Advanced Immunotechniques

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The Hybridoma—An Immunochemical Laser


The advent of hybridoma technology has provided the immunotechnologist a finely tunable instrument that should permit a marked advance in the immunological sciences. The ability to choose the precise antibody required and the virtually unlimited availability of easily purified antibodies have already resulted in the simultaneous immunoassay and potential reagents for immunoscintigraphy and immunotherapy. Using an antibody with a specific affinity and recognition for a specific antigenic determinant can greatly influence the shape and range of a radioimmunoassay calibration curve. With monoclonal antibodies, assay systems based on immune complexes (e.g., turbidimetric assays and counterimmunoelectrophoresis) can be made more precise, thus permitting study of the basic physical-chemical principles underlying the antigen–antibody reaction and the development of greatly improved quantitative assays. On the other hand, the ability to select an antibody exhibiting specific characteristics implies the necessity to select. One no longer has the luxury of using a mixture of antibodies, hoping to take advantage of the fact that some will have desirable secondary characteristics such as electrophoretic mobility or the ability to adsorb to plastics.

Additional Keyphrases: immunoassay · turbidimetry · counterimmunoelectrophoresis · immunoengineering · immunoradiometric assay

It is perhaps a wry trick of fate that would turn cancer upon cancer. Monoclonal “antibodies” have existed for many years in the form of myeloma proteins—products of lymphoid cell cancers. Now the myeloma cell has provided us a mechanism for producing reagents that, among other things, may well be used to detect and perhaps destroy other malignant cells.

The potentials of monoclonal antibodies in scintigraphic and therapeutic techniques have received considerable attention of late (see, for example, references 1 and 2). We will concentrate upon the immunochemical and diagnostic potentials of hybridoma-derived antibodies, emphasizing the advantages homogeneous antibodies offer over polyclonal antibodies.

Attempts to prove the monoclonal antibody nature of myeloma proteins took several years of immunochemical analyses (3). During this time antigenic specificities were demonstrated for many myeloma proteins, both of human and murine origin. In general, however, affinities were low and specificities were purely a matter of chance. It was virtually impossible to utilize the myeloma system—even in mice, where myeloma could be induced—to provide immunodiagnostics tools. Myeloma proteins remained a tool of the immunologist, for structural analysis of immunoglobulin and chemical dissection of the hypervariable region of the antibody molecule to determine the nature of the “active site.”

In 1968 Fleischman et al. (4) described a means of producing myeloma-like proteins, antibodies of restricted heterogeneity directed against specific antigenic determinants. By using rigorous immunization schedules, it was possible to produce in rabbits homogeneous antibodies directed against streptococcal polysaccharide antigens. These antibodies could be induced in concentrations as great as 60 g/L in serum, but required frequent antigenic stimulation and often became less homogeneous during the course of an immunization schedule. Nevertheless, these experiments provided much information regarding the genetics of the immune response, as well as reagents for further elucidation of antibody structure and the nature of specificity.

Montgomery and Pincus (5) later began to engineer the homogeneous antibody response further by conjugating c-dinitrophenyllysine to bacterial polysaccharides and using this system to produce antibodies of restricted heterogeneity against the dinitrophenyl hapten. Affinities of these antibodies were, however, quite low (around $10^4$ L/mol or less).

In 1978 Zurawski et al. (6) took one step further and developed homogeneous rabbit antibodies against the diagnostically significant cardiac glycoside digoxin. Again, the hapten was conjugated to bacterial polysaccharides, rabbits were subjected to rigorous immunization schedules, and “monoclonal” antibodies resulted. Once again, however, the antibodies that were elicited exhibited low affinities. In retrospect, one can see that the stimulation of a clone of cells to proliferate wildly appeared to be somewhat random with respect to the affinity of that clone, and the resulting antibodies were of average (low) affinity. The technique was clever, but the results disappointing in that a sensitive and specific assay did not follow.

Of the homogeneous (monoclonal) antibody-producing systems described above, only myeloma cells were demonstrated to proliferate in tissue culture.

Perhaps the ultimate era in immunoengineering was initiated when Kohler and Milstein reported that an antibody-secreting spleen cell could be fused with an “immortal” myeloma cell to yield a hybridoma cell line (7). Consequently, it is now possible to produce an unlimited supply of antibodies displaying literally any desired specificity.

