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REVIEW ARTICLE

Some properties and applications of monoclonal antibodies

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Introduction

A monoclonal antibody is the antibody produced by a single clone of B lymphoid cells: all the antibody molecules have the same amino acid sequence and hence the same binding properties. A myeloma is a tumour of antibody-secreting cells, apparently derived from a single cell, and is thus a clone of cells producing a monoclonal antibody. For some years it has been possible to grow myelomas of rodents as permanent cell lines in tissue culture or as tumours in animals, but in general they produce antibody to unknown or uninteresting antigens. Kohler & Milstein (1976; see also Milstein, 1980, for historical background) found a way to construct hybrid myelomas—'hybridomas'—that make antibody to an antigen of choice. In their technique, lymphocytes from an animal making useful antibodies are fused with cells of a stock myeloma cell line to give hybrid myeloma cells. Each hybrid produces the antibody specified by the lymphocyte that took part in the fusion; the myeloma confers on the hybrid cell the ability to grow in culture or as a tumour in an animal.

Production of monoclonal antibodies

Outline

A mouse (or rat) is immunized with antigen, say human erythrocytes. Some 3–4 days after a final immunization, the animal's spleen contains many lymphocytes making antibody to erythrocytes. Cells from the spleen are mixed with stock myeloma cells, in the presence of poly(ethylene glycol), to make hybrid cells. The stock myeloma cell line used is usually a selected variant that has lost the ability to produce its own immunoglobulin or at least the immunoglobulin heavy chain (Table 1). The hybrids would otherwise produce this immunoglobulin as well as that of the immunized lymphocyte. In order that the hybrids can be grown in culture without being swamped by unhybridized myeloma cells, the myeloma cell line employed is also a mutant that lacks hypoxanthine phosphoribosyltransferase (EC 2.4.2.8; HPRT or HGPRT), an enzyme of a salvage pathway of nucleic acid metabolism. These cells die in medium containing hypoxanthine, aminopterin and thymidine ('HAT medium'; Littlefield, 1964) because the aminopterin blocks nucleic acid biosynthesis and they are unable to utilize the hypoxanthine. Hybrid cells, however, are wild-type and they survive and grow. Unhybridized spleen cells die out in a few days. Fusions yield hundreds, sometimes thousands, of individual hybrid cell lines, typically 10% of which make antibody to the antigen used to immunize the mouse, fewer with poor antigens. After 10–14 days the growing hybrids are tested for the production of antibody to the antigen, in this case erythrocytes. Culture medium in which the hybrids have been growing and secreting antibody is incubated with erythrocytes, the erythrocytes are washed and the antibody that has bound to them is then detected, for example by binding of radiolabelled rabbit anti-(mouse immunoglobulin) antibody. Interesting hybrids are identified and cloned.

Antibody may be obtained from the hybrids as 'culture supernatant' or ascitic fluid (see Table 2 below). Culture supernatant is medium in which the cells have been growing and secreting. It contains antibody, usually together with foetal calf serum (which may contain bovine immunoglobulin), but no irrelevant mouse or rat immunoglobulin. (It is now possible to use serum-free culture medium: Iscove & Melchers 1978; Chang *et al.*, 1980; Klinman & McKearn, 1980.) Ascitic fluid is produced when hybrids are grown in the peritoneal cavity of mice or rats as appropriate. It contains a variety of serum proteins and irrelevant immunoglobulin, but they do not interfere with most applications. Some 1–5 ml of ascites fluid can be obtained from a mouse, so that 100 mg batches of antibody can be made without difficulty. Rats give much larger volumes of ascites fluid.

The hybrid cells can be stored in liquid N_2 and recultured indefinitely, provided variant cells that no longer produce antibody are eliminated when necessary by recloning.

