Monoclonal Antibody Analysis of Complex Biological Systems

COMBINATION OF CELL HYBRIDIZATION AND IMMUNOADSORBENTS IN A NOVEL CASCADE PROCEDURE AND ITS APPLICATION TO THE MACROPHAGE CELL SURFACE*

To simplify the construction of monoclonal antibody (MAb) libraries to complex biological systems, the use of a cascade procedure has been investigated. The procedure continually restricts MAb production to those antigens previously so unidentified. Murine macrophage membranes were detergent-solubilized and purified by *Lentil, culinaris* lectin affinity chromatography. Prior to immunization of rats, the previously recognized heat-stable antigen and common leukocyte antigen were removed with MAb immunoadsorbents. Analysis of serum antibody concentrations to common leukocyte antigen, heat-stable antigen, and Mac-1 (a previously recognized antigen which served as a positive control for nondepleted antigens) demonstrated the efficacy of the procedure. The ratios of anti-Mac-1 to anti-common leukocyte antigen and anti-Mac-1 to anti-heat-stable antigen antibodies were over 100-fold higher when purified, MAb immunoadsorbent-depleted material rather than whole peritoneal exudate cells were used for immunization. P3-NS1/1-Ag4-1 γ-chain nonsecretor myeloma cells were fused with spleen cells from rats immunized to immunodepleted antigen and hybrid culture supernatants were screened for immunoprecipitation of 125I (lactoperoxidase)-labeled material. The common leukocyte antigen was among the most immunodominant antigens on whole peritoneal exudate cells. However, immunization with immunoadsorbent-depleted antigen did not elicit any hybrid cultures precipitating the 200,000 M, common leukocyte antigen. Eighteen cultures immunoprecipitated four different types of macrophage cell surface antigens. Clones identifying previously undescribed macrophage surface polypeptides of 32,000 M, (Mac-2) and 110,000 M, (Mac-3) have been isolated.

The myeloma-hybrid technique of Kohler and Milstein (1975) has recently given great impetus to the analysis of complex biological systems. For example, whole cells of one species such as the mouse may be injected into another species such as the rat (Springer et al., 1978), or vice versa (Williams et al., 1977). The resultant multispecific response to a large array of different cell surface molecules may then be resolved by cloning into a set of hybrid lines each secreting a monoclonal antibody recognizing a single antigenic determinant on a single cell surface molecule. The technique is so powerful that it frequently leads to the identification and study of previously unknown surface structures. One such antigen described by this laboratory is Mac-1, a granulocyte- and monocyte-specific antigen containing 105,000 and 190,000 M subunits which is particularly richly expressed on macrophages (Springer et al., 1979).

One disadvantage of this approach is that a vast array of cell surface antigens elicit responses, while the experimenter may be interested in only one or a few of these. This necessitates laborious and often complex screening of hundreds, thousands, or even more hybrid cultures. Furthermore, clones recognizing certain (immunodominant) antigens are found with much higher frequencies than others. Thus, screening procedures which distinguish between uninteresting clones occurring at high frequency and interesting clones occurring at low frequency are often required. Of ten previously described rat anti-mouse clones, five recognized a heat-stable antigen while two reacted with a 200,000 M, common leukocyte antigen (Springer et al., 1978). In three subsequent hybridization experiments, these clones have also outnumbered others, and thus appear to be immunodominant.

If the clonal response to previously identified antigens could be eliminated, 1) screening procedures could be greatly simplified, 2) the frequency of clones responding to nonimmunodominant antigens should be increased (Pross and Eidinger, 1974), and 3) novel cell surface antigens could be identified in an efficient and orderly manner. Therefore, in this report, a cascade procedure for accomplishing this has been explored in connection with the identification of further macrophage-specific antigens. Peritoneal exudate cell membranes were detergent-solubilized, and the previously identified common leukocyte antigen and heat-stable antigen which are shared with peritoneal exudate cells and lymphocytes were then removed with MAb immunoadsorbents before immunization for the hybridization experiment. Removal of the antigens was confirmed by radioimmunoassay and by the serological response to immunization. Serum antibodies to specific antigens were also measured to compare the efficacy of this procedure to immunization with either whole cells or MAb-coated cells. Two previously unknown macrophage-specific antigens of 32,000 and 110,000 M, have been identified in this study. The procedure can be extended by arranging further immunoadsorbent depletions and cell fusions in a cascade series and is readily applicable to the monoclonal antibody analysis of many other multicomponent biological complexes.

