Proc. Nat. Acad. Sci. USA
Vol. 72, No. 4, pp. 1427–1430, April 1975

Variants of a Mouse Myeloma Cell Line That Synthesize Immunoglobulin Heavy Chains Having an Altered Serotype

(mutagenesis/immunoglobulin assembly/cultured cells/IgG_{2b} and IgG_{2a})

JEAN-LOUIS PREUD'HOMME*, BARBARA K. BIRSHTEIN, AND MATTHEW D. SCHARFF

Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461

Communicated by Harry Eagle, January 24, 1975

ABSTRACT **Cells of the MPC-11 mouse myeloma cell line, which produces an IgG_{2b} immunoglobulin, were subjected to mutagenesis with Melphalan or with ICR-191, after which they were cloned in soft agar. Approximately 0.5% of the clones produced altered heavy chains that: (*i*) were the same size as or larger than the parent; (*ii*) no longer were recognized by antibody specific for the parent γ2b subclass; (*iii*) were recognized by antibody specific for the γ2a subclass; (*iv*) were recognized by antibody against the Fab (NH_2-terminal half) of the parental immunoglobulin, but lacked some of the antigenic determinants of the Fc (COOH-terminal half) of the heavy chain; (*v*) lacked many of the tryptic/chymotryptic peptides found in the parent; (*vi*) contained tryptic/chymotryptic peptides that were not present in the parent but were present in an unrelated γ2a myeloma heavy chain; and (*vii*) assembled with light chains by a pathway typical of IgG_{2a} myelomas.**

To study the genetic control of antibody synthesis, we have been examining a cultured line of mouse myeloma cells for variants that synthesize altered immunoglobulins. Variants that have lost the ability to synthesize heavy (H) chains occurred spontaneously at a rate of 10^{-3}/cell per generation (1), but only after mutagenesis have we found variants that produce altered H chains. Some of these variants synthesized H chains that were smaller than those of the parent; these variants were discussed in a preceding paper (2). Here, we describe a group of variants that synthesize normal-sized or large H chains having serological, chemical, and assembly characteristics of a different subclass from the parent.

MATERIALS AND METHODS

The cell line 45.6 and its subclones 45.6.2.4, 45.6.3.1, and 45.6.3.2 were derived from the MPC-11 mouse myeloma tumor, which produces an IgG_{2b} immunoglobulin (3). Maintenance of the cell cultures (2), mutagenesis with ICR-191 (2) or with Melphalan (4), and subsequent cloning in soft agar (5) have been described. Clones were overlaid with rabbit antibody against either the Fc region (COOH-terminal half) of the MPC-11 H chain or the completely reduced and alkylated MPC-11 H chains (2). Although variants have been detected using either antibody, we feel that the anti-Fc antibody is a more reliable probe and have used it in most experiments.

"Unstained" variant clones, i.e., those clones not covered by a visible antibody-antigen precipitate, were retrieved from the agar and grown to mass culture. The presence of heavy chains was detected by Ouchterlony analysis of cytoplasmic contents and secreted immunoglobulins using antisera to the MPC-11 H chain and its fragments and antisera directed against other mouse subclasses (Meloy Laboratories). Variants were also characterized by electrophoresis of radiolabeled immunoglobulin molecules on acrylamide gels containing sodium dodecyl sulfate and by peptide analysis using ion exchange chromatography. All these techniques have been described (2).

RESULTS

Isolation of Variants. In a representative experiment cited in detail previously (2), a subclone (45.6.2.4) of the MPC-11 cell line was treated with ICR-191 at a concentration of 1 μg/ml. Approximately 60% of the cells survived; these were cloned in soft agar, and examined with anti-Fc antibody. Of 19 "unstained" clones (presumptive variants) grown to mass culture, four were found to be serologically like the parent, 11 had discontinued H chain synthesis, two synthesized H chains smaller than the parent, and two synthesized H chains of normal size. The incidence of the various types of variants was quite similar in other experiments after mutagenesis with either ICR-191 or Melphalan. The variants that had discontinued H chain synthesis (5) and those synthesizing H chains smaller than the parent (2) have been described. Ouchterlony analysis of cytoplasmic lysates showed that the chains of normal size were not recognized by antiserum against the (parental) IgG_{2b} serotype, but rather were recognized by an antiserum against the IgG_{2a} subclass. The derivation of all such variants synthesizing H chains of the γ2a subclass is presented in Table 1.

