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HOST RESPONSE AND INFLAMMATION

Platelets Enhance Dendritic Cell Responses against *Staphylococcus aureus* through CD40-CD40L

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ABSTRACT *Staphylococcus aureus* is a major human pathogen that can cause mild to severe life-threatening infections in many tissues and organs. Platelets are known to participate in protection against *S. aureus* by direct killing and by enhancing the activities of neutrophils and macrophages in clearing *S. aureus* infection. Platelets have also been shown to induce monocyte differentiation into dendritic cells and to enhance activation of dendritic cells. Therefore, in the present study, we explored the role of platelets in enhancing bone marrow-derived dendritic cell (BMDC) function against *S. aureus*. We observed a significant increase in dendritic cell phagocytosis and intracellular killing of a methicillin-resistant *Staphylococcus aureus* (MRSA) strain (USA300) by thrombin-activated platelets or their releasates. Enhancement of bacterial uptake and killing by DCs is mediated by platelet-derived CD40L. Coculture of USA300 and BMDCs in the presence of thrombin-activated platelet releasates invokes upregulation of the maturation marker CD80 on DCs and enhanced production of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 12 (IL-12), and IL-6. Overall, these observations support our hypothesis that platelets play a critical role in the host defense against *S. aureus* infection. Platelets stimulate DCs, leading to direct killing of *S. aureus* and enhanced DC maturation, potentially leading to adaptive immune responses against *S. aureus*.

KEYWORDS platelets, dendritic cells, *S. aureus*, CD40L, phagocytosis

Platelets are megakaryocyte-derived anucleate circulating cells, conventionally recognized in the context of thrombosis and hemostasis (1). Beyond their traditional role, accumulating evidence suggests that platelets play a vital role in innate immunity against viral, bacterial, and parasitic pathogens (reviewed in references 2 and 3). Upon activation, platelets release numerous preformed or synthesized inflammatory and immune mediators into the circulation, which provide necessary signals to other immune cells and initiate or augment immune responses (2). Platelets express a wide variety of cell surface molecules that can directly interact with pathogens (pattern recognition receptors [PRRs]) and other immune cells (selectins and integrins), leading to the possibility that platelets can orchestrate innate immune cells to fight against invading pathogens (2).

Platelets are capable of recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns through such PRRs as Toll-like receptors (TLRs), lectin receptors, and sialic acid receptors (4–7). Ligation of PRRs causes a rapid response of platelets, resulting in thrombosis, killing of the pathogen, or recruitment of other innate cells (7, 8). In addition to PRRs, other platelet receptors facilitate interaction with bacterial pathogens; they include complement receptors, FcγRIIa, GPIb, and GPIIb-IIIa. Moreover, many plasma proteins bind to pathogens and can act as scaffolds, leading to platelet activation through such receptors as αIIbβ3 (GPIIb-IIIa) (9), GPVI (10), and others (11). The mechanisms by which platelets interact

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with bacteria can result in direct killing of the pathogen or functional enhancement of other innate cells (macrophages and neutrophils) and adaptive cells (B and T cells).

Platelets enhance the activity of macrophages (12) and neutrophils (13, 14) and can influence dendritic cell (DC) maturation and activation (15). One important mechanism by which platelets can augment leukocyte activities is through release of soluble CD40L (sCD40L, or CD154) (16). CD40L, a member of the tumor necrosis factor (TNF) superfamily, is a type 2 transmembrane protein that can be cleaved to yield a bioactive soluble form (17). In fact, the majority of sCD40L in blood is derived from platelets (18). Activated platelet-derived sCD40L is also capable of stimulating DC maturation and augmenting antigen presentation by DCs. Dendritic cell CD40 ligation by CD40L promotes overexpression of costimulatory molecules on DCs and increases cytokine production by DCs, both of which are associated with DC maturation (15).

Staphylococcus aureus is a well-known Gram-positive opportunistic bacterium that can cause mild to severe infection with significant morbidity and mortality (19). *S. aureus* is able to evade immune attack by utilizing a number of tactics, which result in high pathogenicity of the bacterium (20, 21). More importantly, the emergence of methicillin-resistant *S. aureus* (MRSA) has resulted in serious public health concerns (22, 23). Community-acquired MRSA, especially strain USA300, is of special concern to clinicians and investigators, as it can cause persistent and aggressive infection that can spread systemically and provoke life-threatening complications (24). Therefore, therapeutic approaches that boost the host immune system may resolve the problems posed by *S. aureus* infection.

Even though *S. aureus* is considered an extracellular pathogen, numerous reports suggest that it can enter and survive within nonprofessional phagocytic cells, including fibroblasts (25), keratinocytes (26), epithelial (27) and endothelial (28, 29) cells, osteoblasts (30) enterocytes (31), and possibly platelets (32). Surprisingly, *S. aureus* can also survive and/or replicate in the intracellular compartments of such professional phagocytic cells as neutrophils (33, 34) and macrophages (35, 36). However, very little is known about intracellular survival or killing of *S. aureus* in DCs. Since DCs are important components of both innate and adaptive immunity and we know that platelets can enhance DC maturation, we sought to study the effect of activated platelets on DC function after *S. aureus* infection.

DCs are reported to phagocytose many pathogens, including *S. aureus* (37), *Mycobacterium tuberculosis* (38), and *Aspergillus fumigatus* (39), suggesting a role of DCs in early response to infections. DCs can be stimulated by *S. aureus*, leading to expression of surface activation markers and release of proinflammatory cytokines, resulting in a predominantly Th1-skewed immune response (40). It is reported that DCs do not kill *S. aureus* directly, but depletion of DCs in CD11c-DTR transgenic mice completely abolished interleukin 12 (IL-12) production, resulting in severe inflammation and higher mortality (37). These observations suggest that DCs play an important role by coordinating the inflammatory responses against *S. aureus* infection. We previously reported that platelets provide protection against *S. aureus* infection in mice (41). We also reported that platelets can enhance macrophage phagocytosis of *S. aureus* and restrict intracellular growth of the bacteria (36). With this convincing effect of platelets on macrophage activation, we opted to explore the effect of platelets and platelet releasates on DC activation.

We observed increased uptake of *S. aureus* by DCs in response to activated platelets or platelet releasates. Interestingly, we did not observe any intracellular growth of *S. aureus* in DCs. However, we found significant killing of the bacteria when cocultured with thrombin-activated platelets or releasates. We observed significantly increased expression of the maturation marker CD80 on DCs. We also found enhanced production of proinflammatory cytokines (TNF-α, IL-12, and IL-6) released by DCs when cocultured with bacteria and thrombin-activated platelets or releasates. Finally, we discovered that CD40L is the key component of activated platelet releasates for the enhancement of bacterial uptake and killing by DCs. Overall, our findings on the effect of platelets in DC

