

# Antibodies

Moncef Zouali, *Institut National de la Recherche et de la Santé Médicale, Paris, France*

Antigens are antigen-reactive proteins, designated immunoglobulins, present in the plasma and in extracellular fluids. They bind their nominal antigens with exquisite specificity and potentially neutralize their harmful effects.

## Introduction

Antibodies were the first elements of the immune system to be identified. They are antigen-reactive proteins present in an immune serum, called antiserum, and obtained after exposure of the vertebrate host to a given antigen, called an immunogen. By contrast, normal serum, also called preimmune serum, does not contain antibodies specific for that antigen. As the overwhelming majority of antibody activity proved to be associated with the  $\gamma$ -globulin peak seen in fractionation of serum proteins, immune globulins were designated immunoglobulins. Characteristically, antibodies, which are found in the plasma and in extracellular fluids, bind their nominal antigens with exquisite specificity and potentially neutralize their harmful effects.

## Antibody Classes and Subclasses

Serological studies have led to the description of three important determinants on the antibody molecule. First, idiotypes reflect the uniqueness of structural features of the variable region determinants specific for an individual antibody, or idiotypic. Second, allotypes are antibody characteristics reflecting allelic polymorphisms that differ

between individuals of the same species. Third, isotypes designate antigenic determinants present on antibodies shared by all individuals of a given species.

In humans, five classes of immunoglobulins, also called isotypes (IgG, IgM, IgA, IgD and IgE), differ in their physicochemical and serological properties, and in the amino acid sequence of their constant regions (**Table 1**). In their monomeric form, all classes exhibit the same basic structure: two heavy (H) and two light (L) chains. While IgG, IgD and IgE consist of a single monomeric unit, the IgM and IgA classes are composed of monomers that associate to form pentamers and dimers, respectively. Formation of these polymers requires an additional polypeptide chain termed the J chain. Serological analysis of immunoglobulins of the same class revealed the existence of additional heterogeneity and resulted in the subdivision of classes into subclasses. In addition to the five major classes, there are four subclasses of IgG in humans (IgG1 to IgG4) and in mice (IgG1, IgG2a, IgG2b and IgG3), and two subclasses of IgA in humans (IgA1 and IgA2).

At the genomic level, the human H-chain isotypes are encoded by nine constant loci:  $\mu$ ,  $\delta$ ,  $\gamma 1$ ,  $\gamma 2$ ,  $\gamma 3$ ,  $\gamma 4$ ,  $\alpha 1$ ,  $\alpha 2$  and  $\epsilon$ . Each H-chain locus codes for a constant region of the antibody molecule, the variable regions being encoded for by a distinct, large library of gene segments. Although

### Introductory article

#### Article Contents

- Introduction
- Antibody Classes and Subclasses
- Cellular Origin
- Structure
- Function

**Table 1** Characteristics of human immunoglobulin isotypes

	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	$\gamma 1$	$\gamma 2$	$\gamma 3$	$\gamma 4$	$\mu$	$\alpha 1$	$\alpha 2$	$\delta$	$\epsilon$
No. of constant domains	3	3	3	3	4	3	3	3	4
Light chain	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$
Molecular weight (kDa)	150	150	165	150	970	160	160	175	190
Serum concentration (mg mL <sup>-1</sup> )	9	3	1	0.5	1.5	3	0.5	0.04	0.0003
Half-life (days)	23	23	7	23	5	6	6	3	2.5
Placental transfer	+++	+	+++	+++	—	—	—	—	—
Binding to macrophages and other phagocytic cells	+	—	—	+	—	—	—	—	+
Binding to basophils and mast cells	—	—	—	—	—	—	—	—	+
Complement activation (classical pathway)	++	+	+++	—	+++	—	—	—	—
Complement activation (alternate pathway)	—	—	+	—	—	+	—	—	—

the different subclasses belonging to the same class are encoded by different exons, they show greater sequence homology in their constant regions and exhibit a common overall molecular structure. The L chain comprises two isotypes ( $\kappa$  and  $\lambda$ ) and the  $\kappa/\lambda$  ratio varies in different species (humans = 1.5; mice = 19). In humans, there is one C $\kappa$  locus and four C $\lambda$  loci (C $\lambda$ 1 to C $\lambda$ 4), which comprise four  $\lambda$  subclasses.

