Immunological complications of blood transfusion

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SUMMARY

In the developed world, most of the reported complications of transfusion have an immunological basis. Although the media and the public are worried about the infectious risks of transfusion, hemovigilance reports show that antigen–antibody reactions are responsible for the vast majority of acute and delayed transfusion reactions. Among the immediate complications of transfusion, the most common and serious are intravascular hemolytic transfusion reactions because of ABO incompatibility caused by giving the wrong blood to a patient (e.g. group A blood to a group O recipient). Fortunately, the vast majority of ABO-incompatible transfusions do not lead to major morbidity or mortality. Another important cause of severe immediate transfusion reactions is transfusion-related acute lung injury (TRALI), caused by white cell antibodies in donor plasma. The most common, although not severe, acute tansfusion reactions are urticaria and febrile, nonhemolytic, mostly preventable by leukodepletion and leukoreduction. Delayed transfusion reactions are: (i) hemolytic, caused by anamestic responses to red cell antigens, causing hemolysis days after the transfusion; (ii) post-transfusion purpura, caused by an anmnestic response to platelet antigens; (iii) graft-versus-host disease, caused by engrafted donor lymphocyte reacting against the recipient; and (iv) immunological refractoriness to platelet transfusions, caused mostly by human leukocyte antigen antibodies destroying transfused platelets. The diagnosis of most of these complications can now be made by immunohematologists, with the aid of specialist reference laboratories, thus enabling prompt therapy as required.

INTRODUCTION

It is impossible to attain zero risk from blood transfusion, as indeed it is from any therapeutic intervention in medicine. There is increasing public awareness of the possible complications of blood transfusion with intense media attention focusing particularly on the risk of transfusion transmitted infections. The result is that the public perceive that blood transfusion is becoming more and more unsafe, whereas the reality is that blood

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transfusion has probably never been safer. This public concern is also misdirected, as the majority of potential blood recipients are aware of possible infectious complications but oblivious to the risks of incompatible transfusion. In the developed countries, transfusion transmitted infections carry an extremely low risk, while the risk of immunological complications of blood transfusion is somewhat higher. This risk tends to receive rather less media attention although some fatal cases of the wrong blood being transfused into a patient occasionally reach the newspaper headlines. However, the public and the media have a great deal of influence on blood transfusion services, resulting in demands for both improvements in the microbiological safety of blood, and for increased availability of alternatives to blood transfusion.[1]

In this article, the immunological complications of blood transfusion will be discussed; the problems of viral transmission and bacterial contamination are discussed by Kitchen and Barbara in this issue of the journal. In the UK, 2,250,000 of red cells are issued to hospitals in the course of 1 year, together with 300,000 units of fresh frozen plasma and 255,000 units of platelets. Between the years 1996 and 2007, the Serious Hazards of Transfusion (SHOT) report, a centralized anonymous hemovigilance data collection scheme to which all UK hospitals contribute,[1,2] included 4334 reports of SHOT.

Figure 1 shows a pie chart illustrating the breakdown of causes of these transfusion reactions reported to the UK national hemovigilance system, SHOT. Transfusion of the incorrect blood component (IBCT) accounted for 62.7% of the cases reported (2716 cases). This underlines the fact that procedural errors resulting in the blood of the incorrect group or specification being transfused, including incorrect ABO and Rh D groups, are responsible for the majority of complications of transfusion. Incorrect prescription, administration of components, handling and storage are also included in the IBCT category. In fact, in only 24 cases in 11 years, was the transfusion of the IBCT causal or contributory to death; fortunately only 5–10% of recipients of ABO incompatible blood suffer serious morbidity of mortality. It is estimated in the USA and the UK that about 1 in 30,000 units of red cells transfused are ABO incompatible, while deaths because of ABO incompatibility are of the order of 1 in 500,000 to 600,000.[3,4] This fatality rate, although very small, is much higher than the residual risk of acquiring HIV infection by transfusion in the UK, i.e. less than 1 in 5,000,000 units transfused (see the article by Kitchen and Barbara in this issue of the journal).

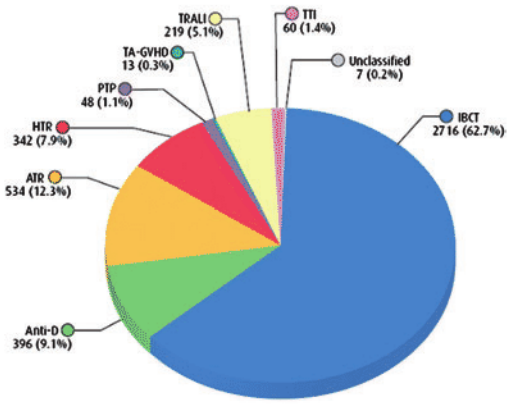

Figure 1. Cumulative numbers of cases reviewed by SHOT, the UK hemovigilance system 1996–2007 ($n = 4335$) (Before 2006 the HTR category was referred to as delayed transfusion reactions.) ATR, acute transfer reactions; HTR, hemolytic transfusion reaction; IBCT, incorrect blood component; PTP, post-transfusion purpura; TA-GVHD, transfusion-associated graft-versus-host disease; TRALI, transfusion-related acute lung injury; TTI, transfusion-transmitted infection.

Other immune-mediated causes of transfusion fatalities and of serious morbidity are generally less predictable or preventable. These include white cell antibody-mediated transfusion-related acute lung injury (TRALI), extravascular acute and delayed hemolytic transfusion reactions (HTR) because of non-ABO antibodies, transfusion-associated graft-versus-host disease (TA-GVHD), post-transfusion purpura (PTP) and anaphylactic reactions because of IgA in plasma transfused to IgA-deficient recipients with anti-IgA (Table 1).

