MINIREVIEW

A Function for Ly-1$^+$ B Cells (43129C)

JANET A. HARDIN, KOEN VOS, YUTAKA KAWANO, AND DAVID H. SHERR
Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

During the last decade, there have been a number of documented sightings of Ly-1$^+$ (CD5$^+$/Leu-1$^+$) B cells in both murine and human experimental systems. Despite these sightings and despite the circumstantial evidence linking Ly-1$^+$ B cells to disease, the contribution of this B cell subset to normal or pathologic immune responses has not been defined. In this report, we will consider some of the unusual characteristics of Ly-1$^+$ B cells and try to demonstrate that most of these traits can be accommodated by the working hypothesis that Ly-1$^+$ B cells can serve as regulators of B cell development and/or function.

Before constructing this hypothesis, we will review some of the peculiarities of Ly-1$^+$ B cells. Special emphasis will be placed on (i) Ly-1$^+$ B cell tissue distribution and ontogeny, (ii) restrictions governing Ly-1$^+$ B cell immunoglobulin heavy or light chain usage and repertoire development, (iii) the theory that Ly-1$^+$ B cells represent a separate B cell lineage, and (iv) the association of Ly-1$^+$ B cells to autoimmunity, lymphoproliferation, and transformation.

Ly-1$^+$ Cell Distribution and Ontogeny

Before 1980, the expression of cell surface antigens by lymphocyte subpopulations was determined primarily by cytotoxicity studies or by immunohistochemistry. By using these relatively nonquantitative methods, Ledbetter *et al.* (1) first suggested in 1980 that cells residing in B cell areas of lymph nodes expressed low levels of the Lyt-1 antigen. The subsequent identification of a number of murine B cell lymphomas which stained with monoclonal anti-Lyt-1 antibodies confirmed that B cells could express this formerly T cell-associated glycoprotein (2). With the advent of flow cytometry, it was possible to quantitate the expression of Lyt-1 on normal B lymphocytes. In 1982, Manohar *et al.* (3) documented the coexpression of IgM and Lyt-1 (then referred to as Ly-1) on splenocytes from normal mice (3) and demonstrated that Ly-1 expression on B cells was more subtle (up to 1 log duller staining) than on T cells. The consensus was that Ly-1$^+$ B cells constituted about 1–2% of the splenic lymphocyte population in most strains of normal mice. Interestingly, it was noted that the frequency of Ly-1$^+$ B cells was significantly increased in the spleens of autoimmune NZB mice (3).

Organ distribution studies revealed that, unlike conventional B cells, Ly-1$^+$ B cells were not detectable in adult murine bone marrow. However, they represented 25–50% of the B cell population in the peritoneal cavity (4). Recent studies indicated that Ly-1$^+$ B cells were disproportionately represented in gut-related mesenchymal areas such as Peyer's patches (5), thymus (6, 7), coelom (8), and omentum (9). The level of Ly-1$^+$ (CD5$^+$) B cells in humans may be somewhat higher than that in the mouse, CD5$^+$ B cells constituting up to 18% of the peripheral B cell pool (10).

A number of experimental results argued that Ly-1$^+$ B cells were frozen in a relatively immature state. They were present and somewhat expanded early in the ontogeny of normal mice (11). Similarly, they represented a major proportion of the lymphocyte pool in human cord blood (10), up to 40–60% of the splenic B cells in the human fetus (11), and a preponderance of B cells in humans shortly after bone marrow transplantation (12). Furthermore, Ly-1$^+$ B cells appeared as large, lymphoblastic B cells (13), expressing the immature B cell marker J11D (14), high levels of IgM and low levels of IgD (4). The genetic or epigentic forces which appear to limit Ly-1$^+$ B cell differentiation are unknown.

Immunoglobulin Expression and Repertoire Development

Several investigators demonstrated that the V gene repertoire of Ly-1$^+$ B cells was markedly restricted. Thus, Ly-1$^+$ B cell hybridomas and normal Ly-1$^+$ B cells preferentially expressed a restricted set of V_H genes (15–17). Indeed, in one system, in which bromelain-treated RBC-specific B hybridomas were generated

0037-9727/90-1952-0172$2.00/0

from Ly-1$^+$ B-enriched populations, it was shown that most hybridomas used V_H11 genes, a V_H family composed of only two members (18, 19). Analogous V_H gene restrictions were observed in human CD5$^+$ (Ly-1$^+$) tumors (17).

Light chain selection also appeared to be atypical. Up to 50% of the peritoneal Ly-1$^+$ B cells expressed λ light chains (20). In another system, all of the Ly-1$^+$ B cells capable of participating in idiotype specific interactions expressed λ light chains (14). This result was surprising in view of the fact that only about 5% of the B cell population in normal mice expressed λ light chains. Of the Ly-1$^+$ B cells which employ κ light chains, a restricted repertoire of V_k genes was shown to be utilized. Thus, the bromelain-treated RBC-specific hybridomas mentioned above employed a single V_k gene, V_k9 (18). Similar results have been obtained in human systems. Although the molecular mechanisms and the selective pressures leading to restricted Ig repertoire usage are not known, it has been postulated that at least some Ly-1$^+$ B cells are capable of employing novel mechanisms for V_H and V_L rearrangements (21, 22).

Are Ly-1$^+$ B Cells Part of a Separate B Cell Lineage?

In 1985, it was reported that irradiated recipients reconstituted with Ly-1$^+$ B cells from the peritoneal cavity regenerated only Ly-1$^+$ B cells in the peritoneal cavity (23). Injection of Ly-1$^-$ bone marrow cells resulted in reconstitution of the conventional Ly-1$^-$ B cell, but not the Ly-1$^+$ B cell, compartment. These and other similar experiments (24) were taken as evidence that conventional bone marrow stem cells, which readily gave rise to conventional B cells, were incapable of differentiating into Ly-1$^+$ B cells and, therefore, that Ly-1$^+$ B cells represented a distinct B cell lineage (20).

However, additional studies suggested alternative interpretations. For example, irradiated CBA/N mice treated with cyclosporine A and reconstituted with autologous bone marrow developed high levels of Ly-1$^+$ B cells (25). Thus, under the right conditions, Ly-1$^+$ B cells could be elicited from bone marrow populations. Recently, Ly-1$^+$ B cells were shown to be generated when young, but not old, mice with severe combined immunodeficiency disease were reconstituted with normal bone marrow cells (C. Martinez-A, personal communication). Similarly, reconstitution of irradiated human recipients with bone marrow cells resulted in expression of CD5$^+$ (Ly-1$^+$) B cells (12). That Ly-1 may represent a differentiation antigen was supported by a number of studies in which Ly-1 expression was modulated under a variety of conditions (26, 27).

There is another complication in using Ly-1 as a marker for a putative B cell lineage. An Ly-1$^-$ "sister population" exists which, like the Ly-1$^+$ population, expresses Mac-1, high levels of IgM, and low levels of IgD, perpetuates in irradiated recipients and produces autoantibodies (20). It is not known at this time if this subset represents another B cell lineage, a subset of conventional B cells, and/or a precursor population to Ly-1$^+$ B cells. Until these issues are clarified, it is not possible to assume that all Ly-1$^-$ cells are distinct from and unrelated to Ly-1$^+$ B cells. Therefore, even Ly-1 may not be a good marker for the "Ly-1 B cell lineage."

In summary, none of the experiments performed to date definitively prove or disprove the theory that Ly-1 marks a separate B cell lineage. Despite the apparent ability to induce Ly-1 expression on Ly-1$^-$ cells and the similarity of Ly-1$^-$ "sister cells" to Ly-1$^+$ B cells, it is still possible that both populations are part of a distinct lineage. Evaluating B cell development by isolating precursor and, ultimately, stem cell populations and testing if they are capable of differentiating into conventional Ly-1$^-$ and/or Ly-1$^+$ B cells (28) may be the only way to resolve this issue. Such studies may prove that both theories are correct, i.e., that Ly-1 marks a differentiation marker on a distinct B cell lineage.

