THE PUTATIVE MECHANISM OF THE IMMUNOMODULATING EFFECT OF HLA-DR SHARED ALLOGENEIC BLOOD TRANSFUSIONS ON THE ALLOIMMUNE RESPONSE

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ABSTRACT

Pretransplant blood transfusions have shown to improve organ allograft survival. However, the immunological mechanism leading to this beneficial effect in clinical transplantation is still not clear. The observation that blood transfusions sharing an HLA-DR antigen with the recipient are more effective than HLA mismatched transfusions, has led to the hypothesis that CD4+ regulatory T (Treg) cells that recognize a foreign peptide in the context of the shared HLA-DR molecule play an important role in down-regulation of the immune response toward the graft. Available experimental evidence supports this hypothesis. Furthermore, these CD4+ Treg cells are able to modulate antigen-presenting cells, which in their turn will induce Treg cells. As long as clinical transplantation tolerance by blood transfusions is not a reality, further studies on the mechanisms of the beneficial effect of pretransplant allogeneic blood transfusions are needed to obtain an effective protocol for the induction of clinically relevant Treg cells.
INTRODUCTION

Blood transfusions can induce seemingly opposite immunological effects in the recipient. They can lead either to immunization or to induction of immunological tolerance. Immunization is reflected by appearance of alloantibodies (1,2), of which HLA antibodies can cause febrile transfusion reactions and hyperacute rejection of mismatched organ transplants.

Clinical phenomena that have been associated with the induction of immunosuppression by blood transfusion include increased susceptibility for postoperative infections (3), whereas immunologic tolerance induced by blood transfusions is held responsible for successful pregnancies in women with recurrent abortions and reduction of rejection of solid organ transplants (4,5).

In this review, we would like to restrict ourselves to the alloimmune response against the HLA alloantigens and the relevance of pretransplant blood transfusions in clinical transplantation. We will show that the degree of HLA matching between blood donor and recipient is a determining factor for the final immunologic effect of a blood transfusion and that the induction of CD4+ regulatory T (Treg) cells may be essential for the induction of the immunomodulatory effect of pretransplant blood transfusions.

Historical and clinical data of pretransplant blood transfusions

In 1963, Medawar (6) reported the reduction of the immunologic rejection of skin homografts in mice after the infusion of antigenic tissue extracts. In 1964, Halasz et al. (7) reported that dogs pre-treated with blood from the donor had a prolonged survival of a renal allograft. A beneficial effect of blood transfusions on graft outcome was also suggested in man (8,9) and was demonstrated in mice (10), rats (11-13) and primates (14). In 1973, Opelz et al. (15) identified, in a multivariate analysis of the outcome of cadaver kidney graft transplantation, a significant enhancement of kidney graft survival in recipients of multiple pretransplant allogeneic blood transfusions compared with nontransfused recipients. Subsequently, Persijn et al. (16) demonstrated that this beneficial effect only occurred when the blood transfusion was not leukocyte depleted and that even patients who received only one single blood transfusion had a kidney graft survival of 80% after 8 months, whereas transplanted patients who received one or three leukocyte-free blood transfusion(s) had a graft survival of only 25%. Thus, apparently, leukocytes are important for the induction of the tolerating effect of allogeneic blood transfusions.

The appearance of transfusion-transmitted HIV at approximately the same time as the improvement of graft survival by advanced patient care, better histocompatibility matching, and the introduction of cyclosporine questioned the need for a possible role for pretransplantation allogeneic blood transfusions in the 1980s. Indeed, in some
observational studies, pretransplant blood transfusions did not lead to better graft survival compared with that seen in nontransfused patients (17,18); but in a prospective randomized study, the beneficial effect of pretransplant allogeneic blood transfusions could still be demonstrated in patients who received very efficient immunosuppressive drugs (19).

The beneficial effect of pretransplant blood transfusions is restricted not only to kidney transplants. Also in heart transplantation (20,21) and combined pancreas-kidney transplantation (Waanders et al, manuscript in preparation), pretransplant blood transfusions are associated with a lower risk of rejection.

The need for HLA compatibility between blood donor and recipient

The aforementioned studies from the 1980s suggested that white blood cells play an essential role in the induction of transplantation tolerance. In addition, retrospective analysis of graft survival showed that HLA-DR compatibility between patient and blood donor is of great importance. Lagaaij et al. (20) demonstrated that patients transfused with HLA-DR shared blood had better kidney graft survival compared with patients who received completely HLA-DR mismatched transfusions. For this effect to be observed, an additional requirement seems to be HLA class II disparity on the other haplotype (22).

