Monoclonal Antibodies Reveal the Structural Basis of Antibody Diversity

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Summary. Hybridoma technology has made it possible to introduce into continuous culture normal antibody-forming cells and to obtain large amounts of the immunoglobulin produced by each of these cells. Examination of the structure of a number of monoclonal antibodies that react with a single antigen has provided new information on the structural basis of the specificity and affinity of antibodies. Comparisons of families of monoclonal antibodies derived from a single germ line gene revealed the importance of somatic mutation in generating antibody diversity. Monoclonal antibodies that react with variable regions of other monoclonals allow the further dissection and modulation of the immune response. Finally, the continued somatic instability of immunoglobulin genes in cultured antibody-forming cells makes it possible to determine the rate of somatic mutation and to generate mutant monoclonal antibodies that may be more effective serological reagents.

Immunologists have studied antibodies for two quite different reasons. On the one hand, antibodies are the final products of an immune response that evolved to protect vertebrates from an environment filled with a seemingly infinite number of life-threatening infectious and toxic agents. An essential property of the immune response is its ability to generate enormous sequence diversity in antibody molecules: an individual can produce more than 10^8 different antibodies, each with a different amino acid sequence. To determine the genetic and molecular mechanisms responsible for this diversity, immunologists examine the primary structure of individual antibody molecules and the genes encoding them. On the other hand, scientists in many areas have recognized the usefulness of antibodies as reagents that can be used to identify, locate, and quantitate macromolecules in complex biological mixtures. However, the production of homogeneous antibodies that can be used as reagents for accurate and reproducible immunoassays has proved difficult.

The problem was to some extent solved when it was recognized that the disease multiple myeloma is a malignancy of antibody-forming cells. The large amount of paraprotein in the serum of patients or animals with this malignancy is a monoclonal antibody that is produced by a transformed antibody-forming cell growing in an uncontrolled fashion. Much of what we know about the structure of antibody molecules and the organization and structure of the immunoglobulin genes came from the analysis of myeloma immunoglobulins and cells. However, only a few of these myeloma proteins were found to react with known antigens and it was not possible to use myelomas to precisely dissect the enormous repertoire of antibodies or to harness the disease to produce homogeneous antibodies that would be useful serological reagents. Relatively large amounts of homogeneous antibodies could be produced by immunizing animals with some bacterial polysaccharides (*1*); small amounts of a wider variety of antibodies could be obtained by limiting dilution cloning of antibody-forming cells in the spleens of irradiated animals and subsequent analysis of fragments of such spleens in short-term culture (*2*). Both approaches provided important information but were limited in their usefulness.

A method for routinely producing large amounts of a wide variety of homogeneous antibodies was discovered in 1975 by Kohler and Milstein (*3*). These workers were using cultured mouse myeloma cells to study the regulation of immunoglobulin gene expression in somatic cell hybrids. By fusing cultured mouse myeloma cells to normal spleen cells from immunized mice, they were able to introduce individual antibody-forming cells into long-term tissue culture (*3*). Since immunization selectively increases the number of spleen cells producing antibody reactive with the immunizing antigen, a significant percentage of the hybrids, or hybridomas, were producing the desired antibody. Furthermore, the clonal progeny of each hybridoma synthesized monoclonal antibodies all with the same amino acid sequence. The hybridomas retained the malignant properties of the myeloma parent, causing tumors when injected into mice. The ascites fluid and serum of such tumor-bearing mice contained large amounts of the monoclonal antibody, making it possible to obtain ten to hundreds of milligrams of a desired antibody. The hybridoma cells could also be frozen and recovered at will and the exact same monoclonal antibody could be renewed when needed and was available indefinitely (*3*). Thus, Kohler and Milstein's discovery simultaneously satisfied the need for large amounts of chemically defined homogeneous antibodies that were easily renewable for immunoassays and allowed the immunologists to repeatedly sample the repertoire of cells making antibody against a particular antigen and to study the protein and nucleic acid structure of representatives of this repertoire. This has led to a better understanding of the genetic and molecular events responsible for antibody diversity.

