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Human Leucocyte Antigen Sensitisation and Its Impact on Transfusion Practice

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Keywords

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Abstract

Human leucocyte antigen (HLA) sensitisation, including the formation of antibodies against HLA, can cause serious effects in patients receiving blood. Under certain circumstances, donor HLA antibodies in the blood product can trigger the patient's granulocytes to release mediators that cause transfusion-associated lung injury (TRALI), a serious complication of transfusion. The HLA systems of both donor and patient are involved in transfusion-associated graft-versus-host disease, which is a rare disease with a high mortality. Patient HLA antibodies can destroy incompatible platelets and may cause refractoriness to platelet transfusion. Identification of a patient's HLA antibody specificities is necessary for issuing compatible platelets to overcome refractoriness. Many techniques for the detection and identification of HLA antibodies have been developed, including complement-dependent cytotoxicity assay, bead-based assays, the platelet adhesion immunofluorescence test, and the monoclonal antibody-specific immobilisation of platelet antigens assay. Different strategies for the selection of HLA-compatible platelets are applied. These strategies depend on the breadth of antibody reactivity and range from avoiding single HLA antigens in the platelet concentrates issued to apheresis of platelets from HLA-identical donors. The mechanisms of HLA sensitisation and the efforts made to provide compatible blood products to sensitised patients are reviewed in this article from the perspective of clinical transfusion medicine.

Introduction

Several groups reported on antileucocyte antibodies in patient sera in the year 1957 [1–3]. In the following years the human leucocyte antigens (HLA) were discovered step by step, and their relevance for the transplantation of solid organs and for the transplantation of haematopoietic stem cells was recognised.

The HLA system did not play a role for transfusion of blood. Blood had been transfused for decades without realising what an immunological barrier the HLA system can actually be for the transfer of allogeneic cells. The major reason for unimpeded transfusions is that erythrocytes, which were by far the largest and clinically most relevant cell population in the transfused blood components, whether whole blood in the beginning or red cell concentrates later on, carry only very few HLA molecules on their surface. This low antigen density protects the red cells from the attack by HLA antibodies or specific T cells and allows transfusion to sensitised patients without haemolysis. It took until 1969 to establish a connection be-

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tween erythrocytes and the HLA system because a clinical relevance was missing: the recently described Bennett-Goodspeed red cell antigen [4] was identified 6 years later to represent the HLA-B7 antigens on red cells [5]. At that time, most clinically relevant blood group systems had already been discovered [6].

Increasing treatment of patients with leukaemia required more and more platelets for the treatment of bleeding or for prophylactic transfusion. Platelets have a short lifespan (8–12 days in vivo) [7], which is why transfused platelets cause an only short increase of the patient's platelet count. The platelet count repeatedly fails to increase after transfusion in some of these patients, a condition which is called refractoriness to platelet transfusion. HLA antibodies are the cause in about 20% of refractoriness. As little as HLA interfere with red cell transfusion, for successful platelet transfusion paying attention to HLA antigens and HLA antibodies may become crucial. In addition, HLA antibodies are involved in a very rare but dramatic transfusion reaction, transfusion-associated lung injury (TRALI). Plasma, platelets, and even red cell concentrates containing only minimal volumes of donor plasma can transfer HLA antibodies from the donor into a patient. Under certain conditions the patient's granulocytes are triggered by HLA antibodies and release cytotoxic mediators. Pulmonary oedema may develop within minutes and often requires intubation and intensive care.

Numerous efforts were made to investigate the mechanisms of HLA sensitisation and to find ways to prevent it. Laboratory methods for detection and identification of HLA antibodies were developed. The apheresis technique was used and further developed in order to yield therapeutic doses of platelets from HLA-compatible donors. These and other aspects will be reviewed in this article.

Causes and Mechanisms of HLA Sensitisation

HLA Sensitisation during Pregnancy

HLA sensitisation results from exposure to alloantigens, as it occurs in transplantation of solid organs, after transfusion of blood, or during pregnancy. Pregnancy is a major cause of HLA sensitisation. Half of a foetus' genes are allogeneic and so are many of the cellular antigens. The mother's immune system tolerates the foetal tissue growing in the uterus, but can simultaneously mount a humoral and cellular immune response against paternal antigens, including the HLA. This "immunological paradox of pregnancy" [8] is still not understood. The cells of the cytotrophoblast and of the syncytiotrophoblast form the border between embryonic and maternal tissue. They lack HLA-A and HLA-B, but express HLA-G, an atypical class I HLA molecule that protects the embryonic cells from lysis by natural killer cells [9]. This and other mechanisms help the embryonic or foetal cells to evade the recognition by the maternal immune cells. However, during growth of the placenta, cells of the syncytiotrophoblast become apoptotic and will be degraded and shed cellular debris, including foetal DNA, into the maternal circulation. Foetal DNA can be found in maternal plasma from 7 weeks after gestation; its concentration is high enough to enable foetal genotyping [10]. It is not known whether the debris of these immunologically camouflaged cells can elicit an immune response to classical class I HLA, but there are other cells escaping from the uterus into the maternal circulation: foetal blood vessels in the chorionic villi are separated from maternal blood in the intervillous spaces by a thin cell layer. Foetomaternal haemorrhage can occur if this cell layer loses its integrity and foetal erythrocytes and leucocytes are released into the maternal blood [11]. It is well known that RhD-positive foetal red cells can cause the formation of anti-D in an RhD-negative mother. The mother's immune response to the foetal cells is very effective; high titres of maternal anti-D can cause severe and even fatal haemolytic disease of the foetus and the newborn. In the same way, foetal leucocytes may elicit maternal antibody formation against leucocyte antigens, including the HLA. In contrast to antibodies directed against, e.g., red cell antigens, platelet-specific glycoprotein antigens, or granulocyte antigens, maternal HLA antibodies normally do no harm to the foetus.

