

Blood Group Incompatibility

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Blood group antibodies present in plasma can bind with blood group antigens on red cells and cause a reaction (blood group incompatibility). Antigen–antibody reactions can occur as a result of transfusion (incompatible donor cells) or pregnancy (incompatible fetal cells).

Introduction

Incompatibility in the context of blood groups is due to the binding of plasma antibody with red cell antigen, thereby causing a reaction. In laboratory tests this reaction is most commonly visualized by agglutination of the red cells. In the body, an antigen–antibody reaction can occur as an adverse consequence of blood transfusion or pregnancy, resulting in accelerated red cell destruction. It is therefore important to detect incompatibility between the plasma of a patient and the red cells of a potential blood donor before transfusion, to avoid a transfusion reaction. Incompatibility occurs in pregnancy when the mother is immunized by cells of the fetus which cross the placenta. This immunization only occurs when the fetus has inherited a blood group antigen from the father which is ‘foreign’ to the mother. By far the most important blood groups in relation to blood transfusion are those within the ABO system and, in pregnancy, the D antigen of the Rh system.

Antibodies may be naturally occurring or immune in type. The term ‘naturally occurring’ is used for blood group antibodies produced in individuals who have never been transfused with red cells carrying the relevant antigen or been pregnant with a fetus carrying the relevant antigen. Explanations for the existence of these antibodies include the possibility that some cells may be capable of making specific antibody in the absence of antigenic stimulus or that antibodies, such as anti-A and anti-B, may be produced as an immune response to substances in the environment which are antigenically similar to human blood group substances. An example might be glycoproteins on the surface of bacteria present in the gut, some of which are antigenically similar to the A and B antigens. Immune antibodies are those produced in response to antigenic stimulus by a foreign (nonself) antigen as a result of either blood transfusion or pregnancy. Any blood group can stimulate immune antibody production but those of the Rh system are the most common, notably anti-D. Antibodies that are capable of causing an adverse reaction to transfusion or are harmful to a fetus are said to be clinically significant.

Blood group antigens are inherited characters which are detected by specific antibodies and may be protein or carbohydrate in nature. The blood groups that will be referred to in this text are those intrinsic to the surface of red cells, although some are present on other cells and

tissues of the body. The genetic mechanism involved in the production of protein and carbohydrate antigens is different. Protein antigens (i.e. Rh) are direct products of the appropriate gene. However, the genes controlling the attachment of an immunodominant sugar to a cell membrane component encode transferase enzymes. Therefore, carbohydrate antigens (i.e. ABO) are indirect products of the defining gene. Some of the blood group-active proteins in the red cell membrane are shown in **Figure 1**. There are over 250 blood group antigens which belong to one of the 25 genetically independent blood group systems but only those with the most clinical relevance, ABO and Rh, will be described in this article.

ABO Blood Group System

The ABO blood groups were the first blood groups to be discovered and still remain the most important in transfusion practice today. In 1900, the Austrian scientist Karl Landsteiner found that the red cells of some of his colleagues, when mixed with the plasma of some others, clumped together. This agglutination was due to the ABO antibodies, which are naturally occurring and regularly present in the plasma of all adult individuals when the corresponding antigen is absent from the red cells; it is this aspect that makes the ABO groups so important.

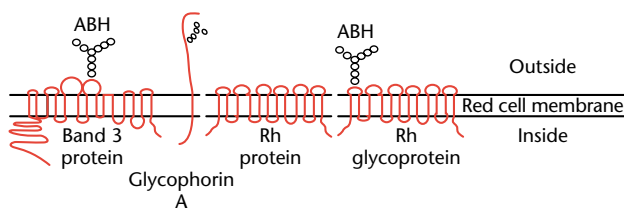


Figure 1 Some blood group-active proteins in the red cell membrane. Circle complexes depict carbohydrate structures (ABH).

Introductory article

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Table 1 ABO blood groups

ABO type	Antibody present
A	Anti-B
B	Anti-A
AB	None
O	Anti-A,B

At its most basic level the ABO system consists of the A and B antigens. Because some individuals express neither A nor B on their red cells, and a few express both, the ABO system gives rise to the following phenotypes: A, B, AB and O. **Table 1** shows the four main ABO blood groups and the corresponding antibodies present in the plasma. Because the antibodies are naturally occurring, as opposed to being the result of immunization by transfusion or pregnancy, severe and immediate adverse reactions, often fatal, can occur if ABO incompatible blood is transfused. This topic will be discussed in more detail in the section dealing with red cell antibodies and blood transfusion.

