Monoclonal Antibodies in Drug Developmnet

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Dedication

John Jacob Abel was appointed in 1893 as the first Chairman of Pharmacology at the newly formed School of Medicine of the Johns Hopkins University. The name of the new department was significant: "Materia medica" previously designated only studies of the clinical efficacy of available drugs. "Pharmacology" encompassed the experimental study of basic biochemical and physiological interactions of cells and organ systems with natural and foreign substances.

The basis of Abel's program throughout was a vigorous effort in fundamental research. "It was experiments of this type—born from the interaction of chemistry, physics, and physiology—that demonstrated . . . and buttressed the view that pharmacology must encompass and exploit basic science . . . ." These efforts led Abel to found the *Journal of Biological Chemistry* in 1905 (with Dr. Christian Herter) and the *Journal of Pharmacology and Experimental Therapeutics* in 1909.

This Symposium is dedicated to the memory of John Jacob Abel.

"Monoclonal Antibodies: The Basis for a New Pharmacology" is the title of one of the manuscripts in this symposium (Haber, p. 22). It remains to be proven that this prediction is correct, but there is no lack of enthusiasm for the concept.

The basis of this enthusiasm, the gift of Köhler and Milstein to biomedical research, is the ability of scientists to control and apply one of nature's biological wonders: the antibody response to foreign molecules. The essence of this procedure is to obtain a clonal population of cells producing a single antibody molecule. Provided that a substance is antigenic, an investigator can now produce an immortal cell that secretes a highly specific and sensitive chemical probe to identify, purify, and characterize the target antigen. This may be accomplished within a few months with virtually any strong antigen. Moreover, because the clonal hybridoma cell line is selected to react with a specific antigen defined by the selection technique employed, such pure antibody probes can be prepared from impure or crude mixtures of antigens. Herein is one of the great advantages of the monoclonal technology: the ability to prepare a specific chemical probe to a defined antigen selected from a complex mixture of antigens, without prior purification or even identification of the antigen. This may apply to individual antigenic sites within a single molecule, such as antigenic epitopes or domains within a protein (Parham, p. 61), or to a specific molecule within a complex structure, such as a differentiation antigen on the cell surface (Hughes and August, p. 49). Many of the initial concerns relating to the function of monoclonal antibodies, possible problems of affinity or specificity, have now been put aside. Provided the appropriate selection procedure is applied in identifying the desired immunoglobulin, molecules of high affinity (10^9 to $10^{10}M^{-1}$) and strict specificity are readily obtained. This biological approach thus rivals, and even exceeds, chemical or physical procedures for the specific detection and quantitative analysis of molecules.

A further advantage of a biological system is the possibility of genetic manipulation. Antibodies can be "tailor-made" by the selection of mutant immunoglobulins for specific functions (Scharff, p. 3). The frequency of spontaneous heavy chain switch or gene modification in hybridomas is on the order of 10^{-5} to 10^{-4}; with mutagenesis, between 0.1% and 1.0% of the hybridomas will contain deletions. Therefore, modified immunoglobulins may readily be obtained. Scharff reminds the reader that some problems persist. One obvious *sine qua non* is that the desired target be antigenic. Antigens in crude mixtures must give rise to a reasonable frequency of responder B cells. The problem is that the yield of variable hybridomas in the

usual fusion reaction is about 10^{-5}. Therefore, beginning with 10^8 cells, a cell producing antibodies against a given antigen must comprise at least 0.1% of the splenocyte population, or a large number of fusions may be necessary. If the antigen is relatively pure, there are chemical modifications that can be used to make the most molecules reasonably antigenic.