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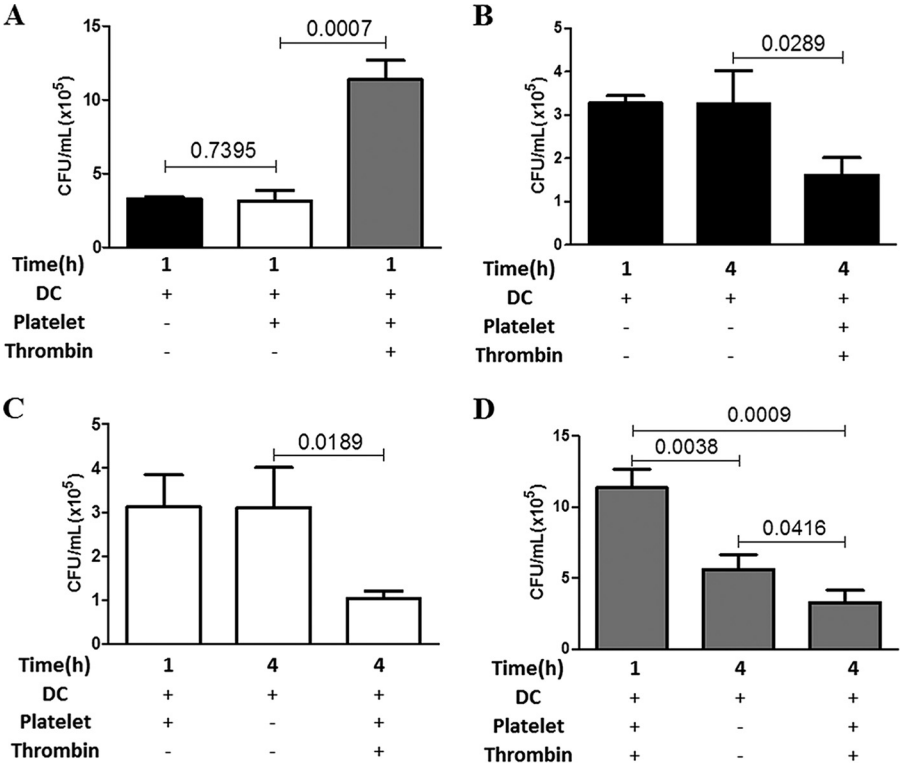

FIG 1 Effect of thrombin-activated platelets on DC phagocytosis and killing of *S. aureus*. BMDCs were incubated with MRSA (at an MOI of 10) in sterile DPBS$^{-/-}$ (black bars) in the presence or absence of unstimulated (white bars) or thrombin-activated (gray bars) platelets for 1 h ($t = 1$). The DCs were washed with DPBS$^{-/-}$, lysed with Triton, and plated on TSA plates to obtain internalized CFU. (A) MRSA CFU obtained from DC lysates ($t = 1$). (B to D) DCs from panel A (bars of corresponding shades) were incubated for a further 3 h ($t = 4$) in the presence or absence of thrombin-activated platelets. Intracellular bacteria were obtained from lysed DCs, and CFU were enumerated. All the values are presented as means and standard deviations (SD) (each experiment was performed in triplicate samples and repeated on at least 3 different days).

activation in response to *S. aureus* support the importance of platelets in the host defense against *S. aureus* infection.

RESULTS

Uptake and intracellular killing of *S. aureus* by BMDCs are enhanced by activated platelets. *S. aureus* is a well-recognized human skin pathogen that can cause infection of almost all tissues and organs (19). A number of reports suggest that neutrophils are critical to clear *S. aureus* infection (42, 43). It has also been observed that platelets enhance neutrophil activities, including neutrophil extracellular-trap production and bacterial killing (13). In our previous study, we reported that platelet-depleted mice succumbed to *S. aureus* (USA300) infection more rapidly than wild-type mice (41). We also discovered that platelet IL-1β enhances macrophage responses against *S. aureus* (36). To assess the role of platelets in dendritic cell uptake and intracellular killing of *S. aureus*, bone marrow-derived dendritic cells (BMDCs) were incubated with USA300 (multiplicity of infection [MOI], 10) for 1 h in the presence or absence of platelets or thrombin-activated platelets prior to lysis. CFU from the lysed cells were enumerated. As shown in Fig. 1A, coculture with activated platelets significantly increased the internalization of MRSA by DCs, as indicated by the number of CFU per milliliter at 1 h. It is also notable that quiescent platelets (without thrombin) did not stimulate DCs to ingest more bacteria.

To test whether platelets can enhance *S. aureus* killing by DCs, BMDCs (Fig. 1A, $t = 1$ h) were cocultured in the absence or presence of thrombin-activated platelets for an additional 3 h (Fig. 1B, $t = 4$ h), and CFU from the lysed cells were enumerated. In the

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absence of platelets, CFU obtained from DCs after 4 h of incubation remained the same as that at 1 h (Fig. 1B and C), suggesting that bacteria survive within DCs but fail to replicate. However, in the presence of thrombin-activated platelets, significant reductions of CFU were observed in all cases (Fig. 1B to D). This observation revealed that activated platelets are able to stimulate DCs to more efficiently kill *S. aureus*. Interestingly, the signals provided by platelets that enhance phagocytosis of MRSA (Fig. 1A, gray bar) also enhance the bacterial-killing capabilities of DCs in the intracellular-killing assay (Fig. 1D), even after the platelets had been washed out (Fig. 1D). However, addition of fresh platelet releasate was able to further improve killing.

Releasates from thrombin-stimulated platelets enhance BMDC uptake and intracellular killing of *S. aureus*. Platelets can directly interact with phagocytes and enhance the uptake of pathogens, thereby contributing to pathogen clearance. In our previous report, we validated the notion hat platelet releasates can enhance macrophage phagocytosis and restrict intracellular growth of *S. aureus* by releasing IL-1β (36). Therefore, we wanted to determine if platelet releasates could enhance DC phagocytosis and killing of *S. aureus*. To do this, parallel experiments were performed as for Fig. 1, along with microscopy to show that bacteria are internalized by DCs (Fig. 2A). We incubated dendritic cells with fluorescein isothiocyanate (FITC)-conjugated anti-*S. aureus* antibody (Ab) at 37°C for 1 h and then with tetramethyl rhodamine isocyanate (TRITC)-conjugated anti-IgG secondary Ab. Afterward, the cells were observed by confocal microscopy to distinguish intracellular ($FITC^+$ $TRITC^-$) from extracellular ($FITC^+$ $TRITC^+$) bacteria. We noticed that most of the *S. aureus* bacteria were $FITC^+$ $TRITC^-$, indicating that they were internalized by DCs (Fig. 2A).

Remarkably, platelet releasates significantly enhanced DC uptake of bacteria by about 46% (Fig. 2B) and induced intracellular killing of *S. aureus* by DCs (Fig. 2C and D). Interestingly, similar to intact platelets, releasates from activated platelets provided sufficient signals to DCs during the phagocytosis assay ($t = 1$ h) to enhance DC killing of *S. aureus* at 4 h (~22%), even in the absence of releasates (Fig. 2D).

Phagocytosis of *S. aureus* by BMDCs is cytoskeleton dependent. Phagocytosis is typically considered a cytoskeleton-dependent process (44). Cytochalasin D, a metabolite known as an actin and myosin depolymerization agent (45, 46), inhibits phagocytosis (47). To test if enhanced phagocytosis of *S. aureus* by DCs was cytoskeleton dependent, we employed cytochalasin D. We incubated DCs in 200 μM cytochalasin D prior to incubating them with thrombin-activated platelet releasates (Dulbecco's phosphate-buffered solution, pH 7, without calcium or magnesium [$DPBS^{-/-}$]) and bacteria. We observed significant decreases (~38%) in the numbers of CFU collected from cytochalasin D-treated DCs. Similarly, platelet releasate-mediated enhancement of DC phagocytosis was also diminished by cytochalasin D, by ~66% (Fig. 2E). These observations suggest that phagocytosis of *S. aureus* by DCs is cytoskeleton dependent.

