Recipient antigen-processing pathways of allogeneic platelet antigens: essential mediators of immunity

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Clinical studies have convincingly demonstrated that WBC reduction of blood components can reduce the incidence of primary HLA antigen alloimmunization at least in patients with acute myeloid leukemia.[1] However, despite receiving WBC-reduced platelets, approximately 19 percent of acute myeloid leukemia patients still became alloimmunized.[1] In addition, it is not known whether WBC reduction will prevent HLA antigen alloimmunization among those recipients who have an intact immune system (e.g., general surgery patients). The biologic mechanisms responsible for immune stimulation by blood components are still incompletely understood but are probably related to both donor and recipient factors. Animal models have proven indispensable for elucidating the basic mechanisms of immunity against foreign antigens and several such models have been developed to study various aspects of transfusion immunobiology.[2-4] Our laboratory has developed a murine transfusion model that has allowed us to focus on recipient factors affecting the immune response to WBC-reduced platelets. This article will describe how the murine immune response to platelet transfusion is critically dependent on recipient antigen-processing and-presentation pathways and how these pathways can be exploited to modulate immunization against WBC-reduced platelets.

ABBREVIATIONS: APC(s) = antigen-presenting cell(s); ER = endoplasmic reticulum; iNOS = inducible nitric oxide synthase; NO = nitric oxide; Th = T helper cell; Th1 = T helper cell 1; Th2 = T helper cell 2.

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BACKGROUND

Alloimmunity is defined as a recipient's immune response against tissues from a genetically dissimilar individual or donor from the same species.[5,6] It can be measured by monitoring either the generation of cytotoxic T cells or the development of IgG against donor antigens.[6] These effector alloimmune responses are primarily responsible for the increased rejection or destruction of transplanted or transfused cells, respectively. Two recipient T cell recognition mechanisms have been shown to be critical for the initiation of alloimmunity.[7] The direct pathway occurs when recipient T-helper (Th) cells directly interact with MHC molecules on donor antigen-presenting cells (APCs).[7] The indirect recognition pathway, on the other hand, is analogous to the normal immune response.[7] Indirect recognition occurs when allogeneic donor molecules are processed by recipient APCs and presented to recipient Th cells. Within the context of indirect allorecognition, interactions between donor antigen and recipient APCs are critical to T-cell activation and subsequent antibody formation.[5,7]

T cells recognize protein antigens that are degraded or processed within APCs and combined with molecules encoded by the MHC.[8] Antigen processing is critical for generating protein determinants, which can be loaded and bound within the antigen-binding grooves of either MHC class I or MHC class II molecules.[8] The spectrum of antigen processing ranges from the simple unfolding of conformational determinants to the proteolytic exposure of primary structure by pH-dependent enzymes (e.g., cathepsins).[8] Generally, APCs process exogenously derived proteins via endosomal compartments that shunt the processed peptides to intracellular compartments rich in MHC class II molecules.[8] This pathway is necessary for the activation of CD4+ Th cells and help for eventual IgG antibody production.[8] Endogenous APCs proteins (e.g., virally derived proteins), in contrast, are generally processed by large-molecular-weight proteosomes within the cytosol of the APCs and are subsequently transported

to the luminal surface of the endoplasmic reticulum (ER) for loading onto MHC class I molecules.[8] This pathway is responsible for the stimulation of CD8+ T cells.[8] For both pathways of processing, the loaded MHC molecules are subsequently expressed on the surface of the APC and are available for presentation to circulating T cells. If a T cell has a receptor with sufficient affinity for the peptide-MHC combination (first signal) and various costimulatory (second signal) events are met, the T cell will be activated and differentiate into an effector cell.[9] Cytokines such as IL-2, IL-4, and IFN-α secreted from the activated Th cells stimulate donor MHC class I-primed B cells to differentiate into plasma cells that ultimately secrete IgG antibodies.[9] Experimentally, the major distinction between the MHC class II- and class I-processing pathways has been that exogenous antigen processing is generally susceptible to pH-raising lysomotrophic agents such as chloroquine, whereas the endogenous antigen pathway is not.[8] Understanding antigen-processing pathways of clinically relevant protein antigens (such as HLA antigens carried on platelets) may be fundamental to developing efficacious antigen-specific therapies for alloimmunization.

