

Monoclonal Versus Polyclonal Antibodies: Distinguishing Characteristics, Applications, and Information Resources

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Abstract

Antibodies are host proteins that comprise one of the principal effectors of the adaptive immune system. Their utility has been harnessed as they have been and continue to be used extensively as a diagnostic and research reagent. They are also becoming an important therapeutic tool in the clinician's armamentarium to treat disease. Antibodies are utilized for analysis, purification, and enrichment, and to mediate or modulate physiological responses. This overview of the structure and function of polyclonal and monoclonal antibodies describes features that distinguish one from the other. A limited review of their use as specific research, diagnostic, and therapeutic reagents and a list of printed and electronic resources that can be utilized to garner additional information on these topics are also included.

Key Words: antibody; immunoglobulin; immunology; monoclonal; polyclonal;

Introduction

Antibodies are host proteins found in plasma and extracellular fluids that serve as the first response and comprise one of the principal effectors of the adaptive immune system. They are produced in response to molecules and organisms, which they ultimately neutralize and/or eliminate. The ability of antibodies to bind an antigen with a high degree of affinity and specificity has led to their ubiquitous use in a variety of scientific and medical disciplines. As a reagent, there is no other material that has contributed directly or indirectly to such a vast array of scientific discoveries. Their use in diagnostic assays and as therapeutics has had a profound impact on the improvement of health and welfare in both humans and animals.

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This manuscript provides an overview of antibody structure and function as well as the use of antibodies as research, diagnostic, and therapeutic reagents. Differences between polyclonal and monoclonal antibodies, with respect to their function and use, are also addressed briefly. For additional information on these topics, we refer the reader to a host of comprehensive references, including textbooks (Benjamini et al. 1996; Janeway et al. 2001), laboratory manuals (Coligan et al. 2005; Cooper and Paterson 1995; Harlow and Lane 1988, 1999; Yokoyama 1995), reference texts (Goding 1996; Gosling 2000; Zola 1999), and the National Research Council committee report (NRC 1999). We also provide in the Appendix a list of printed and electronic resources that can be used to garner additional information.

Antibody Structure and Function

Antibodies are glycoproteins secreted by specialized B lymphocytes known as **plasma cells**. Also referred to as immunoglobulin (Ig¹), because they contain a common structural domain found in many proteins, antibodies are composed of four polypeptides. Two identical copies of both a heavy (~55 kD) and light (~25 kD) chain are held together by disulfide and noncovalent bonds, and the resulting molecule is often represented by a schematic Y-shaped molecule of ~150 kD (Figure 1). Depending on the Ig class, up to five structural molecules may be combined to form any one antibody. In mammals, there are five classes of Ig (IgG, IgM, IgA, IgD, and IgE); and in avians, there are three classes (IgY, IgM, and IgA). In select mammals, IgG and IgA are further subdivided into subclasses, referred to as isotypes, due to polymorphisms in the conserved regions of the heavy chain. Ig class determines both the type and the temporal nature of the immune response.

¹Abbreviations used in this article: ADCC, antibody-dependent cellular cytotoxicity; ADEPT, antibody-directed enzyme pro-drug therapy; CDR, complementarity-determining region; ELISA, enzyme-linked immunosorbent assay; Fab, monovalent antibody fragment; Fc, crystallization fraction; FDA, US Food and Drug Administration; HRP, horseradish peroxidase; Ig, immunoglobulin; MAb, monoclonal antibody; NK, natural killer; PAb, polyclonal antibody; PET, positron emission tomography; RIS, radioimmunosciintigraphy; RIT, radioimmunotherapy; ScFv, single chain fragment variable; SPECT, single photon emission computerized tomography; TNF, tumor necrosis factor; URL, uniform resource locator.

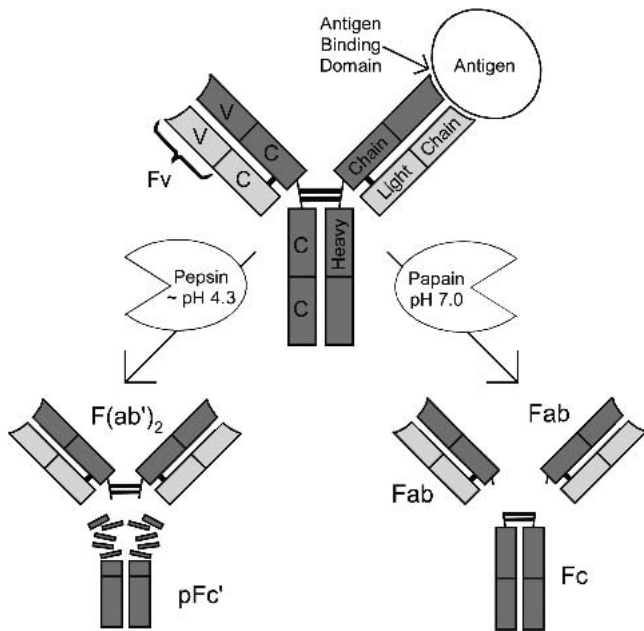


Figure 1 The basic structural molecule of an antibody consists of a “Y”-shaped structure composed of two identical heavy and light chains. Each of these chains contains multiple constant (C) and one variable (V) regions linked by disulfide bonds. The antigen-binding domains reside at the tip of the arms; their effector domains reside in the tail. For most antibodies, these domains can be separated from each other by proteolytic digestion. Under physiological pH, papain is capable of fragmenting all isotypes, irrespective of species, into Fab (monovalent for antigen binding) and Fc (effector domains) fragments by cleaving the heavy chain above the disulfide bonds that hold them together. However, pepsin cuts the molecule below this linkage, giving rise to the F(ab')₂ (bivalent for antigen binding) and various fragments of the Fc region, the largest of which is called pFc' (Andrews and Titus 1997 [see text]).