HTR

HTR are the clinical consequence of the immune destruction of transfused red cells. This typically occurs when antigen-positive red blood cells are transfused into a patient who has a clinically significant alloantibody to that antigen. Severe acute HTR (AHTR) which occur within 24 hours of the offending transfusion are largely due to intravascular hemolysis caused by complement fixing IgM antibodies. However, less severe AHTR can be

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Table 1. Immunological complications of blood transfusion

Acute or immediate immunological complications of blood transfusion (occur within 1–2 hours)
Hemolytic transfusion reactions with symptoms (intra- or extravascular)
Febrile, non-hemolytic transfusion reactions
Uticarial reactions
Anaphylactic reactions
Transfusion-related acute lung injury
Delayed immunological complications of blood transfusion
Delayed hemolytic transfusion reactions with symptoms; always extravascular
Post-transfusion purpura
Graft-versus-host disease
Immunological refractoriness to platelet transfusions

caused by extravascular red cell destruction by IgG antibodies, such as, anti-D, anti-K in patients sensitized by previous transfusions or pregnancy. The incidence of these is reported to be approximately 1 in 25,000 transfused units of blood.[5] Delayed HTR (DHTR) occur after 5–8 days following transfusion and are due to anamnestic or secondary immune responses in previously sensitized ('primed') patients in whom no antibody can be detected in the pre-transfusion sample leading to extravascular hemolysis. The incidence of these is frequently reported to be 1 in 2500 transfused units.[6]

The clinical presentation is a spectrum of symptoms, including fever and chills, which are the most common symptoms in both AHTR and DHTR.[7] Hypotension, tachycardia, nausea and vomiting are more likely to occur in acute reactions, as are loin pain and chest pain, although these may also occur in delayed reactions. A leukocytosis may be noted in any HTR. The intravascular hemolysis of the AHTR produces the classic signs of hemoglobinemia and hemoglobinuria, which are pathognomonic of this condition (they may occur in extravascular HTR if very potent antibodies lead to red cell destruction by cytotoxicity). These signs are not a feature of DHTR in which the hemolysis is largely extravascular and jaundice is characteristic. Disseminated intravascular coagulation (DIC) and renal failure are much more common in AHTR but may also sometimes occur in DHTR, and death may ensue in either case, although more frequently after acute intravascular HTR. In certain circumstances, particularly in patients who are under anesthesia, the typical symptoms may be masked by the paralysis and unconsciousness of the patient. An HTR may then be first noted from the hemoglobinuria and excessive bleeding because of DIC. Biochemical tests may reveal hemoglobinemia, elevated lactic dehydrogenase, renal failure and hyperbilirubinemia. Haptoglobin may become depressed in both kinds of reactions but is more important in intravascular cases. There is of course a danger in this situation that further blood may be transfused to keep up with blood loss, and this may be incompatible blood if the cause of the problem has not yet been fully identified.

Occasionally, an HTR may result from transfusion of non-red cell blood components such as fresh frozen plasma or platelets. In this event, the plasma, which is usually group O transfused to A, B or AB recipients, may contain sufficient potent antibodies directed against A or B antigens on the recipient's red cells to cause hemolysis. Even more rarely, incompatibility between red cells from one donor and plasma from another donor may result in hemolysis when the two are mixed in the recipient ('interdonor' incompatibility). In some instances of DHTR, the apparent loss of circulating red cells exceeds what would be expected if only the antigen-positive transfused cells were cleared from peripheral blood. This may be because of complement deposition on autologous red cells which become positive on the direct antiglobulin test (DAT). This phenomenon has been called 'bystander hemolysis'.[8] This reaction can be differentiated from autoimmune hemolytic anemia by the absence of anti-IgG reactivity in the DAT and a lack of an autoantibody in the eluate.

The characteristics of the alloantibodies and their specificity determine the course and severity of an HTR. Red cell alloantibodies are primarily IgG and less often IgM. IgM antibodies readily fix complement and it is these that classically cause the intravascular AHTR. IgG antibodies typically cause extravascular HTR which can be acute (if there is antibody present in sufficient quantity at the time of the incompatible transfusion), or delayed (if antibody is absent in the pre-transfusion testing, 'boosted' by the transfusion and becomes apparent 5–8 days later leading to red cell destruction). Table 2 shows the specificities associated with intravascular or extravascular hemolysis. Other red cell antibodies may rarely cause mild extravascular hemolysis but not intravascular hemolysis, and are very unlikely to be life-threatening.

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Table 2. Red cell antibody specificities associated with hemolysis

Blood group system	Intravascular hemolysis	Extravascular hemolysis
ABO, H	A, B, A_1, H	
Lewis	Le^a Le^b	
P	$P + P_1 + Pk$ (Tj^a)	P_1
I/i	I, i	
Rh		All
Duffy		Fy^a Fy^b
MNS		M, S, s, Mi, N
Lutheran		Lu^b
Kell		K, k, Kp^a Kp^b Js^a Js^b
Kidd		Jk^a Jk^b Jk^3
Vel	Vel	Vel

Pathophysiology

Intravascular HTR (e.g. caused by anti-A; -B; -A,B; anti-Le^a anti-PP_1P^k)

The most frequent cause is ABO incompatibility because of procedural errors, such as identification mistakes or laboratory errors. Most deaths because of incompatibility are caused by the transfusion of group A or B red cells to group O recipients, because anti-A,B is significantly more potent than anti-A or anti-B in group B or A subjects, respectively. There is activation of the full complement cascade by potent ABO IgM antibodies, leading to hemoglobinemia and hemoglobinuria. C1 to C9 activation leads to liberation of anaphylatoxins C3a and C5a, which are responsible for a significant proportion of the signs and symptoms of immune intravascular hemolysis (e.g. hypotension, shock, renal failure, DIC), and are usually far more serious than those of non-immune intravascular hemolysis or than those of immune extravascular hemolysis.

C3a and C5a act on mononuclear phagocytic cells and neutrophils to stimulate the respiratory burst and to enhance the expression of C3b receptors on these cells. As anaphylatoxins, C3a and C5a trigger the mast cell and basophilic release of mediators already preformed in their granules (e.g. histamine, platelet activating factor, tumor necrosis factor (TNF), IL-1, IL-3, 4, 5 and 6) or newly synthesized through the metabolism of arachidonic acid (e.g. leukotrienes, prostaglandins). In addition, mononuclear phagocytic cells are activated by phagocytosis per se and by C5a with the consequent secretion of mediators of the acute inflammatory response: TNF, IL-1, IL-8, PGE2, neutrophil-activating factor (NAP-1) and neutrophil chemotactic factor. Thromboplastic substances released by hemolysis and the activation of complement lead to activation of the extrinsic pathway of the coagulation cascade, contributing to DIC.