Ly-1$^+$ B Cells in Disease

Perhaps the strongest association of Ly-1$^+$ B cells with disease is with autoimmunity. Some of the earliest flow cytometry experiments demonstrated an increased frequency of Ly-1$^+$ B cells in autoimmune NZB mice (3, 13). Fluorescence cell sorting of these cells demonstrated that they produced the bulk of the spontaneously secreted autoantibodies in these mice. Subsequently, it was shown that the B cell population of another autoimmune mouse strain, me^v/me^v motheaten viable mice, was composed exclusively of Ly-1$^+$ B cells (29). Ly-1$^+$ B cell autoantibodies specifically bound DNA, thymocytes, immunoglobulin constant and variable regions, and common cell membrane components such as phosphatidyl choline (13, 15, 30). One intriguing observation was that the number of Ly-1$^+$ B cells in autoimmune MRL and BXSB mice could be affected by diet (31).

Similarly, human CD5$^+$ B cells were shown to be more frequent in the blood of Sjogren's syndrome patients and in the synovial fluid of rheumatoid arthritis patients (32). Indeed, the CD5$^+$ B cells of rheumatoid arthritis patients secreted rheumatoid factors (33–35). Interestingly, CD5$^+$ B cells were expanded relatively early in the onset of acquired immunodeficiency syndrome (AIDS) (36). The role of these cells to AIDS-related autoimmune hemolytic anemia or to other autoimmune phenomena, believed by some to contribute to the pathology seen in AIDS (37), is unknown. Similarly, the relative ease with which some human and murine retroviruses infect CD5$^+$/Ly-1$^+$ B cells may be of some import in disease processes (38, 39).

Ly-1$^+$ B cells exhibited a propensity for spontaneous proliferation and transformation. They were dis-

proportionately represented as spontaneous lymphomas in mice and humans. For example, Ly-1$^+$ B cells comprised 94% of malignant human B cell chronic lymphocyte leukemias (40, 41). Consistent with the association of Ly-1$^+$ B cells with autoimmunity, B-CLL have been shown to produce autoantibodies *in vivo* (42). Similarly, human and murine lymphomas frequently expressed the Ly-1/CD5 marker (40, 43). Of note was the fact that a number of murine B cell lymphomas used to study B cell development and activation, such as BCL_1 and the CH lymphoma series, expressed Ly-1$^+$. In at least one instance, Ly-1$^+$ cells were shown to grow spontaneously from primary B cell cultures (44). Furthermore, Ly-1$^+$ B cells were occasionally seen in large clonal foci in aged mice (45). One could speculate that the frequency with which Ly-1$^+$ B cells proliferated and transformed was an undesirable consequence of their constant stimulation with autoantigens.

Ly-1$^+$ B Cell Function

The bulk of the evidence summarized above indicated that Ly-1$^+$ B cells were associated with, if not causally related to, autoimmunity. These observations, in combination with the propensity of Ly-1$^+$ B cells to transform, would appear to engender them with physiologically undesirable qualities. What, then, could be the selective value of this autoimmune B cell subset? This question has been particularly vexing in view of the poor reactivity of Ly-1$^+$ B cells to conventional T-dependent and T-independent antigens (4, 13, 46) and their contribution to the bulk of "natural" autoreactive antibodies in serum (4, 47). Results obtained in several laboratories, including our own, suggest that Ly-1$^+$ B cells can act as regulators of immunity.

Okumura *et al.* (48) were the first to suggest that Ly-1$^+$ B cells could help antigen-specific responses of conventional Ly-1$^-$ B cells. These investigators demonstrated that nylon-wool-adherent, anti-Thy-1 antibody plus C-treated Ly-1$^+$ B cells from naive mice (B′ cells) could augment 2,4-dinitrophenol (DNP) hapten-specific secondary antibody responses when cocultured with DNP-primed B cells and suboptimal numbers of carrier primed T cells. Although the mechanism responsible for this activity was not elucidated, the requirement for IgH homology between the B′ and the primed responder B cell donors implicated a role for IgH-linked idiotypic determinants in maintaining the specificity of the helper activity. Similar results were obtained by Ono *et al.* (49). In addition, Ono *et al.* (49) provided data supporting the theory that regulatory Ly-1$^+$ B cells could themselves be the targets of antigen-specific T suppressor factors, thereby implying a further complexity of Ly-1$^+$ B cell-dependent immune homeostasis.

The notion of regulatory subpopulations of B lymphocytes was further supported by Yamamoto *et al.* (50). In these studies, idiotype-specific B cells, generated by immunization with MOPC 104E antibody, were shown to help dextran-specific, MOPC 104E id$^+$ B cell responses. While Ly-1 was never formerly detected on these putative helper cells, their inclusion in the Ly-1$^+$-related subset was supported by other phenotypic (e.g., preferential λ light chain expression) and functional characteristics (e.g., H-2-restricted helper activity).

Recent work from the laboratory of Kearney *et al.* (9) suggests that Ly-1$^+$ B cells play a role in the development of the B cell repertoire. Having demonstrated a network of interconnecting idiotypic and anti-idiotypic antibodies in neonatal animals (51) and noting the increased frequency of Ly-1$^+$ B cells in fetal and neonatal mice, these investigators recently demonstrated that the idiotypic network, which predominated in young animals, consisted primarily of Ly-1$^+$ B cells. The appearance of this idiotypic network in ontogeny before conventional antigen-reactive B cells supported the intriguing hypothesis that Ly-1$^+$ B cells helped drive maturation of the B cell repertoire before challenge with exogenous antigens.

While these and other studies (52) support an important role for Ly-1$^+$ B cells in B cell development and immune regulation, they do not detail the mechanisms by which Ly-1$^+$ B cells functioned or the relationship of Ly-1$^+$ B cells to autoimmunity. Having characterized an *in vitro* model system of immunity (53), our laboratory was in a position to confirm the postulated regulatory role of Ly-1$^+$ B cells and to begin to investigate the conditions under which Ly-1$^+$ B cells develop.

Ly-1$^+$ B Cell-Dependent Regulation of Secondary Ly-1$^-$ B Cell Responses to NP Hapten: Ly-1$^+$ B Cell Effector Function

T cell-depleted spleen cell populations from 4-hydroxy, 3-nitrophenyl acetyl-Keyhole limpet hemocyonin (NP-KLH)-primed IgH^b mice produced significant numbers of NP-specific plaque-forming cells (PFC) after challenge with the T-independent antigen NP-Ficoll. The predominance of a family of serologically related NP^b idiotypic determinants expressed by the responding NP-specific B cells could be detected by plaque inhibition with NP^b idiotype-specific antibodies. Prodded by the findings of Okumuara *et al.* (48), we investigated the possibility that an Ly-1$^+$ B cell subset, present in the NP-primed responder population, was responsible for the observed preferential expression of NP^b idiotype-positive PFC (54).

NP-primed responder cells treated with anti-Thy-1 antibodies + C to eliminate T cells produced significant numbers of NP-specific PFC, usually in the range of 2000–3000 PFC/culture, after *in vitro* challenge with NP-Ficoll. Of these, approximately half expressed NP^b idiotypic determinants as assessed by PFC inhibition

with anti-NP^b antibodies. However, when T cell-depleted responder populations were further treated with anti-Ly-1 antibody + C or panned on petri dishes coated with purified anti-Ly-1 antibody, the level of NP^b idiotypic expression dropped to 0%. Two interpretations were entertained: (i) Ly-1$^+$, Thy-1$^-$ B cells produced all of the NP^b idiotype-positive NP-specific PFC or (ii) Ly-1$^+$, Thy-1$^-$ B cells were required, perhaps as idiotype-specific regulatory cells, for the preferential expression of NP^b idiotype-positive, Ly-1$^-$ PFC responses.

Addition of nylon-wool-adherent, Thy-1$^-$ splenic "B cell" populations from normal mice to Thy-1$^-$, Ly-1$^-$ responder cells challenged with NP-Ficoll resulted in the reconstitution of the NP^b idiotype-positive response (Table I). Expression of maximal levels of NP^b idiotype-positive responses (41–45% NP^b) after reconstitution with anywhere from 10^4 to 2×10^6 nylon-wool-adherent, Thy-1$^-$ B cells indicated that B cells added to responder cultures were not themselves making NP^b idiotype. This experimental protocol (summarized in Fig. 1) was adopted to test the working model (Fig. 2) that idiotype-specific Ly-1$^+$ B cells could preferentially help NP^b idiotype-positive B cell subsets.

By specifically depleting lymphocyte populations with lytic antibodies or by positively selecting populations on purified antibody-coated petri dishes, the B cell subset present in the nylon-wool-adherent fraction required and sufficient for the transfer of NP^b idiotype-specific helper activity was shown to express Ly-1, IgM, IgD, LFA-1, Ia, and λ light chain determinants (14, 54).