Antibodies perform two essential roles:

1. Antibodies bind to an epitope on an antigen with the arms of the Y. Each arm or monovalent antibody fragment (Fab¹) domain contains a binding site, making each antibody molecule at least bivalent.
2. The Fc domain of the Y imparts the antibody with biological effector functions such as natural killer cell activation, activation of the classical complement pathway, and phagocytosis.

Amino termini of the light and heavy chains associate to form an antigen-binding domain, and the carboxy terminal regions of the two heavy chains fold together to form the Fc domain. Light chains consist of a variable amino terminal portion of 110 amino acids and a constant region of equivalent length. Similarly, the heavy chains are also divided into variable and constant regions; however, the heavy chain has one variable and at least three constant regions, each approximately 110 amino acids long. The variable regions of

both chains bind together to form the antigen-binding domain. The three hypervariable regions in both the light and heavy chains, each five to 10 amino acids in length, constitute the actual epitope binding sites or **complementarity-determining regions** (CDRs¹). X-ray diffraction analysis has revealed that each of the variable regions forms three short loops of amino acids (hypervariable regions), with select loops from both the heavy and light chains forming the binding site.

Various mechanisms interplay to generate the sequence diversity necessary to bind a diverse spectrum of antigens, including the following: the combination of different heavy and light chains to produce the antibody's binding site, genetic recombination within hypervariable regions, imprecise joining during recombination, and a high somatic mutation rate. These mechanisms contribute to produce a vast array of coding regions and transcription of unique CDRs. Estimates indicate that mammals can produce antibodies with as many as 10¹² distinct binding domains.

The two arms (Fab) of the antibody molecule containing the antigen-binding domains and the tail (Fc¹) or crystallizable fraction are connected by a region rich in proline, threonine, and serine, known as the **hinge**. This region imparts lateral and rotational movement to the antigen-binding domains, providing the antibody the ability to interact with a variety of antigen presentations. This region, which contains the principal disulfide linkages between the heavy chains, is susceptible to proteolysis with papain or pepsin. Fragmentation of the molecule with papain, which cuts the antibody above the disulfide bridge, generates two Fab fragments and a single Fc fragment (Figure 1). In contrast, pepsin cleaves the antibody below the disulfide bridge, generating a single F(ab')₂ fragment containing both antigen-binding domains as well as a partially digested Fc region (Figure 1).

Antigen interaction is central to the antibody's natural biological function as well as its use as a research or therapeutic reagent. The specificity of the antibody response is mediated by T and/or B cells through membrane-associated receptors that bind antigen of a single specificity. Following binding of an appropriate antigen and receipt of various other activating signals, B lymphocytes divide, which produces memory B cells as well as terminally differentiating into antibody secreting plasma cell clones, each producing antibodies that recognize the identical antigenic epitope as was recognized by its antigen receptor. Memory B lymphocytes remain dormant until they are subsequently activated by their specific antigen. These lymphocytes provide the cellular basis of memory and the resulting escalation in antibody response when re-exposed to a specific antigen (see McCullough and Summerfield 2005, also in this issue).

Because most antigens are highly complex, they present numerous epitopes that are recognized by a large number of lymphocytes. Each lymphocyte is activated to proliferate and differentiate into plasma cells, and the resulting antibody response is **polyclonal**. In contrast, **monoclonal**

antibodies (MAbs¹) are antibodies produced by a single B lymphocyte clone. MAbs were first recognized in sera of patients with multiple myeloma in which clonal expansion of malignant plasma cells produce high levels of an identical antibody resulting in a monoclonal gammopathy. In the mid-1970s, Köhler and Milstein devised the technique for generating monoclonal antibodies of a desired specificity, for which they were awarded the Nobel prize (Köhler and Milstein 1975). They fused splenic B cells with myeloma cells with the resulting immortal hybridomas, each producing a unique MAb.

Antibodies recognize epitopes of varying size and may bind the epitope using some or all of its six CDRs. Binding of an epitope to its antibody is reversible and depends on precise antibody-antigen configuration. Relatively minor changes in antigen structure can markedly affect the strength of the interaction. Because antibodies recognize a relatively small component of an antigen, they can cross-react with similar epitopes on other antigens, but usually with less affinity. Antibody cross-reaction may serve as a useful research tool in that it can serve as the basis for identifying related antigens; however, this method can be confounding when recognizing epitopes on unrelated antigens. The **specificity** of an antibody refers to its ability to recognize a specific epitope in the presence of other epitopes. An antibody with high specificity would result in less cross-reactivity. With respect to native protein antigens, the binding affinity of most antibodies is influenced by conformational determinants, and antibodies may not bind the same protein in a denatured state (Nelson et al. 1997). This characteristic is particularly true of MAbs, which target a single epitope. Conformation may be altered by any number of factors, including association with other proteins, post-translational modification, temperature, pH, salt concentration, and fixation. The impact of conformational change is of less concern when using polyclonal antibodies (PAb^s). PAb^s recognize multiple epitopes, some of which are likely to be linear, and conformational changes may not influence all epitopes to the same degree.

The measure of the binding strength of an antibody for a monovalent epitope is referred to as **affinity**. The interaction adheres to thermodynamic principles and is described by the affinity constant K_A . The affinity constant describes the amount of antigen-antibody complex forming at equilibrium. Precise affinities can be ascertained for MAbs because of their homogeneous nature; however, affinity can only be estimated with PAb^s because they are composed of numerous antibodies of varying affinities. The affinity of an antibody response improves as the immune response matures due to somatic mutation in the hypervariable regions and subsequent selection and proliferation of B lymphocytes, which bind antigen with higher affinity. Antibodies with high affinity bind larger amounts of antigen with a greater stability in a shorter time than those with low affinity and are preferable for immunochemical techniques.