THE INDIRECT RECOGNITION PATHWAY AND THE IMMUNE RESPONSE TO PLATELET TRANSFUSIONS

Indirect allorecognition is critically involved when a recipient mounts an IgG alloimmune response against HLA antigens on transfused platelets.[10] In the murine circulation, platelets harbor the greatest amount of MHC class I molecules and approximately two-thirds of these platelet molecules are adsorbed from the plasma (JW Semple, unpublished observations). Thus, when 10^8 C57BL/6 mouse platelets are transfused into allogeneic BALB/c recipient mice, the host is bombarded with a huge dose of donor MHC class I molecules and IgG MHC class I antibodies to the donor antigen can be identified in all the recipients by five weekly transfusions.[10] These data suggested that platelet-derived MHC class I molecules, whether soluble or associated with the platelet, are pinocytosed or phagocytosed, respectively, by cells of the reticulendothelial system. These initial uptake mechanisms would allow for recipient splenic macrophages and dendritic cells to potentially act as APCs that can stimulate the host's adaptive immune system.

The immune response to allogeneic platelets in BALB/c mice was found to be dependent upon the recipient's ability to produce the diffusible diatomic gas nitric oxide (NO).[11] BALB/c mice treated with aminoguanidine, a selective inhibitor of inducible nitric oxide synthase (iNOS), were incapable of mounting an IgG alloimmune response against transfused allogeneic WBC-reduced platelets.[11] This suggested that NO production was a critical recipient factor responsible for platelet alloimmunization. To study the processing pathways in recipient APCs, we developed an in vitro system in which adherent APCs from recipient BALB/c mice were pulsed with allogeneic donor C57BL/6 platelets and then transfused weekly into naïve BALB/c mice.[12] Our results demonstrated that in vitro APCs pulsing could induce the production of IgG antibodies by the second transfusion. The antibodies to donor antigen were found to be primarily composed of the murine isotypes IgG1 and IgG2a and their specificity was found to be against donor MHC class I molecules.[12] These results confirmed that the in vitro platelet pulsing step could mimic the indirect recognition of allogeneic platelet MHC antigens. It appeared that the transfused platelet-pulsed APCs acted to stimulate recipient CD4+ Th cells in vivo whereas the small amount of contaminating donor platelets (a source of intact MHC class I molecules) within the pulsed APC preparations primed recipient MHC class I-specific B cells for the T-cell help.

PROCESSING MHC ANTIGENS ON PLATELETS AND THE GENERATION ANTIBODIES TO DONOR ANTIGENS

By use of various metabolic processing inhibitors during the in vitro pulsing period, we could dissect which processing pathways were responsible for the antibody production to donor antigen. When aminoguanidine was added to the pulse, it was found that iNOS activation within the pulsed APC was essential for generating the IgG antibody isotypes to donor antigen.[12] Although the exact nature of the iNOS effect is unknown, NO can significantly promote F-actin rearrangements and intracellular membrane movements,[13] which could physically shunt platelet antigens to sites rich in MHC molecules. Preliminary data from our laboratory suggest that this may be the mechanism of action of aminoguanidine-mediated inhibition of platelet immunity (J.S., unpublished observations). Taken together, this murine model supports the concept that manipulating NO levels within recipient APCs may be an effective immunotherapy for the total reduction of alloimmunization against WBC-reduced platelets.

In addition to the above results, we discovered that pathways of platelet antigen processing were not only responsible for antibody production to donor antigen, but also actively controlled the amount and isotype profiles of the antibodies produced.[12] For example, when the lysomotrophic agent chloroquine was used during the in vitro platelet pulsing period, the production of Th1 [T helper-cell 1]-associated IgG2a antibodies was significantly elevated while Th2 [T helper cell 2]-associated IgG1 isotypes were suppressed.[9] Cytokine analyses of recipient sera confirmed that the chloroquine-treated APCs

