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Prospects for Human Monoclonal Antibodies: A Critical Perspective

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Monoclonal antibodies have proved useful in detecting antigenic variation at single determinant sites on complex antigens, a fact which seems to have engendered a common misperception that a monoclonal antibody is necessarily a "monospecific" agent. Yet there exists considerable evidence that individual antibody molecules are multispecific in their binding capabilities, and that the amount of functional and genetic redundancy in mammalian immune systems may be quite large. The implications of this data for the future of monoclonal antibodies are emphasized, along with a brief review of present progress toward human monoclonal antibodies.

In a festschrift honoring Dr. Dorothy Horstmann, it seems particularly appropriate to attempt a critical analysis of a popular subject. Those who have known Dr. Horstmann respect her reverence for the study of basic scientific and pathogenetic principles, rather than therapeutic adventurism based upon variant interpretations of conventional wisdom. It is in this spirit that the present review is undertaken.

Academic readers are not likely to have escaped the conventional wisdom about monoclonal antibodies, which have been called "the smart bombs of biology" [1]. But few in medicine have a basis in training or experience for forming a critical appreciation of the likely uses and limitations of these new agents. In this paper, I will discuss relevant studies in biophysical chemistry and immunogenetics which suggest that monoclonal antibodies, particularly in complex biomedical applications, may often behave considerably less specifically than traditional heterogeneous antisera. Then I will briefly review present progress toward obtaining human monoclonal antibodies *in vitro*. Finally, based upon the reviewed evidence, I will suggest some cautionary views toward their use in medicine.

A MONOCLONAL ANTIBODY MAY NOT BE A MONOSPECIFIC ANTIBODY

Precise knowledge of antibody structure, function, and molecular variability is essential to an intelligent evaluation of the future of monoclonal antibodies. Detailed studies in the biophysical chemistry and the genetics of antibody molecules, which have traditionally been conducted in separate quarters, are beginning to sound similar refrains. Data from both types of analysis have tended to refute the traditional notion of the antibody molecule as being, somehow, the most specific of

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all biologic ligands. This notion arose, in part, from the wonderfully exacting work of Landsteiner [2], who demonstrated that antisera raised in animals to any of a variety of inducing antigens exhibited, in general, exquisite specificity for the inducing antigen, when tested in serologic reactions with that antigen and with related or unrelated antigens. From Landsteiner's work, many inferred that individual antibody molecules, which comprise the antisera, must themselves be exquisitely specific in their binding activity. In light of the apparent ability to induce specific antisera to an almost unlimited variety of antigens, a problematic implication of this classical notion about antibody molecules was that their variety must be almost infinite.

The first investigator to publicly refute the classical notion of the specificity of antibody molecules was Talmage, who in 1959 articulated the idea that individual antibody molecules are likely to be multispecific in their binding functions [3]. Talmage argued that an antiserum, which comprises many different antibody molecules, appears specific for the inducing antigen simply because each of the antibodies in the antiserum has a significant binding affinity for determinants on the inducing antigen, while other binding capabilities of each of the individual antibody molecules are generally not seen because they are generally not held in common by many of the molecules, so can not be detected above the threshold of activity measurable in the usual serologic assays.

The idea that multispecific antibodies can, collectively, yield a pauci-specific antiserum has been slow to assume a prominent place in everyday immunologic thinking, in part because actual data supporting it was not readily available, and the traditional concept, based on Landsteiner's work, was more firmly entrenched and perhaps more attractive. However, experimental progress on this issue has been made in the past 10 to 15 years, and because of the obvious implications for monoclonal antibody work, the evidence offered for antibody multispecificity by biophysical chemists and immunogeneticists will be briefly reviewed here.

In trying to show by straightforward combinatorial arithmetic that as few as 5,000 different antibody molecules could account for the essentially unlimited number of binding "specificities" seen with traditional antisera, Talmage made the assumption, based on little data at that time, that each distinct antibody molecule might be able to combine with 0.01–1.0 percent of all possible antigens, "at some arbitrarily low level of affinity" [3]. Several types of experimental data have shown this estimate to be surprisingly accurate. Perhaps the best evidence comes from the study of purified myeloma immunoglobulins. During the past 15 years, a number of myeloma proteins from human and murine sources have been screened for binding activity to a wide assortment of relatively simple compounds. Even with the relatively low number of myeloma proteins tested (about 1,000–1,500), some binding functions have been noted to occur repetitively, with the two most common types being binding to dinitrophenyl compounds and phosphorylcholine derivatives [4]. Furthermore, the extensive work of F.F. Richards and co-workers at Yale, has led Richards to estimate that, for a given antibody, about one in 20 "compounds off the shelf" will exhibit weak binding activity with the antibody combining site (association constant $K_o \sim 10^3\ M^{-1}$), while about one in 140 to 200 compounds will bind with a fairly high affinity to the antibody ($K_o \geq 10^5\ M^{-1}$) [5,6,7; and F.F. Richards, personal communication]. These energies of interaction are in the range of those which occur during the exercise of biologic function. The multispecificity of an antibody combining site can occur because different antigens may bind at different, though