Table I. Titration of NP^b Idiotype-Specific Helper Activity Transferred by Nylon-Wool-Adherent, Thy-1$^-$ B (B_H) Cells[a]

Normal B (B_H) cells added to Thy-1$^-$, Ly-1$^-$ NP-primed responder cultures[b]	% NP^b idiotypic PFC[c]
None (8)	3 ± 6
2×10^6 (6)	45 ± 7[d]
10^4 (3)	41 ± 3[d]
10^3 (3)	5 ± 12

[a] Responder B cell populations were obtained from mice primed 4 weeks previously with NP-KLH. Spleen cells from primed mice were treated with a pool of anti-Thy-1.2 antibody + C and panned on petri dishes coated with purified monoclonal anti-Ly-1.2 antibody. Nonadherent cells (7.5×10^6) were challenged with 50 ng of NP-Ficoll. The number of experiments is in parentheses.

[b] Nylon-wool-adherent cells were treated with anti-Thy-1.2 antibodies + C. These B_H populations contained no T cells as detected by phenotypic (FACS) or functional (responsiveness to concanavalin A) analyses.

[c] Four days after challenge with NP-Ficoll, cultures were harvested and assayed for direct NP-specific PFC responses. Percentage of NP^b idiotype-positive B cells was determined by plaque inhibition with monoclonal and purified polyclonal NP^b idiotype-specific antibodies.

[d] Value indicates a significant increase in NP^b idiotypic PFC ($P < 0.01$).

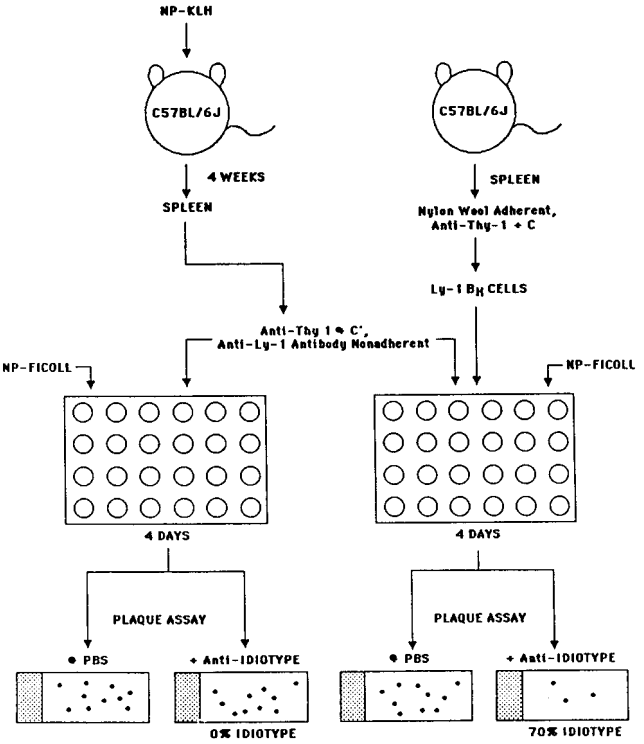

Figure 1. A bioassay for idiotype-specific, Ly-1$^+$ B helper (B_H) cells. C57BL/6 (IgH^b) mice are immunized with NP-KLH. Four weeks later, their spleens are depleted of T cells by treatment with a cocktail of anti-Thy-1.2 antibodies + C. Potentially contaminating T cells and Ly-1$^+$ B cells are depleted by panning on petri dishes coated with purified anti-Ly-1 antibody. Nonadherent cells (7.5×10^6) are plated in Mishell/Dutton cultures and challenged with NP-Ficoll. B_H cells are obtained by passing spleen cells from normal mice over nylon-wool. The B cell-enriched adherent fraction is then treated with an anti-Thy-1.2 cocktail + C to ensure the absence of T cells. These populations are phenotypically (by FACS) and functionally (no response to concanavalin A) negative for T lymphocytes. B cells (10^4 – [2×10^6]) are then added to NP-Ficoll-challenged responder cultures. Four days later, the percentage of NP-specific B cells expressing NP^b idiotypic determinants is determined by inhibition of plaque formation with monoclonal and polyclonal anti-NP^b antibodies.

Similarly, IgM, Ly-1$^+$ B cells sorted by fluorescence-activated cell sorting (FACS) (Fig. 3A, upper box) were sufficient for the transfer of NP^b idiotype-specific helper activity to responder B cell populations (71 ± 16% NP^b idiotype). This Ly-1$^+$ B helper population was referred to as B_H. It is important to note that these results, along with those obtained in a number of protocols, including purification of Ly-1$^+$ B_H cells from athymic mice (55), mitigated against a role for T cells in the observed helper activity.

Expression of Ly-1 was predicted by the initial results in which depletion of Ly-1$^+$, Thy-1$^-$ cells from responder cultures ablated NP^b idiotypic expression. The relative high frequency (at least 1 in 10^4 nylon-wool-adherent B cells) of Ly-1$^+$ B_H cells from *unprimed* mice underscored the potential importance of what might be called a "regulatory idiotope" (56) associated with the NP^b idiotype and further suggested that Ly-1$^+$ B cells played a critical role in the dominance of this

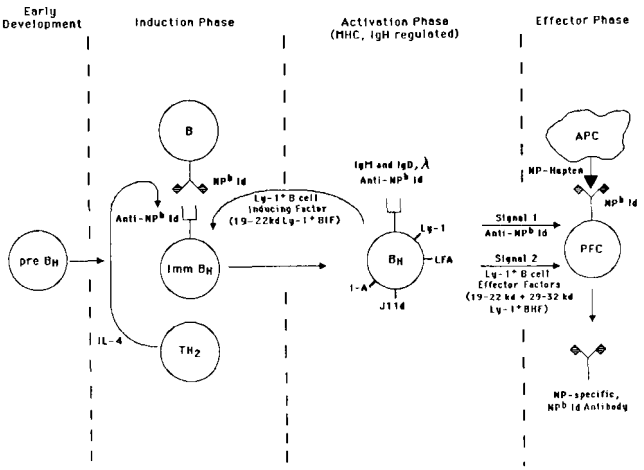

Figure 2. A working model of Ly-1$^+$ B cell development and function. Precursor B_H cells are viewed as surface Ig$^-$ lymphocytes. They develop into immature B_H cells (immB_H) which express idiotype-specific Ig receptors. Splenic immB_H are induced to mature into Ly-1$^+$ B helper cells (B_H) within 40 hr of the addition of IL-4 or Ly-1$^+$ B cell-derived BIF (19- to 22-kDa Ly-1 BIF). Idiotypic B cells appear to play a role in the stimulation and/or selection of idiotype-specific B_H cells. Adult bone marrow B cell populations contain few, if any, mature Ly-1$^+$ B_H cells but do contain preB_H or immB_H cells capable of developing into B_H cells after exposure to IL-4. The spleen contains B_H and immB_H cells which develop into Ly-1$^+$ B cells under the influence of lymphokines. Mature B_H cells express Ly-1, J11d, LFA-1, and I-A antigens. Their surface receptor in the NP-hapten system is idiotype-specific antibody (IgM and IgD, λ light chain). Activation of the B_H population is regulated at least in part by MHC class 2 genes. Once activated, B_H cells secrete two types of synergizing activitites: (i) anti-idiotype which delivers early signals, concomitant with antigen, to idiotypic, NP-specific Ly-1$^-$ responding B cells (PFC) and (ii) BHF (19–22 and 29–32 kDa), which delivers a late-acting signal to responding B cells. It is not known if 19- to 22-kDa BIF is identical to 19- to 22-kDa BHF.