Extravascular HTR (caused by anti-Rh; -K; etc.)

Adherence of red cells coated with IgG1 or IgG3 antibodies and/or C3b, to Fc receptors (FcγR1, FcγR2 and FcγR3) and to complement (CR1) receptors on mononuclear phagocytic cells or lymphocytes, leads to either phagocytosis and/or cytotoxicity of red cells. Cytotoxicity is mediated mostly by lysozymal enzymes released by the mononuclear phagocytic cells when red cells bind to them, heavily coated with IgG1 and/or IgG3 antibodies. Moderate coating of red cells leads to phagocytosis. Those IgG antibodies that fix complement, such as anti-Jk^a are not as efficient as IgM antibodies and will only activate the cascade up to C3. C3b alone does not mediate cytotoxicity or phagocytosis but greatly enhances IgG-induced phagocytosis or cytotoxicity through adherence to the complement receptors (for C3b only) on mononuclear cells. Free IgG in plasma inhibits the binding of IgG red cell antibodies to Fc receptors on mononuclear phagocytic cells. Hence, red cells coated with non-complement fixing IgG1 or IgG3 antibodies (e.g. anti-D, -E, -c) are destroyed mainly in the spleen where there is hemoconcentration and large numbers of macrophages. C3b, with a very short half-life on red cells, abolishes the inhibitory effect of free IgG in plasma on Fc receptors of mononuclear cells: as there is no free C3b in plasma, cells coated with C3b will easily bind to CR1 receptors wherever they are present. For this reason, red cells coated with IgG and C3b (e.g. most

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anti-Jk[a], -Jk[b], many anti-K, many anti-Fy[a], etc.) are destroyed predominantly in the liver where there are abundant phagocytic cells and a good blood flow. This destruction occurs generally more rapidly and efficiently than when cells are coated only with IgG antibodies. Most clinically important IgG red cell antibodies are composed of subclasses 1 and 3, which are the subclasses with greatest destructive power as Fcγ receptors recognize only these two subclasses. Those IgM antibodies which do not activate complement do not seem to cause red cell destruction. Red cell alloantibodies composed only of IgA have not been found.[7]

Clinical signs and symptoms of HTR

Fever and chills are usually the first signs of HTR, and it is impossible to distinguish them from febrile non-HTR (FNHTR). They are due to the release of anaphylatoxins and other mediators. *Back or loin pain* is very common and the cause is unknown. *Feelings of unrest* and *dyspnea* are caused by lung perivascular and peribronchial oedema. *Hypotension, shock and renal failure* occur in up to 10% of patients who have intravascular HTR but are rarely seen in extravascular reactions. Complement activation is likely to be a significant factor in these cases, and the anaphylatoxins C3a and C5a are probably the most important. In addition, the cytokines TNF and IL-1 can cause hypotension and shock. Renal failure may occur in either type of HTR although it is more common in the acute variety. Mildly affected patients may have elevated serum urea and creatinine levels but no symptoms. In more severe cases the patient may become anuric and require dialysis, with hypotension and DIC contributing to the renal impairment. Pathologically, the initial response is acute tubular necrosis, but there is also some thrombus formation in renal arterioles which may cause cortical infarction. With full supportive care, many patients regain normal renal function.

DIC is seen in intravascular AHTR but is very rare in extravascular HTR. It is probably due to complement activation and the release of thromboplastic substances caused by intra-vascular red cell destruction as well as by inflammatory cytokines. It may be difficult to distinguish from other causes of coagulopathy, which may occur in massive transfusion or in liver disease. Any patient who has been transfused and has micro-vascular bleeding must be considered to have an AHTR and be investigated accordingly. Coagulation tests and platelet counts are useful in guiding management.

Investigation of HTR based on AABB standards[9]

(a) Each blood bank or transfusion service must have a system for detection, reporting and evaluation of suspected complications of transfusion. A responsible physician and the transfusion department/laboratory must be notified immediately and the reactions must be evaluated promptly.

(b) If there are symptoms or findings suggestive of an HTR, the transfusion must be stopped and the following must be performed:

1 Checking of all labeling on blood containers and documentation. If it is discovered at this stage that the wrong blood has been transfused, leading to an ABO incompatible transfusion, there is no need to continue investigating any further, except for investigating for signs of hemolysis and monitoring for severe signs and symptoms. The destination of the units of blood intended for that patient must be ascertained in order to avoid an incompatibility to another patient.
2 A properly labeled blood sample must be obtained from the patient and sent to the transfusion laboratory along with the original transfusion bag and giving set.
3 Post-reaction serum must be inspected for evidence of hemoglobinemia. A DAT must be performed and if positive must be compared with a pre-reaction sample.
4 Visual inspection of the urine for hemoglobinuria.

Additional testing must be carried out as necessary:

1 Repeat ABO and Rh testing on pre- and post-transfusion samples and on donor units.
2 Repeat the crossmatches on pre- and post-transfusion samples using the anti-globulin technique.
3 Repeat antibody screen on pre-and post-transfusion samples with antibody identification. Supplementary immunohematological tests may be necessary.

(c) Hematology tests for confirming hemolysis:

1 Red cell osmotic fragility.
2 Peripheral blood film.
3 Reticulocyte count.

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Table 3. Differential diagnosis of hemolytic transfusion reactions

Autoimmune hemolytic anemia
Cold hemagglutinin disease
Non-immune hemolysis
Incompatible fluids
Improper storage
Malfunctioning blood-warmer
Hereditary hemolytic anemias
G6PD deficiency
Hereditary spherocytosis
Hemoglobinopathies, e.g. sickle cell disease
Drug-induced hemolysis
Microangiopathic hemolytic anemia
Thrombotic thrombocytopenic purpura
Hemolytic uremic syndrome
HELLP syndrome
Mechanical heart valve dysfunction
Paroxysmal nocturnal hemoglobinuria
Bacterial contamination
Clostridium welchii
Malaria
Babesiosis

G6PD, glucose-6-phosphate dehydrogenase; HELLP, hemolysis, elevated liver enzymes and low platelets syndrome.