Molecular Basis of Antibody Diversity and Specificity

Ever since it became obvious that antibodies specific for different antigens differed from each other in the amino acid sequence of their variable (v) region (Fig. 1), there have been debates about whether each individual animal inherited in its germ line all of the genes required to code for the many antibody molecules they would produce during their lifetime, or if they inherited only a few germ line genes which subsequently underwent somatic changes in their base sequence. Early studies by Weigert and Cohn and their colleagues on λ light chains produced by mouse myeloma cells suggested that somatic mutation played a major

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role in antibody diversity (*4*). Because very few λ chains are normally produced by mice, it was thought that there might be something peculiar about their genetic control. Even when Tonegawa and his colleagues (*5*) confirmed the findings of Weigert *et al.* by showing that there was only one germ line V region gene of λ1 light chains and that its sequence changed in myelomas producing variant λ chains, many immunologists were unwilling to generalize these findings to κ light chains and heavy (H) chains which were much more heterogeneous and abundant. However, evidence soon began to accumulate that somatic mutation also played a role in the generation of the sequence diversity of κ chains (*6–9*).

The genetic basis for the heterogeneity of both light and heavy chains has now been elucidated by a large number of studies on the immunoglobulin genes. These studies have been recently reviewed (*10*). Briefly, they reveal that there are 100 to 300 V region genes which, in the case of the light chains, code for the first 95 amino acids of the V region including the first two hypervariable (HV) regions and their surrounding framework (FW) residues (Fig. 1). Each of these germ line V region genes can recombine with any of four functional joining (J) region minigenes to complete the V region of the protein (Fig. 1). In the case of heavy chains, there are also 100 to 300 V region germ line genes, which can associate to any of 12 or more D region minigenes which code for the third HV region, and any of four J minigenes which code for the remaining amino acids in the V region (Fig. 1). These combinatorial rearrangements could generate many different antibody sequences from a limited amount of genetic material. Furthermore, in the course of the VDJ or VJ rearrangement, recombination may occur at different sites and bases are sometimes inserted or deleted, producing even further "junctional" sequence diversity in and around the third HV regions. This sequence diversity generated in the individual chains is further amplified by the fact that a given heavy chain can be expressed with any one of a number of different light chains.

The relative roles of the multiple germ line genes, DNA rearrangements, and junctional diversity in generating antibody diversity have yet to be determined. However, the analysis of even a small number of monoclonal antibodies specific for a few antigens has revealed that all of these events contribute to the specificity of antigen binding. Monoclonal antibodies that bind the hapten phosphocholine (PC) have the same heavy chain V and J but differ from each other in the third HV region which is coded for by D, suggesting that V and J region sequences are crucial for PC binding (*11*). Similarly, many of the antibodies produced by mice of the A/J strain against the hapten *p*-azophenylarsonate (Ars) share the same heavy chain V and J (*12, 13*). Monoclonal antibodies that contain the Ars-binding heavy chain V region but a different J do not bind Ars, indicating that the sequences in both V and J are important for the binding of this hapten. (*14*). Antibodies that bind dextran contain a few different heavy chain V and J regions and a D segment composed of two amino acids. The size of the D seems to be important in forming the dextran binding site and the sequence of the third hypervariable region may determine the fine specificity of antigen binding (*15*). In some cases a particular sort of junctional diversity seems to be required for antigen binding. For example, the light chains of the major family of antibodies to Ars all have an arginine in the first codon of J (*16*). This requires that recombination between V and J occur after the second base of the first triplet of J. A similar restricted recombination is found in the heavy chain of monoclonal antibodies against oxazolone (*17*). Finally, the pairing of certain heavy and light chain V regions determines antigen binding specificity.

