0031-6997/82/3401-0115$02.00/0
PHARMACOLOGICAL REVIEWS
Vol. 34, No. 1
Printed in U.S.A.

Hybridomas: A Revolution in Reagent Production

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JUST six years ago, Kohler and Milstein (7) developed a technique by which single antibody-secreting cells can be immortalized into permanent cell lines, called hybridomas, by fusion with plasmacytoma cell lines. The growing importance of this technology in immunology is indicated by its extensive use; for example, it is not unusual now for 25% to 50% of articles occurring in immunology journals to use hybridoma antibodies, and, more importantly, a portion of the studies could not have been done without the hybridomas. Similar trends are seen in new projects evaluated by national funding agencies. What are the advantages of hybridoma antibodies that have captured the imagination, not only of immunologists, but of scientists in all fields that use antibodies?

In this brief review, I wish to call attention to some of these advantages and in addition to point out potential disadvantages of monoclonal antibodies. Further, selected examples of the uses of monoclonal reagents will demonstrate the value of this technology. The reader wishing a more complete review of the current applications should consult several recent articles (5, 6).

Specificity, a hallmark of antibodies, is the ability of antibody binding sites to distinguish molecules with even subtle chemical differences; proteins differing by single amino acids, for example, can be readily distinguished by antibodies. However, antibodies are themselves extremely polymorphic, even when directed to a single antigenic determinant, so that normally produced antisera generally consist of large families of antibodies, each member of which binds to the determinant in different ways. This heterogeneity of conventional antisera has several drawbacks; antibodies with unwanted specificities must be removed, and no two antisera will be identical, two features that make reproducibility of reagents difficult.

Monoclonal reagents solve the reproducibility problem. Early attempts to produce monoclonal reagents by virally transforming normal antibody secreting cells were not satisfactory because of a very low transformation rate and the small amounts of antibody produced by the transformants (3). Cotton and Milstein (4) found that Sendai virus-induced fusion of two antibody-secreting cell lines derived from mice with myeloma generated hybrid cells that expressed large amounts of both immunoglobulins. Kohler and Milstein (7) then showed that normal antibody-secreting cells could fuse with a malignant plasmacytoma cell line to produce hybrids that now produced the normal antibody. Two technical modifications of this procedure, described in figure 1, have improved the methods greatly. First, fusion with a malignant cell line that has lost the ability to secrete immunoglobulin assures that the hybrid cell will produce only the normal antibody (12). Second, the use of Littlefield's drug selection technique has provided a means for selecting only hybrids produced by the fusion of a normal cell with a malignant cell (9). Figure 2 demonstrates the application of hybridoma technology to the dissection of the murine response to streptococcal group A carbohydrate (GAC) into several clones. Mice immunized with GAC produce an antibody response that is heterogeneous and unreproducible by isoelectric focusing; out of 17 antisera raised in genetically identical mice, no two are alike (right upper panel). Fusion of spleen cells from immune mice generates hundreds of hybrids, some of which produce anti-GAC antibodies that are detected by screening of culture supernatants (left upper panel). Cloning of the hybrids in soft agar is then performed (left lower panel). These clones can then be grown indefinitely in tissue culture or as tumors in vivo. Thus, a library of cell lines producing homogeneous antibodies can be generated (right lower panel). Hybridomas can be grown indefinitely and thereby produce unlimited supplies of homogenous antibodies. Secondly, hybridomas generally produce large amounts of proteins so that relatively small amounts of culture fluid or ascites can provide considerable reagent. The result of this should be that scientists in different laboratories will be able to use exactly the same hybridoma-produced reagents now and in the years to come. Further, industries that require standard reagents in bulk, like hospital diagnostic laboratories, will be able to do so with hybridomas.

Are monoclonal reagents better than heterologous antisera for the diagnostic laboratory? We examined that question by comparing one of the anti-GAC hybridoma proteins with a standard, commercially available, rabbit antiserum in the diagnostic microbiology laboratory [table 1 (10)]. The rabbit antiserum mistyped group A streptococci about 4% of the time, whereas the monoclonal reagent made no mistakes in 262 different isolates. This should not be interpreted that heterologous antisera will always be less accurate, but only that reagents currently used in diagnostic laboratories can be improved upon.

