ABO Blood Group Antibodies

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Abstract

In this chapter, the characteristics of an antibody, ABO blood group antibodies, and the detection of blood group antibodies are discussed. ABO blood group antibodies, the most important antibodies in the human body, have many common features with general antibodies in terms of basic functions and biological characteristics. Clearance of ABO blood group antibodies (mainly IgG and IgM) is the key to ABO-incompatible (ABOi) transplantation. Antibody removal technology includes plasma exchange technology, immuno-adsorption technology, and plasma double filtration technology.

Keywords

Antibody · ABO blood group antibody · Antibody removal technology

1 Introduction

The term "antibody" was first proposed in the conclusion of the paper "Experimental Studies on Immunity" published by Paul Ehrlich in October 1891, which states that "if two substances lead to the production of two different antibodies, the two substances must be different." In the early stages of immunological development, bacteria or their exotoxins were injected into animals. After a certain period of time, in vitro experiments proved that there is a component in serum which can specifically neutralize exotoxin, known as antitoxin, and a component that can specifically agglutinate bacteria, known as lectin. After that, this component with a specific

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response in serum was called an antibody (Ab), and the substance that can stimulate the body to produce antibody is called antigen (Ag), thus establishing the concept of antigen and antibody.

2 The Antibody and Its Characteristics

2.1 General Concept of the Antibody

The antibody, also known as immunoglobulin (Ig), is a type of Y-shaped protein that is produced by the proliferation and differentiation of B lymphocytes or memory B cells into plasma cells, which can specifically bind to the corresponding antigen, which is used by the immune system to identify and neutralize foreign substances such as bacteria and viruses. It is only found in body fluids, such as blood, of vertebrates, and on the B-cell membrane surface [1]. Antibody molecules have binding sites (binding clusters) that can bind to the corresponding antigenic determinants. After antibodies bind to different antigens, a different response is produced, so antibodies are often given different names, such as lectin, precipitin, antitoxin, hemolysin, and lysozyme. Antibodies are mainly distributed in the serum, as well as in tissue fluids and exocrine fluid.

At first, some researchers had proved via electrophoresis that antibody activity in the serum occurred in the γ-globulin. Therefore, the antibody was collectively referred to as γ-globulin. Later, it was found that not all antibodies were in the γ-area, and the globulin in the γ-area did not always have antibody activity. In 1964, in a special conference of the World Health Organization, globulin with antibody activity and antibody-related globulin were collectively referred to as *immunoglobulins*, such as abnormal Ig in the serum of patients with myeloma, macroglobulinemia, and cryoglobulinemia, and the natural Ig subunits in healthy people. Thus, Ig is a concept in structural chemistry, whereas the antibody is a concept of biological function. It can be said that all antibodies are Igs but not all Igs are antibodies.

2.2 Structure of the Antibody

An antibody is a globular blood protein macromolecule, weighing about 150 kDa. An antibody is also an Ig, because some amino acid residues contain sugar chains. The basic unit that can exert function is an Ig. The monomer of an antibody is a Y-shaped molecule with a symmetrical structure of four polypeptide chains, of which two chains are longer heavy chains (H chains) with a relatively high molecular weight, and two chains are shorter light chains (L chains) with a relatively low molecular weight. Interchain disulfide bonds and noncovalent bonds bind and form a monomer molecule composed of four polypeptide chains [2]. The whole antibody molecule can be divided into a constant region and a variable region. According to the composition of the constant region, there are two kinds of L chain (κ and λ), and five kinds of H chain (μ, δ, γ, ε, α). The variable region is located at

the ends of both arms of the "Y." In the variable region, a small part of amino acid residues has a high degree of variability. The composition and sequence of these amino acid residues are more prone to variation, so it is known as the hypervariable region. The variable region outside the hypervariable region is called the framework region. Each H or L chain has three hypervariable regions and adjacent regions and four framework regions. The hypervariable region is located on the surface of molecular space structure, and the antibody space structure, determined by its amino acid sequence, is a part where the antibody specifically binds to the antibody, so it is also known as the complementary determining region. The hypervariable region consists of up to 17 amino acid residues, or only 2 or 3 residues. The two H (or L) chains on one antibody molecule are the same. When papain hydrolyzes the antibody, the N end (i.e., between the first and second constant regions) of its hinge region is cut, forming two identical antigen-binding fragments at the ends of both arms, known as the antigen-binding fragment (Fab). The "Y" handle is called the crystalline fragment (FC).

2.3 Function and Production Regularity Law of Antibodies

Activated B cells can differentiate into two kinds of cells with different functions: plasma cells, which produce soluble antibodies, and memory B cells, which are used to memorize the antigens contacted. The latter can exist in the body for many years, and when the body is reexposed to the antigen, it reacts more quickly. Antibodies in the fetus and newborn are provided by the mother, as a kind of passive immunity. Within 1 year after birth, newborns

can produce many different kinds of antibodies on their own. Because antibodies can be dissolved in the bloodstream, they are part of the humoral immune system. Antibodies in the circulation of body fluid are generated by B-cell descendants that respond to a particular antigen, such as a viral capsid. The main function of the antibody is to combine with antigens (both foreign and self) to effectively remove foreign bodies, such as microorganisms and parasites that are invading the body, and to neutralize the toxins released by these, or to remove some autoantigens, maintaining the normal balance of the body. However, sometimes an antibody also causes pathological damage to the body, such as anti-nuclear antibodies, anti-double-stranded DNA antibodies, or anti-thyroglobulin antibodies, leading to autoimmune diseases.

2.3.1 Biological Activity of Antibodies

Antibody function is closely related to biological activity, and the biological activity of an antibody is mainly manifested in the following aspects. (1) An antibody binds a specific antigen: the main difference of the antibody from other Ig molecules is that the antibody can bind to a specific antigen, causing physiological or pathological effects in the body; it produces various direct or indirect visible antigen–antibody binding reactions in vitro. Antibodies bind to the antigen by relying on the specific binding sites on the molecule. (2) Antibodies activate complement: after the antibody binds to the corresponding antigen, the complement system is activated by

means of the exposed complement-binding sites, and the immunological action of complement bacteriolysis and cytolysis is also stimulated. (3) The antibody binds cells: different types of Ig can bind to different kinds of cells involved in immune response. (4) IgG can pass from the mother into the fetal blood through the placenta, forming a natural passive immunity for the fetus. IgA is a major factor in local mucosal immune response through the digestive tract and respiratory mucosa. (5) Antigenicity: the antibody molecule is a kind of protein and also has the ability to stimulate the body to produce an immune response. Different Ig molecules have different antigenicity. (6) Antibody resistance to physical and chemical factors is different from that of general globulin: it is not heat resistant and can be destroyed at 60°–70 °C. A variety of enzymes and substances that produce protein coagulation and degeneration can destroy the effects of antibodies. Also, antibodies can be precipitated by neutral salts. In production, ammonium sulfate or sodium sulfate can often be used to precipitate antibody-containing globulin from the immune serum, which is then purified via dialysis.

2.3.2 Antibody Function

The functions of antibodies are mainly manifested in the following aspects. (1) Antibodies can inhibit the growth and reproduction of pathogens; for example, antibacterial antibodies can agglutinate the invading bacteria and separate the bacteria from nutrients so that the bacteria cannot breed. (2) Antibodies can stop viruses or bacteria from getting close to human cells, so the invaders cannot reach or adhere to the cells; as a result, the invader cannot damage the cells or form an infection. Similar to plant seeds, they can only take root and germinate when they fall into the soil. Antibodies do not allow the virus to adhere to the cells, just like seeds floating in the air can never take root. (3) Antibodies can neutralize viruses, such as hepatitis B virus (HBV); there is a layer of protein structure on the surface that adheres to the surface of human liver cells, which is followed by access into the liver cells. However, it is very important for the antibody to meet with HBV before it binds to the surface, changing the protein on the surface of HBV and losing its ability to adhere to the hepatocytes, so that it cannot enter the hepatocytes, let alone replicate. The neutralizing effect of antibodies, such as "acid–base neutralization," causes HBV to be no longer infectious and it may soon be cleared by the body. (4) Antibodies can also identify the type of microorganisms. Each pathogenic microorganism that invades the human body automatically generates different antibodies according to different antigenic epitopes of microorganisms; for example, if a severe acute respiratory syndrome (SARS) virus invades the human body, that body will produce SARS antibodies; the human body will also produce a series of anti-hepatitis B antibodies in the case of HBV invasion.