The rate of HLA sensitisation that can be found depends on several variables when women with pregnancy in their history are studied; it increases with the number of pregnancies, it depends on the sensitivity of the method used, i.e., bead-based solid-phase assays will find higher rates than complement-dependent cytotoxicity (CDC) assays, and it depends on what time after delivery the mothers are investigated. After their first pregnancy, 1–4% of women were found to have class I HLA antibodies when tested with an agglutination assay [12] or with the CDC assay [13]. Multiparous women were found to have sensitisation rates ranging from 20 to 50% [12–15]. HLA class II antibodies were found by CDC assay in the sera of 14% of primigravidae and in the sera of 28% of multigravidae [15]. In one study, 294 women were retested 3 months after delivery, and it was found that the sensitisation rate as tested by CDC assay had increased from 18 to 21% [14]. When the same group of women was tested with a bead-based assay, the rate at delivery was 45% and increased to 54% when they were tested 3 months later. Of these women, 20% had class I antibodies, 8% had class II antibodies, and 26% had both class I and class II antibodies. Over years and decades antibody titres may fall below the detection threshold. In another study, 3,992 parous women were tested years after delivery [16]. The women were grouped according to the time since their last delivery: within the last 10 years, between 10 and 20 years, between

20 and 30 years, and more than 30 years since their last delivery. The sensitisation rates found by a bead-based method declined from 31 to 26, 22, and 18%, respectively.

HLA Sensitisation by Platelet Concentrates

Blood components transfer large amounts of HLA molecules into the recipient: red cells, platelets, and contaminating leucocytes carry HLA molecules in their membranes, and plasma carries soluble HLA molecules. Red cells are not the major source of transfused HLA; only about 90 HLA molecules/red cell (range 40–550) were found on their surface [17, 18]. Platelets, in contrast, carry way more HLA (between 50,000 and 120,000 HLA molecules/cell), although their surface is markedly smaller than that of red cells [19]. Platelets only express class I molecules, predominantly HLA-A and HLA-B [20, 21]. It was suggested that platelets absorb HLA molecules that have been shed by other cells into the plasma [22, 23]. In a recent investigation, however, HLA-A*0201 molecules were prepared from platelets that presented different peptides in their antigen recognition site, including peptides that had derived from glycoprotein IX, a molecule which is predominantly expressed by platelets. The authors concluded that these HLA-A*0201 molecules, therefore, must have been assembled in a megakaryocyte or its precursor cell [24].

HLA Sensitisation by Plasma

HLA molecules are present in abundance in plasma. HLA class I protein was found in concentrations between 0.25 and 4.1 μg/mL [25, 26]; 1 μg of HLA class I protein equals about 1×10^{13} HLA molecules when calculating with a molecular weight of 55 kDa. For HLA class II molecules similar concentrations up to 11 μg/mL were reported [27, 28]. Therefore, between 0.25×10^{13} and 4.1×10^{13} HLA class I molecules/mL fresh frozen plasma, up to 9.6×10^{10} molecules/mL albumin preparation, and up to 2.09×10^{11} HLA class I molecules/mL immunoglobulin preparation are transfused into patients [29]. The role of soluble HLA in sensitisation has not been investigated exhaustively. It has been stated that in patients with renal insufficiency who had received plasma only, soluble HLA elicited the production of HLA antibodies [30]. Soluble HLA class I molecules may, for example, integrate into low-density lipoproteins [31] and may be phagocytosed, degraded, and presented by macrophages and other phagocytic cells of the recipient. B cells and T cells may recognise the presented peptides and mount a humoral immune response to the allogeneic HLA. Others observed an immunosuppressive effect for soluble HLA, at least in mice. When the mice were pretreated with purified, recombinant soluble HLA-B7, their humoral response to a challenge with leucocytes carrying HLA-B7 on their membrane was significantly blocked [32].

HLA Sensitisation by Leucocytes

Leucocytes present in red cell and platelet concentrates became the main suspects [33, 34]. Peripheral blood lymphocytes carry up to 250,000 HLA class I molecules on their cell surface [35, 36]. Other than platelets and red cells, leucocytes also carry HLA class II molecules. They may shed HLA molecules into the patient's plasma, which are taken up and presented by antigen-presenting cells of the patient. More important is probably the fact that leucocytes also carry costimulatory proteins. Patient lymphocytes, which are specifically directed against the HLA of the donor, recognise the allogeneic HLA molecules and bind to them. The costimulatory molecules on the donor's leucocytes stimulate and activate the patient's lymphocytes. When early work showed that the use of leucocyte-reduced platelets and red cells prevented HLA sensitisation [33, 37], efforts were made to reduce the number of leucocytes in blood components.