A similar differential impact of HLA compatibility was observed with respect to the humoral alloimmune response to allogeneic blood transfusions. The incidence of HLA alloantibody formation was significantly higher after completely HLA-DR mismatched transfusions compared with (partly) HLA-DR shared transfusions. Furthermore, if antibodies are formed, HLA-DR shared allogeneic transfusions are associated with the production of IgM alloantibodies, whereas HLA-DR mismatched transfusions lead to IgG alloantibodies. Several other groups confirmed the beneficial effect of HLA-DR sharing between blood donor and recipient and observed fewer post transplant rejection episodes in renal and cardiac allograft recipients and improved patient survival after kidney transplantation (21,23-25). However, Christiaans et al. (26) did not observe differences in development of alloantibodies or graft survival between recipients of an HLA-DR matched and HLA-DR nonmatched transfusion.

In vitro monitoring of T cell alloreactivity of patients after pretransplant blood transfusions has led to controversial observations. A differential influence of HLA-DR shared versus mismatched allogeneic transfusions on induction of donor-specific cytotoxic T lymphocytes (CTLs) was described. HLA-DR shared transfusion led to a marked reduction of the number of CTLs, whereas HLA-DR mismatched transfusions increased the CTL precursor frequency (27). This finding was confirmed in a mouse model in which semi-allogeneic blood transfusions resulted in less sensitization (cellular and humoral) than fully allogeneic transfusions (28). Moreover, it was found
that an HLA mismatched allogeneic blood transfusion led to high-affinity CTLs against immunogenic class I mismatched antigens (29). However, other studies showed that both HLA-DR shared and mismatched transfusions activate the alloreactive T cell compartment (30,31). The discrepant effect on T cell responses may be explained by a different composition of pretransplant allogeneic blood transfusions, differences in storage time of the blood, and in the time interval between transfusion and the in vitro assays.

In summary, although clinical data such as HLA alloantibody formation and graft survival suggest that (partly) HLA-DR shared blood transfusions lead to down-regulation of the alloimmune response in the recipient, the effect on the T cell compartment is less clear.

Mechanisms of transfusion-induced immunomodulation

Although the effect of a pretransplant allogeneic blood transfusion has been recognized for some decades, there are hardly any data available with respect to the immunologic mechanism leading to the beneficial effect in clinical transplantation. The observation that the beneficial effect of pretransplant allogeneic blood transfusions was not seen in patients who received antithymocyte globulin as induction therapy, suggests that T cells may play an important role (32).

Experimental data in animals support several mechanisms, including clonal deletion, anergy, anti-idiotypic antibodies, veto cells, and the induction of Treg cells. Also in man, several explanations for the immunomodulatory effect of blood transfusions have been suggested. For an efficient induction of an alloimmune response it is essential that the antigen-presenting cells (APCs) in the blood carry both allogeneic HLA antigens (signal 1) and costimulatory molecules (signal 2). Only if both signals are present will this lead to activation of alloreactive T cells. After storage of blood, there is a lower expression of costimulatory molecules on the donor leukocytes. Subsequent transfusion of these impaired APCs will induce anergy rather than activation of the donor specific T cells in the recipient (33). It has also been shown that transfusions may lead to long-term (micro)chimerism leading to 2-way tolerance (34). Moreover, soluble HLA, Fas and other molecules accumulate in donor blood and can modulate the alloimmune response of the recipient (35). Binding of the soluble molecules to the alloreactive T cells will lead to apoptosis rather than to activation. However, these putative mechanisms do not explain why HLA-DR sharing between blood donor and recipient plays such an important role.
In our opinion, the shared HLA-DR antigens may be the clue to the mechanism involved in the beneficial effect of pretransplant allogeneic blood transfusions. But how can a shared HLA-DR antigen affect the recipient's immune system? There are 2 distinct pathways of allorecognition by T cells. In direct allorecognition, T cells recognize intact allogeneic HLA molecules on the surface of donor APCs in the graft (Figure 1A). Indirect allorecognition is based on T cell recognition of an allogeneic peptide in the context of recipient HLA class II molecules after they have been processed and presented by recipient APCs (Figure 1B), which is similar to the T cell response to bacterial or viral antigens. With respect to the immunomodulating effect of HLA-DR shared blood transfusions, it is essential that the immune system of the recipient recognizes the shared HLA-DR antigen, which is only possible if it contains a foreign peptide. As the shared HLA-DR antigen is present on donor APCs, it is indeed likely that the shared HLA-DR antigen contains many foreign allopeptides. These allopeptides may be derived from mismatched HLA class I molecules, minor transplantation antigens or any polymorphic protein present in the donor and not in the recipient (36).Recipient CD4+ T cells that recognize this foreign peptide in the context of the shared HLA-DR molecule may then be activated by the blood transfusion (Figure 2).