Immunoengineering. The concept of immunoengineering suggests that one can produce an antibody specific for any given determinant on an antigen. Indeed, we have succeeded in identifying a series of determinant regions on each antigen we have studied; most determinants yield several antibodies.
with a range of affinities. There are, of course, immunodominant determinants that result in more numerous antibodies and (or) having higher affinity than other antibodies. Determinants exhibiting greater "foreignness" probably result in more antibodies, whereas the presence of certain amino acids in the determinant, such as tyrosine, would be likely to yield higher-affinity antibodies than would other amino acids such as serine or glycine. For example, we have identified four major determinant areas on carcinoembryonic antigen and at least seven on human alpha-fetoprotein. In each case certain determinants yield more antibodies than do others, and some yield antibodies of higher average affinity. The surface of each molecule can be mapped according to the degree of steric interference of binding between antibodies directed against the different areas.

The most immediately obvious potential of such capability is the engineering of a homogeneous immunoassay based on concepts previously described (8), in which free antigen competes with enzyme-linked antigen for antibody binding. For example, if an enzyme can be attached covalently to a specific region of an antigen, an antibody directed against an adjacent determinant may cause a consistent and predictable inhibition or enhancement of enzymatic activity; free antigen (in a patient's serum, for example) would inhibit the effect of the antibody. Moreover, all antibodies in the monoclonal population recognize the same precise determinant; only a small fraction of antibodies present in rabbit antiserum would be expected to be specific for that determinant. Furthermore, hybridoma cell lines live forever—rabbits don't.

A second implication of hybridoma technology is that by choosing an antibody of desired affinity one can engineer a radioassay with a desired range of sensitivity. As will be seen later, the combination of antibodies chosen for the two-site sandwich (IRMA) assay can permit very fine tuning of the sensitivity of such an assay.

A third advantage derived from the option of choosing antibodies specific for individual antigenic determinants is realized through engineering an IRMA from two antibodies directed at sterically distinct determinants. Because neither antibody interferes with the other, the binding of either can occur first without affecting the binding of the second. This concept is illustrated in Figure 1. Thus the advent of the completely controlled simultaneous IRMA and hence the derivation of the TANDEM™ (Hybritech, Inc., La Jolla, CA 92037) concept: It is now possible to mix together the solid-phase antibody against determinant a of the antigen and the labeled (with radioisotope, enzyme, fluorochrome, etc.) antibody against sterically distinct determinant b in the same tube at the same time (in any order) and obtain data that compare well with those obtained when one carries out the conventional two-step reaction. In the sequential assay protocol the solid-phase antibody is first exposed to the antigen, and the resulting complex is washed and then exposed to the labeled antibody; this additional washing and incubation step takes additional time, effort, and expense. Finally, an IRMA functions most efficiently when purified antibodies are used; monoclonal antibodies can be purified without the necessity of affinity chromatography.

In the conventional assay, even after laborious immunization and (or) antibody purification and adsorption protocols, some of the resulting antibodies are still likely to interfere with each other sterically, resulting in an assay that is most efficient and reproducible only when carried out in the two-step protocol. With a carefully engineered monoclonal antibody assay the optimum conditions are the single-step, single-incubation protocol.

Other assay systems involving monoclonal antibodies are beginning to appear, for example, turbidimetric assays (9, 10) and counterelectrophoretic analyses (see below). In the former the appropriate "blend" of monoclonal antibodies can yield a quantitative assay, and in the latter the proper choice of a monoclonal antibody and of an antigen possessing enzymatic activity will provide a highly sensitive electrophoretic assay.

We obtained the data presented below, describing the use of monoclonal antibodies in RIA and IRMA procedures, by using human IgE as an antigen. In general, similar data have been derived from all other antigen systems studied.

Materials and Methods

Monoclonal antibody production. Spleen cells obtained from immunized Balb/c mice were fused with myeloma cell lines essentially as described by Kohler and Milstein (7). The resulting fusion products were plated out into 96-well microtiter plates, and the resulting hybridomas were screened according to their ability to bind 125I-labeled IgE (Kallestad Laboratories, Chaska, MN 55318), or 125I-labeled prostatic-origin acid phosphatase (PAP) (New England Nuclear, Boston, MA 02118). Hybridoma lines secreting antibodies that exhibited acceptable affinities, as determined by saturation analyses (11), were further subcloned to ensure monoclonality. Those antibodies selected for further characterization and for use in assay protocols were obtained in large quantities from

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1 Nonstandard abbreviations used: CIEP, counterimmunoelectrophoresis; IRMA, immunoradiometric assay; IgE, human immunoglobulin E; PAP, prostatic acid phosphatase (EC 3.1.3.2); RIA, radioimmunoassay.
the ascites fluid of mice bearing peritoneal implants of the appropriate hybridoma cell lines.

