

= Antibody =

An antibody (Ab) , also known as an immunoglobulin (Ig) , is a large , Y @-@ shaped protein produced mainly by plasma cells that is used by the immune system to identify and neutralize pathogens such as bacteria and viruses . The antibody recognizes a unique molecule of the harmful agent , called an antigen , via the variable region . Each tip of the " Y " of an antibody contains a paratope (analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen , allowing these two structures to bind together with precision . Using this binding mechanism , an antibody can tag a microbe or an infected cell for attack by other parts of the immune system , or can neutralize its target directly (for example , by blocking a part of a microbe that is essential for its invasion and survival) . Depending on the antigen , the binding may impede the biological process causing the disease or may recruit macrophages to destroy the foreign substance . The ability of an antibody to communicate with the other components of the immune system is mediated via its Fc region (located at the base of the " Y ") , which contains a conserved glycosylation site involved in these interactions . The production of antibodies is the main function of the humoral immune system .

Antibodies are secreted by B cells of the adaptive immune system , mostly by differentiated B cells called plasma cells . Antibodies can occur in two physical forms , a soluble form that is secreted from the cell to be free in the blood plasma , and a membrane @-@ bound form that is attached to the surface of a B cell and is referred to as the B @-@ cell receptor (BCR) . The BCR is found only on the surface of B cells and facilitates the activation of these cells and their subsequent differentiation into either antibody factories called plasma cells or memory B cells that will survive in the body and remember that same antigen so the B cells can respond faster upon future exposure . In most cases , interaction of the B cell with a T helper cell is necessary to produce full activation of the B cell and , therefore , antibody generation following antigen binding . Soluble antibodies are released into the blood and tissue fluids , as well as many secretions to continue to survey for invading microorganisms .

Antibodies are glycoproteins belonging to the immunoglobulin superfamily . They constitute most of the gamma globulin fraction of the blood proteins . They are typically made of basic structural units ? each with two large heavy chains and two small light chains . There are several different types of antibody heavy chains that define the five different types of crystallisable fragments (Fc) that may be attached to the antigen @-@ binding fragments . The five different types of Fc regions allow antibodies to be grouped into five isotypes . Each Fc region of a particular antibody isotype is able to bind to its specific Fc Receptor (except for IgD , which is essentially the BCR) , thus allowing the antigen @-@ antibody complex to mediate different roles depending on which FcR it binds . The ability of an antibody to bind to its corresponding FcR is further modulated by the structure of the glycan (s) present at conserved sites within its Fc region . The ability of antibodies to bind to FcRs helps to direct the appropriate immune response for each different type of foreign object they encounter . For example , IgE is responsible for an allergic response consisting of mast cell degranulation and histamine release . IgE 's Fab paratope binds to allergic antigen , for example house dust mite particles , while its Fc region binds to Fc receptor ? . The allergen @-@ IgE @-@ FcR? interaction mediates allergic signal transduction to induce conditions such as asthma .

Though the general structure of all antibodies is very similar , a small region at the tip of the protein is extremely variable , allowing millions of antibodies with slightly different tip structures , or antigen @-@ binding sites , to exist . This region is known as the hypervariable region . Each of these variants can bind to a different antigen . This enormous diversity of antibody paratopes on the antigen @-@ binding fragments allows the immune system to recognize an equally wide variety of antigens . The large and diverse population of antibody paratope is generated by random recombination events of a set of gene segments that encode different antigen @-@ binding sites (or paratopes) , followed by random mutations in this area of the antibody gene , which create further diversity . This recombinational process that produces clonal antibody paratope diversity is called V (D) J or VJ recombination . Basically , the antibody paratope is polygenic , made up of three genes , V , D , and J . Each paratope locus is also polymorphic , such that during antibody

production, one allele of V, one of D, and one of J is chosen. These gene segments are then joined together using random genetic recombination to produce the paratope. The regions where the genes are randomly recombined together is the hyper variable region used to recognise different antigens on a clonal basis.

Antibody genes also re-organize in a process called class switching that changes the one type of heavy chain Fc fragment to another, creating a different isotype of the antibody that retains the antigen specific variable region. This allows a single antibody to be used by different types of Fc receptors, expressed on different parts of the immune system.

== Forms ==

The membrane bound form of an antibody may be called a surface immunoglobulin (sIg) or a membrane immunoglobulin (mIg). It is part of the B cell receptor (BCR), which allows a B cell to detect when a specific antigen is present in the body and triggers B cell activation. The BCR is composed of surface bound IgD or IgM antibodies and associated Ig α and Ig β heterodimers, which are capable of signal transduction. A typical human B cell will have 50,000 to 100,000 antibodies bound to its surface. Upon antigen binding, they cluster in large patches, which can exceed 1 micrometer in diameter, on lipid rafts that isolate the BCRs from most other cell signaling receptors. These patches may improve the efficiency of the cellular immune response. In humans, the cell surface is bare around the B cell receptors for several hundred nanometers, which further isolates the BCRs from competing influences.

