Immune Haemolytic Anaemia

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The immune haemolytic anaemias comprise a set of diseases characterized by shortened red blood cell survival (haemolysis) mediated by the action of antibodies and serum complement. The haemolysis is evidenced by a raised reticulocyte count in the absence of blood loss. Activity of the immune system is diagnosed by the presence of antibody or fragments of complement components (mainly C3 and C4) on the red blood cell surfaces.

Introduction

The great majority of acquired haemolytic anaemias are mediated by autoantibodies. In contrast, congenital haemolytic anaemias, such as hereditary spherocytosis, are due to an intrinsic defect in red blood cell (RBC) structure. During the first half of the twentieth century, clinicians experienced difficulty in distinguishing acquired from congenital haemolytic anaemias. Elegant cross-transfusion experiments using the Ashby differential agglutination technique to determine RBC lifespan *in vivo* permitted the first distinction between these two disorders (reviewed by Mollison, 1959). In these experiments, RBCs from normal individuals exhibited shortened survival in the circulation of patients with acquired haemolytic anaemia, suggesting that an extrinsic defect was responsible for the haemolysis. In contrast, RBCs from patients with congenital haemolytic anaemia exhibited shortened survival in the circulation of normal individuals, suggesting the presence of a defect intrinsic to the RBC. The development of the antiglobulin test (Coombs *et al.*, 1945) and its application to patients in the 1940s (Boorman *et al.*, 1946; Loutit and Mollison, 1946) provided the defining distinction between acquired and congenital haemolytic anaemias, namely the presence of antibody or complement fragments on RBCs in the former, and their absence in the latter. **See also**: Anaemia: overview; Autoimmune disease; History of immunology

Autoimmune haemolytic anaemia is classified in two complementary ways: by the temperature (warm or cold) at which autoantibodies bind most efficiently to patients' RBCs, and according to the absence or presence of a related underlying disease (primary or secondary). A third category, drug-induced immune haemolytic anaemia, may involve true autoantibodies directed against patient RBC antigens, or antibodies directed against drugs or their metabolites. The classification schema is shown in **Table 1**. **See also**: Antigen–antibody binding

Pathophysiology of the Disease

Mechanisms of RBC destruction differ among warm antibody, cold antibody and drug-induced immune haemolytic

Table 1 Classification of the immune haemolytic anaemias

Warm-reactive autoantibodies
Primary (also termed 'idiopathic')
Secondary
    Collagen vascular disorders (particularly systemic lupus erythematosus)
    Lymphoproliferative disorders
    Certain nonlymphoid neoplasms (particularly ovarian tumours)
    Certain inflammatory disorders (particularly ulcerative colitis)

Cold-reactive autoantibodies
Primary cold agglutinin disease
Secondary cold agglutinin disease
    Lymphoproliferative disorders
    Infections (particularly infectious mononucleosis, *Mycoplasma pneumoniae*)
    Paroxysmal cold haemoglobinuria
    Primary
    Secondary, associated with syphilis
    Donath–Landsteiner haemolytic anaemia, associated with viral syndromes

Drug-induced immune haemolytic anaemia
    Drug adsorption mechanism
    Ternary complex (immune complex) mechanism
    True autoantibody induction

anaemias. Each type will be discussed separately in this section.

The antiglobulin (Coombs) test

The diagnosis of immune haemolytic anaemia requires demonstration that the immune system is involved in the process of RBC destruction. This is achieved clinically by

means of the direct antiglobulin (Coombs) test. The antiglobulin reagent is an antiserum that contains antibodies directed against human immunoglobulin and complement components. The history of its development is both interesting and informative. **See also**: Agglutination techniques for detecting antigen–antibody reactions

Before 1945, it had been observed that the serum from some patients with acquired haemolytic anaemia possessed the ability to agglutinate RBCs in saline suspension. These agglutinins were primarily cold-reactive, and later were demonstrated to be immunoglobulins, chiefly of the immunoglobulin M (IgM) class. IgM molecules, being pentavalent, are physically able to span the distance between two or more RBCs and agglutinate them together. Other patients' serum exhibited the ability to haemolyse RBCs *in vitro* when fresh serum was present as a source of complement; the haemolysis-promoting activity was found to reside either in saline agglutinins or in immunoglobulins of the IgG class, termed haemolysins. However, serum from most patients with acquired haemolytic anaemia contained neither agglutinins nor haemolysins. Through a set of insightful experiments, it was found that the cells from these latter patients could be agglutinated by goat or rabbit antiserum to human IgG or complement proteins. This indicated that the cells from these patients had been coated *in vivo* with either antibody or complement, or both. The test was called the direct antiglobulin test because it detected globulin (either immunoglobulin or complement components) on cells taken directly from the patient. With some refinements, the test exists and is employed today much as it was almost 60 years ago. **See also**: Antibody classes

The indirect antiglobulin test detects free antibody in the plasma of patients with warm antibody autoimmune haemolytic anaemia. The indirect antiglobulin test can also be used to detect alloantibodies formed in patients who have been exposed to RBC antigens through pregnancy or blood transfusion. That it is an alloantibody may be inferred from the absence of a positive direct antiglobulin reaction, and by demonstrating specificity of the antibody for an antigen not present on the patient's RBCs.