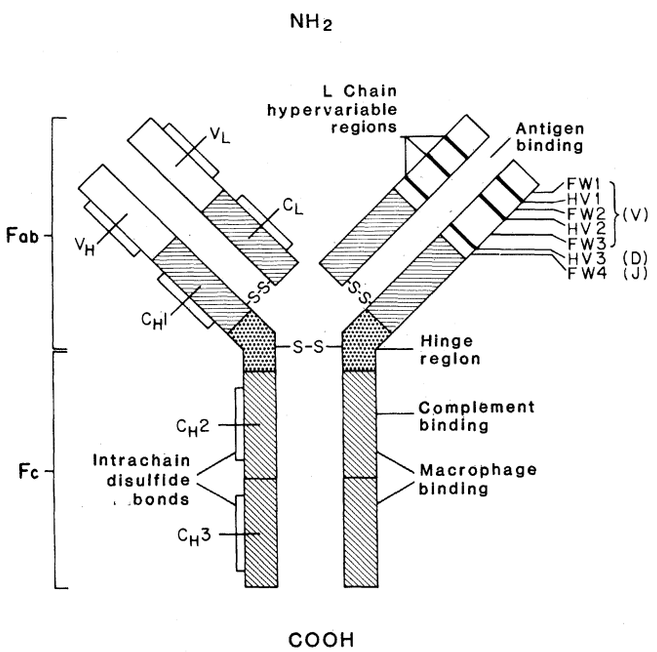

Fig. 1. The structure of an antibody molecule. The different parts of the heavy chain V region are indicated on the right and the segments of the gene which encode them are shown in parentheses.

Role of Somatic Mutation in Antibody Diversity

At first it seemed that the availability of multiple germ line genes, gene rearrangements, and junctional diversity could provide all of the sequence diversity that existed. However, detailed analysis of families of PC- and Ars-binding monoclonal antibodies has already revealed that, just as with λ light chains, somatic mutation of both κ light and heavy chains also contributes significantly to the sequence diversity and affinity of antibody molecules. The importance of somatic mutation was established by examining the heavy and light chains of closely related families of PC- and Ars-binding monoclonals, each of which is coded for by a single germ line variable region gene. For example, Gearhart *et al.* have studied 11 immunoglobulin M (IgM) and 9 immunoglobulin G (IgG) monoclonal antibodies from BALB/c mice that react with PC (*11*). The heavy chain V regions of these antibodies are all very similar and are all rearranged to the J_H1 minigene. All of the IgM antibodies were identical in the NH_2-terminal sequence of their V regions. Further, they reflected the exact sequence of one of the four V region genes which form the T15 family of cross-hybridizing germ line genes in BALB/c mice. All of the IgG antibodies were products of the same germ line gene but contained one or at most a few amino acid substitutions in the HV region or FW residues. These changes were best explained by somatic mutation (*11, 18, 19*), although alternative genetic mechanisms such as gene conversion (*20*) have not been completely ruled out. Since IgM heavy chains are made early and IgG heavy chains later in B-cell

differentiation, these studies suggested that the mechanism that produces base changes is either turned on during B-cell differentiation or that mutations accumulate during the expansion of B-cell clones.

Although there is considerable debate about the latter point (*17, 21, 22*), studies on a few other families of monoclonal antibodies, each apparently derived from a single germ line gene, confirm that many antibodies are derived by a few base changes from the germ line sequence. For example, a number of workers have analyzed the CRI family (*23*) of monoclonal antibodies that react with the hapten Ars (*12, 13*). Just as with the T15 family, it has now been shown that many of these Ars-binding antibodies are derived from a single germ line heavy chain V region gene but differ from it by a few amino acid substitutions in the HV or FW parts of the V region. Since no two of the IgG PC-binding monoclonals analyzed by Gearhart *et al.* (*11*) were identical, and there are few repeats in the Ars (*12, 13*) and other systems that have been studied, there must be even more sequence diversity than was previously suspected. This has led to the suggestion that an individual makes 10^8 or more antibodies in a lifetime. Analysis of the types of base changes which occur in monoclonals does not reveal any significant predominance of transitions or transversions or of particular base changes (*6, 7, 11–13, 19*). Gearhart and Bogenhagen (*24*) reported that the base changes occur in clusters and suggest that an error-prone polymerase is involved.

