MINIREVIEW

CD5$^+$ B Lymphocytes (43250)

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Various T lymphocyte subsets can be segregated on the basis of discrete cell surface molecules, other than those (T cell receptors) deputed to the specific recognition of antigen. Combinations of these molecules define function-related phenotypes of T cell subsets. In contrast, the B cell repertoire has long been regarded as a homogeneous pool of lymphocyte clones which are identical in all respects except for the unique specificity of the antibodies they produce (1). A novel and discrete (CD5$^+$) B lymphocyte subset, however, has recently been identified. CD5$^+$ B cells are committed to the production of antibodies, mainly immunoglobulin (Ig) M, binding a variety of self and foreign antigens, including soluble protein molecules, single-stranded DNA (ssDNA), and cellular components, as well as bacterial components and products (polyreactive antibodies) (2, 3). The polyreactivity of these antibodies contrasts with the monoreactivity and specificity of the high affinity antibodies, mainly IgG, elicited in the course of an immune response to a foreign antigen. Most likely, these polyreactive antibodies identify with the class of Ig termed "natural antibodies." Owing to the anti-self reactivity of the antibodies they produce, it has been postulated that CD5$^+$ B cells may, under certain circumstances, play a role in the establishment of autoimmune phenomena. Moreover, these lymphocytes constitute the cellular elements characteristic of at least one important human B cell neoplasm, chronic lymphocytic leukemia (CLL), the most common adult leukemia in Western societies. In the present review, we will (i) discuss the phenotypic and ontogenic features of CD5$^+$ B cells; (ii) consider the characteristics of the antibodies produced by CD5$^+$ B cells and their expressed variable (V) gene segments; (iii) delineate the possible role of CD5$^+$ B lymphocytes in the normal B cell repertoire and in autoimmune patients; and (iv) speculate on the contribution of CD5$^+$ B cells to the specific antibody response elicited by a defined antigen. Much of the information presented here is directly derived from our experimental findings in the human. Data from studies of murine Ly-1$^+$ (CD5$^+$) B cells will be discussed when they complement or are at variance with information gained in human studies. We refer the reader to some recent and thorough reviews for a more comprehensive discussion of murine CD5$^+$ B cells (4–7).

Phenotype, Distribution, and Ontogeny of CD5$^+$ B Cells

The CD5 Molecule and Identification of CD5$^+$ B Cells. The CD5 molecule (T1, Leu 1) is a 67-kDa transmembrane glycoprotein (8, 9). Its complete amino acid sequence has been deduced from the nucleotide sequence of complementary DNA clones constructed using human T cell mRNA, and shown to be similar (63% identical) to the deduced amino acid sequence of the murine pan-T cell molecule, Ly-1 (10). The predicted secondary structures of the human and murine CD5 molecules display features common to many growth factor receptors, including a high cysteine content in the extracellular domain and a large cytoplasmic region (8, 10). The carboxy-terminal regions (cytoplasmic domain) of the murine and human CD5 proteins are 90% identical (10) and include a potential tyrosine phosphorylation site (8, 10). Although CD5 is found on all T cells, its level of expression varies among T lymphocyte subsets (11) and is modulated during cellular activation (12). CD5 may be involved in T cell proliferative responses to antigenic or mitogenic stimuli (12), as suggested by the ability of anti-CD5 monoclonal antibodies (mAb) to enhance signal transduction by the CD3-T cell receptor complex (13, 14). The CD5 antigen found on the surface of B cells is immunologically cross-reactive with the CD5 molecule expressed on T cells, and displays a similar molecular weight (15). Moreover,

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0037-9727/91/1973-0226/$3.00/0

CD5-specific cDNA synthesized using mRNA from a T cell tumor line efficiently cross-hybridizes with mRNA extracted from phorbol myristate acetate-activated human B cells (15).

Among human cells of the B lineage, CD5 was first identified on the surface of circulating B cells from patients with CLL (16–19). The search for expression of CD5 on normal B lymphocytes led to the definition of the novel $CD5^+$ B cell subset in healthy subjects (20, 21), which is thought to represent the physiological equivalent of the neoplastic $CD5^+$ CLL B cell (22). $CD5^+$ B cells express no other surface markers typical of T cells, including CD3, CD4, or CD8 (17), but they do express all the typical B cell surface antigens, including CD19, CD20, and CD21 (23, 24). In contrast to their $CD5^-$ counterparts, $CD5^+$ B cells express the surface $C3b_i$ receptor, CD11b (Mac-1), a marker characteristic of cells of the myelomonocytic lineage (24, 25). Human $CD5^+$ and $CD5^-$ B lymphocytes do not differ in expression of cell surface IgM and IgD, although reduced expression of these molecules has been found in some $CD5^+$ CLL B cells (26). Most human $CD5^+$ B cells form rosettes with mouse red blood cells (MRBC) (21). Murine $CD5^+$ B cells display most of the features of their human equivalents (24), except for their characteristically higher surface expression of IgM and lower IgD as compared to their $CD5^-$ counterparts (27).

CD5 is expressed at a much lower level on B cells than on T cells (5, 20, 24, 26–30). In fact, using a B cell-specific mouse mAb (anti-CD20) and a mouse mAb to CD5 in dual fluorescence flow cytometric analysis, $CD5^+$ B lymphocytes can be identified, but not completely resolved, as a cell fraction separate from the remaining ($CD5^-$) B lymphocytes (Fig. 1, panel A). Therefore, among B lymphocytes, $CD5^+$ B cells are conventionally defined as those cells that bind CD5-specific mAb, but do not bind isotype-matched mAb controls with irrelevant specificity (28).

Various agents influence the level of surface CD5 expression on human B cells. B cell activation induced by treatment with phorbol myristate acetate (15, 31–33) or exposure to T cell supernatant (34) results in enhanced CD5 expression, leading to the suggestion that CD5 is an activation marker (35, 36). However, treatment of B cells with anti-IgM antibodies or bacterial lipopolysaccharides, two potent B cell activators, does not result in increased CD5 expression (4, 15, 31). Furthermore, human $CD5^+$ B cell proliferation induced by IL-4 (18, 23) or Epstein-Barr virus (EBV) (35, 37) is consistently associated with a decrease in expression of the surface CD5 molecule, possibly due to a block in the transcription of the CD5 gene (15, 35). In our hands, only a minor proportion (less than 5%) of freshly prepared $CD5^+$ B cells display the features of activated cells, i.e., low density and large size (37, 38). The vast majority of $CD5^+$ B cells display the features of resting cells, i.e., high density and small size (38). Thus, most of the experimental findings available thus far do not support the hypothesis that $CD5^+$ B cells constitute a subset of activated B lymphocytes.

Anatomical Distribution of $CD5^+$ B Cells. $CD5^+$ B cells cannot be detected in adult human bone marrow (20, 31) or, to our knowledge, in human peritoneum. They constitute, however, a significant fraction (10–20%) of total B lymphocytes in human peripheral blood (28, 29), and are consistently found in all major secondary lymphoid organs, including lymph nodes (5%), spleen (17–18%), and tonsils (5–10%) (18, 28, 29). In contrast to the nearly ubiquitous distribution of $CD5^+$ B cells in human blood and lymphoid organs, mouse $CD5^+$ B cells are mostly segregated within the peritoneum, where they can account for up to 45% of total local B cells in adult animals (4, 36). $CD5^+$ B cells, however, are very rare in the mouse peripheral blood,

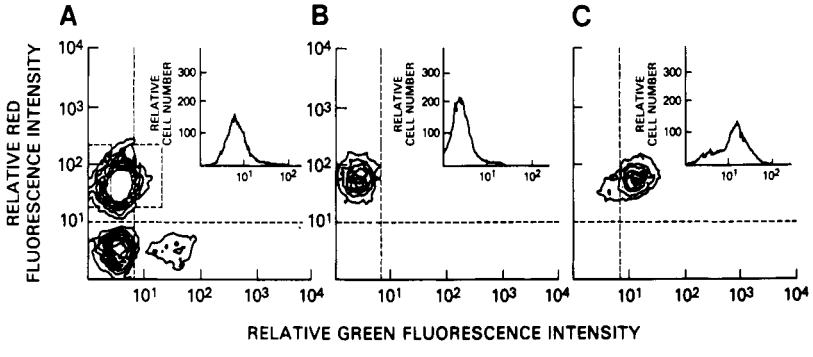

Figure 1. Fluorescence-activated cell sorting of $CD5^+$ and $CD5^-$ peripheral blood B lymphocytes of a healthy subject. Enriched B lymphocytes were simultaneously reacted with phycoerythrin-conjugated mAb to CD20 and biotinylated mAb to CD5, and then reacted with fluorescein isothiocyanate-avidin. (A) Cells included within the left dotted lines (20% of total B cells) and right dotted lines (10% of total B cells) were considered $CD5^-$ and $CD5^+$ B lymphocytes, respectively. These two cell fractions were sorted, collected, and reapplied to the fluorescence-activated cell sorter to evaluate the degree of separation (B and C). Contourgrams and profiles show that the sorted $CD5^+$ and $CD5^-$ B cells fell back within the coordinates established for sorting and overlap only marginally.

spleen, or lymph nodes (24). Interestingly, $CD5^+$ B cells have been found in normal murine thymus, where they account for 70–80% of the very few thymic B lymphocytes (about 1% of total cells) (39). Similar to the human, $CD5^+$ B cells are undetectable in murine bone marrow (27, 36). The skewed anatomical distribution of murine $CD5^+$ B cells suggests that they may not represent, in all respects, the equivalent of human $CD5^+$ B cells.

Ontogeny and Malignancies of $CD5^+$ B Cells. $CD5^+$ B lymphocytes appear in early development, constituting a high proportion of the total fetal and neonatal B cells (6). Approximately 50–75% of B cells found in human fetal spleen (31, 40), and more than 90% of those found in cord blood (30, 41), express CD5. The decrease in the percentage of $CD5^+$ B cells observed in the adult (20, 28, 29) may be secondary to a vigorous expansion of $CD5^-$ B cells, rather than a diminution in absolute numbers of $CD5^+$ B cells (4, 24, 36).

In the human, $CD5^+$ B cells are the first B lymphocytes to repopulate the host following bone marrow transplantation, appearing during the first 2–4 weeks and preceding the emergence of $CD5^-$ B cells by several weeks (42). Thus, although human bone marrow does not contain mature $CD5^+$ B lymphocytes in a detectable number, it does, presumably, contain their precursor cells. The early appearance of $CD5^+$ B cells in development and after bone marrow transplantation suggests that they constitute an "immature" B cell population that can give rise to "conventional" ($CD5^-$) B cells. Alternatively, consistent with their unique expression of myelomonocytic markers and their distinctive anatomical distribution in the mouse, $CD5^+$ B cells may constitute a lineage separate from $CD5^-$ B cells (4, 6, 43, 44). Supporting the hypothesis of a distinct $CD5^+$ B cell lineage are the original experiments by several investigators showing that, in contrast to human bone marrow transplant, the transfer of adult bone marrow into irradiated recipient mice results in reconstitution of $CD5^-$, but not $CD5^+$, B cells (4, 45, 46). Reconstitution of $CD5^+$ B cells in irradiated recipient mice can be achieved, however, by transferring donor cells derived from a source rich in $CD5^+$ B cells, such as adult peritoneum, or neonatal spleen or liver (45). Preliminary observations by Kearney *et al.* (personal communication) show that the omentum may be the primary source of $CD5^+$ B cell precursors in the mouse. These observations are consistent with the hypothesis that murine $CD5^+$ and $CD5^-$ B cells arise from discrete precursors (4–6, 46).