**Antibody purification.** Monoclonal antibodies were purified from murine ascites fluid by two sequential precipitations with sodium sulfate (180 g/L). The antibodies were dissolved in borate-buffered saline (per liter, 10 mmol of sodium borate and 150 mmol of NaCl, pH 8.0), then dialyzed vs the same buffer. These reagents were then stored at either 4 or -20 °C.

Analytical polyacrylamide gel electrophoresis was carried out essentially according to the method of Ornstein (12) and Davis (13), but with a 5 to 20% acrylamide gradient. Antibodies prepared in this manner appeared to be at least 90 to 95% pure. When radiolabeled and tested for immunoreactivity, at least 60 to 80% were capable of binding to excess insoluble antigen, and were quite acceptable for use in immunometric assays.

**Radioiodination.** We radiolabeled the monoclonal antibodies with 125I or 131I (Amersham Corp., Arlington Heights, IL 60005), using solid-phase lactoperoxidase as previously described (14). Specific activities obtained were usually about 10 to 15 Ci/g for 125I-labeled antibodies and approximately 3 to 5 Ci/g for 131I-labeled antibodies. There were no apparent differences related to the nature of the isotope or the specific activity of the iodinated antibodies. Before use in the assay, radiiodinated antibodies were diluted into 0.1 mol/L sodium phosphate, pH 7.0, containing, per liter, 300 mL of horse serum and 1 g of NaNS.

**Calibrators and control sera.** IgE calibrators were prepared by diluting serum obtained from an individual exhibiting extremely high IgE concentrations into horse serum (Colorado Serum Co., Denver, CO 80216). Calibrator concentrations were confirmed by standardization against the 1st British Standard for IgE (75/302 World Health Organization). PAP calibrators were prepared by diluting seminal plasma into bovine serum (Colorado Serum Co.), and were confirmed by comparison with New England Nuclear calibrators. PAP control sera I (11.8 μg/L), II (24.4 μg/L), and III (53.7 μg/L) were purchased from Ortho Diagnostics, Raritan, NJ 08869.

**Radioimmunoassay.** Saturation analyses were carried out with a solid-phase semi-automated assay system previously described (15). Briefly, the reactions were carried out in the wells of microtitr plates by sequential addition of 25 μL of sample (antigen), 25 μL of 125I-labeled antigen, and 20 μL of antibody diluted to bind approximately 50% of the 125I-labeled antigen. After incubating this mixture for 2 h at 37 °C, we added 20 μL of insoluble horse anti-murine IgG suspension, and rotated the resulting mixture at approximately a 45° angle for at least 6 h at room temperature. Samples were then processed with a 24-sample cell harvester (J. M. Specialty Parts, San Diego, CA 92122). Bound 125I-labeled antigen was quantitated with a gamma scintillation spectrometer (Model 1285; Tracer Analytic, Inc., Elk Grove Village, IL 60007).

**Immunoradiometric assays.** IRMAs were performed in 12 X 75 mm disposable plastic gamma-counter tubes (Clinical Plastics, Leominster, MA 01453). Monoclonal antibodies specific for one antigenic determinant were bound (via proprietary procedure) to polystyrene balls, 10 μm diameter (Precision Plastic Ball Co., Chicago, IL). A monoclonal antibody specific for a sterically distinct antigenic determinant was radiiodinated as described above. The solid-phase bound antibody was usually added to the assay tube first, followed by 200 μL of tracer diluent containing approximately 10 ng of 125I- or 131I-labeled antibody, then 50 μL of IgG calibrator. Some experiments included about 0.2 ng of 125I-labeled IgE and 131I-labeled antibodies. After incubation at 37 °C we aspirated the unbound reagents and washed each ball twice with 2 mL of 80 mmol/L NaCl solution containing, per liter, 2 g of Tween 20 and 0.2 g of NaNS. Bound radiolabeled antibody and 125I-labeled IgE were quantitated as described above. In the double-label experiments (125I and 131I) the radioactivity of the samples was counted with a dual-channel gamma scintillation spectrometer (Tracer Analytic Model 1191).

