Evolutionary and Somatic Selection of the Antibody Repertoire in the Mouse

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The repertoire of antibody variable (V) regions has been subject to evolutionary selection, affecting both the diversity of V region genes in the germline and their expression in the B lymphocyte population and its subsets. In ontogeny, contact with an antigen leads to the expansion of B cells expressing antibodies complementary to it. In a defined phase of B cell differentiation, new sets of V regions are generated from the existing repertoire through somatic hypermutation. Cells carrying advantageous antibody mutants are selected into the memory compartment and produce a stable secondary response upon reexposure to the antigen.

THE CELL HYBRIDIZATION TECHNIQUE DEVELOPED BY Köhler and Milstein (*1*) makes it possible to immortalize individual antibody-producing cells taken from the animal at various stages of ontogeny and of the immune response. The molecular analysis of the immunoglobulin loci carried by such cells has improved our understanding of how antibodies are selected and somatically modified in the immune system. In this article we discuss this subject, concentrating on the immune system of the mouse.

Genetic and Structural Basis for the Selection of Antibody Specificity

Antigen binding specificity is conferred to the antibody molecule through its variable (V) regions, which differ from antibody to antibody. Each V region is encoded by two or three different genetic elements, V_L and J_L for immunoglobulin light (L) chains, and V_H, D, and J_H for the heavy (H) chains. Multiple copies of these elements are organized in the H and L chain gene loci into multigene families in which elements of a given kind lie next to each other. In the course of differentiation of B lymphocytes, a series of somatic gene rearrangements takes place in which V, D, and J elements are joined through site-specific recombination. As a result, each B cell expresses a particular combination of V_L and J_L elements as the V_LJ_L gene encoding the V region of the L chain (V_L) and a particular combination of V_H, D, and J_H elements as the V_HDJ_H gene encoding the V region of the H chain (V_H) (*2, 3*). Thus, as postulated by the clonal selection theory (*4*), the cells are precommitted to the expression of a single antibody V region, which differs from cell to cell.

The antigen binding site in the antibody V region is largely composed of well-defined stretches of polypeptide chains that loop out from the V region backbone, a densely packed globular domain of β pleated polypeptide sheets (*5*). The loops forming the antibody binding site are called complementary determining regions (CDRs) and express pronounced sequence variability. There are six CDRs per antibody molecule, three per V_L region and three per V_H region (*6*).

Most of the CDRs expressed in newly generated B cells are encoded by the germline. This is strictly true for CDR1 and CDR2 of H and L chains, which are encoded by V_H and V_L elements, respectively. CDR3 of the L chain is also mostly encoded by V_L, but additional joining diversity is introduced by imprecision of the V to J joining process. CDR3 of the H chain is encoded by sequences contributed by a D element and sequences at its borders, which are introduced somatically, in the processes of D to J_H and V_H to DJ_H joining (*3, 7*). Here, the germline contribution to the structure of the CDR is sometimes minor.

Taken together, the antigen binding sites in the repertoire of antibody V regions generated by gene rearrangements are to a large extent germline encoded in that most of the elements from which these binding sites are made (the CDRs) are carried in the germline in sets of two or three (V_H and V_L genes). These are expressed in antibodies in different combinations (combinatorial diversity).

The V region genes in the germline must have been strongly selected in evolution. While one principle of selection would be to maximize binding site diversity, another would be the selection of V region genes encoding particularly useful CDRs (D elements) or combinations of CDRs (V genes). The latter kind of selection could operate at different levels. Certain V and D elements could be selectively retained in the germline. In addition, mechanisms could evolve by which such elements are expressed in the B cell population at different frequencies. Indeed, early in ontogeny, certain V_H genes appear to be more frequently expressed than others (*8*). While this could be controlled by a developmental program of gene rearrangements, it could also reflect or be combined with selection via cell-cell interactions (*9*). Thus, V region genes expressed by a given cell could affect the expression of other such genes in other cells if these cells are interconnected by complementary receptor structures (idiotypes and anti-idiotypes) as postulated by the network theory (*10*). The experiments of Vakil and Kearney (*11*) suggest that self-stimulatory interactions of this type take place early in ontogeny and determine the later expression of certain antigen binding specificities. In addition, self-antigens other than V regions may be involved in the expansion of certain antibody specificities (*12*), although specificities are also deleted through the various mechanisms of immunological tolerance (*13*).