A series of more recent experiments supports the hypothesis that $CD5^+$ and $CD5^-$ B cells follow separate maturation pathways and suggests that the $CD5^+$ B cell pool in the neonate is self-replenishing, and sustains itself throughout the life of the animal (6, 45, 47). Lalor and colleagues (4, 47) investigated murine $CD5^+$ B cell development in the offspring of a cross between C.B-17 mice bearing two distinct Ig allotypes. Newborns were treated with anti-IgM mAb against the paternal allotype, effectively depleting both $CD5^+$ and $CD5^-$ B cells bearing this allotype. Following cessation of mAb treatment, the paternal allotype $CD5^-$ B cells reappeared, indicating their generation from an IgM^- progenitor cell. $CD5^+$ B cells expressing the paternal allotype, however, were permanently depleted, suggesting that no IgM^- progenitor cell was able to replenish this pool. Further experiments indicated that lack of recovery of paternal allotype $CD5^+$ B cells following mAb treatment was due to the continued presence of $CD5^+$ B cells of the maternal allotype (48). A feedback loop appeared operative whereby development of new $CD5^+$ B cells was inhibited by the presence of mature $CD5^+$ B cells.

Further support for an inhibitory role of "mature" $CD5^+$ B cells on the emergence of new $CD5^+$ B cells has been proposed by Riggs *et al.* (49), who reconstituted C.B-17 severe combined immunodeficiency (SCID) mice with splenic and/or peritoneal B cells of distinct immunoglobulin allotypes from normal animals. Donor peritoneal B cells, which include a high proportion of $CD5^+$ B cells, prevented antibody secretion by donor splenic B cells, which are primarily $CD5^-$, when simultaneously engrafted into SCID mice. Cells of the "suppressed" allotype were found in the spleen but not in the peritoneal cavity of allotype chimeric SCID mice, possibly indicating preferential inhibition of engraftment as well as antibody production at certain sites. This "suppression" by peritoneal cells was not due to regulatory T lymphocytes, and could be overcome by several means, including (i) increasing the ratio of splenic to peritoneal allotype cells engrafted; (ii) adding spleen cells at a later time than peritoneal cells; or (iii) injecting spleen cells by a different route than peritoneal cells. In each case, the manipulation resulted in the dominance of cells bearing the allotype of the donor providing splenic, rather than peritoneal, lymphocytes. These findings indicate that dominance of the peritoneal cells was not due to an intrinsic property of this cell type. They also suggest that important regulatory interactions exist between different B cell subsets and they question, to some extent, the interpretation of some of the experiments (4, 6, 45–48) that originally led to the conclusion that $CD5^+$ B cells belong to a lineage separate from "conventional" $CD5^-$ B cells.

Taken together, the grafting studies reviewed here suggest that reconstitution of either SCID or irradiated mice with a lymphoid source rich in $CD5^+$ B cells results in $CD5^+$ B cell repopulation. Reconstitution of similar mice with a lymphoid source rich in $CD5^-$ B cells results in $CD5^-$ B cell repopulation. This contrasts with the observations made in humans transplanted

with bone marrow. In these patients, the donor marrow, a tissue putatively lacking $CD5^+$ B cells, would give rise to both $CD5^+$ and $CD5^-$ B cells. These observations may suggest that important differences exist between human and murine $CD5^+$ B cells. Further studies are required to identify the precursors of $CD5^+$ B cells and to define the regulatory interactions underlying the emergence and development of $CD5^-$ and $CD5^+$ B cells.

The self-replenishing and self-maintaining nature of $CD5^+$ B cells may be an important factor in the tendency of these lymphocytes to give rise to malignancies. $CD5^+$ B "lymphoid malignancies" appear at high frequencies in normal-aged mice and are a common cause of mortality in adult autoimmune New Zealand Black mice (50). In these autoimmune mice, the $CD5^+$ B cell malignant transformation stage may be preceded by a "hyperdiploid" (premalignant) stage of an oligoclonal or monoclonal $CD5^+$ B cell population (7). In aging normal mice, $CD5^+$ B cell lymphomas may emerge following persistent antigenic stimulation, as suggested by the lymphomas arising in aging B10.H-2^aH-4^bp/Wts mice adoptively transferred with syngeneic spleen cells after hyperimmunization with sheep erythrocytes (51, 52). Consistent with the bone marrow-independent origin of normal $CD5^+$ B cells, these murine B cell lymphomas are often characterized by marked splenomegaly, lymph node enlargement, and lack of primary thymus or bone marrow involvement.

In the human, the most common malignancy of the $CD5^+$ B cell is CLL. Similar to normal $CD5^+$ B cells, $CD5^+$ B cells of CLL express markers of the myelomonocytic lineage (24). CD5 may also be expressed on solid tissue human B cell lymphomas, particularly the small-cell lymphocytic variety (24). A relatively high incidence of B cell malignancies occurs in patients with Sjögren's syndrome, an autoimmune disease associated with the presence of antibodies to nuclear antigens, and, to a lesser extent, in patients with rheumatoid arthritis (53, 54). Both diseases are characterized by high levels of circulating $CD5^+$ B cells (30, 55). In Sjögren's syndrome patients, an expanded B cell population carrying the cross-reactive idiotype identified by the 17.109 mAb has been observed (56). The 17.109 mAb was originally raised against an IgM rheumatoid factor (RF) idiotype (57) that is shared by IgM associated with a variety of CLL and Waldenström IgM paraproteins (56–58). It is possible that the expansion of an activated $CD5^+$ B cell subset that occurs in patients with Sjögren's syndrome or rheumatoid arthritis (see also $CD5^+$ B Cells and Autoimmune Diseases: Rheumatoid Arthritis) represents an important stage preceding and leading to the malignant transformation of these cells.

Antibody Production by $CD5^+$ B Cells

$CD5^+$ B Cells are Committed to the Production of Polyreactive Antibodies. The construction of human and murine monoclonal cell lines capable of producing antibodies, mainly IgM, binding to a variety of self antigens, including soluble hormones, nucleic acids, structural cellular and tissue components, etc., was reported by several investigators (59). The first indication that such autoantibodies may be produced by a discrete B cell subset came from the studies by Hayakawa *et al.* (60) on $CD5^+$ B cells in autoimmune New Zealand Black Mice. These mice naturally develop autoimmune pathology and display an unusually high proportion of $CD5^+$ B cells, which spontaneously secrete Ig *in vitro*. These antibodies are of the IgM class and bind many self-antigens, including surface thymocyte antigens, and ssDNA (34, 60). The production of antibody binding to self-antigen is not an exclusive prerogative of $CD5^+$ B cells from autoimmune mice. Sorted $CD5^+$ B cells from normal BALB/c mice can be activated to produce IgM binding to bromelain-treated MRBC (60). Additional observations suggesting a role for $CD5^+$ B cells in the production of autoantibodies came from the study of C57BL/6J mice with the viable motheaten mutation, which display high levels of $CD5^+$ B cells (>95% of total B cells) (61), high titers of circulating IgM and IgG2a autoantibodies (61, 62), and extensive autoimmune pathology (63).

Based on the original observation in mouse $CD5^+$ B cells (60), we investigated the possibility that, in the healthy human, antibodies endowed with anti-self reactivity could be produced by the $CD5^+$ B cell subset. Using dual fluorescence flow cytometry, we sorted $CD5^+$ and $CD5^-$ B cells from a B lymphocyte-enriched peripheral blood mononuclear cell fraction (28). The efficiency of discrimination of the sorting procedure was assessed by reanalysis of the sorted cell fractions (Fig. 1, panels A, B, and C). To investigate the antibody-producing potential of purified $CD5^+$ and $CD5^-$ B cells, we infected them with EBV. Owing to the resting state of most lymphocytes in the normal B cell repertoire, the use of a polyclonal B cell activator is essential to express their antibody-producing potential. In human B lymphocytes, the only efficient B cell activator is EBV, which serves as both a potent B cell activation stimulus and an efficient transforming agent (64). We have thoroughly demonstrated that EBV is equally efficient in transforming B cells bearing surface μ-, γ-, and α-heavy (H) chains to produce IgM, OgG, and IgA, respectively (2, 64–67). EBV-infected purified $CD5^+$ and $CD5^-$ B cells were cultured in limiting dilution before determination of the antigen-binding activity of the produced antibody. We found that the vast majority of $CD5^+$ B lymphocytes produced antibodies binding to a variety of self-antigens, including IgG Fc fragment

(RF), ssDNA, thyroglobulin, and insulin (2, 28, 59, 65) (Fig. 2). These antigen-binding activities encompassed several exogenous antigens as well, such as β-galactosidase from *Escherichia coli*, and tetanus toxoid (28, 59, 65). In contrast, in healthy subjects that had been vaccinated previously but not boosted recently with tetanus toxoid, the lymphocytes committed to producing IgG to tetanus toxoid segregated within the $CD5^-$ B cell subset (28). To formally demonstrate that the polyreactivity detected in fluids from cultured EBV-transformed $CD5^+$ B cells was due to the multiple antigen-binding property of individual antibody molecules, these lymphoblasts were fused with a preconstructed non-Ig-secreting human-mouse fusion partner (2, 66–68). The resulting cell hybrids were sequentially cloned and found to produce high levels of mAb, suitable for fine immunochemical characterization. Although the vast majority of these mAb were IgM, polyreactive IgG and IgA mAb were also generated using $CD5^+$ B cells (67, 68). Each mAb bound a variety of self- and foreign antigens, including bacterial components and products (2, 3, 37, 66, 67). Competitive antigen-binding studies established that the binding of each mAb to a given solid-phase antigen was inhibited in a dose-dependent fashion not only by the homologous soluble antigen but also by heterologous antigens (2, 3, 66, 67). Moreover, each mAb displayed different affinities for different antigens, and different mAb displayed different affinities for the same antigen (2, 3, 66, 67) (Fig. 2). The K_d values of these mAb ranged from 10^{-4} to 10^{-7} M, consistent with low to moderate intrinsic binding affinities (2, 3, 66, 67).

The (polyreactive) autoantibody-producing potential of human $CD5^+$ B cells is reflected in the ability of monoclonal CLL $CD5^+$ B cells to secrete autoantibodies following activation by phorbol myristate acetate or T cell-dependent stimuli. In a thorough study, Stoegher *et al.* (69) showed that the majority of monoclonal $CD5^+$ B cells from 19 patients with CLL and a patient with well-differentiated lymphocytic lymphoma produced IgM or IgG with strong RF activity, in many cases also binding to ssDNA and double-stranded DNA. These findings may explain the occurrence of circulating autoantibodies and autoimmune symptoms in patients with CLL or other B cell lymphomas (24, 31, 70). The interaction of a variety of self antigens with antigen receptors on $CD5^+$ B cells may play a role in initiating or enhancing the clonal expansion of these lymphocytes in lymphoproliferative disorders (see also Phenotype, Distribution, and Ontogeny of $CD5^+$ B Cells: Ontogeny and Malignancies of $CD5^+$ B cells and $CD5^+$ B Cells and Autoimmune Diseases: Rheumatoid Arthritis).