The structure and biosynthesis of the ABO antigens is well understood as a result of the pioneering work, during the 1950s, of Morgan and Watkins in England and Kabat in the USA. The A and B antigens are carbohydrate determinants of glycoproteins and glycolipids (**Figure 1**) and are distinguished by the type of sugar molecule added to the backbone of the antigen (the immunodominant sugar): *N*-acetylgalactosamine for group A and galactose for group B. The *A* and *B* genes, on chromosome 9, encode glycosyltransferases which catalyse the transfer of the appropriate immunodominant sugar from a nucleotide donor to an acceptor substrate, which is known as the H antigen. The H antigen is the structural backbone of the A and B antigens and is present in almost all people. The *O* gene does not produce an active transferase and so the H backbone remains unaltered and is not antigenic. Hence only anti-A and anti-B occur and never anti-O. The number of A and B antigenic determinants per red cell is in the order of 1×10^6 .

Rh Blood Group System

The first descriptions of the Rh system were in the early 1940s. The antibodies of the Rh system were originally named Rhesus after their discovery during experiments in which blood was transfused into rhesus monkeys, although the term Rhesus is no longer in use. Antibodies to Rh antigens are usually caused through immunization by red cells, although apparently naturally occurring antibodies can and do occur.

The Rh system is far more complex than the ABO system and currently comprises 54 antigens. The D antigen was the first Rh antigen to be described and remains the most clinically important within this system, although other Rh antigens can cause clinical disease. The Rh system at its most basic level can be described in terms of five main antigens: D, C, c, E and e, giving rise to the eight gene complexes shown in **Table 2**. **Table 3** lists the seven most common genotypes found in the United Kingdom. The number of D antigen sites on the different Rh genotypes is approximately in the range $1-3 \times 10^4$.

The Rh antigens are encoded by two highly homologous (i.e. very similar) genes situated very close together on the short arm of chromosome 1. The fact that they are so close together means that they are inherited together. One of these, the *RHD* gene, produces the D antigen and its many variants. The *RHD* gene has only one allele and hence there is either a D antigen produced or nothing; an antithetical d antigen does not exist. The *RHCE* gene produces the C, c, E and e antigens; C and c are antithetical, as are E and e antigens. The C, c, E and e antigens result from nucleotide changes in the *CE* gene giving rise to minor amino acid differences in the *CE* protein, the remainder of the protein being homologous. The D antigen is more immunogenic than the other Rh antigens. The absence of product of an entire *RHD* gene in D negative people, whereas in contrast the difference between C, c, E and e positive and negative is due to small changes in an otherwise identical protein, has been postulated as a reason for the high immunogenicity of the D antigen.

Table 2 Rh system notation

Notation	Rh gene complexes							
CDE	CDe	cde	cDE	cDe	Cde	cdE	CDE	CdE
Shorthand	R ₁	r	R ₂	R ₀	r'	r''	R _z	ry

Blood Group Antibodies (Immunoglobulins)

All antibodies are immunoglobulins (Igs) belonging to a family of structurally related proteins which have two functions: (1) to combine with antigen; and (2) to mediate various biological effects, including the destruction of nonself antigens. All immunoglobulin molecules are made up of two types of polypeptide chains, heavy (H) and light (L), which are held together by disulfide bonds (S–S). A basic structural unit of immunoglobulin is shown in **Figure 2a**. The IgG molecule can be split by digestion with the proteolytic enzyme papain into three fragments (**Figure 2b**). The two Fab fragments are identical and are each composed of one light chain and part of one heavy chain. Each Fab fragment carries an antigen-binding site. The third fragment, the Fc fragment, consists of the remaining parts of the two heavy chains. The Fc fragment carries the sites for complement activation by the classical pathway, for attachment to the surface of other cells, i.e. macrophages (via Fc receptors), and for attachment to placental tissue, which allows transfer of IgG across the placenta. There are five different classes of immunoglobulin, IgG, IgM, IgA, IgD and IgE, but the most important immunoglobulin classes of blood group antibodies in