Whereas the affinity of an antibody reflects its binding energy to a single epitope, **avidity** reflects the overall bind-

ing intensity between antibodies and a multivalent antigen presenting multiple epitopes. Avidity is determined by the affinity of the antibody for the epitope, the number of antibody binding sites, and the geometry of the resulting antibody-antigen complexes. For example, IgG is bivalent, whereas IgM is decavalent and therefore has a higher avidity. Avidity is also assay specific and differs when the same antibodies are used in different techniques. Antigens may be multivalent, presenting multiple identical epitopes (homopolymeric), or they can present multiple distinct epitopes. Low-affinity antibodies may yield high avidity because of multivalent interactions and still be useful. MAbs function well with homopolymeric antigens when epitopes are presented in a manner that does not sterically inhibit binding. Similarly, PAb^s are useful for immunoprecipitation for complex antigens because the antibodies can bind more than one antigen molecule with the resulting antibody-antigen complex, forming a large precipitating lattice. Lattice formation is dependent on the concentration of antibody and antigen because either concentration in excess will inhibit complex formation. High-avidity antibodies present multiple sites for secondary reagent binding, an essential component of most immunochemical techniques.

Species selection is an important consideration when immunizing with mammalian proteins because a phylogenetically divergent species will generate antibodies to a larger array of foreign epitopes than closely related species. Immunization of closely related species generally results in a predominant IgM response due to the lack of T cell recruitment; however, this response may be mitigated by binding antigen to carriers or by immunizing with an adjuvant. Choice of species is relevant, particularly when producing PAb^s, because the quantity of antibody harvested is dependent on animal size. Rabbits, sheep, and goats are the most commonly used mammals based on their size, ease of vascular access, and the nature and robustness of their immune response. Of these mammals, rabbits are used most frequently to generate antibodies for research because they are easier and less expensive to house. However, their immune response is reportedly less consistent and necessitates immunization of multiple animals with the same antigen to ensure a suitable response (Harlow and Lane 1988).

As a nonmammalian species, chickens offer a number of advantages, among them phylogenetic divergence as well as the ability to easily harvest antibodies, equivalent to mammalian IgG, from the yolk (IgY) of the egg without blood collection. The quantity of IgY harvested from a week's worth of eggs is significantly greater (up to 10-fold) than that obtained from rabbit blood collected during an equivalent period (Gassmann et al. 1990). Whereas mice are the predominant species used to generate MAbs, they are used less frequently to generate PAb^s because of their small size and associated blood volume. However, a technique has been described for generating PAb^s as ascites in mice by injecting tumor cells intraperitoneally into immunized mice (Kurpisz et al. 1988; Overkamp et al. 1988).

Polyclonal Versus Monoclonal Antibodies

The decision regarding whether to use a PAb or MAb depends on a number of factors, the most important of which are its intended use and whether the antibody is readily available from commercial suppliers or researchers. PABs can be generated much more rapidly, at less expense, and with less technical skill than is required to produce MABs. One can reasonably expect to obtain PABs within several months of initiating immunizations, whereas the generation of hybridomas and subsequent production of MABs can take up to a year or longer in some cases, therefore requiring considerably more expense and time. The availability of an "off the shelf" reagent eliminates the issues of time and, frequently, cost.

The principal advantages of MABs are their homogeneity and consistency. The monospecificity provided by MABs is useful in evaluating changes in molecular conformation, protein-protein interactions, and phosphorylation states, and in identifying single members of protein families. It also allows for the potential of structural analysis (e.g., x-ray crystallography or gene sequencing) to be determined for the antibody on a molecular level. However, the monospecificity of MABs may also limit their usefulness. Small changes in the structure of an epitope (e.g., as a consequence of genetic polymorphism, glycosylation, and denaturation) can markedly affect the function of a MAB. For that reason, MABs should be generated to the state of the antigen to which it will eventually need to bind. In contrast, because PABs are heterogeneous and recognize a host of antigenic epitopes, the effect of change on a single or small number of epitopes is less likely to be significant. PABs are also more stable over a broad pH and salt concentration, whereas MABs can be highly susceptible to small changes in both. Another key advantage of MABs is that once the desired hybridoma has been generated, MABs can be generated as a constant and renewable resource. In contrast, PABs generated to the same antigen using multiple animals will differ among immunized animals, and their avidity may change as they are harvested over time. The quantity of PABs obtained is limited by the size of the animal and its lifespan.

PABs frequently have better specificity than MABs because they are produced by a large number of B cell clones each generating antibodies to a specific epitope, and polyclonal sera are a composite of antibodies with unique specificities. However, the concentration and purity levels of specific antibody are higher in MABs. The concentration of specific antibody in polyclonal sera is typically 50 to 200 $\mu\text{g/mL}$, and the range of total Ig concentration in sera is between 5 and 20 mg/mL . In comparison, MABs generated as ascites or in specialized cell culture vessels are frequently 10-fold higher in concentration and of much higher purity.

MABs are not generally useful for assays that depend on antigen cross-linking (e.g., hemagglutination) unless dimeric or multimeric antigens or antigens bound to a solid phase are used. Additionally, they may not activate comple-

ment readily because activation requires the close proximity of Fc receptors. Modification of antibodies by covalently linking a fluorochrome or radionuclide may also alter antibody binding. This potential is less of a concern when using PABs, which recognize a host of epitopes, but it can be significant for MABs if the change affects its monospecific binding site.