Abbreviations used: SDS, sodium dodecyl sulphate; Ig,

Table 1. *Some myeloma cell lines used for making hybrids*

Species	Myeloma cell line	Immunoglobulin produced	Reference
Mouse	NS1	Light chain only	Kohler & Milstein, 1976
	Sp_20	None	Schulman *et al.*, 1978
	F0	None	Fazekas de St. Groth & Scheidegger, 1980
	X63.Ag8.653	None	Kearney *et al.*, 1979
Rat	Y3	Light chain only	Galfre *et al.*, 1979
Human	SK0-007	IgE	Olsson & Kaplan, 1980
	GM1500 6TG-A1 2	IgG	Croce *et al.*, 1980
	LICR LON/HMy2	IgG	P. A. W. Edwards & M. J. O'Hare, unpublished work

made, fusing lymphocytes with a myeloma of the same species (Table 1). Some stable hybrids were made between rat or human lymphocytes and mouse myelomas before rat and human myelomas were available (Howard *et al.*, 1980; Schlom *et al.*, 1980). No successful hybrids have been made with rabbit lymphocytes (Yarmush *et al.*, 1980). For large scale production, rat hybrids are preferable to mouse, but for research purposes, mouse hybrids have the advantage that many more reagents and procedures for mouse immunoglobulin are available (Hudson & Hay, 1980). Human hybrids are desirable for the study of human immune responses, and perhaps for producing human antibody for therapeutic applications if rodent antibody is not available or not acceptable.

The production of a new monoclonal antibody requires a lot of time and a wide range of experience, materials and equipment, both for making and for characterizing the antibody. The antigen or mixture of antigens used must be known to give a good conventional immune response, and a sufficiently reliable, sensitive and rapid assay must be available for screening. However, in experienced hands, an appropriately designed fusion experiment is usually successful.

Some notes on methods

For an extensive review of methods the reader is referred to Goding (1980). Fazekas de St. Groth & Scheidegger (1980) give a useful protocol for fusion, while Hudson & Hay (1980) is an indispensable manual of laboratory procedures.

Plating strategy and cloning. Cloning a single hybrid under adverse conditions is as time-consuming as performing a fusion. Also, if one is screening for an antibody that discriminates between two antigens, a specific clone will be missed if it is mixed with a clone that produces non-specific antibodies. Hence fusion protocols should be designed to generate clonal hybrids from the outset, usually by plating on feeder cells (non-dividing cells such as macrophages or thymocytes that provide a 1980) in a large number of wells in microtest plates so that only about 10% of the wells contain hybrids (e.g. Fazekas de St. Groth & Scheidegger, 1980). An alternative is to plate the hybrid cells in agar so that they grow as clones (Sharon *et al.*, 1979; Edwards, 1980). Individual positive clones can be identified by a replica-plating assay (Sharon *et al.*, 1979), or the agar can be in 24-well plates so that testing for antibody is performed conventionally; clones are picked from wells containing antibody and retested after isolation (Edwards, 1980).

Hybrids should be recloned by limiting dilution, rather than in agar (for protocols, see Goding, 1980, or Hudson & Hay, 1980), since agar gives a lower cloning efficiency and so may select for unwanted cells that are more vigorous than the desired hybrid. In addition, colonies of cells picked from agar are not always pure clones.

Screening. When screening, it is essential to identify desirable hybrids quickly, as the labour of maintaining and characterizing them is greater than that needed to perform another fusion. The screening assay must be sensitive enough to give a strong response for about 1μg of antibody/ml if concentration methods are not to be used. As discussed below, some assays do not usually work with monoclonal antibodies, for example, cytotoxicity tests (Howard *et al.*, 1979).

Antibodies are most often detected by binding them to the target antigen, followed by detection with ^{125}I-labelled or enzyme-linked antibody to mouse immunoglobulin (Williams *et al.*, 1977; Goding, 1980). However, as far as possible, the antibody should be tested in the way that it is to be used, and a more direct approach may save labour. For example, immune precipitation of antigen has been used for screening after a preliminary rapid test for abundant antibody binding to Protein A of *Staphylococcus aureus* (Brown *et al.*, 1980). Staining of tissue sections or cells by immunofluorescence (Goding, 1980) or immunocytochemical methods (e.g. Schlom *et al.*, 1980) are suitable screening methods for antibodies that

intermediate filaments; scarce cells, that may not be abundant enough to be a target for a binding assay, can be detected this way (McIlhinney *et al.*, 1981).