Role of Natural Polyreactive Antibodies. Polyreactive antibodies most likely identify with the type of Ig present in the circulation of healthy individuals and previously termed "natural antibodies" (2, 3), as sug-

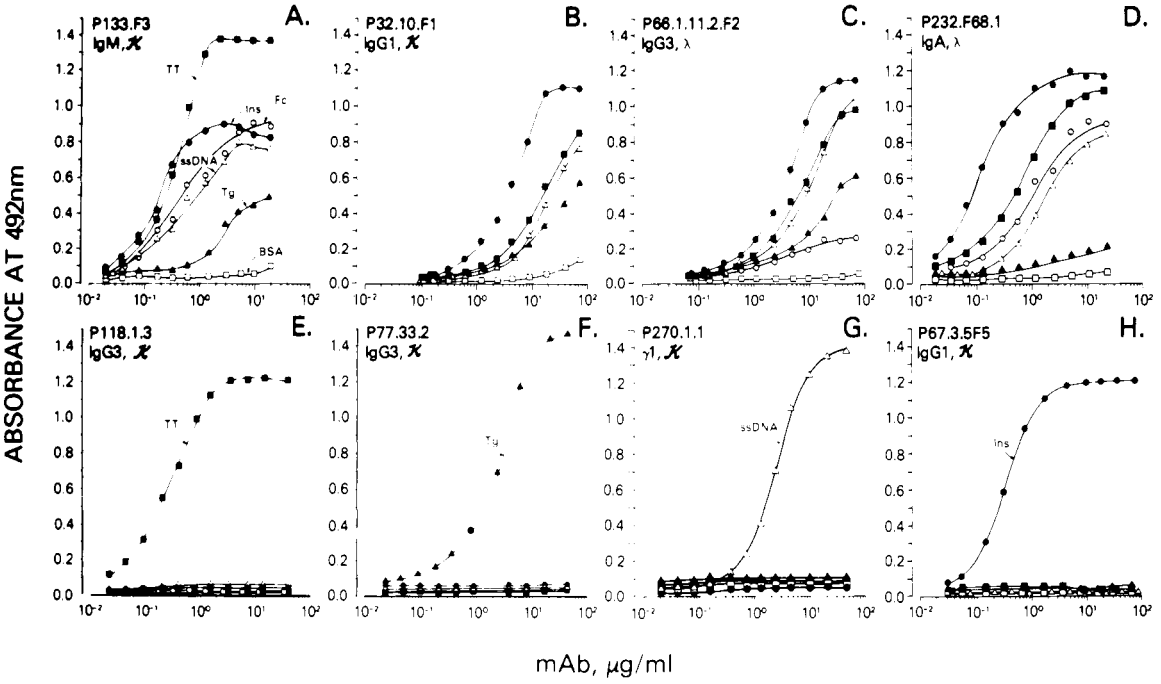

Figure 2. Dose-dependent binding of eight mAb to solid-phase antigens: insulin (●), thyroglobulin (Tg) (▲), tetanus toxoid (TT) (■), IgG Fc fragment (○), ssDNA (△), or bovine serum albumin (BSA) (□). (A–D) Polyreactive IgM, IgG, and IgA generated from B cells or healthy subjects. (E) Anti-TT IgG derived from a healthy subject immunized with TT. (F) Anti-Tg IgG derived from a patient with Hashimoto's disease. (G) Anti-ssDNA IgM derived from a patient with SLE. (H) Anti-insulin IgG derived from a patient with insulin-dependent diabetes mellitus (Reprinted from the Journal of Immunology (Ref. 67), with permission).

gested by their ability to bind lipopolysaccharide from gram-negative bacteria (66) and polysaccharides from gram-positive bacteria (66), as well as viruses, including rabies virus (71), herpes simplex virus, and human immunodeficiency virus-1 (Harindranath *et al.*, manuscript in preparation). *In vivo*, polyreactive antibodies may contribute to the first line of defense against infections by mediating complement-dependent lysis and by enhancing opsonization and phagocytosis. The activation of $CD5^+$ B cells by pathogenic microorganisms to secrete natural (polyreactive) Ig is suggested by the high levels of circulating IgM binding to IgG Fc fragments and other self-antigens found in patients with viral, bacterial, and parasitic infections (72–74). In addition to directly binding antigens on invading microorganisms, polyreactive IgM may amplify the biological activity of specific IgG already bound to pathogens through the recognition of the IgG Fc fragment. Specific IgG aggregated by their binding to microbial antigens may dramatically boost RF production during the immune response (72, 73–78). It has been proposed that in contrast to the preimmune (polyreactive) RF and the RF appearing at early stages of a primary Ig response, some of the RF arising during the specific secondary response display a higher affinity for IgG fragment (77, 78). The dramatic amplification of a specific IgG antibody response by RF is exemplified by the ability of RF arising in rats during a secondary response to *Trypanosoma lewisi* to terminate the *T. lewisi* infection in animals displaying trace amounts of circulating specific IgG (79).

In addition to enhancing the clearance of invading microorganisms, $CD5^+$ B cells may play an important role in the rejection of xenografts, as suggested by the efficient binding of polyreactive mAb (generated from human $CD5^+$ B cells) to a variety of antigens on porcine platelets and endothelial cells (80). Among these antigens, those recognized by "natural" polyreactive human mAb are identical to those recognized by the xenoreactive antibodies naturally occurring in human sera (80). Thus, at least two of the most important biological functions of the immune system are displayed, although less than specifically, by the antibodies derived from $CD5^+$ B cells: (i) the defense of the individual against invading pathogens; and (ii) the preservation of the individuality of the species by the rejection of xenogeneic components. These two functions alone would likely suffice to account for the high representation of polyreactive antibodies in primordial phylogenetic stages: polyreactive IgM are the only antibodies in sharks and torpedo fish (81).

Immunoglobulin Gene Expression by $CD5^+$ B Cells. A major mechanism by which the diversity of the antigen-combining site of antibodies is generated is the combinatorial joining of the gene segments that encode the Ig V region. The full rearrangement of the H chain V region results from the juxtaposition of the diversity (D) and the joining (J_H) segments, followed by the V_H segment. Similarly, the rearrangement of the light (L) chain V region results from the juxtaposition of the V_L and the J_L gene segments. These gene rearrangements are mediated by V_H-D-J_H and V_L-J_L recombinases, the specificity and regulation of which are still poorly understood. In the absence of other known contributory influences, utilization of different V_H genes is thought to be random, resulting in stochastic expression of the members of the various gene families.

Nonstochastic V_H gene expression has been found to occur in early stages of development of the human (82–84) and the mouse (85–89), when $CD5^+$ B lymphocytes constitute the major proportion of the B cell repertoire. In murine fetal pre-B cells, there is a highly preferential utilization of the members of the V_H7183 family, particularly the V_HE415 gene (88–90). In the neonatal mouse, the nonstochastic V_H gene expression includes biased utilization not only of genes of the V_H7183, but also of the V_HQ52 family (91). V_H7183 and V_HQ52 constitute the most J_H-proximal V_H gene families (92). In contrast to the human, in which members of a given V_H gene family are highly interdispersed throughout the 5′ portion of the V_H locus (chromosome 14), most murine V_H gene families map as discrete units within the H chain locus (chromosome 12), perhaps as a result of an extensive process of homologous recombination brought about by thorough inbreeding. Recent studies suggest that the biased utilization of the V_HQ52 and V_H7183 families observed in the murine neonatal B cell repertoire is mainly due to their selective expression by $CD5^+$ B cells, although some overutilization of V_H7183 genes is also observed in $CD5^-$ B cells (91). In adult mice, $CD5^+$ B cells still preferentially express members of the V_HQ52 family and, to a lesser extent, the V_H7183 family, whereas $CD5^-$ B cells express members of the V_H7183 family at a much lower frequency (91, 92), in favor of a more pronounced expression of the members of the most 5′ distal and largest family, V_HJ558 (91, 93–95). Thus, in the adult mouse, some degree of "normalization" in the expression of the V_H gene repertoire takes place (96–98). Such normalization is likely brought about by the repetitive and extensive stimulation of B cell clones by a large number of foreign and, perhaps, self-antigens. Among the latter, cryptic determinants on autologous red cells, as revealed by treatment with the proteolytic enzyme bromelain, may be responsible for the amplification of the mouse peritoneal $CD5^+$ B cell clones expressing V_H11 gene segments in conjunction with J_H1 (99) and producing IgM to bromelain-treated MRBC (99–101). It has been suggested that the developmentally related nonrandom expression of DJ_H-proximal V_H genes is due to a position-dependent rearrangement mechanism, possibly involving a one-dimensional tracking

system (88, 89, 102). However, as V_H7183 and V_HQ52 are highly interdispersed and DJ_H-proximal (92), and since "conventional" $CD5^-$ B cells in the neonatal mouse express preferentially V_H7183, but not V_HQ52, genes (91), it is unlikely that position-dependent rearrangement is responsible for the observed skewing in V_H gene family expression (91). The fact that $CD5^+$ B cells preferentially utilize V_HQ52 segments regardless of their ontogenic stage (91) suggests that expression of these genes is an inherent feature of these lymphocytes. Owing to the under-representation of $CD5^+$ B cells in adult mice, their V_HQ52 gene overutilization would not result in a marked skewness of the overall B cell repertoire.

As discussed above (Phenotype, Distribution, and Ontogeny of $CD5^+$ B Cells: Ontogeny and Malignancies of $CD5^+$ B Cells), the $CD5^+$ B cell is the predominant cell type in the fetal and neonatal B cell repertoires. This is concomitant in the human fetus, at 49 days of gestation, with a skewed expression of gene members of the V_HV and V_HVI families (83). These are the smallest and most DJ_H proximal of the six human gene families, which together encompass fewer than 100 V_H genes (103–108). Later in fetal life, from Day 104 to 130, the vast majority of B cells express genes of the largest V_HIII family, predominantly its 30pl member (82, 109, 110).

Recent findings suggest that, even in the adult B cell repertoire, V_H gene segment expression may not be strictly stochastic and that some gene segments, mainly those of the V_HIII and V_HIV families, are expressed at a frequency at least twice as high as that expected on the basis of their numeric representation in the H chain locus (111). Overexpression of V_HIII and V_HIV family genes may be accompanied by a slight underexpression of members of other families, particularly V_HII (111), and is likely due to overutilization of these genes by $CD5^+$ B cells. We have recently sequenced the V_H genes utilized by 14 polyreactive mAb, some of them formally derived from $CD5^+$ B cells and including 11 IgM, one IgG, and two IgA (Harindranath *et al.*, manuscript in preparation and 113). We found that nine of these polyreactive mAb utilized members of the V_HIV family and five utilized a single member of the V_HIII family, 30pl. Furthermore, probing of the mRNA from 10 $CD5^+$ B cell lines producing polyreactive IgM mAb showed that V_HIV family genes were utilized in nine cases and a V_HIII family gene was utilized in one case (Inghirami *et al.*, manuscript in preparation). The putative preferential expression of the V_HIV gene by human $CD5^+$ B cells may be further supported by the demonstration that over 50% of ($CD5^+$) CLL express gene segments of this family (111) and contrasts with the expression of other V_H gene families, including V_HI and V_HIV, by $CD5^-$ B cells producing high affinity monoreactive mAb in a secondary specific antibody response (71). Overutilization of the (V_HIII) 30pl gene by adult $CD5^+$ B cells is consistent with the marked overutilization of this gene segment in fetal life (82, 110). This gene represents the human V_H segment with the highest similarity to the segment most commonly expressed in fetal and, perhaps, adult murine life, the V_HE415 gene, a member of the V_H7183 family. Although additional experiments are necessary to unequivocally establish whether the relatively biased usage of genes of the V_HIII and V_HIV families in the adult human B cell repertoire is due to their frequent expression by $CD5^+$ B cells, the wide usage of 30pl suggests that this gene may play an important biological role. V_HIII-like genes may have been positively selected in phylogenesis in response to antigens present on common pathogens, as suggested by findings in other species. Both birds and rabbits have multiple V_H gene segments, but all are V_HIII-like (114–116).

