Antibodies

Introductory article

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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.

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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 γ-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, idiotopes reflect the uniqueness of structural features of the variable region determinants specific for an individual antibody, or idiotype. Second, allotopes are antibody characteristics reflecting allelic polymorphisms that differ between individuals of the same species. Third, isotopes 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: μ, δ, γ1, γ2, γ3, γ4, α1, α2 and ε. 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

Table 1 Characteristics of human immunoglobulin isotypes

	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ1	γ2	γ3	γ4	μ	α1	α2	δ	ε
No. of constant domains	3	3	3	3	4	3	3	3	4
Light chain	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Molecular weight (kDa)	150	150	165	150	970	160	160	175	190
Serum concentration (mg mL^{-1})	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 (κ and λ) and the κ/λ ratio varies in different species (humans = 1.5; mice = 19). In humans, there is one Cκ locus and four Cλ loci (Cλ1 to Cλ4), which comprise four λ 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 immune system. It is the H-chain isotype that determines the locations of the antibody and its half-life *in vivo*. For example, binding of IgG isotypes to placental Fc receptors facilitates transport of maternal IgG into the fetus. On the other hand, antibodies of the IgE isotype bind to specific receptors on mast cells and basophils and, upon cross-linking by antigen, induce release of mediators and cytokine production by these cells. Because of this unique property, IgE antibodies play an important role in allergic disorders and parasitic infections. Consequently, the isotypic composition of an antibody response imparts the tissue distribution of the immunoglobulins produced and their biological functions, each isotype contributing distinctively to protection against pathogens. IgG subclasses, for example, exhibit distinct structures and effector functions, and are differentially expressed in response to specific stimuli. In humans, IgG1 predominates in antiviral responses; specific IgG4 levels rise in allergic responses; and IgG2 antibody production is triggered in response to carbohydrate antigens.

Cellular Origin

The human body contains approximately 2×10^{12} T and B lymphocytes. Unlike T lymphocytes, which must migrate through the thymus, B lymphocytes originate from the bone marrow, migrate to secondary lymphoid organs (spleen, lymph nodes, Peyer patches, tonsils, appendix) and produce immunoglobulins. Each B lymphocyte, or B-cell clone, is committed to the synthesis of one antibody specificity characterized by the uniqueness of its variable regions and the pairing of its H and L chains. This combinatorial diversity generates a repertoire of lymphocytes bearing receptors that can recognize virtually all possible antigens. Upon interaction with its target antigen, the B-cell clone is activated and divides rapidly, thus expanding the antigen-specific clone.

The progeny of the stimulated B lymphocyte eventually differentiate into antibody-secreting cells, called plasma cells. The membrane of the B lymphocyte bears antibodies of the same antigen-binding specificity as those secreted by the lymphocyte progeny after cell activation and proliferation. Once a B lymphocyte is committed to antibody secretion, the combination of its variable region genes will be expressed by all members of the progeny. At early stages of B-cell development, it is the Cμ exon, which lies closest to the immunoglobulin H-chain variable region locus, that will be assembled to variable region genes, so that the first isotype to be expressed at the B-lymphocyte surface is IgM. Even though membrane-bound and secreted IgM have the same specificity, the two forms produced by the cell clone are not completely identical. Membrane-bound IgM has a μ chain that is 21 amino acids longer than that of the secreted form; most of the extra residues are uncharged and some of them are hydrophobic, enabling anchorage of the μ chain in the membrane.

With antigen stimulation, B cells proliferate, differentiate and may recombine another constant exon (Cγ, Cα or Cε), giving rise to IgG, IgA or IgE antibodies. This process, called isotype switching or class switching, enables antibodies of a given specificity to acquire a different constant region and, hence, to change their effector function. It is remarkable that antibodies of different isotypes can express exactly the same variable region. Isotype switching usually takes place during the secondary immune response, i.e. following several rounds of exposure of the antigen to the immune system by deliberate immunization or following infection. Different cytokines preferentially induce switching to different isotypes by making the switch recombination sites that lie 5′ to each H-chain constant exon accessible to switch recombinases. As the κ and λ exons are located on different chromosomes, there is no class switching for the L chain.