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2 The abbreviations used are: Mab, monoclonal antibody; BSA, bovine serum albumin; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; i.v., intravenous; Ns, P3-NS1/1-Ag4-1 γ-chain nonsecretor myeloma; PAGT, polyacrylamide gel electrophoresis; PBS, 0.14 M NaCl, 0.01 M NaPO4, pH 7.0; FMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate.
MATERIALS AND METHODS

Cell Lines—M1/69, M1/93, M1/89.18, and M1/70 have been deposited with and may be obtained from the Cell Distribution Center, Sachs Institute, P. O. Box 890, San Diego, Ca. Their properties are described in Table 1. The NS1 myeloma line was obtained from the same center. All hybrid lines were subcloned at least once. Clones are designated numerically, i.e. M1/70.15 and M1/70.15.1 are a clone and a subclone, respectively, of the M1/70 culture, but such designations have been omitted in most cases for brevity. Spent culture supernatants containing the antibodies (50-200 pg/ml) were obtained after sodium deoxycholate (5%) in 10% BSA-PBS were incubated with 5 pl of antigen for 45 min, washed, and transferred to tubes for assay. "Antigen titer" is defined as the inverse of the dilution of antigen which showed a plateau break at about 1:100 dilution, below which the amount of bound second antibody became approximately proportional to the concentration of MAb. Therefore, MAb were used at 1:100 in the inhibition assay. Serial 3-fold dilutions of antigen (10 pl, maximum of 0.5% sodium deoxycholate) were added, plates shaken at 4°C for 45 min, cells were washed, and transferred to tubes for gamma counting. "Antigen titer" is defined as the inverse of the dilution of antigen, in this assay volume after antibody addition, giving 50% inhibition of (125I)anti-IgG binding.

Purification of Macrophage Membranes—Peritoneal exudate cells from 25 C57BL/6 mice (Jackson Laboratory, Bar Harbor, Me.) were collected 3 days after injection of 1.5 ml of Brewer's thioglycollate medium (Difco). Cells (8 x 10^7 total, 84% macrophages, 2.5% neutrophils, 7.5% eosinophils, 4.5% lymphocytes, and 1.5% red blood cells by Wright's stain) were washed and suspended in 20 ml of PBS containing 0.5 M sodium chloride, pH 7.8 (30°C), to a final concentration of 5 x 10^6 cells/ml. One ml of the cell suspension was added to 0.5 ml of peritoneal exudate cells for M1/69 heat-stable antigen, and M1/70 Mac-1 antigen was obtained by bacterial lysis followed by washing. Animals were bled on day 28.

Other methods were as previously published (Springer et al., 1979).

RESULTS

Purification and Assay of Antigens—Macrophage surface glycoproteins were purified as described under "Materials and Methods" according to the scheme of Fig. 1. Purification and/or deletion of the M1/93 common leucocyte antigen, the M1/69 heat-stable antigen, and M1/70 Mac-1 antigen was monitored by inhibition of the indirect binding assay. (For brevity, these antigens will be named according to their defining MAb. See Table 1) Inhibition curves obtained with antigens at different stages of purification were parallel, and showed excellent dose-response characteristics (Fig. 2), suggesting no change in antigen avidity during purification. A glycoprotein-enriched fraction was obtained by L. culinaris lectin affinity chromatography (Fig. 3). The majority of the M1/93 antigen and the M1/70 antigen material was bound to the lectin column; the remainder was retarded relative to the protein peak. After elution with methyl mannoside, the M1/93 and M1/70 glycoproteins were obtained in 75 and 81% yield, respectively, while only 5% of the total protein was bound and eluted (Table III). This suggests that other macrophage surface glycoproteins were similarly purified (also see below). About two-thirds of the M1/69 putative glycolipid heat-stable antigen was unretarded by the lectin column, while one-third was bound and eluted (Fig. 3). M1/69 antigen is expressed in very low amounts on macrophages (Springer et al., 1979), and it is likely that much of the M1/69 antigen in these preparations was derived from small percentages of granulocytes, lymphocytes, and red blood cells in the peritoneal exudate cells (see "Materials and Methods").