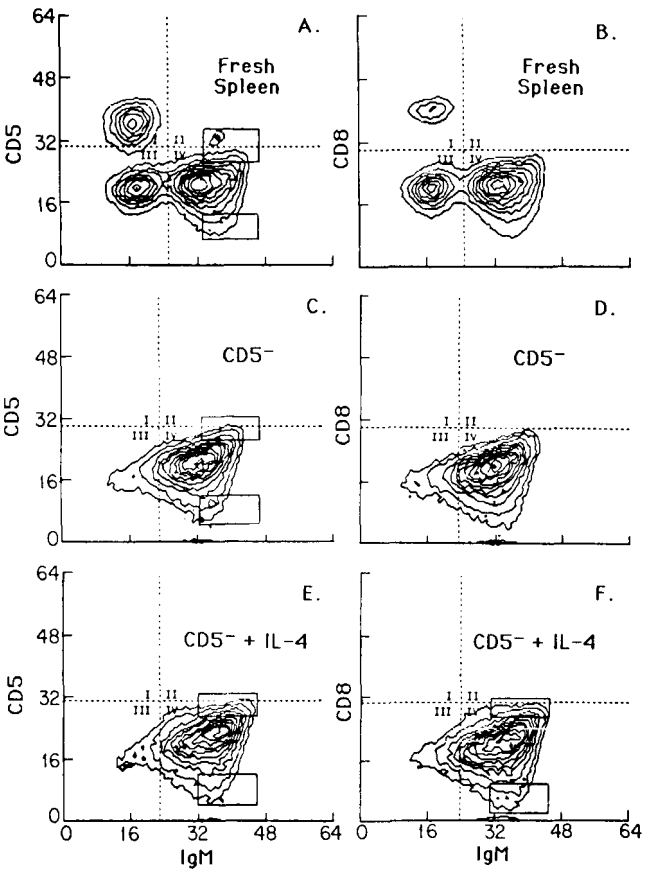

Figure 3. Fluorescence cell sorting of Ly-1$^+$ (CD5$^+$) B cells from untreated spleen and from IL-4-supplemented B cell cultures. Fresh spleen cells (A and B) and CD5$^-$ B cell populations cultured for 2 days in media alone (C and D) or in media supplemented with 20 units/ml rIL-4 (E and F) were passed on Ficoll-Hypaque to remove dead cells. Viable cells were treated with fluorescein-conjugated F(ab')$_2$ goat anti-mouse IgM and either biotin-conjugated rat anti-CD5 or biotin-conjugated rat anti-CD8 antibody plus R-phycoerythrin-conjugated avidin, washed, and analyzed in a Becton Dickinson FACS 440. Monocytes and red blood cells were excluded from analysis and sorting on the basis of 90° and forward light scatter parameters. The data are presented as three-dimensional contour plots in which the logarithmic scales are divided into a 64 × 64 decade grid. Sixteen decades represent 1.5 logs of relative fluorescence. Quadrants were drawn using the anti-CD8 antibody-treated cell populations as isotype controls for nonspecific staining. Boxes superimposed on contour plots represent subsets sorted for functional analysis (Table IV).

and perhaps other idiotypes in primary responses. The coexpression of IgM and IgD on B_H cells was consistent with data from other laboratories in which members of the "Ly-1$^+$ B cell lineage" had been defined as IgM^{high}, IgD^{low} cells (20).

An intriguing finding of these early studies was the exclusive expression of λ light chains on B_H cells. While expressed only by 5–10% of normal murine B cells, disproportionate expression of λ light chains by Ly-1$^+$ B cells had frequently been noted (4). Indeed, the expression of functional λ light chains in at least one Ly-1$^+$ lymphoma *already* possessing successfully rearranged and expressed κ light chains had been documented (22).

By coculturing B_H with responder B cells from H-2 congenic mice, we noted that B_H helper activity was controlled at least in part by major histocompatibility complex (MHC)-encoded determinants (55) (Table II). This apparent H-2 restriction mapped to the I-A subregion. While an unusual finding, an apparent H-2 restriction on B-B cell interactions was not without precedent. As noted above, Bitoh *et al.* (57) demonstrated that the activity of idiotype-induced Ly-1$^+$-like helper cells was H-2 restricted. Several studies implying that B cells could recognize MHC class II determinants by means other than MHC-specific antibody (58) predated those of Bitoh (59, 60). Of particular note was the observation that B cells from autoimmune systemic lupus erythematosus patients spontaneously proliferated and secreted antibody when cultured with autologous B cells and that this proliferation required Ia recognition (61). Given the increased frequency and activity of Ly-1$^+$ B cells in systemic lupus erythematosus patients, it would be interesting to determine if these proliferating B cells expressed CD5.

The simplest explanation for the MHC influence on B_H activity is that idiotype-specific B cell populations

Table II. Helper Activity Mediated by B_H Cells but not by Ly-1$^+$ BHF is H-2 Restricted[a]

Added to responder cultures	Responder cell donor	% NP^b idiotype[b]
C57BL/6 B_H cells	C57BL/6	64 ± 9[c] (2)
C57BL/6 B_H cells	B10.BR	6 ± 18 (2)
Control supernatant	C57BL/6	−16 ± 17 (4)
C57BL/6 B_H hybridoma supernatant	C57BL/6	76 ± 15[c] (3)
Control supernatant	B10.BR	11 ± 20 (3)
C57BL/6 B_H hybridoma supernatant	B10.BR	96 ± 5[c] (4)
C57BL/6 B_H hybridoma supernatant	B10.S	100 ± 0 (1)

[a] Responder cells were prepared from NP-KLH-primed C57BL/6 (H-2^b), B10.BR (H-2^k), or B10.s (H-2^s) mice as described in Table I. Thy-1$^-$, nylon-wool-adherent B_H cells were prepared as described in Table I. B_H cells (5×10^5) or 20 μl of control or Ly-1$^+$ B_H hybridoma supernatant were added to the responder cultures with NP-Ficoll. Four days later, cultures were assayed for NP^b idiotypic PFC responses.

[b] Arithmetic mean ± SE. The number of experiments is indicated in parentheses.

[c] Value indicates a significant level of NP^b idiotype expression as assayed by plaque inhibition with monoclonal and polyclonal NP^b idiotype-specific antibodies ($P < 0.01$).

Table III. B_H Cells Bind NP^b Idiotypic Monoclonal Antibodies[a]

B_H cells added to responder cultures	Treatment of B_H population	% NP^b Idiotype
−	NA	−1 ± 7
+	Untreated	47 ± 6[b]
+	4C2 (NP^{b-})	38 ± 12[b]
+	MOPC 104E (NP^{b-})	48 ± 7[b]
+	B1-8 (NP^{b+})	−13 ± 5

[a] Responder and B_H populations were prepared as described in Table I. Where indicated, B_H populations were further treated with NP^b idiotype-positive or negative monoclonal (IgM) antibodies + C.

[b] Value indicates a significant level of NP^b idiotype ($P < 0.01$).

are contaminated with requisite, MHC-restricted T helper populations. Several results mitigate against but do not exclude this possibility: (i) B_H activity is found in athymic mice; (ii) a low number (10^4) of nylon-wool-adherent, anti-Thy-1 plus complement-treated spleen cells, which by functional (concanavalin A responsiveness) and phenotypic (Thy-1 expression) parameters appear to be depleted of T cells, are required to transfer helper activity; (iii) spleen cells sorted by FACS for IgM$^+$, Ly-1$^+$ populations transfer helper activity; (iv) Ly-1$^-$ B cell populations mixed with T cell populations do not transfer helper activity; and (v) helper activity can be enriched on ligand (NP^b idiotype)-coated petri dishes. Although the means by which MHC control on B_H function is manifest are not known, one simple hypothesis is that the Ig receptor on B_H cells binds idiotype in the context of MHC-encoded molecules with a higher avidity than idiotype alone and that this high-avidity binding is required for B_H activation and subsequent lymphokine production (see below).

The idiotype-specific helper activity observed in the NP system could most easily be explained in terms of idiotypic networking if the autoreactivity of Ly-1$^+$ B cells in general was manifest as idiotype-specific reactivity. The hypothesis was supported by the specific recovery of NP^b idiotype-specific helper activity on NP^b idiotype-coated petri dishes (54) and the specific ablation of helper activity with monoclonal NP^b idiotypic antibody + C (Table III).

Carrying the analogy further, we postulated that if idiotype-specific Ly-1$^+$ helper cells represented the nonpathologic counterpart of autoimmune B cells, then Ly-1$^+$ B_H cells should be present in increased frequency in autoimmune mice. Motheaten viable (me^v/me^v) mice were chosen to test this hypothesis since the B cell population of this severely autoimmune strain was shown to be composed almost exclusively of Ly-1$^+$ B cells (26) and since its *H-2* and *IgH* genotypes (*H-2^b*, *IgH^b*) allowed us to observe B_H help provided to normal C57BL/6 (H-2^b, IgH^b) responder B cells. As few as 10^3 B cells from me^v/me^v provided NP^b idiotype-specific help to normal responder B cells (62). This result represented at least a 1 log increase in NP^b idiotype-specific B_H cells in this autoimmune strain.