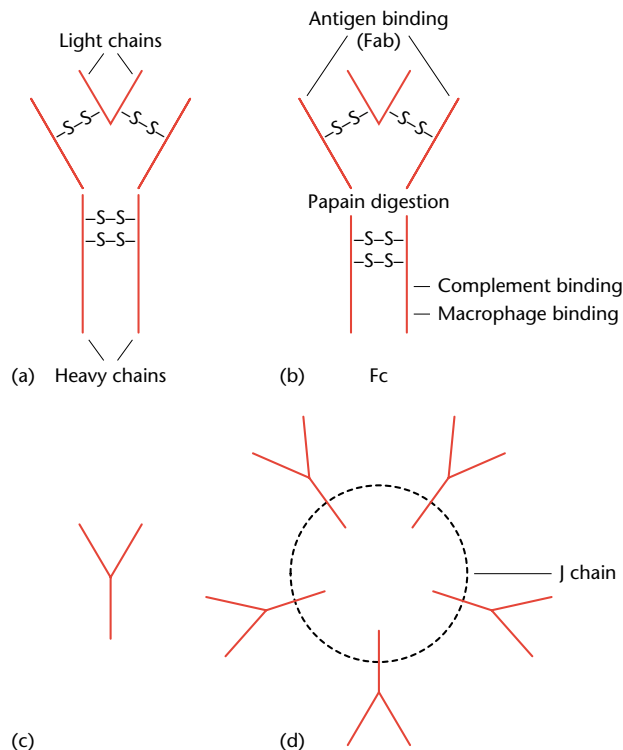


Figure 2 (a) Basic structural unit of immunoglobulin (Ig); (b) Papain digestion of IgG molecule creating Fab and Fc fragments; (c) IgG monomer; (d) IgM pentamer. S-S, disulfide bond.

Table 3 Frequency of the most common Rh genotypes in the UK

Rh genotypes	Approximate frequency (%)
CDe/cde (R ₁ r)	33
CDe/CDe (R ₁ R ₁)	18
cde/cde (rr)	15
CDe/cDE (R ₁ R ₂)	12
cDE/cde (R ₂ r)	11
cDe/cde (R ₀ r)	2
cDE/cDE (R ₂ R ₂)	2

relation to both pregnancy and transfusion are IgG and IgM. IgG is found as a monomer (**Figure 2c**) and comprises about 75% of circulating immunoglobulins. There are four different subclasses of IgG (IgG1, 2, 3 and 4). Red cell alloantibodies are predominantly IgG1 and IgG3, both of which activate complement strongly and therefore have the most clinical importance. IgG antibodies are the most important in pregnancy because this is the only class of immunoglobulin that is capable of crossing the placenta from mother to fetus. IgM is a pentamer with 10 antigen-binding sites (**Figure 2d**) and comprises about 10% of circulating immunoglobulins. The additional polypeptide, the J chain, is required for the polymerization of the basic immunoglobulin units. IgM is particularly efficient at binding the first component of complement (C1), thereby activating the complement cascade, which can lead to lysis of foreign cells.

Immune (Antibody-mediated) Destruction of Red Cells

Antibodies bound to red cells in the body can cause red cell destruction by two major mechanisms: intravascular and extravascular.

Intravascular

Red cells are destroyed in the bloodstream, with consequent release of haemoglobin into the circulation (**Figure 3a**). The antibodies that can cause this type of reaction, i.e. IgM or IgG anti-A or anti-B, cause rapid activation of the complement cascade, usually by the classical pathway. A single IgM antibody molecule can activate complement, but at least two of the binding sites must combine with antigen to initiate complement activation. Two molecules of IgG must be close enough together on the red cell membrane to form a doublet before they can activate the complement cascade. When red cells are coated with a complement-activating antibody, antigen–antibody complexes are formed which activate the first

