Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2926–2930, May 1987
Immunology

Somatic diversification of S107 from an antiphosphocholine to an anti-DNA autoantibody is due to a single base change in its heavy chain variable region

(antibody diversity/point mutation)

ANGELA M. GIUSTI, NADINE C. CHIEN, DONALD J. ZACK, SEUNG-UON SHIN, AND MATTHEW D. SCHARFF*

Department of Cell Biology and Irvington House Institute, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461

Contributed by Matthew D. Scharff, December 30, 1986

ABSTRACT **The S107 myeloma cell line expresses the germ-line sequence of the T15 antiphosphocholine (*P*-Cho) antibody, which is the major antibody made by BALB/c mice in response to *P*-Cho, either on a variety of bacterial polysaccharides or when attached to a protein carrier. We have previously reported that a somatic mutant of the S107 cell line produces an antibody that has lost the ability to bind *P*-Cho and has acquired binding for double-stranded DNA. This antibody has a substitution of an alanine for a glutamic acid at residue 35 in the heavy chain variable region. We now show that this amino acid substitution is due to a single A-C transversion, which is the only nucleotide change in the heavy and light chain variable regions. Further, it appears that this change is due to somatic mutation rather than to gene conversion.**

Studies carried out over the last decade have revealed that a great deal of antibody diversity comes from the availability of multiple germ-line variable (V) region genes and their ability to rearrange to any one of a small pool of diversity (D) and joining (J) region elements for heavy chains and multiple J elements for light chains (1). Further diversity is generated by variations in the recombination sites between these elements and the introduction of N sequences not encoded in the germ-line between germ-line elements during gene rearrangement (1). The recent comparison of the sequences of many monoclonal antibodies with what appear to be the relevant germ-line V region sequences has led to the conclusion that even further sequence diversity is generated somatically in already rearranged and expressed V region genes as B cells progress from the primary through the secondary immune response (2–11). It was initially thought that most of this somatic diversification was caused by some form of hypermutation (3). However, recurring uncertainty about the actual number of germ-line V region genes (12) and proven examples of gene conversion (13, 14) and V region replacement (15–17) have raised some question about the importance of point mutations in generating antibody diversity.

Whatever the genetic mechanism of sequence diversity, libraries of closely related monoclonal antibodies, all reacting with a single antigen, should reveal the structural basis of the changes in affinity and fine specificity that occur during the immune response. Unfortunately, most monoclonal antibodies differ from the germ line and from each other by many amino acid substitutions (2–11). In contrast, most somatic mutants generated from cultured antibody-forming cell lines have only a single amino acid substitution and thus provide an excellent opportunity to evaluate the structural basis of changes in antigen binding.

We have previously reported that the S107 cell line spontaneously and frequently generates variant subclones that produce antibodies with single amino acid substitutions in the heavy chain V region (18–20). One such somatic mutant, U4, is of particular interest because, associated with a single amino acid substitution in its heavy chain V region, it has lost the ability to bind phosphocholine (*P*-Cho) and at the same time acquired the ability to bind double-stranded (ds)DNA (21). In this paper we describe the molecular characterization of the U4 mutant and show that its changes in specificity and affinity are due to a single base change in the heavy chain V region.

MATERIALS AND METHODS

Cell Lines. The S107.3.4 (S107) cell line has been described (18). It was derived from a BALB/c myeloma tumor and continues to secrete an IgA κ-chain antibody of the T15 idiotype that binds *P*-Cho and has the germ-line sequence of the T15 V_H1 heavy chain and $V_κ22$ light V regions (22). The U4 mutant arose spontaneously in tissue culture (20). Both cell lines were maintained in suspension in an atmosphere of 8% CO_2 and air at 37°C in Dulbecco's modified Eagle's medium (H-21, GIBCO) supplemented with 10% fetal calf serum.