Furthermore and more significantly, supernatants from unstimulated me^v/me^v (but not normal C57BL/6) B cell cultures or me^v/me^v sera were capable of substituting for Ly-1$^+$ B cells in providing NP^b idiotype-specific help (62). Production of soluble helper activity *in vitro* was dependent on Ly-1$^+$, λ light chain-bearing B cells. Thus, me^v/me^v B_H cells were not only present in higher frequencies in this autoimmune mouse strain but they appeared to be hyperactive.

Following from theories of idiotypic networking, we initially assumed that the soluble factor constitutively produced by me^v/me^v B cells would prove to be NP^b idiotype-specific antibody. To test this assumption, me^v/me^v B cell supernatants or sera were fractionated on NP^b idiotype-coupled Sepharose columns. As expected, column filtrates failed to augment NP^b idiotypic PFC responses. To our surprise, the column eluates were similarly incapable of providing helper activity. Only a combination of NP^b idiotypic column filtrates and eluates could substitute for B_H cells. We concluded that at least two activities—NP^b idiotype-specific antibody, which presumably imparted the specificity of the help, and a nonimmunoglobulin lymphokine—were required for NP^b idiotype-specific helper activity. Rather than attempt to identify and purify the putative lymphokine(s) from supernatants of heterogeneous populations of hyperactivated B cells, we directed our attention toward a similar soluble factor produced by Ly-1$^+$ B cell hybridomas (30).

A number of hypoxanthine-aminopterin-thymi-

dine-resistant Ly-1$^+$, IgM$^+$ hybridomas were produced and shown to constitutively produce B_H-replacing helper factor(s). When supernatants from these hybridomas were fractionated on NP^b idiotype-coupled Sepharose columns, again only combinations of column filtrates and eluates provided NP^b idiotype-specific helper activity (30). NP^b idiotype-positive PFC were induced in responder B cultures only when the NP^b idiotype-coupled Sepharose eluates were added at the time of challenge with NP-Ficoll. In contrast, addition of the filtrate, containing the putative lymphokine(s), to responder B cell populations within 4 hr of the PFC assay (and 4 days after anti-NP^b idiotypic antibody) still resulted in preferential expression of NP^b idiotype-positive PFC, suggesting that the lymphokine(s) functioned at the level of B cell differentiation. Thus, the helper activity of B_H cells appeared to be mediated by a combination of idiotypic networking (signal 1) and lymphokine-mediated cell activation (signal 2) mechanisms (Fig. 2). The Ly-1$^+$ B cell-derived helper factor was referred to as Ly-1$^+$ BHF (63).

With this working model of Ly-1$^+$ B_H cell helper activity, it was possible to begin to assess the level at which MHC-encoded genes regulated B_H function. B_H cells were shown to provide NP^b idiotype-specific help to IgH-syngeneic, H-2-allogeneic responder populations when hybridoma-derived BHF (i.e., signal 2) was added. This result indicated that signal 1 (i.e., anti-NP^b idiotypic antibody) could be delivered regardless of the H-2 compatibility of the B_H and responder cell donors. Similarly, hybridoma-derived BHF was shown not to be H-2 restricted when added to responder cultures along with anti-NP^b idiotypic antibody (Table II). Finally, T cell-derived lymphokines could bypass the apparent H-2 restriction when added to cultures of H-2-mismatched B_H and responder cells (data not shown). Since B_H- or T cell-derived lymphokines, but not idiotype-specific antibody, bypassed H-2 restriction, it was concluded that H-2-encoded genes controlled B_H cell activation and/or lymphokine production.

Our ability to enrich the hybridoma-derived, protease-sensitive, glycosylated BHF 1000-fold on lectin columns enabled us to begin its characterization.

The first question to be addressed was whether BHF activity was mediated by one or a combination of known B cell-activating cytokines. Fractions containing as much as 2×10^4 units of BHF failed to exhibit cytokine-specific activity in assays for interleukin 1 (IL-1), IL-2, IL-3, IL-4, IL-6, granulocyte-macrophage colony-stimulating factor (CSF), granulocyte CSF, or γ-interferon (63). In addition, RNA isolated from BHF-producing Ly-1$^+$ B hybridomas failed to hybridize with probes specific for IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, granulocyte-macrophage CSF, or γ-interferon (63). RNA from all Ly-1$^+$ hybridomas tested, including the 750-6 fusion partner and one hybridoma which failed to produce BHF, hybridized with tumor growth factor-β probes. Within the limits of detectability, these and other functional studies indicated that BHF activity was not mediated by one or a combination of these cytokines.

In recent months, a new lymphokine, tentatively referred to as IL-10, has been described (64, 65). This 17-kDa molecule is produced by Ly-1$^+$ lymphomas and lipopolysaccharide-stimulated Ly-1$^+$ B cells. It appears to be capable of (i) increasing survival in culture of B cells, (ii) increasing Ia expression on resting B cells, and (iii) inhibiting lymphokine production by T_{H1} cells. BHF-containing fractions have failed to induce Ia on B cells or to increase B cell viability after 2–7 days in culture. Nevertheless, the exact relationship of IL-10 to BHF awaits cloning of the BHF gene.

Finally, gel filtration of BHF enriched from serum-free supernatants revealed two peaks of BHF activity, one at 19–22 kDa and a second at 29–32 kDa.

Ly-1$^+$ B Helper Cell Induction

Since autoreactive Ly-1$^+$ B cells and their secreted factors may play an important role in disease as well as in normal immune responses, we sought to determine under what conditions they matured. By using the B_H system as a sensitive assay for mature Ly-1$^+$ B cells, the ability of T cell-derived IL-4 to induce the maturation of Ly-1$^+$ B_H cells was studied. This lymphokine was chosen because of its documented effects on B cell activation and differentiation.

Nylon-wool-adherent, Thy-1$^-$ B cells from normal mice were depleted of B_H activity by panning on anti-Ly-1 antibody-coated petri dishes. Flow cytometric and functional analyses of the nonadherent fraction revealed that these populations were at least 99.6% depleted of B_H cells. Incubation of these Ly-1$^-$ B cells with rIL-4 for 40 hr resulted in the induction of significant levels of NP^b idiotype-specific helper activity observed on transfer to cultures of NP-primed B cells challenged with NP-Ficoll. That this induced activity was mediated by NP^b idiotype-specific, Thy-1$^-$, Ig$^+$, Ly-1$^+$ B_H cells was supported by depletion of the induced helper activity with lytic antibodies and by positive selection of helper activity on antibody-coated petri dishes.

A curious but consistent result was obtained when the phenotype of IL-4-induced cells was assessed by flow cytometry (Fig. 3 and Table IV). Ly-1$^-$ B cells were subcultured for 40 hr in media alone or media supplemented with rIL-4. Cells were then stained for coexpression of IgM and Ly-1 (CD5) using isotype-matched Lyt-2-specific antibody as a specificity control. Staining of control splenic populations revealed CD5$^+$ T cells (Fig. 3A, quadrant 1), CD5$^-$, IgM$^+$ B cells (Fig. 3A, quadrant 4), and a small fraction (2.7 of the lymphocytes) of double-staining cells (Fig. 3A, quadrant 2).

