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Isolation of myeloma variants with predefined variant surface immunoglobulin by cell sorting

(somatic cell variants/immunoglobulin genes/cell surface antigens/fluorescence-activated cell sorter)

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ABSTRACT **We describe a procedure for the isolation of somatic cell variants in which gene products are expressed on the cell surface that are not expressed in the wild type. Cloned cells of the myeloma line MPC 11, which expresses an IgG2b protein, were incubated with an antiserum specific for IgG1 and IgG2a. Cells reacting with this antiserum were stained with a fluorescent anti-antiserum and enriched in three cycles of sorting in the fluorescence-activated cell sorter and subsequent growth *in vitro*. From the enriched population two variants were isolated by cloning in soft agar. One of them expressed a variant immunoglobulin that typed serologically as an IgG2a but whose variable portion was idiotypically related to that of the MPC 11 wild-type protein.**

The differentiated state of higher cells is characterized by a specific pattern of gene activation and suppression. In the analysis of the rules by which differentiation proceeds, it will be valuable to have methods for the isolation of somatic variants whose phenotypes are characterized by expression of gene products that are not expressed in the wild type. In a random selection technique such variants will be hard to find in the overwhelming majority of other variants. In myeloma cells, for example, the vast majority of variants in immunoglobulin (Ig) production may represent nonsecretors (1, 2) or light chain-secretors (3, 4). Of more interest for the analysis of lymphocyte differentiation are variants in which new sets of genes involved in Ig production are turned on. We describe a procedure for the isolation of such cells. Myeloma cells are treated with fluorescent antibodies specific for Ig structures not expressed in the wild type, and positive cells, i.e., variants reacting with the antibodies, are sorted out with the fluorescence-activated cell sorter (FACS). The method allows the detection of infrequent variants since up to 10^8 cells can be sorted in one experiment. In quantitative terms this compares favorably with the agar-overlay technique (3, 4), which can, in principle, be used for the isolation of myeloma cell variants secreting a predefined variant Ig (5). In addition, the present technique is not limited to surface constituents that are secreted by the cell, but can be applied to cell surface structures in general.

MATERIALS AND METHODS

Animals. Mice of strains BALB/c and NMRI were obtained from Zentralinstitut für Versuchstierzucht, Hannover, Federal Republic of Germany. Rabbits and guinea pigs came from local breeders.

Myelomas and Myeloma Proteins. All myelomas were of BALB/c origin. The myeloma line MPC 11 (secreting an IgG2a with κ light chains) was a gift of S. Tonegawa, Basel, Switzerland. Sublines of the myeloma MOPC 21 (IgG1, κ), namely, X-63 and D1/20 (a nonsecretor), were given to us by C. Milstein, Cambridge, United Kingdom. All these lines had been adapted to growth in cell culture. We passaged them *in vivo* or cultured them in Dulbecco's modified Eagle's medium (Seromed, Munich, Federal Republic of Germany) containing 10% newborn calf serum (Flow Laboratories, Bonn, Federal Republic of Germany), 100,000 units of penicillin, 100 mg of streptomycin, and 3.7 g of $NaHCO_3$ per liter. The cultures were kept at 37° in standard plastic petri dishes (Greiner, Nürtingen, Federal Republic of Germany) in humidified atmosphere containing 10% CO_2. The myeloma lines HOPC 1 (IgG2a, λ^1), RPC 5 (IgG2a, κ), MOPC 195 (IgG2b, κ), and MOPC 31C (IgG1, κ) were obtained from Litton Bionetics, Kensington, MD, USA; myeloma line MOPC 70A (IgG1, κ) was from the Salk Institute, San Digeo, CA, USA. These lines had not been adapted to growth *in vitro*. For passage *in vivo* and preparation of large amounts of myeloma protein the myeloma cells were grown in the peritoneal cavity of BALB/c mice that had received an intraperitoneal injection of 0.5 ml of Pristane (Roth, Karlsruhe, Federal Republic of Germany) 3–60 days before the myeloma cells were injected (6). Ascites fluids and sera of tumor-bearing mice were screened for the presence of myeloma proteins by microzone electrophoresis (Beckman Instruments, Munich, Federal Republic of Germany). Myeloma proteins were purified by precipitation twice with 1.5 vol of 3 M ammonium sulfate followed by chromatography on DE 52 equilibrated with 0.01 M sodium phosphate (pH 8) with a gradient of 0.01–0.1 M sodium phosphate. The variant protein 1B6 (see *Results*) was purified by agarose block electrophoresis at 130 V for 24 hr in 300 ml of a 0.5% gel (18 × 24 × 0.7 cm) in barbiturate buffer (pH 8.6). After the block was sliced into 21 fractions, the protein was eluted by centrifuging the agarose at 36,000 × *g* followed by two washes with buffer. Purification was monitored by microzone electrophoresis. Proteins were concentrated by ultrafiltration in Amicon chambers (Amicon, N.V., Oosterhout, Netherlands) or collodion bags (Sartorius, Göttingen, Federal Republic of Germany). The concentration of purified immunoglobulins was calculated assuming that the A^{1cm}_{280nm} of a solution of 1 mg/ml is 1.4. Molecular weights were determined by sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis in 10% gels (7).