Immunoassays. To compare the ability of S107 and U4 to react with antiidiotypic antibodies, competition assays were carried out. AB1 is a mouse monoclonal anti-T15 antibody kindly provided by John Kearney (University of Alabama Medical School). Its binding to S107 is inhibited by *P*-Cho–bovine serum albumin (23). Rabbit antibody reactive with the *P*-Cho binding site of S107 was prepared as described by Claflin and Davie (24). ELISA plates were coated with 50 μl of S107 protein (10 μg/ml), washed, and blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS). Concentrations of the monoclonal or polyclonal antibodies that bound 80–100% of the available S107 sites on the plate were preincubated with various concentrations of S107 or U4 proteins for 2 hr at room temperature. Fifty microliters of each sample was added to duplicate wells coated with S107 and incubated for 1 hr at 37°C. The amount of the bound AB1 was determined by using an alkaline phosphate-conjugated anti-mouse IgG antiserum (Zymed Laboratories, Burlingame, CA), and the bound rabbit anti-binding site antibody was measured by using an enzyme-linked anti-rabbit IgG antiserum (Zymed Laboratories). Optical densities were read at 405 nm.

The relative binding of U4 and S107 to *P*-Cho was determined by incubating 50 μl (10 μg/ml) of *P*-Cho–bovine serum albumin (4.9 mol of *P*-Cho per mol of bovine serum albumin) with ELISA plates and blocking with bovine serum albumin. Increasing amounts of purified antibody were incubated in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: V, variable; D, diversity; J, junction; H, heavy; *P*-Cho, phosphocholine; dsDNA, double-stranded DNA.

*To whom requests for reprints should be addressed.

duplicate wells for 2 hr at room temperature and washed, and the amount of IgA bound was determined with an enzyme-linked anti-IgA antiserum (Zymed Laboratories).

Nucleic Acid Sequencing. The sequence of the S107 and most of the U4 heavy chain V region, including the A-C transversion that distinguished U4 from S107, was initially determined by dideoxy sequencing of cDNA cloned into M13. Poly(A)$^+$ mRNA was primed with a 15-base-pair (bp) oligonucleotide (5′ TGGGTAGATGGTGGG 3′) homologous to the C_H1 domain and cDNA was prepared using reverse transcriptase (Life Sciences, St. Petersburg, FL) (25, 26). The second strand was synthesized using the Klenow fragment of polymerase I (Bethesda Research Laboratories). After gel filtration on Sephadex G-50 (Pharmacia) and ethanol precipitation, the cDNA was digested into two approximately equal-sized fragments with *Mbo* II, which also removes the 5′ untranslated region and the hairpin loop. The *Mbo* II fragments were treated with polymerase I (Bethesda Research Laboratories) to eliminate the 3′ OH and then blunt-end-ligated into *Hin*cII-cut M13mp8 with T4 ligase. M13 was transfected into JM103 and white plaques containing the desired inserts (27) were identified by filter hybridization with a T15 V_H region probe (28). Single-stranded DNA was prepared from purified and amplified plaques and the inserts were sequenced by the dideoxy method of Sanger and Coulson (29) using a 15-bp mp8M13 oligonucleotide primer (27).

The heavy chain nucleic acid sequence was confirmed and completed and the light chain was sequenced from mRNA. Total mRNA was extracted with guanidinium thiocyanate and purified on a CsCl gradient (30). Poly(A)$^+$ mRNA was then isolated by passage over an oligo(dT)-cellulose column and eluted with low salt. Subsequent sequencing reactions were carried out according to Geliebter *et al.* (31). Twenty micrograms of poly(A)$^+$ mRNA was hybridized to 0.1 μg of ^{32}P-labeled oligonucleotides. The oligonucleotides used are illustrated in Figs. 1 and 2. $J_\kappa5$ was primed with a 23-bp oligonucleotide, 5′ AGCACTTTTCAGCTCCAGCTTGG 3′, and the sequence was continued with the V region oligonucleotide 5′ AGGGACCCCAATGTATC 3′. The same strategy was used for sequencing the heavy chain VDJ regions of U4 and S107 using three oligonucleotides: 5′ C_H1 domain-specific oligonucleotide (5′ TGGGTAGATGGTGGG 3′); U4-specific oligonucleotide (5′ GCGGACCCACGCCATGTAGAA 3′); and an S107-specific oligonucleotide (5′ GCGGACCCACTCCATGTAGAA 3′). Following priming with the above oligonucleotides, the extension reaction was carried out using reverse transcriptase (Life Sciences) in the presence of dNTPs and terminated with dideoxy NTPs (29, 31). The sequence was then resolved on a 6% acrylamide gel.