Table IV. Fluorescence-Activated Cell Sorting of IgM^+, $CD5^+$ ($Ly\text{-}1^+$) Helper Cells from IL-4-Supplemented B Cell Cultures[a]

Cell fraction	Figure 3 representation	% NP^b idiotype[b]
No cells added		1 ± 6 (6)
0.5–2 × 10^4 IgM^+, $CD5^+$ (whole spleen)	3A, upper window	71 ± 16[c] (3)
0.8–8 × 10^4 IgM^+, $CD5^-$ (whole spleen)	3A, lower window	0 ± 17 (3)
0.6–2 × 10^4 IgM^+, "$CD5^+$" (media cultured)	3C, upper window	8 ± 8[c] (2)
0.9–2 × 10^4 IgM^+, $CD5^-$ (media cultured)	3C, lower window	28 ± 14 (2)
2 × 10^4 IgM^+, $CD5^+$ (IL-4 cultured)	3E, upper window	97 ± 6[c] (2)
2 × 10^4 IgM^+, $CD5^-$ (IL-4 cultured)	3E, lower window	−25 ± 27 (2)
2 × 10^4 IgM^+, "$CD8^+$" (IL-4 cultured)	3F, upper window	100 ± 0[c] (2)
2 × 10^4 IgM^+, $CD8^-$ (IL-4 cultured)	3F, lower window	86 ± 18[c] (2)

[a] Responder cells were prepared as described in Table I. Thy-1^-, $CD5^-$ B (nylon-adherent, anti-Thy-1 +-C treated, and anti-CD5-nonadherent) cells were subcultured for 2 days in media alone or media containing 20 units of rIL-4. Whole spleen cells, cells cultured in media alone, or media containing 20 units/ml rIL-4 were passed on Ficoll-Hypaque to remove dead cells and were treated with fluorescein-conjugated affinity-purified $F(ab')_2$ goat anti-mouse IgM. Cells were washed and reincubated with biotin-conjugated monoclonal rat CD5- or CD8-specific antibody in the presence of 10-fold excess normal rat Ig followed by incubation with R-phycoerythrin-conjugated avidin. Samples were analyzed and sorted in a Becton Dickinson FACS 440 or a Coulter EPICS V flow cytometer. Probability contours and approximate sorting gates for a representative experiment are presented in Figure 3. Approximately 10% of the total lymphoid population is represented in each window.

[b] Arithmetic mean ± SE. The number of experiments is indicated in parentheses.

[c] Value indicates a significant increase in NP^b idiotypic PFC ($P < 0.001$).

Thy-1^-, Ly-1^- cells cultured for 2 days in media alone (Fig. 3C and D) failed to stain with CD5-specific antibody. Given the data on induced B_H function, it was surprising that B cells cultured in the presence of IL-4 did not express a clearly resolvable $CD5^+$, IgM^+ population (Fig. 3E). It was hypothesized that the induction of only a subset of autoreactive Ly-1^+ B cells and the relatively low expression of CD5 on splenic B cells precluded the visualization of a distinct $CD5^+$ subpopulation. Nevertheless, it was postulated that $CD5^{dull}$ B cells were present in the IL-4-cultured population. To test this hypothesis, B cells cultured with IL-4 were sorted for putative $CD5^{dull}$ (Fig. 3E, upper box) and $CD5^-$ (Fig. 3E, lower box) subpopulations. Equal numbers (2×10^4) of sorted cells were added to $CD5^-$ responder cultures and tested for NP^b idiotype-specific helper activity. Cells presumed to be $CD5^{dull}$ significantly augmented NP^b idiotype-positive PFC responses (Table 4; 97% NP^b). In contrast, $CD5^-$ cells failed to affect NP^b idiotypic expression.

In comparison, both subpopulations selected from anti-IgM and anti-CD8 antibody-treated cells transferred significant helper activity, indicating that Ly-1^+ B_H helper cells were distributed throughout the anti-CD8 antibody-treated population and that, as previously shown (54), 2×10^4 cells were sufficient for helper activity. In addition to supporting the previous conclusion that IL-4-induced $CD5^+$/Ly-1^+ B helper cell activity, these data highlight the sensitivity of the assay for functionally mature Ly-1^+ B helper cells.

The Ly-1^+ B_H cell population was shown to be expanded and hyperactive in autoimmune me^v/me^v mice (62). Experiments with Ly-1^+ B_H hybridoma-derived BHF suggested that Ly-1^+ B cells produced B cell-activating factors. These observations indicated that Ly-1^+ B_H cells may secrete B cell-activating factors which affect Ly-1^+ B cell maturation. To test for such autocrine factors, BHF-containing G-75 Sephadex fractions were added to Thy-1^-, Ly-1^- B cell populations as described above for IL-4. Two days later, cells were tested for induced B_H activity (66). Indeed, a 19- to 22-kDa fraction, which was shown to contain BHF activity (63), was also capable of inducing Ly-1^+, NP^b idiotype-specific B helper cells. Interestingly, a 29- to 32-kDa fraction, which contained BHF activity, failed to induce Ly-1^+ B_H cells. The Ly-1^+ B hybridoma-derived, Ly-1^+ B cell-inducing factor is referred to as BIF.

Finally, it was noted that if Thy-1^-, Ly-1^- B cell populations were depleted of NP^b idiotypic B cells before being subcultured with rIL-4 or if NP^b idiotype-specific antibody was added along with rIL-4 to the subcultures, then NP^b idiotype-specific B_H cells were not induced. Thus, cross-linking of the surface Ig receptor resulting from recognition of self (idiotypic-determinants was required for the maturation of Ly-1^+ B helper cells. Whereas recognition of autoantigens generally results in B cell deletion or tolerance, recognition of autoantigens (idiotypes) is essential for Ly-1^+ B cell development. One is then left to consider the as yet undefined mechanisms by which Ly-1^+ B cells become immune to self tolerance.

Synthesis

Despite an abundance of data describing the unusual Ly-1^+ B cell population, clarification of their evolutionary value is still forthcoming. However, given current information, it is possible to construct a unifying theory of B cell development and function. Such a working model would have to embrace the following concepts: (i) Ly-1^+ B cells secrete autoantibodies, but these antibodies have yet to be proven to be pathologic, (ii) Ly-1^+ B cells are expanded early in ontogeny, (iii)

reconstitution experiments with irradiated normal and severe combined immunodeficiency disease recipients suggest differences in the developmental pathway of conventional Ly-1$^-$ and Ly-1$^+$ B cells, (iv) recognition of self-determinants is required for Ly-1$^+$ B cell maturation, (v) the tissue distribution of Ly-1$^+$ B cells differs from that of Ly-1$^-$ B cells, (vi) an Ly-1$^-$ sister population resembles Ly-1$^+$ B cells in most aspects except the expression of Ly-1 antigen and idiotype-specific helper activity, (vii) Ly-1$^+$ B cells have increased proliferative potential and frequently undergo transformation, (viii) Ly-1$^+$ B cells utilize a restricted V_H and V_L gene repertoire, and (ix) Ly-1$^+$ B cells make novel B cell-directed lymphokines.

Data obtained in the NP system suggest that at least one selective advantage of autoreactive Ly-1$^+$ B cells is to provide idiotype-specific activation signals to antigen-specific Ly-1$^-$ B cells. In this system, the autoreactivity of Ly-1$^+$ B cells empowers them with the ability to specifically regulate idiotype-positive, antigen-specific B cell responses (i). Ly-1$^+$ B cell activation of NP-primed but not unprimed (data not shown) B cells suggests that this regulatory activity may preferentially affect secondary immune responses. Similarly, the apparent participation of idiotype-specific Ly-1$^+$ B cells in antigen-independent B cell repertoire development (51) is consistent with the theory that Ly-1$^+$ B cells "talk to" and regulate immature Ly-1$^-$ B cell populations as well as memory B cells. This would at least in part explain the abundance of Ly-1$^+$ B cells early in ontogeny (ii).

With regard to idiotypic networking, skeptics have pointed out that such a system of intercellular communication would require that a specific subpopulation of idiotype-reactive B cells bypass self tolerance. It is amusing, therefore, to speculate that Ly-1$^+$ B cells represent the distinct lineage/pathway of B cell development which has evolved for the purpose of idiotype-dependent immune regulation (iii).

Immunity to self-tolerance would offer this B cell subset the opportunity to expand or mature rather than be deleted upon exposure to self-antigens. This outcome is exactly what was noted in our studies on IL-4- or BIF-dependent induction of Ly-1$^+$ B_H cells in which idiotypic recognition was shown to be critical for Ly-1$^+$ B_H maturation (iv). Furthermore, a requirement for Ly-1$^+$ B cells to encounter self-antigens during their development could contribute to the unusual tissue distribution of Ly-1$^+$ B cells. Thus, whereas conventional B cell populations expand their repertoire on exposure to exogeneous antigens in the periphery, autoreactive Ly-1$^+$ B cells may prefer to mature in cell compartments richer in particular autoantigens or maturational factors such as IL-4 and BIF (v). This maturational process may represent a lymphokine- and autoantigen-dependent conversion from Ly-1$^-$ sister cells to mature Ly-1$^+$ B cells (vi). Continued stimulation with autoantigen could result in clonal expansion and a higher chance of accumulation of genetic changes which result in transformation (vii).