One major application of monoclonal antibodies is to cancer biology. The search for tumor-specific antigens continues, and a number of new cell suface proteins and glyco-sphingolipids have been described (Koprowski, p. 130; Kennett et al., p. 91; Hakomori et al., p. 177). It has become increasingly clear that even monoclonal antibodies exhibiting a very restricted pattern of binding to tumor cells often react with more than one cell type (Kennett et al., p. 91; Brown et al., p. 120). The biochemical basis for these cross reactivities is unknown. Alternative possibilities that must be examined are that different cell types contain the same molecule, the same antigenic determinant is present in different molecules, or that different antigenic determinants are cross-reactive. Despite this problem, antigens have been identified that are either tumor-specific, tumor-enriched, or tissue-specific. Research is now directed at the therapeutic efficacy of such monoclonal antibodies. A prototype demonstration of monoclonal antibodies recognizing tumor-specific antigens and their application to therapy is the use of anti-idiotype antibodies directed against the hypervariable sequence of immunoglobulins produced by clonal populations of B cell lymphomas (Levy et al., p. 108). Other initial trials of monoclonal antibody therapy are in progress with a number of model and human tumor systems. IgG2a monoclonal antibodies acting with effector cells, probably macrophages, *in vivo*, are cytolytic to the targeted cell (Koprowski, p. 130; Badger and Bernstein, p. 151). There also is widespread effort to enhance cytotoxicity by conjugating the monoclonal antibody to toxic compounds (Scheinberg et al., p. 159; Uhr et al., p. 172; Hakomori et al., p. 177). One new approach, applicable both for targeting and treatment of tumors, is to conjugate the antibody with a radiometal chelate capable of accepting a variety of isotopes (Scheinberg et al., p. 159). Indium-chelate conjugated antibodies were shown to provide high resolution tumor imaging of relatively small tumors. These conjugates may also include metal isotopes releasing alpha particles of high energy, short path lengths, and short half-life. Such a conjugated antibody could selectively kill neighboring cells with high efficiency and thus eliminate modified cells that may escape killing by a drug acting only on the target cell.

Other important applications of monoclonal antibodies are to infectious diseases, bacteria, viruses, and parasites. This research will permit more rapid diagnosis of infectious organisms (Polin and Harris, p. 203) and will lead to the identification and characterization

of antigens involved in the host immune response to the organism. Extensive results already have been obtained with the malaria and schistosoma parasites (Rener et al., p. 217; Strand et al., p. 223).

This symposium series has been organized to focus on recent advances in biology and chemistry as they apply to pharmacology, particularly to drug development. The organizers are indebted to representatives of the pharmaceutical and chemical industries for the generous support that makes this series possible.

J. Thomas August

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MONOCLONAL ANTIBODIES: THE PRODUCTION OF TAILOR-MADE SEROLOGICAL REAGENTS

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Immunological assays have been used for years to detect, quantify, and localize small amounts of antigen in complex biological mixtures. The various uses of antibodies in the research laboratory are too numerous to categorize but range from detecting mutant enzymes in bacteria to analyzing the causes of malignancies in man. In the diagnostic laboratory immunologic assays are used routinely to determine levels of hormones, enzymes, plasma proteins, and drugs in the blood; to identify and classify microorganisms in body fluids and tissue samples; and for many other diagnostic tasks. In addition, antibodies have been used therapeutically in man to immunize passively against infectious agents, as antidotes to toxins, and to prevent the rejection of transplanted tissue.

Antibodies are so useful because special structural characteristics allow them to bind tightly and specifically to one of many possible antigens and simultaneously to carry out a few effector functions. The amino-terminal or variable part of each antibody contains sequences, called hypervariable regions, that fold to generate a unique antigen binding site (Fig. 1). Current estimates suggest that an individual can make between a million and a billion different antibody molecules, each with different variable-region sequences, and that the genetic mechanisms required to generate this diversity are unique to the immunoglobulin genes. On the other hand, antibodies must carry out only a few effector functions such as complement fixation and binding to macrophages. These functions are mediated by the carboxy-terminal or constant part of the molecule (Fig. 1), which is much more highly conserved than the amino-terminal part of the molecule. In fact, antibodies have only eight possible constant regions, each of which represents an immunoglobulin class or subclass that can carry a subset of effector functions. Whether antibodies are used as scientific probes, diagnostic reagents, or therapeutic tools, both antigen binding and biological function are important, and both determine the usefulness of the antibody as a reagent.