While the ability to sample the repertoire of antibodies through the analysis of monoclonal antibodies can provide important insights, some questions are hard to answer with this approach. The monoclonals obtained are the product not only of B-cell differentiation but also of selection by antigen and by helper and suppressor T cells. It is likely that these immunoregulatory pressures select for variants with higher affinity than the germ line sequence. In fact all of the monoclonal antibodies shown by Gearhart and Gefter and their colleagues to differ from the germ line sequences have higher affinities for hapten than the germ line antibodies (*25, 26*). Such selective pressures make it difficult to use monoclonal antibodies to determine the exact rate of instability of V region genes in vivo and to recover all of the progeny of the mutational or other processes that are occurring.

Another approach to these questions is to examine the molecular genetics of the immunoglobulin genes in cultured myeloma and hybridoma cells using the methods that have been so effectively exploited in bacterial genetics. This is quite feasible since we and others have developed techniques to identify somatic cell mutants of immunoglobulin-producing cell lines and have shown that such mutants are found in cultures at astonishingly high frequencies of 0.05 to 1 percent (*27*). Such cultured cell lines are clearly different from normal cells in that they carry a variety of viruses, grow continuously in culture, and can form tumors in animals. Information obtained from such cultured cells may or may not be relevant to normal events. However, the high rate of spontaneous mutation appears to be restricted to the immunoglobulin genes since other proteins such as thymidine kinase and hypoxanthine phosphoribosyl transferase undergo mutations at the expected frequencies of 10^{-6} to 10^{-7} (*27, 28*).

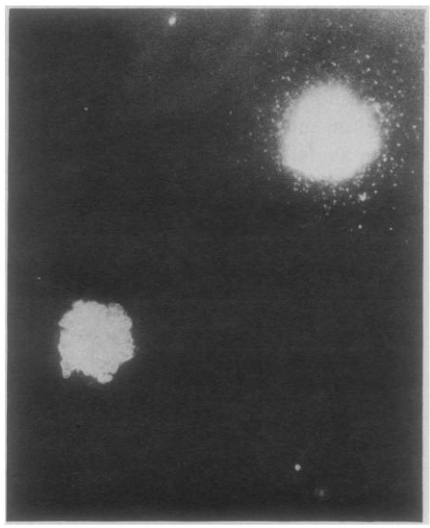

Fig. 2. Identification of antigen-binding mutants. Cells are cloned in soft agar and overlaid with antigen as described in (*34*). The clone on the right is surrounded by an antigen-antibody precipitate. The clone on the left is not surrounded by a visible precipitate and is a presumptive mutant.

Our own studies have concentrated on the T15 heavy chain V region gene as it is expressed in the S107 myeloma cell line and in hybridomas. This is the same PC-binding V region that was studied in hybridomas by Gearhart *et al.* (*11*) and discussed above. It is an extremely well studied antibody because it is produced by many mouse myelomas and is the predominant heavy chain V region expressed in mice immunized with pneumococcal and other bacterial polysaccharides or with PC attached to protein carriers (*29*). The heavy and light chain V regions and the genes that code for the S107 (T15) V region have been completely sequenced (*18, 30, 31*) and the family of closely related germ line heavy chain V regions has also been studied (*18*). In addition, Davies and his colleagues have determined the three-dimensional structure of a related PC-binding antibody and identified the residues that contact the hapten and are essential for the conformation of the hapten binding site (*32*).

When S107 myeloma cells are cloned in soft agar and overlaid with antigen [PC attached to the protein keyhole limpet hemocyanine (PC-KLH)], the antibody secreted by the cells reacts with the antigen in the surrounding agar to form a visible antigen-antibody precipitate around the clones (*33, 34*) (Fig. 2). Clones that are not surrounded by a precipitate are presumptive mutants and are recovered from the agar and characterized. Those presumptive mutants that are producing antibody with a changed ability to bind antigen are considered mutants and are further characterized (*34–36*). The spontaneous frequency of such antigen-binding mutants is 0.05 to 1.0 percent. In the seven mutants analyzed thus far, the defect in antigen binding resides in the heavy chain (*34–36*). Figure 3 shows the amino acid sequences of the heavy chain V region of the parent and two such mutants. Both differ from the parental germ line sequence by only a single amino acid. In U_4, an alanine has replaced the glutamic acid at residue 35 and in U_1 an alanine has replaced the aspartic acid in the fifth residue of the J segment (*35, 36*).