At the functional level, differences in the H-chain constant regions of the isotypes confer distinct properties to the antibody molecule, and each antibody isotype has been adapted to function in a different compartment of the

the protective effect of antibodies present at low concentrations, particularly those directed to soluble antigens active at low concentrations, such as bacterial toxins.

The major sites of formation of high-affinity antibodies are the lymph nodes and the spleen, where antigen-presenting cells degrade the protein antigen into peptides that bind to class II molecules of the major histocompatibility complex (MHC) for presentation to T cells. Activation of B lymphocytes requires antigen recognition through the B-cell receptor, but also a second signal delivered by T cells. Most of the interaction between T and B cells occurs in the primary follicle of secondary lymphoid organs. After cell interaction, lymphocytes transform into blasts and begin to divide, leading to formation of germinal centres. When they recognize antigen, helper T cells activate B cells to proliferate and differentiate into antibody-producing cells and memory lymphocytes. Secreted antibodies are transported via efferent lymphatics into the venous system and circulate through the peripheral blood to accomplish their effector functions.

Some antigens are able to reach the mucosa-associated lymphoid tissue (present in the gut, lung, mammary, lachrymal and salivary glands) in a form capable of stimulating an IgA response. The bulk of IgA found in the secretions, called secretory IgA (sIgA), is produced by plasma cells residing in the lamina propria. It predominates in exocrine secretions of humans and most mammals. It is a polymeric form of IgA containing an additional glycoprotein chain, designated 'secretory component (SC)'. Secretory IgM (sIgM), a form of IgM containing bound SC, also occurs in secretions. The SC is synthesized in epithelial and glandular cells. It is first expressed as a basolateral membrane receptor for J chain-containing polymeric immunoglobulins, referred to as the polymeric immunoglobulin receptor (pIgR). During transepithelial transport, polymeric IgA is disulfide bonded to the SC, which is cleaved from its transmembrane segment before release of sIgA. The rest of the IgA produced at the mucosal sites is released to the systemic circulation where it will constitute, together with IgA produced in secondary lymphoid organs, systemic IgA. Circulating IgA does not contain SC, but may occur in both monomeric and J-chain polymeric forms. IgA antibodies neutralize toxins at the surface of mucosal tissues.

Because antibody production to some antigens requires the help of T lymphocytes, these antigens are called helper T cell dependent. However, a second group of antigens, called helper T cell independent, can trigger an antibody response in the absence of T lymphocytes. Antigens of the second group activate B cells by different mechanisms and fall into two classes. Helper T cell-independent type 1 antigens can induce proliferation and differentiation of immature and mature B cells, a process called polyclonal activation. However, they cannot induce isotype switching, affinity maturation and generation of memory B cells. The prototype of these antigens are bacterial lipopolysac-

charides, which play an important protective role against extracellular pathogens. The second class, termed helper T cell-independent type 2 antigens, can activate only mature B cells. The response is dominated by a subset of B cells bearing the CD5 marker. They generally comprise a repetitive structure (pneumococcal polysaccharide, *Salmonella*-polymerized flagellin). The antibodies produced play an important early protective role against bacterial pathogens with cell wall polysaccharides.

## Structure

Each millilitre of normal serum contains approximately  $10^{16}$  immunoglobulin molecules. Even antibodies specific for the same epitope are heterogeneous. An antiserum raised against a given immunogen contains many different antibodies which bind to the antigen in slightly different fashions, and some of them may cross-react with related antigens and even with antigens exhibiting no obvious structural similarity with the immunogen. Because of this heterogeneity, study of the immunoglobulin structure requires use of homogeneous antibodies produced either by neoplastic plasma cells, termed myeloma cells, or by hybridoma cells. Myeloma cells proliferate and secrete immunoglobulins indefinitely and their products are referred to as myeloma proteins, found in the serum of diseased subjects and animals. Hybridoma cells are obtained experimentally by fusing a myeloma cell line to a normal B lymphocyte obtained from a human or an animal. The hybridoma grows continuously and secretes only one kind of antibody, referred to as a monoclonal antibody.