Another feature of hybridoma technology that is of considerable benefit is that impure antigens can be used equally effectively as pure preparations. Animals can be immunized with crude antigens and yet generate monoclonal reagents after hybridization. In fact, antigens that

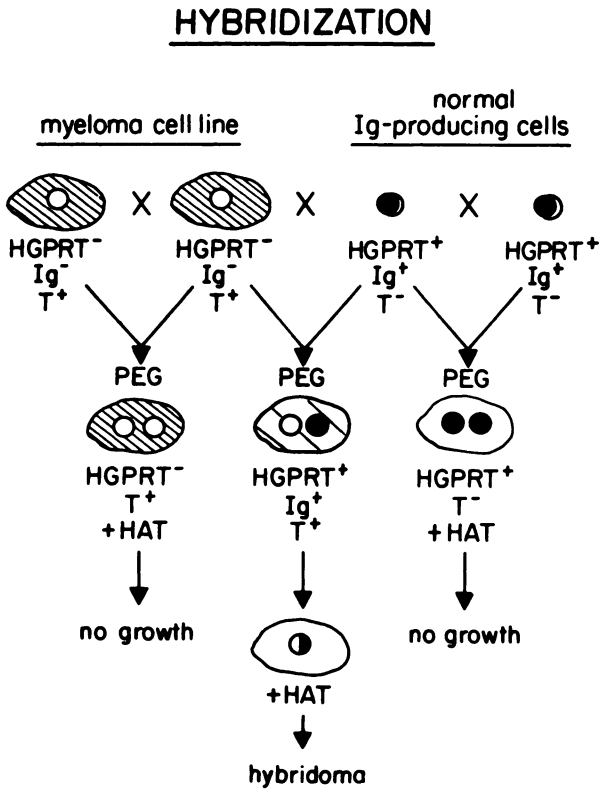

FIG. 1. Production of hybridomas. Normal lymphoid cells from immune animals are mixed with myeloma cells in culture in the presence of polyethylene glycol (PEG), which causes cell membranes to fuse and cell hybrids to form. Normal cells will not grow in culture for long (T^-) so that hybrids formed by the fusion of two normal cells will not grow. Hybrids formed by the fusion of two myeloma cells or one myeloma fused with one normal cell will grow indefinitely (T^+). Littlefield's selection method (9) allows the growth only of hybrids formed by the fusion of normal cells with myeloma cells. This is achieved by fusion with myeloma cells that lack hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme critical for the salvage pathway of DNA synthesis. The HAT (hypoxanthine-aminopterin-thymidine) selection medium blocks de novo synthesis of nucleotides and thereby forces cells to use the salvage pathway. Only those hybrids that possess both the $HGPRT^+$ and T^+ phenotype will grow; i.e., those resulting from fusion of normal and myeloma cells. An additional improvement in the technology is achieved when myeloma cells that had lost the ability to produce immunoglobulin (Ig^-) are used. This results in hybrids that produce only Ig derived from the normal cell.

are difficult to purify can be purified with monoclonal reagents generated by impure antigen preparations.

These advantages of monoclonal reagents are summarized in table 2. Also listed are several potential disadvantages. Unlike carbohydrates, which have large numbers of repeating antigenic determinants, proteins probably have only one determinant of each kind per subunit. This is no concern when using heterologous reagents with mixtures of antibodies to a variety of determinants, but use of monoclonal reagents may pose problems, particularly if the assay used requires cross-linking of determinants, such as immune precipitation or hemagglutination.

The second possible disadvantage is as yet poorly defined. It is known that monoclonal antibodies selected for binding to small haptenic determinants often permit the binding of other unrelated haptens to different subsites of the binding site, a phenomenon called polyfunctional binding sites (11). Richards reasoned that each antibody may be multispecific but that heterogeneous antisera are functionally monospecific because the antibody population shares a single subsite. If this idea is true, then monoclonal reagents may in a sense be less specific than heterogeneous antisera. Unexpected reactivities have been described by us in studies on the specificity of antibodies directed to the variable regions of immunoglobins (so-called idiotypic determinants) [table 3 (2)]. It is apparent that the monoclonal antibody appears to be less specific than the heterologous reagent even though it is likely that both recognize the same determinant.

