**Lec(3) Advanced Serology**

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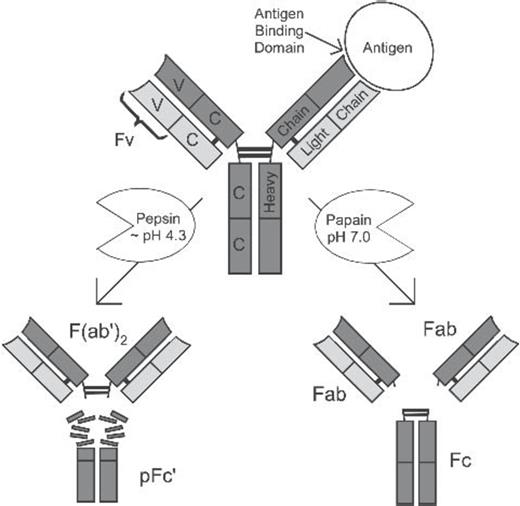
**Monoclonal Versus Polyclonal Antibodies: Distinguishing Characteristics, Applications, and Information Resources**

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.

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.

## Antibody Structure and Function

Antibodies are glycoproteins secreted by specialized B lymphocytes known as **plasma cells** . Also referred to as immunoglobulin (Ig 1 ), 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.

Fig(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′ .

**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 (Fab 1 ) 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** (CDRs 1 ). 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 10 12 distinct binding domains.

The two arms (Fab) of the antibody molecule containing the antigen-binding domains and the tail (Fc 1 ) 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 .

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 (MAbs 1 ) 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 . 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 (PAbs 1 ). PAbs 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 PAbs 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 binding 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, PAbs 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 PAbs, 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 .

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 . Whereas mice are the predominant species used to generate MAbs, they are used less frequently to generate PAbs because of their small size and associated blood volume. However, a technique has been described for generating PAbs as ascites in mice by injecting tumor cells intraperitoneally into immunized mice .

**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 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.

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 complement 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.

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 | | Immunoimaging (SPECT b and PET b ) | |
| Immunoprecipitation | |  | |  | |
| Sandwich ELISA b | | Immunofluorescence | |  | |
| 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 | |

**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

**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.

**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 . 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. 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.

**Radioimmunotherapy** (RIT 1 ) 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, .

**Radioimmunoimaging** , also known as **radioimmunoscintigraphy** (RIS 1 ), 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 . Significant advances have occurred subsequently; however, problems similar to those observed with RIT (e.g., patients developing antiantibody antibodies) have been reported.