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B Cell Subsets and the Preimmune Repertoire

The B cell population of an immunologically mature mouse consists of approximately 10^8 cells, most of which express antibodies of the immunoglobulin M (IgM) isotype on the surface. Physiologically, the population is heterogeneous. About 5×10^7 cells are born in the bone marrow per day, and many of these live only for a few days. Other cells remain in the system for weeks or months (*14*). Although B cells have the capacity for substantial clonal expansion (see below), their capacity to repopulate a B cell–depleted immune system is poor (*15*).

The majority of the B cell population in the animal thus consists of cells with a limited lifetime and is constantly renewed in the bone marrow from stem cells not yet expressing immunoglobulin. We might call these classical B cells, as opposed to the cells of a small B cell subset, Ly1-B. Ly1-B cells, which after their initial description (*16*) were characterized mainly by Leonore Herzenberg and her colleagues (*17*), appear to be seeded into the immune system early in ontogeny and to propagate in the mouse throughout its lifetime (*17–19*). These cells carry IgM on the surface, like most B cells, but also carry a distinctive marker, the Ly1 antigen. They represent only 1 percent of the B cells in the spleen but up to 50 percent in the peritoneal cavity. Cell transfer experiments indicate that surface IgM-positive Ly1-B cells have self-renewing capacity and repopulate the Ly1-B cell compartment in B cell–depleted recipients (*18, 19*). A major fraction of the natural antibodies in the serum, in particular of IgM antibodies, is produced by these cells (*17, 19*). Ly1-B cells are responsible for the production of a variety of autoantibodies. While they do not seem to participate in the antibody response to various haptens and protein antigens, they do respond to certain bacterial antigens such as α-1,3-dextran (Dex) and phosphorylcholine (Pc), and, thus, may play a role in the immunological defense against infection (*17, 19, 20*). The generation of the Ly1-B cell population early in ontogeny, its self-propagating capacity, and the peculiar pattern of antibody specificities expressed by Ly1-B cells make it tempting to speculate that it is this cell population (perhaps together with subsets of classical B cells) whose V region repertoire is selected early in ontogeny by autostimulatory network interactions within the population (*11*). Such interactions have been shown to predispose the animal for later, efficient antibody responses to Dex and Pc (*11*). In addition, Ly1-B cells may exert a regulatory influence on immune responses of the conventional B cell population (*21*).

Which repertoire of V regions do B cells from an unimmunized mouse express? This repertoire is often called preimmune, indicating that it has not been disturbed by intentional immunization. When antibodies of the preimmune repertoire were analyzed in molecular terms, it was found that they largely expressed unmutated V region genes (*8, 22*). Whereas the expression of V_H genes located at the 3′ end of the V_H gene cluster predominates early in ontogeny (*8*), a broad spectrum of V region genes is expressed in the splenic B cells of adult mice. Although there may be exceptions at the level of individual V genes (*8, 23*), the expression of the various V_H gene families in this cell population is random in a first approximation (*24*).

To what extent and in which way are particular specificities in the preimmune repertoire specifically expanded? To study this problem it would seem appropriate to analyze the antibodies expressed by long-lived B cells rather than by the overall B cell population. We have started an attempt in this direction, concentrating on the repertoire expressed by Ly1-B cells, with mice that had been given immunoglobulin allotype-congenic peritoneal B cells at birth. The only B cells of donor allotype detectable in these mice bear the Ly1 antigen (*19*). At the age of 9 months, a mouse reconstituted in this way was injected intraperitoneally with 10 μg of bacterial lipopolysaccharide, and 2 days later peritoneal and spleen cells of the animal were fused to the nonproducer myeloma line X63.Ag8.653 (Fig. 1). Thirteen hybridomas expressing IgM of donor allotype, ten originating from the spleen and three from peritoneal cells, were analyzed in molecular terms by partial sequencing of the antibody V regions at the level of messenger RNA (*19*) (Fig. 2). In the collection of hybridoma antibodies, there were, in the limits of the sequence analysis, two pairs of identical repeats (3B9PC and 5D6S, 1B3S and 12B11S). Presumably, we isolated identical progeny from two different clones of B cells that had grown in the animal to large sizes. [If the cell fusion frequency is 1×10^{-3} to 1×10^{-4} (*25*), then a clone must have a few thousand to 10,000 members to allow us to pick more than one member cell.] In one case, one of the cells came from the peritoneal cavity, the other from the spleen (Fig. 2). Three other antibodies (again derived partly from the peritoneal cavity and partly from the spleen) expressed identical V_H and V_L genes, although their DJ_H sequences demonstrated that they were of different clonal origin (1A1PC, 12F10S, and 7E9S). Two other antibodies, which were also clonally unrelated, expressed identical V_H but different V_L genes (1B1S, 13G3S). The restricted pattern of V gene combinations cannot be explained by a limited number of precursors as a consequence of the cell transfer. It indicates that the donor B cells in the animal were strongly selected for the expression of certain V genes and hence antigen binding specificities. Several of the V genes expressed in the antibodies had either been identified as germ-line V genes before or had been found expressed identically in other antibodies (and thus probably represent germline sequences as well). Overall, among 1458 nucleotides, which could be compared to nucleotides in the same positions of a corresponding germline gene, we have not identified a single somatic point mutation. (The number of nucleotides rises to 2310 if one includes all positions of identical V regions.)