Immunoadsorbent Removal of M1/93 and M1/69 Antigens—In order to increase the likelihood of obtaining macrophage-specific MAb, it was desired to remove antigens shared with other types of cells. Therefore, the widely distributed 200,000 M1/93 antigen and the putative glycolipid M1/69 antigen were removed by immunoadsorbent procedures. The lectin-purified material was first passed through an M1/69 immunoadsorbent column. This resulted in complete removal of M1/69 antigen but had no effect on M1/93 or M1/70 antigen (Table II). The M1/93 antigen was removed using M1/89.23 MAb-Sepharose. The confirmed the previous suggestion (Springer et al., 1978) that M1/93 and M1/89.23 MAb recognize determinants on the same antigen. Passes through two M1/89.23 columns were required to completely remove M1/93 antigen (Table II), presumably because the capacity of the first column was exceeded. The specificity of M1/69 and M1/93 antigen removal was demonstrated by comparison to M1/70 antigen, which was only slightly affected by these procedures (Table II).

Serological Studies of Rats Immune to Immunoad-
FIG. 1. Arrangement of cell hybridization experiments and removal of previously recognized antigens using MAb-immunoadsorbents in a cascade. MAb are obtained to different sets of antigens in each successive hybridization experiment, and the immunogenic stimulus is continually narrowed to those antigens which are yet to be recognized using MAb. Portions of the scheme are separated by dotted lines which correspond to previously described work utilizing whole cell immunization (Springer et al., 1978).

TABLE I

<table>
<thead>
<tr>
<th>Clones</th>
<th>Cellular recognition</th>
<th>Antigen</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1/69*</td>
<td>Mouse red blood cells, granulocytes, monocytes, B lymphocytes, T lymphocytes, but not peripheral T lymphocytes</td>
<td>Heat-stable, no iodinated or [35S]methionine-labeled component, putative glycolipid</td>
<td>Heat-stable antigen</td>
</tr>
<tr>
<td>M1/9.3*</td>
<td>Leukocytes</td>
<td>~200,000 M,*</td>
<td>Common leukocyte antigen, T200*</td>
</tr>
<tr>
<td>M1/70</td>
<td>Granulocytes and mononuclear phagocytes</td>
<td>190,000 and 105,000 M,</td>
<td>Mac-1</td>
</tr>
<tr>
<td>M3/31, M3/38, M3/84</td>
<td>Mononuclear phagocytes</td>
<td>32,000 M, 110,000 M,</td>
<td>Mac-2, Mac-3</td>
</tr>
</tbody>
</table>

* Stable at 120 °C for 15 min.
* The molecular weight of this antigen depends on the source from which it is isolated: 230,000 M, from B lymphocytes or 206,000 and 180,000 M, from T lymphocytes (Springer, 1980).

To determine whether immunoadsorbent purification of macrophage antigens was advantageous, the responses of animals immunized to purified antigen, whole cells, or antibody-coated cells presently described work, and hypothetical extension of the procedure into the future. MAb, = M1/69; A = heat-stable antigen; MAb, = M1/89.23; B = common leukocyte antigen. MAb M1/70 was generated in the first hybridization experiment, but its corresponding antigen was not removed prior to the second hybridization. This allowed M1/70 antigen to serve as a positive control for elicitation of macrophage-specific antibodies.

FIG. 2. Inhibition of the indirect binding assay for M1/70 by sodium deoxycholate-solubilized membrane fractions. Serial dilutions of fractions (10 μl) were incubated with 5 μl of 1/100 M1/70 culture supernatant in 30% BSA-PBS (5 μl) and tested in the indirect 125I-anti-rat IgG-binding assay as described under "Materials and Methods."
Fig. 3. *L. culinaris* lectin affinity chromatography of sodium deoxycholate-solubilized macrophage membranes. The 100,000 × g supernatant from sodium deoxycholate-solubilized peritoneal exudate cell membranes was applied to an *L. culinaris* lectin-Sepharose CL-4B column and washed and eluted as described under "Materials and Methods." Indicated fractions (7-9) were pooled and concentrated for immunoadsorbent chromatography.