(d) Biochemical tests to confirm hemolysis:

1 Haptoglobins.
2 Methemalbumin.
3 Lactate dehydrogenase.
4 Bilirubin.
5 Tests for hemoglobinuria and hemosiderinuria.

If none of these tests are positive and involvement of the local reference laboratory yields no further information, then one must consider non-immunological causes of hemolysis. These include bacterial contamination, physical damage to transfused cells or recipient's cells, destruction of recipient's abnormal cells, e.g. in G6PD deficiency or hemoglobinopathy, or infectious causes such as gram-negative sepsis or malaria (Table 3).

Treatment

The treatment of an HTR must be guided by the clinical manifestations in the patient. The patient with minimal symptoms may be managed by careful observation, but in a severe reaction, early vigorous intervention may save life. Generally, the severity of a hemolytic reaction is directly related to the volume of incompatible blood transfused (although some deaths have been reported after ABO-incompatible transfusions of < 30 mL of blood), so early recognition and stopping the transfusion is paramount in preventing severe morbidity and mortality. If there is ABO incompatibility and severe hemolysis, exchange transfusion may be necessary to prevent death and may be considered if large quantities of incompatible red cells are known to have been transfused. However, it is not always appropriate to expose the patient to further donated units with the associated risks of transfusion-transmitted disease if the hemolytic process is being well tolerated. Renal failure may be prevented by maintaining urine output with intravenous fluids and diuretics such as frusemide, and pressor support may be necessary for hypotension. Care must be taken to avoid fluid overload, especially in oliguric renal failure or patients with cardiac impairment.

DIC should be treated according to local protocols as there is still some controversy in the management of this condition. Plasma, cryoprecipitate and platelets may be necessary; they should be prescribed against the clinical condition of the patient and the results of frequent monitoring of the coagulation screen, fibrinogen, FDP (fibrinogen degradation products) levels and platelet count. Some authors advocate the use of heparin in the management of DIC. Intravenous immunoglobulin (IVIG) has occasionally been used as a pre-treatment for a patient when incompatible blood has had to be given to an alloimmunized patient, because the IgG antibody is acting against a very common antigen and no compatible blood can be found promptly. This has successfully prevented extravascular haemolytic transfusion reactions.[10] Selection of red cells for a patient bleeding severely following an extra-vascular HTR may be very difficult. It is of course paramount that no patient is allowed to bleed to death for lack of red cells in the face of serological incompatibility. If possible, red cells should be obtained which lack the known antigens to which the patient has developed clinically significant antibodies.

Prevention

Proper patient identification is of ultimate importance in the prevention of AHTR because of ABO incompatibility, as the vast majority of these are the result of errors.[1,2]

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These generally occur at the bedside prior to administration of the blood component or when taking samples for pre-transfusion testing.[1,2,11] Laboratory errors are less common. The majority of serious transfusion errors arise from breaches of current and established codes of practice in each institution. Human error is inevitable, but systems must be in place to minimize the possibility of harm to the recipient. Current standard operating procedures for accurate identification of patients must be in place both for taking of blood for pre-transfusion testing and for bedside documentation immediately before transfusion.[12] Staff must be fully trained in the recognition of transfusion reactions and in taking prompt appropriate action. Simple protocols should be established, implemented and adhered to at all hospitals administering blood transfusions.[13]

FNHTR

FNHTR are the most common adverse effect of blood transfusion and have a plethora of causes. When non-leukoreduced or -leukodepleted red cells or platelets are transfused, they occur with an incidence of 1% and 5–10%, respectively.[9] This reaction is usually immune mediated, because of the reaction of white cell antibodies in the recipient's plasma with the leukocytes in the transfused component. However, some evidence suggests that plasma proteins may also have an etiological role in FNHTR.[14]

Definition and differential diagnosis

The definition of FNHTR includes a rise in temperature of at least 1°C (sometimes 1.5–2°C), which is not accounted for by the patient's clinical condition. The fever occurs in association with the transfusion and may be accompanied by chills, rigors and a feeling of discomfort. It usually responds to antipyretic medication and hemolysis does not occur. FNHTR are not life-threatening, but repeated episodes may make the patient very apprehensive and reluctant to have subsequent blood components. To avoid these problems, premedication (with paracetamol) is often used to prevent febrile reactions.[7] However, in many multitransfused patients, premedication will not be sufficient and the white cell load of cellular components will need to be reduced. The differential diagnosis may be difficult, especially when comorbid disorders such as infection or malignancy, and certain treatments, may cause a similar spectrum of symptoms. Fever may also accompany other acute transfusion reactions, including AHTR, infusion of a bacterially contaminated blood component or TRALI. The diagnosis of FNHTR must therefore be a diagnosis of exclusion. If in doubt, a direct anti-globulin (Coombs) test and a test for the presence of free hemoglobin in plasma may be performed. Bacterially contaminated blood components usually cause a rapid and profound febrile hypotensive reaction occurring much earlier than FNHTR. On the other hand, TRALI, which can be severe and life-threatening, is associated with dyspnea and cyanosis as well as hypotension and therefore a clinical diagnosis may be possible.

Antibody-mediated reactions

Evidence has been available since the 1950s to support the hypothesis that FNHTR are associated with the presence of white cell antibodies in the recipient reacting with leucocytes in the transfused component.[15] Also, it was noted that many patients who developed FNHTR had received previous blood transfusions or had been pregnant. Such white cell antigen–antibody interactions result in the stimulation and release of endogenous pyrogens, e.g. IL-1β, IL-6 and TNF cytokines, from the donor leukocytes. FNHTR may also result from cytokines released by the activation of the recipient's macrophages rather than the donor leukocytes. In these cases, the antibody–white cell interaction leads to the activation of complement and it is the antigen–antibody–complement interaction that may cause activation of macrophages in the recipient resulting in the production of endogenous pyrogens.[16]

Antibodies against white cells are found in 70% or more of patients who suffer from FNHTR.[17] These include HLA and granulocyte antibodies. Leukodepletion or leukoreduction of blood components to below a threshold of 5×10^6 leukocytes per component significantly reduces the incidence of FNHTR. However, not all FNHTR are due to leukocyte antibodies. In 30% of patients experiencing these reactions, no white cell antibodies are in fact demonstrable. Although white cell antibodies are the main type of antibody implicated, anecdotal cases of strong FNHTR because of the presence of HPA antibodies reacting with incompatible platelets have been reported.