Southern Blot Hybridization. High molecular weight DNA was isolated from S107 and U4 cells and BALB/c liver. The DNA was digested with *Bam*HI and separated by agarose gel electrophoresis. The DNA was transferred to GeneScreen (New England Nuclear) and hybridized with a 21-bp oligonucleotide specific for the U4 sequence (5′ GCGGACCCACGCCATGTAGAA 3′) under conditions described by Zeff *et al.* (32).

RESULTS

We have described the detection of the U4 mutant of S107.3.4 by the immunoplate assay (20). It was one of a few variant subclones of S107 that arose at spontaneous frequencies of 0.01% or higher and had decreased ability to bind *P*-Cho (18). It continued to secrete large amounts of polymeric IgA whose heavy and light chain's electrophoretic migration and covalent and noncovalent assembly were indistinguishable from the parent. The amino acid sequence of its heavy chain V region revealed a single amino acid substitution of an alanine for a glutamic acid at residue 35 of the first hypervariable region (20). Furthermore, antigen binding studies revealed that U4 protein had not only lost the ability to bind *P*-Cho but also acquired the ability to bind dsDNA, phosphorylated protamine, and cardiolipin (21). Since this reactivity pattern resembles that of autoantibodies found in patients with systemic lupus erythematosus and mice with lupus-like syndromes (33, 34), and the *in vitro* mutants could provide a model for the role of somatic mutation in autoimmunity, we have examined the U4 protein and the molecular basis of these changes in more detail.

The nucleic acid sequence of the coding region of the U4 heavy chain was determined by sequencing cDNA directly from mRNA. S107 was sequenced simultaneously as a control. As can be seen from Fig. 1, the only base change in the coding region of the heavy chain V region is a single A-C

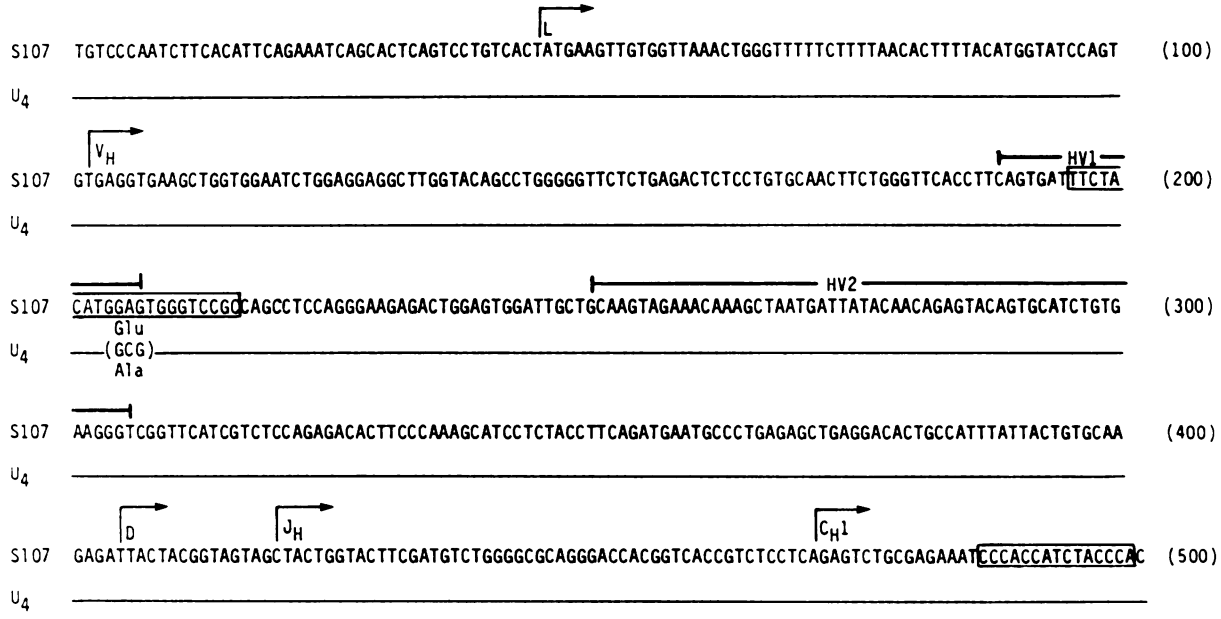

FIG. 1. Nucleic acid sequence of the heavy chain V region of the parent S107 and variant U4. Oligonucleotide primers used to sequence are indicated by the boxed-in sequences.