Efforts Made to Reduce HLA Sensitisation

The awareness that leucocytes are the major cause of HLA sensitisation fostered the development of methods which either inactivated HLA-bearing cells by ultraviolet B irradiation [38, 39] or which reduced the leucocytes in red cell concentrates and in platelets [40, 41]. In addition to lower HLA sensitisation rates, there were other good reasons to reduce the leucocytes in blood components: leucocytes can carry intracellular pathogens, including cytomegalovirus or human T-cell lymphotropic virus, and can release cytokines and other immunologically active substances into the blood component, which may cause febrile or allergic transfusion reactions.

Differential centrifugation is one of the techniques used to reduce leucocytes. It enables the separation of whole blood into red cells, plasma, and a layer between red cells and plasma, which resembled a buffy coat. This buffy coat-like layer contains the major part of the leucocytes and is separated from the red cells and the plasma. Differential centrifugation was further developed by introduction of a special mechanical clamping device to produce leucocyte-depleted red cell concentrates. This device was also used to separate bone marrow haematopoietic stem cells from ABO-incompatible red cells [42]. In the 1980s leucocyte adhesion filters became the standard technique [43]. Prestorage filtration reduces the total number of approximately 3×10^9 leucocytes found in 500 mL of whole blood by more than 99.9% to 1×10^6 or less.

Leucocytes in platelet concentrates were reduced by 81% only in a small study [44]; the difference between the sensitisation rates did not reach statistical significance. The results of several other studies did not reach statisti-

cal significance either (reviewed by Vamvakas [45]). The large TRAP study [39] enrolled more than 500 patients and found a significant reduction in HLA sensitisation in women who had never been pregnant and in men from 32% in the control group (non-filtered platelets) to 9% in the group receiving filtered platelets. When the earlier and smaller studies were reviewed in a meta-analysis, the results of the TRAP study were confirmed [45].

After many countries and institutions had introduced universal pre- or poststorage leucocyte filtration, only few studies on the success of these measures with respect to HLA sensitisation were published. One study investigated 617 patients and found a reduced HLA sensitisation (from 19 to 7%) and refractoriness (from 14 to 4%) [46]. However, two other studies did not find lower rates of HLA sensitisation after introduction of leucocyte filtration [47, 48].

Leucocyte filtration may have reduced, but has not eliminated, HLA sensitisation. Still 2–9% of patients with no pregnancies or transfusions in their history are sensitised by transfusion of filtered red cells or platelets and produce HLA antibodies [39, 46, 49]. Further, sensitisation by pregnancy will be unaffected by these measures.

Laboratory Testing for HLA Antibodies

CDC Assay

The classical and best-known assay for the detection of HLA antibodies is the CDC assay [50]. It was introduced in 1964 and is still the gold standard for the allocation crossmatch procedure in organ transplantation [51]. Trays with 60 or 72 flat wells holding a maximum of 10 μL that fit onto an inverted microscope are used. One microlitre of a lymphocyte suspension is placed in a well and 1 μL of patient serum is added. After incubation, a few microlitres of complement-active human or rabbit serum are added. If patient antibodies have bound to the lymphocytes, they can now activate the added complement and cause the lysis of cells. Addition of a dye that enters only dead cells allows the detection of cytotoxic HLA antibodies. IgM, IgG1, and IgG3 are able to activate complement; IgG2 and IgG4, which do not activate complement, are not detected by the CDC assay. Further, an appropriate density of IgG bound to the lymphocytes is required for binding and activation of C1, the first component of the complement system. The CDC assay will therefore not detect low-titre HLA antibodies. The percentage of reactive wells is given as percent panel reactive antibodies. Low panel reactive antibody values indicate the presence of singular HLA specificities in a serum, whereas highly sensitised patients have panel reactive antibody values up to 100%.

Platelet Adhesion Immunofluorescence Test

With the platelet suspension immunofluorescence test [52], platelet-bound antibodies, including glycoprotein-specific antibodies, isoagglutinins, drug-dependent antibodies, and HLA class I antibodies, can be detected. This test was simplified by taking advantage of the platelets' ability to adhere to glass surfaces [53]. Suspensions of reagent platelets are placed on the marked fields of a glass tray, allowing the platelets to adhere. Excess suspension is removed and the platelets are overlaid with patient serum. Antibodies present in the serum bind to the platelets and are detected using fluorescence-conjugated antihuman IgG antibodies and a microscope (Fig. 1A). Compared to the CDC assay, the platelet adhesion immunofluorescence test (PAIFT) is more sensitive; it detects HLA antibodies with 2- to 3-fold lower titres. The PAIFT is a valuable tool for crossmatching donor platelets.