The U_4 mutation is particularly interesting both with respect to the structural basis of antigen binding and the genetic expression of the T15 family of V region genes. Since the heavy chain has four residues that make contact with hapten and the light chain has one contact residue (*32*), it was surprising that the glutamic acid to alanine change at residue 35 resulted in an almost complete loss of antigen binding. However, the three-dimensional structure suggests that the hydrogen bond between the glutamic acid and the tyrosine at residue 94 of the light chain is important in stabilizing the conformation of the antigen binding site (*32*). This also explains why only this particular member of the T15 family of cross-hybridizing heavy chain V region genes is found in PC-binding antibodies in BALB/c mice; none of the others code for a glutamic acid at residue 35 (*18*). Finally, this is a product of the germ line gene which is "degenerate" in that it no longer binds PC. It is not clear whether it will react with another antigen or represents a sort of wastage. In any case, it is

a product of the gene that would not be detected in screening for hybridomas that bind PC.

The high spontaneous frequency of antigen-binding mutants suggested a very high rate of base changes in the T15 V region gene in the S107 cultured cell line. In order to examine the exact rate of these events, we conducted a fluctuation analysis (*28*) to determine the rate at which the U_1 mutant (Fig. 3) reverts to higher antigen binding. The rate was 1.4×10^{-4} per cell per generation (*37*). A number of independent revertants (*34*), which bound antigen as well as the parent S107 antibody, are being analyzed and are true revertants in that they have both the amino acid and base sequence of the parent (*37*). This means that the mutation rate of U_1 is at least 10^{-4} per cell per generation for the fifth residue of J. It is interesting that a survey of myelomas expressing this same J_{H1} revealed a number of somatic mutations at the residue (*38*), suggesting that it may be unusually unstable. If all the 117 V region residues mutated at the same rate, the rate of mutation for the V region as a whole would be 10^{-2} per cell per generation.

Although the mechanism responsible for the high frequency of base changes both in vivo and in the cultured cells has not been determined, examination of the related V region sequences and of the various monoclonal antibodies suggests that simple recombination is not playing a role and that some sort of hypermutation mechanism is at work (*12, 13, 19, 24*). Rajewsky and his colleagues have been isolating somatic mutants in culture from a hybridoma that produces antibody against the hapten NP (4-hydroxy-3-nitrophenylacetyl) (*39, 40*). One of their mutants differs from the presumed germ line sequence by ten closely linked amino acids. This has led to the suggestion that it arose through the interaction of two closely related V region genes and that a gene conversion-like mechanism could play a role in generating antibody diversity (*39, 40*).

Monoclonal Antibodies to the Variable Region

In the previous sections we have described how the analysis of closely related families of monoclonal antibodies has provided new insights into the structural basis of antigen binding and the molecular mechanism responsible for antibody diversity. Just as investigators in many areas of biology have used conventional antibodies to identify and quantitate the relatedness of different macromolecules, immunologists generated anti-antibodies that were specific for the variable regions of certain myeloma proteins, monoclonal antibodies, or normally occurring antibodies that reacted with a particular antigen. Such antibodies to the V region, or antibodies to the idiotype, were usually rendered specific by absorbing the antisera with immunoglobulins from unimmunized animals. It was in fact such conventional antibodies to the idiotypic determinants that made it possible to recognize and study the families of PC-, NP-, and Ars-binding antibodies described in the previous sections (*41*). When the hybridoma technology became available, immunologists wished to exploit the benefits of monoclonal antibodies and began to generate monoclonal antibodies to the idiotypic determinants. This was usually done by immunizing mice with either monoclonal or myeloma immunoglobulins. The antigenic determinants recognized by monoclonal antibodies to the idiotype are called idiotopes. Some idiotopes are within the antigen binding site while others are located elsewhere on the V region. While it has become increasingly difficult to define the different types of antibodies to the idiotype (*41*), idiotopes that are coded for by the germ line V region gene are usually found in all mice that express that gene and are called public or cross-reactive. Idiotopes that are coded for by somatic mutations or rare junctional changes occur intermittently in individual mice and on one or a few monoclonals in a family and are said to be private (*41*).