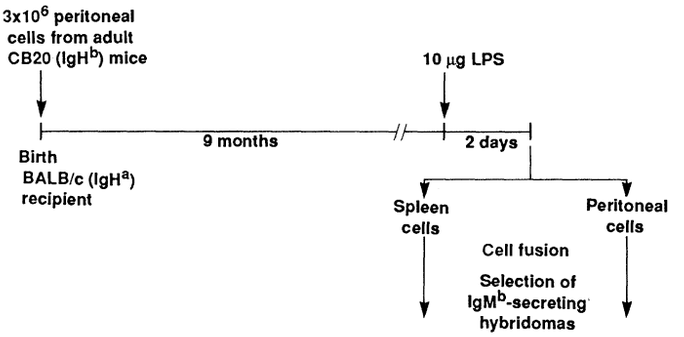

Fig. 1. Outline of the cell transfer experiment. LPS stimulation was performed to increase the fusion efficiency through polyclonal B cell stimulation. Nucleotide sequences of the V region genes expressed by some of the hybridomas are shown in Fig. 2. For experimental details see (*60*).

Since the Ly1 antigen was not expressed by the hybridoma cells, we have no formal evidence that they express antibodies selected in the Ly1-B compartment, although the experimental protocol suggests this. However, it is obvious that we are looking at a section of an antibody repertoire that is strongly selected, through selective expansion of long-lived B cell clones, for the expression of certain specificities encoded in the germline (*26*).

Thus, in the limits of the experimental system, the data support the concept that certain antibody specificities are specifically expanded in the preimmune repertoire. It must be kept in mind that the latter term is merely operational and that we do not know at this point to which extent idiotypic network interactions or antigens other than idiotypes are responsible for this selection. However, the selection differs from that seen in the antigen-driven immune