**Counterimmunoelrohoresis.** We pipetted 10 mL of a solution of 10 g of SEA-KEM HEOE (high electroendosmosis) agarose (Marine Colloids, Inc., Rockland, ME) and 20 g of Dextran T10 (Pharmacia, Piscataway, NJ 08854) per liter onto a 50 X 70 mm sheet of Gelbond film (Marine Colloids). Electrophoresis was carried out at 8 mA for 2 h in 30 mmol/L sodium phosphate, pH 6.5, in a Gelman electrophoresis chamber (Fisher Scientific, Pittsburgh, PA 15219) with ice bags placed on the lid. The slides were stained for PAP activity (16) by immersion in a solution of 50 mmol/L sodium acetate, pH 4.5, containing, per liter, 0.5 g of α-naphthyl phosphate and 0.5 g of GBC-Fast Garnet Red.

**Steric hindrance.** The steric effect of solution-phase antibody on the binding of antigen to the solid-phase antibody was tested by incubating approximately 0.5 ng of 125I-labeled IgE with the solid-phase antibody in the presence of solution-phase antibody, 1 mg/L. Inhibition of binding relative to that in buffer or in the presence of a control antibody of a different specificity indicated steric interference.

**Results.**

**Assay range.** The sensitivity and quantitation range of an RIA can be engineered simply by choosing an antibody of the appropriate affinity. As can be seen in Figure 2, clone E antibody yields a quantitation range approximately one order of magnitude more sensitive than does clone D antibody.

Because two antibodies are necessary for a sandwich-type immunometric assay, a somewhat more complex situation is involved in determining the sensitivity and quantitation range of an IRMA. Immunoradiometric assays involving different combinations of three monoclonal antibodies are illustrated in Figure 3. When radiolabeled clone A antibody is used in conjunction with solid-phase clone B antibody, a highly sensitive but short-range assay is obtained. On the other hand, when these antibodies are reversed and radiolabeled clone B antibody is used with solid-phase clone A antibody, the results are strikingly different: an assay with very low sensitivity but broad range. When radiolabeled clone B antibody is paired with solid-phase clone C antibody, results are intermediate. Thus by choosing appropriate pairs of antibodies for use in an immunometric assay, one can engineer an assay with finely tuned sensitivity.

**Simultaneous incubations.** To test the hypothesis that a simultaneous incubation carried out in an immunometric assay system would yield results similar to those obtained with
two sequential incubation periods, we set up four experiments. Two were simultaneous assays, in which all reagents were mixed together, incubated at 37 °C, and then harvested at either 5 or 24 h. Two were sequential assays: the first incubation, either 5 or 24 h, was followed by a washing procedure, and then by a second incubation period with the radiolabeled antibody for either 5 or 24 h. In all four experiments approximately 0.2 ng of 125I-labeled IgE was added to determine the extent of antigen binding as well as the extent of desorption (in the sequential assays). Antibodies were labeled with 131I. As Figure 4 indicates, the results of the simultaneous assays at both time periods were virtually identical to those of the sequential assays. Approximately 75% of the immunoreactive 125I-labeled IgE had bound within the first 5-h incubation, and approximately 5 to 7% of the bound antigen desorbed during the second incubation period of the sequential assays. The presence of the 131I-labeled antibodies did not alter the binding of the 125I-labeled IgE.

Steric interactions. Figure 5 illustrates the effect of solution-phase antibodies on the binding of 125I-labeled IgE to solid-phase clone C antibody. The control antibody (specific for PAP) had no detectable effect on the binding of the IgE to the insoluble antibody. Similarly, clone D anti-IgE antibody, specific for a determinant sterically distinct from that recognized by the solid-phase antibody, had no detectable effect. Excess soluble clone C antibody (that antibody present on the solid phase) drastically inhibited the binding of the antigen, as would be expected. Clone E antibody partially inhibited antigen binding, which suggested that it was recognizing a distinct determinant, but its presence on the antigen caused some steric inhibition of the binding of the antigen to the insoluble clone C antibody. Interestingly, insoluble clone C antibody can be used with radiolabeled solution-phase clone E or clone D antibody in an IRMA assay. Clone E antibody, however, exhibits a sensitivity only about two- to threefold greater than that exhibited by clone D antibody in this assay. This is in marked contrast to the results obtained when these two antibodies are compared in an RIA (see Figure 2); there, clone E antibody is >10-fold more sensitive than clone D antibody. Thus steric inhibition, caused by the apparent proximity of the antigenic determinants recognized by clones C and E antibodies, significantly affects their performance as an assay pair in an IRMA.