2.3.3 Production Law of Natural Antibodies

Antibodies, as a special kind of immunoglobulins, exhibit a general law when exerting specific immune functions. Mastering the law of antibody production is of great significance in the prevention and diagnosis of disease. For example, the first vaccination should be carried out several weeks before the prevalence of disease, and

people generally should be vaccinated more than two times; when a serological test is performed to diagnose infectious diseases, two samples of serum in the initial and late stages of the disease should be taken for comparative observation. If the serum antibody level at the second testing is more than four times higher than that at the first time, it indicates diagnostic significance.

1. Production of antibodies in primary response: When the antigen enters the body for the first time, it can produce antibodies after a certain incubation period, and not much antibody is produced; it is also maintained in vivo for a shorter time. Its main features are as follows: the latency of primary immune response is long, the concentration of antibody produced is low, and the antibody–antigen affinity is also low, mainly IgM.
2. Production of antibodies in secondary response: When the same antigen enters the body a second time, some of the original antibodies bind to the later antigen initially, so the original antibody amount is slightly reduced. Subsequently, as there is a rapid increase in antibody titer, it can be increased several times over the primary response, and its in vivo retention time is also longer. Its main features are as follows: the latency is usually as short as 1–3 days; antibodies will be produced in the blood, resulting in high antibody concentration; and the antibody–antigen affinity is high, mainly IgG.
3. Production of antibodies in anamnesis reaction: The antibodies produced by the antigens can gradually disappear after a certain period of time. At this point, if the antigen is contacted again, the antibody that had disappeared may be rapidly increased. If the antigen stimulating the body again is the same as that at the first time, it is known as the specific anamnesis reaction; if it is different from that in the primary response, it is known as a nonspecific anamnesis reaction. The increase of antibodies caused by a nonspecific anamnesis reaction is temporary and will decline rapidly in a short time.

2.4 Classification of Antibodies

2.4.1 Classification According to Action Objects

Antibodies can be classified as antitoxin, antibacterial, antivirus, and cytotropic (cell-binding immunoglobulin, such as IgE reagin antibodies in a type I allergic reaction, which can adhere to the target cell membrane).

1. Antitoxin that is produced in the early stage when the immunoglobulin is not clearly classified is a general term for a class of immunoglobulin. It is a specific antibody that can neutralize antibodies of toxin or serum containing this antibody. Also, it can neutralize the toxic effects of the corresponding exotoxin. Pathogens, such as diphtheria, tetanus, gas gangrene, and others that cause the body to produce exotoxin, can produce antitoxin. After formaldehyde treatment, the exotoxin can lose toxicity and maintain immunogenicity, becoming the toxoid. In medical practice, the application of toxoid in prophylactic vaccination

can make the body produce the corresponding antitoxin, preventing disease. In immunotherapy, the commonly used bacteria include exotoxin, toxins, or other poisons (such as snake venom); antitoxin can be produced in the horse after immune injection, and then the serum is drawn, purified, and concentrated into antitoxin, which not only can improve the potency but also reduces side effects. The animal-derived antitoxin serum has a dual effect on the human body: on the one hand, it provides patients with specific antitoxin antibodies to neutralize the corresponding exotoxin in the body, and has a role in prevention and treatment; on the other hand, it has the antigenic heterologous protein, which can stimulate the body to produce anti-horse serum protein antibodies, and hypersensitivity may occur later when receiving the horse immune serum. IgG molecules that have been hydrolyzed by pepsin retain F(ab)2 fragments with antibody activity, and the specific antigenic determinants are removed as much as possible, resulting in a dramatic increase in potency and a lesser chance of hypersensitivity.

2. Antibacterial antibodies and antivirus antibodies, a type of immunoglobulin produced by the body against infection and destruction of pathogens, such as bacteria and viruses, can maintain the function of physiological stability. After the virus breaks through the body's first line of defense, interferons and natural killer cells (NK cells) are important as the second line of defense. After the virus enters into the body, it can stimulate the body's macrophages, lymphocytes, and somatic cells to produce interferon. Interferon, which has a broad spectrum of antiviral effects, can induce antiviral albumin to block the emergence of new viruses, so it can block virus proliferation and proliferation. NK cells are lymphocytes, in addition to T cells and B cells, accounting for 5–10% of the total number of lymphocytes; they perform a "patrol" task in the blood circulation. When abnormal cells are found, the lymphocytes immediately release perforin to kill the abnormal cells. Host cells infected with the virus are abnormal cells and are therefore also within the attack range of NK cells. After the virus escapes from the second line of defense, it faces a third line of defense, namely, specific humoral and cellular immunity, with specific roles in fighting viral infections. Specific antibodies can neutralize viruses outside the host cell. After the antibody binds to the virus, the virus cannot enter the cell by binding to the corresponding receptor on the surface of the infected cell, so the virus is then phagocytosed and degraded by the phagocyte. Antibodies cannot enter an intracellular virus that has entered the infected cells, as in intracellular bacteria, and CD8 cytotoxic T lymphocyte (CTL) cells in cellular immunity are needed for clearing. CTL cells destroy the virus-infected cells. First, CTL cells and virus-infected cells make contact, and CTL cells then release biologically active substances such as perforin and granzyme. The CTL cells then leave and the virus finally infects the cells, leading to rupture and death. The virus that is released can be neutralized by specific antibodies. After the CD8 CTL cells leave, they can attack other infected cells repeatedly in the same manner.
3. A cytophilic antibody is a type of immunoglobulin that binds to cells, such as IgE reaginic antibody in type I allergic reactions, which can bind to mast cells and basophilic granulocytes. IgG can bind to macrophages and adhere to the

target cell membrane. The immunoglobulin that binds to the same type of cell is called a homocytotropic antibody, and immunoglobulin that binds to xenogeneic cells is called a heterocytotropic antibody. The Fc-terminus of a cytophilic antibody can be adsorbed on the immune cells of Fc receptors, including (1) IgE allergic antibodies ecotropic to mast cells and basophils; and (2) IgG antibody that can be adsorbed on killer cells (also known as K cell K). Only K cells with antibodies can effectively kill target cells with the corresponding antigens. (3) Antibodies adsorbed on macrophages can arm macrophages to enhance the ability of phagocytosis and killing target cells.

2.4.2 Classification According to Physical and Chemical Properties and Biological Functions

Antibodies can be divided into IgM, IgG, IgA, IgE, and IgD [3].

1. Immunoglobulin M (IgM) accounts for 5–10% of the total amount of serum immunoglobulin. It is the type of antibody that is present first on the cell membrane after rearrangement of the B-cell antibody gene (as follows), forming the B-cell receptor (BCR). Secretory IgM is a pentamer, and the J chain combines five IgM Fc segments, which is Ig with the highest molecular weight, namely, macroglobulin. Because of its high molecular weight, pentameric IgM usually does not pass through the blood vessel walls and is present mainly in the blood, containing ten F(ab) segments, and thus it has a higher antibacterial activity than IgG; moreover, it has five Fc segments, so it can activate more complement than IgG. The natural blood group antibody is IgM, and inconsistent blood transfusion can cause a serious hemolytic reaction. IgM is the first antibody to be synthesized and secreted during individual development, and the fetus synthesizes IgM at about the 20th week in the embryonic period. IgM in the body cannot pass the placenta, so if IgM appears in newborn blood, it indicates intrauterine infection; moreover, if IgM is increased in the umbilical cord blood, this indicates intrauterine fetal infection (such as rubella or cytomegalovirus infection). IgM, which is also the antibody appearing first in humoral immunity, acts as the "pioneer" of anti-infection, and is also the antibody secreted first in the immune response. After a period of time, the IgM antibody level gradually decreases and then disappears. IgM initiates the cascade of complement after binding to the antigen, and also connects the intruders and gathers them to be engulfed by macrophages. Therefore, the determination of IgM antibodies has clinical diagnostic value for some infectious diseases such as hepatitis. IgM detected in serum suggests a recent infection and can be used for early diagnosis. In children more than 1 year of age, serum IgM will reach the adult level. Many antimicrobial natural antibodies, such as cognate hemagglutinin (anti-type A and anti-type B blood), rheumatoid factor in rheumatoid diseases, and complement-binding antibodies in syphilis belong to IgM.
2. Immunoglobulin G (IgG) is an immunoglobulin component in serum, accounting for about 75% of the total serum immunoglobulin with a normal value of 9.5–12.5 mg/ml: 40–50% IgG is distributed in the serum and the remaining

resides in the tissues. With a molecular weight of about l50 kDa, IgG in human serum is mainly a monomer, and normal human IgG includes four subtypes: IgG1 (60–70%), IgG2 (15–20%), IgG3 (5–10%), and IgG4 (1–7%). These subtypes have different binding abilities in the classic pathway of complement activation. IgG, synthesized mainly by plasma cells in the spleen and lymph nodes, is the only antibody that can pass through the placenta, having an important role in preventing infections within a few weeks after birth. Its synthesis starts at the third month after birth, reaching the adult level at the age of 3–5 years, and gradually decreases after 40 years of age. IgG content varies widely among individuals and fluctuates in the same individual under different conditions. Most antibacterial, antivirus, and antitoxin antibodies produced by the body under antigen stimulation belong to IgG. Many autoantibodies, such as LE factor in systemic lupus erythematosus, and anti-thyroglobulin antibodies, also belong to IgG. IgG is the main anti-infective antibody in the body: it activates complement and neutralizes a variety of toxins. IgG persists for a long time and is the only antibody that protects the fetus across the placenta during gestation. Moreover, it is also secreted into the colostrum from the breast to protect the newborn. The functional role of IgG in body immunity is mainly protective. Antivirals, antitoxins, and most antibacterial antibodies belong to the IgG class. To handle the numbness of hepatitis A, for example, IgG can effectively prevent the corresponding infectious diseases. Therefore, IgG antibodies are important in the neutralization of toxins and antibacterial and antiviral activities. IgG is the only immunoglobulin that passes through the placenta. Thus, IgG from the maternal body is important in the prevention of diphtheria, measles, and poliomyelitis infections during the first few months of life. The maternal IgG delivered to the fetus has almost completely disappeared by 6 months after birth, whereas that produced by the baby itself is gradually increased from 3 months, so it is susceptible to infection after 6 months.

