

Characteristics of Ly 1 B Cells

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Recent studies demonstrate the existence of two murine B cell lineages: a conventional lineage that contains most of the B cells in the animal and has most of the properties commonly associated with mammalian B cells, and a second lineage, called Ly 1 B because it carries the Ly 1 cell surface marker, that derives from different progenitors and has substantially different properties than conventional B cells (including being self-renewing in adults). Surprisingly, although this Ly 1 B lineage contains relatively few cells and responds poorly to most antigens, it produces much of the serum immunoglobulin (Ig) and most of the autoantibodies or natural antibodies found in normal and many autoimmune mice. In studies with Ly 1 B tumors, we have identified two previously unsuspected genetic mechanisms: V_H gene replacement and λ light chain rearrangement in κ -producing cells. These mechanisms introduce the potential for diversifying antibodies produced by progeny of self-renewing B cells, such as Ly 1 B, that already have functionally rearranged heavy and light chain genes and are actively producing surface Ig.

I. Introduction

Recent studies (Hayakawa *et al.*, 1983, 1984, 1985, 1986) demonstrate the existence of two physically and functionally distinct murine B cell lineages. The more conventional of these lineages contains the vast majority of the B cells in the animal and displays most of the characteristics traditionally associated with mammalian B cells. It derives from (conventional) B cell progenitors in the bone marrow and produces most IgM and IgG antibody responses to exogenous antigens. Both the LyB5⁺ and LyB5⁻ subsets, as they were initially defined in Xid animals (Scher *et al.*, 1977; Mosier *et al.*, 1977), belong to this lineage.

The other lineage, called Ly 1 B because it carries the Ly 1 surface antigen, differs from conventional B cells by size, surface marker expression, anatomical localization, progenitor location, ontogeny, functional properties, and self-renewal capability. Cells in this lineage often comprise the majority of B cells recoverable from the peritoneal cavity; however, they represent less than 1% of the lymphocytes in the spleen and cannot be detected in lymph nodes in normal

animals. Surprisingly, despite this sparse representation, these Ly 1 B cells produce most of the IgM autoantibodies and much of the serum IgM found in normal and (most) autoimmune animals (Hayakawa *et al.*, 1984).

The data upon which these conclusions rest are summarized briefly here and in detail elsewhere (Hayakawa *et al.*, 1986; Herzenberg *et al.*, 1986a,b).

II. Self-Renewing Ly 1 B Cells from the Peritoneum Reconstitute the Ly 1 B Lineage

Progenitors capable of reconstituting Ly 1 B cells in irradiated recipients are consistently found in the peritoneal cavity in adults and are only sporadically found in adult bone marrow. Progenitors for conventional B cells, in contrast, are consistently found in adult bone marrow and are never detectable in the peritoneal cavity. Mixture-transfer studies give no indication that regulatory activity accounts for these findings. Thus, these studies define two distinct B cell developmental lineages (conventional and Ly 1 B) whose progenitors inhabit separate locations in adult animals (Hayakawa *et al.*, 1985).

The Ly 1 B progenitors in adult peritoneum appear to be typical Ly 1 B cells in that they are the same size and carry the same amounts of surface Ig and Ly 1 as most of the Ly 1 B cells in the peritoneum. These progenitors may be contained within a subset of the Ly 1 B population; however, the evidence available at present is consistent with all peritoneal Ly 1 B cells being capable of at least limited self-renewal in an irradiated recipient.

The idea of a self-renewing population of B cells (Ig-bearing lymphocytes) was quite novel when we first began these studies. However, Pink and colleagues (1985) have now shown that avian (chicken) B cells in adults are maintained exclusively by self-renewing B cells that reside in the periphery. Thus the Ly 1 B lineage could be an evolutionarily primitive B cell population that represents the mammalian counterpart of the avian B cell population.

III. Ly 1 B Cells Produce Autoantibodies and Serum Ig (Including IgG)

The assignment of Ly 1 B cells as the principal IgM autoantibody producers in normal and most (or perhaps all) autoimmune mouse strains was initially based on data from experiments in which FACS-sorted spleen cells were either cultured and tested for antibody secretion *in vitro* or were tested directly after sorting for the ability to form hemolytic plaques (PFC) with various kinds of hapten-coupled or enzyme-treated erythrocytes. Results showed that the sorted Ly 1 B population contains all splenic cells producing IgM antibodies to well-known murine

autoantigens such as single-stranded DNA (ssDNA), thymocyte cell surface antigens (NTA), and an autologous erythrocyte antigen revealed by treatment with the enzyme bromelain (BrMRBC) (Hayakawa *et al.*, 1984).