The principles of saline agglutination and the direct and indirect antiglobulin tests are shown in **Figure 1**.

Warm antibody autoimmune haemolytic anaemia

Warm autoantibodies are typically of the IgG class. When bound to the surface of RBCs, some warm reactive antibodies fix complement, leaving complement fragments (mainly C3b, C3bi or C4b) covalently attached to the cell surface. Macrophages in the Billroth cords of the spleen and Kupffer cells in the sinusoids of the liver possess surface receptors for the fragment crystalline (Fc) region of IgG and for complement. When RBCs coated with antibody and/or complement pass through these parts of the circulation, they become trapped and are then partly or completely ingested by the macrophages. The complement sequence is generally aborted after deposition of C3, before formation of membrane attack complexes. Thus, completion of the complement sequence with resultant lysis of RBCs does not occur to any great extent in warm antibody haemolytic anaemia, in spite of the ability of many warm antibodies to fix complement. This is due in part to the presence of complement regulatory proteins on RBC membranes as well as in the plasma. The rate of RBC destruction by this so-called extravascular trapping mechanism is relatively slow, occurring over days or weeks. **See also**: Complement; Fc receptors; Macrophages

Cold antibody autoimmune haemolytic anaemia

Cold antibody autoimmune haemolytic anaemia may be mediated by either cold agglutinins or cold haemolysins, antibodies that are most avid for RBCs at temperatures below 37°C. The clinical threat posed by a cold agglutinin is related to two of its properties: titre and thermal amplitude. Titre is measured by diluting patient serum and is expressed as the greatest dilution at which RBC agglutination is observed. Thermal amplitude is the highest reaction temperature at which agglutination occurs. Healthy individuals may have low thermal amplitude cold agglutinins in titres as high as 1:32 or 1:64. Pathological cold agglutinins capable of mediating haemolysis are present in titres ranging from tens of thousands to over one million. The thermal amplitude of pathological cold agglutinins is high, in the range of temperatures attainable in the body, i.e. 30–37°C.

Cold agglutinins and cold haemolysins both shorten RBC survival in the circulation through their ability to fix complement. Cold agglutinins are generally of the IgM class. Agglutination of RBCs in the colder parts of the circulation is responsible for acrocyanosis (blue extremities), one of the symptoms of cold agglutinin disease. Shortened RBC survival in paroxysmal cold haemoglobinuria and Donath–Landsteiner haemolytic anaemia is mediated by cold haemolysins of IgG class.