3. IgA content in normal human serum is second only to IgG, with serum immunoglobulin content of 10–20%. From the aspect of structure, IgA has monomer, dyad, trisomy, and polymer points. According to immune function, it is divided into two types of serotypes and secretions. Serum IgA is present in the serum, which accounts for about 85% of total IgA. Although serogroup IgA has certain functions of IgG and IgM, it does not show important immune function in the serum. Secretory IgA is composed of catenin and secretory components linked by a J chain, mainly in colostrum, saliva, lacrimal fluid, gastrointestinal fluid, bronchial secretions, and other exocrine fluids. It is the most important factor of mucosal local immunity. Secretory IgA, by binding to the corresponding pathogenic microorganisms (such as poliovirus), inhibits its adsorption onto susceptible cells; secreted IgA can also neutralize toxins such as those of *Vibrio cholerae* and *Escherichia coli*. Neonatal respiratory tract infection and gastrointestinal infections may be related to a lack of IgA synthesis. Chronic bronchitis attacks and secretory IgA reduction also have a certain relationship. IgA is also known as mucosal local antibodies. IgA cannot pass the placenta. Neonatal serum IgA antibodies can be maternal colostrum-secreted IgA passed to infants;

this is also an important natural passive immunization. Eosinophils, neutrophils, and macrophages express FcαR, and serotonergic IgA mediates phagocytosis and antibody-dependent cell-mediated cytotoxicity (ADCC). In addition, secreted IgA functions as an immune exclusion whereby secreted IgA binds to a large number of soluble antigens in the diet and the pyrogen released by normal intestinal microflora or pathogenic microorganisms, preventing them from entering the bloodstream.

IgA is mainly produced by mucosa-associated lymphoid tissues, most of which are synthesized by gastrointestinal lymphoid tissues; a small portion is synthesized by respiratory, salivary, and reproductive tract mucosal tissues. Maternal lactation gland tissue contains a large number of IgA-producing cells; these cells are mainly from the gastrointestinal tract. In humans, a small amount of IgA comes from the bone marrow. IgA is synthesized at 4 to 6 months after birth, and serum levels at 4 to 12 years of age reach the adult human level. Antibody enters the mucosal surfaces of the body, including the respiratory, digestive, reproductive, and other pipeline mucosa, as do infection factors. It is also possible to deliver this antibody, the most abundant and important class of antibodies, to the gut mucosa of the newborn through the colostrum of breast milk.

4. Immunoglobulin E (IgE) is mainly produced by plasma cells in the nasopharynx, tonsil, bronchial, gastrointestinal, and other mucosal lamina propria; molecular weight is 188 kDa, and in normal human serum this is the smallest amount of immunoglobulin, only 0.002% of the total Ig in serum. The secretion of IgE often occurs in response to allergen invasion and type I allergy. Serum IgE levels in patients with allergies and parasites are significantly higher than those in normal subjects. In 1966, the Swedish scholar Johansson and the Japanese scholar Ishizaka first isolated IgE from the serum of ragweed allergy sufferers and proved IgE to be a mediator of allergic reactions. IgE is a pro-type antibody. At the tail, basophils, such as mast cell membrane antibody- and antigen-binding basophils, and mast cells release histamine substances to promote the development of inflammation. This mode is also the path that triggers an immediate allergic reaction.

   The mechanism of IgE synthesis and regulation is not entirely clear. A variety of allergic diseases can be seen in the same patient, indicating the constitution of that allergic susceptibility, and in such patients, compared with normal serum, IgE is significantly increased, as is the number of mast cells, and more IgE receptors occur on the membrane. Studies have confirmed that the allergic constitution is autosomal dominant, but different members of the same family may suffer from different allergies; the nature of the antigen and the way it enters the body also affect the synthesis of IgE, although the antigens enter the body in the same way. Some antigens such as hairstyling products cause a strong allergic reaction, although others cannot, although the exact reason is not clear; but with the antigen itself, especially the characteristics of epitopes recognized by T cells, some drugs such as degraded penicillin, worm antigen, wormwood pollen, ragweed pollen, etc., can cause strong IgE-type allergic

reactions. Antigens entering into the body and the frequency of antigen contact with the body produce antibodies having an impact; the mucosa easily produce the IgE response, and injection is caused by the production of IgG. The greater the number of allergens that are in contact with the body, the greater is the possibility of sensitization.

When the allergen is contacted again, binding to the IgE receptors on the cell membrane causes a series of biochemical reactions, which in turn release various bioactive mediators such as histamine and cause allergy and inflammation. Helper T cells and their contributing cytokines have a key regulatory role in the composition of IgE. Depending on the type of excreted cytokines, helper cells are categorized into two subsets, Th1 and Th2. The cytokines secreted by Th2 cells are mainly important in antibody constitution and allergic reaction. By cytokines and mutual regulation, under healthy conditions, Th1 and Th2 balance each other, and at the same time accept T helper cells in the control of helper T-cell deficiencies or contact with certain foreign proteins or more subtle molecules (such as dust mites, pollen, or seafood). Th2 overactivation results in Th2 cytokine excretion that is too high, prompting increased serum IgE concentration, thus triggering allergies. Supplementation with anti-hyperlipid probiotic strains has been shown to lower the level of serum IgE antibodies. Anti-allergic probiotics use a physiologically acceptable probiotic composition with enhanced anti-allergic properties that effectively alleviates the symptoms of allergy by regulating the secretion of interleukin 12 (IL-12) and interferon 7; this also regulates the Th1-type immune response and inhibits the immunoglobulin IgE Th2-type immune response to improve the phenomenon of excessive allergies. Anti-allergic probiotic bacteria and dendritic cells in the intestinal wall act on receptor binding; activation of intracellular translation of protein is moved to the nucleus and releases a large number of cytokines, a part of innate immunity. Polypeptide diabetes substances such as peptidoglycan, lipopolysaccharide, and polysaccharides in the innate immune system can indeed activate T-cell development.

5. Immunoglobulin D (IgD) is the most mysterious type of antibody known today. The specific role of IgD in the human is not known. IgD mainly appears on the surface of mature B lymphocytes, which may be related to cell recognition that responds to the corresponding pathogens for immune response, and may also be related to the differentiation of B cells. Its main cross-model exists in the B-cell surface, and the level of secretion to the serum is very low. IgD as found in human myeloma protein in 1995 has a molecular weight of 175 kDa and is mainly produced by plasma cells of the tonsils, spleen, etc. IgD concentration in human serum is 3–40 μg/ml, less than 1% of the total serum. The IgD hinge region is very long and sensitive to proteolytic hydrolysis, so IgD half-life is very short, only 2.8 days. The exact immune function of serum IgD is not clear. In differentiation to the mature B-cell stage, in addition to expressing SmIgD, the antigen appears to be immune tolerant after stimulation. SmIgD fades after activation of B cells, either upon activation or upon becoming memory B cells.

2.4.3 Classification According to the Presence of Visible Antigen-Binding Reaction

Antibodies can be divided into a medium reaction with the participation of the visible binding reaction of the complete antibody, the so-called antibody; or if no visible reaction occurs, they can suppress the antigen with its corresponding complete antibody binding to an incomplete antibody.

2.4.4 Classification According to the Origin of Antibodies

By origin, there are natural antibodies and immune antibodies.