The sorted Ly 1 B population did not, however, contain any of the spleen cells producing IgM antibodies to exogenously introduced antigens such as DNP-KLH or sheep erythrocytes. These antibody-producing cells were efficiently recovered in a different FACS-sorted population, clearly distinguishable from Ly 1 B (and more like typical plasma cells) by the absence of surface Ly 1 and IgD and the presence of substantially lower levels of IgM.

These findings have recently been confirmed in studies in which we compared the (allotype-marked) *in situ* response characteristics of each of the two B cell lineages coexistent in the same animal, i.e., in a long-term radiation chimera reconstituted with adult bone marrow from one strain and peritoneal cells (PerC) from a second, allotype congenic strain. Under these conditions, the transferred PerC fully reconstitute Ly 1 B lineage cells and do not detectably reconstitute any other B cells, while the transferred bone marrow (marked with a different allotype) restores the hematopoietic potential and reconstitutes the predominant B cell populations throughout the animal. Bone marrow also occasionally reconstitutes some Ly 1 B cells; however, this sporadic reconstitution is easy to detect and does not interfere with interpretation of the data from the experiment (unpublished observation).

In essence, these chimeric studies show that autoantibody production (BrMRBC PFC) originates exclusively from PerC-derived cells (hence from Ly 1 B). Primary and secondary antibody responses to a typical T-dependent antigen (NP-KLH) commonly used for studies of primary and secondary antibody responses, in contrast, originate almost exclusively from conventional B cells reconstituted from bone marrow progenitors (Lalor *et al.*, 1986).

Reconstituted allotype chimeras have also proven useful for tracing sources of serum Ig. Surprisingly, we have found that although Ly 1 B cells produce virtually no antibody response to DNP-KLH, they nonetheless produce quite large amounts of serum IgM and IgG of all isotypes. These findings recall the old idea that serum Ig in normal, healthy animals contains relatively large amounts of autoantibodies or (as they are sometimes called) natural antibodies that recognize various self-determinants. If so, then it is quite possible that these antibodies derive primarily from Ly 1 B cells.

IV. Ly 1 B Cells and Allotype Suppression

Perhaps it is poetic justice, but when the smoke cleared and we had completed our studies characterizing Ly 1 B representation in various mouse strains at various ages, we noted that the SJL-related mice we have worked with for years

in our allotype suppression studies are unique in that they have low but detectable numbers of Ly 1 B cells prior to weaning but progressively lose Ly 1 B thereafter.

These findings demonstrate a surprising inverse correlation between Ly 1 B frequency and the severity of chronic allotype suppression. That is, the most severe suppression occurs in (SJL \times SJA) F_1 animals in which Ly 1 B levels are very low even in young animals and chronic allotype suppression often begins as early as 2-3 months of age. The least severe suppression, in contrast, occurs in (BALB/C \times SJL) F_1 mice in which Ly 1 B deficits mainly become detectable around 6 months of age, just about the time that chronic suppression normally sets in in these animals. Thus it appears that allotype suppression becomes active when Ly 1 B frequencies drop below some preset level, either because higher Ly 1 B frequencies actually interfere with activation of the suppression mechanism or because the activation of suppression and the reduction of Ly 1 B frequencies are consequences of some other primary physiologic changes that occur as SJL-related mice age.

V. Ly 1 B Cells and Conventional B Cells Are Differentially Sensitive to Neonatal Anti-IgM and Anti-IgD Treatments

Although conventional B cells recover readily following neonatal anti-IgM treatment, allotype heterozygotes treated neonatally with anti-Ig-6b (the paternal IgM allotype) lack detectable numbers of peritoneal Ly 1 B cells expressing Igh^b allotypes throughout life. Furthermore, these mice lose and never regain their normal capability for producing Igh^b allotype autoantibodies (measured as Ig-6b anti-BrMRBC PFC in spleen).

Neonatal treatment with anti-IgD allotype antibodies (Igh-5b), in contrast, selectively depletes the conventional B cell populations from neonates and leaves the Ly 1 B population intact. Mature conventional B cells remain undetectable in the treated animals as long as the treatment antibody is present and recover to readily detectable levels within a month of the disappearance of the antibody. Thus, while the anti-IgD is present, the Ly 1 B cells apparently represent the only functional Igh^b allotype B cells present in the animal (Lalor *et al.*, 1986).