The specificity of the interactions of monoclonal antibodies to the idiotype and monoclonal antibodies and myeloma proteins of known sequence illustrates both the benefits and complexities of trying to use such reagents to identify chemical relatedness. For example, Clevinger *et al.* (*42*) have studied the interaction of monoclonal antibodies with a number of monoclonal and myeloma immunoglobulins that react with α(1–3) dextran. Monoclonal antibodies were found that reacted with the two amino acids that are coded for by the D segment of the heavy chain. However, it is not possible to correlate the various amino acid substitutions that occur with reactivity with monoclonal antibodies to idiotypic determinants (*42*). Similar observations have been made for idiotype–anti-idiotype interactions in the NP and galactan binding systems (*43*). Other observations suggest the importance of conformation in forming the idiotopes recognized by some monoclonal antibodies to the idiotype. Morahan *et al.* (*44*) have described a monoclonal which reacts with the T-15 V region only when it is associated with the α constant region, that is, IgM or IgG antibodies with the same V region are not recognized by this monoclonal antibody.

We have studied a similar situation in which a monoclonal antibody only reacts with variants that have different amino acid sequences from the germ line. We have generated mouse monoclonal antibodies that react with the U_1 mutant of S107. As shown in Fig. 3, this mutant differs from the S107 heavy chain sequence by only a single amino acid at the

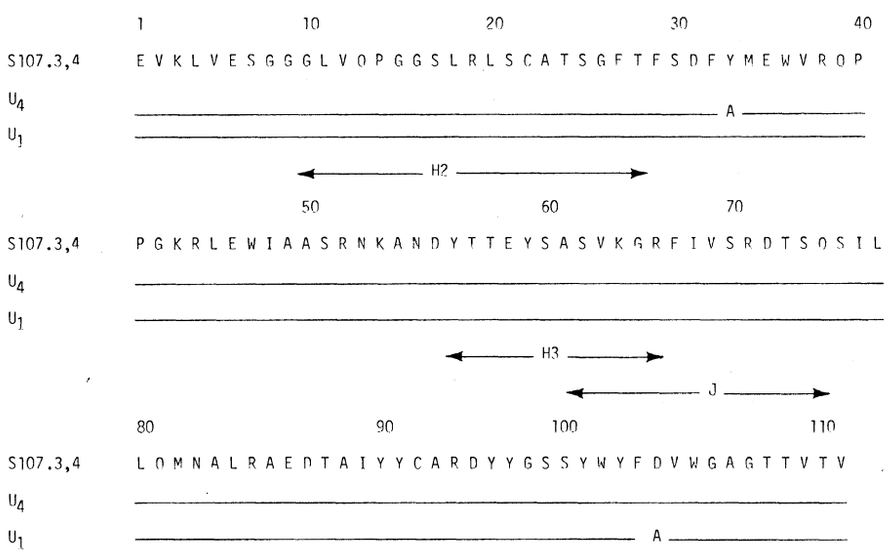

Fig. 3. Amino acid sequence of the heavy chain V region of the S107 parent and two antigen-binding mutants U_1 and U_4. The continuous line indicates sequence homology. The single letter code is used.

fifth residue of J. These monoclonal antibodies react equally well with the U_4 mutant which has a single amino acid change at residue 35, and U_1 which has an amino acid substitution at residue 105 (Fig. 3), but do not react with the S107 parent (Fig. 4). The substitution at position 35 in U_4 is within the hapten binding site while the change at residue 105 in U_1 is probably at the entrance to the site (*32*). Since the antibodies to the mutant do not interfere with the binding of antigen to U_1, we believe that they are recognizing a conformational change that is distant from both substitutions but shared by both mutants. This idiotope is neither private nor public (see above) and illustrates the difficulty of using such definitions.