Platelets enhance *S. aureus* uptake and intracellular killing by BMDCs through CD40L. Activated platelets release several immune mediators capable of moderating leukocyte functions. They release numerous growth factors, microparticles, cytokines, and chemokines (CD40L, RANTES, transforming growth factor β [TGF-β], IL-1β, etc.) (2, 48). In a previous study, we reported that enhancement of macrophage phagocytosis and intracellular growth restriction of *S. aureus* were facilitated by platelet-derived IL-1β. CD40L is an effective bioactive molecule that can mediate DC maturation, T cell activation, and B cell isotype switching (49). CD40L can interact with CD40 of the antigen-presenting cells and trigger inflammatory responses. To explore the mechanism of increased bacterial uptake and killing by platelet-stimulated dendritic cells, we performed phagocytosis and killing assays as described above with platelet releasates, along with replacing the releasates with recombinant sCD40L, IL-1β, or RANTES. Surprisingly, unlike macrophages, uptake of bacteria by dendritic cells was significantly increased (~38%) by the addition of sCD40L (Fig. 3A), but not by IL-1β or RANTES (Fig. 3A). The increased bacterial uptake with sCD40L stimulation is comparable to that with thrombin-activated platelet releasate stimulation (Fig. 3B). Additionally, intracellular-

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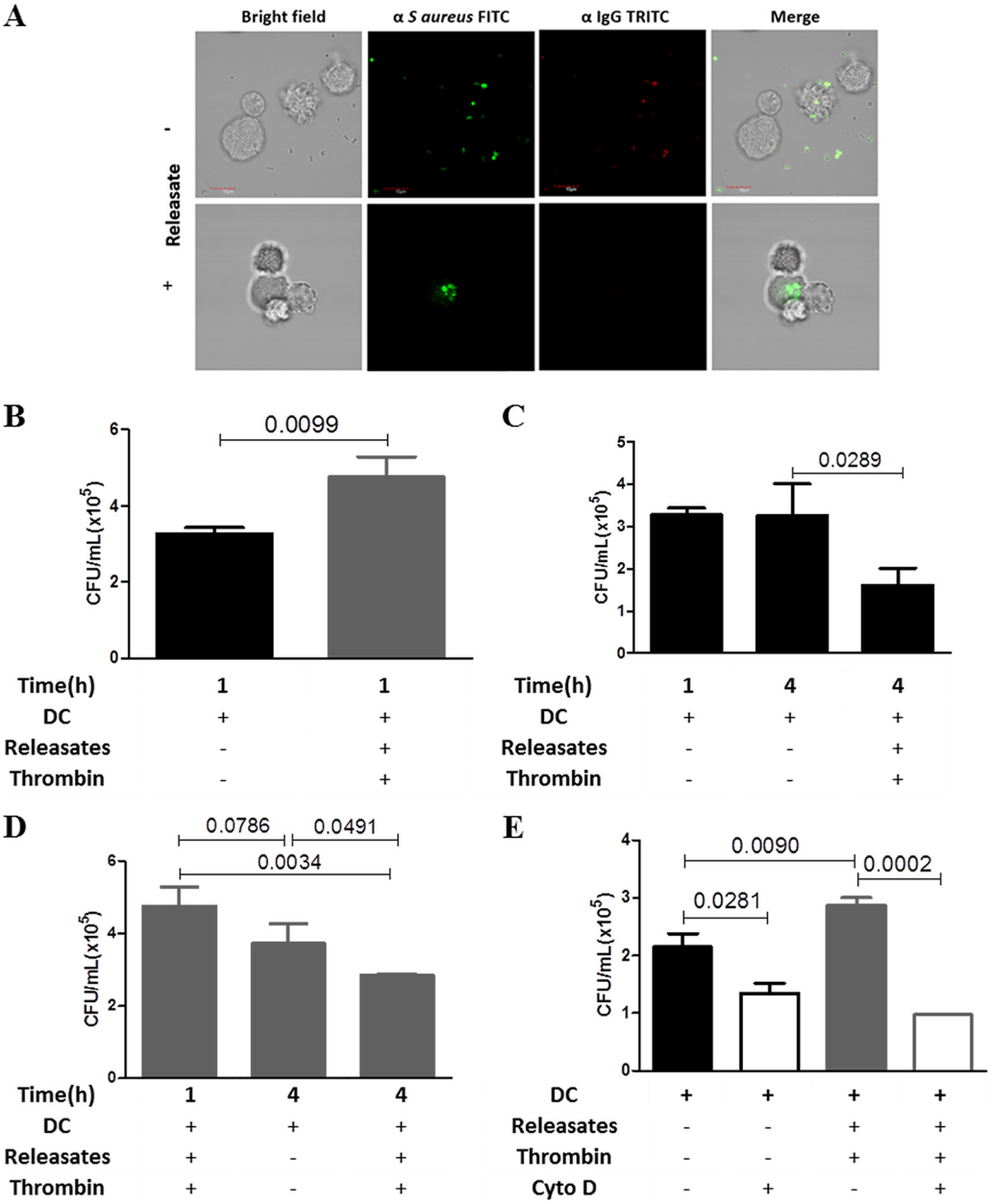

FIG 2 Effect of thrombin-activated platelet releasates on DC phagocytosis and killing of *S. aureus*. (A) Representative confocal images of DCs (t = 1 h). BMDCs were incubated with FITC-labeled MRSA in RPMI 1640 medium in the presence or absence of thrombin-activated platelet releasates for 1 h (t = 1), washed with DPBS$^{-/-}$, and stained with TRITC-conjugated anti-IgG secondary Ab. (B) BMDCs were incubated with MRSA (at an MOI of 10) in sterile DPBS$^{-/-}$ in the presence or absence of thrombin-activated platelet releasates for 1 h (t = 1). The DCs were washed with DPBS$^{-/-}$, lysed with Triton, serially diluted, and plated on TSA plates to obtain internalized CFU (t = 1). (C and D) DCs from panel B were further incubated for 3 h (t = 4) in the presence or absence of thrombin-activated platelets. Intracellular bacteria were obtained from lysed DCs, and CFU were measured. (E) For inhibition of phagocytosis, DCs were incubated with cytochalasin D at a 200 μM final concentration or an equal volume of DPBS$^{-/-}$ for 30 min at 37°C prior to phagocytosis assay. CFU were measured for bacteria obtained from lysed DCs. All measures are presented as means and SD (each experiment was performed in triplicate samples and repeated on at least 3 different days).

killing assays were performed with DCs that were not incubated with platelets or platelet releasates for the first hour. For this, DCs from the 1-h time frame were incubated for an additional 3 h (t = 4 h) in the absence or presence of releasates or sCD40L. Significant killing (~60%) was observed with stimulation by sCD40L, which is consistent with platelet releasate-mediated killing (Fig. 3C). Moreover, platelet releasate-mediated enhancement of phagocytosis can be inhibited by blocking CD40 using an