These variants fall into two groups: four synthesize H chains of normal (55,000 molecular weight) size, and one synthesizes an H chain of 75,000 molecular weight. The molecular weights were assigned according to the mobility of the chains in acrylamide gels containing sodium dodecyl sulfate.

Serological Analysis. Using Ouchterlony analysis with a variety of antisera, we compared variant H chains with the parental H chain (Table 1). As stated, none of these variant chains reacted with antibody specific for the γ2b subclass of

Abbreviations: H and L chains, heavy and light chains, respectively; V region, variable region.

* Present address: Laboratoire d'Immunochimie, Université de Paris, Faculté de Medicine, Institut de Recherches sur les Maladies du Sang, Hôpital St.-Louis, 2, Place du Docteur-Fournier, Paris-Xe, France.

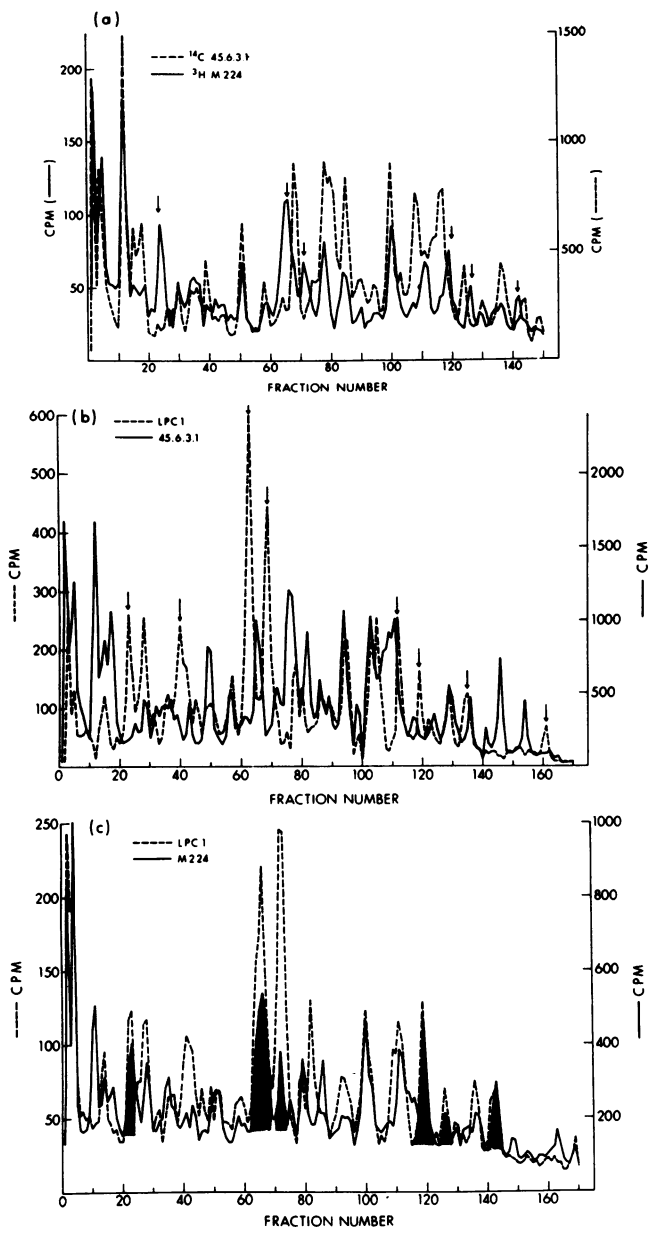

FIG. 1. Comparative peptide maps of (a) ^{3}H-labeled H chains of variant M224 (*solid line*) with ^{14}C-labeled H chains of parent 45.6.3.1 (*dotted line*); (b) ^{14}C-labeled H chains of parent 45.6.3.1 (*solid line*) with ^{3}H-labeled H chains of LPC-1 (γ2a) (*dotted line*); and (c) ^{14}C-labeled H chains of variant M224 (*solid line*) with ^{3}H-labeled H chains of LPC-1 (γ2a) (*dotted line*). H chains were isolated by electrophoresis on acrylamide gels containing sodium dodecyl sulfate. The ^{3}H- and ^{14}C-labeled chains were mixed; the proteins were freed of sodium dodecyl sulfate and then were digested by sequential additions of trypsin and chymotrypsin. The peptides were resolved on a cation exchange resin using a pyridine-acetate gradient. The details are noted in ref. 2. *Arrows* mark differences in the profiles.