Among tricks suggested for streamlining screening are replica-plating methods, using either a blotting technique (Sharon *et al.*, 1979) or a commercially available sample-transferring 96-well plate (Schneider & Eisenbarth, 1979; Bankert *et al.*, 1980*b*), and autoradiography of plates as an alternative to γ-counting after binding ^{125}I-labelled second antibody (Parkhouse & Guarnotta, 1978). Culture fluid can be concentrated by rapidly freezing 1 ml in a narrow tube, then allowing it to thaw while being centrifuged at about 1000 ***g*** at room temperature. The concentrate is found at the bottom of the tube. Samples from 96-well plates can be pooled along rows and columns for preliminary screening (Brown *et al.*, 1980).

Higher yield of useful hybrids. Several attempts have been made to increase the proportion of hybrids that make antibody to the antigen of choice, but none are yet established methods. An improved immunization method for soluble antigens has been claimed by Staehli *et al.*, (1980), and a good yield of antibodies for staining sections of fixed tissue can be obtained by immunizing with fixed cells (R. A. J. McIlhinney, personal communication). Middleton *et al.* (1980), in a preliminary report, induced tolerance to human B cells in mice, then immunized with a T cell fraction and obtained a greater proportion of antibodies specific to T cells. Yelton *et al.* (1978) fused cells that had been selected for their ability to bind the chosen antigen. Particularly interesting is the report of Bankert *et al.* (1980*a*) that spleen cells fuse with hapten-coated myeloma cells to give a high proportion of anti-hapten hybrids.

Problems. It is widely believed that the success of the cell fusion depends primarily on the condition of the myeloma cells used. If the yield of hybrids is low, fresh cells should be obtained from a successful laboratory. The method of routine passage of the myeloma may be critical: high density and low serum or spinner culture are recommended, followed by optimal conditions just before fusion (G. Galfre, personal communication). Another problem is contamination with *Mycoplasma*, which causes cultures to die out gradually. It appears to be controllable by injecting infected cells into mice as for the production of ascites (Hudson & Hay, 1980) and recovering the apparently *Mycoplasma*-free cells when the ascites is drained (P. A. W. Edwards, unpublished work; see also the methods of Marcus *et al.*, 1980 and Schimmelpfeng *et al.*, 1980).

Apparent instability of hybrids, i.e. a loss of antibody production, is thought to be caused by non-secreting variants or unrelated hybrids overgrowing the desired hybrids. It should be possible to

al., 1980; Schlom *et al.*, 1980). Sometimes when a sample of hybrid cells is thawed from storage in liquid N_2 almost all the cells die. Such cultures will usually re-establish if routinely, on thawing, part of the sample is seeded at low cell density, say 10^4 cells/ml, on feeder cells (P. A. W. Edwards, unpublished work; for feeder cell recipes see Goding, 1980).

Special properties of monoclonal antibodies

Monoclonal antibodies are not just exceptionally high quality antibodies. To the research worker, the most important benefit of the monoclonal antibody technique is that it makes possible the identification, assay, marking and purification of antigens that have not been purified, and that are in fact usually completely unknown at the outset. This is because the method generates antibodies to individual components of a mixture. For example, in a mouse immunized with human erythrocytes, individual lymphocytes will be making antibody to individual components of the erythrocyte. When hybrid myelomas are made with the lymphocytes, the hybrids produce these antibodies. From such a hybridization experiment we obtained two monoclonal antibodies that bind specifically to be erythrocyte membrane glycoprotein glycophorin A, each recognizing a different part of the molecule, as well as other antibodies that bind to other molecules on the surface of the erythrocyte (Edwards, 1980; Anstee & Edwards, 1981). The various antibodies can now be used to purify these surface molecules or to detect them on other cells. This example also illustrates the ability of the method to produce antibodies to individual antigenic determinants on a single molecule, which is particularly useful when some determinants are unique to that molecule while other determinants are also present on different molecules.

Of rather specialized interest is the possibility of capturing rare or inaccessible immune responses. It should be possible by screening large numbers of hybrids to obtain good monoclonal antibodies to antigens that give weak or widely cross-reactive conventional antisera, although the effort required should not be underestimated. Antibodies produced to tumours may be studied by making hybrid myelomas with lymphocytes from lymph nodes adjacent to tumours (Schlom *et al.*, 1980).