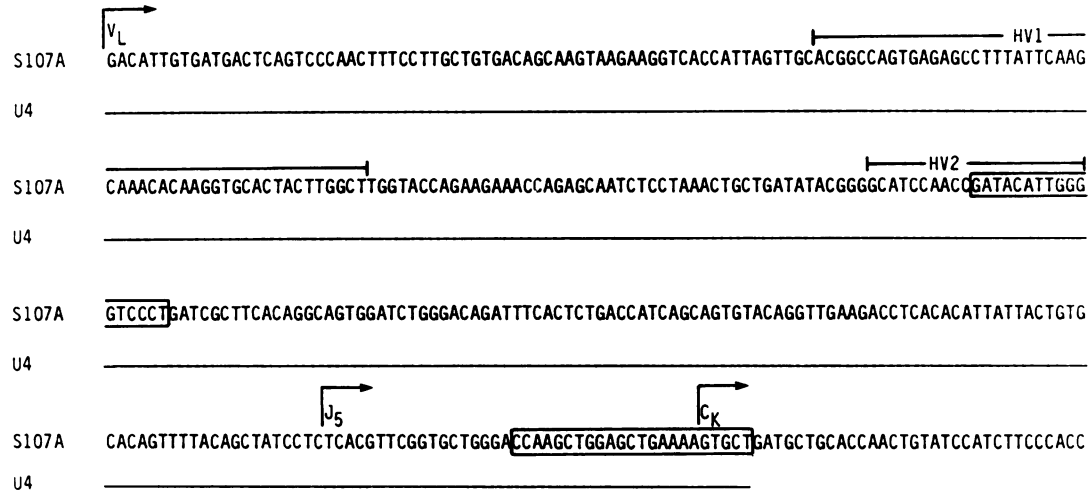

FIG. 2. Nucleic acid sequence of the light chain V region of the parent S107A and variant U4. The boxed-in regions indicate the oligonucleotide sequences used as primers.

transversion in the triplet that encodes residue 35 in the first hypervariable region. Thus, the nucleic acid sequence confirms the earlier protein data that the heavy chain V region of U4 differs from S107 only by the substitution of an alanine for a glutamic acid at residue 35. Further, it shows that there are no silent base changes in the heavy chain V region. The presence of this A-C transversion has been further verified by RNA transfer blot analysis of parental S107.3.4 and U4 mRNA with wild-type- and mutant-specific oligonucleotides (data not shown).

Previously published chain recombination experiments suggested that the loss of *P*-Cho binding was due to the change in the heavy chain and not the light chain (20). This was confirmed by sequencing the mRNA of the U4 light chain V region. S107 expresses equal amounts of two very different light chains (35). However, only the S107A light chain assembles to the heavy chain and is secreted. This light chain contains $J_\kappa 5$, so an oligonucleotide specific for J5 was used as a primer to selectively sequence the S107A V region and half of the J region. As can be seen in Fig. 2, the sequence of the U4 light chain is identical to that of the parent. Since the J5 oligonucleotide hybridizes to the S107A messenger under stringent conditions, we can assume that the 3′ end of J also has the wild-type sequence. This confirms that the amino acid