Finally, because it was suspected that the average antibody has low affinity for its antigenic determinant, there was concern expressed that monoclonal reagents may not be generally useful. This seems not to be a problem, however, because antibodies of a full range of affinities have been described (1).

I would like to describe some selected applications of monoclonal antibodies as examples of the range of uses that this technology has involved. Hybridoma antibodies already have been extremely useful in many areas of immunology. Our understanding of antibody diversity has grown substantially because of hybridomas. It was through the analysis of monoclonal antibodies that the organization of immunoglobulin genes into small pieces was determined and the role of joining the pieces in many combinations to generate diversity was first appreciated (2). Monoclonal antibodies to lymphocytes have disclosed many antigens characteristic of different functional subsets (8). Dr. Stuart F. Schlossman has presented a detailed discussion of these monoclonal antibodies in a previous paper for this workshop. Before monoclonal antibodies understanding of human lymphocyte subsets was primitive at best; now, commercial kits are available that will identify the major functional classes of lymphocytes.

Cell biology in general has also benefitted through better definition of cell populations by hybridomas directed to cell surface antigens. One of the surprising results to come from this research is that antigens that may define a cell population in a species may be expressed in a different cell population in another species (13). The classic example, known before monoclonal antibodies, is the Forssman antigen with a broad, but patchy, cellular distribution that varies from species to species. A variety of other antigens with similar properties have become known as a result of the study of monoclonal antibodies. Thy-1, an antigen associated with T but not B cells in the mouse, is found on both in the rat.

The advantages to biomedicine are only beginning to be realized. Already, however, antibodies to serious pathogens like malaria, hepatitis virus, rabies, and others have been isolated and show promise for better diagnosis and possibly even therapeutic use. Tumor antigens are