Many of the disadvantages of MABs can be overcome by pooling and using multiple MABs of desired specificities. The pooled product is consistent over time and available in limitless quantity. However, it is frequently difficult, too expensive, and too time consuming to identify multiple MABs of desired specificity.

Applications

The ability of antibodies to selectively bind a specific epitope present on a chemical, carbohydrate, protein, or nucleic acid has been thoroughly exploited through the years, as evidenced by the broad spectrum of research and clinical applications in which they are utilized. Applications include simple **qualitative and/or quantitative analyses** to ascertain the following: (1) whether an epitope is present within a solution, cell, tissue, or organism, and if so, where; (2) methods to facilitate **purification** of an antigen, antigen-associated molecules, or cells expressing an antigen; and (3) techniques that use antibodies to **mediate and/or modulate physiological effects** for research, diagnostic, or therapeutic purposes. The applications listed in Table 1 are by no means exhaustive, but serve to illustrate that the versatility of an antibody is frequently limited only by the imagination and determination of the user.

Analysis

Immunoblots and immunoprecipitation are two basic methods by which antibodies are used to establish whether an antigen or related molecule is in a prepared solution (i.e., cell or tissue lysate) (Bonifacino et al. 2001; Gallagher et al. 1998; Harlow and Lane, 1999). Immunoblots involve transferring soluble antigen(s) onto a suitable membrane (nitrocellulose or positively charged nylon/polyvinylidene fluoride [PVDF]), blocking the membrane to prevent subsequent nonspecific binding, and then probing it with an antigen-specific antibody (primary antibody). The primary antibody-antigen complex is then identified by incubating the blot with a secondary antibody against the isotype of the primary antibody, which is conjugated to an enzyme (i.e., horseradish peroxidase [HRP¹]) or radionuclide-labeled antibody to facilitate detection. Western blots (westerns) are immunoblots preceded by protein separation, usually based on size, utilizing a polyacrylamide gel. In immunoprecipitation, the primary antibody binds the antigen in the solution and then the antibody-antigen complex is isolated from the supernatant by centrifugation after addition of inert beads coated with bacterial protein A, G, and/or L, which bind the

Table 1 Research and clinical antibody applications^a

Purpose	Applications relative to antigen context		
	Solubilized	Intact cells/tissue (live/preserved)	Organism (in vivo)
Analysis (qualitative or quantitative)	Immunoblot (Western blot)	FACS ^b analysis	Immunoinaging (SPECT ^b and PET ^b)
	Immunoprecipitation		
	Sandwich ELISA ^b	Immunofluorescence	
	ELISPOT ^b		
	Proteomics/antibody microarray	Immunohistochemistry	
	X-ray crystallography		
Purification and/or enrichment	Immunoaffinity purification	FACS and MACS ^b	
Mediation and/or modulation	Catalysis-abzymes	Neutralize activity	Neutralize activity
		Activate signaling	Deplete cell types to alter phenotype
		Proteomics/intrabodies	Immunotherapy

^aA selective list of applications in which polyclonal (PABs), monoclonal antibodies (MAbs), their fragments and conjugates, which either play an essential role or have had a significant impact in basic research or the clinic. With the exception of imaging, immunotherapy, immunohistochemistry, and x-ray crystallography, the choice whether to use PAB or MAb depends on the context in which the application is being used and the technical abilities of the personnel using them.

^bELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot assay; FACS, fluorescence-activated cell scanning; MACS, magnetic-activated cell sorting; PET, positron emission tomography; SPECT, single photon emission computerized tomography.

primary antibody. The Fc portion of the primary antibody binds these bacterial proteins in a species- and isotype-dependent manner. By combining these techniques, “IP/westerns” can be used to increase the sensitivity of the assay system as well as identify molecules that are associated with the initial antigen.

The enzyme-linked immunosorbent assay (ELISA¹) is another basic application used to analyze soluble antigens (Hornbeck 1991). This approach allows the simultaneous processing of many small samples. It requires two antigen-specific antibodies. One antigen-specific antibody is coated onto a solid substrate (typically a 96-well plate) to capture the antigen from the applied solution while the other is used to detect the immobilized antigen. As with immunoblots, a HRP-conjugated secondary antibody is normally used for detection purposes. The capture and primary antibodies must be different isotypes, if not from different species, so that the secondary antibody will only detect the presence of the primary antibody and correctly indicate that the antigen has been captured in the well.

The enzyme-linked immunospot assay (“ELISPOT”) is an application that determines whether an individual cell is actively secreting a cytokine (Klinman and Nutman 1994). It also utilizes sandwich-based methodology, one antibody for capture and another for detection, both specific for the

same antigen. In contrast to the sandwich ELISA, a membrane support is used. Once the membrane is coated with one of the antibodies and blocked from any further nonspecific binding, live cells are incubated on top of the membrane, where the secreted cytokine is captured by the membrane-bound antibodies immediately under and near each cell. After the cells are removed, the immobilized cytokine can be detected by the other antigen-specific antibody and, if necessary, followed by an appropriate HRP-conjugated secondary antibody. This assay is 20- to 200-fold more sensitive than the ELISA because the cytokine can be captured soon after it is secreted from the cell before it becomes diluted in the media.

Recently, an approach for high throughput proteomic analysis has been developed utilizing an **antibody microarray** platform (Michaud et al. 2003; Nielsen and Geierstanger 2004). A prototype for simultaneously analyzing the tyrosine phosphorylation states of various proteins from a cell lysate has recently been published (Eisenstein 2004; Gembitsky et al. 2004). The technique involves immobilizing antibodies specific for different proteins to distinct locations on a chip, allowing them to capture their respective proteins from the applied cell or tissue lysate, and then probing with a fluorophore-conjugated antiphosphotyrosine antibody.