sometimes spatially overlapping, points within the combining site [6]. Thus, individual antibody combining sites may exhibit significant affinity for a number of different antigenic determinants. These determinants may be structurally related, or completely unrelated. It has been pointed out that what matters is not the closeness of fit, but rather the total interaction energy [7,8]. Richards has proposed the term "linked specificities" to describe the phenomenon of a given antibody species reacting with two or more structurally dissimilar antigenic determinants [6].

In a brief review, it is not possible to cite all the biophysical evidence gathered to date for multispecificity of antibody combining regions, or for the redundancy of particular binding functions within the array of specificities exhibited by murine and human antibodies. For more detailed analysis, the reader is referred to reviews by Richards et al. [7] and by Inman [8]. At this point, however, it is appropriate to review recent immunogenetic studies which demonstrate a considerable degree of redundancy in the structure of the genes that encode antibody molecules.

One of the central facts to emerge from immunogenetics is that antibodies exhibit a high degree of structural homology, both intramolecularly and intermolecularly. The former type of homology is seen as the three-dimensional structural similarity of the various (four to five) 100 amino-acid domains of the antibody molecule to each other. The latter is evident in the amino-acid sequence homology of corresponding domains among different antibody molecules. Among the domains of the antibody molecule, the greatest sequence variability is seen in the amino-terminal domain. This so-called variable-region domain, where antigen binding occurs, comprises the amino-terminal 110 amino-acids of the heavy chain (V_H) and the amino-terminal 110 amino-acids of the light chain (V_L). Extensive amino-acid sequence data on V_H and V_L sequences has shown that different V_H or V_L sequences within the same species infrequently differ by more than 30 percent of the amino-acid residues, and the differences are often considerably less than that [9,10]. Not surprisingly, then, if one uses the nucleic acid sequence of one V_L or V_H region as a probe to identify similar sequences in murine or human germline DNA, the probe will generally cross-hybridize to 4 to 20 genes in the germline DNA [11,12]. Limited data suggest that these closely related, cross-hybridizing genes generally are located close together in the genome [11]. These related genes may be referred to as V_H or V_L subsets. It should be noted that their correspondence to the traditional V-region subgroups, recognized on the basis of amino-acid sequence data, is not yet clear. The number of distinct (non cross-hybridizing) V_H or V_L subsets is presently unknown, but seems likely to be relatively small; current estimates of the total number of V_H or V_L genes vary widely but generally run to no more than one or two thousand genes of each type, among which are scattered an indeterminate number of nonfunctional genes called pseudogenes [11,12,13,14].

The amount of functional diversity observed within a set of related V-region sequences is not yet known. It appears, however, that closely homologous sequences are often capable of qualitatively similar binding functions, as can be seen with antibodies to phosphorylcholine [15]. If there were no somatic contribution to antibody diversity—in other words, if all observed antibody V_H and V_L sequences were found to be encoded in the germline—then the preceding data implies that the number of qualitatively distinct antibody combining regions would be no greater than the product of the numbers of functional V_H and V_L genes, and might more closely resemble the product of the number of distinct V_H and V_L subsets, a much smaller number. Recently, however, there has been increasing evidence for somatic

mutation (the creation of altered antibody V_H and V_L sequences in developing B-cells) [16,17]; the relative contribution of this mechanism to overall antibody diversity is not yet clear, especially with regard to its impact for gross functional diversity.

Why is there so much apparent redundancy in the murine and human genes encoding antibodies? The answer lies in the evolution of antibody genes. It appears likely that all the domains of antibody molecules arose from a single 300-nucleotide (100 amino-acid) sequence which emerged during the evolution of the first vertebrates [18,19].

Thus, taken together, biophysical and genetic studies suggest that the structural diversity of antibodies may be considerably less than was seemingly necessitated by the classical notions of antibody specificity. And the functional diversity among individual molecules is presumably limited by the degree of structural diversity. It should be noted, however, that the apparent functional diversity for a given molecule or set of molecules will depend on the required energy of interaction. Low-energy interactions are common and will give the appearance of little functional distinction among antibodies, while higher energy interactions ($K_o > 10^5$ M^{-1}) will give the appearance of increasingly greater functional diversity among antibody combining regions. The importance of these considerations for monoclonal antibody work is evident, and can be reiterated as follows:

a. A monoclonal antibody may exhibit multiple different binding capabilities, and a "serum" composed solely of this antibody will exhibit each of these binding specificities, as functions of their relative affinities in the test system. A corollary is that these other specificities cannot be removed by absorption.
b. The use of monoclonal antibody preparations in increasingly complex antigenic environments increases the likelihood that unwanted binding activities will be observed, due to interactions with related (cross-reactive) or unrelated ("linked") determinants.