Monoclonal Antibody-Specific Immobilisation of Platelet Antigens

The monoclonal antibody-specific immobilisation of platelet antigens (MAIPA) assay was initially developed to test patient sera for antibodies against human platelet antigen (HPA) [54], but it is also an excellent tool for the investigation of HLA antibodies and crossmatching of platelets. Intact reagent platelets are incubated with patient serum suspected to carry anti-HLA antibodies. The platelets are then washed and incubated with a mouse monoclonal antibody directed against a different epitope on the glycoprotein under investigation, adding up to a bimolecular, or in the presence of patient antibody trimolecular, complex (Fig. 1B). In case of HLA antibody testing the monoclonal antibody is directed against beta-2 microglobulin. After a second washing step the platelets are lysed and the HLA molecules are released from the membrane into the supernatant, which is transferred onto a microtitre plate coated with goat antimouse IgG. The HLA molecules are immobilised and the presence of patient antibody is detected by enzyme-linked antihuman IgG. As it is for the PAIFT, the MAIPA assay is more sensitive for detection of HLA antibodies than the CDC assay [55].

Luminex-Based Bead Assays

The Luminex technology was introduced some years ago. It consists of a flow cytometer with two lasers and a set of up to 200 polystyrene beads which are identifiable by their individual fluorescent dye. One of the lasers (635 nm) induces the beads to emit their bead-specific light, while the second laser (532 nm) causes phycoerythrin (PE)-conjugated substances to emit light at 573 nm. In case of HLA antibody assays the beads are coated with HLA molecules [56]. Beads for antibody screening carry HLA molecules with different specificities, beads for antibody identification carry only one HLA specificity per