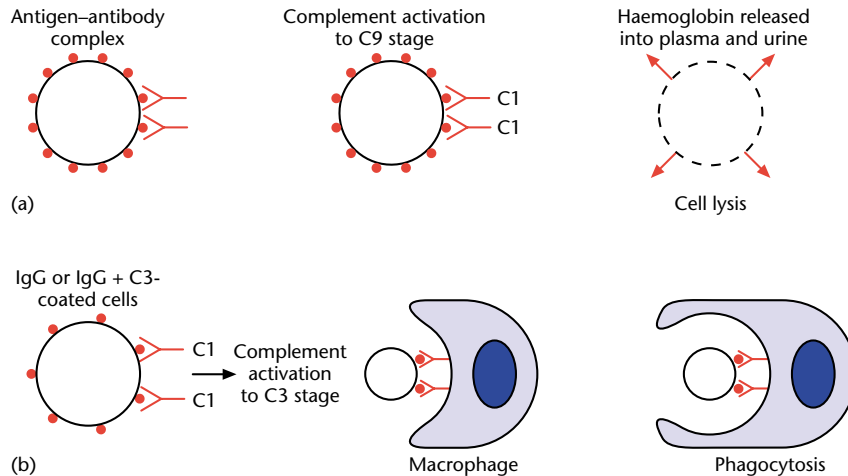


Figure 3 (a) Intravascular and (b) extravascular red cell destruction.

component of complement (C1). Defects occur in the red cell membrane if the activation proceeds sequentially through to the C5b–C9 lytic complex. The membrane defects allow ions to enter the cell, which eventually swells and ruptures, thereby releasing haemoglobin into the plasma. The haemoglobin combines with the plasma protein haptoglobin to form a complex that is cleared by the mononuclear phagocyte system. Excessive haemoglobin may be excreted in the urine.

Extravascular

Intact red cells are removed from the circulation by cells of the mononuclear phagocyte system situated in the liver and spleen. Red cells coated with IgG or sensitized with complement to the C3 stage, but which do not proceed through the cascade to the C5b–C9 lytic complex, may interact with mononuclear phagocytes, notably the macrophage (**Figure 3b**). Attachment of antibody *per se* does not mean that the red cell will be destroyed, but the rate of destruction is related to the number of IgG molecules that bind per red cell and the number of copies of antigen involved. Macrophages have surface receptors which recognize the Fc region of the bound IgG (IgG1 and IgG3) molecule and the complement component C3b. On attachment to the macrophage, the sensitized red cells undergo distortion and may become engulfed by the macrophage. Engulfment may be complete, in which case the red cells are destroyed internally, or partial, in which case the remainder of the red cell circulates as a spherocyte. Spherocytes are more rigid than normal cells, due to loss of protein and lipids, and are susceptible to early destruction. Extravascular red cell destruction results in breakdown products of haemoglobin, such as bilirubin and urobilinogen, in the plasma and urine. This type of red cell

destruction can be caused by IgG anti-D and other Rh antibodies.

Laboratory Detection of Antigen–Antibody Reactions

Blood group antibodies can be detected by a number of methods, known as serological techniques, most of which utilize plasma or serum from the patient. The most commonly used indicator of antigen–antibody interaction in blood grouping is that of agglutination, although haemolysis also indicates that antigen–antibody interaction has taken place. For agglutination to occur, the repulsive forces that normally keep red cells apart must be overcome and, essentially, the multivalent IgM and bivalent IgG molecules crosslink the red cells. The structure of the IgM pentamer with 10 binding sites allows for crosslinking more readily than the IgG monomer. IgM blood group antibodies are capable of acting as ‘direct’ agglutinins; therefore, if serum containing antibody is mixed with red cells possessing the appropriate antigen the cells will clump together ‘directly’, without the addition of anything else, as depicted in **Figure 4**. This is because the pentameric structure ensures that the antibody molecules are close enough to link with antigens on two red cells at once and so bond them together. Although most IgG antibodies do not act as direct agglutinins, there are certain exceptions, notably IgG anti-A and anti-B. This may be due to the number of A/B antigen sites on red cells, which is about 100 times greater than D antigen sites. Agglutination of IgG-sensitized cells can be achieved with the use of various potentiators, such as proteolytic enzymes, or by the indirect antiglobulin technique (IAGT).