Monoclonal antibodies also have some revolutionary practical advantages over conventional antisera, but they also have limitations. Table 2 summarizes the differences. All the molecules of a monoclonal antibody are identical in amino acid sequence and hence in binding properties; the antibody can thus have exceptional specificity. A monoclonal antibody is insignificantly contaminated by other irrelevant antibodies, and it will therefore give a low back-

Table 2. *Summary of the properties of monoclonal and conventional antibodies*

Property	Conventional antiserum	Monoclonal antibody	
		Ascites fluid	Culture supernatant
Useful antibody content	0.1–1.0 mg/ml	0.5–5 mg/ml	5–25 μg/ml
Irrelevant immunoglobulin	10 mg/ml	About 0.5–1 mg/ml†	In principle, none*
Other serum proteins	Present	Some present†	Normally 10% (v/v) foetal bovine serum*
Binds to	All antigenic determinants of all components of immunizing material	One antigenic determinant of one component of immunizing material	
Reproducibility of specificity and affinity	Varies between batches	Invariant	
Cross-reactions with other antigens	Partial with antigens bearing common antigenic determinants	Usually absent but complete if binds to a common determinant	
Applicability of conventional immunological procedures	Applicable	May not work	
e.g. precipitation of antigen	Yes	No	
Class and subclass of immunoglobulin	Typical mixture of all	One only; may be any	
Physical properties of immunoglobulin	Typical spectrum	Individual property of antibody	
Kinetics of binding	Typical spectrum though average behaviour varies	Wide variation expected: selected by screening method	

* See text.
† Variable: contains a filtrate of serum plus a variable amount of whole serum due to haemorrhage.

usually be purified easily so that radioactive labelling and conjugation are easily accomplished and, in contrast with conventional antisera, it is easy to incorporate ^{3}H, ^{14}C or ^{35}S into the antibody molecules biosynthetically, by growing the cells in medium containing labelled amino acids. A monoclonal antibody can be reproduced and distributed indefinitely and its properties will always be the same. Hence radioimmunoassays and related techniques may be permanently standardized on an international basis. The antibody may even replace the antigen as a standard. Although monoclonal antibodies are expensive to make and characterize in the first instance, the cost of continued production is comparable with, or probably less than, the cost of producing conventional antisera. Quality control may also be easier because the presence of the monoclonal antibody can be checked, for example, by its characteristic isoelectric focusing pattern and its activity measured by a convenient titration. So, if the initial cost can be covered, it is to be expected that monoclonal antibodies will gradually replace many conventional commercial antisera.

Limitations

Monoclonal antibodies have some important limitations. For example they generally do not work in that most basic of immunological tests, the Ouchterlony double-diffusion assay, in which antigens and antibody are allowed to diffuse towards each other in agar to form a precipitate. However, their limitations can usually be overcome provided they are understood.

A classical antiserum contains antibodies to a number of antigenic determinants on its target antigen, whereas a monoclonal antibody will only bind to one determinant. An antiserum therefore provides quite a precise identification of its target antigen: an unknown molecule that can compete for all its antibody molecules, as in a classical radioimmunoassay, will almost certainly be identical to the known target antigen. In contrast, a monoclonal antibody is unable to distinguish between a group of different molecules that all bear the antigenic determinant it recognizes, or even between determinants that have a sufficient structural similarity to bind the antibody. In a radioimmunoassay for a peptide hormone, where part of the peptide's sequence may be common to several hormones, such complete cross-reactions might be a serious problem (Bundesen *et al.*, 1980). Also, monoclonal antibodies will not usually precipitate their target antigen because they can only cross-link antigens into dimers rather than form a lattice–hence the failure of Ouchterlony assays. Classical antisera fix complement more readily than do monoclonal antibodies because complement requires at least two bound antibody molecules on neighbouring determinants (Howard *et al.*, 1979; see below). A solution to these problems is to use suitable blends of monoclonal

antibodies; for both precipitation (Jefferis *et al.*, 1980) and cytotoxicity (Howard *et al.*, 1979) it has been shown that a mixture of two monoclonal antibodies is sufficient.

