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Platelets Present Antigen in the Context of MHC Class I

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Platelets are numerous (~200,000/µl in humans), anucleate, megakaryocyte-derived, multifunctional blood cells. They are the primary cellular mediator of hemostasis, but platelets also have important immune roles. When activated, platelets secrete preformed and synthesized immune mediators, and initiate interactions with quiescent leukocytes, making platelets the most numerous circulating cell with an immune function (1–4). Most of what is known about platelet–leukocyte interactions has focused on platelet interactions with monocytes and neutrophils, but platelets also have a role in T cell responses. In ischemia-reperfusion studies, platelets augment the recruitment of T cells to liver sinusoids (5), and activated platelets enhance intrahepatic accumulation of CD8+ T cells in viral hepatitis (6). Our studies using the experimental cerebral malaria (ECM) mouse model have established that the platelet-derived chemokine PF4 (CXCL4) increases T cell CXCR3 expression and T cell trafficking to the brain (7). Platelet and T cell interactions are also important in amplifying acquired immune responses. For example, platelets are a major source of CD154 (CD40L), and platelet-derived CD154 assists in germinal center development, Ig isotype switching, and in driving allograft rejection (8–10).

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We now report that platelets directly present Ag to T cells and stimulate naive T cell responses in the context of MHC class I (MHCI). Although platelets have not been previously associated with processing or presenting Ag, they are known to express many of the molecules necessary for these functions. Platelets have an active proteasome, an endoplasmic reticulum (ER) and Golgi, TAP, calnexin, calreticulin, and ERP57 (11–15). mRNA for MHCI subunits such as β2-microglobulin (β2M) and HLA are also highly expressed in platelets (15). In addition, T cell costimulatory and adhesion molecules associated with APCs are also present on platelets, including CD40, CD44, ICAM-2, and DC-SIGN (16–19), further indicating a potential Ag-presenting role for platelets.

Cerebral malaria (CM) is a severe complication of Plasmodium falciparum infection. More than 1 million people die each year of malaria, and 90% of the deaths occur in children (20). The pathogenesis of CM is the result of a combination of vascular and immune system dysfunction, with autopsy specimens typified by multifocal cerebral capillary obstruction with infected RBCs (iRBCs), platelets, and leukocytes (21). Our laboratory and others have demonstrated that platelets adversely impact the development of ECM by accelerating and supporting its disease course (7, 22–26). We have shown in mice infected with the CM-causing parasite P. berghei that platelet activation occurs in this disease model before any signs of illness, and that platelet-derived inflammatory molecules (such as PF4) contribute significantly to the immune responses leading to ECM (7, 26). CD8+ T cells are necessary for the brain vascular damage seen in ECM (27, 28). Despite its fairly rapid clinical course, there is direct evidence using OVA-transgenic (Tg) P. berghei that blood-stage infection leads to Ag-specific CD8+ T cell infiltration of the brain by day 5 postinfection (29). Using the ECM model, we now have evidence that platelets directly activate naive T cells through the ability of platelets to present Ag in the context of MHCI.

Materials and Methods

Reagents

Abs against MHCI and MHCI, CD40, CD86, CD80, ICOS ligand (ICOSL), MHCI–OVA complex, CD8, CD3, CD38, and CD69, and appropriate isotype controls were purchased from eBiosciences. Ova-pentamer was purchased from ProImmune. All ELISAs were purchased from R&D Systems. SIINFEKL peptide was purchased from Bachem. Platelet-specific Abs were purchased from a second party. Platelet-depleting and control IgG Abs were purchased from BioLegend.
OVA, PGE2, and thrombin were purchased from Sigma and U46619 from Tocris. DQ-OVA was purchased from Invitrogen.

**Procedures**

All mice used were on a C57Bl6/J background and purchased from Jackson Labs. Platelets from mice and humans were isolated as we have described elsewhere (30–32) using University of Rochester School of Medicine-approved animal and human protocols. For all experiments using mouse platelets, platelets were prepared as washed platelets as we have published previously (31), and resuspended in calcium- and magnesium-free Tyrodes solution. Human platelet-rich plasma was isolated and resuspended in Tyrodes solution at a 1:20 ratio as we have described previously (31, 32). Platelets were incubated with Abs for 20–30 min at room temperature and fixed with 2% formalin before flow cytometry using a BDFacs Canto instrument and analyzed using FlowJo software.