VI. Ly 1 B Tumors and Cell Lines

Although Ly 1 B cells constitute a very small proportion of the total B cell population in a normal adult mouse, there are a large number of tumors that carry the Ly 1 surface antigen. In fact, the detection of Ly 1 on several B cell tumors represents the first evidence for the existence of the Ly 1 B lineage (Lanier *et al.*,

1981a,b). Ly 1 is expressed on many of the classical pre-B cell tumors induced by Abelson virus infections in neonates. It is expressed on the 70Z tumor and on several pre-B neoplasms isolated by Davidson *et al.* (1984). Whether the presence of Ly 1 on these tumors reflects their origin in the Ly 1 B lineage has yet to be determined; however, we feel this is likely since we have shown that Ly 1 is not a marker for LPS activated B cells and is not present on a variety of other B cell tumors (unpublished).

The currently identified Ly 1 B tumors include several that are arrested at a relatively early developmental stage and lack a variety of markers (e.g., Ia, IgD) present on mature, normal Ly 1 B cells. Other Ly 1 B tumors express all of the markers found on mature Ly 1 B cells and express these markers at levels that are comparable to the levels of the mature Ly 1 B cells present in the peritoneum. Curiously, at least two Ly 1 B tumors [70Z (Paige *et al.*, 1978) and NFS-5 (Hardy *et al.*, 1986)] undergo differentiation *in vitro* and pass from less mature to more mature stages, particularly following LPS stimulation.

Several cloned Ly 1 B cell lines were established by Braun (1983). These show surface phenotypes that are comparable to mature Ly 1 B and, except for their ability to grow indefinitely in culture, appear to be similar to typical Ly 1 B cells in adult mice (R. R. Hardy, K. Hayakawa, L. A. Herzenberg, and J. Braun, unpublished observations).

In humans, all chronic B lymphocytic leukemias (B-CLL) carry the Leu 1 antigen, which is structurally homologous to the Ly 1 antigen in mice. The normal counterpart of these CLL cells may be in the Leu 1 B cell subpopulation, which represents from 10 to 50% of peripheral B cells in normal adults. The frequency of this subpopulation tends to be elevated in a proportion of patients with rheumatoid arthritis (Hardy, 1986), particularly in patients with high rheumatoid factor titers. Furthermore, as in the mouse, there is clear genetic control of Leu 1 B frequencies in normal human subjects: identical twins and triplets have very similar, stable Leu 1 B frequencies while sibs and unrelated individuals vary enormously in this respect (T.J. Kipps and L.A. Herzenberg, unpublished observations). Thus, the human Leu 1 B subpopulation may be the human homolog of mouse Ly 1 B.

VII. Antibody Specificity and V_H Gene Usage in Ly 1 B Cells

Evidence from several studies indicates that Ly 1 B cells preferentially express V_H genes located within the 7183, Q52, and T15 ("first") V_H gene families, which lie closest to the Ig heavy chain constant region genes on the IgH chromosome. Yancopoulos *et al.* (1984) have shown that Abelson pre-B tumors derived from prenatal and neonatal sources tend to express genes from these first V_H

families. Similarly, several laboratories have shown that fusions with B cells from Ly 1 B-enriched sources (neonatal spleen, adult peritoneum) yield hybridomas that frequently express V_H genes from the first families (J. Kearney, A. Coutinho, and C. Bona, personal communication).

Kearney and colleagues have also shown that the specificities of antibodies produced by these neonatally derived hybridomas are similar to the specificities of hybridomas produced by fusions with peritoneal cells from adult animals. C. Bona, C. L. Sidman, and colleagues have demonstrated similar specificity and V_H gene usage in hybridomas produced by fusions with Ly 1 B-enriched cell populations from autoimmune animals (NZB-related viable motheaten) (personal communication).

Avrameas (1986), working with fusions of cells from unimmunized adult mice, has also generated a large series of hybridomas that fall into this V_H gene usage and specificity category. These hybridomas, which produce so-called "natural" antibodies that react with self-determinants, are obtainable only from organs that contain Ly 1 B cells (e.g., spleen but not lymph node). Thus, as in the cases cited above, these studies support the idea that Ly 1 B cells preferentially use the first V_H gene families.

Kearney and colleagues (1986) have made a curious observation concerning the sequential expression of V_H genes during neonatal development. In essence, by using hybridoma technology to sample the expressed V_H genes present in animals of various ages, they have shown that the first expressed V_H genes (just prior to birth) are drawn from the V_H gene family that is located closest to the IgC_H region and that progressively more distal genes tend to be used more and more frequently as the animals age. Studies presented below introduce a novel V_H gene replacement mechanism that could potentially account for these findings.

