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Ly-1 B CELLS AND AUTOIMMUNITY

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Reconstitution studies in lethally irradiated mice now distinguish two B cell lineages. One of these derives from progenitors that are consistently present in adult bone marrow and includes most of the B cells in spleen, lymph node and peripheral blood. The other derives from progenitors consistently present in adult peritoneum rather than in bone marrow (BM) and is mainly detectable in spleen and peritoneum. Although numerically small, this latter (Ly-1 B) lineage surprisingly contains most of the cells responsible for producing the commonly studied murine IgM autoantibodies in normal and (NZB-related) autoimmune mice, e.g., antibodies to bromelain-treated mouse erythrocytes, ssDNA, NTA (1).

Multiparameter analysis and sorting studies with the Fluorescence Activated Cell Sorter (FACS) identify Ly-1 B cells as lymphocytes that express the Ly-1 surface marker in conjunction with characteristic amounts of classical B cell surface markers, e.g., high IgM, low IgD and intermediate Ia (2). These cells are rare in spleen and undetectable in lymph nodes and Peyer's patches from normal animals. Nevertheless, they represent about half of the B cells (10-20 per cent of total lymphocytes) recoverable from the peritoneal cavity in normal mice (3) and a considerably higher proportion of peritoneal and other lymphocytes in certain autoimmune animals, e.g., NZB (1) and Motheaten viable mice (4).

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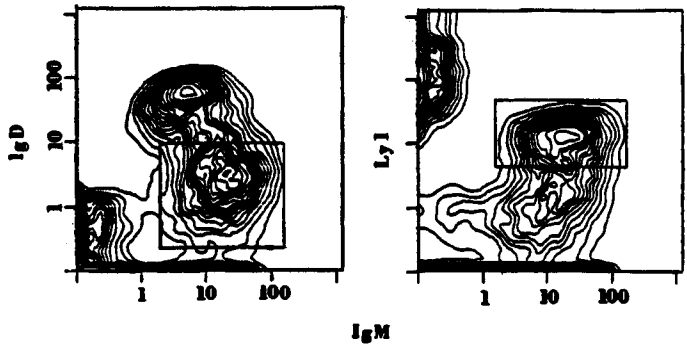
TABLE I. Ly-1 B Cell Characteristics

Phenotype:	Usual B cell surface markers plus Ly-1 Lambda light chain expression frequent	
Location:	Peritoneum >> spleen >> nodes (undetectable)	
Ontogeny:	Highly enriched in neonates	
Tumors:	NFS-1 and NFS-5 (5) 70Z (most isolates) (6) BCL-1 (6) Abelson pre-B tumor lines CH tumor series (7)	
Cell lines:	Long term cultured lines (8)	
Genetics:	Exclusive	Motheaten
	Elevated	NZB-related autoimmune mice
	High	BALB-related mice (in peritoneum)
	Medium	C57Bl/10, CBA, C3H
	Low	SJL-related
	Missing	CBA/N, DBA/2Ha
Source:	Ig^+ cells in peritoneum Ig^+ cells in neonatal spleen Adult bone marrow (sporadic)	

FIGURE 1. LY-1 B CELLS IN BALB/C PERITONEUM



Ly-1 B cells are indicated by boxes in each of the plots

The conclusion that Ly-1 B cell progenitors are distinct from progenitors for typical ($Ly\text{-}1^-$) splenic and lymph node B cells rests on data from a series of reconstitution experiments in which cells from various progenitor sources were transferred into lethally-irradiated allotype (Igh) congenic recipients and the resultant B cell populations were characterized in FACS and functional analyses one to six months later (9). Briefly summarized, these studies show:

1) that reconstituting irradiated Igh^a allotype animals with a mixture of syngeneic (Igh^a) BM and allotype congenic (Igh^b) PerC from adult donors creates stable allotype chimeras in which all of the known B cell populations are maintained at essentially normal frequencies for at least 6 months after transfer;

2) that most of the B cells in these animals express the Igh^a allotype of the BM donor and thus derive from traditional B cell progenitors in the bone marrow;

3) that Ly-1 B cells, in contrast, tend to express the Igh^b allotype of the PerC donor and thus must mainly derive from peritoneal progenitors that do not reconstitute other B cell populations; and,

4) that the donor peritoneal cells that reconstitute the Ly-1 B lineage in irradiated recipients are contained within the FACS-sorted Ly-1 B cell population and thus are themselves IgM^+,$Ly\text{-}1^+$ cells.