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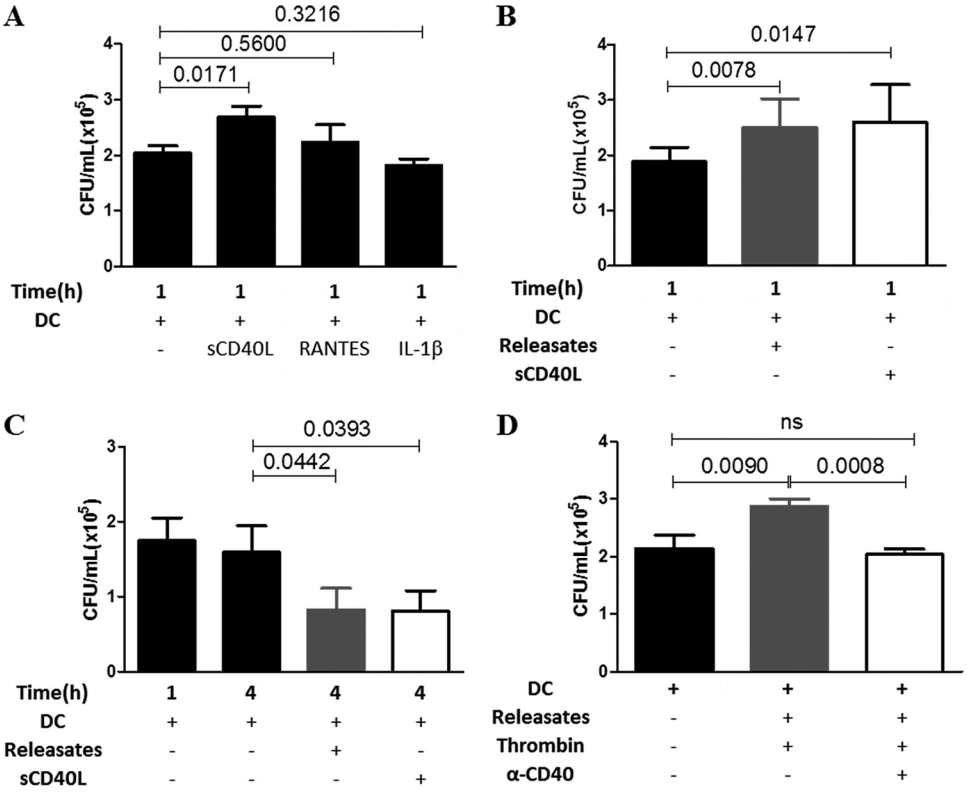

FIG 3 Effect of sCD40L on DC phagocytosis and killing of *S. aureus*. (A) BMDCs were incubated with MRSA (at an MOI of 10) in sterile $DPBS^{-/-}$ in the presence of sCD40L, RANTES, IL-1β, or $DPBS^{-/-}$ for 1 h ($t = 1$). The DCs were washed with $DPBS^{-/-}$, lysed with Triton, and plated on TSA plates to obtain internalized CFU. (B) BMDCs were incubated with MRSA (at an MOI of 10) in sterile $DPBS^{-/-}$ in the presence or absence of thrombin-activated platelet releasates or sCD40L for 1 h ($t = 1$). The DCs were washed with $DPBS^{-/-}$, lysed with Triton, and plated on TSA plates to obtain internalized CFU. (C) DCs (panel B, first bar from left) were further incubated for 3 h ($t = 4$) in the presence or absence of thrombin-activated platelet releasates or sCD40L. Intracellular bacteria were recovered from lysed DCs, and CFU were measured. (D) DCs were pretreated with anti-CD40 antibody or $DPBS^{-/-}$, followed by incubation with MRSA (at an MOI of 10) in sterile $DPBS^{-/-}$ in the presence or absence of thrombin-activated platelet releasates for 1 h. Bacteria obtained from lysed DCs were plated, and CFU were enumerated. All measures are presented as means and SD (each experiment was performed in triplicate samples and repeated on at least 3 different days).

anti-CD40 monoclonal antibody (Fig. 3D). These observations suggest that activated platelets enhance bacterial uptake and killing by DCs in an sCD40L-dependent manner.

Platelet releasates enhance DC activation in response to *S. aureus*. To investigate whether platelet releasates can upregulate DC activation in response to *S. aureus*, expression of the DC activation marker CD80 was analyzed by flow cytometry. Exposure of BMDCs to *S. aureus* for a 3-h infection period at an MOI of 10 in the presence or absence of thrombin-activated platelet releasates led to the enhanced maturation of DCs (Fig. 4A). It should be noted that platelet releasates in the absence of bacteria did not result in increased CD80 expression (Fig. 4A, white bar). These observations suggest that platelet releasates play a crucial role in maturing dendritic cells when the dendritic cells encounter *S. aureus*.

Another marker of DC activation is production of proinflammatory cytokines when stimulated by antigens (40). These cytokines can orchestrate immune responses by activating other immune cells, such as B cells, T cells, and natural killer cells. We hypothesized that dendritic cells would release more proinflammatory cytokines upon *S. aureus* infection with added stimulation by platelet releasates. To test this, we performed cytokine release assays and analyzed the production of such inflammatory cytokines as TNF-α, IL-12, and IL-6, known to be produced by DCs. Dendritic cells were allowed to phagocytose bacteria overnight (24 h) in the presence or absence of platelet

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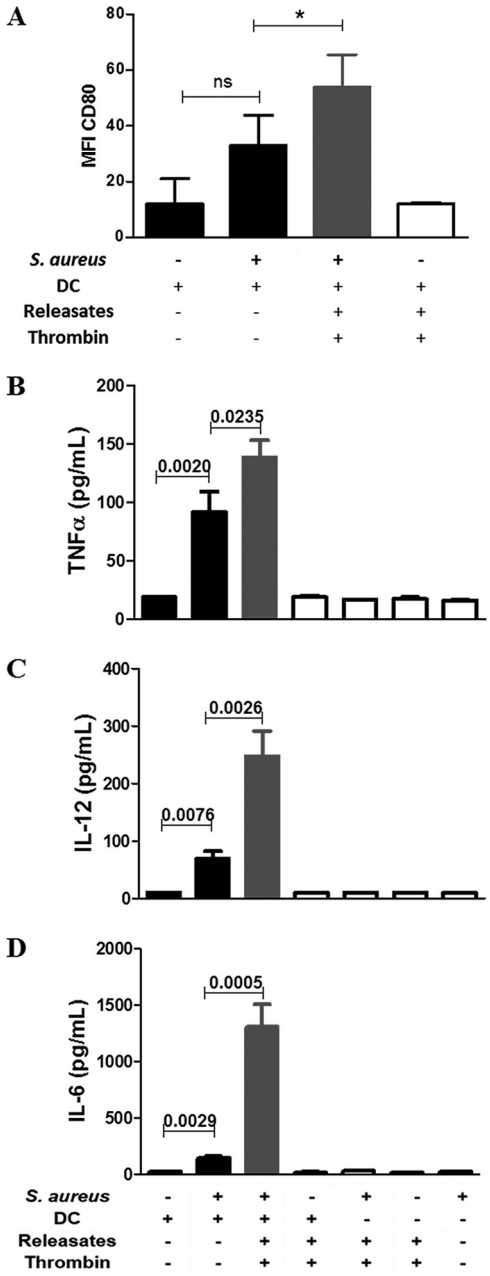

FIG 4 Effect of thrombin-activated platelet releasates on DC activation. (A) BMDCs were incubated with MRSA (at an MOI of 10) in sterile DPBS$^{-/-}$ in the presence or absence of thrombin-activated platelet releasates for 3 h. The cells were harvested, washed with DPBS$^{-/-}$, and stained with PE-CD11c Ab and FITC-CD80 Ab for flow cytometry analysis. Mean fluorescence intensity (MFI) values were obtained by analyzing the data using FlowJo. Cells were gated on CD11c^{+} dendritic cells and analyzed for expression of CD80. (B to D) BMDCs were incubated with MRSA (at an MOI of 10) in sterile DPBS$^{-/-}$ in the presence or absence of thrombin-activated platelet releasates for 2 h. T cell media containing 1% pen-strep were added to the cells and incubated overnight. Cytokines in the culture supernatants were quantified by ELISA. All measures are presented as means and SD (each experiment was performed in triplicate samples and repeated on 3 different days).

releasates. Supernatants from the cells were obtained to quantify the cytokine levels by utilizing commercially available cytokine detection enzyme-linked immunosorbent assay (ELISA) kits. Without exposure to platelet releasates, we observed a 5-fold increase in TNF-α production (Fig. 4B) and ~6-fold increases in both IL-12 (Fig. 4C) and IL-6 (Fig. 4D) production by DCs when infected with *S. aureus*. Surprisingly, exposure to platelet releasates in the presence of *S. aureus* resulted in a further significant increase in all the

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cytokines tested (TNF-α, ~1.5-fold; IL-12, ~3.5-fold; and IL-6, ~8.5-fold). These observations of enhanced DC activation are consistent with the CD80 expression shown in Fig. 4A.