X-ray crystallography has also benefited from the use of antibodies. The binding of Fab fragments to a multitransmembrane ion channel has made it easier to crystallize the channel. It is thought that the tight structure of the Fab provided a scaffold on which the presumably floppy ion channel could align and form a cocrystal (Jiang et al. 2003; Zhou et al. 2001). MAbs are preferred for this application because a homogeneous reagent is necessary.

Antibodies are also powerful tools for analyzing antigens in the context of cells, either as a single cell suspension or as immobilized cells/tissue sections. With flow cytometry or **fluorescence-activated cell scanning** ("FACScan") analysis, suspensions of individual cells are stained with a variety of distinct fluorophore-conjugated antibodies that are subsequently streamed past a laser and detector system capable of exciting and determining which fluorophores, and thus which antigens, are expressed on/in individual cells (Givan 2001). Because cell surface protein profiles of hematopoietic cells are well characterized, one can assess the number and various types of hematopoietic cells with relative accuracy in both the research and clinical settings.

The spatial expression of an antigen relative to an individual cell, or in the context of whole tissue, can be analyzed with antibodies using **immunofluorescence** and **immunohistochemistry**, respectively (Harlow and Lane 1999). Both applications involve preparing samples (cells or tissue sections) in a manner that retains their three-dimensional structure, immobilizing them on glass slides, probing them with antibodies and visualizing the antigen-antibody microscopically. The antibodies used in these applications are either conjugated to fluorophores that emit light when excited by light of the appropriate wavelength or are conjugated to an enzyme, such as HRP, which produces a detectable color when a chromagen is present. PABs are used more frequently in these applications for two main reasons: (1) They recognize multiple independent epitopes and therefore have a better chance of binding epitopes that are still available in fixed samples; and (2) it is generally impractical to screen hundreds to thousands of cultures for MAbs that work in immunohistochemistry. The use of PABs can result in nonspecific background staining; however, affinity purification, using the desired antigen immobilized on a solid support, can be used to minimize or eliminate the problem. Background staining may also result from binding of the antibody's Fc region to Fc receptors in the sample. In this circumstance, Fab or F(ab')₂ fragments that lack the Fc region are useful since they cannot be bound by the Fc receptor. In addition, a panel of MAbs can be tried.

Purification/Enrichment

Antibodies are also used in the **purification/enrichment** of antigens, antigen-associated molecules, or cells expressing the antigen. For soluble proteins and associated molecules, purified antibodies are usually covalently linked to an inert resin and incubated with the sample from which they are to

be purified. After washing away unbound molecules, the proteins are stripped off the resin using conditions that minimize protein denaturation. This technique can be performed in batch or by chromatography (Harlow and Lane 1999; Springer 1996). Subpopulations of cell suspensions can also be positively (enrichment) or negatively (depletion) selected by using antibodies against specific cell surface antigens. For example, **panning** (Hollenbaugh et al. 1995) captures cells using antibody-coated plates. If depletion is required, the plate is coated with an antibody against the unwanted population and the unbound desired population is washed off the plate. If enrichment is the goal, antibodies directed against an antigen expressed on the desired population are used to capture the desired cells on the plate. Most of the unbound cells are removed by gentle washing while an enriched population of the bound cells are subsequently recovered by vigorous pipetting.

Fractionating populations of cells into different groups based on the antigens they express can also be accomplished using a **fluorescent-activated cell sorter** ("FACSsort"). An appropriately configured instrument can distribute cells with a desired fluorescent profile into predetermined pools, thus purifying various cell subpopulations based on the specific antigen-antibody-fluorophore complex(es) on their surface (Givan 2001). Another approach, known as **magnetic-activated cell sorting** ("MACS"), uses superparamagnetic particles coupled to MAbs (microbeads) to separate antibody-bound cells from other populations (Horgan and Shaw 1995; Miltenyi et al. 1990; Thornton 2003). A magnetic field is applied to retain the microbead bound cells while unbound cells are washed away. The desired cells/microbeads are released by removing the magnetic field. An advantage is that this approach can be accomplished in a fraction of the time required by a cell sorter.

Mediation/Modulation

One of the more remarkable applications for antibodies involves a category of antibodies referred to as **abzymes** or **catalytic antibodies**. Since the mid-1980s, abzymes capable of mediating the catalysis of specific synthetic organic reactions have been generated by immunizing animals with a chemical structure that mimics the energetically unfavorable transition state. Because small chemicals like haptens cannot stimulate an immune response themselves, the chemical immunogen is coupled to a "carrier" molecule like keyhole limpet hemocyanin protein, a respiratory pigment found in molluscs and crustaceans that is highly immunogenic in vertebrates. An overview of the current strategies used to generate as well as screen for abzymes has recently been published (Xu et al. 2004). MAbs are most frequently used because of their homogeneous nature and high specificity; however, PABs have also been used as catalytic antibodies.

Antibodies can also be designed to target an antigen within live/intact cells. This process is accomplished by

genetically engineering the antibody to be expressed intracellularly rather than being secreted, thus availing the antibody an opportunity to knock out a specific protein or molecule within a cell functionally. Antibodies expressed under these conditions are referred to as **intra-antibodies** or **intrabodies**. One promising application using this strategy involves expressing single chain fragment variable (scFv¹) antibodies intracellularly, which are genetically manipulated antibodies that contain only the variable regions of the heavy and light chain responsible for antigen binding in one continuous polypeptide chain. This technique can be accomplished by transfecting tissue culture cells with a gene coding for the scFv. Studies are under way to generate functional intrabodies systematically from established hybridomas (MAb source), hyperimmunized animal spleens (PAb source), or phage display antibody libraries. The goal is to knock out intracellular pathways in a highly targeted manner, rather than knocking out the gene or the RNA with iRNA. Antibodies can be utilized to target an individual epitope or post-translational modification rather than the entire protein (Visintin et al. 2004).