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Reactions mediated by accumulation of biological response modifiers during storage

Among recipients of platelet transfusions, over 20% of patients suffer FNHTR on the very first exposure, while 55% experience their first reaction within their first three transfusion episodes. The occurrence of reactions to the first ever platelet transfusion in a patient means that such individuals could not have been previously alloimmunized to leukocytes, and therefore some FNHTR are not antibody-mediated. It has become clear from studies following up these data that component storage is an important factor in the occurrence of FNHTR. Also, it has been noted that the frequency of FNHTR is much greater with platelets than with red cells, even though the absolute number of leucocytes being transfused with each component is similar.

Increased concentrations of cytokines, either endogenous or exogenous, are harmful to the host, acting as endogenous pyrogens.[18] Cytokines such as IL-1β, IL-6, IL-8 and TNFα are actively synthesized and released during platelet and red cell storage. Linear correlations exist between cytokine level, white cell content and duration of storage. Cytokines accumulate more at 22°C than at 4°C.[19] Pre-storage leukocyte reduction prevents accumulation of cytokines and is associated with significantly fewer FNHTR. The removal of the buffy coat from red cells or the preparation of platelet concentrates by the 'top-and-bottom' system or buffy-coat method will be sufficient to significantly reduce the production of cytokines in stored blood components; thus, it is not necessary to aim at leukodepletion when leukoreduction will be sufficient for this purpose. The effects of IL-1 include its potent pyrogenic activity, possibly mediated by IL-6 or PGE-2, stimulation of hemopoiesis, and activation of neutrophils and platelets. TNF is also a potent pyrogen, enhances B cell proliferation and activates the extrinsic pathway of coagulation via tissue factor. IL-6 is a pyrogen and also enhances antibody responses and stimulates B cell proliferation and differentiation. IL-8 is a chemokine and a chemotactic factor for neutrophils and T cells. It stimulates neutrophil oxidative bursts and basophil histamine release. These findings support the concept that proinflammatory cytokines play a role in FNHTR, although the strong association is not necessarily proof of causation.

When plasma is exposed to plastic surfaces, complement is activated through the alternative pathway. C3 activation has been detected in both random donor and single donor apheresis platelets after storage for 5 days at room temperature with agitation. The mechanism of C3 activation is not cell-dependent as leukocyte-depleted platelets still have high C3 activation levels. C3a has multiple pathophysiological effects: it promotes mast cell histamine release, it is a vasodilator increasing microvascular permeability and it enhances *in vitro* platelet aggregation and serotinin release. It has been suggested that complement activation may stimulate monocytes in platelet concentrates to produce cytokines thereby contributing to the characteristic symptoms of FNHTR. Some of the newer leukodepletion filters appear to absorb C3a from platelet concentrates and may therefore be able to help reduce the incidence of FNHTR.

Prevention of FNHTR

The most effective way of preventing the majority of FNHTR is by pre-storage leukodepletion of cellular blood components, i.e. red cells and platelet concentrates. Leukodepletion can be achieved by filtration of blood components or by modern apheresis techniques during the collection of platelets. In addition, as a proportion of reactions are mediated by biological response modifiers released by white cells and accumulating in the blood component over the period of storage, interventions to prevent this accumulation will decrease the frequency of FNHTR.[14] Post-storage (bedside) leukocyte reduction is not as effective as pre-storage leukodepletion, as it is not well quality-controlled and cannot remove mediators and biological response modifiers such as IL-1β, IL-6 and TNF. Several groups have demonstrated that removal of leukocytes to a threshold of 5×10^6 per component before storage prevents the accumulation of IL-8 and proinflammatory cytokines such as IL-1β, IL-6 and TNF in both red cell and platelet components.[20] The administration of an antipyretic such as paracetamol may be useful in damping down the symptoms of FNHTR.

ALLERGIC AND ANAPHYLACTIC REACTIONS

These reactions occur in response to plasma proteins in the blood components administered, and represent a type 1 hypersensitivity response, i.e. an immediate allergic reaction following a second or further contact with an antigen which may vary on a scale from urticaria to

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anaphylaxis.[7] Hypersensitivity responses occur very rapidly following contact with the relevant antigens and recur on subsequent occasions. The primary antigen exposure stimulates plasma cells to produce specific IgE. This IgE binds to mast cells via its Fc receptor and sensitizes them. Representation of the antigen causes crosslinking of surface IgE stimulating degranulation of mast cells. The organ systems affected include skin, and the mucosa of the gastrointestinal and respiratory tracts, which are where mast cells are normally distributed. Stimulation of the sensory nerves causes itch and flare reactions while smooth muscle contraction causes vascular leakage and tissue edema. Arterial dilatation may cause headache and hypotension, while bronchoconstriction can cause respiratory distress. The mediators of this response from endogenous sources include histamine, serotonin and bradykinin, the analphylatoxins C3a and C5a, lymphokines and leukotrienes.[21] Cutaneous allergic transfusion reactions occur in 1–3% of plasma-containing blood components, including red cells and platelets. The cardinal signs and symptoms are local erythema, urticaria and pruritus. Soluble proteins in donor plasma are generally responsible but specific etiologies are rarely identified. Occasionally it has been discovered that the donor has ingested a food allergen or drug to which the recipient is sensitized.[21] Treatment includes antihistamines and occasionally hydrocortisone; antihistamines may be used prophylactically 1 hour before transfusion, to prevent future episodes.