TABLE II

<table>
<thead>
<tr>
<th>Purification step</th>
<th>M1/69 antigen</th>
<th>M1/9.3 antigen</th>
<th>M1/70 antigen</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Units* Yield</td>
<td>Units* Yield</td>
<td>Units* Yield</td>
<td>A\textsubscript{50} units</td>
</tr>
<tr>
<td>1. Detergent-soluble supernatant</td>
<td>53 (100)</td>
<td>40 (100)</td>
<td>234 (100)</td>
<td>34.8</td>
</tr>
<tr>
<td>2a. Lectin flow-through</td>
<td>48 (91)</td>
<td>15 (38)</td>
<td>18 (8)</td>
<td>29.4</td>
</tr>
<tr>
<td>2b. Lectin eluate</td>
<td>27 (51)</td>
<td>30 (75)</td>
<td>189 (81)</td>
<td>1.8</td>
</tr>
<tr>
<td>3. M1/69 Immunoadsorbent flow-through</td>
<td>0</td>
<td>32 (80)</td>
<td>171 (73)</td>
<td>ND*</td>
</tr>
<tr>
<td>4. 1st M1/89.23 Immunoadsorbent flow-through</td>
<td>0</td>
<td>13 (33)</td>
<td>192 (65)</td>
<td>ND*</td>
</tr>
<tr>
<td>5. 2nd M1/89.23 Immunoadsorbent flow-through</td>
<td>0</td>
<td>0</td>
<td>151 (65)</td>
<td>ND*</td>
</tr>
</tbody>
</table>

* Units = inhibitory titer × volume (ml).
* Not done.

TABLE III

<p>| Serum antibody specificities after immunization with cascade-purified antigen or whole cells |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Imunogen</th>
<th>Cell-binding titer*</th>
<th>M1/70 titer</th>
<th>M1/9.3 titer</th>
<th>M1/69-like titer, RBC agglutination*</th>
<th>M1/70 inhib. titer/M1/9.3 inhib. titer</th>
<th>M1/70 inhib. titer/M1/69-like titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cascade purified PEC ag.</td>
<td>275</td>
<td>15</td>
<td>186</td>
<td>50</td>
<td>&lt;0.5</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>PEC</td>
<td>47</td>
<td>49</td>
<td>9.6</td>
<td>2</td>
<td>3.3</td>
<td>2</td>
</tr>
<tr>
<td>PEC (antibody-coated)</td>
<td>44</td>
<td>38</td>
<td>6.6</td>
<td>1.5</td>
<td>2.4</td>
<td>1</td>
</tr>
<tr>
<td>Spleen cells (ND)</td>
<td>6</td>
<td>ND</td>
<td>2.3</td>
<td>2</td>
<td>110</td>
<td>2.6</td>
</tr>
<tr>
<td>None (immune serum)</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;0.1</td>
<td>&lt;0.2</td>
<td>&lt;0.5</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

* Sera were tested in the indirect binding assay on 10^3 3-day-thioglycollate-induced PEC or spleen cells. Titer = (dilution giving half-maximal binding)^-1.

* The abbreviations used are: RBC, red blood cells; PEC, peritoneal exudate cells; ND, not done.

* Serially diluted sera were tested for indirect RBC agglutination as previously described (Springer et al., 1978).

Agglutination titer = \left(\frac{\text{serum agglutination titer}}{\text{M1/69 agglutination titer}}\right) × M1/69 concentration (µg/ml)

* Inhibition of binding of ^3H-labeled antibodies to target cells was as previously described (Springer et al., 1978). The concentration of serum antibodies competing for the same site as monoclonal antibody (MAb) = (serum inhibitory titer/homologous MAb inhibitory titer) × MAb concentration. MAb concentration was determined by Mancini radial immunodiffusion (Ouchterlony and Nilsson 1978).

* Indirect immunoprecipitates using 0.2, 1, or 5 µl of sera were subjected to SDS-PAGE, autoradiography, and densitometry (Laskey and Mills, 1977). Precipitation titer = 10 × (volume (µl) giving maximal precipitation)^-1. Bands measured were the 190,000-M, M1/70 polypeptide from spleen cells.