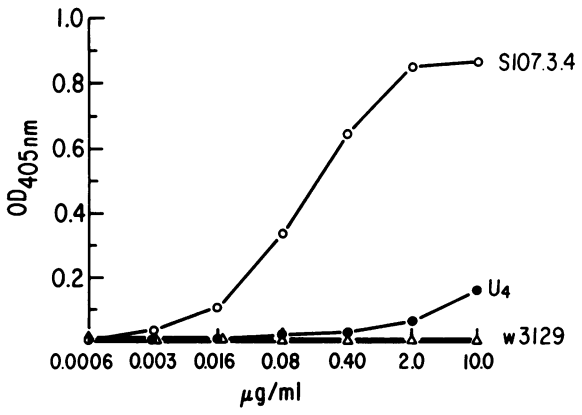

FIG. 3. Direct-binding ELISA of increasing concentrations of S107- (○), U4- (●), and W3129- (△) purified protein to *P*-Cho–bovine serum albumin-coated plates. The amount of binding was determined by using anti-mouse IgA enzyme-linked antiserum and is expressed in optical density (OD) units at 405 nm. W3129 is an anti-dextran myeloma protein that does not bind *P*-Cho.

substitution at residue 35 of the heavy chain is solely responsible for the change in affinity and specificity of U4.

Based on the three-dimensional structure of the *P*-Cho binding site of the McPC 603 protein, Davies and his colleagues suggested that the glutamic acid at residue 35 of the heavy chain V region plays an important electrostatic and conformational role in *P*-Cho binding (36). We have attempted to evaluate the impact of this change serologically. We cannot detect any *P*-Cho binding by equilibrium dialysis. However, U4 will bind to *P*-Cho–bovine serum albumin in a direct binding assay if highly substituted carrier is adsorbed to an ELISA plate and large amounts of U4 protein are incubated with the plate. A comparison of U4 and S107.3.4 binding is shown in Fig. 3. Thus, U4 retains some ability to react to *P*-Cho attached to a protein carrier.

In addition, we have compared the reactivity of the parental S107.3.4 protein with U4 using a variety of T15-specific antiidiotypic reagents. Eight different rat monoclonal antibodies that react with either heavy chain framework residues or conformational determinants requiring heavy and light chains (37) do not distinguish between U4 and S107.3.4 (data not shown). In addition, monoclonal and polyclonal antibodies that react with various parts of the binding site do not distinguish between U4 and S107.3.4. Examples of two such antibodies are shown in Figs. 4 and 5. In Fig. 4, a monoclonal antibody, AB1, whose binding to S107 is inhibited by *P*-Cho conjugated to bovine serum albumin, but not by *P*-Cho alone (23), was examined in a competition assay for the ability of S107.3.4 and U4 to inhibit its binding to plates coated with S107.3.4 protein. In Fig. 5, reactivity of S107.3.4 and U4 with a polyclonal rabbit anti-binding site antibody was studied in a similar fashion. Neither distinguishes between the parental and mutant proteins. This suggests that the dramatic changes in *P*-Cho affinity and the acquisition of binding to dsDNA by U4 is not associated with a major conformational change in the antigen binding site.

Finally, we wished to determine if the single base change in the heavy chain V region arose through somatic mutation, gene conversion, or V gene replacement. The A-C transversion is not present in any of the three other strongly cross-hybridizing members of the T15 heavy chain V region family (22). In an attempt to identify either another germ-line V region gene that might have replaced V_H1 or a possible donor sequence for gene conversion, a 21-base oligonucleotide containing the single base change was synthesized and hybridized to liver, S107.3.4, and U4 DNA. As already noted, this oligonucleotide hybridizes on RNA transfer blots to U4 but not to parental mRNA. As can be seen in Fig. 6,

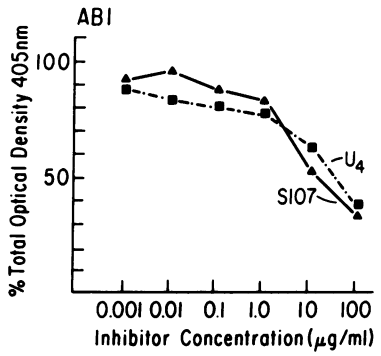

FIG. 4. Inhibition ELISA of S107 (▲) and U4 (■) with an anti-binding site monoclonal antibody, AB1. Purified S107 protein was attached to ELISA plates and increasing amounts of S107 and U4 were preincubated with 50% saturating amounts of AB1. The amount of AB1 bound was determined by using an anti-mouse IgG1 enzyme-linked antiserum and is expressed in optical density units.

this mutant-specific oligonucleotide does not hybridize to liver or S107.3.4 DNA but does hybridize to U4 DNA. Our inability to identify a donor sequence suggests that the single base substitution in the U4 V region arose by somatic mutation rather than gene conversion.