With successive exposures to the antigen, the isotype and the magnitude and affinity of the antibody response change. Following initial antigen injection, termed priming, the recipient mounts a primary immune response, which is influenced by the nature of the antigen, the dose injected, the route of injection, the adjuvant (a preparation that enhances the immune response to an antigen) used and the genetic make-up of the immunized host. After reaching a plateau, the amount of the primary IgM produced will decline progressively. A second exposure to the same antigen, called boosting, triggers a secondary immune (anamnestic) response that differs in several respects from the primary response. It is produced more rapidly, its magnitude is higher, and it persists for longer time periods. Persistence of memory lymphocytes for many years provides the recipient with a potential long-lasting protection against the invader. It is this immunological memory that allows successful vaccination and prevention against pathogens. Secondary antibodies are dominated by IgG of higher affinity for the immunogen than the primary IgM. The isotype-switched cell will evolve to a plasma cell or to a memory cell expressing a unique immunoglobulin receptor. The secondary immune response depends on the presence of T cells and usually involves both memory B cells and memory T cells. This increase in affinity enhances

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 lipopolysaccharides, 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)′2 fragment, of molecular weight double that of one Fab fragment, and capable of binding

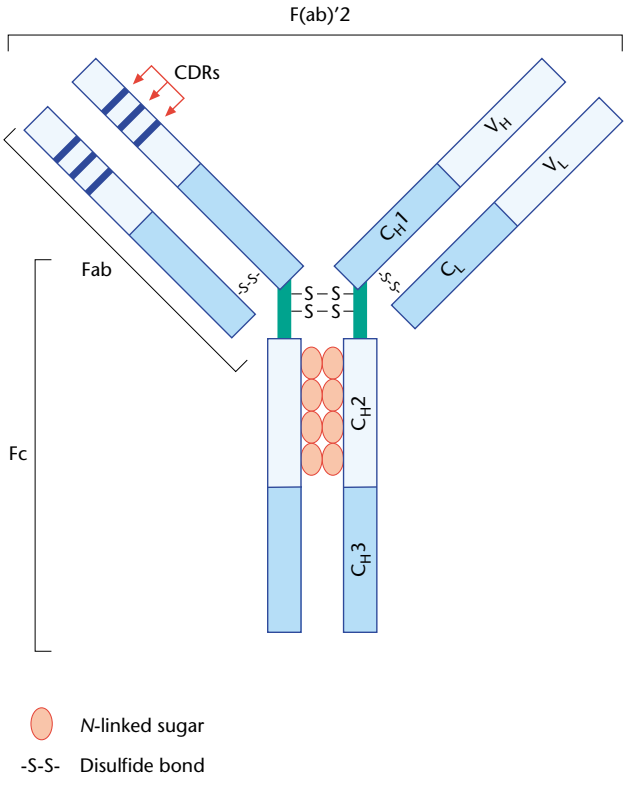

Figure 1 Diagram of a prototypic immunoglobulin (Ig)G monomer. Each rectangle represents an immunoglobulin domain. The complementarity-determining regions (CDRs) are highlighted. The green bars depict the hinge region. The Fc fragment (for 'fragment crystalline') is produced by papain digestion of IgG and can be crystallized from a solution. It is the anchoring site for proteins of the complement system and for receptors of various effector cells. The Fab fragment (for 'fragment antigen binding') is produced by papain digestion of IgG. It possesses one combining site and can bind, but cannot precipitate, the antigen. The F(ab)'2 fragment is produced by pepsin digestion of IgG. Its molecular weight is double that of one Fab fragment, and it is capable of binding and precipitating the antigen.

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 κ and λ 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 γ1 chain to 550 residues for the μ chain. This length variability is due essentially to the presence of additional residues at the C-terminal end of the μ and α 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 composed of two layers of polypeptide chains linked by a disulfide intrachain bridge in the centre of the domain. The chains exhibit a β-strand conformation and form two antiparallel β 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 γ, δ and α chains consist of one variable (V_H) and three constant (C_H1 to C_H3) domains, and the μ and ε 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 residues form interchain disulfide bonds linking the two H chains together. In addition to these interchain bridges, most immunoglobulins comprise a disulfide bridge between the H and L chain. Isotypes differ in the number and location of interchain disulfide bonds and in the number of oligosaccharide moieties attached to the H chain. In addition, the μ and ε chains contain an additional constant region domain that replaces the hinge region found in the γ, δ and α chains.

At the amino acid level, the variable region is comprised of three regions of extreme variability which include residues that may contact the antigen by virtue of mutual complementarity. They are called complementarity-determining regions, or CDRs. Interspersed among the CDRs are framework residues that represent approximately 80% of the variable region and are less variable and more evolutionarily conserved. At the three-dimensional level, the three CDRs of each chain converge to form a combining site (also called a paratope) which recognizes the antigenic determinant (also called an epitope). Because of the complexity of macromolecular antigens, each molecule has many different epitopes. Some of them are composed of a single segment of the molecule and are termed continuous or linear epitopes. Others, called discontinuous or conformational epitopes, involve sites distributed in different parts of the molecule, but brought together in the three-dimensional structure.