Antisera. Class- and idotype-specific antisera were raised in rabbits and guinea pigs and are listed in Table 1. The guinea pigs were rendered tolerant against immunoglobulins of irrelevant class and/or idiotype at the time of immunization (8, 9). They were immunized by injection of 100 μg of the immunogen in Freund's complete adjuvant (Difco Laboratories, Detroit, MI) into the footpads and bled 4 weeks after immunization. An antiserum with specificity for γ_1 chains was raised

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Abbreviations: FACS, fluorescence-activated cell sorter; Ig, immunoglobulin; $NaDodSO_4$, sodium dodecyl sulfate.

Table 1. Class- and idiotype-specific antisera

Serum donor	Immunogen*	Tolerogen	Absorption	Specificity
Guinea pig	X-63 ($\gamma1, \kappa$)	MPC 11	—	$\gamma1, \gamma2a^\dagger$
Rabbit	MOPC 70A ($\gamma1, \kappa$) Fc	—	HOPC 1 MPC 11	$\gamma1$
Guinea pig	HOPC 1 ($\gamma2a, \lambda^1$)	NMRI Ig	MPC 11	$\gamma2a$
Guinea pig	MOPC 195 ($\gamma2b, \kappa$)	MOPC 70A HOPC 1	RPC 5 ($\gamma2a, \kappa$)	$\gamma2b$
Guinea pig	MPC 11 ($\gamma2b, \kappa$)	NMRI Ig	MOPC 195	MPC 11 idiotype

* Purified immunoglobulins in all cases. Chain composition in parentheses.

† This serum was used for the selection of MPC 11 variants. In addition to anti-idiotypic antibodies to the MOPC 21 protein, it contained anti-$\gamma1$ antibodies, 15% of which crossreacted with $\gamma2a$ in radioactive binding assays. In indirect fluorescence, the serum stained X-63 cells brightly but was negative on MPC 11 and D1/20 cells.

in a rabbit by injecting 150 μg of the Fc fragment of MOPC 70A (prepared as described in ref. 10) in Freund's complete adjuvant into the footpads. On days 28 and 30 after priming, 150 μg of the same protein were injected intravenously and the animal was bled on day 40. The specificity of the antisera was determined in radioactive binding and binding inhibition assays (see below, also Fig. 6). If necessary, the sera were absorbed on insolubilized myeloma proteins (11). Fluoresceinated rabbit anti-mouse Ig and anti-guinea pig Ig sera were obtained from Behringwerke, Marburg/Lahn, Federal Republic of Germany.