Anaphylactic transfusion reactions are much less common, occurring once per 20,000–400,000 units of blood or components transfused. The cause is generally an IgG anti-IgA in an IgA-deficient recipient who is transfused with IgA-containing blood products. The formation of IgG/IgA immune complexes leads to the activation of complement and the subsequent release of C3a and C5a anaphylotoxins.[22] The signs and symptoms include a feeling of apprehension and impending doom, generalized flushing, nausea, vomiting, diarrhea and abdominal cramps, laryngeal edema, bronchospasm and dyspnea, profound hypotension, shock and potential cardiopulmonary arrest. The transfusion should be stopped immediately and adrenaline 1 in 1000 (0.3–0.5 mL) given immediately. Supportive therapy for the circulation and respiratory system may be necessary. The differential diagnosis for such an acute and severe transfusion reaction must include ABO-incompatibility with an AHTR, TRALI and perhaps bacterial contamination. IgA deficiency occurs in approximately 1 in 700 of the population in the UK and is defined as less than 0.05 mg/dL of IgA. The frequency of IgA deficiency with IgA antibodies is 1 in 1200; often anti-IgA is found in subjects who have never been pregnant or received a blood transfusion. Less than 20% of suspected cases of anti-IgA in a recipient reveal this to be the cause of the reaction.

Diagnosis must be made by reliably demonstrating deficiency of IgA and the detection of an anti-IgA. Once the diagnosis has been made, the patient must be clearly identified both on the hospital notes and on a wrist band or bracelet, and he/she must be fully informed of the implications. In future such patients should receive only IgA-deficient components which are collected from a special panel of IgA-deficient donors. In the absence of IgA-deficient donors, washed red cells may be administered with appropriate prophylactic measures taken beforehand in case of a mild reaction. Autologous transfusion may be considered in appropriate circumstances.

TRALI

TRALI is a life-threatening complication of transfusion which may have a very dramatic clinical presentation indistinguishable from adult respiratory distress syndrome.[23] In most cases it begins within 2 hours of transfusion but may be up to 4 or 6 hours following administration of a plasma-containing blood component. Symptoms generally include fever, hypotension, chills, cyanosis, non-productive cough and dyspnea. Chest X-ray shows severe bilateral pulmonary edema or perihilar and lower lung field infiltration, without cardiac enlargement or involvement of the vessels. The X-ray findings may be much more severe than the auscultatory changes on examination. Severe hypoxia is usual, with very low arterial oxygen tensions, and the patient frequently requires mechanical ventilation. In contrast to patients with circulatory overload, those with TRALI have normal central venous pressure and normal or low pulmonary wedge pressure. It is possible that milder cases of TRALI may occur and not be recognized. Approximately 80% of patients with TRALI improve both clinically and physiologically within 2 or 3 days with adequate supportive care. Overall mortality appears to be in the region of 5–8%, in contrast to ARDS (adult respiratory distress syndrome), which has a mortality rate of 40–50%. Differential diagnosis

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includes circulatory overload, anaphylactic transfusion reaction and bacterial contamination.[7,9]

The true incidence of TRALI is unknown although there is a much quoted figure of 1 in 5000 plasma-containing transfusions.[24] This figure is likely to be an underestimate as milder cases may pass relatively unnoticed and severe cases may still be misdiagnosed, being attributed to circulatory overload. The precise mechanism involved in the development of TRALI is not clear, but two possible mechanisms have been postulated: an antibody-mediated and a soluble mediator-mediated. These mechanisms both involve the activation of granulocytes and the triggering of an inflammatory process, leading to the sequestration of neutrophils in the lung. In the vast majority of cases, investigators have demonstrated the presence of HLA class I and class II or granulocyte-specific antibodies in the donor.[25] In about half the cases studied, the HLA antibodies in the implicated donor correspond with one or more of the HLA antigens in the recipient. In other cases, neutrophil-specific antibodies (HNA1, HNA-3a) have been identified in the plasma of implicated units.[25,26] These antibodies are most commonly found in the donations of multiparous women. It seems that the granulocytes interact with activated complement causing aggregation and blockage of the pulmonary microvasculature. Pulmonary leukosequestration leads to transient changes in vascular permeability and pulmonary edema. In a small number of reported cases, similar antibodies are found in the pre-transfusion serum of the recipient and in such cases TRALI is more frequent after granulocyte transfusions.[27] In some cases clinically diagnosed as TRALI, no antibody has been identified. It has been suggested that in these cases the granulocyte activation is mediated by a soluble lipid substance, which accumulates during the storage of the products.[28] In any case, it is likely that a number of factors determine the final clinical response of a patient and these may include the characteristics of the antibody, nature and distribution of the related antigen, the extent of complement activation (in particular liberation of C5a) and the immune status of the recipient.

Diagnosis and treatment

There is no diagnostic test or pathognomonic finding for TRALI, so the diagnosis is one of exclusion. Other causes of respiratory distress and pulmonary edema in the transfusion setting must be fully investigated, including myocardial infarction, circulatory overload and bacterial infection. The measuring of central venous and pulmonary wedge pressures is very helpful. A proper work-up of TRALI should include testing of the donor and recipient sera for granulocyte (HNA) and lymphocyte (HLA) antibodies.

Antibody specificity can be determined and HLA or HNA typing of the recipient's or donors' cells can also be carried out. The presence of a positive reverse lymphocyte crossmatch between donor serum and patient lymphocytes provides further supportive evidence. The treatment of TRALI includes intensive respiratory and circulatory support.[23] In almost all cases, oxygen supplementation is necessary, although mechanical ventilation may not always be required. Some reports suggest that the administration of corticosteroids may be beneficial.

Prevention

It is recommended that donors who have been implicated in TRALI and who are found to have granulocyte or lymphocyte antibodies should be withdrawn from the donor panel unless their components are to be issued as deglycerolized or washed red cells.[23] Exclusion of all multiparous women from the donor panel would result in a huge (5–30%) loss of blood donors, but it is advisable not to use their plasma for the manufacture of FFP or for suspension of platelet concentrates. Routine donor testing for HLA and granulocyte antibodies is time-consuming and much too costly to be implemented. In the UK, efforts are made to use only male donations for the preparation of FFP and for suspension of pooled platelets.

TA-GVHD

Acute GVHD is a recognized complication of allogeneic hemopoietic progenitor cell transplantation. It results from the presence of viable lymphocytes in the allograft recognizing the host HLA antigen type as foreign, resulting in a characteristic immune response.[29] The clinical syndrome includes fever, diarrhea, abnormal liver function tests and a characteristic rash particularly affecting the palms. A similar picture may result from the transfusion of viable lymphocytes into immunosuppressed recipients in the absence of an allogeneic stem cell transplant. In this situation bone marrow aplasia and pancytopenia also result.