Figure 1: Direct and indirect pathways of T cell allorecognition. (A) In direct allorecognition, T cells directly recognize an allogeneic HLA molecule on the surface of donor antigen presenting cells (APCs) in the graft. (B) In indirect allorecognition, T cells recognize an allogeneic peptide in the context of recipient HLA class II molecules, after processing and presentation by recipient APCs.
Figure 2: Allorecognition after an HLA-DR matched blood transfusion. T cells of the recipient recognize a donor-derived peptide in the context of a shared HLA class II molecule, leading to T cells with a similar specificity as the ones described in Figure 1B.

The next question is, how can these CD4+ T cells mediate down-regulation of the alloimmune response to a donor kidney or heart from a complete different individual than the blood transfusion donor? We hypothesize that this can be possible if the organ donor shares at least 1 of the allopeptides with the blood transfusion donor. When recipient T cells get activated by the graft, they start to express HLA class II molecules and may present an allopeptide shared between the organ donor and the blood transfusion donor. In this way, the same complex of allopeptide and self-HLA class II molecule will become available after transplantation and may serve as a specific target for the CD4+ T cells induced by the allogeneic blood transfusion (Figure 3). The CD4+ T cells which down-regulate the immune response toward the graft are presumably Treg cells (37,38).

In the first 5 months after transplantation, organ donor-derived passenger APCs are also present (39). In case there is HLA-DR sharing with the allogeneic blood transfusion donor and the recipient, APCs from the graft may present the specific allopeptide-HLA complex. In this special case, APCs derived from the graft may also serve as a specific target for CD4+ Treg cells, which are induced by the blood transfusion (not shown in figures). However, the blood transfusion effect is independent of the HLA-DR phenotype of the organ donor.
Figure 3: Proposed role of alloreactive T cells in antigen presentation after transplantation. (A) T cells of recipient origin may directly recognize allogeneic HLA molecules on the surface of the transplanted organ, which results in (B) expression of HLA class II molecules. Alloreactive T cells can pick up and present the donor-derived peptides to CD4^+ Treg cells induced by the blood transfusion, which may lead to down-regulation of the immune response toward the graft.

Figure 4: Proposed role of recipient DCs in antigen presentation after transplantation. After processing, organ donor-derived peptides are presented in the context of HLA class II molecules on recipient DCs and serve as a target for CD4^+ Treg cells induced by the blood transfusion.
Putative mechanism of pretransplant blood transfusions

Several months after transplantation, in all recipients, the APCs of the organ donor are replaced by APCs (ie, dendritic cells or DCs) of the recipient. Recipient DCs can also process and present organ donor-derived peptides and function as professional APCs (40). Recipient CD4+ T cells induced by the allo-ergic blood transfusion may indirectly recognize organ donor-derived peptides presented by recipient DCs, which may lead to down-regulation of the immune response toward the graft (Figure 4).

Besides the possibility that the CD4+ Treg cells with indirect allospecificity down-regulate the alloimmune response to the organ donor, these CD4+ Treg cells may also lead to modulation of the DCs. These modulated DCs may induce non-responsiveness of the potential donor reactive T cells of the recipient. Modulation of DCs through maturation in the presence of interleukin (IL) 10, can lead to APCs which preferentially induce a regulatory immune response, rather than a destructive immune response (41,42).

Figure 5: Proposed action of IL-10 in modulating the immune response toward the graft. (A) IL-10 produced by CD4+ Treg cells facilitates the generation of modulated DCs. (B) The modulated DCs promote the differentiation of naive alloreactive T cells into Treg cells via IL-10. (C) In turn, the newly formed Treg cells can affect newly developing DCs by their IL-10 production, which may modulate immune responses toward the graft.
In Figure 5, it is shown that IL-10 production by the CD4+ Treg cell may facilitate the generation of modulated DC. In turn, the modulated DC may promote differentiation of naive alloreactive T cells into Treg cells (by production of IL-10 or reduced stimulation or costimulation). These Treg cells can produce IL-10 again and may affect the function of newly developing DCs. Such an inhibitory feedback loop between tolerogenic DCs and Treg cells has also been described in the transplantation tolerance seen in a mouse model (43).

The CD4+ Treg cells in this hypothesis are probably different from the naturally occurring Treg cells (CD4+CD25+), which are involved in the prevention of autoimmune reactions and the maintenance of peripheral tolerance to self-antigens (44). Blood transfusion-induced CD4+ Treg cells are only found after confrontation with the allogeneic blood transfusion donor APCs carrying the combination of shared HLA class II and a foreign peptide. Whether these cells are expanded from the naturally occurring Treg cells or are newly induced Treg cells remains to be established.