1. Natural antibodies are also known as normal antibodies, that is, various antibodies that naturally occur in humans without significant infection by humans or animals or by artificial injection of antigens. Natural antibodies include not only antibodies against pathogenic microorganisms and their products, but antibodies against nonpathogenic substances, such as blood group antibodies. In addition to blood type, antibodies are mainly affected by genetic factors; the other natural antibodies may result from latent infection or common antigen stimulation. Because people or animals are constantly exposed to various antigens after birth, some observers think that natural antibodies are correct and are just customary. In a given region, the average level of natural antibodies for the diagnosis of disease has a certain reference value. The most important natural antibody is IgM.
2. An immune antibody occurs by the stimulation of antigen substances, by B-cell differentiation into plasma cells generated, with the corresponding antigen-specific binding reaction of immunoglobulin. Antibodies can be combined with antigens, both foreign and foreign, to effectively remove foreign bodies such as pathogenic microorganisms and parasites that invade the body, to neutralize the toxins they release, or to remove certain autoantigens to keep the body in good condition and balance. However, immune antibodies such as anti-nuclear antibodies, anti-double-stranded DNA antibodies, and anti-thyroglobulin antibodies can cause harm, sometimes pathological lesions, to the human body.

2.4.5 Classification According to Antibody Preparation Methods

The categories are polyclonal antibodies and monoclonal antibodies.

1. A polyclonal antibody (PcAb) (referred to as polyclonal), as its name implies, is a relatively “monoclonal” antibody in terms of antigen and is usually composed of multiple antigenic determinants that stimulate the body. An antibody produced by a B lymphocyte that receives the antigen is called a monoclonal antibody. By a variety of antigenic determinants to stimulate the body, corresponding to produce a variety of monoclonal antibodies, these monoclonal antibodies are mixed together as a polyclonal antibody; the body produces antibodies that are polyclonal antibodies in addition to antigenic determinants. Diversity is the same type of antigenic determinant but also stimulates the body to produce antibodies such as IgG, IgM, IgA, IgE, and IgD. The correct understanding of monoclonal

antibodies, in turn, allows further understanding of the significant role of polyclonal antibodies. Polyclonal antibodies from the body are from the outside of the heterologous antigen (macromolecular antigen, hapten, etc.) to stimulate the body's immune response; the body of plasma cells secrete a group of special immunoglobulin. Polyclonal antibodies are widely used in research and diagnosis because of their ability to recognize multiple epitopes, precipitating reactions, short preparation times, and low cost.

2. Monoclonal antibody (McAb) (referred to as monoclonal antibody, MAb) is known as the tumor "biological missile," resembling a drug that can be directly directed to the tumor; it is produced by a lymphocyte hybridoma, only for a single antigen molecule, with antigenic determinants of specific antibodies. A lymphocyte hybridoma is an artificial method to make myeloma cells (ascites tumor-like plasma cells of pure mice) fuse with lymphocytes that have been sensitized with antigens and can secrete certain antibodies (commonly used are sensitized animal spleen cells, which act as one of the B cells). After addition of HAT selection medium, unfused B cells die because they cannot survive for long periods of time, whereas myeloma cells cannot synthesize DNA through the DNA remediation synthesis pathway because of the lack of HGPRT enzyme and therefore also die. Survival of fusion cells, the final acquisition of monoclonal cells, compares the potency of antibodies between different monoclonal cells to select the appropriate monoclonal strain. The fusion cells not only have a large number of tumor cells, the characteristics of unlimited reproduction, but also the ability of B cells to synthesize and secrete specific antibodies. Antigens that can be used to produce monoclonal antibodies can be human or animal T cells, B cells, erythrocytes, tumor cells, or various microorganisms or proteins, nucleic acids, and other molecules.

   Because a B-cell clone produces antibodies to only one antigenic determinant, a single hybridoma cell from a B cell fused with a myeloma cell produces only a single antibody. The hybridoma cells are isolated by suitable means and subjected to a single cell culture to multiply in large quantities so that the cell clones that proliferate in the culture medium produce only perfectly uniform and monospecific antibodies, that is, monoclonal antibodies. Monoclonal antibodies of high purity, specificity, and high potency, and the use of different cells and microbial strains or strains, can be eliminated from serological cross-reaction, which greatly improves diagnostic specificity and sensitivity. In addition, these have a very important role in studying cell-surface markers, purifying soluble antigens, and further studying the structure and function of antibodies.

   In 1975, British scientists Kohler and Milstein fused antibody-producing lymphocytes and tumor cells and successfully established this monoclonal technology, winning the Nobel Prize in physiology or medicine in 1984. Since its advent in 1975, the monoclonal antibody has opened a new chapter in clinical immunology. It not only opens up a new path for the preparation of antisera, but also greatly improves the diagnosis and treatment of many clinical diseases.

Monoclonal antibody technology is an important tool in modern life science research, having an indispensable role in the study of the structure and function of genes and proteins. So far, monoclonal antibody technology is still irreplaceable in the immunological diagnosis of humans, animals, and plants. In recent years, monoclonal antibodies have been widely used in tissue and organ transplantation, which greatly improves the curative effect of transplantation and provides strong technical support for the rapid development of transplantation. Common clinical monoclonal antibodies include rituximab, cetuximab, and trastuzumab.

3. Single B-cell polymerase chain reaction (PCR) expression of antibodies in vitro is similar to the aforementioned polyclonal or monoclonal antibody; after immunizing a mouse or a rabbit with the antigen of interest, the animal's peripheral blood or spleen tissue is removed to prepare a cell suspension and incubated with the antigen of interest labeled with fluorescein. As the surface of the memory B cells that expresses specific IgG or IgM can express the desired antigen-specific antibody, it will bind to the antigen, which has been labeled with fluorescein; thus, memory B cells that bind the antigen will produce fluorescence under the laser-specific excitation of a flow cytometer. Cell sorting and lysis, single-cell messenger RNA reverse transcription into complementary DNA (complement DNA, cDNA), and the use of antibody variables and constant region primers for PCR increase the specific antibody sequence. It is then cloned into the antibody expression vector and transfected into common 293 cells to express the monoclonal antibody in vitro.

   The single-cell PCR method in vitro expression method rather than the traditional monoclonal antibody technology has certain advantages. (1) There is no hybridoma formation process, greatly saving time and cost. In addition, certain low abundance antibody information may be lost because of the relatively low efficiency of hybridoma technology. (2) Peripheral blood can also be used in this method for antibody expression. It is also possible to extract B cells from blood samples of infected individuals to obtain fully humanized monoclonal antibodies such as anti-SARS virus antibodies and AB blood group antibodies.

2.4.6 Classification According to the Antigen–Antibody Reaction in Various Manifestations

(1) Precipitin refers to the antigen and the precipitation reaction can occur with the antibody. (2) Lectin refers to the combination of particulate antigen aggregation antibody. (3) Lysin: with cell membrane antigen binding, the complement can make the cells appear as dissolved antibodies, such as lysozymes or hemolysin. (4) Complement fixation antibody refers to the combination of antigens that can activate complement antibody. (5) Opsonin refers to the role of conditioning with the combination of microorganisms that can promote swallowing by phagocyte antibodies. (6) When neutralizing antibodies are combined with the virus, the virus can lose the infectious antibodies.

2.5 Diversity of Antibodies and Its Production Mechanism

2.5.1 Diversity of Antibodies

The diversity of antibodies is the heterogeneity of antibodies. Antibody composition is extremely complex and consists of thousands and thousands of Ig molecules. These Ig molecules are both similar and different in shape, size, structure, and amino acid composition and arrangement. Their differences cause their electrophoretic activity to vary greatly. As the antibody has a binding site (antigen-binding cluster) corresponding to the antigenic determinant, the binding of the antibody to the antigen is specific. On the other hand, the antibody itself is a protein, with its own amino acid composition, arrangement, and three-dimensional structure; in the case of xeno animals, it is an antigen. Each Ig has an antigen specificity that is detectable by serological methods and shows different serological types. There are three specific Ig antigens. (1) In those isotype specific, all from the same species have common antigen specificity. Human Ig can be divided into five major categories (IgM, IgG, IgA, IgD, IgE), and two types (lambda and kappa), as well as several subclasses, subtypes, groups, and subgroups. However, the specificity of the binding of the antibody to the antigen is not related to the antibody class, subclass, type, etc. (2) In allotype specificity, the antigenic determinant is shared by some individuals of the same species (such as the human), which is determined by the genetic category, some of the heavy chain and light chain constant regions, and the replacement of individual amino acids to form Gm, Am, and Km, of each type. (3) Unique type specificity arises from a single B-cell clone of Ig molecules with unique antigenicity. This determinant is in the variable regions of the heavy and light chains, especially in the hypervariable regions of the variable regions. Because each individual antibody-forming cell is composed of polyclones, the idiosyncratic specificity is extremely large.