In spite of these problems, monoclonal antibodies to idiotypic determinants are being used extensively to study the expression and genetics of V region genes. Since it is impossible to sequence heterogeneous populations of antibodies or very large numbers of monoclonal antibodies, the determination of the frequency of rare antibodies or somatic variants in vivo or among monoclonal antibodies will require serological analysis. This has been done with collections of monoclonal antibodies to idiotypes that recognize different public idiotopes encoded by the germ line sequence. Monoclonal antibodies that no longer react with one or a few of those anti-idiotypic monoclonals are presumptive variants. Monoclonals that react with private idiotopes on somatic variants have been used to determine the frequency with which that variant appears in immunized animals (*41*). Such analyses are complicated by the fact that many of the idiotopes recognized by monoclonal antibodies require the interaction of both heavy and light chain V regions, so changes in either V region could cause the loss of the antigenic determinant (*41*). We have overcome this problem by immunizing rats with mouse immunoglubulins expressing the T15 germ line sequence and obtaining rat-mouse hybridomas (*45*). Four out of eight monoclonal antibodies reacted as well with free heavy chains, or heavy chains associated with an irrelevant light chain, as with the intact antibody indicating that they were recognizing heavy chain V region determinants. We have used these to examine a question discussed earlier, that is, do PC-binding IgM antibodies undergo somatic mutations in their V regions? So far, one of 70 IgM monoclonal antibodies does not react with one of the four heavy chain specific monoclonals. Preliminary DNA sequencing suggests that the amino acid sequence changes in this somatic variant are not due to changes in D or J.

Table 1. Structural and functional characteristics of Ar13.4 mutant monoclonal antibodies. The size of the heavy chains was measured on unglycosylated proteins synthesized in the presence of tunicamycin. Complement fixation was measured with H_2L_2 molecules of ArM1 and ArM2.

Cell line	H chain size (kd)	Domain predominately affected	Assembly		Binding to Fc receptor	Fixation to complement
			H_2L_2 (%)	HL (%)		
Ar13.4	55		100	0	+	+
ArM1	39	C_H3	27	73	−	+
ArM2	41	C_H3	18	83	−	+
ArM16	39	C_H2	100	0	−	−
ArM20	39	C_H2	100	0	−	−

Antibodies to idiotypic determinants have also been used to modulate the expression of antibodies expressing particular V regions in the intact animals. Such experiments were originally carried out with conventional antibodies made in other species with sometimes conflicting results. When mouse monoclonal antibodies to idiotypic determinants became available, they provided more useful reagents both because antibodies that recognized either public or private idiotypes could be used and syngeneic antibodies were being administered to mice. The results of these experiments have been reviewed (*41*) and there seem to be some generally accepted conclusions. When certain strains of mice are immunized with PC, Ars, NP, and presumably other haptens, the antibody response is usually dominated by a single idiotype which is coded for by a single heavy and light chain germ line gene. If newborn animals are injected with small amounts of antibody to the idiotype and then subsequently immunized with antigen, they no longer express the predominant idiotype but ultimately do make antibodies coded for by other germ line genes. In addition, if adult mice are injected with 10 to 100 nanograms of antibody to idiotype, increasing amounts of idiotype are expressed. However, the injection of 10 micrograms of antibody to the same idiotype suppresses the production of that idiotype in response to antigen, at least for a time (*41*). The mechanisms of these effects are not fully understood. However, they do provide a means to further analyze both the germ line repertoire and somatic mutation. For example, animals have been suppressed for the expression of a public idiotope and then analyzed for the production of antibodies that bear other idiotopes expressed by the germ line gene (*46–48*). Monoclonal antibodies generated from such mice can then be analyzed to determine if they are the product of different germ line genes or of somatic mutation of a single germ line gene (*48*).