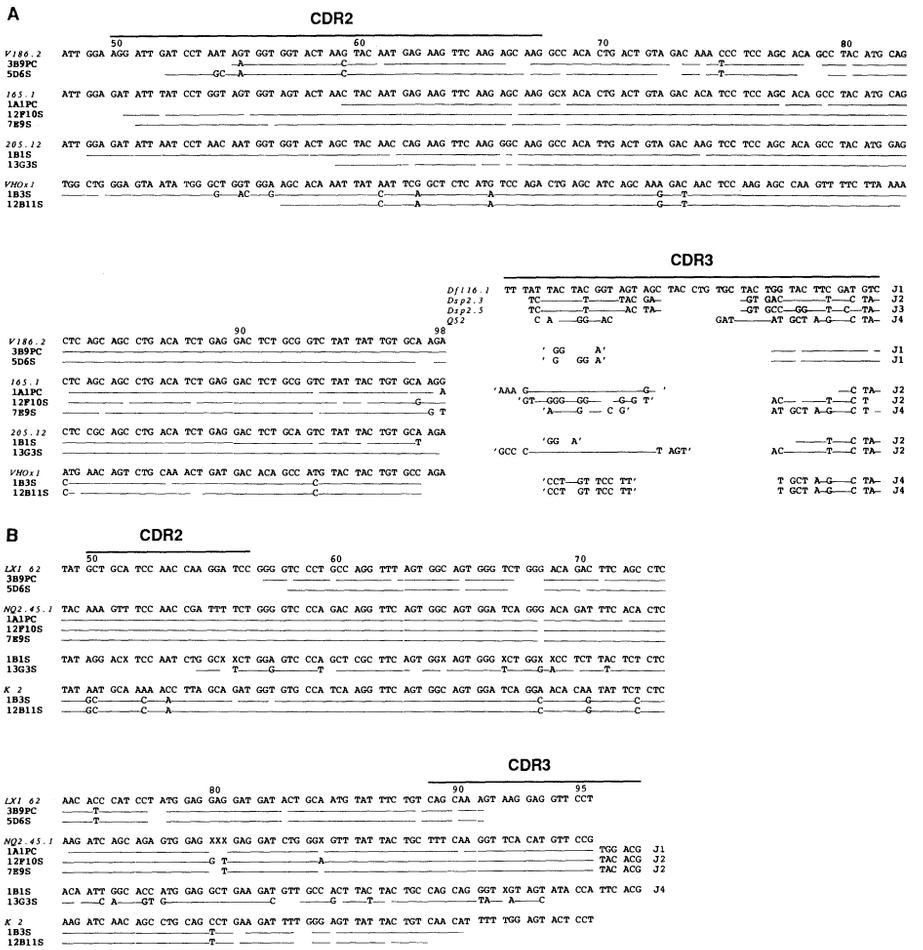

Fig. 2. Partial nucleotide sequences of the expressed (**A**) V_HDJ_H and (**B**) V_K genes from 9 of 13 randomly selected hybridomas from fusions of either splenic (S) or peritoneal cells (PC). Sequences were compared to known germline genes (*61*). Identities are represented by solid lines, nucleotide differences are shown explicitly, and gaps represent sequence uncertainties. The length of the D elements is indicated by dashes (').

responses described below in which the antibodies are somatically modified through isotype switching and somatic hypermutation.

Somatic Modification of Antibodies: Isotype Switching and Hypermutation

As already described, most of the cells in the preimmune repertoire express on their surfaces IgM (or IgM and IgD) antibodies with unmutated V regions. However, in most immune responses, antibodies of other isotypes, such as IgG and IgA, predominate. At the cellular level this is achieved by isotype switching. As a consequence, a cell expresses a different constant (C) region of the H chain (C_H) (a different isotype) while keeping its V_H region and L chain unchanged. The genes encoding the various C_H regions are organized in a cluster that is 3′ of J_H. Isotype switching involves recombination between special switch (S) regions in stretches of DNA that separate the C_H genes from each other. As a result, the V_HDJ_H gene is brought into the proximity of a downstream C_H gene through deletion of upstream C_H genes. Because the process can involve various S regions, and even within an S region generate different recombination breakpoints, switch events can be distinguished from each other in molecular terms (*3, 27*).

A second molecular event that may change the properties of the antibody expressed by a B cell in the course of its differentiation is somatic mutation of its V region. Following the initial observations of somatic V region mutants (*28*), it became clear that without exception antibody V regions expressed in secondary (2°) and hyperimmune responses are heavily somatically mutated. In contrast, antibodies of primary (1°) responses are mutated only rarely (*29–31*). Mutations are also seen in the sequences surrounding mutated rearranged V_LJ_L and V_HDJ_H genes (*32*) and in nonproductively rearranged V region genes of cells expressing mutated antibodies (*33*). This, together with the observation of silent and other mutations without obvious selective value, make it clear that a special mechanism of hypermutation is operating at some step of B cell development, rather than that rare, naturally occurring mutants are selected in the system.

Sequences of V_HDJ_H regions of antibodies isolated from C57BL/6 mice undergoing a 1° or a 2° response to the hapten 5-hydroxy-3-nitro-phenylacetyl (NP) coupled to a protein carrier are depicted in Fig. 3. All these antibodies express the λ1 L chain (not shown) and a particular V_H gene, V186.2. While somatic mutations can hardly be seen in the 1° response antibodies, they are numerous in the V regions of the 2° response antibodies. The mutations are spread over the entire V_H gene and also D, J_H, and V_L (*31*). A comparison of the exchanges in the 2° response antibodies with the sequences of all other known V_H genes of the mouse suggests that the vast majority of the mutations represent point mutations. Recombinations (conversions) appear to occur rarely, if at all (*31*), in agreement with observations by others (*29, 30*).