DISCUSSION

The germ-line S107 (T15) V1 heavy chain V region gene is rearranged to the DFL 16.1 D and J_H1 gene segments to form an antibody that reacts with *P*-Cho. This rearranged heavy chain V region gene dominates the response of BALB/c mice to pneumococcal C polysaccharides and is an important protective antibody against this and other endogenous bacteria (38). The results presented above show that a single somatically generated base substitution in the already rearranged and expressed heavy chain V gene can lead to a dramatic change in the affinity and specificity of this important antibody in the mouse.

A variety of data in the literature indicate that the glutamic acid at residue 35 plays a crucial role in *P*-Cho binding. In the course of their analysis of the three-dimensional structure of the related McPC 603 anti-*P*-Cho antibody, Davies and

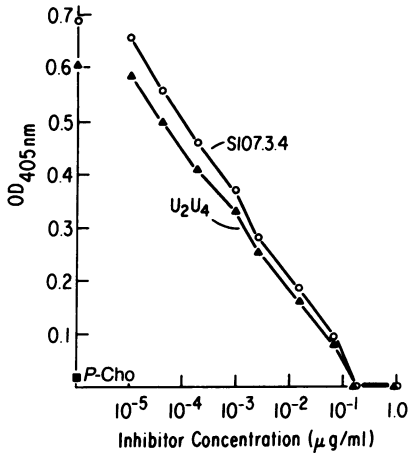

FIG. 5. Inhibition ELISA of S107 (○) and U4 (▲) with an anti-binding site polyclonal antiserum. Purified S107 protein was attached to ELISA plates and increasing amounts of S107 and U4 protein were incubated with 50% saturating amounts of the polyclonal antiserum. The assay was developed by using an anti-rabbit IgG enzyme-linked antiserum and the amount of inhibition was expressed in optical density (OD) units. *P*-Cho (■) completely inhibited the binding of the anti-binding site antibody to S107.

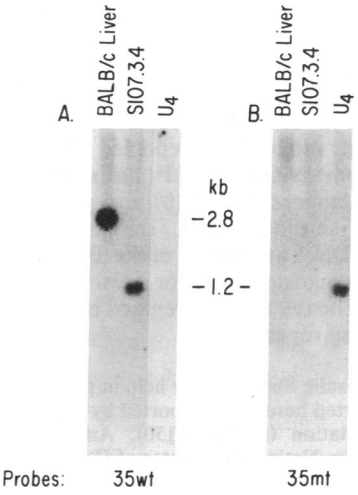

FIG. 6. Southern blot analysis of BALB/c liver, parental S107, and variant U4 DNA using an S107-specific oligonucleotide (*A*) and a U4-specific oligonucleotide (*B*). The DNA was digested with *Bam*HI, transferred to GeneScreen, and hybridized under stringent conditions. kb, Kilobases; wt, wild type; mt, mutant.

colleagues (36) suggested that the glutamic acid at residue 35 forms a hydrogen bond with the tyrosine at residue 94 in the light chain to stabilize the choline moiety in the *P*-Cho binding site. A survey of many *P*-Cho binding antibodies also suggests the importance of the glutamic acid at residue 35 (39). This is confirmed by the fact that V_{11} and V_{13}, the two other very homologous members of the S107 V region family, do not encode a glutamic acid at residue 35 (22) and have not been observed to be responsible for *P*-Cho binding antibodies in BALB/c mice.

It is of interest that the changes in affinity and specificity in the U4 antibody are not associated with a major conformational change. In fact, U4 is indistinguishable from the parental S107 antibody when assayed with a variety of anti-T15 antiidiotypic antibodies. Even monoclonal and polyclonal antibodies that react with the *P*-Cho binding site react identically with U4 and S107.