A conventional antiserum contains a variety of antibody molecules of different immunoglobulin class, with different physical properties, different affinities for antigen, etc. Many practical procedures rely on this—for example, some of the antibodies will normally be IgG and a proportion will have high affinity. A monoclonal antibody has a unique structure and so it may not satisfy some of these conditions. It may, for example, be unusually susceptible to denaturation by freezing or iodination, or it may elute from a DEAE-cellulose column at an atypical point (Mason & Williams, 1980). In assays with monoclonal antibodies it is particulary important to select appropriate concentrations of antibody and antigen, and the time and temperature of the reaction must be carefully chosen. Mason & Williams (1980) have studied the binding of three monoclonal antibodies to the rat lymphocyte. Under typical assay conditions, binding was determined by rates of association and dissociation, and did not reach equilibrium in 1h at 4°C. The antibodies had association rate constants of about 10^5–$10^6\,M^{-1}\cdot s^{-1}$, slow enough for the slowest to have a half-time of binding of 5h when the concentration of antigen was 0.5nM (10^7 thymocytes/ml). The antibodies dissociated about ten times faster at room temperature than at 4°C, though association rates changed only about 2-fold. So for example, if a high concentration of antigen is used in a screening assay, antibodies may be obtained that will bind too slowly for rapid detection of low concentrations of antigen. Mason & Williams (1980) also showed that an antibody that binds satisfactorily to a cell surface, where it can bind bivalently, may nevertheless dissociate rapidly from antigen that has been solubilized from the membrane, to which it can only bind monovalently. Such an antibody may not be suitable for purification of the antigen by affinity chromatography.

Some applications

I have chosen examples showing the variety of uses of monoclonal antibody technology.

The cell surface

To date, the fields most affected by monoclonal antibody technology have been the cell surface and the related area of separating and analysing cell populations from tissues by using cell surface antigens. The ability to make antibodies that recognize only one antigenic determinant in a mixture has made it possible to obtain antibodies to individual components of the cell surface most of which have not previously been described. The this way, and individual cell types within a population can be isolated for functional studies. A good example is the work on the rat T lymphoid cell surface by Williams and co-workers (Brown *et al.*, 1981; Brideau *et al.*, 1980; Williams, 1980).

When the plasma membrane of the rat thymocyte (immature T lymphocyte in the thymus) is analysed by SDS/polyacrylamide-gel electrophoresis, three main bands that stain for carbohydrate and are of apparent molecular weights 25000, 95000 and 150000 are seen. They are known respectively as Thy-1, W3/13 and the leucocyte-common antigen. Only Thy-1 could be purified by conventional methods. Monoclonal antibodies were made by immunizing mice with thymocyte plasma membrane, or glycoprotein fractions from it. Individual antibodies were obtained to all the three major glycoproteins and to the rat Ia antigens, but in addition antibodies to four previously unknown components of the thymocyte surface were obtained. The antibodies to the leucocyte-common antigen and W3/13 were used with remarkable success to purify the respective glycoproteins; W3/13 glycoprotein was purified 7000-fold simply by affinity chromatography on the monoclonal antibody, followed by gel filtration (Brown *et al.*, 1981).

These antibodies have also been used to determine the number of copies present per thymocyte of the glycoproteins to which they bind, and to detect them, or at least their antigenic determinants, on other cell types, i.e. brain, kidney, liver and lymphocytes, myeloid cells and erythrocytes (Williams, 1980). The three major carbohydrate-bearing glycoproteins of rat thymocytes occur on a quite restricted range of cell types: Thy-1 and W3/13 are not even present on all leucocytes; leucocyte-common antigen is not a substantial constituent of kidney, liver or brain. All of the other four glycoproteins identified are at least as restricted in their distribution (Williams, 1980).

Two of the antibodies obtained to as yet unidentified antigens, MRC OX8 and W3/25, were found to bind to complementary subpopulations of T lymphocytes (Brideau *et al.*, 1980). All mature T cells bound either W3/25 or MRC OX8, but not both. So without any prior knowledge of this subdivision of T lymphocytes, two reagents were obtained that could be used to identify and separate the two types of cell using the fluorescence-activated cell sorter*. Their role in the immune response

* In the fluorescence-activated cell sorter (FACS) a stream of cell suspension is broken into minute droplets each of which contains a single cell. A laser illuminates the stream, and the light-scattering and fluorescence characteristics of each illuminated cell are instantly determined. A cell with particular characteristics can be selected by deflecting the fall of its droplet with an electric

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