The diversity of antibodies is controlled by the B-cell system genes. The peptide chain is encoded by two different genes, respectively, of the variable or constant region; the constant region of the gene (C gene) is limited, although it can determine the type and subclass of Ig molecules, to cause Ig molecular diversity. However, the main reason for the diversity of immunoglobulin molecules lies in the heterogeneity of the variable region.

2.5.2 Antibody Diversity Mechanism

Antibody diversity has the following mechanisms [3].

1. VDJ gene fragment combination: The antibody forms a Y-structure from two identical heavy chains and two identical light chains. The specificity of the antibody for the antigen is determined by the variable region of the antibody. The heavy chain variable region of the human antibody consists of three VDJ gene segments located on chromosome 14: about 45 V gene segments, 23 D gene segments, and 6 J gene segments. During maturation of B cells to produce antibodies, the region of the antibody gene that contains the VDJ gene is rearranged; one for each of the VDJ fragments to form the variable region of the antibody, and

one of many constant region gene fragments is then taken to form the antibody heavy chain. As a result, 45 × 23 × 6 = 6210 kinds of antibodies are possible. The human light chain genes are k and λ, located on chromosomes 2 and 12, respectively. The light chain variable region consists of two VJ gene fragments: the number of Vk and Jk fragments is 40 and 5, and the number of Vλ and Jλ fragments is 30 and 4, respectively. Thus, possible k species are 40 × 5 = 200 and the possible k type is 30 × 4 = 120 species. The antibody consists of a heavy chain and a light chain such that there are 6210 × (200 + 120) = 1,198,700 species possibilities for the number of antibodies that result from the light and heavy chain V (D) J rearrangements.

2. Diversity of junctional connections among VDJ: Although the antibody diversity caused by the combination of VDJ gene fragments is about 2 × 106, the actual situation is far more than that, suggesting that there are other diversity mechanisms. The V (D) J gene fragment leaves the 3′-end of the sense strand and the 5′-end of the antisense strand before ligation, such as before the V and D gene fragments are ligated. Similarly, similar to the V gene fragment, the D gene fragment ends (the head end, the end to be linked to the V gene) are also dissociated by a recombinase to form a hairpin. When the V gene is linked to the D gene, the hairpin structures of the two gene segments are resected. However, the site of cleavage is not necessarily the original position, resulting in one strand in the double strand of the V (D) gene being longer than the other. The shorter one is filled to the blunt end and then to the D (V) gene fragment. Completed nucleotides are called P-nucleotides. The P-nucleotide is present between the DJ gene segments or the light chain VJ gene segments as the result of this mechanism. The presence of P-nucleotides greatly enriches the diversity of antibodies. In addition, after P-nucleotides are added, and also when terminal deoxynucleotidyl transferase (TdT) is active, the terminal transferase may add nucleotides after the P-nucleotide up to 20 nucleotides; these additions are called N-nucleotides. In addition to adding P- or N-nucleotides, deletions of nucleotides can also be made at the ends between the VDJ fragments of the antibody. The insertion and deletion of this nucleotide greatly enriches the diversity of antibodies.
3. Somatic high-frequency mutation of mature B cells in addition to the VDJ gene junction region and the precursor cells or other cells in the body is different; it was found that the VDJ gene is also different, suggesting a mature B-cell VDJ gene mutation, called somatic hypermutation. High-frequency somatic mutations have a crucial role in the diversity of antibodies, especially the specificity and affinity of antibodies. Through the combination of diversity and P-, N-, and other nucleotides, changes mostly produce low-affinity antibodies. Affinity maturation at this time has a function in the peripheral lymphoid organs (mainly lymph nodes) germinal center; in B cells with low-affinity binding antigen in the VDJ nucleotide, mutation occurs by mitosis, at a mutation frequency as great as one mutation per 1000 nucleotides. Mutated cells with more affinity to the antibody gain preponderance of growth, thereby greatly increasing the affinity of the antibody produced by the B cells.

3 ABO Blood Group Antibodies

3.1 Introduction

The ABO blood group antibody, which is one of the most important antibodies in the human body, has many features in common with general antibodies in terms of basic functions and biological characteristics. The relationship between ABO blood group antibodies and common antibodies is individuality and commonness. As the basic rules and characteristics of antibodies are mentioned earlier, this section focuses on the ABO blood group antibody personality characteristics.

3.2 Type of ABO Blood Group Antibodies

In the human ABO blood group system, there are blood group antigens on the erythrocyte membrane, the most important of which are the A antigen and the B antigen. The carbohydrate-linked glycoproteins or glycolipids of these two antigens are distributed on the surface of most tissues, including the vascular system of endothelial cells. Blood can be divided into four kinds of ABO blood groups according to the existence of A antigen and B antigen on the erythrocyte membrane: four blood types, namely, A, B, O, and AB. The surface of the human type A red blood cell contains A antigen (also known as agglutinogen), the serum contains anti-B antibody; the type B human erythrocyte surface contains B antigen, and the serum contains anti-A antibody. In the AB type of human red blood cells, the cell surface contains two antigens, A and B, but the serum has neither anti-A antibody nor anti-B antibody. The type O human erythrocyte cell surface has neither A antigen nor B antigen; the serum contains anti-A antibody and anti-B antibody. The ABO blood group system can be divided into several subtypes: for type A blood, there are at least two subtypes, A1 and A2, and in the A1 erythrocyte membrane two antigens, A and A1, in the A2 type erythrocyte membrane. Antigen B is only contained in the sera of type A sera, which contains only the A antigen. The serum of type A2 sera contains anti-B and anti-A1 antibodies. The A type is divided into A1 and A2 types, making AB type, which is also divided into A1B type and A2B type, or two subtypes. Among these blood types, the antigenicity of each blood group antigen is different according to the distribution of the antigen on the erythrocyte membrane of each blood group: the antigenicity of the AB blood group is strongest, the antigenicity of O-type blood is the weakest, and in the others, the blood group antigenicity is A1B > A > A2B > B > A2.

Inheritance of the human ABO blood group system is controlled by the alleles IA, IB, and i on chromosome 9. Only two of these three genes may appear on a pair of chromosomes, one for each offspring (except for the rare CisAB blood group). Three genes can be composed of six genotypes, because the A and B genes are genetically dominant. O-type genes are recessive, so in the blood type of expression of only four, the same blood type of their genetic type is not certain (Table 3.1). For example, in the phenotype of type A blood, the genetic type may be A1A1 or A1O;

Table 3.1 Genotype and phenotype, antigen and antibody, of ABO blood groups

Phenotype	Genotype	Erythrocyte antigen	Natural antibodies in serum
Al	Al Al	A1	Anti-B
	A1 O	A1 + H	
A2	A2 A2	A2	Anti-B (about 10% people have anti-A1)
	A2O	A2 + H	
B	BB	B	Anti-A
	BO	B + H	
A1B	AB	A1 + B	None
A2B	Al B	A2 + B	(About 25% people have anti-A1)
O	O	H	Anti-A + B

Note: All erythrocytes in the foregoing list generally have a certain H antigen on their surface and therefore do not produce anti-H antibodies (although some reports suggest that approximately 3% of A1B-type blood donors contain anti-H antibodies). To indicate that A2 antigen is weaker than A1, the H antigen is not marked in these blood types

in phenotype A2 type blood, the genetic type may be A2A2 or A2O; but for the red blood cells on the phenotype O, the gene type can only be OO. Because phenotypes A or B may originate from the AO and BO genotypes, respectively, it is entirely possible that parents of type A or B blood types will give birth to children whose phenotype is type O.

In the United States, about 20% of blood group A individuals showed A2, whereas most showed A1. In contrast, A2 is extremely rare in Japan [4]. However, although type A2 and A2B Han people in China accounted for a smaller proportion of type A and type AB, respectively, type A1 and type A2 A2B are agglutinated by type A1 erythrocytes, and the RBCs are less antigenic than A1 and A1B erythrocytes. Therefore, in view of this characteristic of blood type A2, in ABOi (ABO-incompatible) organ transplantation, studies have shown [4, 5] that the kidneys from the A2 blood group donors have corresponding preoperative and postoperative treatment. The extent to which antibody-mediated graft injury is undergone by technology is less severe than that of other blood donor donors. To some extent, this experimental result confirms the idea that the antigenicity of type A2 erythrocytes is weaker than that of A1 erythrocytes and has a role in future development of transplantation.