Basically, the immunoglobulin molecule is a Y-shaped tetrameric protein characteristically composed of two H and two L polypeptide chains held together by covalent (disulfide) and noncovalent bonds (**Figure 1**). Treatment of immunoglobulins with enzymes and chemical reagents capable of cleaving peptide bonds breaks them up into fragments. Three types of treatment are particularly important in understanding antibody structure. First, agents that cleave disulfide bonds generate two H-chain polypeptides of approximately 50 kDa and two L chains of 25 kDa. Second, papain splits the basic molecule into three fragments of approximately 45 kDa. Two of them retain the antibody ability to recognize the antigen and are referred to as Fab fragments (for 'fragment antigen binding'). Each fragment possesses only one combining site and can bind, but cannot precipitate, the antigen. The third fragment produced by papain digestion can be crystallized from a solution and is therefore referred to as Fc fragment (for 'fragment crystalline'). Finally, treatment with pepsin gives rise to several small fragments and a large fragment, the F(ab')<sub>2</sub> fragment, of molecular weight double that of one Fab fragment, and capable of binding

composed of two layers of polypeptide chains linked by a disulfide intrachain bridge in the centre of the domain. The chains exhibit a  $\beta$ -strand conformation and form two antiparallel  $\beta$  sheets that pack together. An L chain is composed of one variable ( $V_L$ ) and one constant ( $C_L$ ) domain. For the H chain, the  $\gamma$ ,  $\delta$  and  $\alpha$  chains consist of one variable ( $V_H$ ) and three constant ( $C_{H1}$  to  $C_{H3}$ ) domains, and the  $\mu$  and  $\epsilon$  chains exhibit an extra constant domain ( $C_{H4}$ ). Carbohydrate chains of variable shape and length, ranging from two sugar residues to a dozen or more units, are almost always present. Simple or branched, the carbohydrate chains normally attach to the Fc portion (the  $C_{H2}$  domain) of the immunoglobulin. This domain organization appears to be an important feature shared by immunoglobulins from all species studied. The sequence similarity among various domains suggests that they are phylogenetically related, having evolved from a single ancestral gene.

Linking the Fab and Fc regions of the immunoglobulin is a short segment of the H chain between the  $C_{H1}$  and  $C_{H2}$  domains, known as the 'hinge', with no sequence similarity to any of the other domains. This proline- and cysteine-rich, nonglobular portion of the polypeptide backbone allows segmental flexibility of the Fab arm and Fc region relative to one another. This flexibility is important for antigen binding and effector functions. The cysteine

and precipitating the antigen. The Fab is the antigen-binding constituent and the Fc fragment is the anchoring site for proteins of the complement system and for receptors of various effector cells.

In humans, both  $\kappa$  and  $\lambda$  chains of the L chain consist of approximately 250 amino acid residues. By contrast, the different types of H chain vary considerably in length, from 446 amino acid residues for the  $\gamma 1$  chain to 550 residues for the  $\mu$  chain. This length variability is due essentially to the presence of additional residues at the C-terminal end of the  $\mu$  and  $\alpha$  chains. These extra amino acids participate in polymerization of immunoglobulins.

Each antibody chain is composed of a constant and a variable region. In addition, comparison of antibody amino acid sequences has revealed the existence of homology regions, or domains, each of approximately 110 amino acids. Each domain is a globular structure

combining site, and have confirmed the domain-type organization of the immunoglobulin initially deduced from amino acid sequence analysis. They have provided evidence that antigen–antibody interactions are noncovalent and that the antibody-binding site requires contributions from the H- and L-chain variable domains of the Fab fragment. Amino acids from the CDRs, also called loops, between strands of the  $\beta$  sheets in the variable domains provide the specificity of the interaction. The shape and size of the combining site can vary, depending on the particular antigen–antibody complex analysed, from a shallow groove of  $15 \times 6 \text{ \AA}$  in size to a conical pocket of approximately  $10 \text{ \AA}$  in diameter. The interactions occur over large sterically and electrostatically complementarity areas. Hydrophobic determinants of the antigen interact with hydrophobic determinants of the antibody-binding site, charged side-chains interact with side-chains of opposite charge, and proton donors and acceptors are involved in hydrogen bonding. Thus, electrostatic forces between charged amino acid side-chains (salt bridges), hydrogen bonds, van der Waals forces and hydrophobic forces, together with surface complementarity, impart antibody-specific recognition.