DISCUSSION

Apart from their conventionally recognized functions in hemostasis and thrombosis, there is increasing evidence to support the notion that platelets play a critical role in inflammation and immunity (2). Upon activation, platelets release a number of immune mediators, initiate interactions with leukocytes, and eventually may bridge innate and adaptive immunity. Moreover, we reported that platelet-depleted mice succumbed to *S. aureus* (USA300) infection more rapidly than wild-type mice, demonstrating the role of platelets in protection against *S. aureus* blood infection (41). Platelets can protect against *S. aureus* infection via two seemingly unrelated mechanisms: (i) direct killing of *S. aureus* (which has been demonstrated previously [36, 50]) and (ii) enhancing the activities of leukocyte populations (2). We showed previously that platelets enhance macrophage activities against *S. aureus*. Additionally, it is known that platelets enhance the activities of neutrophils (13, 14). Interestingly, *S. aureus* infection in DC-depleted (CD11c-DTR transgenic) mice resulted in accelerated mortality and additional severe pathology (37). However, little was known about the ability of platelets to assist dendritic cells in combating pathogens.

Our present effort was directed at determining if activated platelets or their releasates enhance both dendritic cell phagocytosis and intracellular killing of *S. aureus*. We observed that thrombin-activated platelets or their releasates do indeed enhance phagocytosis and intracellular killing of *S. aureus* by DCs, whereas absence of activated platelets or releasates showed less phagocytosis and no killing of the bacteria by DCs. What is striking about this finding is that DCs have not previously been shown to be able to kill *S. aureus* directly (36). Interestingly, Mihret et al. (38) show that *S. aureus* replicates in the DC intracellular compartment at 6 h or 24 h postinfection. We found no bacterial replication in our assays, but our phagocytosis and killing assays extended only to 4 h postinfection. However, our findings clearly indicate the participation of activated platelets during the innate immune response through enhancing DC phagocytosis and killing of *S. aureus*. By microscopic study, we observed inhibition by cytochalasin D of DC internalization of *S. aureus*, which disrupts cytoskeletal polymerization and ultimately results in decreased bacterial uptake by eukaryotic cells (47). Therefore, our findings, along with the established literature, suggest that *S. aureus* internalization by BMDCs is cytoskeleton dependent.

We also investigated the mechanism associated with the enhanced DC activation by releasates from thrombin-activated platelets. Our data reveal that the enhancement of bacterial uptake and killing in DCs is mediated by activated platelet-derived sCD40L. Involvement of sCD40L is further supported by the observation that anti-CD40 antibody diminishes the platelet releasate-mediated enhancement of *S. aureus* phagocytosis by DCs. In this case, anti-CD40 antibody abrogated CD40-CD40L ligation (51), leading to failure of platelet releasates to stimulate DC phagocytosis. Activated platelet-derived CD40L induces endothelial cell activation by CD40-CD40L ligation (16). Elzey and coworkers reported the contribution of platelet-derived CD40L to DC maturation, B cell isotype switching, and increased $CD8^+$ T cell responses both *in vitro* and *in vivo* (49). Interestingly, diminished activation of DCs was observed in the case of Th2-inducing parasite *Schistosoma mansoni*-infected $CD40L^{-/-}$ mice compared to wild-type mice (52). All of these observations are indicative of the participation of CD40L in activating CD40-expressing cells and are consistent with our observations.

Next, we explored the expression of the activation marker CD80 on BMDCs due to *S. aureus* infection in the presence or absence of thrombin-activated platelet releasates. We observed significantly increased expression of the costimulatory molecule CD80 on DCs exposed to *S. aureus* compared to control DCs. This expression level was further enhanced by platelet releasates. Surprisingly, CD80 expression was not upregulated when DCs were stimulated by platelet releasates alone. These data suggest that our

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findings contradict previous reports that platelets or platelet releasates can efficiently induce dendritic cell maturation over the course of 48 h (15, 53). However, our experiments were performed over a period of only 3 h to induce activation markers, which may explain this discrepancy. However, another study by Kissel et al. reported that activated human platelets do not induce phenotypic maturation of DCs when cocultured for 24 h (54). This result supports our findings that 3-h coculture of DCs and activated platelet releasates is not sufficient to upregulate CD80 on DCs in the absence of *S. aureus*. However, the presence of *S. aureus* dramatically changes the phenotype of DCs as early as 3 h postinfection. These observations imply that DCs respond very quickly upon encountering *S. aureus*, and activated platelets can further augment the response.

Similarly, we determined the DC production of such proinflammatory cytokines as TNF-α, IL-12, and IL-6 in response to *S. aureus* infection and platelet stimulation. Dendritic cells produce a variety of cytokines and chemokines upon activation with pathogens, which are essential for stimulating adaptive immune responses. *S. aureus* is reported to stimulate DCs to secrete many proinflammatory cytokines, such as TNF-α, IL-6, IL-12, and IL-1β (40). However, the effect of platelets on DC-mediated cytokine production during *S. aureus* infection is not known. Consistent with previous reports, we observed significantly increased production of TNF-α, IL-12, and IL-6 by dendritic cells in the presence of bacteria (37, 55). Additionally, we found that cytokine release was further augmented by platelet releasates. Similar to CD80 expression, cytokine levels remained unchanged when DCs were cocultured with activated platelet releasates in the absence of *S. aureus*.

Therefore, platelets can play a vital role in inducing innate and perhaps adaptive immune responses against *S. aureus* infection. Cytokines released from dendritic cells can effectively invoke adaptive immunity. One could postulate that platelets may promote dendritic cells to trigger a balanced T cell response against *S. aureus*, but this needs to be evaluated both *in vitro* and *in vivo*. Overall, platelets are able to play a crucial role in the host defense against the highly pathogenic USA300 strain and less pathogenic methicillin-susceptible *S. aureus* (MSSA) by stimulating DCs to directly kill the bacteria, as well as by enhancing DC maturation to orchestrate other immune cells to respond to the bacteria.

MATERIALS AND METHODS

Mice. Ten- to 14-week-old male and female C57BL/6 mice were housed at the AAALAC-accredited animal facility at the University of Toledo Health Science Campus in a specific-pathogen-free environment. The animals received water and chow *ad libitum*. All mouse experiments were performed according to National Institutes of Health guidelines with approval from the Institutional Animal Care and Use Committee at the University of Toledo. Gender differences in response to *S. aureus* were not observed.

Platelet preparation. Mice were euthanized with CO_2 prior to blood collection via cardiac puncture. Blood was drawn using a 1-ml syringe with a 25-gauge needle containing anticoagulant (22.0 g/liter trisodium citrate, 8.0 g/liter citric acid, and 24.5 g/liter dextrose) and was pooled in 1.5-ml sterile microcentrifuge tubes. Platelets were isolated and purified by centrifugation as described previously (56).