== Antibody ? antigen interactions ==

The antibody's paratope interacts with the antigen's epitope. An antigen usually contains different epitopes along its surface arranged discontinuously, and dominant epitopes on a given antigen are called determinants.

Antibody and antigen interact by spatial complementarity (lock and key). The molecular forces involved in the Fab epitope interaction are weak and non specific: for example electrostatic forces, hydrogen bonds, hydrophobic interactions, and van der Waals forces. This means binding between antibody and antigen is reversible, and the antibody's affinity towards an antigen is relative rather than absolute. Relatively weak binding also means it is possible for an antibody to cross react with different antigens of different relative affinities.

Often, once an antibody and antigen bind, they become an immune complex, which functions as a unitary object and can act as an antigen in its own right, being countered by other antibodies. Similarly, haptens are small molecules that provoke no immune response by themselves, but once they bind to proteins, the resulting complex or hapten-carrier adduct is antigenic.

== Isotypes ==

Antibodies can come in different varieties known as isotypes or classes. In placental mammals there are five antibody isotypes known as IgA, IgD, IgE, IgG, and IgM. They are each named with an "Ig" prefix that stands for immunoglobulin, a name sometimes used interchangeably with antibody, and differ in their biological properties, functional locations and ability to deal with different antigens, as depicted in the table. The different suffixes of the antibody isotypes denote the different types of heavy chains the antibody contains, with each heavy chain class named alphabetically: γ , δ , ϵ , μ , and ν . This gives rise to IgA, IgG, IgD, IgE, and IgM, respectively.

The antibody isotype of a B cell changes during cell development and activation. Immature B cells, which have never been exposed to an antigen, express only the IgM + isotype in a cell surface bound form. The B lymphocyte, in this ready to respond form, is known as a "naive B lymphocyte." The naive B lymphocyte expresses both surface IgM + and IgD +. The co-expression of both of these immunoglobulin isotypes renders the B cell ready to respond to antigen. B cell activation follows engagement of the cell bound antibody molecule with an antigen,

causing the cell to divide and differentiate into an antibody @-@ producing cell called a plasma cell . In this activated form , the B cell starts to produce antibody in a secreted form rather than a membrane @-@ bound form . Some daughter cells of the activated B cells undergo isotype switching , a mechanism that causes the production of antibodies to change from IgM or IgD to the other antibody isotypes , IgE , IgA , or IgG , that have defined roles in the immune system .

= = Structure = =

Antibodies are heavy (~ 150 kDa) globular plasma proteins . They have sugar chains (glycans) added to conserved amino acid residues . In other words , antibodies are glycoproteins . The attached glycans are critically important to the structure and function of the antibody . Among other things the expressed glycans can modulate an antibody 's affinity for its corresponding FcR (s) .

The basic functional unit of each antibody is an immunoglobulin (Ig) monomer (containing only one Ig unit) ; secreted antibodies can also be dimeric with two Ig units as with IgA , tetrameric with four Ig units like teleost fish IgM , or pentameric with five Ig units , like mammalian IgM .

The variable parts of an antibody are its V regions , and the constant part is its C region .

= = = Immunoglobulin domains = = =

The Ig monomer is a " Y " -shaped molecule that consists of four polypeptide chains ; two identical heavy chains and two identical light chains connected by disulfide bonds . Each chain is composed of structural domains called immunoglobulin domains . These domains contain about 70 ? 110 amino acids and are classified into different categories (for example , variable or IgV , and constant or IgC) according to their size and function . They have a characteristic immunoglobulin fold in which two beta sheets create a " sandwich " shape , held together by interactions between conserved cysteines and other charged amino acids .

= = = Heavy chain = = =

There are five types of mammalian Ig heavy chain denoted by the Greek letters : α , β , γ , δ , and ϵ . The type of heavy chain present defines the class of antibody ; these chains are found in IgA , IgD , IgE , IgG , and IgM antibodies , respectively . Distinct heavy chains differ in size and composition ; α and β contain approximately 450 amino acids , whereas γ and δ have approximately 550 amino acids .

Each heavy chain has two regions , the constant region and the variable region . The constant region is identical in all antibodies of the same isotype , but differs in antibodies of different isotypes . Heavy chains α , β and γ have a constant region composed of three tandem (in a line) Ig domains , and a hinge region for added flexibility ; heavy chains δ and ϵ have a constant region composed of four immunoglobulin domains . The variable region of the heavy chain differs in antibodies produced by different B cells , but is the same for all antibodies produced by a single B cell or B cell clone . The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain .

= = = Light chain = = =

In mammals there are two types of immunoglobulin light chain , which are called lambda (λ) and kappa (κ) . A light chain has two successive domains : one constant domain and one variable domain . The approximate length of a light chain is 211 to 217 amino acids . Each antibody contains two light chains that are always identical ; only one type of light chain , λ or κ , is present per antibody in mammals . Other types of light chains , such as the iota (ι) chain , are found in other vertebrates like sharks (Chondrichthyes) and bony fishes (Teleostei) .

= = = CDRs , Fv , Fab and Fc regions = = =

Some parts of an antibody have the same functions . The arms of the Y , for example , contain the sites that can bind to antigens (in general , identical) and , therefore , recognize specific foreign objects . This region of the antibody is called the Fab (fragment , antigen @-@ binding) region . It is composed of one constant and one variable domain from each heavy and light chain of the antibody . The paratope is shaped at the amino terminal end of the antibody monomer by the variable domains from the heavy and light chains . The variable domain is also referred to as the FV region and is the most important region for binding to antigens . To be specific , variable loops of ? @-@ strands , three each on the light (VL) and heavy (VH) chains are responsible for binding to the antigen . These loops are referred to as the complementarity determining regions (CDRs) . The structures of these CDRs have been clustered and classified by Chothia et al. and more recently by North et al. and Nikoloudis et al . In the framework of the immune network theory , CDRs are also called idiotypes . According to immune network theory , the adaptive immune system is regulated by interactions between idiotypes .

The base of the Y plays a role in modulating immune cell activity . This region is called the Fc (Fragment , crystallizable) region , and is composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody . Thus , the Fc region ensures that each antibody generates an appropriate immune response for a given antigen , by binding to a specific class of Fc receptors , and other immune molecules , such as complement proteins . By doing this , it mediates different physiological effects including recognition of opsonized particles (binding to Fc?R) , lysis of cells (binding to complement) , and degranulation of mast cells , basophils , and eosinophils (binding to Fc?R) .

In summary , the Fab region of the antibody determines antigen specificity while the Fc region of the antibody determines the antibody 's class effect . Since only the constant domains of the heavy chains make up the Fc region of an antibody , the classes of heavy chain in antibodies determine their class effects . Possible classes of heavy chains in antibodies include alpha , gamma , delta , epsilon , and mu , and they define the antibody 's isotypes IgA , G , D , E , and M , respectively . This infers different isotypes of antibodies have different class effects due to their different Fc regions binding and activating different types of receptors . Possible class effects of antibodies include : Opsonisation , agglutination , haemolysis , complement activation , mast cell degranulation , and neutralisation (though this class effect may be mediated by the Fab region rather than the Fc region) . It also implies that Fab @-@ mediated effects are directed at microbes or toxins , whilst Fc mediated effects are directed at effector cells or effector molecules (see below) .

= = Function = =

The main categories of antibody action include the following :

Neutralisation , in which neutralizing antibodies block parts of the surface of a bacterial cell or virion to render its attack ineffective

Agglutination , in which antibodies " glue together " foreign cells into clumps that are attractive targets for phagocytosis

Precipitation , in which antibodies " glue together " serum @-@ soluble antigens , forcing them to precipitate out of solution in clumps that are attractive targets for phagocytosis

Complement activation (fixation) , in which antibodies that are latched onto a foreign cell encourage complement to attack it with a membrane attack complex , which leads to the following :

Lysis of the foreign cell

Encouragement of inflammation by chemotactically attracting inflammatory cells

Activated B cells differentiate into either antibody @-@ producing cells called plasma cells that secrete soluble antibody or memory cells that survive in the body for years afterward in order to allow the immune system to remember an antigen and respond faster upon future exposures .

At the prenatal and neonatal stages of life , the presence of antibodies is provided by passive immunization from the mother . Early endogenous antibody production varies for different kinds of antibodies , and usually appear within the first years of life . Since antibodies exist freely in the

bloodstream , they are said to be part of the humoral immune system . Circulating antibodies are produced by clonal B cells that specifically respond to only one antigen (an example is a virus capsid protein fragment) . Antibodies contribute to immunity in three ways : They prevent pathogens from entering or damaging cells by binding to them ; they stimulate removal of pathogens by macrophages and other cells by coating the pathogen ; and they trigger destruction of pathogens by stimulating other immune responses such as the complement pathway . Antibodies will also trigger vasoactive amine degranulation to contribute to immunity against certain types of antigens (helminths , allergens) .