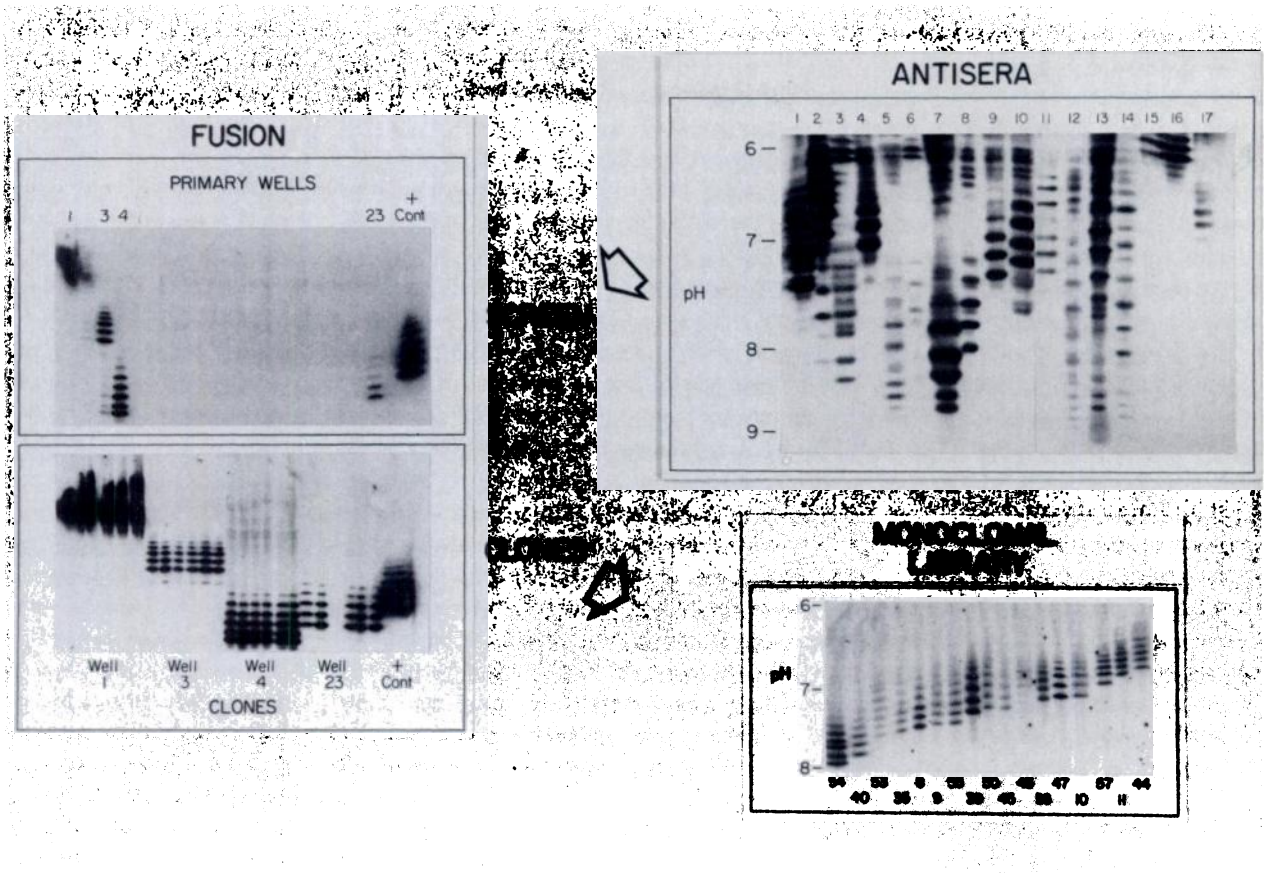

FIG. 2. Application of hybridoma technology to the dissection of the murine response to a simple polysaccharide.

TABLE 1

*Fluorescent-antibody testing of beta-hemolytic streptococci**

Streptococcal Groups (Precipitin Analysis)	F1 Monoclonal Anti-GAC		Commercial FA Reagents	
	Positive	Negative	Positive	Negative
A	113	0	77	2
Non-A	0	149	7	138

* A total of 262 isolates were tested with F1-HGAC-1 reagent, and 224 isolates were tested with the commercially prepared fluorescent-antibody (FA) reagents. Adapted from Nahm et al. (10).

TABLE 2

Monoclonal antibodies

Advantages
Monospecific
Reproducible
Unlimited quantities
Antigen need not be pure or characterized
Disadvantages
Individual antigenic determinants on proteins are rare, so that assays requiring cross-linking of molecules may not be possible.
Unexpected cross-reactions may be encountered because of polyfunctional binding sites.
Average antibody may have low affinity.

TABLE 3

Specificity of heterologous and monoclonal antibodies to a V_H determinant on murine anti- (1→3) dextran antibodies

Protein	V_H Amino Acid at Position 100–101	Anti-V_H (Idiotype IdI (J558))	
		Heterologous	Monoclonal
J558*	Arg Tyr	++	++
Hdex 31	Arg Tyr	++	++
Hdex 9	Arg Tyr	++	++
Hdex 6*	Ser His	−−	++
Hdex 12*	Gly Asn	−−	++
Hdex 3*	Arg Asp	−−	−−
Hdex 4	Lys Asp	−−	−−
Hdex 5	Ser Asn	−−	−−
Hdex 10	Val Asn	−−	−−
Hdex 14	Tyr Asp	−−	−−
M104*	Tyr Asp	−−	−−

* These myeloma and hybridoma antidextran antibodies differ from one another only at positions 100 and 101 of the V_H region. Adapted from Clevinger et al. (2).

just beginning to be examined. Human immunology has benefitted from hybridoma-derived antibodies to different functional subsets of lymphocytes, which now makes feasible the evaluation of immune status of patients. The diagnostic laboratory can expect major benefit from hybridomas; not only better reagents but new methods of testing should be achieved. One can imagine uses of hybridomas in conjunction with radiological evaluation of tumor metastases and heart attacks; organ transplants may be improved as well.

Although new technologies often generate optimism that in time proves unwarranted, hybridomas have already provided a revolution in reagent production. The

technology is now dependent on the imaginations of us all.

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