* Sera from rats immunized for the M1 fusion (Springer et al., 1978).
Monoclonal Antibodies to Immunoadsorbent-depleted, Purified Antigens—After priming with purified antigen in complete Freund's adjuvant as described under "Materials and Methods," one (Lewis × BN)F1 rat received 100 ng of antigen in saline i.v. and its spleen was removed for fusion 3 days later. Fusion with NSI was carried out as previously described (Springer et al., 1978) and the cells were aliquoted into 5 × 96 well microculture plates. The 86 cultures giving the highest binding in the indirect binding assay were saved. However, since concentration-dependent background binding was given by normal rat IgG, the 86 cultures may have included some with high concentrations of irrelevant antibodies. Culture supernatants were next screened for immunoprecipitation of 125I (lactoperoxidase)-labeled peritoneal exudate cell surface antigens (Fig. 4). Supernatants from five of the M3 cultures precipitated 190,000 M₀ and 105,000 M₀ polypeptides and thus appeared to have the same specificity as M1/70. Other supernatants showed specificity for quite different polypeptides. Antibodies from 11 cultures precipitated a 32,000-M₀ polypeptide, and one (M3/37) a 180,000-M₀ polypeptide. Another culture (M3/84) identified a 110,000-M₀ polypeptide (not shown).

Some but not all cultures were successfully cloned as determined by the indirect binding assay and were retested for immunoprecipitation (Fig. 5). Supernatants from the M3/37 culture precipitated a single polypeptide of 180,000 M₀, but lost antibody activity before cloning (Fig. 5, A–E). The M3/84 culture defining a 110,000-M₀ polypeptide was more stable (Fig. 5, F–H). Successful clones (Fig. 5J) and subclones (Fig. 5J) were isolated. M3/37, M3/84, and M1/70 all precipitated distinctive high molecular weight polypeptides as seen by side-by-side comparison (Fig. 5 A–K). Two different cloned lines (M3/31 and M3/38) were obtained which precipitate 32,000-M₀ polypeptides (Fig. 5, M and N). M3/31 is an IgM while M3/38 is an IgG2a as determined by double immunodiffusion with subclass-specific antibodies.
This paper describes a procedure which greatly simplifies the collection of MAb libraries directed toward individual components of complex biological systems. Removal of previously recognized antigens with immunoadsorbent columns has been combined with cell hybridization in a cascade which restricts the immunizing stimulus to previously unrecognized antigens. The cascade of alternating cell hybridization and further immunoadsorbent depletion experiments can be indefinitely extended (Fig. 1) until the library is completed.

In this application of the method, the M1/9.3 common leukocyte and M1/89 heat-stable antigens shared with lymphocytes and peritoneal exudate cells were removed from detergent-solubilized peritoneal exudate cell membranes to help focus the immune response toward macrophage differentiation antigens. Quantitative depletion was demonstrated with a radioimmunoassay. The common leukocyte and heat-stable antigens appear to be the most immunodominant antigens in xenogeneic rat anti-mouse spleen cell immunization. In one anti-spleen (Springer et al., 1978), two anti-T lymphocyte, and two anti-T lymphoblast (unpublished) fusions, clones recognizing these antigens have consistently outnumbered those recognizing H-2 and other antigens. The M1/9.3 and M1/89 MAb were derived from anti-spleen immunizations, but were used here to remove antigens from peritoneal exudate cells. Therefore, it was necessary to test whether in fact the removal of these antigens improved the quality of anti-macrophage responses. This was done by comparing antibodies elicited by immunoadsorbent purified antigens and by whole macrophages. Removal of the common leukocyte and heat-stable antigens resulted in a considerable increase in the macrophage specificity of whole antisera. This suggests that the common leukocyte and heat-stable antigens are among the most immunodominant antigens in rat anti-mouse macrophage as well as in anti-mouse spleen cell immunization. Indeed, similar anti-common leukocyte antigen antibody levels were found after spleen cell and peritoneal exudate cell immunization. Furthermore, whole peritoneal exudate cells elicited almost at a high concentration of anti-common leukocyte antigen as anti-Mac-1 antibodies. Thus, while the cascade-purified material elicited five anti-Mac-1 and zero anti-common leukocyte antigen hybridomas, whole peritoneal exudate cells would have been expected to elicit similar numbers of each.