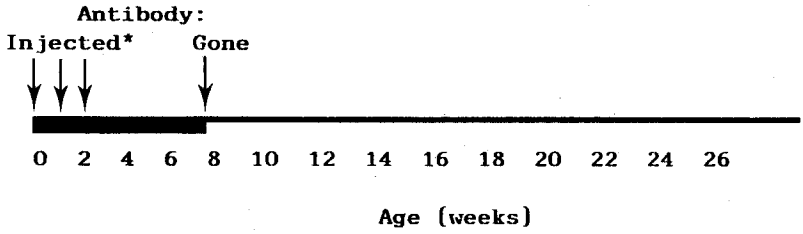
This evidence places Ly-1 B cells in a developmental pathway (lineage) that is distinct from the developmental pathway for other B cells. It is complemented by evidence from recent studies in our laboratory showing that treating neonatal animals with monoclonal antibodies to IgM or to IgD results in markedly different effects on the development of the two B cell lineages (P. Lalor, in preparation). The

effects of these treatments are most clearly discernable in Igh alloytpe heterozygotes treated with monoclonal antibodies to allotypic determinants (Igh-6b on IgM; Igh-5b on IgD). In these animals, anti-allotype antibody treatment does not directly effect the development of cells committed to producing the other parentally-derived allotype. Therefore, the treated mice have at least half their normal number of B cells at all times and thus remain essentially normal physiologically throughout life.

In brief, these studies show that treating neonates with anti Igh-5b (IgD^b) allotype prevents the development of the predominant (Igh^b allotype) B cell populations in lymph node and spleen but does not hamper development of the Ly-1 B population (which has substantially lower levels of surface IgD). Recovery of the depleted B cell populations begins as soon as the anti Igh-5b disappears from circulation and reaches completion two months later. Treatment with anti Igh-6b (IgM^b), in contrast, prevents (short-term) development of all Igh^b allotype B cells while the antibody is present, but permanently blocks the develpment of virtually all Igh^b allotype Ly-1 B cells (P. Lalor et al., in preparation)

TABLE II. Neonatal Treatments with Monoclonal Anti-Ig

Antibodies:	AF6-78.25	anti Igh-6b (IgM allotype)
	AF6-122.2	anti Igh-5b (IgD allotype)
	MOPC-21	isotype control
$Igh^{a/b}$ Mice:	(BALB/c x SJL/J)F1	
	(BALB/c x BAB/25)F1	



* 120ug per injection (i.p.)

TABLE III. Summary of Neonatal Treatments with Monoclonal Anti Ig Allotypes

$Igh^{a/b}$ heterozygotes

* Anti Igh-6b (IgM) depletes all Igh^{b} B cells; Ly-1 B fail to recover

* Anti Igh-5b (IgD) depletes most Igh^{b} B; leaves Ly-1 B; all B cells recover

Igh^{b} homozygotes

* Anti Igh-6b (IgM) depletes all B cells; all B cells recover (Ly-1 B later)

* Anti Igh-5b (IgD) depletes most B; leaves Ly-1 B; all B cells recover

Much of our work, including the antibody treatment studies just described, relies heavily on multiparameter FACS analysis for estimations of Ly-1 B cell and other B cell frequencies in various lymphoid organs. In many expermiments, however, we independently evaluate Ly-1 B cell representation by injecting animals with 10 ug. of LPS intravenously and (2-3 days later) measuring the frequency of splenic cells capable of forming hemolytic plaques when plated on bromelain-treated mouse erythrocytes. Earlier studies show that these well-known autoantibody producing cells (anti-BrMRBC PFC) are all contained within the FACS-sorted Ly-1 B population (1). Thus, not surprisingly, they tend to be well represented in (autoimmune) animals that have high Ly-1 B frequencies and poorly represented in animals that have few Ly-1 B cells (3, P. Lalor, unpublished).

Aside from being a measure of the autoimmune state of the animal, anti-BrMRBC PFC levels have proven particularly useful as an index of Ly-1 B cell presence because the greater

sensitivity of the PFC assay substantially extends the lower limit of Ly-1 B detection. Thus small numbers of anti-BrMRBC PFC may be detectable in animals in which FACS analyses fail to reveal significant numbers of splenic or peritoneal Ly-1 B cells.

