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Leucodepletion and immune response mechanisms

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Introduction

The recipient and donor mechanisms responsible for mediating alloimmunity and immunomodulation during blood transfusions are complex and most probably superimposed thus creating multilevel fields of potential interaction and regulation. For example, at the recipient level, both the innate and adaptive immune systems can be significantly affected by various types of transfusions [1,2]. In addition, the health status of the recipient plays a critical role in determining how the host will immunologically respond to transfusion. On the other hand, donor transfusion products have many characteristics that can potentially influence recipient immunity and its regulation (e.g. age, leucocyte content, etc.). Understanding how these varied parameters culminate in an immune response or not is fundamental to developing safer blood products and better care for recipients.

Many prospective randomized studies have shown that leucoreduction of blood components can reduce the incidence of alloimmunization and platelet refractoriness [3]. Mainly based on these data, leucoreduced components are now recommended for any patient requiring long-term transfusion support such as patients with leukaemia, lymphoma, myelodysplasia or those undergoing stem cell transplantation. Although leucoreduction has shown a clear benefit by reducing the incidence of alloimmunization and refractoriness in these immunocompromised patient groups, it is not clear how leucoreduced blood products may affect the immune response in immunocompetent individuals such as trauma or surgical patients. However, in a recent randomized controlled study with 404 cardiac surgery patients, a single multiunit transfusion of either leucofiltered RBC (stored or not) or buffy coat-reduced RBC induced similar levels of anti-HLA alloimmunization [4]. These results suggest that not all patient groups may benefit from leucoreduced blood products and other treatment modalities may need to be developed to reduce alloimmunization in those patients. Understanding the immune response against leucoreduced blood products in healthy recipients can be accomplished by studying animal models of transfusion. This paper will focus on how, in healthy recipients, leukoreduction may have a 'double-edged sword' effect on alloimmune mechanisms.

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Alloimmunity

Alloimmunity is defined as a recipient's immune response against tissues from a genetically dissimilar donor. It is usually measured by testing the generation of either cytotoxic T cells or antidonor antibodies [5]. These effector alloimmune responses are primarily responsible for the increased rejection or destruction of transplanted/transfused allogeneic tissues or cells, respectively. Two recipient T-cell allorecognition mechanisms are critical for the initiation of alloimmunity. The direct allorecognition pathway occurs when recipient T-helper cells directly interact with class II molecules encoded by the major histocompatibility complex (MHC) on donor antigen presenting cells (APC). This pathway is the strongest known stimulator of immunity and is the one targeted to be removed by leucoreduction strategies. The second pathway called indirect allorecognition occurs when allogeneic proteins are administered to a recipient and involves the processing and presentation of allelic donor antigens (e.g. MHC class I molecules) by recipient APC to recipient T-helper cells [6]. This pathway is approximately 100-fold less potent in stimulating alloimmunity compared with the direct pathway, nonetheless, this latter pathway is capable of generating powerful immune responses against the donor tissue.

Murine models that address leucocyte modulation of alloimmunity

Most animal studies using whole blood transfusions have found immunosuppressive-like responses in recipients and these are primarily due to the leucocyte content of the donor blood [1,7]. With respect to components such as leucoreduced platelets, it also appears that the leucocyte content of the product is critical to modulating immunity [7]. For example, Claas *et al.* [8] showed that allogeneic platelets could induce IgG alloantibody formation only if at least 10^3 contaminating leucocytes were present. Based on blood volumes, this dose

in mice translates to an approximate dose in humans of $2{\cdot}5 \times 10^6$ leucocytes per transfusion. Clinical studies in leukaemic patients subsequently suggested that the minimal threshold of leucocyte contamination in blood products to prevent alloimmunization should be less than $1–5 \times 10^6$ leucocytes [3]. However, several reports subsequently reported that in healthy mice, rats and humans transfused with leucoreduced allogeneic platelets, leucocyte levels as low as 1/μl (approximately 3×10^5 per transfusion) not only activated recipient T cells [9] but also stimulated IgG antidonor alloantibody responses [10–12].