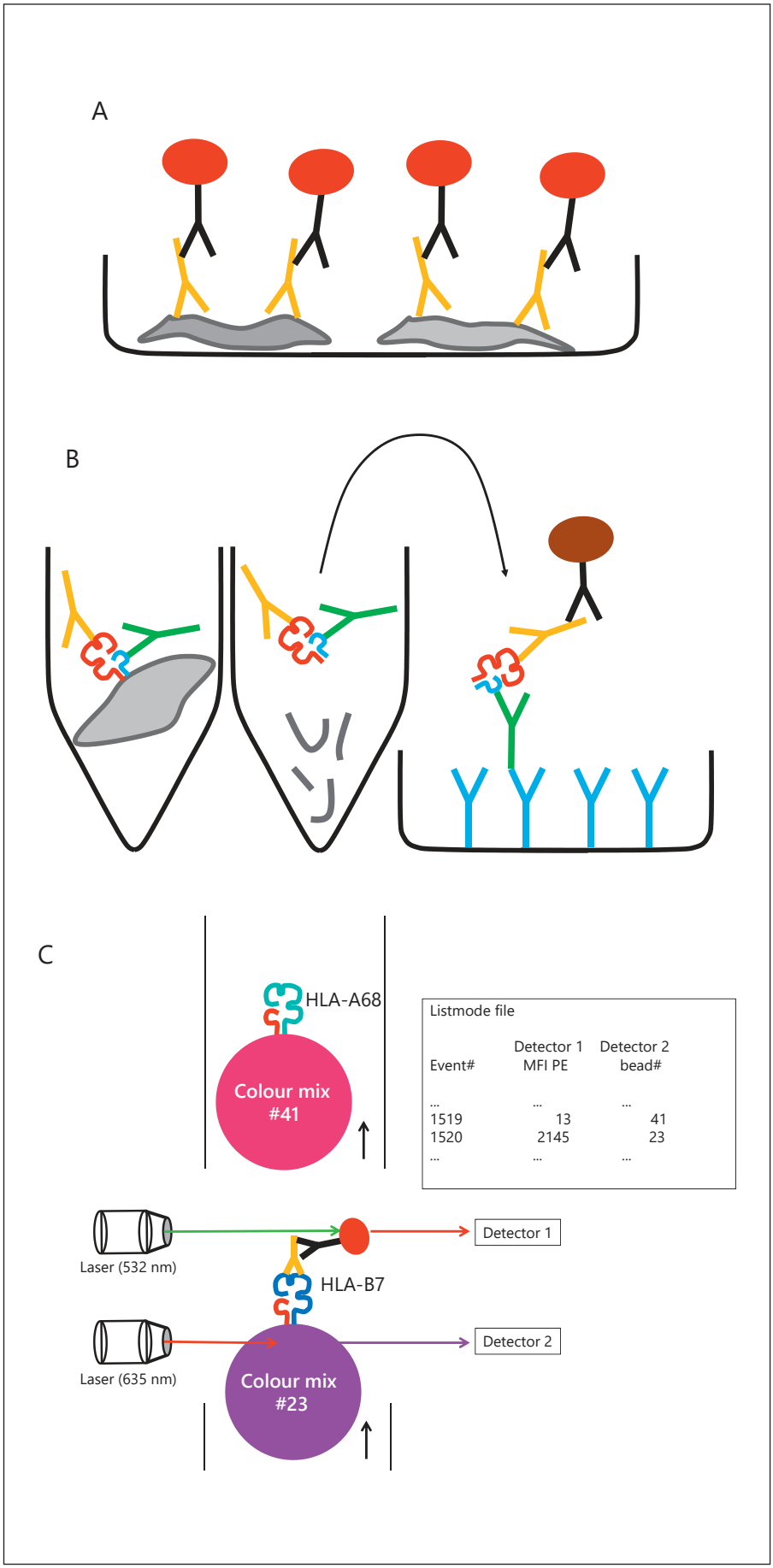

Fig. 1. A PAIFT. Platelets adhere to glass trays and are overlaid with patient serum. Platelet antibodies bind to their cognate antigens and are detected by fluorescence-labelled dyes by using a microscope. **B** MAIPA assay. Reagent platelets are incubated with patient serum containing HLA antibodies. After washing, a mouse monoclonal antibody directed against beta-2 microglobulin (green) is added and the platelets are lysed. Antibody-HLA complexes are centrifuged into the supernatant and carried onto microtitre plates coated with goat antimouse IgG (blue). The HLA molecules are immobilised and presence of patient antibody is detected by enzyme-linked antihuman IgG. **C** Luminex-based bead assays. Polystyrene beads, which are identifiable by their individual fluorescent dye, are coated with HLA molecules. One of the lasers induces the beads to emit their bead-specific light (i.e., the bead's ID), the second laser causes PE-conjugated substances to emit light. For each bead passing the flow chamber its ID and its PE mean fluorescence are saved to listmode files. HLA, human leucocyte antigen; MAIPA, monoclonal antibody-specific immobilisation of platelet antigens; MFI, mean fluorescence intensity; PAIFT, platelet adhesion immunofluorescence test; PE, phycoerythrin.

bead type (single-antigen beads [SABs]). After incubation with patient serum, the beads are washed and PE-conjugated antihuman IgG is added. Beads carrying HLA that have bound patient antibody now also carry PE-conjugated detection antibody. The flow cytometer simultaneously records which type of bead is passing the flow cell and whether the passing bead has the PE-conjugated antibody bound. The ID and the intensity of the PE fluorescence are registered by the system for each bead and can be analysed using a special software (Fig. 1C).

The SAB assay can be easily performed and is very sensitive, but shows two unwanted effects. High-titre antibodies that bind to the beads may activate the complement system. Activated complement components interfere with the detection of the antibodies and cause false low or even negative results [57, 58]. This effect can be circumvented by pretreatment of the serum by dilution, heat inactivation, treatment with dithiothreitol, or by addition of EDTA to the serum [59]. The second effect derives from denaturation of HLA antigens during the manufacturing process of the beads. HLA molecules devoid of beta-2 microglobulin or HLA molecules lacking the peptide [60] in the antigen recognition site may be present on the beads [61, 62]. Such altered molecules may give false-negative results, or they may cross-react with non-HLA antibodies and give false-positive results [63–65]. The mean fluorescence intensity values depend on the manufacturer of the test kit [66], the lot used [67, 68], and the pretreatment of the sample [57]. For these reasons, no consensus cut-off mean fluorescence intensity value exists for clinically relevant antibodies in organ transplantation [69] or platelet transfusion. Another approach was tried to identify clinically relevant antibodies: the HLA-SAB assay had been modified to distinguish between non-complement-fixing and complement-fixing IgG. Exogenous C1q is added to the HLA-SAB and C1q binding to patient HLA antibodies is detected using fluorescent-conjugated antihuman C1q antibody. C1q binding depends on IgG subclass (IgG1 and IgG3), on the level of antibodies bound to the bead [57], and on technical artefacts that interfere with IgG or C1q binding [70]. The use of C1q-SAB has been suggested for both organ transplantation [71, 72] and platelet transfusion [73], but its value for clinical routine is uncertain [74].

Platelet Refractoriness

Clinical conditions may cause increased consumption or destruction of platelets. Based on studies in patients with acute myeloid leukaemia or haematopoietic stem cell transplantation, these conditions include, but are not limited to, bleeding, fever, sepsis, graft-versus-host disease (GVHD), disseminated intravascular coagulation, splenomegaly, HLA antibodies, HPA antibodies, and medications. Transfusion of platelets in such patients may not lead to the expected rise in platelet numbers in the posttransfusion count. Refractoriness is diagnosed when after at least two consecutive transfusions of "fresh" (issued as soon as possible after production, latest within 2 [75] to 3 [76] days) ABO-compatible platelets increase or the platelet count is unsatisfactory. There is lack of agreement on whether the count should be done 1 h or 20–24 h after transfusion and what formula [77] should be used for correcting for the patient's blood volume and the number of platelets transfused [78]. One simple, clinically oriented approach to diagnose refractoriness could be the finding of an increment of less than 10×10^9/L 20–24 h after transfusion [79].

In 16–18% of patients refractory to platelet transfusion HLA antibodies and in 2–4% antibodies against HPAs are detected [80, 81]. These antibodies destroy incompatible platelets and are the cause of platelet refractoriness. Contrary to red cells, the destruction of platelets normally does not cause clinical signs.