VIII. Ig Light Chain κ/λ Shifts in a Cloned Ly 1 B Tumor Cell Line

About 2 years ago, Dr. H. C. Morse brought us a tumor cell line that he and Dr. Wendy Davidson had recently isolated and established as a long-term cultured cell line. Initially, all of the cells in this line expressed B220, a classical B cell surface marker, and a few cells expressed the Ly 1 marker characteristic of the Ly 1 B lineage. However, within 2 weeks of its arrival at Stanford, the cell line began to differentiate and increasing numbers of cells expressing Ly 1 and low levels of surface μ without accompanying light chains began to appear in the cultures. Stimulation with lipopolysaccharide (LPS) induced the line to differentiate further and allowed us to isolate cloned sublines that had substantially more μ on the surface, this time in the form of classical surface IgM molecules

with two heavy chains and two κ light chains. Removal of the LPS resulted in a reduction of surface Ig and a return to the μ chain only form, and restimulation with LPS repeated the cycle (Hardy *et al.*, 1986).

Surprisingly, continued culture of κ -producing cloned cell lines in LPS resulted in the appearance of λ -bearing cells that could be cloned and generally grew thereafter (in the presence of LPS) as λ producers. For the most part, these λ -producing clones no longer produced the κ light chains they had been producing earlier; however, one clone stably produced both light chains. As before, removal of the LPS from the culture resulted in a return to the μ chain only form. Restimulation with LPS restored κ production to a proportion of the cells and λ production to the others.

Genetic analysis demonstrated that the initial μ chain only form in this cell line has a rearranged κ gene that is expressed following LPS stimulation. The λ chain loci in this form are in the germ line configurations and rearrange and become expressed only after growth in LPS. Removal of LPS and restimulation some time later results in the expression of the same rearranged λ gene as was expressed initially (Hardy *et al.*, 1986). Secondary rearrangements of light chain genes occur rarely if at all. None has been observed thus far despite the identification of at least five rerearrangements (V_H gene replacements) among the isolated λ -expressing clones (see below) (D. Tarlinton and L. A. Herzenberg, unpublished).

IX. V_H Gene Replacement in Ly 1 B Tumor Cell Lines

The various NFS-5 κ - and λ -expressing clones isolated and analyzed in these studies also showed evidence of curious rerearrangements of the genes coding for immunoglobulin heavy chains (Hardy *et al.*, 1986). Subsequent studies done in collaboration with Kleinfeld and colleagues (1986) in Philadelphia extend these findings and reveal a previously unsuspected genetic mechanism potentially capable of increasing antibody diversity in self-renewing populations of B cells that have already completed a successful V-D-J-C rearrangement and are actively producing cell surface (or even secreted) Ig. In essence, this mechanism serves to replace an active, previously rearranged V_H gene with a new V_H gene drawn from the same V_H gene family or from an adjacent (more distal) family.

These studies were completed simultaneously with studies by Reth (1986) demonstrating a similar V_H gene replacement mechanism in a neonatally derived Abelson tumor. However, in the Reth studies, an inactive V_H gene in a cell incapable of producing Ig was replaced with an active V_H gene that permitted the initiation of Ig production (Reth, 1986). Thus V_H gene replacement also provides a potential mechanism for rescuing nonfunctional B cells during development.

Current views vary as to the significance of this V_H gene replacement mecha-

nism with respect to normal B cell development. The conservative view sees this as an abnormal mechanism occurring only in tumor cells. The most radical view sees it as a mechanism that could be expected to operate in all B cells (see Marrack and Geffer, 1986). We feel that the operation of this mechanism is most likely restricted to cells within the self-renewing Ly 1 B lineage, which would have much to gain from its operation.

The proportion of Ly 1⁺ B tumors derived from late fetal liver or spleen or from neonatal spleen seems to be higher than from adult bone marrow and perhaps reflects the predominance of mature Ly 1 B cells early in development. This would be consistent with evidence showing that the kinds of anti-self and anti-idiotypic specificities displayed by monoclonal antibodies produced from fusions with neonatal spleen cells are similar to the specificities displayed by monoclonal antibodies produced by fusions with adult peritoneal cells (J. Kearney, personal communication) or with adult spleen cells from motheaten viable (me^vme^v) animals (C. Bona, personal communication) that lack virtually all B cells except Ly 1 B. Thus, there is certainly more evidence for than against the idea that the presence of Ly 1 on a B cell tumor reflects the tumor's origin.

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