the parent and all reacted with antibody specific for the γ2a subclass. When the variants and the parent were compared using antibody against the Fc region of the MPC-11 protein, "spurs" of incomplete identity were seen, indicating that the variants lacked antigenic determinants present in the parent chain. An antiserum directed against the Fab region (NH_2-terminal) of the MPC-11 protein failed to distinguish the

TABLE 1. *Variants of the MPC-11 cell line: Size and serological characteristics of the heavy chains they synthesize*

Cell line	Molecular weight (H chain)	Cytoplasmic lysates				Secretions
		Anti-γ2b	Anti-γ2a	Anti-Fab	Anti-Fc	Anti-γ2a
Parent	55,000	+	−	+	+	−
45.6.3.2.2 M 224*	55,000	−	+	+	+ spur	+
45.6.3.2.2 M 319*	55,000	−	+	+	+ spur	+
45.6.3.1 ICR 11†	55,000	−	+	+	+ spur	+
45.6.3.1 ICR 16†	55,000	−	+	+	+ spur	+
45.6 ICR 9*	75,000	−	+	+	+ spur	−

* These clones were selected by an overlay procedure with rabbit anti-MPC-11 H chains.

† These clones were selected by an overlay procedure with rabbit anti-Fc of MPC-11. The secretions of these clones are positive with the same antiserum in Ouchterlony analysis. This observation indicates the relative insensitivity of the overlay technique, a point which has favored us in the selection of these clones.

variants from the parent. Experiments done by Drs. Rose Lieberman and Michael Potter showed that some variant proteins reacted with anti-idiotypic antiserum prepared against the parent while others did not.

Peptide Analysis. To extend these serological findings, we did comparative ion-exchange chromatography of tryptic/chymotryptic peptides from parental and variant H chains. The variant H chains differed extensively from the parent γ2b H chain. Fig. 1a shows one peptide analysis in which the variant 45.6 M224 shared only 18 of the 34 parental peaks. The analysis also showed that the variant had many "new" peaks (arrows). To try to determine whether some of these latter peaks were specific for γ2a chains, we carried out two other peptide analyses. First, the H chains of a γ2a myeloma protein (LPC-1) were compared with the MPC-11 γ2b H chain (Fig. 1b). Nonidentical peaks (arrows) would include presumptive 2a specific or variable (V) region components. Then the γ2a variant H chain (M224) was compared with LPC-1 to see if any of these presumptive 2a specific or V region components were shared. Many were shared (see shaded peaks in Fig. 1c). Since the V regions of two nonrelated proteins such as LPC-1 and MPC-11 would be unlikely to account for all these shaded peaks, the conclusion is that the variant contains at least some γ2a specific peptides. However, until further structural analysis is done, one can only say that the variant M224 γ2a H chain has some peptides in common with another γ2a protein (LPC-1) that are not contained in a γ2b (MPC-11) protein.

Peptide analysis has also shown that two γ2a variants, 45.6 M224 and 45.6.2.4 ICR 16, are similar but not identical (Fig. 2). The light (L) chains of some variants have also been compared by peptide analysis with the parent: no differences have been seen.

Assembly Pattern. Variant cells differ from the parent in their assembly of H and L chains into immunoglobulin components. After the cells were incubated for 10 min with radioactive amino acids, the newly synthesized and assembled immunoglobulins were immunologically precipitated from