Whether in the context of cell culture, live animals, or human patients, antibodies can **neutralize** (disrupt) or **activate** (stimulate) normal cellular signaling by simply binding their corresponding antigen. For example, to prove that a cytokine is mediating a response in vitro, excessive molar ratios of an antibody can be added such that the antibody outcompetes binding of the native cytokine for its receptor and neutralizes its function (Buza et al. 2004; Sivashanmugam et al. 2004). Although it is also possible to block ligand binding with an antireceptor antibody, the antibody itself may mimic ligand binding and activate the receptor. Alternatively, the bivalent antibody may cross-link the receptors, a mechanism by which many receptors are naturally activated by their ligands. For example, antibodies can be used to activate B and T cells to proliferate in culture (Koike et al. 2003; Kruisbeek et al. 2004; Mond and Brunswick 1991). Incubation of B cells with anti-IgM or T cells with anti-CD3, anti-T cell receptor, or anti-Thy-1 is sufficient to mediate cross-linking of the respective cells surface antigens and stimulate an intracellular signaling cascade, which results in cell growth. This functionality holds true in vivo as well. MAbs have been successfully used to neutralize IL-17 (Linden 2002), anthrax lethal factor (Zhao et al. 2003), and *Clostridium botulinum* neurotoxin type B (Yang et al. 2004). The ability of Remicade® (infliximab) to neutralize tumor necrosis factor (TNF¹)-α in patients makes it potentially valuable in treating Crohn's disease (Kirman et al. 2004).

Antibodies can be used to create animal models that lack one or multiple cell types. There are several advantages of using antibodies in lieu of genetically engineering a “knock-out” model. First, because the antibody is administered to an existing animal, there is no need to generate a genetically engineered model, which may not even have been feasible. Second, the depletion is reversible, assuming the cell type is naturally replenished. This approach has been successfully

used in vivo for T cell depletion using MAbs directed against CD4 (GK1.5), CD8 (53-6.72 or 2.43), CD3 (145-2C11 or KT3), and/or CD2 (BTI-322 as well as its humanized version) (Kruisbeek 2003; Mottram et al. 2002; Snanoudj et al. 2004). The caveats to this approach are that one cannot assume 100% efficacy and that large quantities of antibodies are necessary (100-500 μg/mouse).

If developing a **genetically engineered model** lacking a specific cell population is desirable, the gene coding for an antibody known to bind to and delete the targeted population can be knocked in and permanently expressed to generate mice that are deficient for the appropriate cells. This technique has been used to generate a natural killer (NK¹) cell-deficient mouse by engineering plasma cells to express the heavy and light chains of the anti-NK1.1 MAb PK136, which has been shown to deplete antibody-specific NK cells when administered in vivo (Yuan et al. 2004).

Immunotherapy and Imaging

Despite the vast use of antibodies in basic research, their translation into the clinic, especially as **immunotherapeutics**, has only recently begun to meet the expectations of a “magic bullet” put forth more than a century ago by Ehrlich (Winau et al. 2004). These expectations were based on the proven principle that passive/serum immunotherapy could bestow protection against infectious agents such as *Corynebacterium diphtheriae*. The prospects of transferring polyclonal serum from an immune-protected animal/human to a patient were often hampered by lack of reproducibility, and toxic side effects associated with injecting foreign proteins (Llewelyn et al. 1992). It has taken years to develop the necessary knowledge to begin to harness the power held within the serum, specifically that of the antibody, while reducing adverse effects. To supplement the brief overview of immunotherapy we have provided, we urge the reader to consult the extensive literature, for details (Börjesson et al. 2004; Britz-Cunningham and Adelstein 2003; Casadevall et al. 2004; Chester et al. 2004; Francis and Begent 2003; Kipriyanov 2003; Pelegri et al. 2004; Waldmann 2003).

A variety of strategies have been deployed using antibodies to facilitate death of specific cell types, a central objective in the treatment of cancer. Once the antigen-binding domains have localized the antibody to the target cell, **antibody-dependent cellular cytotoxicity** (ADCC¹) or **complement-mediated lysis** can be initiated through the Fc region. Binding of the Fc-gamma receptor III, which stimulates ADCC, to the Fc region of Rituxan® (rituximab) [anti-CD20] and Herceptin® (trastuzumab) [anti-HER2/neu] has been shown in xenografted mice to be one of the mechanisms by which they may mediate their antitumor effects on non-Hodgkin's lymphoma and breast cancer, respectively (Baselga and Albanell 2001; Cartron et al. 2004; Clynes et al. 1998, 2000; Waldmann 2003). Herceptin also causes down-regulation of the receptor, resulting in a decrease in cell growth. Alternatively, the antibody can be modified to

“carry and deliver” a lethal substance directly to the targeted cells. (Börjesson et al. 2004; Britz-Cunningham and Adelstein 2003; Waldmann 2003).