== = Activation of complement == =

Antibodies that bind to surface antigens (for example , on bacteria) will attract the first component of the complement cascade with their Fc region and initiate activation of the " classical " complement system . This results in the killing of bacteria in two ways . First , the binding of the antibody and complement molecules marks the microbe for ingestion by phagocytes in a process called opsonization ; these phagocytes are attracted by certain complement molecules generated in the complement cascade . Second , some complement system components form a membrane attack complex to assist antibodies to kill the bacterium directly (bacteriolysis) .

== = Activation of effector cells == =

To combat pathogens that replicate outside cells , antibodies bind to pathogens to link them together , causing them to agglutinate . Since an antibody has at least two paratopes , it can bind more than one antigen by binding identical epitopes carried on the surfaces of these antigens . By coating the pathogen , antibodies stimulate effector functions against the pathogen in cells that recognize their Fc region .

Those cells that recognize coated pathogens have Fc receptors , which , as the name suggests , interact with the Fc region of IgA , IgG , and IgE antibodies . The engagement of a particular antibody with the Fc receptor on a particular cell triggers an effector function of that cell ; phagocytes will phagocytose , mast cells and neutrophils will degranulate , natural killer cells will release cytokines and cytotoxic molecules ; that will ultimately result in destruction of the invading microbe . The activation of natural killer cells by antibodies initiates a cytotoxic mechanism known as antibody @-@ dependent cell @-@ mediated cytotoxicity (ADCC) ? this process may explain the efficacy of monoclonal antibodies used in biological therapies against cancer . The Fc receptors are isotype @-@ specific , which gives greater flexibility to the immune system , invoking only the appropriate immune mechanisms for distinct pathogens .

== = Natural antibodies == =

Humans and higher primates also produce " natural antibodies " that are present in serum before viral infection . Natural antibodies have been defined as antibodies that are produced without any previous infection , vaccination , other foreign antigen exposure or passive immunization . These antibodies can activate the classical complement pathway leading to lysis of enveloped virus particles long before the adaptive immune response is activated . Many natural antibodies are directed against the disaccharide galactose ? (1 @,@ 3) -galactose (? @-@ Gal) , which is found as a terminal sugar on glycosylated cell surface proteins , and generated in response to production of this sugar by bacteria contained in the human gut . Rejection of xenotransplanted organs is thought to be , in part , the result of natural antibodies circulating in the serum of the recipient binding to ? @-@ Gal antigens expressed on the donor tissue .

== = Immunoglobulin diversity == =

Virtually all microbes can trigger an antibody response . Successful recognition and eradication of

many different types of microbes requires diversity among antibodies ; their amino acid composition varies allowing them to interact with many different antigens . It has been estimated that humans generate about 10 billion different antibodies , each capable of binding a distinct epitope of an antigen . Although a huge repertoire of different antibodies is generated in a single individual , the number of genes available to make these proteins is limited by the size of the human genome . Several complex genetic mechanisms have evolved that allow vertebrate B cells to generate a diverse pool of antibodies from a relatively small number of antibody genes .

== Domain variability ==

The chromosomal region that encodes an antibody is large and contains several distinct gene loci for each domain of the antibody ? the chromosome region containing heavy chain genes (IGH @) is found on chromosome 14 , and the loci containing lambda and kappa light chain genes (IGL @ and IGK @) are found on chromosomes 22 and 2 in humans . One of these domains is called the variable domain , which is present in each heavy and light chain of every antibody , but can differ in different antibodies generated from distinct B cells . Differences , between the variable domains , are located on three loops known as hypervariable regions (HV @-@ 1 , HV @-@ 2 and HV @-@ 3) or complementarity determining regions (CDR1 , CDR2 and CDR3) . CDRs are supported within the variable domains by conserved framework regions . The heavy chain locus contains about 65 different variable domain genes that all differ in their CDRs . Combining these genes with an array of genes for other domains of the antibody generates a large cavalry of antibodies with a high degree of variability . This combination is called V (D) J recombination discussed below .

== V (D) J recombination ==

Somatic recombination of immunoglobulins , also known as V (D) J recombination , involves the generation of a unique immunoglobulin variable region . The variable region of each immunoglobulin heavy or light chain is encoded in several pieces ? known as gene segments (subgenes) . These segments are called variable (V) , diversity (D) and joining (J) segments . V , D and J segments are found in Ig heavy chains , but only V and J segments are found in Ig light chains . Multiple copies of the V , D and J gene segments exist , and are tandemly arranged in the genomes of mammals . In the bone marrow , each developing B cell will assemble an immunoglobulin variable region by randomly selecting and combining one V , one D and one J gene segment (or one V and one J segment in the light chain) . As there are multiple copies of each type of gene segment , and different combinations of gene segments can be used to generate each immunoglobulin variable region , this process generates a huge number of antibodies , each with different paratopes , and thus different antigen specificities . Interestingly , the rearrangement of several subgenes (i.e. V2 family) for lambda light chain immunoglobulin is coupled with the activation of microRNA miR @-@ 650 , which further influences biology of B @-@ cells .