Preparation of bacteria. A derivative of a previously characterized clinical isolate of community-acquired (CA) MRSA (USA300) (57) and a second laboratory MRSA strain (Sanger 476) were provided by R. M. Blumenthal (University of Toledo, Toledo, OH). The bacteria were grown overnight at 37°C in sterile tryptic soy broth, centrifuged at 1,000 $\times$ *g* for 10 min, and resuspended in 1 ml of sterile Dulbecco's phosphate-buffered solution, pH 7, without calcium or magnesium ($DPBS^{-/-}$). Bacteria were enumerated using a hemacytometer, and counts were confirmed by serial dilution on tryptic soy agar (TSA).

Generation and purification of BMDCs. Dendritic cells were cultured from bone marrow of C57BL/6 mice as previously described (58). Briefly, bone marrow cells were flushed from the tibias and femurs of the mice. Red blood cells were lysed using ACK lysing buffer (Life Technologies) according to the manufacturer's instructions. Bone marrow cells were enumerated and resuspended in T cell culture medium (RPMI 1640 with 2.05 M L-glutamine supplemented with 10% heat-inactivated fetal bovine serum [HyClone Laboratories, Logan, UT] 30 μM 2-mercaptoethanol [2-ME]; 10 mM HEPES buffer; 1$\times$ penicillin-streptomycin [pen-strep]; and medium additions containing folic acid, L-asparagine, L-arginine, L-glutamine, and sodium pyruvate) at a concentration of 10^6 cells/ml supplemented with recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) (Peprotech, Rocky Hill, NJ; 10-ng/ml final concentration) and recombinant murine IL-4 (Peprotech, Rocky Hill, NJ; 10-ng/ml final concentration). The cells were cultured at day 0 in T75 flasks for 6 days at 37°C in a 5% CO_2 atmosphere; additional medium was added at day 3, supplemented with GM-CSF and IL-4, each at a 10-ng/ml final concentra-

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tion. At day 6, floating cells from the flasks were harvested, enumerated, and subjected to purification using CD11c MicroBeads and magnetically activated cell sorting (MACS) columns (Miltenyi Biotec GmbH, Germany), following the manufacturer's protocol. The enriched cells were stained with phycoerythrin (PE)-conjugated anti-CD11c antibody and analyzed by flow cytometry to assess the purity of the dendritic cells. Approximately 80 to 90% of the cells were $CD11c^+$ cells (data not shown), which were immediately utilized for the assays. The remaining 10 to 20% of the cells were characterized as $CD11c^+$ $Ly6G^-$ $F4/80^-$. No additional characterization was performed.

Phagocytosis and intracellular killing of *S. aureus*. Phagocytosis of *S. aureus* by purified BMDCs was performed in the presence or absence of thrombin-activated (0.1 U, final concentration) platelets or their releasates (0.1 U, final concentration). Dendritic cells (1.5×10^5) were mixed with platelets (1.5×10^6) and thrombin or releasates from activated platelets (equivalent to 1.5×10^6 platelets) or an equal volume of $DPBS^{-/-}$ in sterile microcentrifuge tubes, followed by addition of *S. aureus* (1.5×10^6 CFU) in a 25-μl final volume. The cell suspension was then incubated for 1 h in a 37°C water bath. At this time ($t = 1$ h), the cells were washed with $DPBS^{-/-}$ three times by centrifugation at $300 \times g$ for 1 min each time to clear nonphagocytosed bacteria, as well as platelets or their products. The cells were resuspended in 150 μl of $DPBS^{-/-}$, and then 10 μl of cell suspension was lysed with 0.2% Triton X-100 for 10 min, serially diluted, and plated to ascertain the CFU of *S. aureus* as a measure of phagocytosis. For the bacterial-killing assays, the remaining cells from each tube were transferred to two sterile microcentrifuge tubes (20 μl in each tube), and incubation continued for 3 h ($t = 4$ h) in the presence or absence of fresh thrombin-activated platelets or releasates, after which the dendritic cells were lysed with 0.2% Triton X-100, serially diluted, and plated. CFU were counted to determine the intracellular-killing efficiency. In some experiments, dendritic cells were incubated with recombinant IL-1β (10 ng/ml), trimeric sCD40L (100 ng/ml), RANTES (10 ng/ml) (all from PeproTech, Rocky Hill, NJ), or anti-CD40 antibody (clone HM40-3; Biogems, Westlake Village, CA). For inhibition of phagocytosis, dendritic cells were incubated with cytochalasin D (Sigma-Aldrich, St. Louis, MO) at a 200 μM final concentration or an equal volume of $DPBS^{-/-}$ for 30 min at 37°C prior to phagocytosis assay. Each experiment was performed in triplicate, and serial dilutions were plated in triplicate. Each experiment was performed at least three times on different days.

Microscopy. *S. aureus* bacteria were stained with FITC-conjugated anti-*S. aureus* polyclonal primary antibody (Abcam, Cambridge, MA) at 37°C for 30 min. After incubation, the cells were washed and resuspended in $DPBS^{-/-}$. Purified dendritic cells were incubated with FITC-labeled *S. aureus* in RPMI 1640 medium in the presence of 0.1 U thrombin-activated platelet releasate or $DPBS^{-/-}$ for 1 h at 37°C. The cells were washed with $DPBS^{-/-}$ ($300 \times g$ for 1 min; 3 times) to clear nonphagocytosed bacteria. The cells were then stained with TRITC-conjugated anti-IgG secondary antibody (Jackson ImmunoResearch, West Grove, PA) for 30 min at 4°C, washed at $600 \times g$ for 5 min with $DPBS^{-/-}$, resuspended in 10 μl of $DPBS^{-/-}$, placed on coverslips (using Prolong Gold antifade reagent; Invitrogen; P10144), and observed under confocal microscopy to distinguish intracellular ($FITC^+$ $TRITC^-$) from extracellular ($FITC^+$ $TRITC^+$) bacteria.

Microscopy was performed using an Olympus FV1000 confocal microscope. Slides were imaged using the Olympus PlanAPO 603/1.42 oil immersion objective at 2× optical zoom (DC and bacteria). Images were acquired using FV10-ASW v2.1 software. The following fluorescence filter sets were used for image acquisition: FITC (excitation, 488 nm; emission, 519 nm) and tetramethylrhodamine (excitation, 543 nm; emission, 578 nm).

Surface marker expression. Purified dendritic cells (1.5×10^5 cells) were incubated in a 96-well plate with unstimulated or thrombin-activated platelet releasates (harvested from 1.5×10^6 platelets) in $DPBS^{-/-}$ in the presence or absence of *S. aureus* (1.5×10^6 CFU) in a 25-μl final volume. The cells were incubated at 37°C in the presence of 5% CO_2 for 3 h. After incubation, the cells were harvested and fixed with 2% paraformaldehyde (PFA) at 4°C. Finally, flow cytometry was performed to assess the surface expression of the maturation marker CD80 on infected or uninfected dendritic cells. The cells were stained with anti-CD80-FITC (1:50; BioLegend, San Diego, CA) or isotype control for 30 min on ice. After incubation and washing with $DPBS^{-/-}$, the cell-associated fluorescence was measured with a FACSCalibur flow cytometer (BD Biosciences) and analyzed using FlowJo (Tree Star, Ashland, OR).