The molecular basis of the U4 change is certainly somatic since it occurred in an already rearranged and expressed V region. Potential donor sequences were sought by hybridizing a mutant-specific oligonucleotide with S107 and BALB/c liver DNA. No cross-hybridizing DNA was found. This makes it unlikely that another germ-line gene that differs from V_H1 by the single A-C transversion rearranged into the V_H1 site. In fact, neither the two other very homologous productive members of the S107 family nor the strongly cross-hybridizing pseudogene contains this sequence. It is more difficult to rule out gene conversion through a small donor sequence embedded in an otherwise nonhomologous gene. However, the presence of only a single base change and the lack of a detectable donor sequence suggest that U4 arose through somatic mutation rather than gene conversion.

The nature and occurrence of the U4 mutation show that somatic diversification can occur in cultured antibody-forming cells at a relatively high frequency. Previous efforts to find V region mutants in some myeloma and hybridoma cell lines have yielded constant but not V region mutants. Rajewsky and his collegues (40) have identified V region mutants at low frequencies of around 10^{-7}. However, Wabl and colleagues (41, 42) have shown that V region mutants do occur in the Abelson B-cell line 18-81 at a rate of 10^{-5} per bp per generation. The S107 myeloma cell line is a more well-differentiated cell line than 18-81, suggesting that the mechanism for somatic diversification of V regions may be

active in the latter stages of B-cell differentiation as well as in early B cells.

These studies also indicate that heavy chain V regions of the S107 (T15) family can, as the result of a single base change, undergo somatic diversification to become autoantibodies. In fact, others have reported anti-DNA monoclonal antibodies from (NZB/NZW)F_1 autoimmune mice that are encoded by members of the S107 heavy chain V region family (43, 44). The serum of MRL/1pr and BALB/c mice also contains anti-DNA antibodies of the T15 idiotype (44). These findings also suggest that one or a few amino substitutions of this and other heavy chain V regions can greatly increase the antigen binding repertoire.

We thank Rosalie Spata for her help in preparing the manuscript. The work reported here was supported by grants from the National Science Foundation (DCB8316150), American Cancer Society (IM-317), and the National Institutes of Health (CA39838, AI05231, AI10702). A.M.G., N.C.C., and S.-U.S. are supported by a training grant (CA09173) from the National Cancer Institute. M.D.S. is supported in part by the Harry Eagle Chair for Cancer Research from the National Women's Division. The oligonucleotides were synthesized on an Applied Biosystems synthesizer (Foster City, CA) obtained through the National Science Foundation (PCM8400114).