X-ray diffraction crystallographic studies of antibody crystals have revealed that the constant regions of the H and L chains do not contribute to formation of the

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 β 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 15×6 Å in size to a conical pocket of approximately 10 Å 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 opsonization and complement activation (see below). IgG antibodies can diffuse into tissues and bind toxins rapidly and with high affinity.

IgA is the principal isotype in secretions, particularly in the intestinal and respiratory tracts. Its main property is to neutralize foreign antigens and to protect epithelial cells from infectious agents, providing a first line of defence against pathogens. This is illustrated by the crucial role of sIgA present in the mother's colostrum, which contains approximately the same level of sIgA as other external secretions, conferring protection from infections to the newborn. The protective role of IgA is also indicated by observations that individuals deficient in IgA produce high titres of antibodies against various milk antigens. In response to certain pathogens, IgA antibodies predominate in certain locations. For example, cholera toxins trigger few, if any, circulating antibodies but induce the production of IgA in the faeces of the infected individual.

Interaction with superantigens

Recently, a new type of lymphocyte ligand, referred to as a superantigen, has been discovered. Initially described for T

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 described were transition-state analogues. There have since been other ligands, including vasoactive intestinal peptide, deoxyribonucleic acid (DNA) and thyroglobulin. Since, in principle, catalytic antibodies may have applications in biotechnology, and in infectious and autoimmune diseases, studies are carried out to understand the mechanism of their activity and to develop tailor-made antibody catalysts.

Fc-mediated effector functions

The constant portion of antibodies is responsible for several important functions. Through their Fc fragments, antibodies can activate accessory effector cells, including phagocytic cells (macrophages and neutrophils), natural killer cells, eosinophils and mast cells (**Table 2**). These cellular effectors bear Fc receptors for different isotypes, which enables them to identify and eliminate pathogens and their undesirable products.

Complement binding

Binding to determinants of the pathogen leads to the formation of antigen–antibody complexes and activation of the complement cascade, which comprises a series of plasma proteins involved in mast cell degranulation, chemotaxis, phagocytosis and cell lysis. The C1q molecule binds to antibody molecules attached to the surface of a pathogen and triggers the classical pathway of complement activation. As a result, the inflammatory soluble mediators released participate nonspecifically in the elimination of molecules and cells identified as not belonging to the repertoire of self antigens. Although the complement system can destroy some bacteria directly, its main function is to enable phagocytes to destroy bacteria that they would otherwise not recognize. It is important to

Table 2 Immunoglobulin preference for Fc receptors on human effector cells

Fc receptor	Affinity	Preference	Cellular distribution	Effect of engagement
FcγRI (CD64)	High	IgG monomers IgG1 = IgG3, IgG4	Monocytes, macrophages, neutrophils, dendritic cells	ADCC, uptake, phagocytosis
FcγRII-a (CD32)	Low	IgG1 = IgG3, IgG2	Monocytes, macrophages neutrophils, platelets	Uptake, phagocytosis, platelet aggregation
FcγRII-b (CD32)	Low	IgG1 = IgG3, IgG2	B lymphocytes	Inhibition of activation
FcγRII-c (CD32)	Low	IgG1 = 1gG3, IgG2	Monocytes, macrophages, neutrophils, platelets	Uptake, phagocytosis, platelet aggregation
FcγRIII-a (CD16)	Medium	IgG monomers IgG1 = IgG3	Natural killer cells, macrophages	ADCC, uptake, phagocytosis
FcγRIII-b (CD16)	Low	IgG1 = IgG3	Neutrophils	Phagocytosis
FcεRI	High	IgE monomers	Mast cells, basophils	Secretion of granules
FcεRII-a (CD23)	Low	IgE	B cells	Isotype modulation, antigen presentation
FcεRII-b (CD23)	Low	IgE	Eosinophils, platelets, monocytes, macrophages, some B and T cells	Secretion of granules, uptake, phagocytosis
FcαRI (CD89)	Medium	IgA	Monocytes, macrophages, neutrophils, eosinophils	ADCC, uptake, phagocytosis, secretion of granules

ADCC, antibody-dependent cell-mediated cytotoxicity.
Secretion of granules results in the release of inflammatory mediators and cytokines.

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 FcγRI receptor (CD64) exhibits appreciable affinity for monomeric human IgG1 and IgG3, the FcγRII receptor (CD32) present on phagocytic cells binds complexed IgG1 and IgG3, and the FcγRIII-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 FcεRI 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 Fcγ 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 FcεRI, 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.

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