Most of the antibodies of the 2° response have higher binding affinity for the hapten than the 1° response antibodies. This is a classical phenomenon observed in many experimental systems and often accompanied by selection for replacement mutations in CDR1 (*29–31, 34*). Can somatic mutations responsible for the increase in affinity be identified? Inspection of the sequences (Fig. 3) suggests the Trp to Leu exchange in position 33 as the best candidate for a

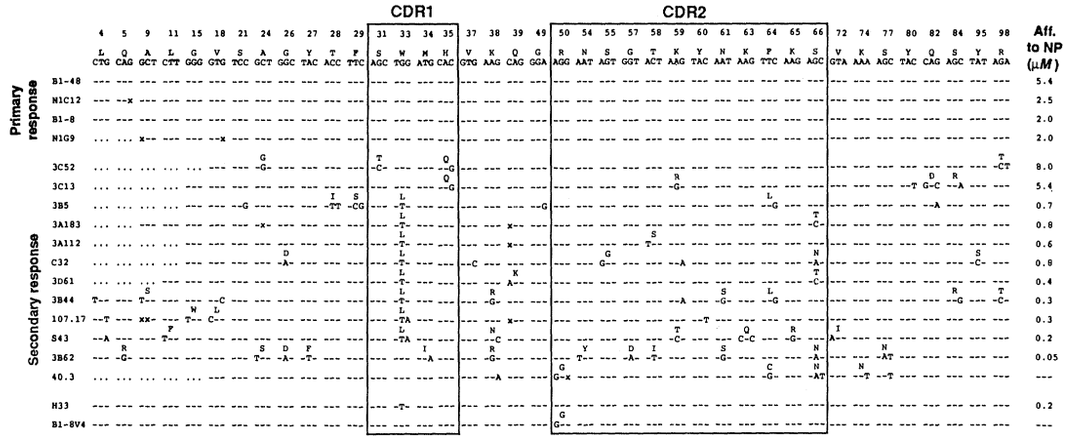

Fig. 3. Nucleotide sequences from unmutated and somatically mutated V186.2 genes expressed in antibodies from the 1° and 2° response to the hapten NP (*62*). The sequences are aligned to the germline sequence of V186.2 (only those codons where sequences differ from the V186.2 gene are shown). H33 is a mutant antibody that was constructed in vitro (*35*) and carries only a point mutation at position 33. The 40.3 is an in vivo hapten-binding loss variant, and B1-8V4 is an antibody mutant selected in vitro that has lost NP-binding specificity because of a point mutation at position 50 (*56*). The affinities of the corresponding antibodies for the hapten NP are shown on the right.

positively selected somatic mutation in the collection. This mutation occurs in 7 of 12 sequences and is in the middle of CDR1. Two antibodies lacking the mutation, 3C52 and 3C13, have an unusually low affinity. The effect of this mutation was directly tested by cloning the V_HDJ_H genes of the canonical, unmutated 1° response antibody B1-8 and of the 2° response antibody 3B44, which carries among other point mutations the Trp to Leu exchange in position 33 and differs from B1-8 in its DJ_H segment. Four additional VDJ_H genes were constructed by genetic engineering: V_H of 3B44 with DJ_H of B1-8; V_H of B1-8 with DJ_H of 3B44; V_HDJ_H of 3B44 without the position 33 exchange; and V_HDJ_H of B1-8, carrying as the only mutation the Trp to Leu exchange in position 33 (Fig. 4). These constructs were put into a suitable expression vector carrying a Cγ3 gene and transfected into J558L cells that express the λ1 L chain. Transfectants were cloned, antibodies were isolated from the supernatants, and their affinities for the NP hapten were determined in a radioactive binding competition assay (*35*). The results (Fig. 4) demonstrate that, of all the mutations in antibody 3B44, the exchange in position 33 alone is responsible for the increased affinity and that the same increase is brought about by inserting this mutation into the germline encoded 1° response antibody B1-8 (*36*). The difference of the two antibodies in DJ_H is apparently of no importance for their hapten binding affinity. We conclude that the replacement mutations in 3B44, other than that in position 33, and perhaps most of the mutations in the collection of 2° response antibodies (Fig. 3) are either selected on different principles or simply represent noise in the system (see below).