Serology and Immunofluorescence. Serological radioactive binding and binding inhibition assays with ^{125}I-labeled protein antigens were performed as described (12). For immunofluorescence studies the cells were incubated with the appropriate antisera (always diluted 1:10) for 30 min at a density of 10^7 cells per ml in Dulbecco's modified Eagle's medium with the $NaHCO_3$ replaced by an equiosmolar amount of NaCl. Total surface Ig was detected in direct immunofluorescence at 25° with fluoresceinated rabbit anti-mouse Ig serum. The class of surface Ig was determined in indirect immunofluorescence. The cells were incubated with the relevant guinea pig antiserum at 25°, then washed and incubated with fluoresceinated rabbit anti-guinea pig Ig serum on ice. The cells were washed again and analyzed in a fluorescence microscope (Orthoplan, Leitz, Wetzlar, Federal Republic of Germany) or in the FACS (FACS-1, Becton, Dickinson, Mountain View, CA), which is described in detail elsewhere (13).

Cell Sorting. The FACS-1 was used at a rate of 1000–3000 cells and 36,000 droplets per sec. Dead cells were excluded from the analysis by light scatter (14). For each fluorescent cell to be separated, three droplets were deflected into a petri dish (6 cm diameter) (Greiner und Söhne, Nürtingen, Federal Republic of Germany) that contained 2–5 ml of culture medium supplemented with gentamycin (200 mg/liter) and neomycin (50 mg/liter). Between 10^4 and 10^5 cells were sorted into each petri dish.

Cloning and Characterization of Variants. Myeloma cells were cloned in soft agar as described (15). Clones were transferred from the agar to 24-well Costar plates (Costar, Inc., Cambridge, MA) and grown to more than 5×10^5 cells. [^{14}C]Lysine-labeled secretion products were obtained as described (1) and analyzed by isoelectric focusing at pH 5–9 (16). The plates were dried and exposed to Agfa Curix Film (Agfa Gevaert, Leverkusen, Federal Republic of Germany) for 2–4 weeks. For karyotype analysis, cell growth was stimulated by addition of fresh medium to the cultures 10 hr before preparation. Cells were then incubated for 12 min in 0.563% (wt/vol) KCl. They were fixed in methanol/acetic acid (3:1 vol/vol) twice for 10 min and once overnight. Metaphase plates were spread by suspending the cells in fixative and letting the cell suspension fall dropwise on cold slides (4°) covered with water. Slides were dried for 1 week and stained for trypsin–Giemsa bands (17).

RESULTS

Enrichment of Variants. Cells of the MPC 11 line were cloned in soft agar, and a few clones were picked and expanded in cell culture over a period of 3 mo. One of the clones was chosen for the isolation of variants that would stain with an antiserum specific for $\gamma1$ and $\gamma2a$ heavy chains (cf. Table 1).

Cells reacting with the selecting serum were enriched by three subsequent cycles of sorting and growth of the sorted cells in culture (Table 2). The data in Table 2 also establish that variants were indeed enriched during the three cycles. As judged by fluorescence microscopy, the frequency of cells staining with the selecting serum was 10^{-4} before sorting and increased 10- to 20-fold after the first and the second round of selection. For unknown reasons, the third round of selection was only marginally effective.

The success of the selection procedure was also evident from fluorescence analysis in the FACS. The fluorescence distribution in the thrice-sorted cell population was shifted towards higher fluorescence intensities as compared to the fluorescence distribution in the wild-type cells (Fig. 1). The thrice-sorted cells were cloned in soft agar, and clones were picked and expanded in cell culture. The secretion products of 59 clones were char-

Table 2. Protocol of MPC 11 variant enrichment

Sorting	% positive cells*	% cells deflected	Total cells sorted	Growth,† days
1st	0.010 (1.7×10^5); 0.012 (1.7×10^5)	1.0	6.3×10^6	30
2nd	0.25 (800); 0.1 (1000)	0.5	2.8×10^7	40
3rd	5.7 (300); 4.2 (380)	2.0	7.2×10^6	—
After 3rd	8.4 (500); 9.3 (300)	—	—	—

* Percent cells in the cell population to be sorted staining with an anti-$\gamma2a$ serum in indirect fluorescence as determined by fluorescence microscopy. Two independent counts. Number of total cells counted in parantheses. An anti-$\gamma2a$ serum was used instead of anti-$\gamma1$ since variants stained equally well or better with the former serum.