Destruction of RBCs in cold antibody haemolytic syndromes may occur by either of two mechanisms. Autoantibody attaches to RBCs as they travel through colder parts of the circulation such as the fingers, toes, nose and ears. The temperature of the blood in these areas may be as low as 30–32°C. Complement fixation, which can occur at those temperatures, becomes even more efficient as the cells circulate to areas where the temperature is 37°C. In cold agglutinin disease, many complement sequences that are thus initiated will be aborted by the cell membrane and plasma complement regulatory proteins, leaving opsonically active fragments of C3 and C4 on the RBC. These complement-coated cells may be partly ingested by
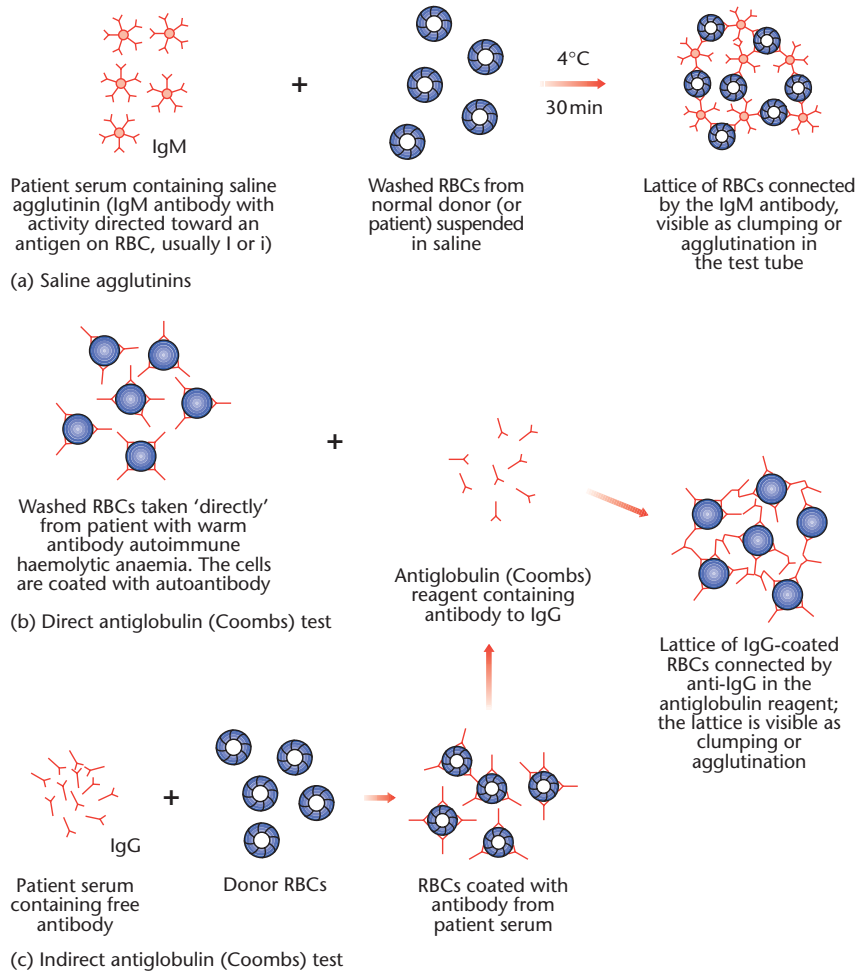
Figure 1 Saline agglutinins and the antiglobulin test. (a) *Saline agglutination*. Antibodies that can agglutinate RBCs are termed saline agglutinins. Most such antibodies are of IgM class and are almost always autoantibodies, usually directed towards RBC antigens I or i. These antibodies react with RBC antigens more efficiently at temperatures below normal body temperature (37°C) and are thus termed cold agglutinins. IgM antibodies can agglutinate RBCs more easily than IgG antibodies, primarily because the molecules are pentavalent and large, which allows them to span the intercellular space and bind as many as five RBCs. (b) *Direct antiglobulin (Coombs) test*. IgG antibodies are in general too small to span between two RBCs, and thus are incapable of causing agglutination. Most patients with acquired haemolytic anaemia have warm antibody autoimmune haemolysis mediated by IgG autoantibodies, with or without complement. IgG autoantibodies and complement components are detected on RBCs by the direct antiglobulin test, often called the Coombs test, after its originator. RBCs taken directly from the patient are washed to remove plasma. Washing does not remove the bound IgG or complement from the RBCs. The antiglobulin reagent, containing antibody to IgG, complement components, or both, is then added to the RBC suspension. The antibodies in the antiglobulin reagent bind to the cell-bound antibody or complement, and agglutinate the cells. Using monospecific antiglobulin reagents, the patterns of IgG alone, complement alone or IgG plus complement may be detected. In the figure, the cells are coated with IgG alone. In cases of warm antibody autoimmune haemolytic anaemia exhibiting complement alone, there is actually IgG present on the RBCs but in quantities too small to detect (about 400 molecules per cell) by standard antiglobulin reagents. Cold antibodies and drug-dependent antibodies of the ternary or immune complex type also exhibit a complement alone pattern. (c) *Indirect antiglobulin test*. The indirect antiglobulin test detects antibody in patient plasma. The test is performed by first incubating patient serum containing free antibody with donor RBCs. Antibody from the serum binds to the RBCs and can now be detected using the antiglobulin reagent. It may be alloantibody formed in response to previous RBC exposure through transfusion or pregnancy, or it may be autoantibody. Alloantibodies do not bind to the patient's own RBCs, thus the direct antiglobulin test will be negative. When autoantibodies are detected by the indirect antiglobulin test, the direct antiglobulin test result must also be positive as well. This is due to overflow: there is more antibody present in the patient's blood than can be absorbed by the patient's own RBCs. The antigen specificity of alloantibodies and autoantibodies can be ascertained by the indirect antiglobulin test using panels of reagent RBCs of defined antigenic phenotype.

macrophages in the liver or spleen, as in warm antibody haemolytic anaemia. Complement sequences on other cells may escape regulation, allowing generation of the C5b–9 membrane attack complex which lyses the cells in the circulation, without help from fixed macrophages. This type of 'intravascular' haemolysis can also occur in cold agglutinin disease, and is the chief mechanism of RBC destruction by cold haemolysins. Haemoglobinuria is one

of the hallmarks of intravascular haemolysis because the rapidity of RBC destruction overwhelms the normal mechanisms of haemoglobin removal from the blood, causing haemoglobin to be filtered and excreted by the kidneys. **See also**: Complement regulatory proteins

Drug-induced immune haemolytic anaemia

It has been understood since the 1950s that certain drugs can injure blood cells by immune mechanisms. It is important to distinguish drug-mediated immune injury of RBCs from drug-mediated injury to intrinsically defective RBCs (e.g. as in glucose 6-phosphate dehydrogenase deficiency). It is further important to differentiate drug-mediated immune processes from autoimmune processes because, once recognized, treatment consists of drug withdrawal and prevention of further episodes is achieved by drug avoidance. **See also**: Inborn errors of metabolism

The mechanisms by which drugs induce antibodies in patients are poorly understood. Most drugs are small molecules that alone are nonimmunogenic. However, according to well-established principles of hapten chemistry, small molecules such as drugs (haptens) attached covalently to larger carrier proteins can induce antibodies with specificity towards the drug. In some cases, the antibody thus elicited may demonstrate specificity not only towards the hapten (drug), but also towards the carrier protein, the so-called 'carrier effect'. Carrier proteins in patients have not been identified with certainty. The antibodies are usually drug-dependent, which means that the drug must be present, either attached to the RBC surface or in the aqueous milieu, in order for the antibody to bind to the RBC. A carrier effect is sometimes suggested, as when drug-dependent antibodies are directed towards a particular RBC membrane antigen. In such cases, the RBC membrane antigen may have played a specific role in antibody induction. In other cases, true autoantibodies indistinguishable from naturally occurring autoantibodies, and which can bind to RBCs in the absence of the drug, may arise during use of certain drugs. **See also**: Antibodies; Antibody function; Haptens