The likelihood of overcoming problems related to *a.* or *b.* above is related to the ability to obtain many possible monoclonal antibodies with the desired binding activity, in order to select the one whose unwanted binding activities are minimally evident in the test system. In general, antibodies with high affinity for the desired determinant are preferable, but the necessity of avoiding ligand activity with other determinants may occasionally compel the choice of a lower-affinity antibody.

HUMAN MONOCLONAL ANTIBODIES

What, then, is the current prospect for obtaining human monoclonal antibodies of such diversity as to be potentially useful? At present, it must be said that cell fusion (hybridoma) technology has yielded limited success with human cell hybrids, although such hybrids have been reported [20,21]. One of the main problems has been the lack of suitable human myeloma carrier lines to which normal antigen stimulated human B-cells could be fused [22,23]. Naturally occurring human myelomas, unfortunately, are not readily cultivable into long-term cell lines, although a few such lines have been derived. Other human B-cell lines exist, but these are either EBV-transformed lymphoblastoid lines or human B-lymphoma lines representing putatively different (generally earlier) stages of B-cell ontogeny than the myeloma cell. The utility of both of these types of human B-cell lines for cell fusion work is under current investigation, but it is possible that neither type will

generally afford the close ontogenetic "match" apparently needed for successful cell fusion work with normal B-cells [23].

The Epstein-Barr virus (EBV) itself immortalizes human B-cells. EBV-transformed lines secreting specific human antibody to various antigens have been derived [24]. Using high multiplicities of infection with transforming EBV, the potential immortalization efficiency of this method appears to exceed that of hybridoma technology [22,25,26]. Another advantage of the EBV method may be that the cell lines tend to be diploid early in culture and are generally quite stable [24,27], while hybridoma lines are aneuploid and tend to lose chromosomes, often with untoward consequences for cell viability or immunoglobulin production [22,28]. The chief disadvantage of the EBV methodology appears to be that the EBV-transformed cell line secretes quantitatively less immunoglobulin (about 2–20 μg/ml of cell culture) than hybridoma cell lines (about 10–100 μg/ml). Also, it is still an open question as to whether EBV-mediated cell transformation will show a preference for "activated" (antigen or mitogen stimulated) B-cells, as appears to be the case for the cell fusion procedure [23].

Another potential method for obtaining human monoclonal antibodies would, of course, be recombinant DNA technology, with translation of isolated immunoglobulin genes either in bacterial host cells or in a cell-free system. However, while a totally artificial system may well be the way of the future, these prospects seem remote at present, for several reasons. Antibodies are large four-chain molecules of eukaryotic origin, whose synthesis and assembly in bacteria is currently fraught with technical problems. Furthermore, antibodies undergo certain post-translational modifications which might be difficult for bacterial cells to duplicate (when necessary). Alternatively, systems based on a cell-free synthesis are relatively inefficient at present, and do not reproduce large molecules well. Work on "artificial" antibody-like ligands is likely to progress, however, as molecular subregions with distinct antigen-binding activity (or other biologic activities of interest) are identified and characterized, allowing cell-free synthetic work with more limited sequences.

CONCLUSIONS

In the present paper, I have reviewed the conceptual framework by which clinicians and biomedical research workers might anticipate some of the problematic aspects of monoclonal antibodies, as they are likely to be observed in biomedical applications. Due to the multispecific nature of antibody combining regions, and the homogeneity of monoclonal antibody preparations, unwanted binding activities are likely to be observed when one attempts to probe increasingly complex biologic systems with them. The adverse significance of these unwanted activities will, of course, vary from one application to another. However, it should be noted that for medical applications in which a monoclonal antibody is injected into a genetically polymorphic species such as man, the occurrence of unwanted binding activities in the individual recipient may be quite unpredictable. These unwanted binding activities may become more evident when monoclonal antibodies are coupled with various toxins. Additionally, the potential exists for novel derangements of the recipient's immune system, arising from anti-allotype and anti-idiotype reactions which, in genetically polymorphic recipients, may be difficult to anticipate and which are likely to be more intense than those seen in reactions to heterogeneous antisera. Thus, safety and efficacy assessments of monoclonal antibody products, for applications in outbred animal species, are likely to prove difficult.

There is no question that monoclonal antibodies have provided a convenient method by which separate determinants of complex biological antigens may be identified and analyzed [29]. The specificity and safety of a monoclonal antibody product, however, needs rigorous evaluation in every application, especially those involving medical therapeutics.

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