RAG proteins play an important role with V (D) J recombination in cutting DNA at a particular region . Without the presence of these proteins , V (D) J recombination would not occur .

After a B cell produces a functional immunoglobulin gene during V (D) J recombination , it cannot express any other variable region (a process known as allelic exclusion) thus each B cell can produce antibodies containing only one kind of variable chain .

== Somatic hypermutation and affinity maturation ==

Following activation with antigen , B cells begin to proliferate rapidly . In these rapidly dividing cells , the genes encoding the variable domains of the heavy and light chains undergo a high rate of point mutation , by a process called somatic hypermutation (SHM) . SHM results in approximately one nucleotide change per variable gene , per cell division . As a consequence , any daughter B cells will acquire slight amino acid differences in the variable domains of their antibody chains .

This serves to increase the diversity of the antibody pool and impacts the antibody 's antigen @-@

binding affinity . Some point mutations will result in the production of antibodies that have a weaker interaction (low affinity) with their antigen than the original antibody , and some mutations will generate antibodies with a stronger interaction (high affinity) . B cells that express high affinity antibodies on their surface will receive a strong survival signal during interactions with other cells , whereas those with low affinity antibodies will not , and will die by apoptosis . Thus , B cells expressing antibodies with a higher affinity for the antigen will outcompete those with weaker affinities for function and survival . The process of generating antibodies with increased binding affinities is called affinity maturation . Affinity maturation occurs in mature B cells after V (D) J recombination , and is dependent on help from helper T cells .

== = Class switching == =

Isotype or class switching is a biological process occurring after activation of the B cell , which allows the cell to produce different classes of antibody (IgA , IgE , or IgG) . The different classes of antibody , and thus effector functions , are defined by the constant (C) regions of the immunoglobulin heavy chain . Initially , naive B cells express only cell @-@ surface IgM and IgD with identical antigen binding regions . Each isotype is adapted for a distinct function ; therefore , after activation , an antibody with an IgG , IgA , or IgE effector function might be required to effectively eliminate an antigen . Class switching allows different daughter cells from the same activated B cell to produce antibodies of different isotypes . Only the constant region of the antibody heavy chain changes during class switching ; the variable regions , and therefore antigen specificity , remain unchanged . Thus the progeny of a single B cell can produce antibodies , all specific for the same antigen , but with the ability to produce the effector function appropriate for each antigenic challenge . Class switching is triggered by cytokines ; the isotype generated depends on which cytokines are present in the B cell environment .

Class switching occurs in the heavy chain gene locus by a mechanism called class switch recombination (CSR) . This mechanism relies on conserved nucleotide motifs , called switch (S) regions , found in DNA upstream of each constant region gene (except in the ? @-@ chain) . The DNA strand is broken by the activity of a series of enzymes at two selected S @-@ regions . The variable domain exon is rejoined through a process called non @-@ homologous end joining (NHEJ) to the desired constant region (? , ? or ?) . This process results in an immunoglobulin gene that encodes an antibody of a different isotype .

== = Affinity designations == =

A group of antibodies can be called monovalent (or specific) if they have affinity for the same epitope , or for the same antigen (but potentially different epitopes on the molecule) , or for the same strain of microorganism (but potentially different antigens on or in it) . In contrast , a group of antibodies can be called polyvalent (or unspecific) if they have affinity for various antigens or microorganisms . Intravenous immunoglobulin , if not otherwise noted , consists of polyvalent IgG . In contrast , monoclonal antibodies are monovalent for the same epitope .

== = Asymmetrical antibodies == =

Heterodimeric antibodies , which are also asymmetrical and antibodies , allow for greater flexibility and new formats for attaching a variety of drugs to the antibody arms . One of the general formats for a heterodimeric antibody is the ? knobs @-@ into @-@ holes ? format . This format is specific to the heavy chain part of the constant region in antibodies . The ? knobs ? part is engineered by replacing a small amino acid with a larger one . It fits into the ? hole ? , which is engineered by replacing a large amino acid with a smaller one . What connects the ? knobs ? to the ? holes ? are the disulfide bonds between each chain . The ? knobs @-@ into @-@ holes ? shape facilitates antibody dependent cell mediated cytotoxicity . Single chain variable fragments (scFv) are connected to the variable domain of the heavy and light chain via a short linker peptide . The linker

is rich in glycine , which gives it more flexibility , and serine / threonine , which gives it specificity . Two different scFv fragments can be connected together , via a hinge region , to the constant domain of the heavy chain or the constant domain of the light chain . This gives the antibody bispecificity , allowing for the binding specificities of two different antigens . The ? knobs @-@ into @-@ holes ? format enhances heterodimer formation but doesn ? t suppress homodimer formation .