† Until subsequent sorting.

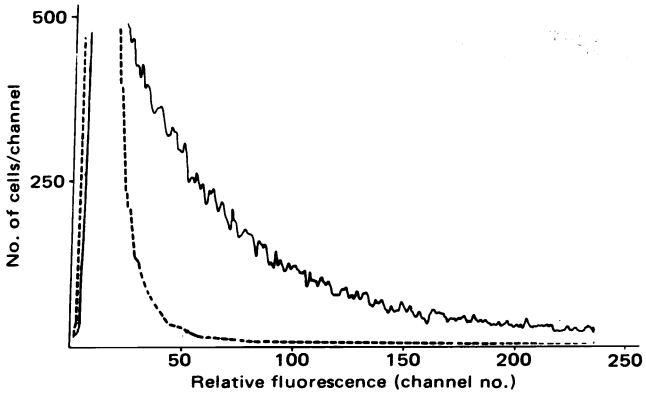

FIG. 1. Fluorescence profiles of MPC 11 wild-type cells (broken line) and MPC 11 cells after the third round of selection (solid line). The cells were stained with the selecting antiserum. Each distribution is based on analysis of 10^4 living cells.

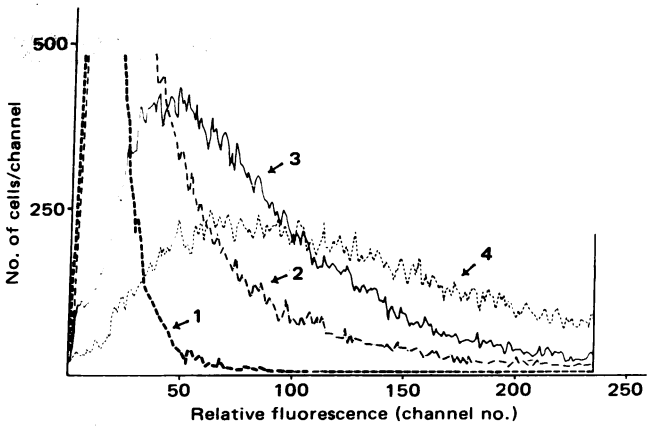

FIG. 3. Fluorescence profiles of cells from MPC 11 (*1*), X-63 (*2*), 1B6 (*3*), and 1A4 (*4*) stained with the selecting antiserum. Each distribution is based on analysis of 10^4 living cells.

acterized by [^{14}C]lysine labeling and subsequent analysis by isoelectric focusing (Fig. 2). Eight clones secreted a product that was indistinguishable from the MPC 11 wild-type protein. Five clones secreted a protein with a different migration pattern, but the pattern was identical for all five clones. For the remaining 46 clones, a secreted protein could not be detected. Two of the variant clones, namely, clone 1B6, which secreted a new protein, and the nonsecretor line 1A4, were selected for further investigation and grown to mass cultures.

The fluorescence intensity distributions of cells of the two variant clones stained with the selecting antiserum are depicted in Fig. 3. Both variant cell populations exhibit higher fluorescence than MPC 11 wild-type cells and lower fluorescence than the X-63 line. 1B6 cells stain more brightly than 1A4 cells. The discrepancy between the high frequency of variants among the cell clones and the low frequency of "positive" cells in the cell population from which the clones were derived (Table 2) can thus be explained by assuming that only 1B6 cells were typed "positive" in the fluorescence microscope. The various cell populations were also stained with the selecting antiserum after it had been absorbed on MOPC 21 protein (fluorescence distributions not shown). This absorption led to a decrease of fluorescent staining of 1B6 and X-63 cells to background levels, but the staining of 1A4 cells was almost unchanged. This indicates that 1B6 cells were selected by the FACS because they carry non-wild-type Ig determinants on the surface and that 1A4 cells were enriched on the basis of a staining reaction, the specificity of which differs from the previous one and is unknown.