Transfusion of Platelets to Patients with HLA Antibodies: Management of Refractoriness

Not all patients with HLA or HPA antibodies become refractory to platelet transfusions [49, 82]. In one of these studies, refractoriness depended on a higher titre and probably on other, unknown factors. In addition, patients receiving chemotherapy or who are otherwise immunocompromised may not be able to booster existing low-titre HLA antibodies.

At the latest when a patient becomes refractory, HLA antibody testing and HLA-A and -B typing is advisable. Even if no HLA antibodies are detected, transfusing HLA-matched platelets is worth a try in order to rule out antibodies below the detection limit or interferences with the assay used which cause false-negative results.

For patients with low panel reactivity, platelets can be selected that avoid the cognate HLA antigens. HLA antigens have private and public epitopes. A public epitope is shared with other HLA antigens, which serologically form a cross-reactive group (CREG) [83]. HLA antibodies directed against a public epitope of an HLA antigen may therefore cross-react with the other antigens of this CREG. In addition to donors expressing the cognate HLA antigens, also donors with HLA antigens belonging to the same CREGs should be ruled out. The selection of compatible donors can be refined by identifying related epitopes using a bead-based single-antigen method for antibody identification in combination with the HLAMatchmaker software [84, 85]. The corrected count increments at 1 h of platelets from donors selected by this latter ap-

proach were comparable to the increments of platelets from donors without HLA-A and -B mismatch.

Another option is to crossmatch platelets from a donor panel and select the crossmatch-negative units [86]. Neither the patient's HLA type nor the antibody specificity needs to be known for this approach. With crossmatch-negative platelets the increment is comparable to that observed with HLA-matched platelets. A large donor panel must be at disposal and must be tested when compatible platelets are searched for patients with a high panel reactivity. This unfavourably increases the workload for the laboratory.

For highly sensitised patients, donors without an HLA-A or -B mismatch (i.e., HLA-identical donors or donors homozygous for one HLA-A or/and one HLA-B antigen) are the first choice. For some phenotypes, compatible donors are very rare and strategies to expand the donor pool were developed. Patients often do not make antibodies against HLA antigens that share public antigens with their own HLA antigens. Donors with HLA antigens from the patient's CREGs can be tried and often expand the number of compatible platelet donors. Further, some donors express the HLA antigens B12, B8, or B35 at very low levels on their platelets [87, 88]. For donor search, these antigens can be added to the permissible antigens.

If HLA-matched platelets do not result in a satisfying increment, the patient should be tested for HPA antibodies [89].

Transfusion of Red Cells to Patients with HLA Antibodies

HLA Expression on Red Cells

Red cells express only few copies of HLA antigens. The antigen density varies between individuals and seems not to be inherited [90]. The antigen density on the red cells of an individual also can vary over time. The reasons for this are not well understood, but an increase in HLA expression on red cells was observed during infections [91], in autoimmune disease [92], and in haematological disease [93]. In addition, individuals with HLA-A28, -B7, -B8, or -B17 express significantly more HLA on their red cells than those without [17, 94]. As with platelets, there is an ongoing discussion whether the HLA on erythrocytes are absorbed from the plasma [95] or are intrinsic red cell antigens [96].

Reagent red cells may react with patient sera containing HLA antibodies. Morton et al. [5, 97] established the connection of HLA specificities with the Bennett-Goodspeed antigens. Bg^a correlated with the presence of HLA-B7 on the red cells, Bg^b with HLA-B18, and Bg^c with HLA-A28 (Table 1). The concordance is not always complete, which can be explained by HLA antibodies reacting with additional antigens within the CREG. For example, anti-HLA-B27 often cross-reacts with Bg(a+) red cells, or a strong-reacting anti-HLA-A2 likely will react with Bg(c+) red cells. Patient sera containing specific HLA antibodies reacting with the "wrong" Bg antigens, or reagent red cells denoted as Bg(a+) reacting with patient sera containing no anti-HLA-B7, often confuse in routine blood bank work.

Table 1. Association of Bennett-Goodspeed antigens with HLA specificities and their CREGs

Bg antigen	Correlation with	Cross-reacting with	CREG [83]
Bg^a	B7	B8, 13, 22 (54, 55, 56), 27, 40 (60, 61), 41, 42, 47, 48, 59, 67, 81, 82	7C
Bg^b	B18	B8, 14 (64, 65), 16 (38, 39), 59, 67	8C
Bg^c	A28 (A68, A69)	A2, 9 (23, 24), B17 (57, 58)	2C

CREG, cross-reactive group; HLA, human leucocyte antigen.

Reagent cells for red cell antibody screening and antibody identification are selected for low or missing HLA expression by testing with HLA antisera. Some distributors use strongly reacting patient sera and denote their cells Bg^a, Bg^b, or Bg^c, others use monoclonal anti-HLA antibodies and mark the reactive cells with "Bg" only. Despite labelling reagent cells positive for Bg antigens and despite these cells reacting in the antiglobulin test, the Bennett-Goodspeed antigens, i.e., the HLA antigens, are not classified as blood group antigens because red cells are unsuitable for HLA phenotyping [98].