For example, transfer of peritoneal cells into lethally irradiated, Igh-congenic mice (rescued by co-transfer of bone marrow of the recipient Igh allotype) establishes Ly-1 B cells of the donor Igh allotype, at levels readily detected by FACS analyses, within the peritoneal cavity but not the spleen of the recipient mice. However, anti BrMRBC PFC of the donor Igh allotype can be detected within the spleen of these mice, representing greater than 90% of the total anti BrMRBC PFC response (9). As a further example, 6 month old Igh^b allotype heterozygous mice, that lack FACS-detectable Igh^b Ly-1 B cells due to neonatal treatment with anti Igh-6b (IgM^b allotype), show low but detectable levels of splenic Igh^b anti BrMRBC PFC (P. Lalor et al., in preparation).

The curious propensity for Ly-1 B cells to produce autoantibodies becomes even more intriguing when considered in the light of the evidence cited above showing that neonatal antibody treatments can significantly alter the levels of Ly-1 B cells throughout life. This evidence, which suggests that a record of neonatal immunological experience may be retained in this B cell population, is consistent with evidence discussed earlier showing that Ig^+ Ly-1 B cells reconstitute the Ly-1 B population in irradiated recipients. Typical splenic and lymph node B cells, in contrast, are continually replenished from self-renewing Ig^- progenitors whose repetoire (apparently) remains unaltered throughout life.

The mechanism(s) that generate and maintain antibody diversity in Ly-1 B cells have yet to be defined. The repetoire of the initial pre-B progenitor pool (fed from non-rearranged progenitors) should be dependent on the same kinds of Ig chromosome (VDJ) rearrangements that introduce diversity into traditional (BM-derived) B cell populations. The repetoire of the progenitor pool in older animals, however, will be decreased to the extent that clones of original progenitor cells have been depleted and increased to the extent that somatic mutation or other diversity-generating mechanisms are able to operate.

Current studies indicate that the repertoire of the Ly-1 B population in adults does not shift radically from that in neonates in that hybridomas made with peritoneal cells from adults have a similar specificity range to hybridomas made from neonates (J. Kearney, C. Bona, personal communication). In both cases, the frequencies of hybridomas producing autoantibodies and anti-idiotype antibodies are substantially increased in comparison with hybridomas prepared from immunized adult spleens. This would be consistent with the maintence of the Ly-1 B populations from cells whose specificity was defined in the neonate; however, it would also mean that the repetoire of such populations in adults would be highly restricted (since the introduction of "new" V_H regions would be rare).

Alternatively, preliminary studies with a murine Ly-1 B tumor line (NFS-5) established by W. Davidson and H.C. Morse (National Institutes of Health, USA), raise the possibility

TABLE IV. History of Kappa/Lambda Expression Changes in NFS-5, a Ly-1 B Cell Tumor Line

Original tumor (NFS-5)	Rearranged Igh and kappa light chain
Culture (no LPS)	Surface Ly-1, no surface Ig
Clones Cultured	Surface Ly-1, mu; no light chains
LPS added	Kappa expression
LPS continued	Lambda rearrangement and expression Lambda only and kappa/lambda clones
LPS removed	Surface Ly-1, mu; no light chains

TABLE V. Summary of Igh-V Segment Replacement in NFS-5, a Ly-1 B Tumor Cell Line

* **Original tumor and cloned cell lines express an Igh V segment from the Q52 family**
* **The expressed V segment is replaced by a V segment from the 7183 family in one clone isolated after LPS culture**
* **V segment replacements have been found associated with the non-expressed Igh genes in several clones**

that V_H substitutions and kappa/lambda light chain switches may serve to increase antibody diversity amongst Ly-1 B (and perhaps other) self-renewing B cells (See tables IV and V). Kappa to lambda switches clearly occur at reasonable frequencies in NFS-5 (10) and lambda light chain expression is clearly increased in Ly-1 B cell populations (3); however, further studies are required to determine whether this increase reflects light chain switches in normal Ly-1 B populations.

The existence of a mechanism that allows V genes to be replaced on the active IgH chromosome once VDJ-rearrangement is complete introduces enormous potential for diversification in self-renewing B cell populations. Thus it is of interest that recent studies have shown that V_H genes can be replaced in NFS-5 cells that have already initiated Ig production (Kleinfeld, Weigert, Tarlinton and Herzenberg, in preparation). However, once again, further studies are required to determine whether such V gene replacements occur in vivo and contribute to diversity in the normal Ly-1 B population.

Taken together, these findings raise interesting questions concerning the cellular and molecular mechanisms operating in the Ly-1 B population and the significance of such mechanisms with respect to auto-immune disease. Hopefully these questions will begin to be answered shortly.

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