Although the molecular basis of antibody polyreactivity has yet to be established, it is thought to rely on the presence of an antigen-combining site capable of accommodating multiple ligands (118). Utilization of V gene segments in unmutated configuration seems to be a characteristic feature of polyreactive antibodies (92, 112, 118), and some germ line V gene segments, e.g., members of the V_HIII and V_HIV families, may contribute to the structural basis of polyreactivity. However, different polyreactive mAb utilize different V_H gene segments. Recent crystallography studies have shown that the V region of the H chain, mainly its complementarity-determining region (CDR) 3, accounts for most of the antigen-binding activity of the whole molecule, particularly in the case of proteinic antigens (119, 120). Consistent with a primary role of the V_H CDR3 segment in determining the polyreactivity of antigen-binding sites, we recently found that the nucleotide sequences of the D segments encoding the CDR3 portion of polyreactive mAb are significantly longer than those of monoreactive molecules (30–60 b and 15–24 b, respectively) (113; Harindranath *et al.*, submitted; Ikematsu *et al.*, manuscript in preparation) (Fig. 3). Although preliminary, these findings raise the possibility that the length of the D region may be an important factor in determining antibody polyreactivity.

As has been found for murine $CD5^+$ B cells, we found that polyreactive mAb-producing cell lines generated using $CD5^+$ B cells from healthy subjects express both κ- and λ-L chains (66, 112). Nonrandom L chain utilization has been consistently observed in RF produced by neoplastic (CLL) $CD5^+$ B cells and in RF isolated from patients with Waldenström's macroglobulinemia and/or mixed cryoglobulinemia (24). These RF, particularly in CLL, preferentially express Cκ- over Cλ-L chain genes (121), most often in conjunction with the unmutated Humkv325 gene, a member of the

A. D Segments of Polyreactive mAbs

mAb18	GGT	GGG	GTT	GAG	CTG	GCA	TCA	ACA	AAA	CCA	TCC	TCT	ATC	TGG	CGA	CTC	AAC	CCA	ATC	AGA
(V_HIII)	G	G	V	E	L	A	S	T	K	P	S	S	I	W	R	L	N	P	I	R
mAb26	CTC	CGT	GTC	TTC	TAT	GAT	AGT	CGT	GGC	CTT	AAA	CAC								
(V_HIV)	L	A	V	F	Y	D	S	R	G	L	K	H								
mAb21	GTT	GCC	AGT	TAC	AGT	TTT	CGT	TTC	CGT	CAC										
(V_HIV)	V	A	S	Y	S	F	R	F	R	H										
mAb63	GGG	GGA	TCG	GTA	TTA	CGA	TTT	TTG	GAG	TGG	TTA	TTA	TAT	CCC	GCT					
(V_HIV)	G	G	S	V	L	R	F	L	E	W	L	L	Y	P	A					
mAb67	GTG	ACG	GGC	TCC	ACA	TTT	TGG	AGT	GGT	TAT	TAT	ACT	CGG	GGT	TAC					
(V_HIV)	V	T	G	S	T	F	W	S	G	Y	Y	T	R	G	Y					
mAb65	TGG	GGC	TAT	TAC	AAT	AGT	AAT	GGT	TCT	CCG	CTG	GTC								
(V_HIV)	W	G	Y	Y	N	S	N	G	S	P	L	V								

B. D Segments of Monoreactive mAbs

mAb61	CTC	GGC	CCT	GAT	GAC	TAT	ACC	CTT
(V_HIV)	L	G	P	D	D	Y	L	T
mAb60	ATC	GGA	GGT	GGG	ACG			
(V_HIV)	I	G	G	G	T			
mAb53	GTG	CAG	AGG	GAT	CCT	GCT	CCT	CGG
(V_HIII)	V	Q	R	D	P	A	P	R

Figure 3. D gene segments utilized in (A) polyreactive mAb (15–24 b in length) and (B) monoreactive mAb (30–60 b in length). mAb18, 26, and 21 were IgM produced by cell lines generated using CD5$^+$ B cells from healthy subjects (66, 112). mAb 63, 65, and 67 were IgM, IgM, and IgA1, respectively, produced by cell lines generated using CD5$^+$ B cells from a rheumatoid arthritis patient (113). mAb 60 and 61 were IgM and IgA1 RF, respectively, produced by cell lines generated using CD5$^+$ B cells from the same rheumatoid arthritis patient used as a source of mAb 63, 65, and 67 (113). mAb 53 was IgG1 to rabies virus produced by a monoclonal cell line generated using CD5$^-$ B cells from a subject vaccinated with inactivated rabies virus vaccine (71; Ikematsu *et al.*, manuscript in preparation).

V_κIIIb subgroup (57, 121, 122) (see also Phenotype, Distribution, and Ontogeny of CD5$^+$ B Cells: Ontogeny and Malignancies of CD5$^+$ B Cells). In contrast, our studies in a single rheumatoid arthritis patient showed that all polyreactive low affinity and monoreactive high affinity RF mAb generated using CD5$^+$ B cells utilized λ-L chains (113). The restricted expression of λ-L chains by CD5$^+$ B cells from this rheumatoid patient is reminiscent of the preferential expression of λ-L chains in autoantibodies associated with systemic lupus erythematosus (SLE) (123), Graves' disease (124), and Sjögren's syndrome (125). The possibility exists that, whereas CD5$^+$ B cells in healthy individuals express either κ- or λ-L chains, CD5$^+$ B cells in autoimmune humans preferentially express λ-L chains. It can be hypothesized that a particular subset (λ-L bearing) of CD5$^+$ B cells is amplified in certain autoimmune conditions. Whether this is the result of a selection mechanism by self-antigens or is due to an inherent feature of the autoantibody-producing (λ-L bearing) CD5$^+$ B cells remains to be determined.

CD5$^+$ B Cells and Autoimmune Diseases

Owing to the broad self-reactivity of the antibodies they produce, CD5$^+$ B cells have been thought to play a role in the pathogenesis of autoimmune diseases. Recognition of self is a prominent feature of the early immune system, as suggested by the high frequency of CD5$^+$ B cells and natural antibodies, mainly IgM, with self-reactivity in the fetus and neonate (84, 126–128). The unmutated V_H gene segments expressed by the early (CD5$^+$) B cells (82) would provide the structural correlate for the high degree of "connectivity" of the idiotypic-anti-idiotypic network in neonatal mice (129, 130). It has been proposed that dysregulation of such a formal idiotypic network can lead to the amplification of selected clones, often sharing common cross-reactive idiotypes (CRI) and producing autoantibodies (130–132). For example, the CRI 17.109, expressed by 2% of circulating IgM from healthy subjects (57), is shared by about 50% of RF paraproteins derived from patients with mixed cryoglobulinemia or Waldenström's macroglobulinemia (58), 40% of circulating RF from patients with Sjögren's syndrome (133), and 25% of κ-positive CLL B cells (134) (see also Phenotype, Distribution, and Ontogeny of CD5$^+$ B Cells: Ontogeny and Malignancies of CD5$^+$ B Cells). Accordingly, the CRI 16/6 (135–137), first identified in various ssDNA-binding mAb (mainly IgM) generated from patients with SLE, was also found at low frequency in mAb generated from healthy subjects, consistent with its representation in the normal idiotypic network (131, 138, 139). Further studies (129), however, revealed that the ssDNA-binding IgM bearing the 16/6 CRI display the features of polyreactive antibodies and differ strikingly from the anti-DNA autoantibodies characteristic of SLE patients. These are mainly monoreactive high affinity IgG and appear at higher titers in the circulation during the active phases of the disease (140). Thus, the relationship between natural "autoantibodies" and the autoantibod-

ies found in autoimmune patients remains to be established.

SLE and Other Autoimmune Diseases. Using EBV in limiting dilution assays, we found that in patients with SLE, a systemic autoimmune disease, and Hashimoto's disease, an organ-specific autoimmune condition directed to thyroid components, the frequencies of circulating cells capable of producing IgM to ssDNA and thyroglobulin, respectively, as well as to exogenous antigens, are similar to the frequencies found in healthy subjects (about 5–10% of the total IgM-producing cell precursors) (37, 67). In contrast, in SLE patients, B cells committed to the production of IgG antibodies to ssDNA are approximately 0.5% of the total IgG-producing cells, that is, at least 15 times greater than that found in healthy subjects and Hashimoto's disease patients (67, 141). Similarly, in Hashimoto's disease patients, B cells committed to the production of IgG to thyroid antigens (i.e., thyroglobulin or thyroid microsomal antigen) are approximately 0.2% of the total IgG-producing cells (67). This is at least 20 times greater than that found in healthy subjects and SLE patients.

By constructing more than 30 mAb-producing hybridomas selected for production of antibodies to insulin, thyroglobulin, ssDNA, or tetanus toxoid, we found that the vast majority of IgM "autoantibodies" detected in SLE patients, Hashimoto's disease patients, and healthy persons are polyreactive (K_d, 10^{-3} to 10^{-7} M) (2, 3, 67, 142). The normal frequencies of circulating cell precursors producing these polyreactive antibodies are consistent with the normal size of the $CD5^+$ B cell subset in these patients. In contrast, the IgG autoantibodies found in SLE and Hashimoto's disease patients are monoreactive and display a high affinity (K_d, 10^{-7} to 10^{-11} M) for thyroid antigens and ssDNA, respectively (67). Thus, the high frequency and affinity of the specific autoantibodies in patients with SLE or Hashimoto's disease mimic those of anti-tetanus toxoid and anti-insulin IgG mAb produced by cell lines constructed using specific B cells from vaccinated healthy subjects and from insulin-treated patients with insulin-dependent diabetes mellitus, respectively (67, 141, 142). Moreover, similar to monoreactive high affinity IgG antibody to tetanus toxoid from tetanus-vaccinated subjects, the monoreactive high affinity IgG autoantibodies to ssDNA in SLE patients are produced by $CD5^-$ B cells. These findings suggest that, in many respects, the high affinity autoantibody responses characteristic of various systemic and organ-specific autoimmune diseases, e.g., SLE, Hashimoto's disease, or insulin-dependent diabetes mellitus, resemble the high affinity response elicited by a foreign antigen through a process of B cell clonal selection and affinity maturation.

Rheumatoid Arthritis. In contrast to SLE, other autoimmune diseases, including Sjögren's syndrome (55) and rheumatoid arthritis (30, 38), are characterized by an increase in the number of $CD5^+$ B lymphocytes. In these patients, $CD5^+$ B lymphocytes can account for up to 50–60% of circulating B cells (30, 38, 143). A study of rheumatoid arthritis patients and their healthy relatives suggested that family members may also have elevated proportions of circulating $CD5^+$ B cells (143). This corroborates the findings of a study in monozygotic twins discordant for rheumatoid arthritis (23). In each case, the healthy twin had a level of $CD5^+$ B cells similar to that of the twin with rheumatoid arthritis. Taken together with our studies of rheumatoid arthritis patients, demonstrating that $CD5^+$ B cell levels do not correlate with RF levels in the serum (37, 38), these findings suggest that $CD5^+$ B cell frequency may not be directly related to disease activity, but rather to genetic background. To determine the state of activation of fluorescence-activated cell sorted $CD5^+$ and $CD5^-$ B cells in patients with active rheumatoid arthritis, we investigated their forward and 90° light-scattering properties and incorporation of [^{3}H]thymidine. We found that most $CD5^+$ B cells, but very few $CD5^-$ B cells, from rheumatoid arthritis patients were low density, large-sized, spontaneously proliferating cells (38). Moreover, these $CD5^+$ B lymphocytes, but not their $CD5^-$ counterparts, spontaneously secreted high levels of IgM, IgG, and IgA RF (37, 38). The fact that $CD5^+$ B cells are activated, proliferating, and producing RF in rheumatoid patients may be consistent with a role for $CD5^+$ B cells in the disease process. Thus, unlike $CD5^+$ B cell frequency, the $CD5^+$ B cell activation state may be an indicator of disease activity.