In contrast to our poor understanding of the induction phase of drug–antibody formation, the mechanisms of RBC injury during the effector phase are better understood. Classification of drug-induced immune haemolytic anaemias is based on distinctions in effector mechanisms, of which there are three. A fourth category, drug-induced nonimmunological adsorption of proteins by RBCs, must be distinguished from the other three. Drugs that have been documented to cause immune haemolysis or a positive direct antiglobulin test are listed by mechanism in **Table 2**.

Drug adsorption mechanism

Certain drugs, exemplified by penicillin, can bind covalently to proteins, including those found on the RBC

Table 2 Drugs that cause immune haemolysis or a positive direct antiglobulin test result

Drug adsorption mechanism	
Penicillins	Carbromal
Cephalosporins	Tolbutamide
Tetracycline	Cianidanol
6-Mercaptopurine	Carboplatin
Ternary complex (immune complex) mechanism	
Stibophen	Quinine
Quinidine	Chlorpropamide
Rifampicin	Antazoline
Thiopental	Tolmetin
Probenecid	Nomifensine
Cephalosporins	Diethylstilboestrol
Amphotericin B	Doxepin
Diclofenac	Etodolac
True autoantibody mechanism	
α-Methyldopa	L-Dopa
Cephalosporins	Cianidanol
Tolmetin	Nomifensine
Teniposide	Mefenamic acid
Latamoxef	Glafenine
Procainamide	Diclofenac
2-Chlorodeoxyadenosine	Fludarabine
Nonimmunological protein adsorption	
Cephalosporins	Cisplatin
Undetermined mechanisms	
Streptomycin	Ibuprofen
Triamterene	Erythromycin
5-Fluorouracil	Nalidixic acid
Sulindac	Omeprazole
Thiazides	Acetaminophen
p-Aminosalicylic acid	Isoniazid
Melphalan	Chlorpromazine
Insecticides	Phenacetin
Mesantoin	

membrane. Patients who receive high doses of penicillin (10–30 million units per day), or those receiving lower doses in the presence of renal failure, achieve high blood levels of drugs that bind covalently to RBC membrane proteins. The drug does not by itself injure the RBCs, but if the patient makes IgG antibody to certain penicillin epitopes the antibody attaches to the penicillin coating the RBCs (**Figure 2a**), which are in turn removed from the circulation by splenic macrophages. The direct antiglobulin (Coombs) test detects IgG on the patient's RBCs, but
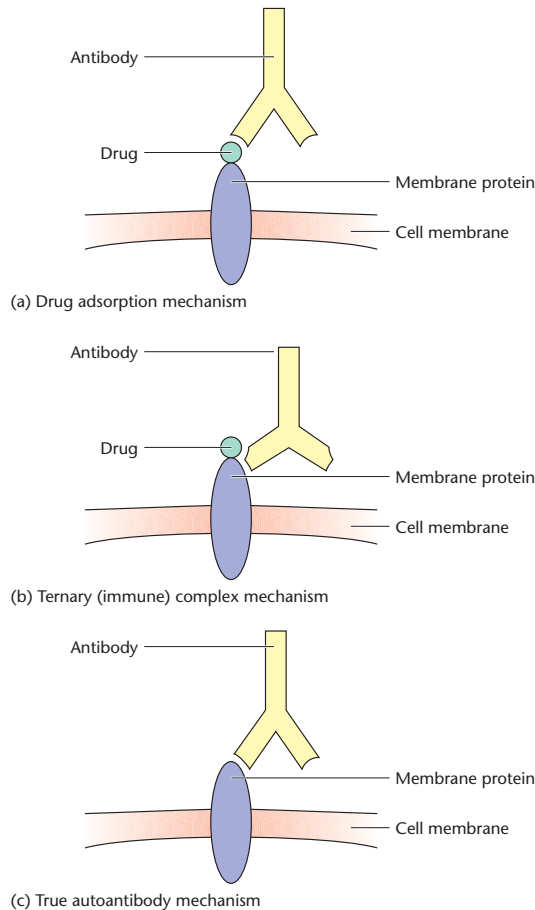
(a) Drug adsorption mechanism