Mouse T cells from spleens and lymph nodes were isolated using a negative selection T cell enrichment kit from Stem Cell Technology. Plates were coated with anti-CD3 Ab before plating T cells and incubating in RPMI 1640 with 5% FBS and penicillin/streptomycin. T cells were incubated with washed platelets in an approximately physiologic ratio of 1:20. In vivo platelet T cell activation assay used washed mouse platelets injected i.v. via the retro-orbital plexus into recipient mice.

In *P. berghei* infections, mice were injected i.p. with ∼500,000 infected mouse RBCs. Mice were platelet depleted by an i.p. injection with 50 mg platelet-depleting Ab or control IgG. This Ab in our experience greatly depresses platelet counts for ∼4 d (33). All mouse plasma was collected by bleeding into EDTA-coated tubes and centrifuging at 2500 g for 10 min.

In platelet vaccine studies, OVA peptide or control PBS was added to washed platelets; then platelets were stimulated with thrombin and incubated for 2 h. Platelets were then washed in the presence of PGE2 and resuspended in Tyrodes buffer before 1 × 10⁷ platelets in a volume of 100 µl were injected into wild-type (WT) mice via the retro-orbital plexus. Parasitemia was measured by isolating RBCs and incubating with Sybr Green to label iRBCs, and the number was determined by flow cytometry using described methods (34) and confirmed by Giemsa-stained blood smears. The number of OVA-specific T cells was determined by flow cytometry using splenocytes with Ab to CD8 and a Pentamer for OVA-specific TCRs.

All statistics are shown as SD, and *p* values were determined by a standard Student *t* test.

**Results**

Platelets express molecules for Ag presentation and T cell costimulation

Platelets possess the molecular machinery needed to process and present Ag to CD8+ T cells. We first confirmed that platelets express MHCI by flow cytometry comparing WT and MHCI-deficient platelets from β2M−/− mice. WT platelets express MHCI (Fig. 1A). Although platelets have been noted to express MHCII in some disease states (35, 36), under normal conditions, platelets do not express MHCII (Supplemental Fig. 1). Other molecules needed for MHCI Ag presentation, including TAP1 and ERp57, are also present in platelets (Supplemental Fig. 2). Not only do platelets express MHCI, they also express T cell costimulatory molecules. Others have described the presence of CD40 on platelets (37). We have confirmed its presence and found that CD40 expression does not increase significantly with platelet activation in response to thrombin receptor-activating peptide.

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**FIGURE 1.** Platelets express Ag presentation molecules. (A) MHCI expression. Platelets from β2M−/− and WT mice (n = 4 ± SD, *p < 0.01 versus β2M−/−). (B) CD40. Resting or activated human platelets (2 μM thrombin receptor-activating peptide or U46619; n = 4 ± SD, *p < 0.01 versus IgG). (C) CD86. Human platelets express CD86, which is increased with stimulation. (D) CD86 quantification (n = 4 ± SD, *p < 0.05, **p < 0.01 versus IgG). (E) Mouse platelets do not express CD86 (n = 4 ± SD). (F) ICOSL. Mouse platelets were incubated with control PBS or activated with thrombin before addition of control IgG or anti-ICOSL Ab (n = 4 ± SD, *p < 0.05). MFI, Mean fluorescent intensity.
μM) or the thromboxane receptor agonist U46619 (2 μM; Fig. 1B, human platelets; mouse platelets, Supplemental Fig. 3). CD86 (B7.2) is a ligand for T cell CD28. Human platelets express CD86, and CD86 surface expression increases slightly with platelet activation (Fig. 1C, 1D). However, mouse platelets do not express CD86 (Fig. 1E), and neither mouse nor human platelets express CD80 (B7.1, data not shown). Other costimulatory molecules, such as ICOSL, are also expressed by both human and mouse platelets (Fig. 1F, mouse platelet example). Taken together, these data demonstrate that platelets express the molecular machinery necessary to activate naive T cells.