By studying the antigen-binding properties of the RF mAb produced by cell lines generated using purified $CD5^+$ B lymphocytes from a number of different rheumatoid arthritis patients, we identified two different types of RF autoantibodies with discrete patterns of antigen reactivity (38). The first type, more frequent, consisted of polyreactive antibodies which bound not only to IgG Fc fragment, but also to other self and non-self antigens, including ssDNA, thyroglobulin, insulin, and tetanus toxoid (38). These RF resemble the polyreactive antibodies produced by $CD5^+$ B cells in healthy subjects. The second type, much less frequent, consisted of monoreactive autoantibodies binding to IgG Fc fragment only and was found exclusively in rheumatoid arthritis patients. The K_d values displayed by the polyreactive RF for the IgG Fc fragment were in the range of 10^{-5} M, similar to the K_d values of polyreactive RF from healthy subjects. In contrast, the K_d values of the monoreactive RF were at least two orders of magnitude lower, 10^{-7} M (higher affinity). Whereas the specific high affinity anti-ssDNA autoantibodies and anti-tetanus toxoid antibodies of SLE patients and tetanus toxoid-vaccinated subjects, respectively, are produced mainly by $CD5^-$ B lymphocytes, the monoreactive high

affinity autoantibody response in rheumatoid arthritis is confined mainly to the $CD5^+$ B cell compartment.

A question that arises from the above considerations is what could be the role of polyreactive natural "autoantibodies" in autoimmune disease. RF and anti-DNA autoantibodies have been classically related to the pathogenesis of rheumatoid arthritis and SLE, respectively, and polyclonal B cell activation has been proposed as one mechanism accounting for the production of autoantibodies in these and other systemic autoimmune diseases (59, 144, 145). According to this model, autoantibody producing B cell clones would be activated because of an impaired T cell suppressor control. Alternatively, they could express such a high degree of intrinsic activation as to be able to override an otherwise normal suppressor T cell mechanism. In either case, this hypothesis predicts that in autoimmune conditions, B cells spontaneously produce autoantibodies, independent of the stimulation by any self-antigen. Moreover, the autoantibodies produced would have the functional features of the anti-self antibodies found in healthy subjects, that is, polyreactivity and relatively low affinity. In fact, polyreactive "autoantibodies" are spontaneously produced *in vivo* by activated B lymphocytes in patients with rheumatoid arthritis and SLE (2, 30, 37, 38). However, the selective antigen-binding activity and the relatively high affinity of the autoantibodies to IgG Fc fragment, ssDNA, and thyroglobulin produced by some of the B cell clones derived from rheumatoid arthritis, SLE, and Hashimoto's disease patients, respectively, support the hypothesis that an antigen-driven process of clonal selection and somatic mutation shapes the anti-self response in these diseases. Along these lines, it was recently reported that in the MRL/lpr and $(NZB \times NZW)F_1$ autoimmune mouse strains, the gene segments encoding a number of different RF and anti-DNA autoantibodies harbor many somatic mutations, mostly in their CDR, suggesting that a nonrandom, antigen-driven clonal selection is operative in their generation (146–148). One may speculate that such antigen-driven clonal selection and somatic mutation could lead polyreactive antibody-producing ($CD5^+$) B cells to produce monoreactive high affinity, potentially pathogenic autoantibodies.

To investigate whether the genetic composition of some high affinity autoantibodies could be consistent with a process of antigen-driven clonal selection and somatic mutation, we recently sequenced the genes encoding the complete V_H and V_L regions of three monoreactive high affinity IgM and IgA1 RF mAb produced by cell lines generated using $CD5^+$ B cells from a patient with rheumatoid arthritis (113). We found that: (i) unlike the polyreactive antibodies produced by $CD5^+$ B cells of normal individuals, these monoreactive RF contained extensive somatic point mutations clustered in the H chain V gene CDR1 and CDR2; and (ii) two clones independently generated from different $CD5^+$ B cells and coding for high affinity RF expressed identical V_HDJ_H and V_LJ_L sequences (Harindranath and Casali, unpublished). Thus, the restricted clonality of these B lymphocyte clones and the CDR-restricted distribution of the somatic mutations expressed in their coding sequences are highly consistent with the hypothesis that these cells were selected through a process of antigen-driven clonal amplification. The high degree of spontaneous proliferation of $CD5^+$ B cells in rheumatoid patients (38) would increase the likelihood that the V_H gene CDR accumulate somatic point mutations. It is tempting to speculate that in rheumatoid patients, the inherent proliferative potential of $CD5^+$ B cells and the stimulation by self antigens synergistically contribute to the establishment of premalignant ($CD5^+$) B lymphoid cells, as is often observed in these diseases (53, 54; see also Phenotype, Distribution, and Ontogeny of $CD5^+$ B Cells: Ontogeny and Malignancies of $CD5^+$ B Cells and Antibody Production by $CD5^+$ B Cells: $CD5^+$ B Cells Are Committed to the Production of Polyreactive Antibodies).

$CD5^+$ and $CD5^-$ B Cells in the Primary and Secondary Antibody Response to Defined Antigens

Both $CD5^+$ and $CD5^-$ B cells contribute to the antibody response to defined antigens. Owing to the polyreactivity of their surface receptors for antigen, $CD5^+$ B cells could provide most of the precursors of the antibody-producing cells recruited in the primary response, e.g., to bacterial or viral components (see Antibody Production by $CD5^+$ B Cells: Role of Natural Polyreactive Antibodies). Activation of $CD5^+$ B cells by antigen would result in production of "low" or "moderate" affinity polyreactive antibodies, similar to those observed in some murine primary responses, e.g., to oxazolone (149, 150).

B lymphocytes originally committed to the production of polyreactive antibodies utilizing unmutated, possibly restricted, V_H and V_L genes may, eventually, give rise to cells producing antibodies with selective binding activity through an antigen-driven selection and somatic point mutation process that may or may not be associated with loss of the surface CD5 molecule. Some findings in the A/J strain of mice (150) suggest that anti-arsonate antibodies induced after active immunization are produced by B cells, thought to be the progeny of virgin B lymphocytes, originally making Ig with V_H segments in unmutated configuration and endowed with multiple "anti-self" reactivities. Maturation of the antibody response to arsonate is associated with class switch from IgM to IgG, an increasing load of point mutations in the Ig V_H gene segments, and loss of anti-self reactivity (151). Thus, in this system, the same V_H segments are used in both the primary and secondary antibody responses and the increase in anti-

body affinity is due exclusively to accumulation of somatic point mutations. The ability of many polyreactive antibody-producing cells to accumulate somatic point mutations and undergo affinity maturation would be consistent with our demonstration that human $CD5^+$ B cells can produce high affinity, somatically mutated autoantibodies (113). A different process takes place in the response of BALB/c mice to oxazolone, in which cell clones expressing new V_H/V_L gene combinations resulting in antibodies with higher affinity for antigen emerge in the secondary response (150). These high affinity antibody-producing cell precursors are likely recruited among the "conventional" $CD5^-$ B cell subset, which consists of clones that express stochastic usage of V_H and V_L gene segments and, therefore, display a high degree of built-in antibody diversity and specificity. Thus, lymphocytes committed to the production of antibodies with a "better" fit for antigen would be available, although at very low frequency, among $CD5^-$ B cells. A third possibility for the articulation of a specific antibody response is the recruitment of a considerable number of the cell precursors of the primary response among $CD5^-$ B cells, followed by a secondary response which may or may not involve different $CD5^-$ B cell clones.

We recently found support for some of these ideas by studying the segregation and frequency of circulating cell precursors producing antibodies to rabies virus in healthy humans before and after sequential immunization with inactivated virus (71). The fine specificity of the IgM, IgG, and IgA mAb produced by cell lines generated at different times of the response was determined (71). $CD5^+$ B cells producing (polyreactive) IgM binding to rabies virus could be isolated from the preimmune blood. These cells utilized exclusively genes of the V_HIII family, mainly the 30pl member (Ikematsu *et al.*, manuscript in preparation). Most of the B cells involved in the primary antiviral response (Days 1 and 7 after the first injection) were also $CD5^+$, expressed V_HIII family genes, and made IgM antibodies that bound not only to rabies virus, but also to various self and exogenous antigens. In the secondary response, additional, predominantly $CD5^-$, B cells were recruited that produced IgG binding exclusively, and with high affinity, to rabies virus. One of these mAb efficiently neutralized the virus *in vitro* and *in vivo*. These mAb utilized a diverse selection of V_H segments, including members of the V_HI, V_HIII, and V_HIV gene families (Ikematsu *et al.*, manuscript in preparation). Although preliminary gene sequencing studies do not allow us to make any conclusions concerning the degree of somatic point mutation of these mAb, they do suggest that B cells producing high affinity Ig to rabies virus are not clonally related to the ($CD5^+$) B cells producing (polyreactive) Ig present in the preimmune blood or those recruited in the primary response. Further experiments are required to establish whether $CD5^+$ B cells are involved in affinity maturation of the human antibody response to other, nonproteinic, antigens, e.g., polysaccharidic or lipopolysaccharidic molecules.

Summary and Concluding Remarks

A major proportion of $CD5^+$ B lymphocytes are committed to the production of a discrete type of antibody that is polyreactive. Polyreactive antibodies appear to use selected V and, perhaps, D gene segments, mostly in unmutated configuration. They generally display relatively low affinity for different antigens and are often part of the primary response to foreign antigens. Polyreactive antibodies probably play a major role *in vivo* as a first line of defense against infectious agents not only by helping to temporarily limit the early stage of infection by directly binding to microorganisms, but also, owing to their RF-like activity, to amplify an ongoing IgG-mediated secondary response. In some cases, however, complete eradication of the invading pathogens may only be accomplished by the high affinity antibodies, mainly IgG, that appear late in the antibody response and are produced, in general, by cells that utilize a more diverse assortment of V_H/V_L gene combinations and/or have undergone an antigen-driven process of somatic point mutation and positive selection.

Our experiments have clearly established that the "autoantibodies" normally produced by $CD5^+$ B cells (polyreactive and low affinity) differ functionally from the autoantibodies that are characteristic of autoimmune diseases such as SLE, Hashimoto's disease, and insulin-dependent diabetes mellitus (monoreactive and high affinity), which are consistently detectable only in autoimmune patients. As with cells that respond to an exogenous antigen, these cells are, in some cases, the progeny of ($CD5^-$) B lymphocytes that underwent an antigen-driven process of somatic point mutation and positive selection. We also established, however, that under some circumstances (e.g., rheumatoid arthritis), monoreactive high affinity autoantibodies, i.e., RF, are produced by $CD5^+$ B cells. Our preliminary experiments showed that the V genes expressed by these cells can be somatically mutated, consistent with an antigen-driven process of clonal selection. These findings do not support the view that $CD5^+$ B cells are primordial cellular elements committed to production of germline antibodies, and suggest that they may accumulate somatic point mutation and play some role in the affinity maturation of an antibody response.