HLA Antibodies and the Transfusion of Red Cells

HLA antibodies normally do not haemolyse transfused red cells. Many patients and many multigravidae have HLA antibodies, and statistically many of these patients receive red cells from donors carrying HLA antigens the patients' antibodies are directed against. For example, the antigen HLA-A2 is found with more than 50% of an European population [99], and anti-HLA-A2 is one of the most prevalent antibody specificities. Thus, many red cell transfusions are incompatible regarding HLA, but only very few reports have been published on reduced survival of the transfused red cells [100] or on acute or delayed haemolysis [101–103]. Activation of the complement system requires bound IgM antibodies or two or more IgG bound in close proximity, which allow binding and activation of C1q. Similarly, initiation of phagocytosis requires complement-coated, IgM-coated [104], or IgG-coated cells. Again, several IgG bound in close proximity initiate or at least enhance phagocytosis [105]. Other than platelets, most red cells do not carry enough HLA

Table 2. Patients at risk of transfusion-associated GVHD

Congenital immunodeficiency
Intrauterine transfusions
Neonate with exchange transfusion
Prior to autologous stem cell collection and after transplantation until reconstitution of the immune system
Allogeneic stem cell transplantation until reconstitution of the immune system
GVHD in progress
Hodgkin's lymphoma
Non-Hodgkin's lymphoma
Therapy with purine analogues
Recipients of HLA-matched blood components
Recipients of blood products donated by relatives

GVHD, graft-versus-host disease; HLA, human leucocyte antigen.

molecules to allow activation of the complement system or phagocytosis by HLA antibodies. In addition, the residual donor plasma present in red cell concentrates transfers soluble HLA, which (partially) inhibit the recipient's HLA antibodies.

Additional antibodies with "undetermined specification" in the column agglutination technique were noticed in one of the reports on haemolysis [101]. In the other two reports, red cell antibody testing was done in the tube technique. It was not investigated whether more sensitive techniques, such as column agglutination technique or the use of polybrene, would have revealed low-titre blood group antibodies as the cause of the haemolysis [106].

It cannot be excluded that in very rare situations red cells with a high HLA antigen density are transfused to patients with high-titre HLA antibodies or to patients with IgM HLA antibodies, and that these antibodies attack and destroy red cells. The possibility of haemolysis by HLA antibodies, therefore, should be kept in mind, but will be a very rare event.

Other Adverse Effects of HLA in Transfusion

Febrile Non-Haemolytic Transfusion Reactions

The main clinical signs of febrile reactions are chills followed by a rise in temperature of 1 or 2 °C during or shortly after transfusion. Pyrogenic cytokines are thought to mediate the fever and other possible symptoms such as rigor, discomfort, and nausea. These cytokines can be released from donor leucocytes during storage into the plasma or the additive solution of the blood component [107, 108]. The release of cytokines may depend on production and storage conditions because not all studies found elevated cytokines in platelet concentrates [109, 110]. Cytokines also can be released by donor leucocytes after transfusion when patient leucocyte antibodies, including HLA antibodies, have activated the cells [111, 112]. In addition, patient leucocytes may release cytokines and cause febrile reactions when donor HLA antibodies are present in the transfused blood product [113]. Without leucocyte reduction, febrile reactions occur in 1 out of 200 transfusions, with platelet concentrates more often involved than red cells [114, 115]. The use of leucocyte-reduced blood products may help decrease the frequency of febrile reactions by 30–50% in red cell transfusion and by 70–90% in platelet transfusion [114, 116]. Febrile reactions occurred in about 0.1% of transfusions despite the use of leucocyte-reduced blood products [114] and are, together with minor allergic reactions, the most frequent adverse effects of transfusion.

Transfusion-Associated Lung Injury

TRALI is defined as an acute respiratory distress syndrome that develops during or within 6 h after transfusion in the absence of other causes of acute respiratory distress syndrome and in the absence of circulatory overload [117]. One explanation for the pathogenesis of a TRALI is the "two-hit-model" [118]. It is thought that clinical events, including recent surgery (<48 h), infection, and massive transfusion (first hit), make a patient susceptible for TRALI, which is triggered by a subsequent transfusion (second hit). In most TRALI cases the triggering blood component contained donor-derived antibodies directed against human neutrophil antigens, HLA class I, or HLA class II. There is clinical and experimental evidence that neutrophilic granulocytes play a central role in the pathogenesis of TRALI [119]. HLA class I antibodies may bind to the endothelium and trap and activate neutrophils via their Fc receptor [120]. Human neutrophil antigen antibodies and HLA class I antibodies also may bind directly to the cognate HLA molecules on the surface of the neutrophils and activate them [121]. Antibodies directed against HLA class II likely bind to and activate the recipient's monocytes. The activated monocytes cause the neutrophils to release mediators including cytokines, oxygen radicals, and proteases, which damage the capillary endothelium and initiate the pathophysiological changes leading to the symptoms of TRALI [122]. Leucocyte antibodies were detected in 80% of TRALI cases; in 20% of cases antibodies were not detected [123] or other substances were suspected to have triggered TRALI [124].