To further improve the function of heterodimeric antibodies , many scientists are looking towards artificial constructs . Artificial antibodies are largely diverse protein motifs that use the functional strategy of the antibody molecule , but aren ? t limited by the loop and framework structural constraints of the natural antibody . Being able to control the combinational design of the sequence and three @-@ dimensional space could transcend the natural design and allow for the attachment of different combinations of drugs to the arms .

Heterodimeric antibodies have a greater range in shapes they can take and the drugs that are attached to the arms don ? t have to be the same on each arm , allowing for different combinations of drugs to be used in cancer treatment . Pharmaceuticals are able to produce highly functional bispecific , and even multispecific , antibodies . The degree to which they can function is impressive given that such a change shape from the natural form should lead to decreased functionality .

= = Medical applications = =

= = = Disease diagnosis = = =

Detection of particular antibodies is a very common form of medical diagnostics , and applications such as serology depend on these methods . For example , in biochemical assays for disease diagnosis , a titer of antibodies directed against Epstein @-@ Barr virus or Lyme disease is estimated from the blood . If those antibodies are not present , either the person is not infected or the infection occurred a very long time ago , and the B cells generating these specific antibodies have naturally decayed .

In clinical immunology , levels of individual classes of immunoglobulins are measured by nephelometry (or turbidimetry) to characterize the antibody profile of patient . Elevations in different classes of immunoglobulins are sometimes useful in determining the cause of liver damage in patients for whom the diagnosis is unclear . For example , elevated IgA indicates alcoholic cirrhosis , elevated IgM indicates viral hepatitis and primary biliary cirrhosis , while IgG is elevated in viral hepatitis , autoimmune hepatitis and cirrhosis .

Autoimmune disorders can often be traced to antibodies that bind the body 's own epitopes ; many can be detected through blood tests . Antibodies directed against red blood cell surface antigens in immune mediated hemolytic anemia are detected with the Coombs test . The Coombs test is also used for antibody screening in blood transfusion preparation and also for antibody screening in antenatal women .

Practically , several immunodiagnostic methods based on detection of complex antigen @-@ antibody are used to diagnose infectious diseases , for example ELISA , immunofluorescence , Western blot , immunodiffusion , immunoelectrophoresis , and magnetic immunoassay . Antibodies raised against human chorionic gonadotropin are used in over the counter pregnancy tests .

= = = Disease therapy = = =

Targeted monoclonal antibody therapy is employed to treat diseases such as rheumatoid arthritis , multiple sclerosis , psoriasis , and many forms of cancer including non @-@ Hodgkin 's lymphoma , colorectal cancer , head and neck cancer and breast cancer .

Some immune deficiencies , such as X @-@ linked agammaglobulinemia and hypogammaglobulinemia , result in partial or complete lack of antibodies . These diseases are often treated by inducing a short term form of immunity called passive immunity . Passive immunity is

achieved through the transfer of ready @-@ made antibodies in the form of human or animal serum , pooled immunoglobulin or monoclonal antibodies , into the affected individual .

= = = Prenatal therapy = = =

Rhesus factor , also known as Rhesus D (RhD) antigen , is an antigen found on red blood cells ; individuals that are Rhesus @-@ positive (Rh +) have this antigen on their red blood cells and individuals that are Rhesus @-@ negative (Rh ?) do not . During normal childbirth , delivery trauma or complications during pregnancy , blood from a fetus can enter the mother 's system . In the case of an Rh @-@ incompatible mother and child , consequential blood mixing may sensitize an Rh- mother to the Rh antigen on the blood cells of the Rh + child , putting the remainder of the pregnancy , and any subsequent pregnancies , at risk for hemolytic disease of the newborn .

Rho (D) immune globulin antibodies are specific for human Rhesus D (RhD) antigen . Anti @-@ RhD antibodies are administered as part of a prenatal treatment regimen to prevent sensitization that may occur when a Rhesus @-@ negative mother has a Rhesus @-@ positive fetus . Treatment of a mother with Anti @-@ RhD antibodies prior to and immediately after trauma and delivery destroys Rh antigen in the mother 's system from the fetus . It is important to note that this occurs before the antigen can stimulate maternal B cells to " remember " Rh antigen by generating memory B cells . Therefore , her humoral immune system will not make anti @-@ Rh antibodies , and will not attack the Rhesus antigens of the current or subsequent babies . Rho (D) Immune Globulin treatment prevents sensitization that can lead to Rh disease , but does not prevent or treat the underlying disease itself .