Because platelets express T cell costimulation molecules, we next determined whether platelets provide costimulation signals to T cells (signal 2). Mouse T cells were incubated overnight with control buffer, platelets only, or anti-CD3 Ab only (signal 1) as negative controls. As a positive control, T cells were incubated with both anti-CD3 and anti-CD28 Ab. T cells were also incubated with anti-CD3 Ab and platelets. As expected, T cells incubated with anti-CD3 and anti-CD28 produced IL-2 and had increased CD69 expression (Fig. 2A, 2B). T cells incubated with anti-CD3 and platelets also had significantly increased IL-2 production and CD69 expression as compared with CD3 alone (Fig. 2A, 2B). These data demonstrate that platelets are able to provide T cell costimulation.

Platelets can present Ag

Platelets can be infected with viruses and bacteria including HIV, dengue virus, and *Staphylococcus* (38–40). Platelets have also been shown to be infected with intact *Plasmodium* and contain structures suggestive of degraded parasite material in electron microscopy (41, 42). Platelets are phagocytic, taking up molecules and proteins from their extracellular environment, and have active proteasomes (43–47). This raises the possibility that platelets may be able to acquire and present Ag to T cells. To determine whether platelets present Ag, we incubated platelets from C57Bl6/J (B6) mice with the OVA octapeptide (SIINFEKL) presented by B6 mouse MHCI haplotype H-2Kb. Resting and thrombin (0.1 U/ml)-stimulated platelets were incubated with buffer or OVA peptide, and MHCI-SIINFEKL was measured by flow cytometry using an Ab that recognizes this specific complex. Resting platelets did not have an increase in MHCI-SIINFEKL, but activated platelets incubated with OVA peptide had a large increase in OVA peptide presentation (Fig. 3A, 3B). Platelets were also mildly stimulated with thrombin in the presence of control buffer or full OVA protein and OVA-MHCI determined. Platelets presented OVA-MHCI from OVA protein (Fig. 3C), and this was greatly reduced by pretreatment of platelets with brefeldin A (Supplemental Fig. 4), indicating that they can process and present Ag from full-length protein. These data show that platelets may have the ability to take up antigenic proteins and present Ag in MHCI.

OVA-Tg mice present OVA Ag complex in MHCI. If platelets can present Ag to T cells, platelets from OVA-Tg mice are pre-
dicted to activate T cells expressing receptors specific for OVA-MHCI. OT-I mice are Tg for TCRs recognizing OVA residues 257–264 in the context of B6 mouse MHCI. To provide an experimental proof of principle that platelets can present Ag to T cells, we incubated WT or OT-I T cells with buffer, platelets from WT mice, or with platelets from OVA-Tg mice. IL-2 and IFN-γ production were measured 24 and 48 h later, respectively. OT-I T cells incubated with platelets from OVA-Tg mice produced greatly increased IL-2 and INF-γ compared with OT-1 T cells and WT platelets (Fig. 4A, 4B). These data demonstrate that platelets present Ag and activate T cells in vitro.

To determine whether platelets are a source of Ag in vivo, we i.v. injected OT-I mice with $1 \times 10^5$ WT or OVA-Tg platelets, and as a control, OVA-Tg platelets were injected into WT mice. Plasma IL-2 was measured 48 h later. Only OT-I mice given OVA-Tg platelets had evidence of T cell stimulation in vivo (Fig. 4C). These data indicate that platelets present Ag to T cells in vitro and are a source of Ag in vivo.

**Platelets promote T cell activation in ECM**

CM is a severe complication of infection with *P. falciparum*, and its pathogenesis is in part a CD8+ T cell-dependent process (26, 28, 48, 49). Platelets also have an important role in initiating and sustaining the pathogenesis of ECM (21, 50). To determine whether platelets have a role in ECM-associated T cell responses, we treated mice with a platelet-depleting Ab that greatly reduces platelet counts for $\sim 4$ d (33) or control IgG 24 h after *P. berghei* infection and measured plasma IL-2 and IFN-γ on day 4 postinfection. Platelet-depleted mice had greatly reduced plasma IL-2 and IFN-γ compared with control IgG-treated mice (Fig. 5A, 5B). These data support our hypothesis that platelets have an in vivo role in *P. berghei* T cell responses.