The efficacy of the cascade immunization procedure was most convincingly demonstrated by comparing antibody levels to the common leukocyte and heat-stable antigens which were removed, and the Mac-1, which was not. The ratios of Mac-1 to common leukocyte antigen and Mac-1 to heat-stable antigen antibodies were more than 100-fold higher in animals receiving immunoadsorbent-purified antigens than in those receiving whole cells. Animals receiving immunoadsorbent-purified antigen also had higher absolute levels of anti-Mac-1 antibodies, but this could have been due in part to the use of complete Freund's adjuvant. In the phenomenon of antigenic competition, co-administered antigens nonspecifically suppressed the immune response to each other (Pross and Eidinger, 1974). Therefore, membrane and glycoprotein purification and immunoadsorbent depletion could also have contributed to the higher absolute Mac-1 antibody response.

Other procedures have also been proposed for narrowing the range of MAb elicited in cell hybridization experiments. Tolerance induction to B lymphocytes has been used to enhance the percentage of T cell-specific hybridomas (Midleton et al., 1980). Blocking surface antigens of one type of cell with whole antisera to another type of cell has also been used to increase specificity (Kennett et al., 1978). These procedures have the disadvantage that they cannot continue to narrow the response to unidentified cell surface antigens in further hybridization experiments. An alternative method of blocking cell surface sites with MAb before injection was used here with little success. MAb differ from conventional sera in that only one out of a large number of potential antigenic sites would be blocked, and this may have contributed to the observed lack of effect. Also, it is likely that a fraction of cellsurface-bound MAb would dissociate after injection and allow immune responses.

Previously, a number of investigators have prepared antimacrophage serum by xenoinmunization with macrophages and exhaustive absorption with other tissues (Unanue, 1968; Hirsch et al., 1969; Gallily and Gornostansky, 1972). Several macrophage-specific antigens have also been defined by monoclonal sera. The Mac-1 antigen containing polypeptide chains of 190,000 and 105,000 M, is present on monocytic and granulocytic but not erythroid or lymphoid lines of differentiation (Springer et al., 1979). An alloantigen, Mph-1, has also been defined by cytotoxicity and by genetic mapping but has not been structurally characterized (Archer and Davies, 1971). The mouse Fc receptor II of 47,000 and 70,000 M, has also been identified with MAb (Mellman and Unkeless, 1980), and is expressed on both macrophages and B lymphocytes. Antigens of 32,000 M, (designated Mac-2, 110,000 M, (designated Mac-3), and an antigen of 180,000 M, have now additionally been described. Further detailed studies on the cell distribution and biochemistry of these antigens show they are present on stimulated macrophages but absent from erythroid, lymphoid, and granulocytic cells and thus appear restricted to the mononuclear phagocyte line of differentiation. Purified macrophages incorporate [35S]methionine into the M3/38 32,000-M, and M3/84 110,000-M, polypeptides, suggesting these cells synthesize as well as express these antigens on their surfaces. These monoclonal antibodies should prove invaluable in studying macrophage subpopulations, differentiation, localization, and the structure and function of these antigens.

Theoretically, the hybridomas approach should allow the complete repertoire of cell surface antigens to be catalogued. The cascade procedure (Fig. 1) allows this to be done in an efficient and straightforward manner, and has three considerable advantages over whole antigen mixtures. First, repetition of the monoclonal pest is avoided, thereby greatly simplifying screening procedures. Second, immunodominant antigens are removed, and therefore should not inhibit the immune response to other antigens by antigenic competition (Pross and Eidinger, 1974). Third, the procedure can be extended at will in a cascade which continually restricts MAb production to previously unidentified antigens. Before each new hybridization experiment, the antigen preparation is simply passed through immunoadsorbent columns containing the most recently isolated MAb. The procedure is not much more difficult than immunization with whole antigen mixtures. If sufficient quantity of antigen can be obtained, preliminary procedures such as membrane isolation, detergent solubilization, and lectin affinity chromatography (if desired) need be done only once. The cascade approach is also applicable to any other complex system where the antigenic components can be separated under nondenaturing conditions, such as tissue extracts, detergent-solubilized organelles, or mixtures of different viruses. When this work was in progress, the theoretical application of this method to cytoplasmic proteins was also independently proposed in a review article (Milstein and Lennox, 1980). The use of cell hybridization-MAb-immuno-
adsorbent cascades should greatly facilitate the analysis of biological complexity using myeloma-hybrid technology.

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REFERENCES