1. Tonegawa, S. (1983) *Nature (London)* **302,** 575–581.
2. Gearhart, P. J., Johnson, N. D., Douglas, R. & Hood, L. (1981) *Nature (London)* **291,** 29–34.
3. Kim, S., Davis, M., Sinn, E., Patten, P. & Hood, L. (1981) *Cell* **27,** 573–581.
4. Kaartinen, M., Griffiths, G. M., Markham, A. F. & Milstein, C. (1983) *Nature (London)* **304,** 320–324.
5. Griffiths, G. M., Berek, C., Kaartinen, M. & Milstein, C. (1984) *Nature (London)* **312,** 271–275.
6. Manser, T., Wysocki, L., Gridley, T., Near, R. I. & Gefter, M. L. (1985) *Immunol. Today* **57,** 94–101.
7. Slaughter, C. A. & Capra, J. D. (1983) *J. Exp. Med.* **158,** 1615–1634.
8. McKean, D., Huppi, K., Bell, M., Staudt, L., Gerhard, W. & Weigert, M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3180–3184.
9. Clarke, S. H., Huppi, K., Ruczinsky, D., Staudt, L., Gerhard, W. & Weigert, M. (1985) *J. Exp. Med.* **161,** 687–704.
10. Gearhart, P. J. & Bogenhagen, D. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3439–3443.
11. Berek, C., Griffiths, G. M. & Milstein, C. (1985) *Nature (London)* **316,** 412–418.
12. Livant, D., Blatt, C. & Hood, L. (1986) *Cell* **47,** 461–470.
13. Krawinkel, U., Zoebelein, G., Buggemann, M., Radbruch, A. & Rajewsky, K. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4997–5001.
14. Reynaud, C.-A., Anquez, U., Behan, A. & Weill, J.-C. (1985) *Cell* **40,** 283–291.
15. Reth, M., Gehrmann, P., Petrac, E. & Weise, P. (1986) *Nature (London)* **322,** 840–842.
16. Kleinfield, R., Hardy, R. R., Tarlinton, D., Dangl, J., Herzenberg, L. A. & Weigert, M. (1986) *Nature (London)* **322,** 843–846.
17. Reth, M. G., Jackson, S. & Alt, F. W. (1986) *EMBO J.* **5,** 2131–2138.
18. Cook, W. D. & Scharff, M. D. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5687–5691.
19. Cook, W. D., Rudikoff, S., Giusti, A. M. & Scharff, M. D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1240–1244.
20. Rudikoff, S., Giusti, A. M., Cook, W. D. & Scharff, M. D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1979–1983.
21. Diamond, B. & Scharff, M. D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5841–5844.
22. Crews, S., Griffin, J., Huang, H., Calame, K. & Hood, L. (1981) *Cell* **25,** 59–66.
23. Kearney, J. F., Bartetla, R., Quan, Z. S. & Quintans, J. (1981) *Eur. J. Immunol.* **11,** 877–883.
24. Claflin, J. L. & Davie, J. M. (1974) *J. Exp. Med.* **140,** 673–686.
25. Buell, G. N., Wicken, M. P., Payver, F. & Schimke, R. T. (1978) *J. Biol. Chem.* **253,** 2471–2482.
26. Wickens, M. P., Buell, G. N. & Schimke, R. T. (1978) *J. Biol. Chem.* **253,** 2483–2495.
27. *M13: Cloning/"Dideoxy" Sequencing* (1980) (Bethesda Research Laboratories, Bethesda, MD).
28. Benton, M. D. & Davis, R. (1977) *Science* **196,** 180–182.
29. Sanger, F. & Coulson, A. R. (1978) *FEBS Lett.* **87,** 107–110.
30. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 196.
31. Geliebter, J., Zeff, R. A., Melvold, R. W. & Nathenson, S. G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3371–3375.
32. Zeff, R. A., Gopas, J., Steinhauer, E., Rajan, T. V. & Nathenson, S. G. (1986) *J. Immunol.* **137,** 897–903.
33. Lafer, E. M., Rauch, J., Audrezejewski, C., Jr., Mudd, D., Furie, B., Schwartz, R. S. & Stollar, B. D. (1981) *J. Exp. Med.* **153,** 897–909.
34. Shoenfeld, Y., Isenberg, D. A., Rauch, J., Madaio, M. P., Stollar, B. D. & Schwartz, R. S. (1983) *J. Exp. Med.* **158,** 718–730.
35. Kwan, S. P., Max, E. E., Seidman, J. D., Leder, P. & Scharff, M. D. (1981) *Cell* **26,** 57–66.
36. Padlan, E. A., Cohen, G. H. & Davies, D. R. (1985) *Ann. Immunol. (Paris)* **136C,** 271–276.
37. Desaymard, C., Giusti, A. M. & Scharff, M. D. (1984) *J. Mol. Immunol.* **21,** 961–967.
38. Briles, D. E., Forman, C., Hodak, S. & Claflin, J. L. (1983) *J. Exp. Med.* **156,** 1177–1185.
39. Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M. & Perry, H. (1983) in *Sequences of Proteins of Immunological Interest* (U.S. Department of Health and Human Services, Washington, DC), pp. 128–133.
40. Bruggermann, M., Rudbruch, A. & Rajewsky, K. (1982) *EMBO J.* **1,** 629–634.
41. Wabl, M., Burrows, P. D., Gabain, A. V. & Steinberg, C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 479–482.
42. Meyer, J., Jack, H.-M., Ellis, N. & Wabl, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6950–6953.
43. Eilat, D., Hochberg, M., Pomphrey, J. & Rudikoff, S. (1984) *J. Immunol.* **133,** 489–494.
44. Davidson, A., Chien, N., Frank, L., Halpern, R., Snapper, S., Zupko, K. & Diamond, B. (1986) *Cell. Immunol.* **99,** 44–52.