Nonprofessional APCs typically increase MHCI expression to facilitate their role in Ag presentation. To determine whether platelets increase MHCI as part of an active role in ECM, we isolated platelets from uninfected control mice and *P. berghei*-infected mice on days 4 and 6 postinfection, and measured platelet MHCI expression by flow cytometry. Four days postinfection, MHCI expression was only slightly increased (data not shown); however, by day 6, there was a population of platelets that expressed greatly increased levels of MHCI we have called MHCI$^{\text{hi}}$ platelets (Fig. 5C, 5D). These data demonstrate that platelets increase MHCI expression during infection.

To more directly demonstrate a role for platelets in the presentation of *Plasmodium* Ag to T cells, we infected MHCI-deficient β2M$^{-/-}$ mice with a *P. berghei* parasite Tg for the C-terminal amino acids 150–386 of OVA (*Plasmodium berghei* ANKA-OVA (PbBA-OVA)) (51). On day 5 postinfection, iRBCs were isolated and incubated with OT-1 T cells alone, with WT platelets, or with β2M$^{-/-}$ platelets (note that we used iRBCs from β2M$^{-/-}$ PbBA-OVA mice to eliminate the potential for contaminating Ag-presenting leukocytes contributing to Ag presentation). T cell stimulation was determined 48 h later by measuring CD25 expression using flow cytometry and IL-2 production by ELISA. OT-I T cells incubated with iRBCs and WT platelets, but not β2M$^{-/-}$ platelets, had significantly increased CD25 expression and IL-2 production (Fig. 6A, 6B). These data demonstrate that platelets can acquire and present parasite-derived Ag to T cells in a platelet MHCI-dependent manner.

To explore whether platelets present *Plasmodium*-derived Ag in vivo, we isolated platelets from uninfected mice and mice infected with PbBA-OVA to determine platelet MHCI–OVA complex expression on days 3, 4, and 6 postinfection. Beginning 4 d postinfection, platelets from PbBA-OVA-infected mice had increased MHCI–OVA complex expression (Fig. 6C).

**Platelets can be used to induce protective T cell responses**

Ag-pulsed dendritic cells (DCs) have been used in clinical trials to induce protective T cell responses for the treatment of cancer and HIV (52, 53). Because platelets can present Ag, they may represent an alternative or adjunctive platform for cell-based vaccines. To test this concept using the ECM model, we incubated washed WT platelets with β2M$^{-/-}$ platelets with OVA peptide (300 μg/ml), activated the platelets with mild thrombin stimulation (0.2 U/ml), and incubated for 2 h. Platelets were then washed, and $1 \times 10^7$ platelets were injected i.v. into WT mice. This was repeated 10 d later, and 21 d after the first platelet injection. WT platelet/OVA, β2M$^{-/-}$/platelet/OVA, and untreated control mice were infected with PbBA-OVA. WT platelet/OVA mice also had greatly improved survival compared with β2M$^{-/-}$ and control mice (Fig. 7A), indicating that the platelet vaccine strategy was protective against the development of ECM in a platelet MHCI-dependent manner.
dependent manner. Mice that received the WT platelet/OVA vaccine also had reduced parasite burdens (\% iRBCs; Fig. 7B, 7C), indicating these mice were able to mount an immune response that minimized parasite expansion. A separate group of mice was sacrificed on day 4 postinfection, and OVA-specific CD8+ T cells in the spleen were quantified using an OVA MHCI pentamer. As expected, PbA-OVA–infected mice that received no platelet treatment (untreated) had few OVA-specific T cells (Fig. 7D). However, mice treated with the WT platelet/OVA regimen had a greatly increased number of OVA-specific T cells compared with mice treated with the \(\beta_2M^{-/-}\) platelet OVA (Fig. 7D), demonstrating that platelets induced a specific T cell response in a manner dependent on platelet MHCI. These data indicate that platelets can be used to present Ag to naive T cells and induce protective T cell responses in a platelet MHCI-dependent manner.