## Function

For the immune system, immunoglobulins present on the B-lymphocyte surface play an essential role in transducing signals to the cytoplasmic and nuclear effectors, and in delivering the antigen to the cell compartment where it is degraded, processed and returned to the cell surface to be presented by MHC class II molecules to antigen-specific T helper cells. In turn, T lymphocytes provide signals for the proliferation and differentiation of B cells. For the organism, antibodies secreted by B lymphocytes are responsible for the humoral immune response, which plays a critical role in destruction of extracellular pathogens, prevention against the spread of intracellular infections, and protection against toxins. The two structural portions of the antibody, i.e. the variable (Fab) and the constant (Fc) fragments, impart distinct biological functions.

## Fab-mediated functions

### Antigen recognition

The immune system is able to generate a large repertoire of antibody-combining sites capable of recognizing virtually all possible antigens present in pathogens and their products (bacteria, viruses, and protozoal and metazoal parasites), and in environmental antigens. Antibodies can be raised against a variety of molecules, including carbohydrates, nucleic acids and phospholipids, but their induction generally requires the association of the antigen to a protein, called a carrier. Remarkably, even epitopes

that do not exist in nature, such as synthetic haptens, can be exquisitely recognized and bound. A strong antibody response requires that the antigen be injected with a substance, called the adjuvant, that enhances immunogenicity. Other factors that influence immunogenicity include the dose injected, the route of immunization (subcutaneous injections are more immunogenic than intravenous injections) and the form of the antigen (soluble or particulate, native or denatured).

### Pathogen neutralization

Antibodies recognize antigens outside the cells, where most bacteria and bacterial toxins are found. They can bind to a pathogen and block its access to cells, thereby preventing infection or destruction of host cells. In addition, antibodies can block the adherence of bacteria to host cells by binding to cell-surface proteins used by the bacterium to adhere to the cell. This binding will inhibit bacterial adherence and prevent infection. Antibodies may also be important for protection against viruses, which will not be able to infect cells and replicate.

### Antibodies as a first line of defence

IgM antibodies are generally encoded by germline-encoded variable region genes and are of low affinity. However, because of their pentameric structure and their rapid generation in the blood, they can bind to multivalent antigens, such as bacterial cell wall polysaccharides. The existence of 10 antigen-binding sites on an IgM molecule enhances the strength of its effective binding. IgM antibodies are also effective in complement activation (see below). IgG is the main isotype in the blood and the extracellular fluid. It plays an important role in opsoniza-

cells, superantigens are a group of microbial proteins known for their potent ability to activate a large number of T cells expressing a common immunoreceptor variable region family. A counterpart of T-cell superantigens has been described for B cells wherein the H-chain variable region determines the specificity of the immunoglobulin for the superantigen. While conventional antigens stimulate a small proportion of B cells, the proportion of B cells responsive to superantigens can be orders of magnitude higher. For example, *Staphylococcus aureus* protein A activates approximately 40% of human polyclonal IgM. Superantigen binding is mediated by regions of the H chain outside the classical antigen-binding site of antibodies. In addition to this bacterial superantigen, there is evidence that viruses can produce B-cell superantigens, and this unconventional mode of interaction with B cells is being investigated for human immunodeficiency virus (HIV) antigens.

### **Catalytic antibodies**

Analogies between antigen–antibody and enzyme–substrate interactions have prompted studies aiming to identify antibodies with catalytic activity. In initial reports, the ligands

emphasize that neither the complement nor phagocytes are specific for the pathogen. Instead, the antibodies involved in complement binding identify the pathogen as foreign.

### Opsonization

Pathogens that replicate outside cells can be eliminated by a mechanism mediated by the interaction of the Fc region of antibodies with specific receptors on the surface of effector cells. By coating the surface of a pathogen, antibodies allow binding of their Fc domains to Fc receptors present on effector cells (**Table 2**). For example, the Fc $\gamma$ RI receptor (CD64) exhibits appreciable affinity for monomeric human IgG1 and IgG3, the Fc $\gamma$ RII receptor (CD32) present on phagocytic cells binds complexed IgG1 and IgG3, and the Fc $\gamma$ RIII-b receptor (CD16) binds polymeric IgG1 and IgG3. Thus, by opsonizing pathogens via specific receptors on macrophages and neutrophils, antibodies trigger the effector functions carried out by these phagocytes (i.e. engulfment followed by internalization and destruction). Through a similar mechanism, eosinophils interacting with IgE can attack metazoal parasites. While the serum IgE level is very low in healthy individuals, that of subjects infested with parasites increases and remains high as long as the infestation lasts. When a large parasite, such as a helminth, is coated with IgE, eosinophils attack it via the high-affinity Fc $\epsilon$ RI receptor. Externalization of internal vesicles induces a complex tissue response to eliminate the undesirable parasite from the body.