ELISA. Cytokines released by dendritic cells were detected in cell-free culture supernatants utilizing commercial sandwich Mini TMB ELISA development kits for TNF-α, IL-12, and IL-6 (PeproTech, Rocky Hill, NJ). To obtain supernatants, purified dendritic cells (1.5×10^5 cells) were incubated in the wells of 96-well plates with unstimulated or thrombin-activated platelet releasates (equivalent to 1.5×10^6 platelets) in $DPBS^{-/-}$ in the presence or absence of *S. aureus* (1.5×10^6 CFU) in a 25-μl final volume. The cells were incubated at 37°C in the presence of 5% CO_2 for 2 h. After incubation, 100 μl of T cell medium containing 1% pen-strep was added to each well to kill the extracellular bacteria. Then, the plate was kept at 37°C in the presence of 5% CO_2 for overnight incubation. After 24 h total incubation, the supernatants were collected and stored at −80°C until they were used for ELISA analysis. To measure TNF-α, 20 μl of supernatant was diluted with 80 μl of diluent (0.05% Tween 20 and 0.1% bovine serum albumin [BSA] in PBS) and added to each well. To measure IL-6 and IL-12, 5 μl of supernatant was diluted with 95 μl of diluent to be added to each well. The assays were performed according to the manufacturer's instructions.

Statistical analysis. All data were plotted using GraphPad (La Jolla, CA) Prism 5.03 for Windows and are expressed as means and SD. Significant differences between two groups were determined using a two-tailed, unpaired Student *t* test (Fig. 2B). Statistical significance was set at a P value of <0.05. All other comparisons were analyzed by one-way analysis of variance (ANOVA) with Bonferroni's multiple-comparison analysis, and statistical significance was set at a P value of <0.05.

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REFERENCES

1. Clemetson KJ. 2012. Platelets and primary haemostasis. Thromb Res 129:220–224. https://doi.org/10.1016/j.thromres.2011.11.036.
2. Lam FW, Vijayan KV, Rumbaut RE. 2015. Platelets and their interactions with other immune cells. Compr Physiol 5:1265–1280. https://doi.org/10.1002/cphy.c140074.
3. Alonso AL, Cox D. 2015. Platelet interactions with viruses and parasites. Platelets 26:317–323. https://doi.org/10.3109/09537104.2015.1025376.
4. Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. 2005. Evidence of Toll-like receptor molecules on human platelets. Immunol Cell Biol 83:196–198. https://doi.org/10.1111/j.1440-1711.2005.01314.x.
5. Aslam R, Speck ER, Kim M, Crow AR, Bang KWA, Nestel FP, Ni H, Lazarus AH, Freedman J, Semple JW. 2006. Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-α production in vivo. Blood 107:637–641. https://doi.org/10.1182/blood-2005-06-2202.
6. Cognasse F, Nguyen KA, Damien P, McNicol A, Pozzetto B, Hamzeh-Cognasse H, Garraud O. 2015. The inflammatory role of platelets via their TLRs and Siglec receptors. Front Immunol 6:83. https://doi.org/10.3389/fimmu.2015.00083.
7. Michels A, Albanez S, Mewburn J, Nesbitt K, Gould TJ, Liaw PC, James PD, Swystun LL, Lillicrap D. 2016. Histones link inflammation and thrombosis through the induction of Weibel-Palade body exocytosis. J Thromb Haemost 14:2274–2286. https://doi.org/10.1111/jth.13493.
8. Savage B, Saldivar E, Ruggeri ZM. 1996. Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. Cell 84:289–297. https://doi.org/10.1016/S0092-8674(00)80983-6.
9. Ali RA, Wuescher LM, Worth RG. 2015. Platelets: essential components of the immune system. Curr Trends immunol 16:65–78.
10. Nieswandt B, Brakebusch C, Bergmeier W, Schulte V, Bouvard D, Mokhtari-Nejad R, Lindhout T, Heemskerk JW, Zirngibl H, Fassler R. 2001. Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. EMBO J 20:2120–2130. https://doi.org/10.1093/emboj/20.9.2120.
11. Hamzeh-Cognasse H, Damien P, Chabert A, Pozzetto B, Cognasse F, Garraud O. 2015. Platelets and infections; complex interactions with bacteria. Front Immunol 6:82. https://doi.org/10.3389/fimmu.2015.00082.
12. Mantovani A, Garlanda C. 2013. Platelet-macrophage partnership in innate immunity and inflammation. Nat Immunol 14:768. https://doi.org/10.1038/ni.2666.
13. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, Devinney R, Doig CJ, Green FH, Kubes P. 2007. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med 13:463–469. https://doi.org/10.1038/nm1565.
14. Ruf A, Schlenk RF, Maras A, Morgenstern E, Patscheke H. 1992. Contact-induced neutrophil activation by platelets in human cell suspensions and whole blood. Blood 80:1238–1246.
15. Czapiga M, Kirk AD, Lekstrom-Himes J. 2004. Platelets deliver costimulatory signals to antigen-presenting cells: a potential bridge between injury and immune activation. Exp Hematol 32:135–139. https://doi.org/10.1016/j.exphem.2003.11.004.
16. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, Kroczek RA. 1998. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 391:591–594. https://doi.org/10.1038/35393.
17. van Kooten C, Banchereau J. 2000. CD40-CD40 ligand. J Leukoc Biol 67:2–17. https://doi.org/10.1002/jlb.67.1.2.
18. Andre P, Nannizzi-Alaimo L, Prasad SK, Phillips DR. 2002. Platelet-derived CD40L: the switch-hitting player of cardiovascular disease. Circulation 106:896–899. https://doi.org/10.1161/01.CIR.0000028962.04520.01.
19. Miller LS, Cho JS. 2011. Immunity against Staphylococcus aureus cutaneous infections. Nat Rev Immunol 11:505–518. https://doi.org/10.1038/nri3010.
20. Foster TJ. 2005. Immune evasion by staphylococci. Nat Rev Microbiol 3:948–958. https://doi.org/10.1038/nrmicro1289.
21. Graves SF, Kobayashi SD, DeLeo FR. 2010. Community-associated methicillin-resistant Staphylococcus aureus immune evasion and virulence. J Mol Med 88:109–114. https://doi.org/10.1007/s00109-009-0573-x.
22. Daum RS. 2007. Clinical practice. Skin and soft-tissue infections caused by methicillin-resistant Staphylococcus aureus. N Engl J Med 357:380–390.
23. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. 2010. Community-associated meticillin-resistant Staphylococcus aureus. Lancet 375:1557–1568. https://doi.org/10.1016/S0140-6736(09)61999-1.
24. Chambers HF, DeLeo FR. 2009. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol 7:629–641. https://doi.org/10.1038/nrmicro2200.
25. Fowler T, Wann ER, Joh D, Johansson S, Foster TJ, Hook M. 2000. Cellular invasion by Staphylococcus aureus involves a fibronectin bridge between the bacterial fibronectin-binding MSCRAMMs and host cell beta1 integrins. Eur J Cell Biol 79:672–679. https://doi.org/10.1078/0171-9335-00104.
26. Nuzzo I, Sanges MR, Folgore A, Carratelli CR. 2000. Apoptosis of human keratinocytes after bacterial invasion. FEMS Immunol Med Microbiol 27:235–240. https://doi.org/10.1111/j.1574-695X.2000.tb01435.x.
27. Bayles KW, Wesson CA, Liou LE, Fox LK, Bohach GA, Trumble WR. 1998. Intracellular Staphylococcus aureus escapes the endosome and induces apoptosis in epithelial cells. Infect Immun 66:336–342.
28. Menzies BE, Kourteva I. 1998. Internalization of Staphylococcus aureus by endothelial cells induces apoptosis. Infect Immun 66:5994–5998.
29. Peacock SJ, Foster TJ, Cameron BJ, Berendt AR. 1999. Bacterial fibronectin-binding proteins and endothelial cell surface fibronectin mediate adherence of Staphylococcus aureus to resting human endothelial cells. Microbiology 145:3477–3486. https://doi.org/10.1099/00221287-145-12-3477.
30. Nair SP, Bischoff M, Senn MM, Berger-Bachi B. 2003. The sigma B regulon influences internalization of Staphylococcus aureus by osteoblasts. Infect Immun 71:4167–4170. https://doi.org/10.1128/IAI.71.7.4167-4170.2003.
31. Hess DJ, Henry-Stanley MJ, Erickson EA, Wells CL. 2003. Intracellular survival of Staphylococcus aureus within cultured enterocytes. J Surg Res 114:42–49. https://doi.org/10.1016/S0022-4804(03)00314-7.
32. Youssefian T, Drouin A, Masse JM, Guichard J, Cramer EM. 2002. Host defense role of platelets: engulfment of HIV and Staphylococcus aureus occurs in a specific subcellular compartment and is enhanced by platelet activation. Blood 99:4021–4029. https://doi.org/10.1182/blood-2001-12-0191.
33. Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, Long RD, Dorward DW, Gardner DJ, Kreiswirth BN, Musser JM, DeLeo FR. 2005. Insights into mechanisms used by Staphylococcus aureus to avoid destruction by human neutrophils. J Immunol 175:3907–3919. https://doi.org/10.4049/jimmunol.175.6.3907.
34. Gresham HD, Lowrance JH, Caver TE, Wilson BS, Cheung AL, Lindberg FP. 2000. Survival of Staphylococcus aureus inside neutrophils contributes to infection. J Immunol 164:3713–3722. https://doi.org/10.4049/jimmunol.164.7.3713.
35. Kubica M, Guzik K, Koziel J, Zarebski M, Richter W, Gajkowska B, Golda A, Maciag-Gudowska A, Brix K, Shaw L, Foster T, Potempa J. 2008. A potential new pathway for Staphylococcus aureus dissemination: the silent survival of S. aureus phagocytosed by human monocyte-derived macrophages. PLoS One 3:e1409. https://doi.org/10.1371/journal.pone.0001409.
36. Ali RA, Wuescher LM, Dona KR, Worth RG. 2017. platelets mediate host defense against Staphylococcus aureus through direct bactericidal activity and by enhancing macrophage activities. J Immunol 198:344–351. https://doi.org/10.4049/jimmunol.1601178.
37. Schindler D, Gutierrez MG, Beineke A, Rauter Y, Rohde M, Foster S, Goldmann O, Medina E. 2012. Dendritic cells are central coordinators of the host immune response to Staphylococcus aureus bloodstream infection. Am J Pathol 181:1327–1337. https://doi.org/10.1016/j.ajpath.2012.06.039.