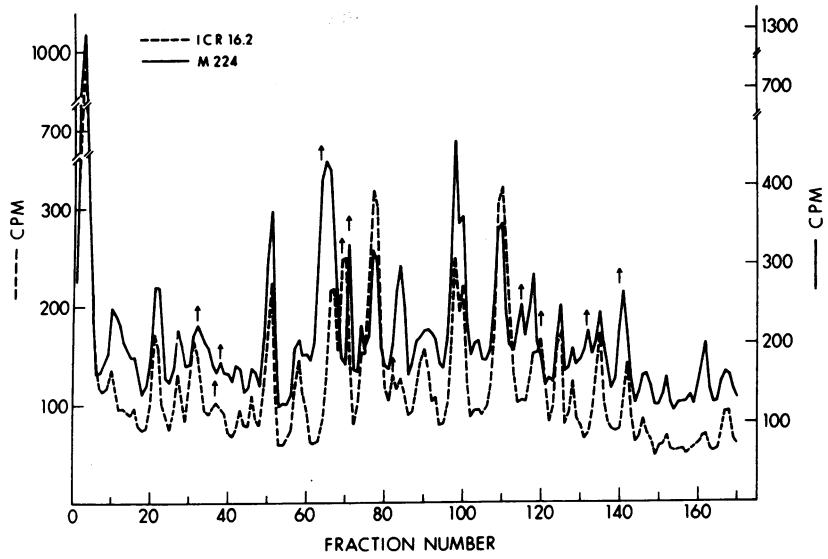

FIG. 2. Comparative peptide map of two variant H chains: ^{14}C-labeled H chains of 45.6 M224 (*solid line*) and ^{3}H-labeled H chains of 45.6.2.4 ICR 16 (*dotted line*). The details are presented in the legend to Fig. 1.

cytoplasmic lysates and analyzed on acrylamide gels containing sodium dodecyl sulfate (Fig. 3). In common with many IgG_{2a} cells, the variant had little HL (8). In addition, the variant assembled H_2L_2 more slowly than the parent.

DISCUSSION

In previous studies (1, 2, 4, 6, 9), we have shown that mutagenesis of an IgG_{2b}-producing mouse myeloma cell line frequently gives rise to clones that have undergone a change in immunoglobulin production. Many of these variants produce heavy chains that differ from the parental heavy chains in their primary sequence. The heavy chains produced by the particular set of variants described above have serological and chemical characteristics of a heavy chain constant region that is different from the one expressed in the parent cells. We can only speculate on the mechanism by which a clone committed to the synthesis of a γ2b H chain can give rise to a clone synthesizing a γ2a H chain. There are two general types of mechanisms: (*i*) changes outside the gene coding for the parental H chain that affect its expression and (*ii*) mutations within the structural gene itself. One example of the former that we cannot exclude is the turning off of one gene and the turning on of another. Another example of a nonmutational event is a translocation (10) of the MPC-11 V region gene from the γ2b constant region gene to the γ2a constant region gene. However, if a strict translocation (10) were taking place, one would expect all the γ2a variants to be identical; i.e., they would have the MPC-11 V region gene and a γ2a constant region gene. In fact, they differ from each other by peptide analysis, by assembly patterns, and by size, thus excluding this possibility.

Contrasted to these possibilities are mechanisms involving changes within the structural genes for the γ2b H chain. Indeed, the variants do appear to be true mutants, since they arise as a result of mutagenesis, are stable, and synthesize an altered H chain. One possible mutational mechanism is mitotic crossing-over, equal or unequal, or some other sort of recombination between related tandem genes. Spontaneously occurring "recombinant" proteins have been observed in

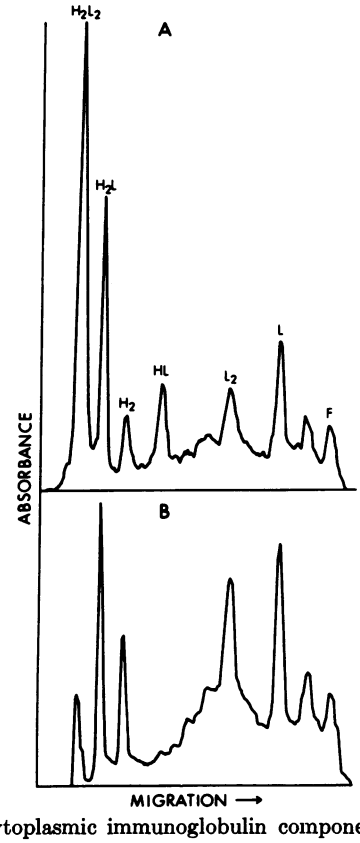

FIG. 3. Cytoplasmic immunoglobulin components of parent 45.6.3.1 (panel A) and variant M224 (panel B). Cells were incubated for 15 min with ^{14}C-labeled valine, threonine, and leucine, after which immunoglobulin was specifically precipitated from the cytoplasmic lysate. The precipitates were analyzed by electrophoresis on 5% acrylamide gels containing sodium dodecyl sulfate. Autoradiography was performed and the resulting films were analyzed by densitometry (Joyce Lobl densitometer). The figure shows the densitometric tracings. Immunoglobulin assembly components are noted. F is the constant region of the L chain (7).