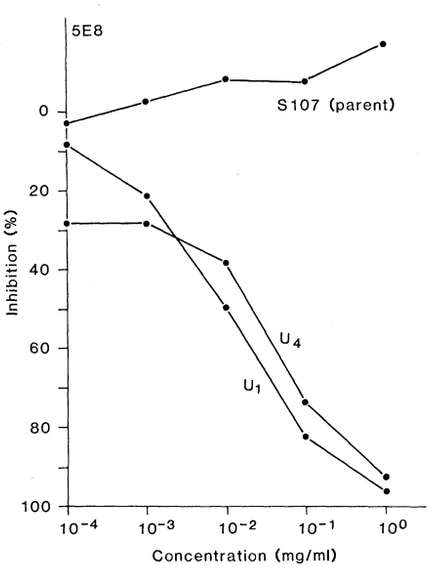

Fig. 4. Radioimmunoassay to compare antigen-binding mutants U_1 and U_4 with the S107 parent. The antimutant monoclonal antibody 5E8 was labeled with [S^{35}]methionine. Mutant immunoglobulin was adsorbed to polyvinyl microtiter wells. Labeled antimutant monoclonal antibody was added to each well in the presence of decreasing amounts of unlabeled mutant and parent proteins. Wells were washed and then counted.

Studies of the sort described above were originally complicated by results which suggested that the class of anti-idiotypic antibodies (that is, the structure of the constant region) determined whether suppression or enhancement occurred (*41*). However, Muller and Rajewsky (*49*) took an IgG_1 mouse monoclonal antibody to a particular idiotype and in culture derived from it a series of antibodies containing the exact same V region but other C regions of the IgG_{2b} and IgG_{2a} subclasses. These sets of antibodies to idiotypes were used to study suppression and enhancement, and it was shown that the subclass of the anti-

body was not important and that the earlier results were probably due to different affinities of the antibodies to idiotypic determinants used (*41*).

Generation of Tailor-Made Antibodies

The studies of Muller and Rajewsky (*49*), in which they generated somatic variants of a single monoclonal antibody in order to obtain a better set of serological reagents, point to another benefit of monoclonal antibodies that is beginning to be exploited. We have already discussed the instability of immunoglobulin genes in cultured myeloma and hybridoma cells. We and others had shown some years ago that mutations and recombinations in the constant region arise frequently in such cells (*27*). The changes observed include deletions, point mutations, and class and subclass switching. As it has become obvious that monoclonal antibodies will not only be used as research reagents but also for in vivo diagnosis and therapy, it has also become clear that we do not know enough about the kinds of properties that will make antibodies most effective in passive immunization against infectious agents and toxins, and for targeting radioactive or cytotoxic agents to tumors. The ability to generate mutant monoclonal antibodies with changes in their constant region sequence provides an opportunity not only to learn more about the structural basis of the effector functions of antibodies but also to learn how to tailor-make monoclonal antibodies that will be more effective serological reagents (*27, 50, 51*).

The techniques for isolating such mutants have been reviewed (*27*). In our own studies we use the same immunoplate assay described earlier (Fig. 2), except that the clones are overlaid with antibodies that react with particular parts of the constant region (*51*). Mutants that are not surrounded by a visible antigen-antibody precipitate are screened for the production of antigen-binding antibodies and then characterized to determine the structural changes in the antibody and its impact on effector functions.

We have recently described a set of mutants of an Ars-monoclonal (Ar13.4) with deletions in the C_H2 and C_H3 (see Fig. 1) domains of their constant regions (*51*). Some of these results are summarized in Table 1. These mutants confirm the role of sequences in both the C_H2 and C_H3 domains for binding to the immunoglobulin (Fc) receptors of macrophages, of the C_H2 domain for the fixation of complement, and of the C_H3 domain for the assembly of heavy chains to form H_2L_2 molecules. It is clear that further refinements of this approach should make it possible to identify the amino acid residues responsible for these functions and to obtain a variety of mutant reagents that can be used to explore the types of structures that should be constructed, probably by the recombinant DNA technology, for in vivo diagnosis and therapy.

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