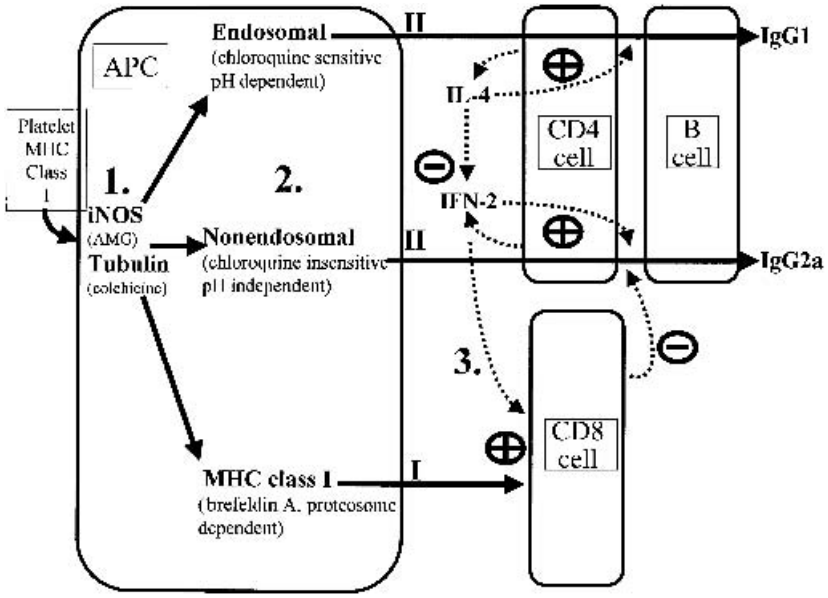

Fig. 1. Model of how donor platelet MHC molecules are processed and how the processing can stimulate and regulate the development of antibodies against the donor. 1) Tubulin polymerization and stimulation of iNOS within the recipient APC are critical requirements for the initiation of platelet antigen processing and eventual immunity. Without these events, the recipient APC cannot initiate the immune response. 2) The iNOS-exposed platelet molecules are shunted to endosomal-, nonendosomal-, or MHC class I-processing pathways. 3) These latter three processing pathways can regulate the strength and isotype of the antibody response to the donor at two levels at least. Endosomal processing, inhibits (–) nonendosomal-dependent production of IFN, which ultimately lowers IgG2a production and the overall antibody response. MHC class I processing can activate (+) recipient CD8+ T cells, which can also negatively regulate nonendosomal-dependent IgG2a production.

reduced IL-4 production while enhancing IFN-γ levels.[12] Thus, it appears that pH-dependent endosomal processing of antigens found on transfused platelets is responsible for Th2-associated antibody responses whereas a nonendosomal pathway is responsible for Th1-related antibodies. The opposing nature of Th1 and Th2 cytokines may explain how endosomal processing of platelet MHC class I molecules counteracts the enhanced IgG2a antibody synthesis by the nonendosomal pathway (Fig. 1). Our data demonstrate an unusual circumstance where an exogenous protein antigen (e.g., donor platelet MHC class I molecules) can be handled by an APC for subsequent MHC class II-dependent antibody production without the requirement for endosomal processing.

To further study the nature of nonendosomal processing of platelet alloantigens, we pulsed the APCs in the presence of either brefeldin A (to inhibit nascent protein transport between the ER and Golgi) or proteosome inhibitors (to inhibit cytosolic processing). Inhibition of these MHC class I-processing pathways similarly suppressed IgG1 synthesis and significantly enhanced IgG2a production.[9] These results suggested the intriguing possibility that processing pathways responsible for the activation of CD8+ T cells are also responsible for normally suppressing Th1-associated platelet antibody production. Recent results from our laboratory by use of recipients depleted of CD8+ cells has confirmed that these cells are intimately involved with the regulation of IgG immune responses directed against platelets.[14] Table 1 summarizes the inhibition studies with respect to IgG production. These findings have allowed us to develop a working model of the platelet processing machinery within recipient APCs and how these pathways modulate donor antibody production (Fig. 1).

In summary, allogeneic platelet antigens have unique antigen-processing requirements to stimulate MHC class I antibody formation against the donor. Platelet-derived MHC class I molecules first need exposure to iNOS activation and then utilize one of three potential intracellular routes: endosomal-, nonendosomal-, or MHC class I-processing pathways. These processing pathways most probably generate peptides that can stimulate and/or regulate recipient T-cell activation and eventual IgG alloantibody formation. It appears that nonendosomal processing is the major stimulatory pathway for Th1-associated IgG2a production whereas endosomal processing counteracts this production by generating Th2-associated cytokines and IgG1 antibodies. Furthermore, the MHC class I-processing machinery allows the APCs to interact with CD8+ T cells that additionally counteract nonendosomal

TABLE 1. Summary of the effects of inhibitors on platelet-pulsed APC immunity*

Inhibitor used (effect)	IgG anti-donor production
None	IgG1, IgG2a
Colchicine (tubulin polymerization)	↓IgG1, ↓IgG2a
Aminoguanidine (iNOS inhibition)	↓IgG1, ↓IgG2a
Chloroquine (raises lysosomal pH)	↓IgG1, ↑IgG2a
Brefeldin A (ER-Golgi protein transport)	↓IgG1, ↑IgG2a
MG115 (proteosome inhibitor)	↓IgG1, ↑IgG2a

* Recipient adherent APC were pulsed with donor platelets for 18 h at 37°C in the presence of the indicated inhibitor, washed, and transfused into naïve recipient mice weekly. Sera were tested for the presence of IgG anti-donor isotypes by flow cytometry as described by Bang et al.[12]

Th1-associated immunity against platelet antigens. Thus, these data support the concept that antigen-processing pathways can be targeted for specific immunotherapies designed to further reduce the alloimmune response to transfused WBC-reduced platelets.

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