to showing that Ly-1 B reside in spleen but not in the lymph node, we recently demonstrated that Ly-1 B are present at quite high frequencies in peritoneal exudate cell (PEC) populations. This finding is consistent with earlier evidence showing that PECs have a high frequency of anti-BrmRBC PFC (particularly after incubation in vitro) (Lord et al., 1975), since (as we have shown) anti-BrmRBC antibody come solely from Ly-1 B.

Analyses of Ly-1 B in PEC reveals clear frequency differences between mouse strains and indicates that the Ly-1 B level in the peritoneum is strongly influenced by one or more genes that map within or quite close to the mouse major histocompatibility complex (MHC). NZB mice have the highest level of Ly-1 B in PEC (40–80% out of IgM^+ cells), and most normal mice (e.g., BALB/c, CBA, CSW) have a frequency of 20–40%. SJL (and its Igh congenic strain, SJA) have a distinctively low Ly-1 B frequency in PEC (1–5%). (BALB/c × SJA)F_1 mice have an intermediate frequency and progeny from the backcross of this F_1 to the SJA parent show either the low (SJA) frequency or the intermediate (F_1) frequency. Linkage analyses on 15 backcross progeny showed that the low phenotype occured only in $H\text{-}2^s/H\text{-}2^s$ homozygotes (4/15) and that the intermediate phenotype occured in all $H\text{-}2^s/H\text{-}2^d$ heterozygotes (10/15) and in one $H\text{-}2^s$ homozygote. The X-linked immunodeficiency in CBA/N (Xid) mice, however, has the most severe effect on the frequency of Ly1 B in PEC: CBA/N mice totally lack Ly-1 B in PEC.

VI. Conclusion

The Ly-1 B cell constitutes a functionally unique B-cell population. That is, only certain antigens are capable of triggering Ly-1 B and the antibody produced by these cells is enriched for autoantibody. Furthermore, the unusual surface antigen expression, tissue localization, and strain distribution of Ly-1 B suggest a distinctive role for this cell population in the immune system.

References

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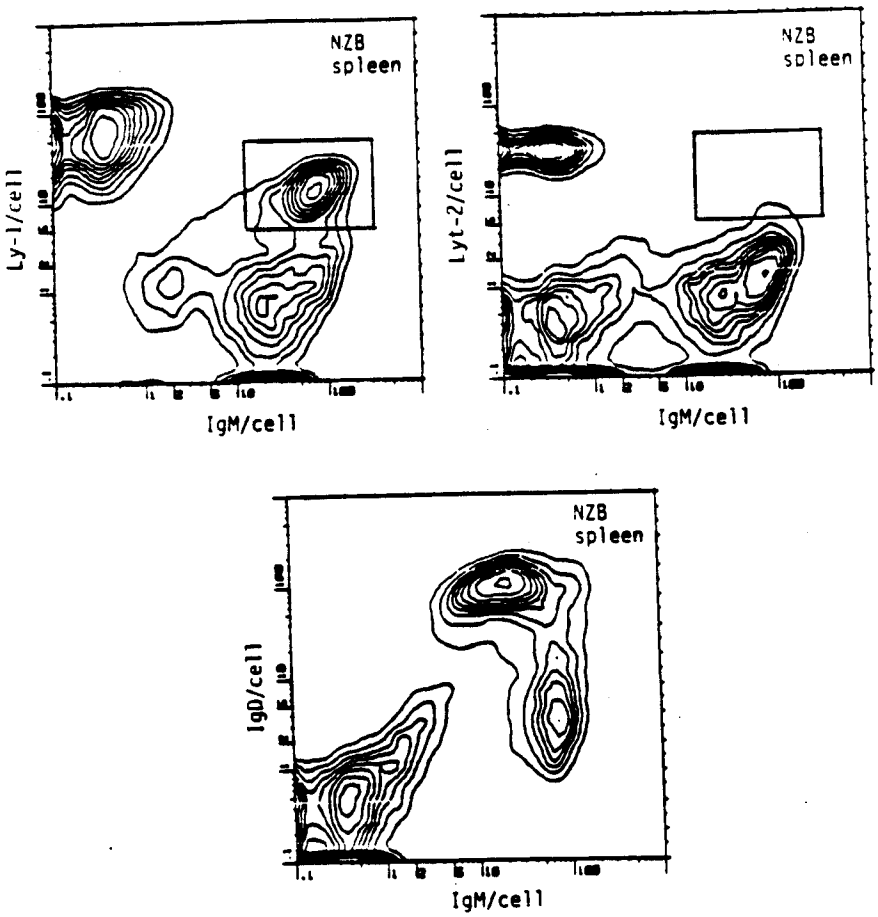

Fig. 1. Ly-1 B in NZB spleen. NZB spleen cells (from 3-month-old mice) were stained simultaneously with fluoresceinated monoclonal antibody specific for mouse IgM and biotinated monoclonal antibody specific for either Ly-1, Lyt-2, or IgD. Biotinated antibody was revealed by staining with Texas Red-avidin. The box enclosed the Ly-1 B population. The increased Ly-1 B population is reflected in the IgM/IgD pattern as a population of IgM-bright/IgD-dull cells.

added mitogens or antigens. It occurs only with Ly-1 B from NZB-related mice and does not occur at detectable levels with Ly-1 B from normal animals. The IgM secreted by NZB Ly-1 B contains autoantibody (e.g., NTA or anti-ssDNA).

The Ly-1 B population in normal mice can also include cells that secrete autoantibodies. Mitogen (LPS) injection *in vivo* in normal mice

gives rise to various kinds of autoantibody in serum. Antibromelain-treated mouse red blood cell (BrmRBC) antibody is one of these autoantibodies (Cunningham, 1974). This antibody specifically reacts with auto (mouse) red blood cell determinant(s) exposed by proteolytic enzyme (bromelain) treatment. Characteristically, the plaque-forming cells (PFC) against anti-BrmRBC appear early after stimulation, and it has been shown that although the production of this antibody does not require cell proliferation it does require protein synthesis. LPS injection *in vivo* does not induce any increase in the Ly-1-bearing IgM^+ cell frequency. However, by sorting out $Ly\text{-}1^+$ and $Ly\text{-}1^-$ B cells from BALB/c spleen cells injected with LPS 1 day previously, we have clearly demonstrated that all anti-BrmRBC PFC reside in the Ly-1 B population (data not shown).

IV. Most Antigens Do Not Trigger Antibody Production in Ly-1 B

By contrast with their responsiveness to autoantigens, Ly-1 B do not respond to most T-dependent and T-independent antigens. Nevertheless, certain T-independent antigens (TI-1) do stimulate these cells to produce IgM antibody. As Table I summarizes, specific anti TNP (or anti-SRBC) PFCs generated in response to TNP-KLH-, TNP-Ficoll, or SRBC were present only in the $Ly\text{-}1^-$ B-cell fraction. Immunization with TNP-*Brucella abortus* (TNP-BA), in contrast, generated significant levels of PFC in the Ly-1 B population as well as in $Ly\text{-}1^-$ B cells.

TABLE I

Restricted Antibody Secretion by Ly-1 B

In vivo stimulation	IgM antibody response	Produced by	
		Ly-1 B	B
SRBC	Anti-SRBC	No	Yes
TNP-KLH	Anti-TNP	No	Yes
TNP-Ficoll	Anti-TNP	No	Yes
TNP-BA	Anti-TNP	Yes	Yes
LPS	Autoantibody[a]	Yes	No

[a]PFC for bromelain-treated mouse red blood cell.