Counterimmunoelectrophoresis. The counterimmuno- electropherogram presented in Figure 6 illustrates the sensitivity of a CIEP assay for PAP when monoclonal antibodies are used. Concentrations of 1 to 2 µg/L of serum can readily be detected here, and the Ortho Diagnostics control sera can be quantitated well in the assay. The monoclonal antibody probably functions to “stop” the migration of the antigen in the electrophoretic field. No actual zone of precipitation can be detected in such an assay, but in the absence of antibody, the PAP enzymatic activity would have appeared much more diffuse and closer to the anode.

**Discussion**

Clearly a new generation of immunoassay systems is about to evolve as a result of hybridoma technology. The opportunity
to engineer an assay by choosing antibodies with precise affinities, determinant specificities, electrophoretic mobilities, and a variety of other physicochemical properties will provide the immunotechnologist with a tool the power of which we probably have not yet to realize. Figure 7 graphically compares three hypothetical monoclonal antibodies and a population of polyclonal antibodies. The physicochemical properties involved could be antigenic determinant-site specificity, affinity, isoelectric point, electrophoretic mobility at a given pH, ability to bind to a polystyrene surface, or virtually any other imaginable measurable quality.

By choosing an appropriate pair of antigen-specific antibodies, one can engineer an assay of utmost simplicity. Classically, to obtain the most reliable results in an immunoradiometric assay, two sequential incubations have been necessary, the initial incubation to allow the antigen to bind to a solid-phase antibody, and the second to permit radiolabeled second antibody to bind to the insolubilized antigen. Furthermore, a washing step is necessary between the first and second incubations, increasing the manual labor involved as well as the time consumed during an assay. The added manipulations would also be expected to introduce additional sources of potential error. Sequential incubation periods are necessary because of the presence of antibody populations in the solution phase that would interfere with the binding of the antigen to solid-phase antibodies, particularly at low antigen concentrations, where an excess of radiolabeled antibodies would saturate the antigen molecule such that binding to the solid phase would be inhibited.

Attempts have been made to select antibody populations specific for distinct regions of an antigen by using proteolytic cleavage and isolation of individual segments of the molecule. This was accomplished for rat IgE (17), leading to an assay of greater sensitivity than that involving unfractonated antibody populations. No attempts were made to develop a simultaneous assay, however. Some antibody populations would still likely hinder one another sterically, and thus limit the efficiency of a simultaneous assay (Figure 5).

Hybridoma technology has made it possible to choose two different antibody molecules to include simultaneously in an incubation mixture, such that the binding of one antibody to the antigen does not interfere in any way with the binding of the antigen to the second antibody. Thus it is now possible to carry out a complete assay with only one incubation period and only one wash step. Moreover, one can choose antibodies or antibody pairs such that specific antigen sensitivity ranges can be achieved. In an RIA one can simply choose an antibody to fit the sensitivity requirements of a specific assay (Figure 2), whereas in an IRMA the choice of two different antibodies or two different configurations (labeled soluble phase or solid phase) of an antibody pair provides additional control over assay sensitivity (Figure 3). The precise reasons for the drastic differences observed in assay performance when the two antibody components are switched to opposite phases is not entirely clear, although relative affinities are certainly involved.

The ability to use a monoclonal antibody in a CIEP assay is probably due to factors other than the precipitation of immune complexes in the agarose. Indeed, such precipitin reactions are very difficult to achieve, if at all, even when the antigen in question is bivalent. More likely, the monoclonal antibody, when it encounters an antigen, inhibits the mobility of that antigen. Thus, as a region of antigenic species encounters a region of antibody molecules, the effect of the antibody population is to halt and concentrate the population of antigen molecules. The results are probably similar to those observed in "disc" electrophoresis (12, 13), in that the alteration of electrophoretic mobility by complex formation and the resulting concentration of the antigen zone provides a highly sensitive tool for detection and quantitation of an enzymic antigen.