$CD5^+$ B lymphocytes may not be the only B cells capable of producing polyreactive antibodies. Studies in the mouse have revealed the existence of a cell population phenotypically similar to $CD5^+$ B cells, but lacking the surface CD5 marker (4, 47). These $CD5^-$ B cells, named the "sister population" to reflect their

putative similarity to $CD5^+$ B cells, are thought to arise from a distinct B cell lineage (152). Although the ability of the murine "sister" ($CD5^-$) B cells to produce antibodies similar to those made by $CD5^+$ B lymphocytes has not been conclusively established (153, 154), we recently obtained evidence for the production of polyreactive antibodies by an analogous ($CD5^-$) "sister" B cell population in human peripheral blood (Kasaian *et al.*, manuscript in preparation). The characterization of the human "sister" ($CD5^-$) B cell population would represent a further step toward the definition of discrete functional and phenotypic layers of specialization and complexity within the B cell repertoire, as proposed by Herzenberg and Herzenberg (150). Many issues concerning the functional features of the "conventional" $CD5^+$ B cell subset still remain to be addressed. Is there a role for $CD5^+$ B cells in antigen presentation or in helping other B cells to produce antibodies (24)? What is the precise contribution of the self-replenishing and self-maintaining nature of $CD5^+$ B cells to the establishment of human $CD5^+$ B cell leukemias or lymphomas? Similar issues would most likely apply to the ($CD5^-$) "sister" B cell subset. It is tempting to speculate that ($CD5^-$) "sister" B cells may contain progenitors of the lymphocytes eventually producing high affinity, mainly IgG, autoantibodies in those autoimmune diseases, e.g., SLE, in which such potentially pathogenic autoantibody-producing cells have been shown to segregate mostly within the $CD5^-$ B cell compartment.

This work was supported in part by NIH Grants CA-16087 and IP30 A127742, and by the Jeanette Greenspan Fund for Cancer Research. Dr. Casali is a Kaplan Cancer Scholar.

1. Jerne NK. The natural selection theory of antibody formation. Proc Natl Acad Sci USA **41**:849–857, 1955.
2. Casali P, Notkins AL. Probing the human B-cell repertoire with EBV: Polyreactive antibodies and $CD5^+$ B lymphocytes. Annu Rev Immunol **7**:513–535, 1989.
3. Casali P, Notkins AL. $CD5^+$ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. Immunol Today **10**:364–368, 1989.
4. Herzenberg LA, Stall AM, Lalor PA, Sidman C, Moore WA, Parks DR, Herzenberg LA. The LY-1 B cell lineage. Immunol Rev **93**:81–102, 1986.
5. Hardy RR, Hayakawa K. Development and physiology of LY-1 B and its human homolog, LEU-1 B. Immunol Rev **93**:53–79, 1986.
6. Hayakawa K, Hardy RR. Normal, autoimmune, and malignant $CD5^+$ B cells: The Ly-1 B lineage? Annu Rev Immunol **6**:197–218, 1988.
7. Raveché ES. Possible immunoregulatory role for $CD5^+$ B cells. Clin Immunol Immunopathol **56**:135–150, 1990.
8. Jones NH, Clabby ML, Dialynas DP, Huang H-JS, Herzenberg LA, Strominger JL. Isolation of complementary DNA clones encoding the human lymphocyte glycoprotein T1/Leu-1. Nature **323**:346–349, 1986.
9. Engleman EG, Warnke R, Fox RI, Dilley J, Benike CJ. Levy R. Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. Proc Natl Acad Sci USA **78**:1791–1795, 1981.
10. Huang H-JS, Jones NH, Strominger JL, Herzenberg LA. Molecular cloning of Ly-1, a membrane glycoprotein of mouse T lymphocytes and a subset of B cells: Molecular homology to its human counterpart Leu-1/T1 (CD5). Proc Natl Acad Sci USA **84**:204–208, 1987.
11. Hollander N, Pillemer E, Weissman IL. Effects of Lyt antibodies on T-cell functions: Augmentation by anti-Lyt-1 as opposed to inhibition by anti-Lyt-2. Proc Natl Acad Sci USA **78**:1148–1151, 1981.
12. McAteer MJ, Lagarde A-C, Gergiou HM, Bellgrau D. A requirement for the CD5 antigen in T cell activation. Eur J Immunol **18**:1111–1117, 1988.
13. Imboden JB, June CH, McCutcheon MA, Ledbetter JA. Stimulation of CD5 enhances signal transduction by the T cell antigen receptor. J Clin Invest **85**:130–134, 1990.
14. June CH, Rabinovitch PS, Ledbetter JA. CD5 antibodies increase intracellular ionized calcium concentration in T cells. J Immunol **138**:2782–2792, 1987.
15. Freedman AS, Freeman G, Whitman J, Segil J, Daley J, Levine H, Nadler LM. Expression and regulation of CD5 on *in vitro* activated human B cells. Eur J Immunol **19**:849–855, 1989.
16. Boumsell L, Bernard A, Lepage V, Degos L. Lemerle J, Dausset J. Some chronic lymphocytic leukemia cells bearing surface immunoglobulins share determinants with T cells. Eur J Immunol **8**:900–904, 1978.
17. Royston I, Majoa JA, Baird SM, Meserve GL, Griffiths JC. Human T-cell antigens defined by monoclonal antibodies: The 65,000-dalton antigen of T cells (T65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulin. J Immunol **125**:725–731, 1980.
18. Kamoun M, Kadin MF, Martin PJ, Nettleton J, Hansen JA. A novel T cell antigen preferentially expressed on mature T cells and also on (B-type) chronic lymphatic leukemic cells. J Immunol **127**:987–991, 1981.
19. Martin PJ, Hansen JA, Siadak AW, Nowinshi RC. Monoclonal antibodies recognizing normal human T lymphocytes and malignant human B lymphocytes: A comparative study. J Immunol **127**:1920–1923, 1981.
20. Caligaris-Cappio F, Gobbi M, Bofill M, Janossy G. Infrequent normal B lymphocytes express features of B-chronic lymphocytic leukemia. J Exp Med **155**:623–628, 1982.
21. Gobbi M, Caligaris-Cappio F, Janossy G. Normal equivalent of cells of B cell malignancies: Analysis with monoclonal antibodies. Br J Haematol **54**:393–403, 1983.
22. Boumsell L, Coppin H, Pham D, Raynal B, Lemerle J, Dausset J, Bernard A. An antigen shared by human T cell subsets and B cell chronic lymphocytic leukemic cells: Distribution on normal and malignant cells. J Exp Med **152**:229–234, 1988.
23. Kipps TJ, Vaughn JH. Genetic influence on the levels of circulating CD5 B lymphocytes. J Immunol **139**:1060–1064, 1987.
24. Kipps TJ. The CD5 B cell. Adv Immunol **47**:117–185, 1989.
25. Herzenberg LA, Stall AM, Braun J, Weaver D, Baltimore D, Herzenberg LA, Grosschedl R. Depletion of the predominant B cell population in immunoglobulin μ heavy chain transgenic mice. Nature **329**:71–73, 1987.
26. Freedman AS. Immunobiology of chronic lymphocytic leukemia. Hematol Oncol Clin North Am **4**:405–429, 1990.
27. 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.
28. Casali P, Burastero SE, Nakamura M, Inghirami G, Notkins AL. Human lymphocytes making rheumatoid factor and anti-

body to ssDNA belong to the Leu-1$^+$ B-cell subset. Science **236**:77–81, 1987.
29. Gadol N, Ault KA. Phenotypic and functional characterization of human Leu 1 (CD5) B cells. Immunol Rev **93**:23–34, 1986.
30. Hardy RR, Hayakawa K, Shimuzu M, Yamasaki K, Kishimoto T. Rheumatoid factor secretion from human Leu-1$^+$ B cells. Science **236**:81–83, 1987.
31. Freedman AS, Boyd AW, Bieber FR, Daley J, Rosen K, Horowitz JC, Levy DN, Nadler LM. Normal cellular counterparts of B cell chronic lymphocytic leukemia. Blood **70**:418–427, 1987.
32. Miller RA, Cralow 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.
33. Defrance T, Vanbervliet B, Durand I, Banchereau J. Human interleukin 4 down-regulates the surface expression of CD5 on normal and leukemic B cells. Eur J Immunol **19**:293–299, 1989.
34. Werner-Favre C, Vischer TL, Wohlwend D, Zubler RH. Cell surface antigen CD5 is a marker for activated human B cells. Eur J Immunol **19**:1209–1213, 1989.
35. Paavonen T, Quartey-Papafio R, Delves PJ, Mackenzie L, Lund T, Youinou P, Lydyard PM. CD5 mRNA expression and autoantibody production in early human B cells immortalized by EBV. Scand J Immunol **31**:269–274, 1990.
36. Hayakawa K, Hardy RR, Herzenberg LA. Peritoneal Ly-1 B cells: Genetic control, autoantibody production, increased lambda light chain expression. Eur J Immunol **16**:450–465, 1986.
37. Burastero SE, Casali P. Characterization of human CD5 (Leu-1, OKT1)$^+$ B lymphocytes and the antibodies they produce. Contrib Microbiol Immunol **11**:231–262, 1989.
38. 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.
39. Inaba M, Inaba K, Adachi Y, Nango KI, 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.
40. 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.
41. Durandy A, Thuillier L, Forveille M, Fischer A. Phenotypic and functional characteristics of human newborns' B lymphocytes. J Immunol **144**:60–65, 1990.
42. Antin JH, Ault KA, Rappeport JM, Smith BR. B lymphocyte reconstitution after human bone marrow transplantation: Leu-1 antigen defines a distinct population of B lymphocytes. J Clin Invest **80**:325–332, 1987.
43. Hardy RR, Hayakawa K, Parks DR, Herzenberg LA, Herzenberg LA. Murine B cell differentiation lineages. J Exp Med **159**:1169–1188, 1984.
44. Lalor PA, Morahan G. The peritoneal Ly-1 (CD5) B cell repertoire is unique among murine B cell repertoires. Eur J Immunol **20**:485–492, 1990.
45. Hayakawa K, Hardy RR, Herzenberg LA. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. J Exp Med **161**:1554–1568, 1985.
46. 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.
47. Lalor PA, Stall AM, Adams S, Herzenberg LA. Permanent alteration of the murine Ly-1 B repertoire due to selective depletion of Ly-1 B cells in neonatal animals. Eur J Immunol **19**:501–506, 1989.
48. Lalor PA, Herzenberg LA, Adams S, Stall AM. Feedback regulation of murine Ly-1 B cell development. Eur J Immunol **19**:507–513, 1989.
49. Riggs JE, Stowers RS, Mosier DE. The immunoglobulin allotype contributed by peritoneal cavity B cells dominates in SCID mice reconstituted with allotype-disparate mixtures of splenic and peritoneal cavity B cells. J Exp Med **172**:475–485, 1990.
50. Stall AM, Farinas MC, Tarlinton DM, Lalor PA, Herzenberg LA, Strober S, Herzenberg LA. Ly-1 B-cell clones similar to human chronic lymphocytic leukemias routinely develop in older normal mice and young autoimmune (New Zealand Black-related) animals. Proc Natl Acad Sci USA **85**:7312–7316, 1988.
51. Lanier LL, Lynes MA, Haughton G, Wettstein P. Novel type of murine B cell lymphoma. Nature **271**:554–555, 1978.
52. Lanier LL, Arnold LW, Raybourne RB, Russel S, Lynes MA, Warner NL, Haughton G. Transplantable B-cell lymphomas in B10.H-2^{a}H-4^{b}p/Wts mice. Immunogenetics **16**:367–371, 1982.
53. Lewis RB, Castor CW, Knisley RE, Bole GG. Frequency of neoplasia in systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum **19**:1256–1260, 1976.
54. Summons DPM, Ahern M, Bacon PA, Hawkins CF, Amlot PL, Jones EL, Scott DL. Lymphoproliferative malignancies in rheumatoid arthritis: A study of twenty cases. Ann Rheum Dis **43**:132–135, 1984.
55. Dauphinee M, Tovar Z, Talal N. B cells expressing CD5 are increased in Sjögren's syndrome. Arthritis Rheum **31**:642–647, 1988.
56. Fox RI, Chen P, Carson DA, Fong S. Expression of a cross-reactive idiotype on rheumatoid factor in patients with Sjögren's syndrome. J Immunol **136**:477–483, 1986.
57. Carson DA, Chen PP, Fox RI, Kipps TJ, Jirik F, Goldfein RD, Silverman G, Radoux V, Fong S. Rheumatoid factor and immune networks. Annu Rev Immunol **5**:109–126, 1987.
58. Silverman GJ, Goldfien RD, Chen P, Mageed RA, Jeffries R, Goni F, Fragione B, Fong S, Carson DA. Idiotypic and subgroup analysis of human monoclonal rheumatoid factors: Implications for structural and genetic basis of autoantibodies in humans. J Clin Invest **83**:469–475, 1988.
59. Casali P, Prabhakar BS, Notkins AL. Characterization of multireactive autoantibodies and identification of LEU-1+B lymphocytes as cells making antibodies binding multiple self and exogenous molecules. Int Rev Immunol **3**:17–45, 1988.
60. 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, 1984.
61. Sidman CL, Shultz LD, Hardy RR, Hayakawa K, Herzenberg LA. Production of immunoglobulin isotypes by Ly-1$^+$ B cells in viable motheaten and normal mice. Science **232**:1423–1425, 1986.
62. Shultz LD, Green MC. Motheaten, an immunodeficient mutant of the mouse. II. Depressed immune competence and elevated serum immunoglobulins. J Immunol **116**:936–943, 1976.
63. Shultz LD, Coman DR, Bailey CL, Beamer WG, Sidman CL. "Viable motheaten," a new allele at the motheaten locus. I. Pathology. Am J Pathol **116**:179–192, 1984.
64. Casali P, Inghirami G, Nakamura M, Davies TF, Notkins AL. Human monoclonals from antigen-specific selection of B lymphocytes and transformation by EBV. Science **234**:476–479, 1986.
65. Inghirami G, Nakamura M, Balow JE, Notkins AL, Casali P. Model for studying virus attachment: Identification and quantitation of Epstein-Barr virus-binding cells by using biotinylated virus in flow cytometry. J Virol **62**:2453–2463, 1988.