(b) Ternary (immune) complex mechanism

(c) True autoantibody mechanism

Figure 2 Mechanisms of interaction between drug, antibody and RBC membrane antigen during the effector phase in drug-immune haemolytic anaemia. (a) *Drug adsorption mechanism*. The drug is tightly bound to the RBCs, presumably to a protein. This occurs *in vitro* or *in vivo*. Antibody directed against the drug may then attach to the membrane-bound drug. As is typical for haptens (in this case the drug), antibody does not bind to the hapten unless it is attached to a protein carrier (in this case, the RBC membrane protein). There is no evidence that the antidrug antibody recognizes any epitope of the membrane protein. A high blood level of the drug is required to achieve RBC membrane coating with the drug (and subsequently antibody) sufficient to cause haemolysis. The direct antiglobulin test is positive for IgG. The indirect antiglobulin test result is also positive if the reagent cells are previously coated with the drug before incubation with patient serum. (b) *Ternary (immune) complex mechanism*. Drugs that mediate immune haemolysis by this mechanism bind only weakly and in small quantity to RBCs. The mechanism by which the drug-dependent antibodies form is not known. The antibodies will not bind drug in the absence of RBCs, and the RBCs cannot be coated with the drug in the absence of the antibody. However, if all three components (RBC, drug and antibody) are present together, a stable trimolecular (ternary) complex consisting of drug, RBC membrane protein antigen and antibody is formed. Ternary complexes mediate haemolysis through the complement system. The direct antiglobulin test is positive for complement, mainly fragments of C3. (c) *True autoantibody mechanism*. Certain drugs elicit autoantibodies against RBCs. The mechanism of antibody induction is unknown. The antibodies, mostly IgG class, are indistinguishable from RBC autoantibodies that arise *de novo*. The likelihood of haemolysis increases with increasing drug dosage, but the antibodies are not drug dependent in that their attachment to RBCs occurs equally well in the presence or absence of the drug.

the indirect antiglobulin test is negative with normal RBCs. However, if normal RBCs are first coated with penicillin *in vitro*, then incubated with patient serum, the indirect antiglobulin test detects IgG on the cells. Only a small proportion of patients who receive penicillin develop the requisite IgG antibodies, and since high-dose penicillin is rarely used any more, this cause of drug-induced immune haemolysis is seen infrequently. Other drugs may also cause haemolysis by the same mechanism. **See also**: Renal failure

Ternary complex mechanism

In sharp contrast to drugs that cause haemolysis by the drug adsorption mechanism, drugs that cause haemolytic anaemia by the ternary complex mechanism bind only weakly to the RBC membrane, and the antibodies that mediate RBC injury seem to recognize both a component of the drug or a metabolite, and an antigen on the RBC membrane. This mechanism is termed 'immune complex' by many authorities, but ternary complex seems to be a more accurate descriptive term. This process is illustrated in **Figure 2b**, in which the drug, antibody and RBC antigen form a stable trimolecular or ternary complex. RBC injury is mediated by activation of the complement system, with resultant intravascular lysis of RBCs. In this type of immune haemolysis a single dose of the drug is sufficient to induce haemolysis in patients who have previously been exposed to the drug and have formed the requisite drug-dependent antibodies.

The responsible antibodies may be either the IgG or IgM class. As in the drug adsorption mechanism, the antibodies require the presence of the drug in order to attach to the RBCs. However, in contrast to the drug adsorption mechanism, the drug must be present in the fluid phase simultaneously with antibody and RBCs in order for antibody attachment to occur. The direct antiglobulin test detects only complement on RBCs taken directly from the patient. However, if drug is included to stabilize the ternary complex during the washing steps for the indirect antiglobulin test, and fresh patient serum is used as the antibody and complement source, both antibody and complement may be detected on the RBC membranes.

The clever idea to include drug in the wash steps of the indirect antiglobulin test, thereby stabilizing the ternary complex and allowing detection of the responsible drug-dependent antibodies, led to observations suggesting that RBC antigens contribute to the epitope recognized by these antibodies. It was noted that, in some cases, antibodies from patients with this type of drug-mediated immune haemolysis were reactive only with RBCs expressing certain blood group antigens such as Kell, Kidd, Rh and others, but nonreactive with RBCs lacking these antigens. These observations suggest that the drug-dependent antibody recognizes a composite neoantigen consisting of the drug and a specific epitope of an RBC membrane antigen. It is

further implied by these observations that the immunogen for the drug-dependent antibody in these cases included the specific epitope recognized by the antibody in concert with the drug. **See also**: Blood groups and transfusion science

A traditional concept in hapten chemistry holds that in order for a hapten to be immunogenic it must be covalently bound to its carrier protein. The weak binding of drug to the RBC membrane of patients with ternary complex immune haemolysis seems to violate that concept, and the mechanism by which such a composite neoantigen may be immunogenic is unclear. The antibodies in these patients are usually strongly drug-dependent: they do not bind to RBCs in the absence of drug. However, in some instances patients produce both drug-dependent and drug-independent antibodies (autoantibodies) and, where an antigen specificity has been identified, it is the same for both types of antibody, providing support for the idea that the RBC antigen played a role in antibody induction.

Drug-induced autoantibodies

Some drugs possess the capacity to induce antibodies that bind to RBCs in the absence of drug. Such antibodies are indistinguishable from spontaneously arising autoantibodies. The mechanisms by which drugs can induce autoantibodies are unknown but, as noted above, in at least some cases drug and membrane antigen may combine to serve as immunogen.

The prototype drug for this mechanism of immune haemolysis is α-methyldopa. α-Methyldopa was in widespread use as an antihypertensive agent during the 1960s and 1970s, but it has been largely supplanted by other drugs.