mouse and human immunoglobulins (11–14). Another possible mechanism would be a deletion involving parts of two neighboring (γ2b and γ2a) genes with the loss of termination of the MPC-11 (γ2b) constant region gene and a readthrough (14) into the adjoining γ2a constant region gene. With this mechanism, one would infer that the γ2b and γ2a genes are adjacent and ordered. This possibility could be tested by examining the polarity of the interconversions of these and other subclasses whose genes are closely linked.

We continue to consider why our variants of all types (those that synthesize no H chains, those synthesizing small H chains, and those synthesizing H chains of a different subclass) occur at such a high frequency and if these variants arise by the same or different mechanisms. Does the generation of variants relate in any way to the commitment of lymphocytes to the synthesis of a single immunoglobulin and the concomitant presence of unexpressed homologous genes? Is there any relationship of the incidence of variants to the genetics of a multi-gene system, the organization of the immunoglobulin genes, or to the generation of antibody diversity? The isolation of additional variants and structural studies of "mutant" proteins may help in the resolution of these questions. Fortunately, variants synthesizing γ2a proteins of normal size secrete copious amounts of these proteins into the serum of tumor-bearing animals, thus facilitating their chemical characterization.

We thank Ms. Lucille Frank and Ms. Theresa Jasek for their expert technical assistance. This work was supported by funds from the National Institutes of Health (AI 5231, AI 10702), the National Science Foundation (GB 29560), and the American Cancer Society. B.K.B. was a fellow of the New York Heart Association. J.L.P. was supported by the French National Institutes of Health [Medical Research (INSERM)] and a National Institute of Health John E. Fogarty International Fellowship (1 FO 5TW-1862).

1. Coffino, P. & Scharff, M. D. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 219–223.
2. Birshtein, B. K., Preud'homme, J. L. & Scharff, M. D. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 3478–3482.
3. Laskov, R. & Scharff, M. D. (1970) *J. Exp. Med.* **131**, 515–541.
4. Preud'homme, J. L., Buxbaum, J. & Scharff, M. D. (1973) *Nature* **245**, 320–322.
5. Coffino, P., Baumal, R., Laskov, R. & Scharff, M. D. (1972) *J. Cell. Physiol.* **79**, 429–440.
6. Baumal, R., Birshtein, B. K., Coffino, P. & Scharff, M. D. (1973) *Science* **182**, 164–166.
7. Kuehl, W. M. & Scharff, M. D. (1974) *J. Mol. Biol.* **89**, 409–421.
8. Baumal, R., Potter, M. & Scharff, M. D. (1971) *J. Exp. Med.* **134**, 1316–1334.
9. Morrison, S. L., Baumal, R., Birshtein, B. K., Kuehl, W. M., Preud'homme, J. L., Frank, L., Jasek, T. & Scharff, M. D. (1974) in *Cellular Selection and Regulation in the Immune Response*, ed. Edelman, G. M. (Raven Press, New York), Vol. 29, pp. 233–244.
10. Gally, J. A. & Edelman, G. H. (1970) *Nature* **227**, 341–348.
11. Warner, N. L., Herzenberg, L. A. & Goldstein, G. (1966) *J. Exp. Med.* **123**, 707–721.
12. Natvig, J. B. & Kunkel, H. G. (1974) *J. Immunol.* **112**, 1277–1284.
13. Kunkel, H. G., Natvig, J. B. & Joslin, F. G. (1969) *Proc. Nat. Acad. Sci. USA* **62**, 144–149.
14. Natvig, J. B., Michaelsen, T. E., Gedde-Dahl, J., Jr. & Fischer, J. (1974) *Immunogenetics* **1**, 33–42.