In order to confirm that the two variant cell lines are indeed derived from the MPC 11 line, we performed karyotype analysis of the three cell lines. Fig. 4 demonstrates that the 1B6 line and MPC 11 share at least two marker chromosomes, which may have arisen from chromosomal rearrangement. The same two marker chromosomes were also present in 1A4 cells (not shown).

Isolation and Characterization of 1B6 Variant Immunoglobulin. Cells of clones 1B6 and 1A4 were injected into BALB/c mice and grown as ascites tumors. Microzone electrophoresis of the ascitic fluids of the tumor-bearing animals showed a strong protein band in the gamma globulin region in the 1B6 cell line. This band was in a different position from that of the wild-type MPC 11 protein. In accord with the biosynthetic labeling experiments, no such band was detectable in the ascites fluids of animals carrying the 1A4 line.

The 1B6 protein was purified from 2.5 ml of ascites fluid and serum from tumor-bearing animals by agarose block electrophoresis. A single major peak appeared in the gamma globulin region, from which 5–10 mg of protein was recovered. The

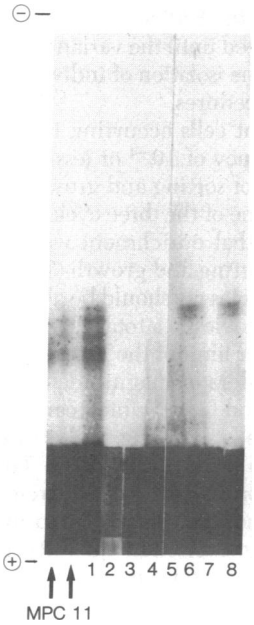

FIG. 2. Isoelectric focusing of the secretion products of some MPC 11-derived clones. Gradient, pH 5–9. Cells from 10 clones were biosynthetically labeled with [^{14}C]lysine and their secreted products were detected on the plates by autoradiography. The pattern of clone 1 is that of MPC 11 wild type; two clones (6 and 8) exhibit the pattern of the variant myeloma protein.

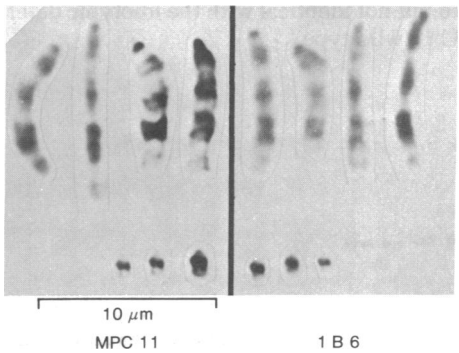

FIG. 4. Identification of two marker chromosomes, M1 (*Upper*) and M2 (*Lower*), of MPC 11 in 1B6 cells. In each case chromosomes found in three or four metaphase plates are shown. M1 is longer than the corresponding normal chromosome 1; M2 is smaller than the normal chromosome 19. The Giemsa banding pattern is different from that of the corresponding normal chromosomes in both cases.

peak fraction contained 3 mg of protein. This sample was further analyzed. Densitometric analysis of microzone electrophoretograms revealed that approximately 90% of the purified protein migrated into the position of the 1B6 band that had been found in the ascitic fluid of animals carrying the corresponding tumor. However, the purity of the preparation is probably considerably better than 90%. This is apparent from the serological analysis, which reveals a contamination with only 2.6% IgG1 and 1% IgG2b (see Fig. 6).