It was realised that TRALI was one of the major causes of transfusion-associated death. Since most TRALI cases were caused by donor leucocyte antibodies, many countries implemented strategies to mitigate the risk for TRALI by producing plasma from selected donors only [125–131]. The selection criteria included male donors only, male donors without transfusion history, female do-

nors without history of transfusions or pregnancies, female donors tested negative for the presence of leucocyte antibodies, or a combination thereof. These measures reduced the plasma-related risk of TRALI by 80–90% [126, 131, 132].

Transfusion-Associated GVHD

Acute GVHD is regularly observed with allogeneic haematopoietic progenitor cell transplantation and, in a mild form, accepted as a graft-versus-leukaemia effect. Transfusion-associated GVHD can develop not only in immunocompromised patients (Table 2), but also in immunocompetent patients after transfusion of leucocyte-containing blood components. For the latter, a certain HLA constellation between donor and recipient is necessary, in which the recipient's lymphocytes do not recognise the transfused leucocytes as allogeneic. For example, the donor is homozygous for an HLA haplotype and the recipient is heterozygous for the same haplotype. In this constellation the recipient's lymphocytes will accept the donor lymphocytes as familiar, but the donor's lymphocytes will mount an immune response against the host. In transfusion-associated GVHD, symptoms including fever, rash, diarrhoea, and liver disease develop within 8–10 days after transfusion. Other than in transplantation-associated GVHD, the mortality of transfusion-associated GVHD is as high as 90%. Transfusion-associated GVHD can be prevented by irradiation of cellular blood components at a minimum of 25 Gy. With this dose the DNA of the donor leucocytes will be severely damaged, which prevents proliferation of the cells and an effective immune response. A similar protective effect can be achieved by application of pathogen inactivation procedures [133, 134]. The risk for transfusion-associated GVHD in immunocompetent patients depends on the genetic variation at the HLA locus in a population. For a European population, the risk for HLA constellations facilitating transfusion-associated GVHD was estimated to be 1/6,900 to 1/48,500 [135], while for the United States this risk was estimated to be 1/17,700 to 1/39,000. In these countries non-directed transfusions are not irradiated, unless the recipient is immunocompromised or at other risk of developing transfusion-associated GVHD (Table 2). In the Japanese population the risk was estimated as 1/1,600 to 1/7,900, which is the reason why cellular blood products are irradiated, even in the non-directed transfusion setting.

A Look into the Crystal Ball

The idea of removing HLA from the membrane of platelets [136–138] or leucocytes [139] has been around for years. Treatment of platelets with acid removed beta-2 microglobulin and reduced HLA expression by 80–90% in a recent study [140]. The treatment prevented binding of patient HLA antibodies to the platelets, it prevented antibody-mediated complement activation, and it reduced antibody-mediated phagocytosis. The physiological functions of the platelets remained intact after treatment for at least 4 h. HLA stripping of platelets (and the residual leucocytes) prior to storage, during the routine preparation of the platelet concentrates, or, if implemented, in combination with a pathogen inactivation process would be advantageous. Additional investigations on platelet function are needed, and the possibility of reassembling HLA molecules [141] or of de novo synthesis and expression of HLA molecules [142] has to be investigated. Finally, clinical studies are required. So far, 5 patients and 2 healthy volunteers have been transfused with acid-treated platelets by four different groups. In the 2 healthy volunteers, recovery was 69 and 75%, respectively with acid-treated platelets versus 71 and 76%, respectively with control platelets [143]. In both volunteers the survival of acid-treated platelets was slightly reduced from 8 to 6 days when compared to control platelets. In patients, acid-treated platelets gave a corrected count increment at 1 h comparable to that of HLA-matched platelets [143] and stopped gastrointestinal bleeding [144, 145]. One group did not observe a significant response to two acid-treated platelet concentrates [146]. In 1 patient a febrile reaction occurred and the observation was stopped 10 min after transfusion [145].

Supplying patients with HLA-stripped platelet preparations could help prevent transfusion-associated HLA sensitisation and overcome refractoriness.

Summary

Evidence of HLA sensitisation can be found in many patients. In rare cases, HLA sensitisation can be the cause of a severe complication of blood transfusion such as TRALI or transfusion-associated GVHD. Sixteen to eighteen percent of patients with refractoriness to platelet transfusion carried HLA antibodies. Blood services successfully implemented measures to mitigate these risks, including provision of HLA-compatible platelets, selection of plasma donors without sensitisation risk, and irradiation of blood products. Efforts to prevent primary HLA sensitisation by reducing the leucocyte load of blood products were not as effective. Furthermore, a large part of HLA sensitisation occurs during pregnancy, which is why we will also have to deal with HLA sensitisation in the future. In order to further decrease the risk of HLA-related serious transfusion reactions, the efforts to be made probably concern refined donor selection or more sophisticated processing of blood components.

Disclosure Statement

C. Weinstock received honoraria from Grifols, S.A. M. Schnaidt has no conflicts of interest to declare.

Author Contributions

C. Weinstock and M. Schnaidt wrote the manuscript.

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