**Experimental and clinical evidence for the hypothesis**

To find support for this hypothesis, we tested whether CD4+ T cells recognizing an allopeptide in the context of self-HLA class II do have regulatory properties. We could generate in vitro CD4+CD25+ T cell clones which down-regulate the alloreactive immune response of autologous cytotoxic T cells, provided the proper peptide was present (45). These CD4+ T cells lyse autologous activated (HLA class II positive) T cells in the presence of a specific peptide, which is supportive of the mechanism described in Figure 3. Besides a putative regulatory function based on lysis of target cells, these CD4+CD25+ T cells produce considerable amounts of IL-10 when confronted with the proper peptide HLA-DR complex. IL-10 is typically one of the cytokines that is produced by Treg cells and not by ordinary type 1 T helper cells (involved in evoking cellular immune responses). The large quantities of IL-10 produced by these CD4+ Treg cells make them particularly suitable for affecting the function of APCs, as shown in Figure 5.

To substantiate these in vitro data with clinical observations, we studied the CD4+ T cells of renal transplant recipients who received HLA-DR shared allogeneic blood transfusions. The reactivity of recipient CD4+ T cells against the transfusion donor in patients who received an HLA-DR shared blood transfusion was compared with the reactivity after an HLA-DR mismatched blood transfusion. An HLA-DR mismatched blood transfusion led to increased donor-specific interferon γ (IFNγ) production without detectable IL-10 production. In contrast, HLA-DR matched transfusions induced significantly less donor-specific IFNγ production, whereas in a proportion of the patients, increased IL-10 production was observed (46). These data are in concordance with the assumption that HLA-DR mismatched allogeneic blood
transfusions are associated with immunization (IFN$\gamma$ production is a parameter for type 1 T helper cell reactivity), whereas HLA-DR shared transfusions are associated with the induction of tolerance (IL-10 production is a marker for Treg cells). However, these observations reflect the immune response to the allogeneic blood transfusions. The impact of HLA-DR shared blood transfusions on the alloimmune response toward the transplanted organ remains to be clarified.

Recently, we have started to analyze the organ donor-specific T cell repertoire of patients with well-functioning kidney grafts, who had received an HLA-DR shared pretransplant blood transfusion. Thus far, these patients were found to have a low organ donor-specific CTL frequency after transplantation. Further studies in vitro showed that this low donor-specific CTL precursor (CTLp) frequency is not due to the absence of donor-specific CTLs, but rather to the effect of CD4$^+$ T cells which prevent the reactivity of donor-specific CD8$^+$ CTLs. When the CD4$^+$ T cells were removed from the leukocyte population, a significant organ donor-specific CTL response could be observed. This phenomenon was donor-specific as no significant effect was observed on the third-party CTL response (46).

CONCLUSIONS

Both clinical studies and in vitro models suggest that allogeneic blood transfusions may lead to down-regulation of the immune response against allogeneic HLA antigens presented by transplanted organs. Some of these effects are dependent on the transfusion dose and the length of storage of allogeneic blood. We further showed that a single transfusion containing viable leucocytes can mediate allograft tolerance, provided that HLA-DR sharing is present between the blood donor and the recipient. We have indirect evidence that this down-regulation of alloreactive cells is the result of the induction of CD4$^+$ Treg cells that recognize an allopeptide in the context of a self-HLA class II molecule.

The fact that an HLA-DR antigen or HLA haplotype should be shared to induce a Treg cell that prevents a destructive alloimmune response, shows similarities with the situation that occurs during pregnancy. Mother and child always share at least 1 HLA haplotype, and allore cognition will always imply indirect recognition of an allopeptide in the context of self-HLA class II. Also in this case, Treg cells may be induced (47).

This is in line with the observation that immunized patients less likely produce alloantibodies against maternal HLA mismatches compared to paternal HLA mismatches and may also explain why renal transplants mismatched for noninherited maternal HLA antigens have a significantly better graft survival than renal transplants mismatched for noninherited paternal HLA antigens (48,49). A recent study reveals that also in bone marrow transplant recipients, the incidence of graft vs host disease is
significantly lower when allore cognition involves the noninherited maternal HLA antigens (50). These data suggest that the induction of regulatory cells by HLA-DR shared APCs is not restricted to allogeneic blood transfusions but may also apply to fetomaternal exchange in pregnancy, in which the effect is long-lasting. Compared with the current immunosuppressive drugs, allogeneic blood transfusions are less toxic. However, as long as allogeneic blood transfusion-induced clinical transplantation tolerance does not become a reality, further studies on the mechanisms of the immunomodulatory effect of allogeneic blood transfusions are needed before an optimal protocol for the induction of stable transplantation tolerance can be realized.

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References


Putative mechanism of pretransplant blood transfusions