= = Research applications = =

Specific antibodies are produced by injecting an antigen into a mammal , such as a mouse , rat , rabbit , goat , sheep , or horse for large quantities of antibody . Blood isolated from these animals contains polyclonal antibodies ? multiple antibodies that bind to the same antigen ? in the serum , which can now be called antiserum . Antigens are also injected into chickens for generation of polyclonal antibodies in egg yolk . To obtain antibody that is specific for a single epitope of an antigen , antibody @-@ secreting lymphocytes are isolated from the animal and immortalized by fusing them with a cancer cell line . The fused cells are called hybridomas , and will continually grow and secrete antibody in culture . Single hybridoma cells are isolated by dilution cloning to generate cell clones that all produce the same antibody ; these antibodies are called monoclonal antibodies . Polyclonal and monoclonal antibodies are often purified using Protein A / G or antigen @-@ affinity chromatography .

In research , purified antibodies are used in many applications . Antibodies for research applications can be found directly from antibody suppliers , or through use of a specialist search engine . Research antibodies are most commonly used to identify and locate intracellular and extracellular proteins . Antibodies are used in flow cytometry to differentiate cell types by the proteins they express ; different types of cell express different combinations of cluster of differentiation molecules on their surface , and produce different intracellular and secreted proteins . They are also used in immunoprecipitation to separate proteins and anything bound to them (co @-@ immunoprecipitation) from other molecules in a cell lysate , in Western blot analyses to identify proteins separated by electrophoresis , and in immunohistochemistry or immunofluorescence to examine protein expression in tissue sections or to locate proteins within cells with the assistance of a microscope . Proteins can also be detected and quantified with antibodies , using ELISA and ELISPOT techniques .

Researchers using antibodies in their work need to record them correctly in order to allow their research to be reproducible (and therefore tested , and qualified by other researchers) . Less than half of research antibodies referenced in academic papers can be easily identified . A paper published in F1000 in 2014 provided researchers with a guide for reporting research antibody use .

= = Regulatory validation of monoclonal antibody products for human use = =

Production and testing :

Traditionally , most antibodies are produced by hybridoma cell lines through immortalization of antibody @-@ producing cells by chemically @-@ induced fusion with myeloma cells . In some cases , additional fusions with other lines have created " triomas " and " quadromas " . The manufacturing process should be appropriately described and validated . Validation studies should at least include :

The demonstration that the process is able to produce in good quality (the process should be validated)

The efficiency of the antibody purification (all impurities and virus must be eliminated)

The characterization of purified antibody (physicochemical characterization , immunological properties , biological activities , contaminants , ...)

Determination of the virus clearance studies

Before clinical trials , studies of product safety and feasibility have to be performed :

Product safety testing : Sterility (bacteria and fungi) , In vitro and in vivo testing for adventitious viruses , Murine retrovirus testing ... Product safety data needed before the initiation of feasibility trials in serious or immediately life @-@ threatening conditions , it serves to evaluate dangerous potential of the product .

Feasibility testing : These are pilot studies whose objectives include , among others , early characterization of safety and initial proof of concept in a small specific patient population (in vito or in vivo testing) .

Preclinical studies :

Testing cross @-@ reactivity of antibody : to highlight unwanted interactions (toxicity) of antibodies with previously characterized tissues . This study can be performed in vitro (Reactivity of the antibody or immunoconjugate should be determined with a quick @-@ frozen adult tissues) or in vivo (with appropriates animal models) . More informations about in vitro cross @-@ reactivity testing .

Preclinical pharmacology and toxicity testing : Preclinical safety testing of antibody is designed to identify possible toxicities in humans , to estimate the likelihood and severity of potential adverse events in humans , and to identify a safe starting dose and dose escalation , when possible .

Animal toxicity studies : Acute toxicity testing , Repeat @-@ dose toxicity testing , Long @-@ term toxicity testing [http : // www.animalresearch.info / en / drug @-@ development / safety @-@ testing /](http://www.animalresearch.info/en/drug@-@development/safety@-@testing/)

Pharmacokinetics and pharmacodynamics testing : Use for determinate clinical dosages , antibody activities (AUC , pharmacodynamics , biodistribution , ...) , evaluation of the potential clinical effects

= = Structure prediction = =

The importance of antibodies in health care and the biotechnology industry demands knowledge of their structures at high resolution . This information is used for protein engineering , modifying the antigen binding affinity , and identifying an epitope , of a given antibody . X @-@ ray crystallography is one commonly used method for determining antibody structures . However , crystallizing an antibody is often laborious and time @-@ consuming . Computational approaches provide a cheaper and faster alternative to crystallography , but their results are more equivocal , since they do not produce empirical structures . Online web servers such as Web Antibody Modeling (WAM) and Prediction of Immunoglobulin Structure (PIGS) enables computational modeling of antibody variable regions . Rosetta Antibody is a novel antibody FV region structure prediction server , which incorporates sophisticated techniques to minimize CDR loops and optimize the relative orientation of the light and heavy chains , as well as homology models that predict successful docking of antibodies with their unique antigen .