ABO blood group antibodies are divided into two categories of natural antibodies and immune antibodies; these blood group antibodies are mainly IgM and IgG class antibodies, with are a very small number of IgA antibodies. The origin of human blood group antibodies cannot be completely elucidated. It is generally considered to be caused by exposure to A and B blood group antigens or their analogues in the environment (bacteria, viruses, animals, plants, etc.). These antibodies can cause human blood group substance reactions, such as when type A individuals exposed to B antigens produce anti-B antibodies; this produces more antibodies to IgM. Pregnancy, blood transfusion, and transplantation have produced more blood group antibodies, which are IgG. In physiological circumstances, the newborn has not yet fully developed, so there is no blood ABO blood group antibody. At 2–8 months after birth the antibody begins to produce, with 18 months needed to

reach a steady state, peaking at 8–10 years of age. Because of its molecular weight, the natural antibody, mostly IgM, cannot pass the placenta; therefore, in pregnant women with fetal blood group discrepancy, the natural antibodies in the body of the pregnant woman will not enter the fetus through the placenta, and will not cause fetal agglutination of red blood cells. An immune antibody in the body to accept their own does not exist in the stimulation of erythrocyte antigen produced. Immune antibodies are mostly IgG antibodies, so by their molecular weight they can enter the fetus through the placenta. Therefore, if the mother in the past has experienced exogenous A or B antigen in the body (such as in blood transfusion) and produces immune antibodies, fetal and ABO blood group incompatibility of pregnant women may be caused by entrance of maternal immune blood type antibodies into the fetus, causing fetal red blood cell damage, the occurrence of hemolytic disease of newborns.

3.3 Blood Group Antibody Removal Technology

Postoperative rejection is a major challenge for ABOi organ transplantation. Acute rejection has been the main factor impeding the long-term survival of ABOi organ transplantation. With the application and development of blood group antibody removal technology in organ transplantation, people have come to realize that this technology will become crucial for determining the success or failure of transplantation. Its significance lies in the ABO blood group antibodies (mainly IgG and IgM) in patients with clearance or reduced in vivo titers, so that ABOi transplant rejection is reduced to achieve effectiveness in enhancing graft survival or prolongation of the patient's life.

Blood group antibody removal technology includes plasma exchange technology, immuno-adsorption technology, and plasma double filtration technology.

1. Plasma exchange technology is a plasma separation device, the use of extracorporeal circulation of the patient's plasma separation and filtration, removal of abnormal plasma, and recovery of the blood to replace the physical components; some of the autoantibodies in plasma are removed in this method. In ABOi organ transplantation, the primary purpose of plasmapheresis is to remove anti-A and anti-B antibodies in patients and to reduce the incidence of postoperative acute rejection. Plasma exchange technology can effectively remove from the body small, medium, and large molecular substances, especially protein-binding substances, such as toxins and antibodies. The filtration effect of plasma exchange on various ABO blood group antibodies is shown in Fig. 3.1. Among the three

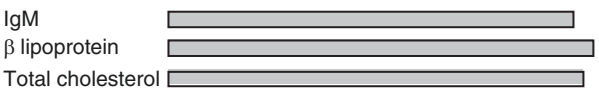

Fig. 3.1 Filtration composition and proportion of ABO blood group antibody in plasma exchange

blood group antibodies, the antibody IgG has the smallest molecular weight and IgM the highest molecular weight, and, therefore, the highest filtration rate is that of the IgG of the blood group antibody, and IgA is second, the lowest rate of IgM antibody filtration. We can see that among the three types of blood group antibodies, the blood group antibody IgM is most likely to be cleared in blood replacement. Plasma exchange technology is widely used in the United States and Japan, with anti-ABO blood group antibodies eliminated by one application reaching about 20%. The main advantage of plasmapheresis in the removal of ABO blood group antibodies is that, for example, freshly freeze-dried type AB plasmapheresis removes blood group antibodies and replenishes clotting factors; its major drawback is the risk of a viral infection. It is prone to allergic reactions, and the treatment is more costly. On the other hand, plasma exchange technology can be used alone to remove blood group antibodies and can also be used in combination with plasma double filtration.

2. Adsorption technology is a blood purification technology developed on the basis of plasma exchange in the past 15 years. The principle is that the separated plasma selectively removes related antibodies through an adsorber. In ABOi organ transplantation, immuno-adsorption occurs through the extracorporeal circulation, the highly specific antigen–antibody and adsorbent material of the adsorption column being selective for removal of antibodies in plasma, thereby reducing rejection after transplantation. Approximately 30% of anti-A and/or anti-B IgM antibodies are cleared by immunosorbent in a single treatment, and clearance of the anti-A and/or anti-B IgG antibody is approximately 20%, which will not cause other antibody changes [6]. In ABO-incompatible organ transplantation, the types and percentages of the immunosorbent-removed blood group antibodies are IgG1, -2, -3 (100%), IgG3 (30–80%), IgM (56%), and IgA (69%). Compared with plasmapheresis, immunosuppressive technology has obvious advantages in terms of curative effect and safety. For example, the adsorption of blood type antibody is specific; no albumin and other plasma component is lost, there is no need of replenishing fluid, and no risk of virus infection. However, immunosorbent technology also has its own shortcomings, such as the need to use specific adsorbers, which are more expensive.
3. Dual plasma filtration technology is the separation of the smaller plasma components through the membrane pores of the separator, and the clearance of the body of large molecular weight proteins, while leaving the small molecular weight albumin and other active ingredients, and adding the supplement liquid and returning it to the treatment. Plasma double filtration technology is a highly selective blood purification technology, which uses two different pore-size filters: one is a plasma separator, by centrifugation or filtration effect, and the other is the plasma filter. The pore size of the filter mainly allows no more than or less than the passage of albumin molecules, and substances greater in size than albumin will be cleared out. In the application of ABOi organ transplantation, because of the different pore size of the blood plasma filter membrane, the inhibition rate of the blood group antibody is also different. Therefore, plasma membrane filters with different pore sizes should be selected according to the molecular weight of

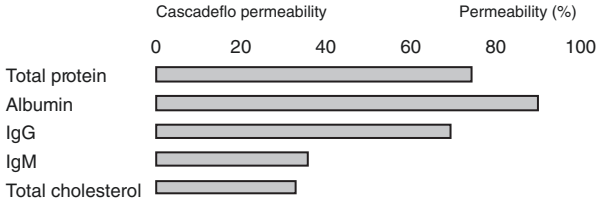

Fig. 3.2 Filtration composition and proportion of ABO blood group antibody in plasma double filtration

the pathogenic substance, to ensure not only the complete elimination of the corresponding antibodies, but also to minimize the loss of albumin. In ABOi organ transplantation, the double filtration technique of plasma filters with the filtration rate and the proportion of different components is shown in Fig. 3.2. Because of the large molecular weight of the blood group antibody IgM, it is difficult to be effectively removed through the blood plasma filter; blood group antibodies IgG have a relatively small molecular weight and pass through the plasma filter relatively more easily than IgM. The main advantage of the plasma double-pass technique in removing blood group antibodies is that the relatively selective elimination of ABO blood group antibodies and HLA antibodies reduces the loss of albumin and can generally be supplemented with albumin solution to effectively reduce the risk of infection, such as viruses, and allergic reactions. The main drawbacks are the large number of coagulation factors and macromolecular protein loss.

Filtration composition and proportion of ABO blood group antibody in plasma double filtration using a certain type of adsorption column are shown in Fig. 3.2.

The plasma exchange technique, immuno-adsorption technique, and plasma double filtration technique are important breakthroughs in the history of ABOi organ transplantation. These three techniques can effectively remove anti-A and anti-B antibodies from patients, when applied alone or in combination, to reduce the incidence of acute rejection after transplantation, and greatly improve the survival rate of graft and patients, thus having an important role in the development of ABOi organ transplantation.

4 Detection of Blood Type Antibody and Its Significance

4.1 Test Method for Blood Group Antibody

In the immune response, cellular immunity and humoral immunity are two closely related and mutually regulated physiological processes. There are many detection methods for both immune responses, but so far in clinical testing, the detection of specific antibodies is the most widely used. Antibody titer refers to the physical state of an antibody and its residence time in the body, and its immune response is expressed by how much it reacts with the antigen.

Both anti-A and anti-B antibodies include IgG and IgM. IgG can be detected by the indirect Coombs test, IgM antibody can be detected by the saline method; the bromelain method can be used to detect IgG and IgM, and has higher sensitivity than that of the other two methods. Micro-column gel Karl's method is a new method to display the reaction of erythrocyte antigen antibody in recent years. It has advantages such as simple operation, easy observation of results, and simultaneous detection of incomplete antibodies.