**Discussion**

Our results demonstrate that platelets present Ag to T cells in a platelet MHCI-dependent manner, and that platelets acquire and present *Plasmodium*-derived Ag to CD8+ T cells both in vitro and in vivo. This represents an important new concept; platelets not only support and promote acquired immune responses, but platelets may also directly participate in the initiation of acquired immune responses. A platelet Ag presentation role may be important in many blood-borne infections and vascular inflammatory diseases.
Thrombocytopenia and inflammation are associated with malaria, dengue, and HIV, among other infectious diseases. Immune-mediated thrombocytopenia can also be the result of cytotoxic T cell responses to platelets presenting self-Ag. The pathogenesis of infection-related and immune-mediated thrombocytopenias are likely multiple, but may, in part, be driven by platelets presenting Ag and being specifically targeted by CD8+ T cells.

Initiation of innate and acquired immune responses by platelets is a potentially important mechanism to combat infectious agents. Following a breach in the skin, for example, platelets limit bleeding, but they also simultaneously recruit and activate "professional" immune cells to limit skin pathogen dissemination. Platelet presentation of foreign Ag derived from the site of the skin lesion may provide an early means to respond to subsequent Ag exposure. Because platelets are much more numerous than leukocytes and tissue-restricted professional APCs, an Ag presentation function may also provide early blood surveillance to begin Ag-specific responses before more specialized cells become involved. Platelets can be activated by and phagocytose bacteria, and can be infected by numerous types of viruses, perhaps indicating a more active role in Ag presentation than previously considered. There is also growing evidence that platelets may traffic in a regulated manner. Platelets have an ER and Golgi (11), as well as other components necessary for MHCI peptide loading, including TAP, calnexin, calreticulin, and ERp57 (12–14), further supporting the concept that platelets process Ag and regulate MHCI expression. Platelet mRNA expression data have shown that compared with other transcripts, platelets have large amounts of Ag presentation-related mRNA, in particular, b2M and HLA mRNA are highly abundant in platelets (15). Our data, the high mRNA expression, and ability of platelets to upregulate protein expression in the periphery (57) may further indicate the importance of regulating MHCI expression and Ag presentation in platelets. Whether platelets present Ag as a result of platelet infection, or whether platelets present Ag they have taken up by phagocytosis is unclear from our studies and requires much further work to clarify.

Because DCs induce robust T cell responses, cell-based vaccine research has focused on DCs, with cancer and HIV the diseases most often targeted. Mature DCs provide optimal immune stimulation, but immature DCs may tolerize T cells. This means that DCs need to be significantly manipulated in culture before in vivo use. On an individual cell basis, platelets may not induce as robust an immune response as professional APCs, and platelets are limited by their expression of only MHCI. But platelets may offer many other unique advantages for cell-based vaccine development, including that platelets are easy to isolate in large numbers and do not require extensive manipulation and differentiation. Much further study is needed to determine whether platelets can be effective in presenting Plasmodium-restricted Ags for vaccine development.
but our data do not demonstrate the ability of platelets presenting Ag to induce T cell activation in vivo. The WT platelet/OVA vaccinated mice eventually developed an increasing parasite burden after about day 15 postinfection, indicating that the mice were protected from the ECM phase, but the parasite eventually escapes the protection offered by the platelet vaccine. CM protection is an important therapeutic goal, and these results point out the complexity of malaria immune responses and disease pathogenesis. Our data also demonstrate that although CD8+ T cells are deleterious in the late stages of ECM and drive ECM pathogenesis (51, 58, 59), an early, robust CD8 response can be protective. Although our data represent an important novel proof of principle, there is, of course, much work that must be done to better understand the long-term therapeutic potential of our results, and any potential adverse effects. Because platelets are proinflammatory and the cellular mediator of thrombosis, unintended side effects must also be given great study and consideration.

Platelets are dynamic cells and represent an early link between the innate and acquired immune responses in many vascular inflammatory processes, including CM. Thrombosis and vascular inflammation represent a continuum of each other; platelet activation incites an immune response and vascular inflammation leads to platelet activation. Our data extend this important paradigm to platelet Ag presentation in the context of MHCI.

Disclosures

The authors have no financial conflicts of interest.

References