### Antibody-dependent cell-mediated cytotoxicity

Antibodies can contribute to elimination of undesirable cells by recruiting killer cells. Following antibody binding to allogeneic cells, the Fc region of the antibody may be recognized by non-T, non-B lymphoid cells, called natural killer (NK) cells, which will initiate an antibody-mediated cytotoxic process that will kill the target cells. NK cells express Fc $\gamma$  receptors, which enable them to recognize the IgG1 and IgG3 subclasses. Activation of NK cells triggers the release of granules containing a membrane pore-forming protein which causes osmotic lysis, a cytotoxin that activates DNA-degrading enzymes within the target cell, and various proteolytic enzymes. This mechanism is used to clear pathogen-infected cells.

### Undesirable functions

Rather than playing a protective role, antibodies may be responsible for undesirable functions.

### Antibody enhancement

This phenomenon has been described for antibodies that, rather than neutralizing a virus, enhance its infectious potential, at least *in vitro*. For example, serum from HIV-1-

infected individuals contains antibodies that enhance infection by HIV-1 *in vitro*. Even though the molecular mechanisms of these antibody enhancements remain unclear, several studies support their possible clinical importance and it has been proposed that vaccine preparations against HIV-1 should exclude immunogenic epitopes for enhancing antibodies. In organ transplantation, instead of contributing to graft rejection, enhancing antibodies may protect the graft from being eliminated by cellular immune effectors.

### Antibody-mediated hypersensitivity reactions

The role of IgE antibodies is reminiscent of a double-edged sword, protecting with one edge and aggressing with the other. In the absence of antigen, basophils and mast cells are capable of binding circulating IgE through species-specific cell surface receptors, designated Fc $\epsilon$ RI, which bind monomeric IgE antibodies with high affinity. Engagement of the cell-bound IgE by a multivalent antigen activates the mast cell, which releases chemical mediators stored in granules and capable of mediating local inflammatory reactions. Degranulation of mast cells occurs within seconds of crosslinking IgE displayed on the cell surface by antigen, releasing the vasoactive amines histamine and serotonin. However, some individuals are unable to control the mechanism of IgE recruitment and the cascade leading to the release of chemical mediators induced by antigens, called allergens. These inappropriate responses cause a group of diseases called allergy, or atopy. Most of them are of type I and are due to soluble antigens. They result from production of IgE antibodies which activate mast cells to release vasoactive amines, leukotrienes and cytokines. These IgE-mediated allergic reactions cause symptoms such as asthma. Thus, there is a correlation between IgE levels and the prevalence and severity of bronchial asthma. In the type II class, such as occurs in some drug allergies, the allergen is cell bound and the IgG or IgM antibody against the tissue in question activates complement and Fc-mediated effector reactions, and the cell is attacked. This scenario occurs in haemolytic anaemia, seen in subjects who produce antibodies to penicillin-coated red blood cells. Finally, in the type III class, the allergen is soluble and immune complexes of IgG antibodies and allergen are formed in the tissue where they cause local inflammation, such as occurs in serum sickness induced by injection of foreign serum.

### Further Reading

- Braden B and Poljak R (1995) Structural features of the reactions between antibodies and protein antigens. *FASEB Journal* 9: 9–16.
- Klein J (1982) *Immunology. The Science of Self–Nonself Discrimination*. New York: John Wiley.

- Milstein C (1993) From the structure of antibodies to the diversification of the immune response. *Scandinavian Journal of Immunology* **37**: 385–398.
- Paul S, Gabibov A and Massey A (1994) Catalytic antibodies. *Molecular Biotechnology* **1**: 109–111.
- Paul W (1993) *Fundamental Immunology*, 3rd edn. New York: Raven Press.
- Rajewsky K (1996) Clonal selection and learning in the antibody system. *Nature* **381**: 751–758.
- Zanetti M and Capra J (eds) (1999) *The Antibodies*, vol. 6. Amsterdam: Harwood Academic.
- Zouali M (ed.) (1996) *Human B-cell Superantigens*. Austin, Texas: RG Landes.
- Zouali M and Hansen DE (1994) Antibodies – no longer just for binding. *Trends in Biotechnology* **12**: 73–74.