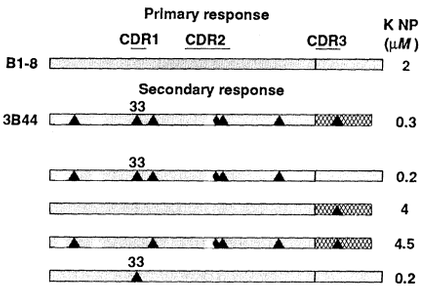

Fig. 4. Schematic representation of the heavy chain V region sequences of the 1° response antibody B1-8 (*62*), the 2° response antibody 3B44 (*62*), and constructs obtained by genetic engineering. The V_H to DJ_H borders are indicated by vertical bars. Closed triangles represent replacement, and open triangles represent silent mutations (Fig. 3). The affinities of the corresponding antibodies for the hapten NP are given on the right. The λ1 L chain of antibody 3B44, although bearing two point mutations (*31*), was shown not to change the affinity of the antibody as compared to the unmutated λ1 L chain. Taken from (*35*).

The Timing of Isotype Switching and Somatic Hypermutation

In the reaction of B cells to an antigen, three fundamental differentiation pathways can be distinguished: the 1° response (in which a section of the preimmune repertoire is expressed), the induction of memory, and the 2° response (in which memory is expressed) (Fig. 5). The induction of memory and of the 2° response are typically restricted to T helper cell–dependent responses, and these pathways may, therefore, be under direct T helper cell control (*25*). In general, all three pathways involve clonal expansion of precursor cells expressing a suitable antibody on the surface. However, only in the course of the 1° and the 2° responses do cells differentiate terminally into antibody-secreting plasma cells.

What is the timing of isotype switching and somatic hypermutation in the three pathways of differentiation? An approach to this problem, pioneered by Weigert and his colleagues (*37*) and subsequently used by several groups (*25, 38, 39*), is the molecular analysis of progeny of individual B cell precursors expanding in vivo under various experimental conditions. The progeny cells are isolated by fusion to a nonsecretor myeloma line. They can be identified because all cells of a clone share as clonal markers unique V_LJ_L and V_HDJ_H rearrangements. Although the cells express only one V_L and one V_H region (allelic exclusion), rearrangements are often also seen in the nonexpressed homologous loci (*2, 3*). The antibodies expressed by different members of the clone may carry identical or different C_H regions, and in the former case the switch recombination breakpoints could be identical or different. Their V regions, although derived from a common ancestor, may express shared or individual somatic point mutations (Fig. 6). The molecular analysis of such cells gives insights into the interrelationship of clonal expansion, somatic hypermutation, and isotype switching in the various differentiation pathways. Indirect information on the extent of clonal expansion in the system is also obtained since, as mentioned above, with cell fusion frequencies on the order of 10^{-3} to 10^{-4}, a clone must comprise many thousands of cells to allow the isolation of several of its members by the hybridoma technique.

A summary of the results obtained in this type of experiment is as follows (Fig. 6): (i) The analysis of clonal progeny of naïve B cells, which had been transferred into irradiated recipients and stimulated with antigen (adoptive 1° response), indicated multiple isotype switch events in the course of clonal expansion and little, if any, somatic mutation (*25*) in accord with earlier data on isotype switching in vivo (*40*) and the molecular composition of the 1°

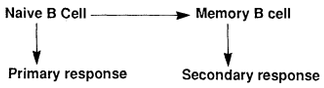

Fig. 5. Schematic representation of B cell differentiation pathways in the course of the immune response.

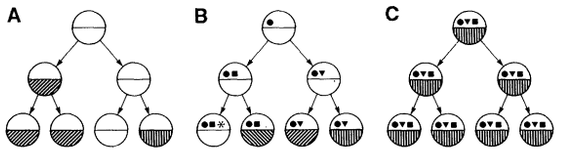

Fig. 6. Schematic representation of clonal B cell expansion in (**A**) the primary response, (**B**) the induction of memory, and (**C**) the secondary response. The black dots, triangles, squares, and stars represent independent point mutations in the V region genes. The different shadings in the circles indicate different isotypes or isotype switch recombinations. Unshaded circles represent IgM producing cells.