The typical patterns of immunoglobulin heavy and light chains appeared upon $NaDodSO_4$/polyacrylamide gel electrophoresis of the reduced and alkylated 1B6 protein. Within the limits of our technique, the heavy and light chains of the variant protein have the same molecular size as the corresponding polypeptide chains of the wild-type MPC 11 protein (Fig. 5). The immunoglobulin class and the idiotypic specificity of the 1B6 protein were determined in serological radioactive binding inhibition assays (Fig. 6). The 1B6 protein carries the full set of γ2a-specific determinants as recognized by our anti-γ2a antiserum (Fig. 6*a*) since it inhibits the binding of an IgG2a myeloma (RPC 5) to the antiserum completely and equally well as RPC 5 itself and another IgG2a protein (HOPC 1). The specificity of inhibition is controlled by two proteins not belonging to the IgG2a class, namely, MPC 11 (IgG2b) and MOPC 70A (IgG1). The slight inhibition obtained by high concentrations of the latter protein is presumably due to contamination with serum IgG2a. The inhibition patterns depicted in Fig. 6 *b–d* demonstrate that the 1B6 protein lacks determinants that are recognized by our anti-γ1 and anti-γ2b antisera on the corresponding heavy chains. [The inhibition pattern in Fig. 6*b* shows that the selecting antiserum is largely specific for γ1 determinants against which it was raised, but that 10–20% of the antibodies crossreact with γ2a (RPC 5 and 1B6). This crossreaction has been verified in binding experiments not documented here.] Again, the slight inhibition seen at high inhibitor concentrations points to a low degree of contamination of the sample by serum Igs and actually allows us to calculate the extent of this contamination.

The 1B6 protein thus consistently types as an IgG2a protein, in contrast to the MPC 11 wild type, which belongs to the IgG2b class (Fig. 6; compare *c* with *a*, *b*, and *d*). With respect to idiotypic determinants, however, the two proteins are interrelated. The binding of MPC 11 to an anti-MPC 11 idiotype is specifically and almost completely inhibited by the 1B6 proteins (Fig. 6*e*). The inhibition curve obtained with 1B6 as inhibitor is flatter than the homologous inhibition curve, suggesting that the variant protein carries idiotypic determinants that are similar to but not identical with the idiotypic determinants of the MPC 11 wild type.

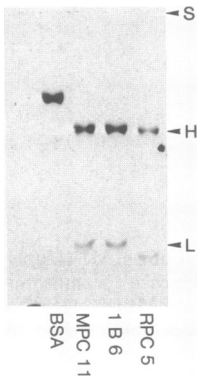

FIG. 5. Analysis of MPC 11 and 1B6 heavy and light chains by $NaDodSO_4$ polyacrylamide gel electrophoresis. The proteins were reduced by addition of 5% 2-mercaptoethanol to the sample buffer. Positions of heavy (H) and light (L) chains are indicated. RPC 5 and bovine serum albumin (BSA) were controls. S, start.