4.1.1 Saline Medium Agglutination Test

The antigen in the saline media agglutination test is a saline erythrocyte suspension. The antigenic determinant on the erythrocytes binds to the antigen-binding site on the corresponding antibody molecule and crosslinks to form a macroscopic agglutination mass. The saline media agglutination test is used to detect, identify, and cross-match blood tests with IgM antibodies as well as to blood group systems identified by saline antibodies such as ABO, MN, P, or S. In the saline medium agglutination test in the determination of blood type and cross-matching blood, results of the observation are available immediately after centrifugation, with the advantages of easy operation, saving time, and low consumption. However, because the distance between erythrocytes in saline solution is about 25 nm, the shortest distance between the two most adjacent Fab ends of IgM antibody molecules is more than 35 nm, and the distance between two Fab ends of IgG antibody molecules is generally less than 25 nm, IgG antibodies do not agglutinate with the red blood cells of the corresponding antibodies in the saline medium, whereas IgM antibodies do agglutinate. However, low detection sensitivity and low sensitivity to incomplete antibodies can lead to serious consequences as well as disadvantages such as unstable results and poor interpretation, and such methods have therefore been either eliminated or used only for preliminary tests of blood compatibility testing.

4.1.2 Colloidal Media Agglutination Test

Agglutination of IgG antibodies usually does not occur in saline media. However, if an erythrocyte suspension is prepared in colloidal medium, the antibodies on erythrocytes agglutinate with the corresponding IgG antibodies in serum, and the colloidal medium affects the second phase of the agglutination reaction. The erythrocyte surface is rich in sialic acid; red blood cells in a neutral environment are negatively charged and because of the same charge repel each other in a suspended state. Electrostatic theory holds that the higher the cell-surface charge, the dielectric constant of the smaller media, the greater the potential, the greater the power between the red blood cells, and the greater the distance between cells; on the other hand, the distance between the red blood cells is small. The main role of colloidal media is to maintain the dielectric constant and shorten the distance between the red blood cells, the IgG antibodies sensitized erythrocytes agglutination phenomenon. The substances used to form the colloidal medium include bovine serum albumin (BSA), human albumin, AB type serum, and gum acacia. It is most convenient to take AB type serum, and the best is bovine albumin, which is also used more often. Colloidal media agglutination tests increase the sensitivity of the reaction, especially for IgG

class antibodies. However, the operation of such methods is complicated and the test requirements are also high; there is also a problem of subjective interpretation. At present, only a few laboratories in the United States use certain BSA-boosting antibody-enhancing solutions: 18% use 22% BSA, and only 3% use 30% BSA. Other methods neutralize the negative charges on the surface of red blood cells by using low ionic strength solution (LISS) or polyethylene glycol (PEG) added with molecular substances, and shorten the distance between the red blood cells to enhance the direct agglutination reaction.

4.1.3 Enzyme-Treated Erythrocyte Agglutination Test

The erythrocyte surface is rich in sialic acid, making it negatively charged in the neutral environment, which is why red blood cells exclude each other. Proteolytic enzymes digest and destroy this sialic acid, reducing the negative charge on the surface of red blood cells, thereby promoting agglutination. Common enzymes are bromelain and papain. The enzyme method can be divided into one-step and two-step methods; the one-step method is not as sensitive as the two-step method, but is easy to operate, and more convenient for cross-matching blood, and the two-step method is generally used for antibody screening and antibody identification if the positive result is too much. The enzyme method can significantly enhance the antigen–antibody reaction of the Rh and Kidd systems. However, proteases can destroy M, N, S, s, Fya, and Fyb antigens and cannot be treated with enzymes for such antigens. Although the enzymatic method is more sensitive, the application of such methods has been limited for some of these reasons. The use criteria recommended by the Swiss Red Cross Blood Transfusion Service (BTSSRC) suggest that one-step enzymatic methods are not sensitive enough to be eliminated, but enhanced two-step enzyme assays are necessary for pregnant women. Gerbr believes that the enzymatic assay is not suitable for routine work; too many positive results are caused by unrelated clinical factors. In Finland, enzymatic methods have been phased out since the 1980s to avoid interfering with meaningless antibodies. In the UK, a survey of 50,776 pregnant women who had "records" showed that the positive rate was 6.6% for initial screening tests, 4.7% was still positive again, and only 1.4% (731 cases) contained identifiable potentially clinically relevant erythrocyte alloantibodies, of which 117 cases were detectable only enzymatically; 54 of 117 patients had antibodies with potential clinical significance to the fetus. Results of these 54 follow-up studies showed that only 3 patients were detected by PEG-IAT at later stages of pregnancy (anti-D 0.9 IU/ml; anti-D 1:32; anti-C 1:1), with only one infant showing signs of hemolytic disease of the newborn (HDN). It can be inferred that the results of the enzyme test are not effective for the detection of the morbidity and mortality of the fetus or the newborn and a role in the prenatal examination. The abolition of the enzymatic test and the introduction of the automatic processing program and data acquisition will probably compress the huge workload. Similarly, the use of enzymatic crossover tests is also not recommended in the UK. The results of the UK National Objective Quality Assessment Program (NEQAS) showed that although the IAT assay is sensitive to detect weak RH antibodies by enzymatic assays, the IAT is weakly detectable in the most sensitive anti-D method, which is based on experimental evidence.

4.1.4 Antiglobulin Test and Its Improved Method

The antiglobulin test, established in 1945 by Coombs et al., is also known as the Coombs test. The establishment of an antiglobulin test is very important for blood group serology. The erythrocyte surface is coated with IgG antibody molecules or complement molecule C3, C4 fragments, but cannot produce agglutination. IgG antibodies and complement are human globulin, and with these human immunoglobulin immunization animals or hybridoma technology can be made of anti-human globulin The specificity of such antibodies against the Fc fragment of IgG molecules or complement C3, C4 fragments can be coated on the red blood cells and antibody complement molecules, agglutination of red blood cells. Anti-human globulin molecules take part in bridging. The antiglobulin test is divided into the direct anti-human globulin test (DAT) and indirect anti-human globulin test (IAT). The traditional method is saltwater as a medium, test tubes by centrifugation, widely used in cross-matching blood, blood type identification, and antibody screening. In the UK, only 82% of the test-anti-D programs in 1985 adopted the indirect anti-human globulin test (IAT), but reaching 98% in 1992 and 100% in 1994 [7]. A survey of 59 hospitals in Ontario conducted by the Toronto Red Cross Society Toronto Center in 1993 showed that two thirds of hospitals used IAT for antibody screening experiments. Although tube centrifugation of anti-human globulin assays is considered an acceptable technique for detecting antibodies to erythrocytes, data suggest that the reaction between 1+ and 2+ is easy because of improper interpretation in standard IAT tube centrifuge assays, being wrong especially more often than 20% on a large scale. Because many clinically significant erythrocytic antibodies are IgG, many improvements have been made to increase the sensitivity of anti-human globulin assays: the medium is changed to low ionic strength solution (LISS), polyethylene glycol (PEG), and the like. These improved tests are sensitive in tubes or microplates. As can be seen from the data provided by NEQAS, almost all laboratories in the UK and Scotland have adopted enhanced anti-human globulin methods, usually using low ionic strength media plus two-step enzymatic methods. These methods are recommended because they improve sensitivity and show good performance in detecting clinically relevant antibodies and also have excellent records in NEQAS experiments in Scotland. Molds believes that in the hands of a skilled serologist, LISS—antiglobulin test (AGT) and PEGAGT—have like sensitivity, but the latter will produce less ambiguous results. LISS-AGT or PEG-AGT experiments are all available if only antiglobulin-reactive antibodies are tested, but both are flawed in the detection of mixed antibodies. From a report from the Netherlands, Overbeeke said that their choice of the PEG-IAT method was based on a comparative study of a large sample of plasma containing known antibodies and a large sample of unknown patients. They found that the PEG-IAT method was more sensitive than the standard centrifuge tube IAT assay using albumin. Several clinically significant antibodies are detected by the PEG-IAT method but not by the albumin IAT method, such as Jka antibodies, Jkb, antibodies and E antibody. The only problem with this technique is that IgM hemolysins (such as anti-Vel antibodies and anti-P antibodies) are detectable using the multispecific antiglobulin reagent IAT but not with PEG-IAT. The main problem with anti-human globulin is the interpretation of the results. When the anti-human

globulin method is used in a large number of sample experiments, and results are negative, it is very difficult to maintain accurate interpretation. In addition, there are some problems such as cumbersome operation, heavy workload, and being time-consuming, which restrict their application.