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Table 4. Patient groups at risk of transfusion-associated graft-versus-host disease (TA-GVHD)

Patient groups at risk of TA-GVHD
Congenital immune-deficiency disorders
Hodgkin's disease
Neonates with erythroblastosis fetalis
Recipients of intrauterine transfusions
Recipients of stem cell transplants
Recipients of blood components donated by relatives
Recipient-donor pairs from genetically homogeneous populations
Recipients of HLA-matched cellular products
Premature neonates
Patients possibly at risk
Non-Hodgkin's B cell lymphomas
Solid tumors
Potential at-risk group
Full term neonates
Patients with AIDS
Patients receiving immunosuppressive medication

HLA, human leukocyte antigen.

TA-GVHD is typically evident from 8–10 days post transfusion. It is almost uniformly fatal, with death occurring within 1 month in over 90% of cases. The features discussed above are not substantially different from those of a variety of viral illnesses or drug reactions.[30] Comorbid conditions may obscure the clinical features of TA-GVHD, particularly if the clinician has a low index of suspicion. Cases are most certainly underreported because of lack of recognition or the absence of definitive diagnostic studies in many cases. Cases were originally recognized in patients with severe combined immunodeficiency or Wiskott–Aldrich syndrome, neonates with hydrops fetalis, and in patients with Hodgkin's disease. The incidence is unknown, but TA-GVHD is estimated to occur in up to 1% of patients with hematological malignancies or lymphoproliferative diseases.

In addition to defined populations at risk as in Table 4, TA-GVHD has also been reported in non-immunocompromized hosts, particularly pregnant women, people undergoing cardiovascular and abdominal surgery, patients with active rheumatoid arthritis, and trauma cases.[31] Clearly not all immunocompromized individuals develop TA-GVHD and there must be additional risk factors pre-disposing patients to this condition. The main requirements for the development of GVHD are: shared HLA types between the recipient and donor but with other differences that will make the donor recognize the recipient as foreign, the presence of immunocompetent cells in the transfused blood components, and inability of the host to reject the immunocompetent donor lymphocytes. In a normal recipient, immune cells will far outnumber donor-derived T cells, which are therefore eliminated by a host-versus-graft reaction. However, if a small number of functional T lymphocytes are transfused which derive from a donor who is homozygous for one of the recipient's HLA haplotypes, the recipient will not recognize these cells as foreign. The donor T cells will, however, recognize the host as foreign, undergo clonal expansion and establish TA-GVHD. This situation is referred to as a one-way HLA match and TA-GVHD may be expected to occur regardless of the host immune status.[32] Recent experiments in selective depletion of recipients' CD4+, CD8+ and NK cells have suggested that CD4+ cells may be involved in the pathogenesis of TA-GVHD, while CD8+ and NK cells appear to be protective. This may explain why TA-GVHD is not reported in patients suffering from AIDS.

Diagnosis

Only the documentation of donor-derived lymphocytes in a recipient's circulation or tissues can confirm the presence of TA-GVHD. Characteristic histological changes may be seen in a skin biopsy which may reveal degeneration of the basal cell layer with vacuolization, dermal epithelial layer separation and bulla formation. Liver biopsy may reveal degeneration and eosinophilia, and bone marrow aspiration may reveal aplasia with lympocytic infiltration. Several methods are used to make a positive diagnosis of TA-GVHD, and these are based on demonstrating donor cells or DNA in the patient, as shown in Table 5.[33–35]

Treatment

There is no effective treatment of TA-GVHD and the mortality rate is extremely high. Immunosuppressive therapies have been used with little success, including steroid therapy, antithymocyte globulin, cyclosporin, cyclophosphamide and anti-T cell monoclonal antibodies. These treatments are sometimes useful in GVHD

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Table 5. Diagnostic techniques for graft-versus-host disease

HLA typing of donor and recipient, ideally using DNA-based techniques
Molecular genetic evaluation using short tandem repeats probes
Identification of donor T cells using the above techniques in skin biopsy samples

HLA, human leukocyte antigen.

after stem cell transplantation but are ineffective in TA-GVHD. In the light of the absence of any effective treatment, the prevention of this condition is essential. This is particularly the case following the recent UK hemovigilance report (SHOT), which shows that TA-GVHD is a significant cause of mortality from transfusion while other causes are gradually being eliminated.[1]

Prevention

The irradiation of cellular blood components renders the donor lymphocytes non-viable and protects the recipient from potentially developing TA-GVHD. Guidelines have been produced by the American Association of Blood Banks in the USA and the British Committee for Standards in Haematology in the UK recommending for which patients gamma-irradiated products should be available.[36–38] At the moment, because of the low incidence of TA-GVHD in immunocompetent patients receiving donated blood from unrelated donors, gamma irradiation is not applied to all transfused cellular blood components. This decision is based upon the extremely low risk in such recipients, and the costs and logistics of universal irradiation, plus the effect on other measurable parameters in components such as potassium content and shelf life. Leukocyte reduction to a level of less than 5×10^6 residual leukocytes per unit is not an effective way of preventing the occurrence of TA-GVHD, as sufficient viable lymphocytes are still present to cause this syndrome. The introduction of universal leukodepletion in the UK has resulted in a significant reduction in the number of reported cases in the past years.[24]

PTP

PTP is characterized by the development of severe, sudden and self-limiting thrombocytopenia occurring 5–10 days after a blood transfusion. The recipients always have a history of sensitization, mostly by pregnancy, and occasionally by blood transfusion. The diagnosis rests on the demonstration of potent antiplatelet reactivity in the patient's serum for a specific platelet antigen, usually HPA-1a. PTP therefore is a disease of adults, with no patients younger than 16 years of age being reported in the literature.[39] The female-to-male ratio is 5:1. The epidemiological findings are due to the requirement that a patient has previously been exposed to platelet-specific antigens before PTP can develop following a subsequent transfusion.