66. 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, 1988.
67. Nakamura M, Burastero SE, Ueki Y, Larrick JW, Notkins AL, Casali P. Probing the normal and autoimmune B cell repertoire with Epstein-Barr Virus: Frequency of B cells producing monoreactive high affinity autoantibodies in patients with Hashimoto's disease and systemic lupus erythematosus. J Immunol **141**:4165–4172, 1988.
68. Larrick JW, Chiang YL, Sheng-Dong R, Senyk G, Casali P. Generation of specific human monoclonal antibodies by *in vitro* expansion of human B cells: A novel recombinant DNA approach. In: Borrebaeck CAK, Ed. *In Vitro* Immunization in Hybridoma Technology. Amsterdam: Elsevier Science Publishers, pp231–246, 1988.
69. Stoegher ZM, Wakai M, Tse DB, Vinciguerra VP, Allen SL, Budman DR, Lightman 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.
70. Rosenthal MC, Pisciotta AV, Kommios ZD, Goldenberg H, Dameshek W. The autoimmune hemolytic anemia of malignant lymphocytic disease. Blood **10**:197–227, 1955.
71. Ueki Y, Goldfarb IS, Harindranath N, Gore M, Koprowski H, Notkins AL, Casali P. Clonal analysis of a human antibody response: Quantitation of precursors of antibody-producing cells and generation and characterization of monoclonal IgM, IgG, and IgA to rabies virus. J Exp Med **171**:19–34, 1990.
72. Casali P, Perrin LH, Lambert PH. Immune complexes and tissue injury. In: Dick G, Ed. Immunological Aspects of Infectious Diseases. Baltimore: University Park Press, pp295–342, 1979.
73. Kissick WL. Studies of the epidemiology of the rheumatoid factor. Arthritis Rheum **4**:424, 1961.
74. Welch MJ, Fong S, Vaughn J, Carson D. Increased frequency of rheumatoid factor precursor B lymphocytes after immunization of normal adults with tetanus toxoid. Clin Exp Immunol **51**:299–304, 1983.
75. Coulie PG, Van Snick J. Rheumatoid factor (RF) production during anamnestic immune responses in the mouse. III. Activation of RF precursor cells is induced by their interaction with immune complexes and carrier-specific helper T cells. J Exp Med **161**:88–97, 1985.
76. Nemazee DA, Sato VL. Induction of rheumatoid antibodies in the mouse: Regulated production of autoantibody in the secondary humoral response. J Exp Med **158**:529–545, 1983.
77. Van Snick J, Coulie P. Rheumatoid factors and secondary immune responses in the mouse. I. Frequent occurrence of hybridomas secreting IgM anti-IgG1 autoantibodies after immunization with protein antigens. Eur J Immunol **13**:890–894, 1983.
78. Nemazee DA. Immune complexes can trigger specific, T cell-dependent, autoanti-IgG antibody production in mice. J Exp Med **161**:242–256, 1985.
79. Clarkson BA Jr, Mellow GM. Rheumatoid factor-like immunoglobulin M protects previously uninfected rat pups and dams from *Trypanosoma lewisi*. Science **214**:186–188, 1981.
80. Turman MA, Casali P, Notkins AL, Bach FH, Platt JL. Polyreactivity and antigen specificity of human xenoreactive serum natural antibodies and monoclonal antibodies. Transplantation (in press).
81. Gonzalez R, Charlemagne J, Mahana W, Avrameas S. Specificity of natural serum antibodies present in phylogenetically distinct fish species. Immunology **63**:31–36, 1988.
82. Schroeder HW Jr, Hillson JL. Perlmutter RM. Early restriction of the human antibody repertoire. Science **238**:791–793, 1987.
83. Cuisinier AM, Guigou V, Boubli L, Fougereau M, Tonnelle C. Preferential expression of V_H5 and V_H6 immunoglobulin genes in early human B cell ontogeny. Scand J Immunol **30**:493–497, 1989.
84. Chen PP, Soto-Gil RW, Carson DA. The early expression of some human autoantibody-associated heavy chain variable region genes is controlled by specific regulatory elements. Scand J Immunol **31**:673–678, 1990.
85. Jeong HD, Teale JM. Comparison of the fetal and adult functional B cell repertoires by analysis of V_H gene family expression. J Exp Med **168**:589–603, 1988.
86. Jeong HD, Teale JM. V_H gene repertoire of resting B cells. Preferential use of D-proximal families early in development may be due to distinct B cell subsets. J Immunol **143**:2752–2760, 1989.
87. Reth MG, Jackson S, Alt FW. V_HDJ_H formation and DJ_H replacement during pre-B differentiation: Non-random usage of gene segments. EMBO J **5**:2131–2138, 1986.
88. Malynn BA, Yancopoulos GD, Barth JE, Bona CA, Alt FW. Biased expression of J_H-proximal V_H genes occurs in the newly generated repertoire of neonatal and adult mice. J Exp Med **171**:843–859, 1990.
89. Yancopoulos GD, Desiderio SV, Paskind M, Kearney JF, Baltimore D, Alt F. Preferential utilization of the most J_H proximal V_H segments in pre-B cell lines. Nature **311**:727–733, 1984.
90. Perlmutter RM, Kearney JF, Chang SP, Hood LE. Developmentally controlled expression of immunoglobulin V_H genes. Science **227**:1597–1601, 1985.
91. Jeong HD, Teale JM. Contribution of the CD5$^+$ B cell to D-proximal V_H family expression early in ontogeny. J Immunol **145**:2725–2729, 1990.
92. Brodeur P, Osman GE, Mackle JJ, Lalor TM. The organization of the mouse Igh-V locus: Dispersion, interdispersion, and the evaluation of V_H gene family clusters. J Exp Med **168**:2261–2278, 1988.
93. Andrade L, Freitas AA, Huetz F, Poncet P, Coutinho A. Immunoglobulin V_H gene expression in Ly-1$^+$ and conventional B lymphocytes. Eur J Immunol **19**:1117–1122, 1989.
94. Kastner DL, McIntyre TM, Mallett CP, Hartman AB, Steinber AD. Direct quantitative *in situ* hybridization studies of Ig V_H utilization: A comparison between unstimulated B cells from autoimmune and normal mice. J Immunol **143**:2761–2767, 1989.
95. Tarlinton D, Stall AM, Herzenberg LA. Repetitive usage of immunoglobulin V_H and D gene segments in CD5$^+$ Ly-1 B clones of (NZB × NZW) F1 mice. EMBO J **7**:3705–3710, 1988.
96. Dildrop R, Krawinkel U, Winter E, Rajewsky K. V_H gene expression in murine lipopolysaccharide blasts distributes over the nine known V_H-gene subgroups and may be random. Eur J Immunol **15**:1154–1156, 1985.
97. Yancopoulos GD, Malynn BA, Alt FW. Developmentally regulated and strain-specific expression of murine V_H gene families. J Exp Med **168**:417–435, 1988.
98. Schulze DH, Kelsoe G. Genotypic analysis of B cell colonies by *in situ* hybridization: Stoichiometric expression of three V_H families in adult C57BL/6 and BALB/c mice. J Exp Med **166**:163–172, 1987.
99. Carmack CE, Shinton SA, Hayakawa K, Hardy RR. Rearrangement and selection of V_H11 in the Ly-1 B cell lineage. J Exp Med **172**:371–374, 1990.
100. 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: Definition of the V_H11 family. J Immunol **142**:3643–3651, 1989.
101. Poncet P, Huetz F, Marcos M-A, Andrade L. All V_H11 genes expressed in peritoneal lymphocytes encode anti-bromelain-treated mouse red blood cell autoantibodies but other V_H gene