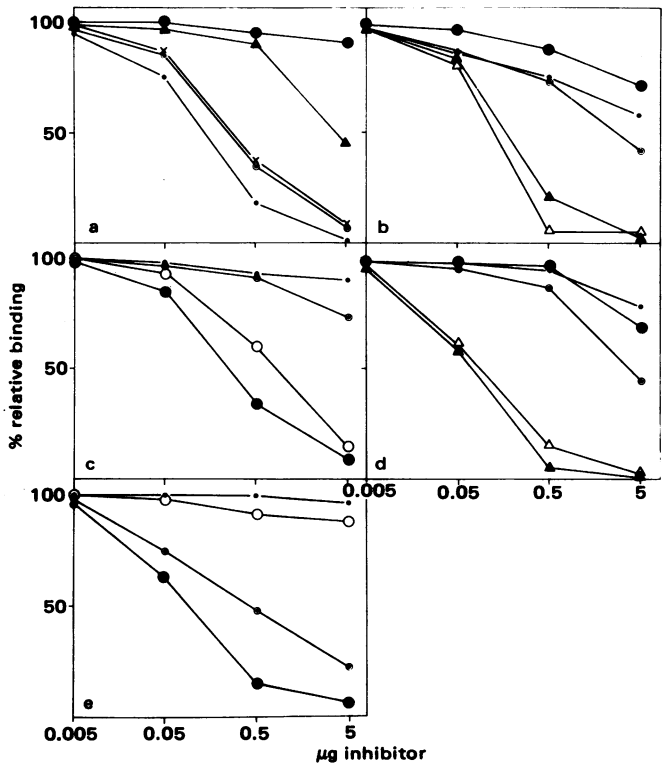

FIG. 6. Serological analysis of the 1B6 protein and of MPC 11 wild type in binding inhibition assays. 100% relative binding was binding without inhibitor. Binding of (*a*) RPC 5 to anti-γ2a, (*b*) MOPC 70A to the selecting serum, (*c*) MPC 11 to anti-γ2b, (*d*) MOPC 70A to anti-γ1, and (*e*) MPC 11 to anti-MPC 11 idiotype was inhibited by (△) MOPC 21 (IgG1, κ), (▲) MOPC 70A (IgG1, κ), (•) RPC 5 (IgG2a, κ), (×) HOPC 1 (IgG2a, κ), (⊙) 1B6, (●) MPC 11 (IgG2b, κ), and (○) MOPC 195 (IgG2b, κ).

DISCUSSION

The method described here permits the isolation of somatic cell variants that express on the cell surface a known gene product that is not expressed in the wild type. The variants are detected by specific antibodies that either themselves carry a fluorescent tag or are stained by fluorescent anti-Ig antibodies. The fluorescent cells are then separated out in the FACS and grown in tissue culture. This procedure is repeated until the variant cells are enriched to an extent that allows the isolation of individual variants by conventional cloning procedures.

In the present experiments, variant cells occurring in the wild-type cell population at a frequency of 10^{-4} or less were enriched by three subsequent cycles of sorting and growth to a frequency of approximately 10^{-1}. One of the three cycles was inefficient for unknown reasons, so that enrichment was essentially achieved in two rounds of sorting and growth (Table 2). Thus, with a limited number of cycles we should be able to isolate variants present in only a few copies in a total cell input of 10^8 cells, which represents the upper limit of the FACS. The present method thus appears 2–3 orders of magnitude more efficient than the agar-overlay method (3, 4), which can also be used for isolation of myeloma cells secreting predefined variant Ig (5). The two methods also differ in principle. The agar-overlay method analyzes gene products that are secreted by the cells. Cell sorting as described here can be applied to any cell surface constituent expressed in sufficient quantity to permit fluorescent labeling. This may (as for our variant 1B6) or may not include products that the cells secrete.

The most important limitation of the present technique is due to the fact that even genetically homogeneous cell populations exhibit a rather broad distribution of fluorescence intensities upon fluorescent labeling of surface antigens. Since the level of fluorescence in a variant cell is known only after its isolation, it is always possible that the brightest wild-type cells will be as bright as or even brighter than certain variants. We arbitrarily decided to select in each cycle about 1% of the total population. By choosing this high percentage we reduce the chance of missing faintly staining variant cells, but make it also obligatory to go through several cycles of enrichment and growth in order to enrich sufficiently for variants occuring at low frequency in the wild-type cell population. Variant cells with a longer cell cycle than that of the wild type will often be lost or not sufficiently enriched during selection.

We have isolated two variants of MPC 11 with entirely different properties. The isolation of variant 1A4 demonstrates another limitation of procedure, namely, a limitation in terms of specificity. It is likely that this variant cell does not carry surface Ig of the $\gamma 1$ or $\gamma 2a$ class for which we selected since it stains with various fluorescent anti-Ig reagents in an undefined way. It still appears that this undefined staining reaction reflects a variation of Ig synthesis since the 1A4 cells do not detectably secrete the MPC 11 protein or any other immunoglobulin.