Clinical presentation

In the majority of cases (over 80%), the platelet count drops around 1 week following the transfusion to less than 10×10^9 per liter. If random platelets or specific antigen-negative platelets are transfused, the increment is generally very poor or nonexistent.[40] One or two reports suggest that HPA-1a-negative platelets may be beneficial, and in cases of severe bleeding platelet transfusion should be considered.[41] Hemorrhage may occur from the gastrointestinal tract and epistaxis is common. Intracranial hemorrhage is responsible for the mortality rate which is around 9%. The differential diagnosis of PTP includes immune thrombocytopenic purpura (ITP), sepsis and DIC, bone marrow failure, drug-induced thrombocytopenia and thrombotic thrombocytopenic purpura (TTP). Drugs, infection and DIC are common causes of thrombocytopenia and these must be excluded. In straightforward alloimmunization to platelet, red cell or lymphocyte antigens, only the incompatible cells bearing the relevant alloantigen are destroyed by the reaction. The unique feature of PTP is destruction of autologous antigen-negative platelets in the presence of a platelet-reactive alloantibody. A source of indirect evidence of PTP as opposed to straightforward alloantibody-mediated platelet destruction is the response to therapy. IVIG infusion or plasma exchange has little effect on simple alloantibody-mediated platelet destruction, but these therapies are effective in PTP. The difficulty in proving a diagnosis of PTP means that the incidence of PTP is unclear, especially in the group of

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long-term platelet-dependent patients. The clinical spectrum of PTP may be very broad and mild cases may also not be noticed. Calculations of the theoretical frequency of occurrence of PTP based on the incidence of HPA-1a and other platelet antigens in the population, and on the frequency of alloimmunization through pregnancy, suggest a high incidence of PTP. In fact, it is quite rare, and it may be that other immune response factors may be necessary for individuals exposed to incompatible platelet antigens to develop the syndrome whereby autologous platelets are destroyed.

Mechanism

Several theories have been put forward to explain the destruction of autologous antigen-negative platelets in PTP. The first suggests that immune complexes are formed by the interaction of soluble platelet-specific antigen in donor plasma and platelet antibody in the patient.[42] These complexes then bind to autologous platelets through a high affinity Fc receptor mediating platelet destruction. A second theory maintains that an auto-antibody is developed in response to exposure to an incompatible platelet antigen and this antibody reacts not only with HPA-1a-positive cells but also with antigen-negative cells in the recipient. A third suggestion is that the soluble platelet antigen in donor plasma adsorbs onto the recipients' platelets, converting them to antigen-positive targets which are then destroyed by the alloantibody.[43] Soluble HPA-1a substances have certainly been identified in the plasma of HPA-1a-positive donors, however platelet antigen–antibody complexes have not been demonstrated in the serum of PTP patients. In support of the auto-antibody theory, platelet-associated IgG is increased in PTP. In addition, acute-phase PTP serum contained reactivity against a protein present in both HPA-1a-positive and HPA-1a-negative platelets. This reactivity occurred concurrently with anti-HPA-1a activity and disappeared after the acute phase of the illness, although the anti-HPA-1a persisted. Certainly, the response to therapy of PTP is similar to that of ITP, in which steroids, IVIG and splenectomy may be associated with elevations of the platelet count and decreases in platelet-associated IgG.

The diagnosis of PTP depends upon the finding of severe thrombocytopenia of less than 10×10^9/L approximately a week to 10 days post-transfusion. Normal red cell morphology rules out the possibility of TTP. Platelet antibody assays reveal serum antibody with HPA-1a specificity in most cases, although antibodies to other platelet-specific antigens are sometimes implicated.[44] Such patients frequently have antibodies to red cell and white cell antigens as well, and it may be that some individuals mount a generalized immune response encompassing a number of targets.

Therapy

Treatments for PTP are hard to evaluate as the condition is generally self-limiting and untreated patients recover in approximately 2 weeks. Most patients with PTP are treated with corticosteroids during the acute phase at a dose of 2 mg/kg of prednisolone, or an equivalent dose of an alternative preparation.[45] There is little evidence of the efficacy of this treatment, although steroids may inhibit reticuloendothelial cell function or alternatively may result in a decreased antibody production. The most effective therapy for PTP is plasma exchange using some fresh frozen plasma as a replacement.[46] Recently, infusions of IVIG have become the first line in therapy for PTP, and a large proportion of patients respond well.[47] Only those unresponsive to IVIG now go on to plasma exchange. Recovery from PTP occurs 3–4 days after initiation of treatment with IVIG 0.5 g/kg for 2 days.

Prognosis

Prognosis is good with spontaneous recovery occurring in all cases. Mortality rates relate to the incidence of intracranial hemorrhage in a few patients. The incidence of recurrence of PTP with subsequent transfusions in an individual patient is extremely low, although, because of the potential severity of the reaction, patients with a documented history of PTP should receive antigen-negative blood products when at all possible.

IMMUNOLOGICAL REFRACTORINESS TO PLATELET TRANSFUSIONS

Platelet transfusions play a major role in the management of thrombocytopenia in hematological and oncologic patients. However, a proportion of these patients become refractory to the transfusion of platelets from random donors, because of immunological and/or

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non-immunological causes. Immunological refractoriness is primarily caused by HLA antibody-mediated destruction of transfused platelets, although HPA and high-titer ABO alloantibodies have occasionally been implicated. The non-immunological causes of platelet refractoriness, because of the destruction/consumption of transfused platelets, include sepsis, disseminated intra-vascular coagulation in the patient and certain drugs, such as amphotericin B, vancomycin and ciprofloxacin. Patients with confirmed immunological refractoriness because of the presence of HLA antibodies require transfusions of HLA-matched or HLA compatible platelets.

The laboratory investigations to identify these cases involve an HLA antibody screening of the patient's serum, and if positive, the identification of antibody specificity. This is followed by the HLA typing of patients and the selection and issue of HLA compatible platelets. An alternative approach, in the absence of a panel of HLA-typed donors, is to provide crossmatch-compatible platelets. It is important to document the post-transfusion increments in order to evaluate the efficacy of the transfusion and the donor. Although platelets express HLA-A, -B and -C antigens, only matching at the HLA-A and -B locus antigens is considered to be relevant.

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