Radioimmunotherapy (RIT¹) involves labeling an antibody, or antibody fragment, with a radioactive isotope that causes DNA damage wherever it localizes. The stable beta emitters iodine 131 and yttrium 90 are commonly used because they exhibit desirable tissue penetrations of 2.4 and 11.9 mm, respectively. Cellular toxins such as ricin A, saponin, and *Pseudomonas* sp. exotoxins can also be attached to the antibody, which serves as a homing device. Because internalization of only a few toxin molecules is necessary to damage a cell permanently, neighboring cells are not affected directly. This method is less likely to affect neighboring “bystander” cells, which may be destroyed inadvertently with the latter method. However, a potential disadvantage to the use of an **immunotoxin** is that each cell may need to be bound by an antibody to have a curative effect. Another strategy for achieving directed cell toxicity involves **antibody-directed enzyme pro-drug therapy (ADEPT¹)**. ADEPT is a multistep technique that is initiated with the administration of an enzyme-conjugated antibody directed against the cell to be targeted. After sufficient time has elapsed to allow for clearance of unbound antibody, a pro-drug that is toxic only when converted by the enzyme is administered. Thus, therapy-induced toxicity is restricted to the site where the antibody-enzyme has been bound by the target antigen.

Although most therapeutic antibody applications have focused on endogenous targets (e.g., tumor antigens for cancer, IgE for asthma, and TNF- α for inflammatory bowel disease), **passive antibody therapy** is also being reconsidered for treating infectious diseases, specifically hepatitis B virus, rabies virus, respiratory syncytial virus, vaccinia virus, echovirus, enterovirus, and *Clostridium tetani* and *botulinum*. Minimally, this expansion of immunotherapy targets is very timely in light of the growing number of antibiotic-resistant microbes. One therapeutic MAb, CROFABTM, is even used as a rattlesnake venom antidote. To date, the US Food and Drug Administration (FDA¹) has licensed more than 12 MAbs for therapeutic purposes, and many more are the subject of active clinical evaluation/development.

Specificity, homogeneity, and the “limitless” supply of MAbs may have given new life to the concept of immunotherapy; however, rodent MAbs are not an ideal reagent for clinical applications because their constant regions are unable to activate human effector functions fully. In addition, patients frequently develop antimouse antibodies that effectively neutralize the therapeutic MAb by rapidly clearing the antibodies before it has a chance to find its target. One tactic taken to overcome this obstacle was to humanize murine MAbs by grafting the mouse regions responsible for antigenic specificity into the context of a human antibody. Other strategies have been used to address this problem, including generating MAbs in transgenic mice that carry human immunoglobulin genes and using phage display li-

braries generated from human sequences (Bleeker et al. 2004; Francis and Begent 2003; Ranson 2003).

A shortcoming of immunotherapy, when treating a chronic disease, is that therapy entails multiple administrations of the therapeutic antibody over time. Studies are under way to evaluate the possibility of having the MAb expressed within the patient either in cells grown in encapsulated “vessels” through which the MAb diffuses or, alternatively, transfecting the patient’s cells *ex vivo* to express the gene coding for the antibody stably, and then reintroducing the cells into the patient (Pelegrin et al. 2004).

Radioimmunoimaging, also known as **radioimmuno-scintigraphy (RIS¹)**, uses radionuclide-labeled antibody or antibody fragments to target cells in a patient in an antigen-dependent manner. This application was first attempted in 1948, when antitumor PABs linked to radioactive iodine were administered. This attempt was unsuccessful because the PAB failed to localize to the tumor to any significant level relative to normal tissue (Oriuchi and Yang 2001). Significant advances have occurred subsequently; however, problems similar to those observed with RIT (e.g., patients developing anti-antibody antibodies) have been reported. The FDA has approved several radiolabeled MAbs for RIS, and many more are currently in development. Oncoscint[®] (satumomab pendetide), carcinoembryonic antigen (CEA)-scan[®] (arcitumomab), VerlumaTM (nofetumomab merpentan), and ProstaScint[®] (capromab pendetide) are just a few of the reagents approved to detect ovarian and colorectal carcinoma, small cell lung cancer, and prostate cancer, respectively (Börjesson et al. 2004). Single photon emission computerized tomography (SPECT¹) and positron emission tomography (PET¹) are used for imaging. The isotopes of choice for SPECT imaging are generally ones that produce low-energy emissions and have a relatively short half-life, including iodine 131, iodine 123, indium 111, and technetium 99m. In contrast, PET utilizes positron emitters such as gallium 68 and fluorine 18 (Börjesson et al. 2004).

Information Resources for Monoclonal and Polyclonal Antibodies

A vast amount of information is available on both MAbs and PABs, including basic concepts in immunology and antibody response; antibody structure and function; antibody production techniques and protocols; adjuvants used for immunization; animal welfare concerns related to antibody production techniques; associated regulations; guidelines, policies, and proceedings; and, existing and emerging uses of antibodies in research, diagnostics, and therapeutics. Our recent “Google” searches (<http://www.google.com>) using the search terms “monoclonal antibodies” and “polyclonal antibodies” resulted in 1.59 million and 496 thousand hits, respectively. In addition to the information presented in this issue of *ILAR Journal*, a selected list of information resources is provided below. Where available, uniform resource locators (URLs¹) have been provided. Many of these

web sites provide additional URL links to other resources including text books, bibliographies, journal articles, meeting and committee proceedings, regulations, policies and guidelines, suppliers of antibody reagents and services, applications for use of antibodies, and organizations that compile information related to antibody production and use. The *Antibody Resource Page* (<http://www.antibodyresource.com>) is comprehensive and particularly valuable because it is broad in scope and provides detailed information. Additional web sites and a brief summary of the information provided on each site are also included in the Appendix.

Conclusion

Antibodies have provided and will continue to provide scientists and clinicians an extraordinarily powerful and important tool for use in the research laboratory and clinic. The unique molecular structure of the antibody by which it bivalently binds to a broad array of antigenic epitopes (on, e.g., proteins, carbohydrates, and nucleic acids) serves as the foundation of its utility. Antibodies are commonly utilized to “tag” molecules, both in vitro and in vivo, for a diverse array of functions that include identification, isolation, modification, and destruction. Antibodies can directly mediate effector functions such as cytotoxicity, or can be used to deliver effectors such as radionuclides, immunotoxins, or enzymes, which can be bound to the molecule.