4.1.5 Low-Ion Polybrene Test

In 1980, Lalezari and Jiang first applied polybrene technology to blood bank operations. Polybrene is a high-priced cationic quaternary ammonium salt polymer that can produce much positive charge when dissolved to redirect the surface of the negative charge, so that the distance between the red blood cells can be reduced; the normal erythrocyte can cause reversible nonspecific agglutination. If the antibody-sensitized erythrocytes are agglutinated by agglutinants, the agglutination is irreversible. In the polybrene test that detects the activity of antibodies, erythrocytes and serum are first incubated in a low-ionic medium to promote the binding of antibodies to erythrocytes, followed by the addition of polybrene to agglutinate the cells. After centrifugation, the agglutinated cells are no longer suspended, added to the resuspension, agglutination of polybrene can be neutralized by addition of sodium citrate, the agglutination of normal erythrocytes dissolved, and antibody-induced agglutination still exists. Chen Heping et al. improved the technique of agglomerating amines. The modified polybrene technique is characterized as fast, simple, and sensitive. Especially for Rh system antibodies, its sensitivity is higher than that of the anti-human globulin method; for example, the detection of anti-D is possible to 1 ng/ml, much higher than the antiglobulin method of about 10 ng/ml. This feature provides a powerful tool for the detection of weak Rh system antibodies. In 1983, Fisher used the saltwater method, papaya enzyme method, low ionic saline anti-human globulin method, the polybrene method, and other four different methods to detect the ability of alloantibodies and found that the polybrene method detected alloantibodies with higher sensitivity than other methods. Since then, European and American blood bank laboratories have adopted this method. However, the coagulation amine method still cannot detect all the incomplete antibodies, such as anti-k antibody, so its application has been limited.

4.1.6 Microtube Gel Test

The micropipette gel detection method has gradually emerged in recent years as a new immunoassay method. First invented by Dr. Yves Lapierre in France in 1986, it combines a gel column with an anti-human globulin method, etc., and is a product of the combination of biochemical gel filtration and immunological antigens. The gel is the key to microtubule gel detection technology; the concentration of gel controls the size of the gel gap so that the gap can only allow the passage of free red blood cells. So, free red blood cells and red blood cells gather to distinguish the reaction. If by centrifugation, erythrocyte sedimentation in the bottom of the test tube indicates that no agglutination of erythrocytes is a negative reaction; if the red blood cells are in the upper part of the gel band, it indicates agglutination of red blood cells, a positive reaction. In addition, the gel has been added to anti-human

globulin and various types of blood group antibodies, thus simplifying the operation; just add the sample. This type of technology in blood type identification, cross-matching of blood, and antibody screening and identification has a wide range of applications. Gel detection technology is a new technology, currently in the stage of improvement and promotion, that is also gradually being taken seriously. Some hospitals in Canada use gel detection technology to experiment as they think this technology is simple and the results easy to determine. Gel detection technology has also drawn considerable attention in the United States. Garratty stated that the study of alloantibodies produced the detection of very good results. He believes (and hopes) to automate all compatibility experiments quickly. ABO and D blood group identification and antibody screening by blood recipients and blood donors in the United States will be automated and electronic: that is, computerized cross-matching will be used, and gel cards can easily achieve this goal. In 1994 NEQAS received feedback from 380 hospital blood banks which showed that approximately 65% of the cross-match blood tests were performed with gel technology using low-ion-concentration solution centrifugation (BTSSRC) in antibody studies based on 6-month results of the comparative experiment and the subsequent operational experience. Several smaller studies of other technologies have also consolidated their choices. In addition to high sensitivity and high specificity, gel experiments also seem to have high reproducibility, relying on experimental staff: small, long-term preservation of experimental results can be reviewed at any time, which may have so far unknown advantages. Two different techniques were also chosen, traditional centrifugation tube antiglobulin and gel technology, based on the pathological changes of plasma in some patients, such as plasma protein disorders and strong cold or thermal antibodies. Specimens, known as HLTA antibodies, are more easily detectable by the tube method than the gel method. As a reference laboratory, problematic samples are very common: more than 50% of cross-matched blood shows unpredictable responses in different blood types. Approximately one-third of Finnish hospital laboratories, including three-fifths of the university hospitals, chose the condensate method. The data obtained from KUOPIO University Hospital show that screening with the gel method has important clinical implications for increasing the percentage of antibodies identified. Many published data show that this gel anti-human globulin approach meets or exceeds conventional anti-human globulin approaches when examining weak antibodies. Voak and Ouvehand have developed rapid validation techniques for evaluating new methods and using the LISSIA method correctly. This work, as well as many articles covering the new IAT method, shows that the effects of the new antiglobulin method, that is, gel technique and the polyethylene glycol (PEG) centrifuge antiglobulin method, are at least equivalent to the LISS tube centrifugation globulin method. Therefore, with the correct use of high-quality screening cells, they can meet the antibody screening experiments. In addition, data from the NEQAS in the UK demonstrated this, with 38 of the 188 subjects using the IVTG assay for 1 weak anti-antibody missing, whereas only 1 of 53 using the microtubule method missed the check. Many advantages of gel detection techniques are increasingly being applied because of their sensitivity, ease of operation, and long timeframe for interpreting results. As the key to gel technology

is control of the quality of the gel, variations in gel cards have also drawn the same attention. Data from two Finnish Red Cross Blood Centers at university hospitals using DiaMed-ID gel cards indicate that the number of positive anti-human globulin experiments in cross-matched blood tests is unbelievably high (1:400), whereas that from the third laboratory using the traditional method was low (1:3000), and they concluded that at least some batches of DiaMed experiments are overly sensitive to very weak IgGs on the erythrocyte surface. Gel card variations and ABO compatibility (i.e., B plasma and A2B cells compatible) may cause gel cards to miss-check; this was first discovered in sophisticated experiments in the UK and has been confirmed by Cummuns and Downham. They believe that the application of gel microcolumn technology in cross-matching experiments remains to be further studied. Under Spain's recently enacted law, the saline method and the traditional anti-human globulin method will be eliminated to detect ABO and other incompatibilities when cross-matching blood. Because of the advantages and automation of gel cards, their use will be greatly increased. This result suggests that the widespread use of gel technology in other countries to detect red blood cell antibodies will become a trend.

4.2 Significance of Blood Group Antibody Test

4.2.1 Significance in Blood Transfusion

Because each person has his or her own blood type and corresponding antibody, when blood transfusions are needed clinically for surgery, it is necessary to first identify the blood type of recipients and donors, and then cross-match the blood type. When a different type of blood is infused, it can cause severe hemolysis that can endanger the recipient's life. Because O-type blood has neither A nor B antigens, many people think that O-type blood is a universal donor, and this idea is not desirable. Because type O blood plasma contains antibodies A and B, in a small amount of blood transfusion the antibody may be diluted in the recipient blood and the secretion of blood type substances and will not have a significant impact, and when a large number of infusions take place, antibodies in the plasma can react with hematopoietic cells of the recipients to cause hemolysis. In addition, studies have shown that some O-containing anti-A or anti-B antibodies that cannot be neutralized by blood group substances can cause a hemolytic reaction. Similarly, that AB is a universal recipient of blood is not correct, in addition to the need to be careful as to A1 and A2 subtypes. Therefore, only the same group of blood is infused, and a different group is used as a last resort.

4.2.2 Significance in Organ Transplantation

The ABO blood group is a strong antigen and therefore needs to be considered for donor ABO blood grouping in organ transplants or hematopoietic stem cell transplants, which otherwise cause hyperacute rejection, especially skin grafts. With the development of science and technology, it has been possible to overcome the ABO

blood group disorder. For removal of AB antibodies (such as removal of donor erythrocytes by centrifugation during hematopoietic stem cell transplantation) by immunosorbent, plasmapheresis, or similar methods preceding transplantation, and successful transplantation of T (B) cells using immunosuppressive agents after ABO blood incompatible transplantation, see the following chapter.

4.2.3 Significance in Neonatal Hemolysis

In humans, about half a year after babies are born, because of contact with the nature of the A or B antigens the AB antibody begins to appear in the blood, mainly including IgM and IgG. Neonatal hemolysis occurs in the mother when the blood type is O, and the most common fetal blood group is A or B, and caused by heavier jaundice; others such as the mother's blood group A, fetal blood group B or AB; mother's blood group B, fetal blood group A or AB are less common, and cause less jaundice. Because of the presence of A/B antigens in nature, ABO hemolysis can occur in the first child. Although Rh-negative mothers develop only when the second fetus is Rh-positive and the first fetus is also Rh-positive, although the first child, a history of blood transfusions could cause the mother to produce Rh antibodies.

4.2.4 Other Significance

The ABO blood groups can also be used for kinship identification and forensic identification. Second, the ABO blood group is also associated with disease. The study found that in type A, B, and AB individuals, the probability of squamous cell carcinoma is lower than in the individual with O-type blood. The probability of individual type O blood pancreatic cancer is lower than in other blood types. In terms of coagulation, individuals with type O blood and individuals with level VI and vWF are lower than those with other blood types and tend to have bleeding. Accordingly, studies have shown that A1 and B increase the risk of venous thrombosis, whereas A2 and O decrease the risk.

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