Another criticism of idiotype networking is that, in order to prevent a series of self-perpetuating cell interactions in which the specificity of the interactions degenerates with each interaction, idiotype-anti-idiotypic interactions must be limited to a subset of "regulatory idiotopes" (56). Perhaps this requirement would best be satisfied by a subpopulation of B cells expressing a restricted Ig repertoire. Ly-1$^+$ B cells would neatly fit this bill (viii). Indeed, the general failure of Ly-1$^+$ B cells to express heavily mutated V genes and the select usage of certain V_H and V_L genes could reflect selective pressures in favor of maintaining regulatory idiotope specificity and against generating pathologic autoantibodies (67) (i).

The production of novel B cell-activating lymphokines by Ly-1$^+$ B hybridomas (BIF/BHF) or by lipopolysaccharide-stimulated Ly-1$^+$ B cells (provisional IL-10; 64) suggests that cross-linking of Ig receptors (by antigen and/or anti-idiotype) followed by lymphokine-mediated growth and/or differentiation signals is a common B cell-activating strategy employed in both T and Ly-1$^+$ B helper cell-dependent responses (ix). The identification of Ly-1$^+$ B cell-derived, B cell-directed lymphokines further suggests that autoimmunity may be exacerbated by Ly-1$^+$ B cell factors. The BIF autocrine may represent a subset of these lymphokines which feeds the cycle by further expanding or activating autoreactive Ly-1$^+$ B cells.

The challenge for the future will be to determine how much of this working model is fanciful and how much is fact.

This research was supported by Grant AI 23978-02 from the National Institute of Allergy and Infectious Diseases, Grant IM-570 from the American Cancer Society, and Grant 810 from the March of Dimes and by Genetics Institute.

1. Ledbetter JA, Rouse RV, Micklem HS, Herzenberg LA. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. Two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. J Exp Med **152**:280–295, 1980.
2. Lanier LL, Warner NL, Ledbetter JA, Herzenberg LA. Expression of Lyt-1 antigen on certain murine B cell lymphomas. J Exp Med **153**:998–1003, 1981.
3. Manohar V, Brown E, Leiserson WM, Chused TM. Expression of Lyt-1 by a subset of B lymphocytes. J Immunol **129**:532–538, 1982.
4. Hayakawa K, Hardy RR, Herzenberg LA. Peritoneal Ly-1 B cells: Genetic control, autoantibody production, increased lambda light chain expression. Eur J Immunol. **16**:450–456, 1986.
5. Kroese FG, Butcher EC, Stall AM, Lalor PA, Adams S, Herzen-

berg LA. Many of the IgA producing plasma cells in murine gut are derived from self-replenishing precursors in the peritoneal cavity. Int Immunol **1**:75–84, 1989.
6. Farinas MC, Adkines B, Stall AM, Herzenberg LH, Weisman I, Strober S. B cell infiltration of the thymic medulla in NZB, NZW, and NXB/NZW mice: Effect of total lymphoid irradiation (TLI) [Abstract 80–12]. Berlin: International Meeting on Immunology, p495, 1989.
7. Inaba M, Inaba K, Adachi Y, Nango K-I, Ogata H, Muramatsu S, Ikehara S. Functional analysis of thymic $CD5^+$ B cells. Responsiveness to major histocompatibility complex class II-restricted T blasts but not to lipopolysaccharide or anti-IgM plus interleukin 4. J Exp Med **171**:321–326, 1990.
8. Marcos MAR, Huetz F, Pereira P, Martinez-A C, Coutinho A. Ontogeny of coelomic-associated B-cell populations [Abstract M121]. J Cell Biochem Suppl **14D**:206, 1990.
9. Kearney J, Solvason N, Vakil M, Lehuen A, Hamilton AM. Development of B cell subsets and antibody repertoires [Abstract M-011]. J Cell Biochem Suppl **14D**:189, 1990.
10. Gadol N, Ault KA. Phenotypic and functional characterization of human LEU1 (CD5) B cells. Immunol Rev **93**:23–34, 1986.
11. Antin JH, Emerson SG, Martin P, Gadol N, Ault KA. Leu-1^+ ($CD5^+$) B cells. A major lymphoid subpopulation in human fetal spleen: Phenotypic and functional studies. J Immunol **136**:505–510, 1986.
12. Ault KA, Antin JH, Ginsburg A, Orkin SH, Rappeport JM, Keohan ML, Martin P, Smith BR. Phenotype of recovering lymphoid cell populations after marrow transplantation. J Exp Med **161**:1483–1502, 1985.
13. Hayakawa K, Hardy RR, Parks DR, Herzenberg LA. The "Ly-1 B" cell subpopulation in normal, immunodefective, and autoimmune mice. J Exp Med **157**:202–218, 1983.
14. Sherr DH, Ju S-T. Idiotype-specific helper cells (B_H) express immunoglobulin markers and employ T cell function associated molecules. J Immunol **137**:3406–3413, 1986.
15. Mercolino TJ, Locke AL, Afshari A, Sasser D, Travis WW, Arnold LW, Haughton G. Restricted immunoglobulin variable region gene usage by normal Ly-1 ($CD5^+$) B cells that recognize phosphatidyl choline. J Exp Med **169**:1869–1877, 1989.
16. Tarlinton D, Stall AM, Herzenberg LA. Repetitive usage of immunoglobulin V_H and D gene segments in $CD5^+$ Ly-1 B clones of $(NZB \times NZW)F_1$ mice. EMBO J **7**:3705–3710, 1988.
17. Humphries CG, Shen A, Kuziel WA, Capra JD, Blattner FR, Tucker PW. A new human immunoglobulin V_H family preferentially rearranged in immature B-cell tumours. Nature **331**:446–449, 1988.
18. Hardy RR, Carmack CE, Shinton SA, Riblet RJ, Hayakawa K. A single V_H gene is utilized predominantly in anti-BrMRBC hybridomas derived from purified Ly-1 B cells. J Immunol **142**:3643–3651, 1989.
19. Conger JD, Sage HJ, Corley RB. Combining site diversity of murine monoclonal antibodies specific for bromelain-treated isologous erythrocytes [Abstract 45-5]. Berlin: Seventh International Congress of Immunology, p250, 1989.
20. Herzenberg LA, Stall AM, Lalor PA, Sidman S, Moore WA, Parks DR, Herzenberg LA. The Ly-1 B cell lineage. Immunol Rev **93**:81–102, 1986.
21. Kleinfeld R, Hardy RR, Tarlinton D, Dangl J, Herzenberg LA, Weigert M. Recombination between an expressed immunoglobulin heavy-chain gene and a germline variable gene segment in a Ly-1^+ B cell lymphoma. Nature **322**:843–846, 1986.
22. Hardy RR, Dangl JL, Hayakawa K, Jager G, Herzenberg LA, Herzenberg LA. Frequent lambda light chain gene rearrangement and expression in a Ly-1 B lymphoma with a productive kappa chain allele. Proc Natl Acad Sci USA **83**:1438–1442, 1986.
23. Hayakawa K, Hardy RR, Herzenberg LA, Herzenberg LA. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. J Exp Med **161**:1554–1568, 1985.
24. Forster I, Rajewsky K. 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, 1987.
25. DeLa Hera MAR. Marcos ML, Toribio C, Marquez ML, Gaspar Martinez-A C. Development of Ly-1^+ B cells in immunodeficient CBA/N mice. J Exp Med **166**:804–809, 1987.
26. Palacious R, Stuber S, Rolink A. The epigenetic influences of bone marrow and fetal liver stroma cells on the developmental potential of Ly-1^+ pro-B lymphocyte clones. Eur J Immunol **19**:347–356, 1989.
27. Miller RA, Gralow J. The induction of Leu-1 antigen expression in human malignant and normal B cells by phorbol myristic acetate (PMA). J Immunol **133**:3408–3414, 1984.
28. Hardy RR, Kishimoto T, Hayakawa K. Differentiation of B cell progenitors *in vitro*: Generation of surface IgM^+ B cells, including Ly-1 B cells, from Thy-1^- asialo GM_1^+ cells in newborn liver. Eur J Immunol **17**:1769–1774, 1987.
29. Sidman CL, Shultz LD, Hardy RD, Hayakawa K, Herzenberg LA. Production of immunoglobulin isotypes by Ly-1^+ B cells in viable motheaten and normal mice. Science **232**:1423–1425, 1986.
30. Sherr DH, Braun J, Dorf ME. B helper cell hybridomas. Idiotype specific Ly-1 B cell mediated helper activity. J Immunol **138**:2057–2062, 1987.
31. Ogura M, Ogura H, Lorenz E, Ikehara S, Good RA. Undernutrition without malnutrition restricts the numbers and proportions of Ly-1 B lymphocytes in autoimmune (MRL and BXSB) mice. Proc Soc Exp Biol Med **193**:6–12, 1990.
32. Dauphinee M, Tovar Z, Talal N. B cells expressing CD5 are increased in Sjogren's syndrome. Arthritis Rheum **31**:642–647, 1988.
33. Burastero SE, Casali P, Wilder RL, Notkins AL. Monoreactive high affinity and polyreactive low affinity rheumatoid factors are produced by $CD5^+$ B cells from patients with rheumatoid arthritis. J Exp Med **168**:1979–1992, 1988.
34. Hardy RR, Hayakawa K, Shimizu M, Yamasaki K, Kishimoto T. Rheumatoid factor secretion from human Leu-1^+ B cells. Science **23**:81–83, 1987.
35. Plater-Zyberk C, Maini TN, Lam K, Kennedy TD, Janossy G. A rheumatoid arthritis B cell subset expresses a phenotype similar to that in chronic lymphocytic leukemia. Arthritis Rheum **28**:971–976, 1985.
36. Moody DJ, Hollander H, Wang YJ, Stites DP. Human immunodeficiency induced hyperglobulinemia and its association with CD5 (Leu-1) expression on B cells. Washington, DC: The Third International Conference on AIDS. **30**:30, 1987.
37. Hoffmann G, Grant M, Kion T. Is AIDS provoked autoimmunity? Canadian Research, September, p16, 1988.
38. Mann DL, DeSantis P, Mark G, Pfeifer A, Newman M, Gibbs N, Popovic M, Sarngadharan MG, Gallo RC, Clark J, Blattner W. HTLV-I associated B-cell chronic lymphocytic leukemia: Indirect role for retrovirus in leukemogenesis. Science **236**:1103–1106, 1987.
39. Klinken SP, Fredrickson TN, Hartley JW, Yetter RA, Morse HC III. Evolution of B cell lineage lymphomas in mice with a retrovirus-induced immunodeficiency syndrome, MAIDS. J Immunol **140**:1123–1131, 1988.
40. Anderson KC, Bates MP, Slaughenhoupt BL, Pinkus GS, Schlossman SF, Nadler LM. Expression of human B cell associated antigens on leukemias and lymphomas: A model of human B cell differentiation. Blood **63**:1424–1433, 1984.
41. Caligoris-Cappio F, Gobbi M, Janossy G. Infrequent normal B lymphocytes express features of B-chronic lymphocytic leukemia. J Exp Med **155**:623–628, 1982.
42. Sthoeger ZM, Wakai M, Tse DB, Vinciguerra VP, Allen SL,