families contribute to this specificity. Eur J Immunol **20**:1583–1589, 1990.
102. Alt F, Blackwell K, Yancopoulos G. Development of the primary antibody repertoire. Science **238**:1079–1087, 1987.
103. Berman JE, Mellis SJ, Pollock R, Smith CL, Suh H, Heinke B, Kowal C, Surti U, Chess L, Cantor CR, Alt FW. Content and organization of the human Ig V_H locus: Definition of three new V_H families and linkage to the Ig V_H locus. EMBO J **7**:727–738, 1988.
104. Kodaira M, Kinashi T, Umemura I, Matsuda F, Noma T, Ono Y, Honjo T. Organization and evaluation of variable region genes of the human immunoglobulin heavy chain. J Mol Biol **190**:529–541, 1986.
105. Lee KH, Matsuda F, Kinashi T, Kodaira M, Honjo T. A novel family of variable region genes of the human immunoglobulin heavy chain. J Mol Biol **195**:761–768, 1987.
106. Rechavi G, Ram D, Glazer L, Zakut R, Givol D. Evolutionary aspects of immunoglobulin heavy chain variable region (V_H) gene subgroups. Proc Natl Acad Sci USA **80**:855–859, 1983.
107. Matthyssens G, Rabbits TH. Structure and multiplicity of genes for the human heavy chain variable region. Proc Natl Acad Sci USA **77**:6561–6565, 1980.
108. Sasso EH, Van Dijk KW, Milner ECB. Prevalence and polymorphism of human V_H3 genes. J Immunol **145**:2751–2757, 1990.
109. Schroeder HW, Walter MA, Hofker MA, Ebens A, Van Dijk KW, Lia LC, Cox DW, Milner CB, Perlmutter RM. Physical linkage of a human immunoglobulin heavy chain variable gene segment to diversity and joining region elements. Proc Natl Acad Sci USA **85**:8196–8200, 1988.
110. Schroeder HW, Wang JY. Preferential utilization of conserved immunoglobulin heavy chain variable gene segments during human fetal life. Proc Natl Acad Sci USA **87**:6146–6150, 1990.
111. Mayer R, Logtenberg T, Strauchen J, Dimitriu-Bona A, Mayer L, Mechanic S, Chiorazzi N, Borche L, Dighiero G, Mannehimer-Lory A, Diamond B, Alt F, Bona C. CD5 and immunoglobulin V gene expression in B-cell lymphomas and chronic lymphocytic leukemia. Blood **75**:1518–1524, 1990.
112. Sanz I, Casali P, Thomas JW, Notkins AL, Capra JD, Nucleotide sequences of eight human natural autoantibody V_H regions reveals apparent restricted use of V_H families. J Immunol **142**:4054–4061, 1989.
113. Harindranath N, Goldfarb IS, Ikematsu H, Burastero SE, Wilder RL, Notkins AL, Casali P. Complete sequence of the genes encoding the V_H and V_L regions of low and high affinity monoclonal IgM and IgA1 rheumatoid factors produced by $CD5^+$ B cells from a rheumatoid arthritis patient. Int Immunol (in press).
114. Tutter A, Riblet R. Conservation of an immunoglobulin variable-region gene family indicates a specific, noncoding function. Proc Natl Acad Sci USA **86**:7460–7464, 1989.
115. Parvari R, Avivi A, Ziv E, Tel-Or S, Burnstein Y, Shechter I, Chicken immunoglobulin γ-heavy chains: Limited VH gene repertoire, combinatorial diversification by D gene segments and evolution of the heavy chain locus. EMBO J **7**:739–744, 1988.
116. Bernstein KE, Reddy EP, Alexander CB, Mage RG. A cDNA sequence encoding a rabbit heavy chain variable region of the V_Ha2 allotype showing homologies with human heavy chain sequences. Nature **300**:74–76, 1982.
117. Amit AG, Mariuzza RA, Phillips SE, Poliak RJ. Three-dimensional structure of an antigen-antibody complex at 2.8 A resolution. Science **233**:747–753, 1986.
118. Richards RR, Konigsbert WH, Rosenstein RW, Varga JM. On the specificity of antibodies. Science **187**:130–137, 1975.
119. Baccala R, Quang TV, Gilbert M, Ternynck T, Avrameas S. Two murine natural polyreactive autoantibodies are encoded by nonmutated germ-line genes. Proc Natl Acad Sci USA **86**:4624–4628, 1989.
120. Stanfield RL, Fieser TM, Lerner RA, Wilson IA. Crystal structure of an antibody to a peptide and its complex with peptide antigen at 2.8 A. Science **248**:712–719, 1990.
121. Kipps TJ, Tomhave E, Chen PP, Carson DA. Autoantibody-associated κ light chain variable region expression in chronic lymphocytic leukemia with little or no somatic mutation: Implications for etiology and immunotherapy. J Exp Med **167**:840–852, 1988.
122. Radoux V, Chen PP, Sorge JA, Carson DA. A conserved human germline V_κ gene directly encodes rheumatoid factor light chains. J Exp Med **164**:2119–2124, 1986.
123. Reeves WH, Ali SS. Preferential use of lambda L chains in laminin B autoantibodies. J Immunol **143**:3614–3618, 1989.
124. Jasani B, Smith C, Williams ED. Lambda light chain restriction in the diffuse thyroid infiltrate in untreated Grave's disease. J Endocrinol Invest **9**: (suppl 3):101, 1986.
125. Jasani B. Immunohistologically definable light chain restriction in autoimmune disease. J Pathol **154**:1–5, 1988.
126. Kipps TJ, Robbins BA, Carson DA. Uniform high frequency expression of autoantibody-associated crossreactive idiotypes in the primary B cell follicles of human fetal spleen. J Exp Med **171**:189–196, 1990.
127. Pisetsky DS, Jelinek DF, McAnally LM, Reich CF, Lipsky PE. *In vitro* autoantibody production by normal adult and cord blood B cells. J Clin Invest **85**:899–903, 1990.
128. Lydyard PM, Quartey-Papadio R, Broker B, Mackenzie L, Jouquan J, Blaschek MA, Steele J, Petrou M, Collins P, Isenberg D, Youinou PY. The antibody repertoire of early human B cells. I. High frequency of autoreactivity and polyreactivity. Scand J Immunol **31**:33–43, 1990.
129. Kearney JF, Vakil M, Nicholson N. Non-random VH gene expression and idiotype anti-idiotype expression in early B cells. In: Kelsoe G, Schulze D, Eds. Evolution and Vertebrate Immunity: The Antigen Receptor and MHC Gene Families. Austin, TX: Texas University Press, pp175–190, 1987.
130. Coutinho A. Beyond clonal selection and network. Immunol Rev **110**:63–87, 1989.
131. Zouali M, Stollar BD, Schwartz RS. Origin and diversification of anti-DNA antibodies. Immunol Rev **105**:137–159, 1988.
132. Pennell CA, Mercolino TJ, Gardina TA, Arnold LW, Haughton G, Clarke SH. Biased immunoglobulin variable region gene expression by Ly-1 B cells due to clonal selection. Eur J Immunol **19**:1289–1295, 1989.
133. Fong S, Chen PP, Gilbertson TA, Weber JR, Fox RI, Carson DA. Expression of three cross reactive idiotypes on rheumatoid factor autoantibodies from patients with autoimmune diseases and seropositive adults. J Immunol **137**:122–128, 1986.
134. Kipps TJ, Fong S, Tomhave E, Chen PP, Goldfien RD, Carson DA. High frequency expression of a conserved kappa variable region gene in chronic lymphocytic leukemia. Proc Natl Acad Sci USA **84**:2916–2920, 1987.
135. Isenberg DA, Schoenfeld Y, Madaio MP, Rauch J. Anti-DNA antibody idiotypes in systemic lupus erythematosus. Lancet **2**:417–421, 1984.
136. Isenberg D, Collins C. Detection of cross-reactive anti-DNA antibody idiotypes on renal tissue-bound immunoglobulins from lupus patients. J Clin Invest **76**:287–294, 1985.
137. Young F, Tucker L, Rubenstein D, Guillaume T, Andre-Schwartz J, Barrett KJ, Schwartz RS, Logtenberg T. Molecular analysis of a germ line-encoded idiotypic marker of pathogenic human lupus autoantibodies. J Immunol **145**:2545–2553, 1990.
138. Datta SK, Naparstek Y, Schwartz RS. *In vitro* production of an anti-DNA idiotype by lymphocytes of normal subjects and patients with systemic lupus erythematosus. Clin Immunol Immunopathol **38**:302–318, 1986.

139. Madaio MP, Schattner A, Schattner M, Schwartz RS. Lupus serum and normal serum contain anti-DNA antibodies with the same idiotypic marker. J Immunol **137**:2535–2540, 1986.
140. Hall GM, Highton J, Kalmakoff J, Palmer DG. Changes in anti-DNA antibody affinity during exacerbation of systemic lupus erythematosus. Scand J Rheumatol **15**:243–250, 1986.
141. Casali P, Burastero SE, Balow JE, Notkins AL. High affinity antibodies to ssDNA are produced by $CD5^-$B cells in systemic lupus erythematosus patients. J Immunol **143**:3476–3483, 1989.
142. Casali P, Nakamura M, Ginsberg-Fellner F, Notkins AL. Frequency of B cells committed to the production of antibodies to insulin in newly diagnosed patients with insulin-dependent diabetes mellitus and generation of high affinity human monoclonal IgG to insulin. J Immunol **144**:3741–3747, 1990.
143. Youinou P, Mackenzie L, Katsikis P, Merdrignac G, Isenberg DA, Tuaillon N, Lamour A, LeGoff P, Jouquan J, Drogou A, Muller S, Genetet B, Moutsopoulos HM, Lydyard PM. The relationship between CD5-expressing B lymphocytes and serologic abnormalities in rheumatoid arthritis patients and their relatives. Arthritis Rheum **33**:339–348, 1990.
144. Hang L, Slack JH, Amundson C, Izui S, Theofilopoulos A, Dixon FJ. Induction of murine autoimmune disease by chronic polyclonal B cell activation. J Exp Med **157**:874–883, 1983.
145. Klinman DM, Steinberg AD. Systemic autoimmune disease arises from polyclonal B cell reactivation. J Exp Med **165**:1755–1760, 1987.
146. Schlomchik MJ, Marschak-Rothstein A, Wolfowicz CB, Rothstein TL, Weigert MG. The role of clonal selection and somatic mutation in autoimmunity. Nature **328**:805–811, 1987.
147. Radic MA, Mascelli MA, Erikson J, Shan H, Shlomchik M, Weigert M. Structural patterns in anti-DNA antibodies from MRL/lpr mice. Cold Spring Harbor Symp Quant Biol **54**:933–946, 1989.
148. Marion TN, Bothwell ALM, Briles DE, Janeway CA Jr. IgG anti-DNA autoantibodies within an individual autoimmune mouse are the products of clonal selection. J Immunol **142**:4269–4274, 1989.
149. Berek C, Milstein C. Mutation drift and repertoire shift in the maturation of the immune response. Immunol Rev **96**:24–41, 1987.
150. Griffiths GM, Berek C, Kaartinen M, Milstein C. Somatic mutation and the maturation of immune response to 2-phenyl oxazolone. Nature **312**:271–275, 1984.
151. Naparstek Y, Andre-Schwartz J, Manser T, Wysocki LJ, Breitman L, Stollar D, Gefter M, Schwartz RS. A single germline V_H gene segment of normal A/J mice encodes autoantibodies characteristic of systemic lupus erythematosus. J Exp Med **164**:614–626, 1986.
152. Herzenberg LA, Herzenberg LA. Toward a layered immune system. Cell **59**:953–954, 1989.
153. Klinman DM, Holmes KL. Differences in the repertoire expressed by peritoneal and splenic Ly-1 $(CD5)^+$ B cells. J Immunol **144**:4520–4525, 1990.
154. Hiernauz JR, Goidl EA, Martin McEvoy SJ, Stashak PW, Baker PJ, Holmes KL. Characterization of the immunodeficiency of RIIIS/J mice. I. Association with the CD5 (Ly-1) B cell lineage. J Immunol **142**:1813–1817, 1989.