In 1998, Kao *et al.* [13] demonstrated that when MHC class II positive APC were depleted from murine peripheral blood mononuclear cells and 10^5 depleted cells were transfused weekly into fully allogeneic recipients, the anti-MHC class I antibody response was significantly reduced. This suggests that when relatively small doses of MHC class I molecules are transfused, direct allorecognition of MHC class II + leucocytes may be necessary to enhance the recipient alloimmune response. However, this may not be the case for transfused allogeneic platelets since they themselves deliver a significantly higher dose of donor MHC class I molecules compared with leucocyte transfusions. This exposes the recipient to high doses of MHC class I molecules that can significantly stimulate alloimmunity via indirect allorecognition. We addressed this by using mice with severe combined immunodeficiency (SCID) as platelet donors. SCID mice do not have circulating T or B cells and thus platelets prepared from their plasma can be consistently rendered extremely leucoreduced (< 0·05 WBC/μl) [14]. The SCID mouse platelets expressed high levels of MHC class I molecules and despite undetectable MHC class II positive APC, they were significantly more immunogenic than control platelets containing 1 leucocyte/μl when transfused into allogeneic CBA recipients. Thus, as the class II positive APC numbers are lowered within the platelet product, a biphasic anti-MHC class I antibody response is observed (Fig. 1). This response pattern suggests that leucoreduction may be an active process in that it produces a dose of MHC class II positive APC that can suppress the antibody response against platelets. When these cells are totally depleted, the immunosuppression is apparently relieved and indirect allorecognition of donor platelet antigens proceeds unchecked. The recipient immune mechanism(s) responsible for this WBC-induced reduction of platelet immunity are unknown but may include the long-term engraftment of donor-derived haematopoietic cells (microchimerism), a limited sublethal graft-vs.-host reaction or the transfer of potentially tolerogenic costimulatory molecule-deficient APC resulting in operational tolerance.

To further test how direct allorecognition of donor leucocytes affects platelet MHC class I immunity, we first pulsed recipient BALB/c APC with donor C57BL/6 platelets *in vitro* to allow for uptake and processing (the indirect pathway) and then transfused them into BALB/c recipients together with varying numbers of intact donor MHC class II positive APC. Similar to the experiments with platelets derived from SCID mice, the donor leucocytes significantly reduced the pulsed APC immunity in a dose dependent manor (J. W. Semple, unpublished). These results confirmed that during leucoreduced platelet transfusions, the direct allorecognition pathway against donor MHC class II positive APC significantly affects the indirect pathway of platelet antigen processing and presentation. This suggests that small numbers of donor leucocytes within leukoreduced platelet concentrates actually suppress immunity directed towards the MHC class I positive platelets.

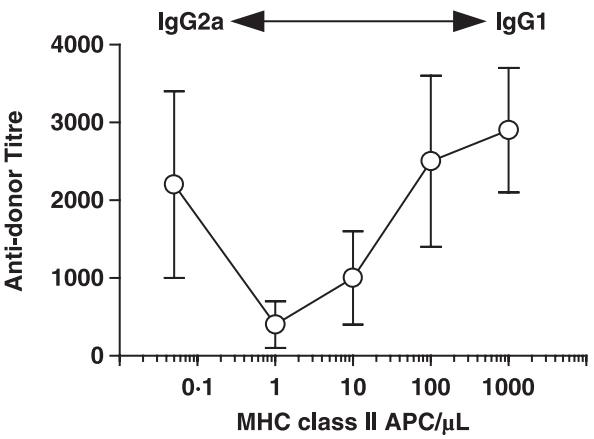

Fig. 1 Relationship between MHC class II APC content in platelets and their immunogenicity. APC were titrated into stock platelets from SCID mice and transfused into CBA recipients weekly and IgG antidonor antibodies were measured by flow cytometry. The data is expressed as the mean ± SD of week 5 titres from 10 recipient CBA mice. The arrow at the top represents the shift in IgG antidonor isotype production dependent on the APC content.

In summary, murine models of leucoreduced platelet immunity support the notion that leucoreduction significantly reduces alloimmunization against platelets. What the animal models additionally suggest is that in recipients with a healthy immune system, leucoreduction may not be effective in reducing immunity against platelet-derived alloantigens because normal immunity (indirect allorecognition) is intact. Understanding the nature of this leucocyte-dependent regulation of platelet humoral immunity may be fundamental to designing more effective antigen-specific immunotherapies for those patients who can respond to platelet antigens.

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