The ability to describe the antibody through binding affinity to the antigen is supplemented by

information on antibody structure and amino acid sequences for the purpose of patent claims .

= = History = =

The first use of the term " antibody " occurred in a text by Paul Ehrlich . The term Antikörper (the German word for antibody) appears in the conclusion of his article " Experimental Studies on Immunity " , published in October 1891 , which states that , " if two substances give rise to two different antikörper , then they themselves must be different " . However , the term was not accepted immediately and several other terms for antibody were proposed ; these included Immunkörper , Amboceptor , Zwischenkörper , substance sensibilisatrice , copula , Desmon , philocytase , fixateur , and Immunisin . The word antibody has formal analogy to the word antitoxin and a similar concept to Immunkörper (immune body in English) . As such , the original construction of the word contains a logical flaw ; the antitoxin is something directed against a toxin , while the antibody is a body directed against something .

The study of antibodies began in 1890 when Kitasato Shibasabur? described antibody activity against diphtheria and tetanus toxins . Kitasato put forward the theory of humoral immunity , proposing that a mediator in serum could react with a foreign antigen . His idea prompted Paul Ehrlich to propose the side @-@ chain theory for antibody and antigen interaction in 1897 , when he hypothesized that receptors (described as " side @-@ chains ") on the surface of cells could bind specifically to toxins ? in a " lock @-@ and @-@ key " interaction ? and that this binding reaction is the trigger for the production of antibodies . Other researchers believed that antibodies existed freely in the blood and , in 1904 , Almroth Wright suggested that soluble antibodies coated bacteria to label them for phagocytosis and killing ; a process that he named opsoninization .

In the 1920s , Michael Heidelberger and Oswald Avery observed that antigens could be precipitated by antibodies and went on to show that antibodies are made of protein . The biochemical properties of antigen @-@ antibody @-@ binding interactions were examined in more detail in the late 1930s by John Marrack . The next major advance was in the 1940s , when Linus Pauling confirmed the lock @-@ and @-@ key theory proposed by Ehrlich by showing that the interactions between antibodies and antigens depend more on their shape than their chemical composition . In 1948 , Astrid Fagreaus discovered that B cells , in the form of plasma cells , were responsible for generating antibodies .

Further work concentrated on characterizing the structures of the antibody proteins . A major advance in these structural studies was the discovery in the early 1960s by Gerald Edelman and Joseph Gally of the antibody light chain , and their realization that this protein is the same as the Bence @-@ Jones protein described in 1845 by Henry Bence Jones . Edelman went on to discover that antibodies are composed of disulfide bond @-@ linked heavy and light chains . Around the same time , antibody @-@ binding (Fab) and antibody tail (Fc) regions of IgG were characterized by Rodney Porter . Together , these scientists deduced the structure and complete amino acid sequence of IgG , a feat for which they were jointly awarded the 1972 Nobel Prize in Physiology or Medicine . The Fv fragment was prepared and characterized by David Givol . While most of these early studies focused on IgM and IgG , other immunoglobulin isotypes were identified in the 1960s : Thomas Tomasi discovered secretory antibody (IgA) ; David S. Rowe and John L. Fahey discovered IgD ; and Kimishige Ishizaka and Teruko Ishizaka discovered IgE and showed it was a class of antibodies involved in allergic reactions . In a landmark series of experiments beginning in 1976 , Susumu Tonegawa showed that genetic material can rearrange itself to form the vast array of available antibodies .

= = Antibody mimetic = =

Antibody mimetics are organic compounds that , like antibodies , can specifically bind antigens . They are usually artificial peptides or proteins with a molar mass of about 3 to 20 kDa . Nucleic acids and small molecules are sometimes considered antibody mimetics , but not artificial antibodies , antibody fragments and fusion proteins are composed from these . Common advantages over

antibodies are better solubility , tissue penetration , stability towards heat and enzymes , and comparatively low production costs . Antibody mimetics such as the Affimer and the DARPin have being developed and commercialised as research , diagnostic and therapeutic agents .