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38. Mihret A, Mamo G, Tafesse M, Hailu A, Parida S. 2011. Dendritic cells activate and mature after infection with Mycobacterium tuberculosis. BMC Res Notes 4:247. https://doi.org/10.1186/1756-0500-4-247.
39. Czakai K, Dittrich M, Kaltdorf M, Muller T, Krappmann S, Schedler A, Bonin M, Duhring S, Schuster S, Speth C, Rambach G, Einsele H, Dandekar T, Loffler J. 2017. Influence of platelet-rich plasma on the immune response of human monocyte-derived dendritic cells and macrophages stimulated with Aspergillus fumigatus. Int J Med Microbiol 307:95–107. https://doi.org/10.1016/j.ijmm.2016.11.010.
40. Wu X, Xu F. 2014. Dendritic cells during Staphylococcus aureus infection: subsets and roles. J Transl Med 12:358. https://doi.org/10.1186/s12967-014-0358-z.
41. Wuescher LM, Takashima A, Worth RG. 2015. A novel conditional platelet depletion mouse model reveals the importance of platelets in protection against Staphylococcus aureus bacteremia. J Thromb Haemost 13:303–313. https://doi.org/10.1111/jth.12795.
42. Mölne L, Verdrengh M, Tarkowski A. 2000. Role of neutrophil leukocytes in cutaneous infection caused by Staphylococcus aureus. Infect Immun 68:6162–6167. https://doi.org/10.1128/IAI.68.11.6162-6167.2000.
43. Verdrengh M, Tarkowski A. 1997. Role of neutrophils in experimental septicemia and septic arthritis induced by Staphylococcus aureus. Infect Immun 65:2517–2521.
44. Sarantis H, Grinstein S. 2012. Subversion of phagocytosis for pathogen survival. Cell Host Microbe 12:419–431. https://doi.org/10.1016/j.chom.2012.09.001.
45. Casella JF, Flanagan MD, Lin S. 1981. Cytochalasin D inhibits actin polymerization and induces depolymerization of actin filaments formed during platelet shape change. Nature 293:302.
46. Schliwa M. 1982. Action of cytochalasin D on cytoskeletal networks. J Cell Biol 92:79–91.
47. Gottlieb TA, Ivanov IE, Adesnik M, Sabatini DD. 1993. Actin microfilaments play a critical role in endocytosis at the apical but not the basolateral surface of polarized epithelial cells. J Cell Biol 120:695–710.
48. Antczak AJ, Singh N, Gay SR, Worth RG. 2010. IgG-complex stimulated platelets: a source of sCD40L and RANTES in initiation of inflammatory cascade. Cell Immunol 263:129–133. https://doi.org/10.1016/j.cellimm.2010.03.009.
49. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, Stein CS, Nieswandt B, Wang Y, Davidson BL, Ratliff TL. 2003. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. Immunity 19:9–19.
50. Tang YQ, Yeaman MR, Selsted ME. 2002. Antimicrobial peptides from human platelets. Infect Immun 70:6524–6533. https://doi.org/10.1128/IAI.70.12.6524-6533.2002.
51. Karimi MH, Ebadi P, Pourfathollah AA, Moazzeni SM. 2010. Tolerance induction by CD40 blocking through specific antibody in dendritic cells. Iran J Allergy Asthma Immunol 9:141–147.
52. Straw AD, MacDonald AS, Denkers EY, Pearce EJ. 2003. CD154 plays a central role in regulating dendritic cell activation during infections that induce Th1 or Th2 responses. J Immunol 170:727–734. https://doi.org/10.4049/jimmunol.170.2.727.
53. Hamzeh-Cognasse H, Cognasse F, Palle S, Chavarin P, Olivier T, Delézay O, Pozzetto B, Garraud O. 2008. Direct contact of platelets and their released products exert different effects on human dendritic cell maturation. BMC Immunol 9:54. https://doi.org/10.1186/1471-2172-9-54.
54. Kissel K, Berber S, Nockher A, Santoso S, Bein G, Hackstein H. 2006. Human platelets target dendritic cell differentiation and production of proinflammatory cytokines. Transfusion 46:818–827. https://doi.org/10.1111/j.1537-2995.2006.00802.x.
55. Hu L, Bray MD, Osorio M, Kopecko DJ. 2006. Campylobacter jejuni induces maturation and cytokine production in human dendritic cells. Infect Immun 74:2697–2705. https://doi.org/10.1128/IAI.74.5.2697-2705.2006.
56. Worth RG, Chien CD, Chien P, Reilly MP, McKenzie SE, Schreiber AD. 2006. Platelet FcgammaRIIA binds and internalizes IgG-containing complexes. Exp Hematol 34:1490–1495. https://doi.org/10.1016/j.exphem.2006.06.015.
57. McCullough AC, Seifried M, Zhao X, Haase J, Kabat WJ, Yogev R, Blumenthal RM, Mukundan D. 2011. Higher incidence of perineal community acquired MRSA infections among toddlers. BMC Pediatr 11:96. https://doi.org/10.1186/1471-2431-11-96.
58. Nishat S. 2016. Syntheses and immunological evaluation of zwitterionic polysaccharide (PS A1) based vaccines. University of Toledo, Toledo, OH.

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