All of the uses of antibodies in biology and medicine have been complicated by the difficulty of generating large amounts of specific

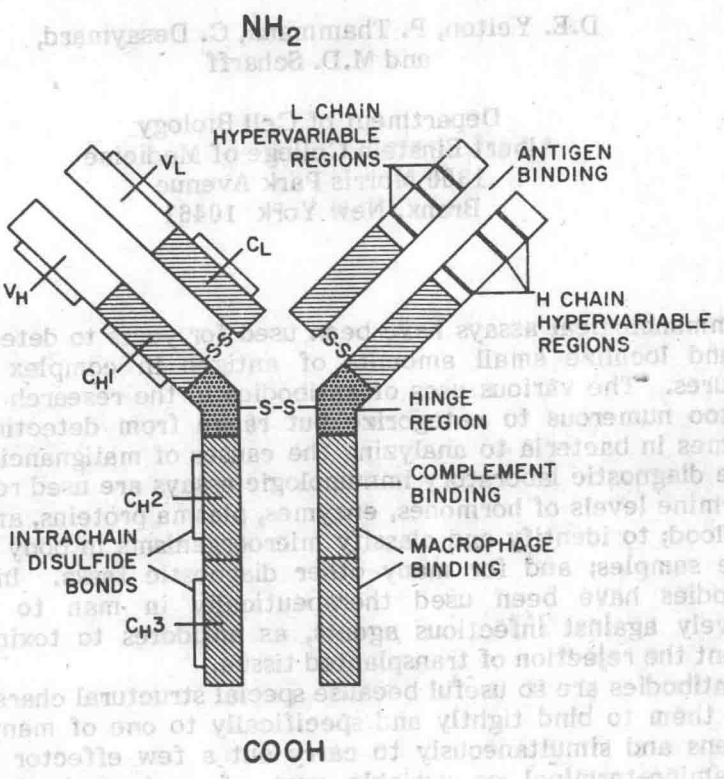

Fig. 1. The structure of immunoglobulin molecules.

antisera by conventional immunization (Table 1). Upon immunization, most individuals or animals respond with the production of different antibodies containing many different variable regions, each covalently linked to some or all of the constant regions. Because of each individual's unique history of past exposure to environmental antigens and because of the complexity of the cellular events and interactions that make up the normal immune response, the production of specific serological reagents is extremely unpredictable. Most antigen preparations are impure, and immunization results in varying amounts of antibody directed against contaminating substances. Even if a purified antigen is readily available, animals or humans immunized with it will generate a very heterogeneous array of antibodies that differ in affinity, cross reactivity with structurally related antigens, and effector functions. Such unpredictability and heterogeneity are obvious when one compares the antisera obtained from different animals or individuals and even when one compares different bleedings of the same animal.

These unavoidable differences in antisera have made it difficult to standardize immunoassays and to accumulate large amounts of antisera that can be used as reference reagents. This is especially true if the antigen is a weak immunogen and does not induce production of large amounts of antibody in recipients or if the antigen is impure or poorly characterized. Tumor antigens provide an example of these problems (1). The biology of malignancy has led investigators to believe that there are chemical differences in the surface components of malignant and normal cells. To confirm this and to obtain a simple way of identifying malignant cells, such cells have been injected into heterologous hosts such as rabbits. The rabbit produces antibodies against the many surface and intracellular components foreign to it. The resulting antiserum is then absorbed extensively with normal cells, preferably with those having the same genetic constitution as the malignant cells. Any residual (unabsorbed) antibody is assumed to be tumor-specific, an assumption that can be confirmed by examining the antibody's reactivity with other malignant and normal cells. Because of the many antigens present and their relatively poor immunogenicity, the amount of residual tumor-specific antibody is small and, after it is used in the experiments required to confirm its specificity, there is usually not enough antibody left to share with other investigators or to use in clinical trials. While some of these problems have been overcome by immunizing large animals and pooling sera, in the end there is never enough antibody, and this has discouraged many investigators from pursuing the problem.

While the heterogeneity and unpredictability of the immune response result from its inherent complexity, the problem can be thought of as arising from the polyclonality of the normal antibody response. Each antibody-forming cell and its progeny make antibodies with identical variable regions, and many different clones of cells respond to a given antigen, resulting in many different antibodies. One potential solution to the heterogeneity of the immune response was to reproducibly stimulate only one of the many clones that could produce antibody reactive with a given antigen. An animal

Table 1. Problems with conventional immunization

1. Unpredictability of the immune response
2. Immunogenicity of minor contaminants
3. Heterogeneity of even highly specific antibodies
a. Affinity
b. Cross reactivity
c. Effector functions
4. Supply often limited