For small antigens with only one or two identical subunits the opportunity for developing a precipitation-type reaction, such as would be required for immunodiffusion analysis or a nephelometric assay, is somewhat more difficult. Polyclonal antiserum is, however, nothing more than a large number of monoclonal antibodies all thrown together in a somewhat random manner. Thus artificial synthesis of a "polyclonal" antibody population should be possible by mixing in a defined manner several monoclonal antibodies specific for different determinants. Indeed, Steensgaard et al. (9) and Jefferis et al. (10) have developed turbidimetric assays for human IgG, using appropriate mixtures of monoclonal antibodies. The obvious advantage of a mixture of monoclonal antibodies over polyclonal antiserum is the precision with which the mixture can be replicated from batch to batch.

Another major advantage of monoclonal antibodies, aside from the opportunity to engineer a desired assay, derives from the consistency and unlimited availability of antibody molecules, as well as the simplicity in obtaining purified populations of such molecules. A hybridoma cell line, if maintained
properly, can easily be made to produce antibodies in kilogram quantities, and the antibodies can be isolated by simple physicochemical techniques. On the other hand, no two animals are likely to produce exactly the same population of antibody molecules, nor for that matter is one rabbit likely to produce a consistent mixture of antibodies from one bleeding to another.

Furthermore, many immunochemical techniques, such as the IRMA, require the acquisition of purified antibodies. To obtain purified antibodies from polyclonal antisera requires the use of affinity-purification techniques. Aside from the labor and expense involved, two main problems can result from most available affinity-purification procedures: In the first place, antibodies having very high affinity may be lost, because they bind to an affinity adsorbent so tightly that even the strong denaturing conditions usually used to remove antibodies cannot adequately break the antigen–antibody bonds. Furthermore, these harsh conditions often result in somewhat denatured antibody molecules, which may lead to altered specific binding characteristics or somewhat greater non-specific binding.

The opportunity to select monoclonal antibodies that exhibit certain desirable physicochemical properties is unfortunately accompanied by the necessity to select antibodies that are compatible with the particular system in which one wishes to use them. CIEP procedures, for example, that involve a polyclonal antiserum can take advantage of the fact that this antiserum contains numerous populations of antibody molecules, some of which exhibit optimum electrophoretic mobilities under a given condition. For instance, at pH 6.5 certain antibody molecules will be carried by electroendosmosis toward the cathode. Therefore, one does not have to subfractionate a polyclonal antiserum but merely relies on the conditions of the assay system to perform that selection for him. Such a luxury does not exist when the tool of choice is a monoclonal antibody. For a population of monoclonal antibodies, either the whole population moves in the right direction at the right rate in an electrophoretic field or it doesn’t. A specific monoclonal antibody must be selected that exhibits not only the correct antigen specificity and affinity but also the correct electrophoretic mobility under these conditions. The result of such a selection procedure is the acquisition of a single population of antibody molecules with a very precise electrophoretic mobility. Complexing an antigen with a member of such a population of antibody molecule results in a precise alteration of the antigen’s electrophoretic mobility. Because the polyclonal antiserum consists of populations of molecules with somewhat differing electrophoretic mobilities, the effect is very likely to be a less precise alteration of antigen mobility, resulting in a more diffuse band of immune complexes. Results of assays for detecting such immune complexes will be less sensitive than when monoclonal antibodies are used.

In another example, immunization of an animal for the purpose of producing RIA-grade antisem results in what is often referred to as a “high-affinity” antisem. What this term actually means is that within this antisem certain populations of antibody molecules exhibit high affinities for the antigen. When the antisem is diluted under the conditions of an RIA, only the high-affinity antibodies are functional (see discussion section of reference 11). Thus the conditions of the assay “select” the appropriate class of antibodies for the assay. Once again, one does not have this luxury when using monoclonal antibodies. All members of a population of monoclonal antibodies either have a high affinity or they do not.

Along with the advantages of hybridoma technology, one must accept certain drawbacks. The result, however, when used properly, is the availability of an extremely powerful tool.

The advent of the laser, a means of producing very precisely defined monochromatic (“monoclonal”) light, opened up a flow of new technological capabilities spanning a variety of disciplines from physics to biology. Similarly, hybridoma technology has provided a means of producing a number of precisely defined components of the immune response, which probably will also profoundly affect the biomedical and physicochemical sciences.

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