Antibodies induced by α-methyldopa are of the IgG class, with specificity for core components of the Rh antigen, not unlike the autoantibodies seen in warm antibody autoimmune haemolytic anaemia. The direct antiglobulin test is positive in 8–36% of patients receiving this drug, but only about 1% exhibit haemolytic anaemia. The direct antiglobulin test may turn positive 3–6 months after starting the drug, the length of time being inversely proportional to the drug dosage. When the drug is discontinued, the direct antiglobulin test reverts to negative over several months to 2 years, but the haemolytic anaemia ceases promptly, even while the antiglobulin test is still positive. Patients who are rechallenged with α-methyldopa will again develop a positive direct antiglobulin test result, but there is no anaemnestic response, i.e. the length of time to develop a positive antiglobulin test is the same with rechallenge as it was following the first challenge.

Drug-induced nonimmunological protein adsorption

A fourth type of drug-induced antibody attachment to RBCs is termed nonimmunological protein adsorption. Certain drugs mediate nonspecific attachment of plasma proteins to RBCs, including albumin, transferrin, fibrinogen and antibodies. Special antiglobulin reagents may be used to detect these nonimmune proteins on the RBC. The protein binding does not injure RBCs, but a positive direct antiglobulin test result must be interpreted with caution in patients receiving drugs known to cause this phenomenon. **See also**: Autoimmune disease: pathogenesis; Haematopoiesis

Major Clinical Features, Course and Complications

The annual incidence of autoimmune haemolytic anaemia is estimated at one to two cases per 100 000 population. About 80–90% of these are of the warm antibody type, the remainder being mediated by cold-reactive antibodies. There are no good estimates of the incidence of haemolytic anaemia mediated by drug-dependent antibodies, but such cases are not rare. The peak incidence is in the seventh decade, and most patients are over 40 years of age, although cases in children are well described. Very rarely does autoimmune haemolytic anaemia occur in families. The disease recognizes no sex, racial or ethnic group difference.

Interestingly, approximately 1 in 10 000 normal volunteer blood donors exhibits a positive direct antiglobulin test result, a frequency about 10 times that observed for autoimmune haemolytic anaemia. The autoantibody is usually a warm reactive IgG, which exhibits serological features similar to those of the autoantibodies found in patients with warm antibody autoimmune haemolytic anaemia. A few of these patients develop overt autoimmune haemolysis but most do not, even though the positive direct antiglobulin test result may persist indefinitely. It is possible that many or most cases of clinically overt autoimmune haemolysis develop in a subset of these healthy individuals who exhibit a positive direct antiglobulin test result, but thus far there is no evidence to support this hypothesis.

Clinical features

History and examination

The symptoms experienced by patients with autoimmune haemolytic anaemia are influenced largely by the rapidity of the haemolysis. Most patients with warm autoantibody-mediated haemolysis, some patients with cold autoantibody-mediated haemolysis and those with drug-immune haemolysis of the drug adsorption or true autoantibody types experience a gradual onset of anaemia, and thus their symptoms appear gradually. In these patients, RBC destruction occurs primarily in the spleen (extravascular haemolysis). Such patients may complain of easy fatigue, dyspnoea on exertion, rapid heart beat, malaise, pale skin or jaundice. All these symptoms, except jaundice, are

typical for anaemia of any cause. Patients with cardiovascular disease or limited cardiac reserve may experience angina or symptoms of congestive heart failure. **See also**: Heart failure

Intravascular haemolysis mediated by cold antibodies or by drug-dependent antibodies that work by the ternary complex mechanism is typically more rapid in onset, and symptoms are accordingly more acute and severe. In addition to the symptoms listed above, such patients often complain of dark urine, which is due to haemoglobinuria. Cardiac decompensation is more likely to occur in the elderly or in those with cardiac compromise. In these patients, a history of exposure to cold temperature or recent ingestion of a new drug may be given.

Common physical findings include pallor, jaundice and a mildly or moderately enlarged spleen. In patients for whom the onset of anaemia was rapid, signs of heart failure and orthostatic hypotension may also be present. Patients with severe anaemia may also exhibit tachypnoea and tachycardia. **See also**: Anaemia: adaptive mechanisms and consequences

Laboratory features

Virtually all patients present with anaemia, which may be mild and asymptomatic, or severe and life threatening, with haemoglobin levels below $5\,g\,dL^{-1}$. The blood smear holds important clues to the diagnosis. Polychromasia, which indicates increased egress of reticulocytes from the marrow, is often present. Spherocytes are an important diagnostic hallmark that should prompt strong consideration of immune haemolysis. RBC fragments and nucleated RBCs may also be seen on the blood smear. The reticulocyte count is usually increased, although early in the process as many as one-third of patients may exhibit reticulocytopenia. In these latter patients, the reticulocyte count usually increases during the first few days of observation. Leucocytes, primarily neutrophils, are often increased, and the platelet count is typically normal. The serum bilirubin concentration is usually mildly increased (up to $5\,mg\,dL^{-1}$), chiefly the unconjugated (indirect) fraction. This is due to the liver's inability to conjugate the increased load of bilirubin being presented. Serum lactate dehydrogenase concentration is also increased because the RBC cytoplasm is rich in this enzyme, which is released into the plasma when RBCs are destroyed. The level of haptoglobin, the chief binding protein for free haemoglobin in the plasma, is usually decreased as haptoglobin–haemoglobin complexes are rapidly cleared by the liver. Urinary and stool concentrations of urobilinogen, a colourless intermediate formed by bacterial degradation of bilirubin in the gut, are increased, but their measurement is cumbersome and not necessary for the diagnosis. Bile is not typically found in the urine since unconjugated bilirubin is not excreted by the kidneys. Dark urine in patients with haemolytic anaemia is usually due to haemoglobinuria, which occurs in patients with hyperacute haemolysis. **See also**: Bone marrow; Erythrocytes; Neutrophils; Platelets