The 1B6 cell represents precisely the kind of variant we selected for. It carries on its surface and secretes an Ig that differs from the wild-type MPC 11 protein in that it types serologically as an IgG2a instead of an IgG2b protein. Within the limits of the range of serological specificities recognized by our antisera, the 1B6 protein has lost all $\gamma 2b$ and acquired all $\gamma 2a$ determinants. The straightforward interpretation of this result implies that the cell has switched from the expression of the gene encoding the constant part of $\gamma 2b$ heavy chains ($C\gamma 2b$) to that of the $C\gamma 2a$ gene. Scharff and collaborators (18) have isolated similar variants of the MPC 11 myeloma by screening clones in soft agar after mutagenesis. The variable portion of the 1B6 protein is similar but not identical to that of the MPC 11 protein in terms of idiotypic specificity. This, together with the karyotypic analysis, documents that the 1B6 line and its product are indeed derived from the MPC 11 wild type. However, the idiotypic analysis does not allow us to define the variable portions of the 1B6 protein in any detail. In experiments not documented here we have attempted to compare selectively the idiotypic determinants of the heavy chains of MPC 11 and 1B6. For this purpose recombinant molecules consisting of MPC 11 heavy chains and light chains from MOPC 70A were prepared. The results of these experiments indicate that 1B6 heavy chains have lost some but not all of the idiotypic specificity of MPC 11 heavy chains. Thus, at least part of the variable region of the MPC 11 heavy chain is still present in the 1B6 protein. As to light chain expression, MPC 11 and 1B6 synthesize κ chains of similar size and there is no obvious reason why the chains should not be identical, but this point is not established.

The exact characterization of the genetic event that has generated the 1B6 phenotype requires structural analysis of the 1B6 protein. The evidence suggests that the 1B6 line expresses the $C\gamma 2a$ instead of the $C\gamma 2b$ gene, but that it has retained the expression of at least part of the V gene which encodes the variable region of the MPC 11 heavy chain.

B lymphocytes are thought to express a single heavy chain variable region in conjunction with several constant regions during differentiation into mature plasma cells (19). The present experimental approach, if applied systematically, may establish whether the cell can randomly switch from the expression of one C gene to that of another or whether C gene expression follows a predetermined sequence. It may be significant that, similar to the results of Scharff's group, we found an IgG2a but not an IgG1 variant of MPC 11 although we selected for both types of variants.

It is of interest to ask whether one can also isolate variants of V gene expression. Variants of this type may arise from the wild type by mutation in the V gene itself or, alternatively, by activation of another V gene. A second approach aims at the isolation of variants in expression of products encoded by the major histocompatibility complex.

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1. Cotton, R. G. H., Secher, D. S. & Milstein, C. (1973) *Eur. J. Immunol.* **3**, 135–140.
2. Secher, D. S., Milstein, C. & Adetugbo, K. (1977) *Immunol. Rev.* **36**, 51–72.
3. Coffino, P. & Scharff, M. D. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 219–223.
4. 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, New York), pp. 233–244.
5. Koskimies, S. & Birshtein, B. K. (1976) *Nature (London)* **264**, 480–482.
6. Cancro, M. & Potter, M. (1976) *J. Exp. Med.* **144**, 1554–1567.
7. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
8. Henney, C. S. & Ishisaka, K. (1969) *J. Immunol.* **103**, 56–61.
9. Eichmann, K. & Kindt, T. J. (1971) *J. Exp. Med.* **134**, 532–552.
10. Askonas, B. A. & Fahey, J. L. (1961) *Nature (London)* **190**, 980–983.
11. Avrameas, S. & Ternynck, Th. (1969) *Immunochemistry* **6**, 53–66.
12. Jack, R. S., Imanishi-Kari, T. & Rajewsky, K. (1977) *Eur. J. Immunol.* **8**, 559–565.
13. Bonner, W. A., Hulett, H. R., Sweet, R. G. & Herzenberg, L. A. (1972) *Rev. Sci. Instrum.* **43**, 404–409.
14. Loken, M. R. & Herzenberg, L. A. (1975) *Ann. N. Y. Acad. Sci.* **254**, 163–171.
15. Paul, J. (1970) *Cell and Tissue Culture* (E. & S. Livingstone, London), 4th Ed., pp. 236–237.
16. Awdeh, Z. L., Williamson, A. R. & Askonas, B. A. (1968) *Nature (London)* **219**, 66–67.
17. Seabright, M. (1972) *Chromosoma* **36**, 204–210.
18. Preud'homme, J.-L., Birshtein, B. K. & Scharff, M. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1427–1430.
19. Lawton, A. R., Kearney, J. F. & Cooper, M. D. (1978) in *Biological Regulation and Control: A Comprehensive Treatise*, ed. Goldberger, R. F. (Plenum, New York), Vol. 2, in press.