The decision regarding whether to develop and use PAbs, which are relatively easy to produce in a timely and cost-efficient way, or to develop MAbs, which are homogeneous and available in a limitless supply, despite being time consuming and expensive to generate, frequently depends on the application in which the antibody will be used. Ultimately, however, the decision frequently also involves the element of chance, which dictates whether the antibody will function as desired.

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APPENDIX

General Information Resources for Monoclonal and Polyclonal Antibodies

The Antibody Resource Page (<http://www.antibodyresource.com>)

Comprehensive information resources on antibody structure and function, production, research, and clinical applications to include educational resources, immunology/biotechnology; databases/software; antibody image gallery; journals and books; how to find an antibody, antibody suppliers, custom antibody suppliers, and contract antibody services.

Animal Welfare Information Center—Books and Proceedings (<http://www.nal.usda.gov/awic/pubs/antibody/books.htm>)

Resources on immunology, antibodies, antibody technologies, protocols, and applications.

General Information, Production, and Therapeutic Applications for Use of Monoclonal Antibodies (<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/M/Monoclonals.html>)

Animal Welfare Information Center—Antibody Production Bibliography (<http://www.nal.usda.gov/awic/pubs/antibody/prodbib.htm>)

ILAR Journal Online, Volume 37(3) 1995—Adjuvants and Antibody Production (http://dels.nas.edu/ilar/jour_online.asp?id=jour_online)

Animal Welfare Information Center—Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies (<http://www.nal.usda.gov/awic/pubs/antibody/>)

Using Antibodies: A Laboratory Manual. E. Harlow and D. Lane. 1999. Cold Spring Harbor Laboratory Press. ISBN 0-87969-544-7 (<http://www.cshlpress.com/>)

Chapters include antibody structure and function, antibody-antigen interactions, choosing antibodies, handling antibodies, staining cells, staining tissues, immunoprecipitation, immunoblotting, immunoaffinity purification, tagging proteins, and epitope mapping.

Immunoassays. A Practical Approach. Gosling JP, ed. 2000. Oxford University Press. ISBN 0-19-963710-5 (<http://www.oup.com/>)

Includes basic principles in immunoassay design and development, step-by-step protocols, and information on characterizing antibodies and preparing radioactive, enzymatic, fluorescent, and other labels.

Companies and Institutions That Provide Antibody Reagents and/or Services

The Antibody Resource Page (<http://www.antibodyresource.com>)

Linscott's Directory of Immunological and Biological Reagents (<http://www.linscottsdirectory.com/#>)

The Nature Biotechnology Directory (<http://www.guide.nature.com/>)

Animal Welfare Information Center—A Selected List of Company and Institute Resources Providing New Technologies (<http://www.nal.usda.gov/awic/pubs/antibody/company.htm>)

American Type Culture Collection (<http://www.atcc.org>)

A repository and distributor of cell lines that include hybridoma and myeloma cells, and provide cell culture services.

National Cell Culture Center (<http://www.nccc.com>)

A repository and distributor of cell lines that include hybridoma and myeloma cells, and cell culture services.

General Information, Guidelines, and Recommendations

The Report of the Committee on Methods of Producing Monoclonal Antibodies, ILAR (NRC 1999) (<http://www.grants.nih.gov/grants/policy/antibodies.pdf>)

Includes information on generation of hybridomas, in vitro and in vivo production methods and advantages and disadvantages of each, large-scale production, animal welfare issues, conclusions, and recommendations. To purchase copies of this report from the National Academies Press: <http://www.nap.edu/catalog/9450.html>

Monoclonal Antibodies—The Report and Recommendations of ECVAM Workshop 23, 1997 (<http://www.nal.usda.gov/awic/pubs/antibody/ecvam.htm>)

The Production of Polyclonal Antibodies in Laboratory Animals—The Report and Recommendations of ECVAM Workshop 35, 1998 (<http://altweb.jhsph.edu/publications/ECVAM/ecvam35.htm>)

Monoclonal Antibody Production Supplement—Lab Animal, Autumn 1999

Provides an overview of the NRC report on monoclonal antibody production, perspectives on in vitro production, an IACUC guide to reviewing protocols for in vivo ascites production, small scale in vitro production methods and resources, and a selected list of company and institutional resources providing new technologies for monoclonal antibody production. Available for purchase: <http://www.nature.com/laband/contact/purchase.html>

Alternatives to Animal Testing—Special Section on Monoclonal Antibodies (<http://altweb.jhsph.edu/topics/mabs/mabs.htm>)

Provides general information, in vitro and in vivo production techniques, policies, regulations, guidelines and recommendations, where to obtain in vitro monoclonal antibodies, academic core centers and commercial facilities, other web sites, reports, and proceedings.

Applications and Uses of Antibodies

The Antibody Resource Page (<http://www.antibodyresource.com>)

Emerging Therapies: Spectrum of Applications of Monoclonal Antibodies. Hematology. American Society of Hematology. Education Program Book (<http://www.asheducationbook.org/cgi/content/full/2000/1/394>)

Monoclonal Antibodies and Therapies (<http://www.nature.com/focus/antibodies/>)

Guidance for Industry: Monoclonal Antibodies Used as Reagents in Drug Manufacturing. US Food and Drug Administration (<http://www.fda.gov/cber/gdlns/mab032901.pdf>)

Clinical and Diagnostic Laboratory Immunology. American Society for Microbiology (<http://cdli.asm.org/>)