The antiglobulin (Coombs) test

In patients with autoimmune haemolytic anaemia, three patterns of RBC coating may be identified with monospecific antiglobulin reagents: (1) IgG alone, (2) complement alone and (3) IgG plus complement. All three patterns may be seen in patients with warm antibody autoimmune haemolytic anaemia. Patients with haemolysis mediated by cold-reactive autoantibodies or by drug-dependent antibodies that mediate haemolysis by the ternary complex mechanism generally exhibit a complement-alone pattern. Finally, patients with drug-immune haemolysis of the drug adsorption and true autoantibody types exhibit an IgG-alone pattern. **See also**: Antibody classes; Complement

Course and complications

Patients with warm antibody autoimmune haemolytic anaemia exhibit an unpredictable relapsing and remitting course, in spite of a high initial response rate to treatment with glucocorticoids and splenectomy. Deep vein thrombosis of the lower extremities is common during active haemolysis, and pulmonary embolism, infection and cardiovascular complications have all been documented as causes of death. Recent data on survival are lacking, but an actuarial survival rate of 73% at 10 years has been reported. **See also**: Pulmonary embolic disease; Venous thrombosis

Chronic cold agglutinin disease generally pursues a protracted but benign course. Such patients learn to avoid chilling, which can precipitate a haemolytic episode. The postinfectious forms of cold agglutinin haemolytic anaemia seen in association with *Mycoplasma pneumoniae* and with infectious mononucleosis are generally self-limited, resolving in 1–3 weeks. Paroxysmal cold haemoglobinuria is rarely seen any more; previously, the disease was seen in patients with syphilis. A related disorder, Donath–Landsteiner haemolytic anaemia in children, is usually seen following a viral infection and is self-limited, often clearing after a single episode of haemolysis. **See also**: Syphilis

Drug-immune haemolytic anaemia is usually mild and ceases promptly upon discontinuation of the offending drug. Drugs that mediate haemolysis through the ternary complex mechanism are an exception, occasionally causing acute severe haemolysis leading to renal failure and death. In drug adsorption-mediated and ternary complex-mediated haemolysis, the direct antiglobulin test result becomes negative a few days after the drug is discontinued. However, in cases of haemolysis mediated by true autoantibodies related to α-methyldopa, the direct antiglobulin test may remain positive for many months after the drug is stopped, even though the haemolysis has ceased.

Approaches to Management

Warm antibody autoimmune haemolytic anaemia

RBC transfusion

Patients with warm antibody-mediated haemolysis generally develop anaemia slowly with ample time for cardiovascular compensation, and are thus in little danger of circulatory collapse. The best indicator of the need for transfusion is the patient's clinical condition, rather than a predetermined haemoglobin level. A young, previously healthy, patient may be relatively asymptomatic at a given haemoglobin concentration, whereas an elderly person with cardiovascular disease may be suffering angina and dyspnoea at rest at the same haemoglobin level. Even though the transfused RBCs will be destroyed as rapidly as the patient's own, in the latter case transfusion may be life saving, maintaining the patient until other measures take effect. Patients who present with reticulocytopenia often develop severe anaemia rapidly, because of the failure of the marrow to compensate. Such patients should also be considered for early transfusion. **See also**: Blood groups and transfusion science

It is usually impossible to find truly compatible donor RBCs. The patient's autoantibody usually reacts with all donor RBCs. In that case, it is necessary for the blood bank to provide blood that is least incompatible with the patient's serum in the crossmatch procedure. It is also important for the blood bank to search for possible alloantibodies in the plasma of patients who have been previously transfused or pregnant. Once selected, the RBCs should be infused slowly while the patient is monitored carefully for signs of a haemolytic transfusion reaction.

Glucocorticoids

Glucocorticoids have been the mainstay of treatment for warm antibody autoimmune haemolytic anaemia for over 50 years. Cessation, or at least slowing of RBC destruction, occurs in about two-thirds of patients. Therapy is usually initiated with prednisone $1–1.5\,\text{mg}\,\text{kg}^{-1}$ orally or its equivalent intravenously. Once the haemoglobin level begins to improve, usually in 1–3 weeks, the dose of prednisone is decreased in a stepwise fashion. When the direct antiglobulin test result becomes negative, the patient can be weaned completely from the prednisone. Relapses are common, necessitating close follow-up for several years.