Budman DR, Lichtman SM, Schulman P, Weiselberg LR, Chiorazzi N. Production of autoantibodies by CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia. J Exp Med **169**:255–268, 1989.

43. Davidson WF, Fredrickson TN, Rudikoff EK, Coffman RL, Hartley JW, Morse HC. A unique series of lymphomas related to the Ly-1$^+$ lineage of B lymphocyte differentiation. J Immunol **133**:744–753, 1984.
44. Braun J. Spontaneous *in vitro* occurrence and long-term culture of murine B lymphoblast cell lines. J Immunol **130**:2113–2116, 1983.
45. Seldin MF, Conroy J, Steinberg AD, D'Hoosteleare LA, Raveche ES. Clonal expansion of abnormal B cells in old NZB mice. J Exp Med **166**:1585–1590, 1987.
46. Hayakawa K, Hardy RR, Honda M, Herzenberg LA, Steinberg AD, Herzenberg LA. Ly-1 B cells: Functionally distinct lymphocytes that secrete IgM autoantibodies. Proc Natl Acad Sci USA **81**:2494–2498.
47. Nakamura M, Burastero SE, Notkins AL, Casali P. Human monoclonal rheumatoid factor-like antibodies from CD5$^+$ (Leu-1$^+$) B cells are polyreactive. J Immunol **140**:4180–4186.
48. Okumura K, Hayakawa K, Tada T. Cell to cell interaction controlled by immunoglobulin genes. Role of Thy-1$^-$, Ly-1$^+$, Ig$^+$ (B′) cells in allotype-restricted antibody production. J Exp Med **156**:443–453, 1982.
49. Ono K, Kato F, Taniguchi M. Possible involvement of Ly-1 B cells in the effector phase of IgG suppression mediated by suppressor T cell factor. J Mol Cell Immunol **3**:167–175, 1987.
50. Yamamoto H, Bitoh S, Fujimoto S. Adaptive differentiation of H-2- and IgH-restricted B lymphocytes in tetraparental bone marrow chimera. J Immunol **138**:380–384, 1987.
51. Kearney JF, Vakil M. Idiotype-directed interactions during ontogeny play a major role in the establishment of the adult B cell repertoire. Immunol Rev **94**:49–50, 1986.
52. MacKenzie MR, Paglieroni TG, Warner NL. Multiple myeloma: An immunologic profile. IV. The EA rosette-forming cell is a Leu-1 positive immunoregulatory B cell. J Immunol **139**:24–28, 1987.
53. Dorf ME, Usui M, Sherr DH. Use of suppressor cell hybridomas to dissect T cell interactions. In: Von Boehmer H, Hass W, Eds. T Cell Clones. Amsterdam: Elsevier, pp41–53, 1985.
54. Sherr DH, Dorf ME. An idiotype specific helper population which bears immunoglobulin, Ia and Lyt-1 determinants. J Exp Med **159**:1189–1200, 1984.
55. Sherr DH, Dorf ME. H-2 restricted helper activity mediated by immunoglobulin bearing lymphocytes. J Immunol **134**:2084–2089, 1985.
56. Bona CA, Heber-Katz E, Paul WE. Idiotype-anti-idiotype regulation. I. Immunization with a levan-binding myeloma protein leads to the appearance of auto-anti-anti-idiotype) antibodies and to the activation of silent clones. J Exp Med **153**:951–967, 1981.
57. Bitoh S, Fujimoto S, Yamamoto H. Evidence for idiotypic and antiidiotypic B-B cellular interaction with the use of cloned antiidiotypic B cell line. J Immunol **144**:2046–2052, 1990.
58. Wylie DE, Sherman LA, Klinman NR. Participation of the major histocompatibility complex in antibody recognition of viral antigens expressed on infected cells. J Exp Med **155**:403–414, 1982.
59. Ono S, Takahama Y, Hamaoka T. Ia-restricted B-B cell interaction. I. The MHC haplotype of bone marrow cells present during B cell ontogeny dictates the self-recognition specificity of B cells in the polyclonal B cell activation by a B cell differentiation factor, B151-TRF2. J Immunol **139**:3213–3223, 1987.
60. Hodes RJ, Singer A. MHC-restricted recognition by antigen-specific B cells: fact or artefact? Immunol Today **5**:50–54, 1984.
61. Tanaka Y, Shirakawa F, Suzuki H, Eto S, Yamashita U. Mechanism of spontaneous activation of B cells in patients with systemic lupus erythematosus. J Immunol **140**:761–767, 1988.
62. Sherr DH, Dorf ME, Gibson M, Sidman CL. Ly-1 B helper cells in autoimmune "viable motheaten" mice. J Immunol **139**:1811–1817, 1987.
63. Hardin JH, Kawano Y, Sherr DH. Biochemical and functional characterization of a potentially novel effector lymphokine produced by CD5$^+$ B helper cell hybridomas. Cell Immunol **126**:304–321, 1990.
64. Howard M. UCLA Symp Mol Cell Biol. April 1990.
65. Fiorentino DF, Bond MW, Mosmann MR. Two types of mouse T helper cell IV. T_{h2} clones secrete a factor that inhibits cytokine production by T_{h1} clones. J Exp Med **170**:2081–2096, 1989.
66. Gibson M, Hardin JA, Sherr DH. CD5$^+$ B helper cell hybridomas produce a lymphokine which induces CD5$^+$ B helper cell populations. J Mol Cell Biol **4**:241–253.
67. Shlomchik MJ, Marshak-Rothstein A, Wolfowicz CB, Rothstein TL, Weigert MG. The role of clonal selection and somatic mutation in autoimmunity. Nature **328**:805–811, 1987.