response in the intact animal (Fig. 6A). (ii) Clonally related antibody-producing cells from animals undergoing multiple immunizations expressed antibodies whose V regions differed from one another by somatic point mutations. However, shared mutations were also identified so that genealogical trees could be constructed (*37–39*; Fig. 6B). This provides formal evidence that somatic hypermutation occurs stepwise in clonal proliferation. Assuming that the point mutations are introduced independently of each other and depend on cell division, the rate of mutation must be in the order of 1×10^{-3} per base pair per generation to explain the accumulation of nonselectable mutations in the cells (*41*). Almost all of the clonally related cells analyzed so far that did not express IgM expressed either different isotypes or carried different switch recombination breakpoints (*42*). Therefore, the shared mutations in these cells must have occurred when the cells still expressed IgM, and almost all mutations could have been introduced at that stage (Fig. 6). There are also examples of clonally related hypermutated cells within certain human B cell tumors that express IgM (*43*). (iii) In the analysis of B cell clones isolated from animals under prolonged immunization, one cannot distinguish between cells on the way into the memory compartment and cells recruited into a 2° response (Fig. 5). In an attempt to selectively analyze cells in the latter pathway, the same cell transfer system was chosen as described for the 1° response. B cells in limiting numbers from antigen-primed donors were transferred into irradiated recipients and stimulated with antigen. The clonally related cells isolated from the recipients expressed heavily but identically mutated antibodies and had undergone identical class switch recombination (*25*; Fig. 6C). This demonstrates that B cells that have undergone somatic hypermutation and isotype switching expand and terminally differentiate upon 2° stimulation without further modification of the antibodies that they express. Consistent with this result is earlier work on the stability of the adoptive 2° response and of V region genes in myeloma and hybridoma cells (*44*) and the recent isolation of hybridomas expressing identical, mutated antibodies from intact immune mice (*45, 46*).

Taking the results of this and the previous section together, the scheme of B cell differentiation (Fig. 5) reads as follows: In the 1° response, antigen-specific B cells available in the preimmune repertoire are triggered into expansion and terminal differentiation. Isotype switching occurs in this pathway, but little somatic mutation is seen. At the same time, some B cells, which may well belong to a separate B cell subset, are selected into the pathway of memory cell generation. It is in this pathway that somatic hypermutation occurs in the course of cell proliferation, generating a population of cells with drastically changed antibody V regions from which the ones that best fit the antigen are selected. Memory B cell generation requires not only induction of a hypermutation mechanism, but also continuous selection of the cells by antigen without induction of terminal differentiation. (Antibody secreting plasma cells do not express antibody on the surface and consequently cannot be selected by antigen.) These requirements are distinct from what is required in the induction of 1° and 2° responses. There is reason to believe that differentiation into memory cells is triggered in a particular histological microenvironment, the germinal centers (GC) (*47*). The GC are well-defined accumulations of proliferating B cells, which also contain antigen-presenting cells and T cells. They build up in peripheral lymphoid organs after immunization with T cell dependent antigens, when cells expressing highly mutated antibodies begin to appear in the animal (*48*). The isotype of the antibodies expressed by B cells in GC is mainly IgM. After some time the cells leave the GC, which eventually disappear, and may then represent stable memory B cells, ready to respond to further contact with antigen by proliferation and terminal differentiation in the 2° response (Fig. 5).

There is evidence that most memory B cells (cells responding to 2° immunization by antibody production) have switched to the expression of an isotype other than IgM and carry the isotype that they are going to express, already on their surfaces, before 2° immunization (*49*). This agrees with the clonal analysis of the adoptive 2° response in which the cells were shown not to accumulate further somatic mutations (*25*). We therefore propose that, in contrast to earlier speculations (*29*), the isotype switch may be the molecular event that terminates the hypermutation process. The easiest way to envision this would be to attribute to the membrane-bound IgM molecule, whose H chain has already been shown to be involved at an earlier stage of differentiation in the induction of L chain rearrangement (*50*), a role in the triggering of the hypermutation mechanism in the pathway of memory cell generation (*51*). The deletion of the Cμ gene in the productively rearranged IgH locus of the cell would then terminate the phase of hypermutability in an ordered program of differentiation through gene rearrangements in the B cell lineage. Immunological memory would be maintained by the production of long-lived memory cells that have undergone isotype switch recombination and express a stable antibody repertoire. This could be accompanied by continuing selection of cells in the memory compartment (*52*) that have not yet completed isotype switching (*53*). Clearly, however, any molecular connection between isotype switching and somatic hypermutation remains speculative at the moment, and it cannot be excluded that once they reach a suitable environment, switched mutated cells go through further rounds of hypermutation (which could still be triggered through membrane-bound immunoglobulin), and selection. The elucidation of the molecular mechanism of the hypermutation process is the most urgent next step in the field.

Conclusions

Some simple principles seem to emerge on how antibody specificity is generated and selected in the immune system of the mouse. The germline encodes a large but selected repertoire of antibody-binding sites. This repertoire is constantly displayed in a population of B lymphocytes with a rapid turnover, and specificities from the repertoire can be selected or suppressed according to the needs of the system.