Splenectomy

Patients who chronically require more than 15–20 mg prednisone daily, or those who do not respond to initial therapy, are candidates for splenectomy. There is no reliable way to determine who will respond to splenectomy, so the decision is made on clinical grounds. If the patient relapses repeatedly as the prednisone dose is decreased, if there is no response at all in 3–4 weeks, if the anaemia is severe, or the patient deteriorates, splenectomy should be done sooner rather than later.

The haemolysis will remit in about two-thirds of patients, either partially or completely, but relapses are common. However, many patients who still require glucocorticoid therapy after splenectomy can be maintained with alternate-day therapy or with lower daily doses than before.

Splenectomy itself carries a low risk of morbidity or mortality, but splenectomized subjects are at increased risk for infection with encapsulated organisms. Children are at greater risk than adults, and the risk of sepsis is greatest in the first year or two following splenectomy. However, the increased risk probably persists for life. It is recommended that patients younger than 40 years of age receive vaccination against pneumococcus, meningococcus and *Haemophilus influenza* type b before operation. Patients older than 40 years generally have immunity to meningococcus and *H. influenza* and require immunization only for pneumococcus (Centers for Disease Control and Prevention, 1993). **See also**: Spleen; Spleen: consequences of lack of function

Other treatments

Patients who have not responded to glucocorticoids or splenectomy may respond to immunosuppression with cytotoxic agents such as cyclophosphamide $60\,\text{mg}\,\text{m}^{-2}$ or azathioprine $80\,\text{mg}\,\text{m}^{-2}$ daily. These drugs should be prescribed only by physicians familiar with their side effects. Both agents suppress haematopoiesis and have been associated with the development of secondary acute leukaemia. **See also**: Immunosuppressive drugs

Recently, several case reports and one large prospective series have described the use of rituximab in patients with relapsed or refractory warm antibody autoimmune haemolytic anaemia. Rituximab is a monoclonal antibody to CD20, an antigen expressed on B lymphocytes and is used chiefly to treat patients with lymphoma. The rationale for its use in this setting was to deplete B lymphocytes and thereby decrease autoantibody production. In the prospective series, 12 of 14 children responded (Zecca *et al.*, 2003). Numerous case reports suggest similar efficacy in adults.

Successful use of plasma exchange has been reported in a few cases. High-dose intravenous γ-globulin has also been reported as effective, in case reports and in one series. The nonvirilizing androgen, danazol, has achieved some degree of acceptance based on many case reports and uncontrolled studies. In patients who are poor surgical candidates, danazol may obviate the need for splenectomy. When combined with prednisone, danazol may allow for lower doses and shorter duration of the glucocorticoid, possibly forestalling some of its long-term side effects.

Cold antibody autoimmune haemolytic anaemia

Patients with chronic cold agglutinin disease enjoy considerable symptomatic relief simply by keeping themselves warm, particularly their extremities. Further treatment is usually unnecessary in such patients with mild chronic haemolysis. Splenectomy and glucocorticoids are generally of no use. The cold agglutinin-mediated haemolysis in patients with an underlying lymphoma often responds to treatment of the lymphoma. In a small number of idiopathic cases, successful treatment with chlorambucil or cyclophosphamide has been reported. A remarkable success was achieved in one patient treated with interferon-α_{2b}, but failures have been reported with this drug as well. Plasma exchange may temporarily slow the process in refractory cases by physically removing the cold agglutinin. RBC transfusions may be life saving in cases of symptomatic anaemia, bearing in mind that any benefit is only temporary. Some authorities recommend using washed RBCs to avoid repleting consumed complement components. Postinfectious cold agglutinin haemolysis is usually self limited, requiring only supportive care until recovery occurs in a few days or weeks. **See also**: Interferons: therapeutic uses; Leukaemias and lymphomas

Rituximab has also been used successfully in patients with cold agglutinin disease. Approximately two-thirds of patients respond (Berentsen *et al.*, 2003).

Patients with paroxysmal cold haemoglobinuria may avoid acute attacks by avoiding cold exposure. In cases associated with syphilis, the haemolysis often responds to successful treatment of the syphilis. Donath–Landsteiner haemolytic anaemia in children is usually self-limited, often consisting of a single haemolytic episode.

Drug-immune haemolytic anaemia

The most important measure is discontinuation of the offending drug, which can be life saving in patients with severe haemolysis mediated by the ternary complex mechanism. If a drug is suspected, but the culprit is not immediately obvious – as is often the case – it is prudent judiciously to eliminate any drugs started in the last few weeks while the blood bank completes the serological evaluation. Glucocorticoids are of questionable efficacy but may need to be used empirically if a drug cannot be implicated clearly. Transfusions are useful for symptomatic or life-threatening anaemia, but crossmatching can be a problem as in warm antibody-mediated haemolysis. Haemolysis ceases within a few days of discontinuation of the responsible drug, as soon as the drug clears from the circulation in the case of the ternary complex type, or when the plasma level of the drug lowers in the case of drug adsorption-mediated haemolysis. True autoantibodies induced by drugs are not drug-dependent during the effector phase, i.e. the drug need not be present for the antibody to bind to RBCs. Nonetheless, haemolysis in these patients ceases shortly after the offending drug is stopped, even though the direct antiglobulin test may remain positive for many months.

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