The action of proteolytic enzymes (i.e. papain) on red cells may potentiate agglutination in at least two different

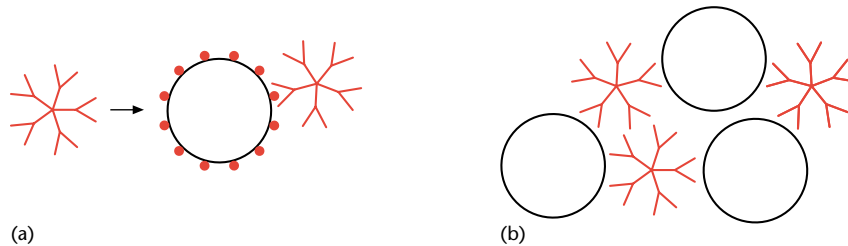


Figure 4 (a) Sensitization of red cell antigen with IgM antibody, and (b) agglutination of cells.

ways: (1) reduces the surface charge and allows red cells to come closer together; and (2) removes structures which sterically interfere with the access of antibody molecules; however, it should be realized that some blood group antigens are destroyed by papain and therefore its use is not suitable for the detection of all blood group antibodies.

The antiglobulin test (AGT) was developed in 1945 and still remains the most important test for detecting clinically significant blood group antibodies. It used to be known as the 'Coombs test' after its inventor. The AGT can be used as an indirect test (IAGT), to determine the presence of antibody in patients plasma, or as a direct test (DAGT), to detect antibody bound to red cells in the body, i.e. cells of babies with haemolytic disease of the newborn or patients with certain types of autoimmune haemolytic anaemia. In the AGT, agglutination is visualized by the addition of antihuman globulin (AHG) to the cells which have antibody on their surface (sensitized), and have been washed in saline to remove residual unbound plasma proteins. The washing procedure is an important step in the AGT because unbound plasma proteins will bind with AHG and inactivate the reagent. AHG reagents contain antibodies to human immunoglobulins but usually contain anticomplement (C3) as well as anti-IgG. Methodology for performing the AGT has evolved since the inception of the test, when an opaque glass tile was used. The tile was superseded by the test tube, which today has largely been superseded by the 'gel test' or 'column agglutination' technology in which plasma proteins do not come into direct contact with the AHG, thereby negating the need for the washing procedure.

Blood Group Antibodies and Transfusion

Blood transfusion is a commonly used form of therapy in hospital practice but it is not without its problems. Adverse reactions to blood transfusion can occur, and the most serious are associated with red cell destruction due to sensitization of red cells by antibody. The most severe of these is the haemolytic transfusion reaction involving intravascular red cell destruction (**Figure 3a**), which is immediate, and is the most likely to be fatal. This can

happen as a result of inadvertent transfusion of A, B or AB blood to a group O patient; A or AB blood to a group B; B or AB to a group A. To avoid such severe haemolytic reactions it is imperative that the correct ABO group of blood is transfused: pretransfusion compatibility tests are carried out to ensure that this happens. Red cell destruction may also occur by an extravascular mechanism (**Figure 3b**) when incompatible blood is transfused to a patient with a red cell antibody that either binds complement slowly or not at all, but the consequences are not as severe.

It is routine practice for all blood transfused to a patient to be compatible within the ABO system and for the D antigen of the Rh system. The minimum requirement for the grouping of patient and donor blood is therefore ABO and D. Matching for D type is especially important for D-negative females before and during childbearing age because of the potential danger of anti-D in pregnancy. A small but significant proportion of patients who receive ABO and D compatible blood will produce antibodies to other blood group antigens that they lack. Donor blood for these patients would be additionally tested for the appropriate antigen and 'antigen-negative' blood given. Patients who are transfused on a regular basis as part of their long-term therapy are more likely to make antibodies because of the repeated exposure to foreign blood group antigens.

Blood Group Antibodies and Pregnancy

Haemolytic disease of the newborn (HDN) is a condition in which the normal lifespan of a fetus's red cells is shortened by the action of a specific red cell IgG antibody which crosses the placenta from the mother. The antibody can be made when the fetus inherits a blood group antigen from the father that is absent from the cells of the mother. Stimulation for antibody production occurs when fetal cells enter the maternal circulation during pregnancy. The events that can result in HDN due to anti-D are shown in **Figure 5**. The cells of the baby, which become coated with IgG, undergo extravascular destruction both before and after birth. Antibody production during a first pregnancy seldom results in HDN because an insufficient amount of