There is evidence that certain specificities in the preimmune repertoire are selectively expanded. A special subset of B cells (Ly1-B) may be mainly responsible for this. These long-lived cells appear to have different growth properties than classical B cells [the Ly1 antigen has a structure reminiscent of a growth factor receptor (*54*)] and may be interconnected in an autostimulatory, idiotypic network

of complementary germline encoded antibody V regions. This would be an efficient way to amplify the expression of selected sets of antigen binding sites whose expression is useful on the basis of evolutionary experience.

Once confronted with a particular antigen, the system, in addition to responding by activation and selective expansion of cells expressing antibodies of the preimmune repertoire, selects new sets of binding sites into the B cell memory compartment to deal more efficiently with that antigen on a second encounter, on the basis of ontogenetic experience. The new sets of binding sites are generated somatically through a special process of hypermutation. From this process, which represents a particular phase of B cell maturation, long-lived memory cells emerge that express high affinity antibodies in the 2° immune response. These cells, as well as those producing 1° responses, behave as postulated by the clonal selection theory in that they are clonally selected for the expression of a given antibody specificity. In contrast, drastic intraclonal diversity is generated in the memory pathway. It seems economical to select high affinity binding sites by modifying sites that already have some affinity for the antigen and expanding those that have acquired advantageous modifications. Random mutation of the entire preimmune repertoire would be wasteful, difficult to control, and antagonize evolutionary selection of antibody specificities.

The efficiency of the system can only be fully appreciated if one takes into account how the various compartments of the immune system act in concert. Thus, speculating further along the lines of the arguments made in this article, an immune response could develop as follows: Depending on the evolutionary experience of the species, an antigen entering the body of a mouse for the first time may encounter in the preimmune repertoire significant levels of antibodies, carried perhaps mainly by Ly1-B cells and also secreted by these cells into the body fluid as "natural" antibodies. These antibodies not only help in the elimination of the antigen directly but also mediate its presentation to T helper cells, either by the Ly1-B cells themselves or by antigen-presenting cells taking up antigen-antibody complexes (*55*). The activated T helper cells in turn select in the population of classical B cells the entire available repertoire of binding sites specific for any of the antigen's antigenic determinants. Cells are recruited both into the primary response and into the memory pathway, where a new repertoire of binding sites with high affinity for the antigen is generated by mutation and selection, ready to interact with the antigen upon a second encounter.

We close with a remark on autoimmunity. Self-reactive antibodies (including those involved in idiotypic interactions) may play an important physiological role in the preimmune repertoire (*11, 12*). The appearance of such antibodies early in ontogeny is presumably carefully controlled. A different problem is posed by the process of somatic hypermutation, which because of the high mutation rate, must generate, in addition to high affinity antibodies, antibodies whose specificity is changed altogether. Such antibody mutants would appear in an unpredictable way on mature, responding B cells. Experimental evidence indicates that this is indeed the case (*56*; Fig. 3) and also that autoreactivity can be generated by somatic mutation (*57*). This is probably the reason why the pathway of memory cell generation, for which hypermutation is characteristic, is dependent on T cells. T cells are strongly selected against autoaggression in ontogeny (*58*) and are, therefore, unable to help B cells in their response to autoantigens. Nevertheless, one might still expect to find somatic mutation involved in autoimmune processes as, indeed, recent evidence indicates (*45, 59*).

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The Chicken B Cell Compartment

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A very unusual molecular mechanism is involved in generating the preimmune repertoire in the chicken bursa of Fabricius. A unique rearranged V gene is diversified through a program of segmental gene conversion with a pool of noncoding pseudogenes being used as donors. A specifically committed progenitor that originates in the embryonic bursa is responsible for long-term maintenance of the B cell population. Both these properties and the characteristics of the peripheral B cell compartment are discussed in terms of the evolution of the T and B immune systems.

A VAST AMOUNT OF INFORMATION CONCERNING THE MAMmalian immunoglobulin (Ig) superfamily has been collected, but several areas of cellular regulation have remained unexplained. For the mouse B cell system, data have pictured a multiple gene family organization comprising several hundred functional variable (V) genes [for both heavy (H) and light (L) chain loci] in association with a massive daily production of B cells. This production, which occurs in the bone marrow, probably involves continuous rearrangement that results in the random expression in each

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