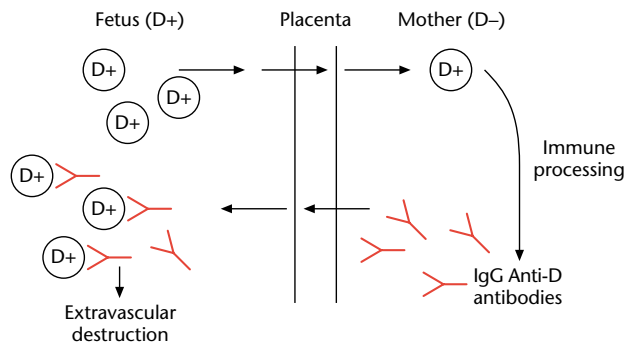


Figure 5 Events leading to haemolytic disease of the newborn.

antibody is produced. However, a second or subsequent pregnancy can result in HDN if the fetus inherits the same antigen and thus boosts the existing antibody. Clinical severity of HDN is extremely variable, ranging from a mild condition that can only be detected in laboratory tests (positive DAGT) on an apparently healthy newborn baby to a severe condition which can cause death in the uterus. Blood used to transfuse the baby, either in the uterus or after birth, should be compatible with the mother's antibody.

HDN due to anti-D tends to be more severe than HDN due to any other antibody, and anti-D used to be the most common antibody implicated in severe and fatal HDN; however, since 1970 all RhD-negative women who give birth to an RhD-positive baby are given an injection of anti-D at the time of birth to prevent the occurrence of HDN due to anti-D in subsequent pregnancies. The rationale for this anti-D prophylaxis is that the anti-D administered to the mother at the time of the first birth binds to and destroys any D-positive fetal cells before they have a chance to prime the maternal immune system to produce endogenous anti-D. Sensitization of the mother is thus prevented. It should be realized that transfer of 'non-red cell stimulated' IgG antibody from mother to fetus is a normal physiological event necessary for protection against infection during the first few weeks of life.

Compatibility Procedures and Selection of Donor Blood

Certain tests are carried out before transfusion to minimize the risks of incompatibility between patient and blood donor. Blood transfusion laboratories in the United Kingdom use guidelines for these tests prepared by the British Committee for Standards in Haematology (BCSH) Blood Transfusion Task Force. The primary purpose of pretransfusion compatibility testing is to ensure ABO compatibility between patient and donor; it is also used to

detect the small number of patients who have clinically significant antibodies other than anti-A and/or anti-B. A brief description of recommended pretransfusion tests and donor selection is as follows:

1. ABO and D grouping of the patient (recipient).
2. Testing the plasma of the recipient for the presence of antibody, or the mother's plasma in the case of transfusion of a newborn baby. The IAGT is considered to be the most suitable technique for the detection of clinically significant antibodies. If an antibody is detected, the specificity should be identified and its clinical significance determined.
3. Computer or manual check of previous records.

These three elements constitute a 'group and screen'.

ABO and D compatible donor blood should be selected wherever possible and a crossmatch performed. The crossmatch is a procedure to exclude incompatibility between donor and recipient and may include serological tests or electronic (computer) crossmatching. Serological tests, in which the prospective donor cells are matched against the patient's plasma, are carried out by the IAGT to detect IgG antibodies or 'immediate spin' to detect directly agglutinating antibodies. The computer crossmatch should only be used when several strict criteria are in place. These criteria include, among others, more than one record of the patient's ABO and RhD type on file, validation of the ABO and RhD type of the donor blood, and known absence of clinically significant antibodies in the patient's plasma. In certain emergencies the recipients need for immediate red cell support may dictate that pretransfusion testing is abbreviated.

If ABO-identical blood is not available, group O blood may be used provided it is plasma-depleted or does not contain high levels of anti-A and anti-B agglutinins. Group AB blood should be used for AB patients but if it is not available group A or B blood may be used. If supplies of RhD-negative blood are limited, RhD-positive blood may be used for RhD-negative recipients; however, it is important that RhD-positive blood is not given to RhD-negative premenopausal females. If patients are found to have a clinically significant antibody in their plasma, blood should be selected which has been tested and found negative for the relevant antigen.

Technical errors and/or inappropriate test systems or administrative errors may result in immediate or delayed haemolytic transfusion reactions. It is extremely important, therefore, that the recommended compatibility procedures are adhered to and adequate quality assurance of those procedures are in place.

Further Reading

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