Of Clots and Granulomas: Platelets are New Players in Immunity to Tuberculosis

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(See the major article by Feng et al on pages 1700–10.)

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Over a century and a half after their first observation under the microscope by the English anatomist and physiologist George Gulliver, platelets, named after their plate-like shape by the American pathologist James Wright in 1910 \cite{1}, are still intensively studied, not only in the context of hemostasis but also in that of host defense against microbial pathogens.

Platelets are small anucleated cells derived from megakaryocytes and constitute 1 of the 3 cell types of the blood, with white blood cells (leukocytes) and red blood cells (erythrocytes). These cells are copiously produced in the bone marrow, with up to $10^{11}$ cells produced daily in humans, and have a life span of about 1 week. In the context of hemostasis, thrombin produced by injured endothelia promotes the rapid recruitment of circulating platelets to the site of injury, where these cells aggregate and promote activation cascades leading to the deposition of fibrin and other tissue substrates, a process that culminates with clot formation. In addition to their role in clotting, platelets have been recognized for their role in antimicrobial host defense for nearly 20 years \cite{2}.

Despite their lack of a nucleus, platelets are not inert cell structures and can translate messenger RNA (mRNA) derived from megakaryocytes and synthesize proteins. Because of their rapid recruitment to the sites of injury, platelets are well positioned to support both hemostasis and microbial recognition and control \cite{3}.

Sullam and colleagues \cite{2} provided one of the first evidences for a role of platelets in host defense against pathogens. In this study, rabbits in which platelets were depleted through administration of anti-platelet antibodies, showed an increased susceptibility to Streptococcus sanguis; the authors also found that platelets can interact and kill the pathogen in vitro, through the production of antimicrobial proteins. Since this seminal discovery, numerous studies have reported a role for platelets in immune defense against various pathogens, including viruses, bacteria, parasites, and fungi \cite{3}.

Platelets recognize pathogens either indirectly, through sensing signals of the acute phase response, such as complement components C3a and C5a, C-reactive protein, and thrombospondin-1, or directly, through recognition of a range of microbial molecular patterns by so-called pattern recognition receptors, including Toll-like receptors (TLRs). Indeed, platelets express TLRs, such as TLR4, and recognize microbial TLR ligands, such as lipopolysaccharide \cite{4}. Upon ligand binding, platelets translate microbial recognition into antimicrobial effector functions, including the production of inflammatory cytokines, such as tumor necrosis factor, of reactive oxygen species, and of a range of so-called platelet microbicidal proteins (PMPs), which include antimicrobial peptides and other microbicidal molecules \cite{5}. In addition to microbial killing, platelets can enhance the function of other immune cells: (1) they recruit neutrophils and promote the formation of neutrophil extracellular traps (NETs) \cite{6}; (2) they activate dendritic cells (DCs) and promote DC maturation and antigen presentation; (3) and they can polarize T and B lymphocytes and thus contribute to the adaptive immune response. Although platelets are now recognized as playing a key part in immune defense against a range of pathogens, their role in immunity to one of the major bacterial pathogens, Mycobacterium tuberculosis, has never been adequately explored.

Worldwide tuberculosis still claims nearly 1.5 million lives every year, and it is estimated that up to one-third of the global population may carry latent M. tuberculosis infection \cite{7}. The recent
development of new therapeutic and preventive strategies to control tuberculosis has clearly benefited from a better understanding of anti-mycobacterial immunity and tuberculosis pathogenesis over the past 2 decades. However, much still remains to be understood. Chief among the pressing issues regarding immunity to tuberculosis is to decipher the qualitative and quantitative nature of an immune response that can be considered protective against the pathogen. Equally important is to understand how *M. tuberculosis* circumvents immune defenses and can establish persistent infections in spite of an apparently functional immunity. Numerous studies have clearly demonstrated the key part played by various immune cells, most notably macrophages and interferon (IFN)-γ-producing T lymphocytes, in protection against *M. tuberculosis* [8, 9]. And yet the roles of other cell types, such as B lymphocytes or platelets in antimycobacterial immunity are far less clear.

In this issue of the *Journal of Infectious Diseases*, Feng and colleagues [10] uncovered an unsuspected role for platelets in immune regulation during tuberculosis as drivers of macrophage transformation in mycobacterial infection. Indeed, through coculture system with human platelets, the authors convincingly demonstrated that monocyte differentiation is tilted toward a macrophage program with an epithelioid-like and anti-inflammatory phenotype, which the authors labeled as monocyte/platelet-derived macrophages (MP-Mac; Figure 1). Moreover, this phenotype was accompanied by important functional changes when facing mycobacteria, such as high rate of phagocytosis and a low ratio of tumor necrosis factor (TNF)/interleukin 10 (IL-10) production. A transcriptomic analysis of MP-Mac confirmed that a profound alteration of the global gene expression profile takes place, including the particular capacity of these cells to express the CXCL5 chemokine, which the authors nicely correlated in the bronchoalveolar lavage from tuberculosis patients. Interestingly, further incubation with platelets drove MP-Mac progression into multinucleated giant cells with a foamy phenotype (platelet/mönocyte-differentiated macrophage multinucleated giant cell [MP-MNGC]), a differentiation program that is exacerbated in the presence of lipoarabinomannan from *M. tuberculosis*. Finally, the authors correlated the expression of platelet-specific and MP-Mac-related cell surface markers in epithelioid macrophages and multinucleated giant cells (MNGCs) typically present in the tuberculous granuloma, implying that the effects of platelets may be ultimately reflected in vivo during the formation and function of these immune-related structures (Figure 1).

There are 2 critical aspects of this study that are worth highlighting. The first one is the novel role of platelets in influencing the process of macrophage polarization in the absence of pathogenic challenge. Macrophage polarization is a highly dynamic process that dictates the functional capacity of these phagocytes to deal with pathogens, among other biological activities [12]. In terms of the macrophage response to bacterial infection, the classical (M1) activation program grants these cells microbicidal properties characterized by a highly toxic intracellular environment. Although M1 macrophages usually become predominant early on at the site of infection, upon control of the pathogen, there is a frequent switch in the macrophage population toward the alternative (M2) program. From a physiological perspective, it is argued that this shift is important to control tissue damage following pathogenic insult and to promote the resolution of inflammation and tissue repair, features typically attributed to M2 macrophages [12]. That platelets contribute to the establishment of the M2 program in the macrophage population suggests they represent an important cellular component in preventing immunopathology. This property, in itself, is a novel biological function for platelets, given their established reputation (although recent) as effector cells promoting immunity against bacterial pathogens [13].

The second aspect is that there is increasing evidence that some pathogens have evolved the capacity to manipulate macrophage polarization to their advantage [12,14]. In the case of *M. tuberculosis*, not only does it inhibit the proinflammatory and microbicidal mediators, but this pathogen also promotes a switch from M1 to M2 macrophages to ensure long-term intracellular survival. Is the ability of platelets to direct monocytes toward a M2 macrophage program subverted by *M. tuberculosis*? Feng and colleagues suggest that this may be the case within granulomas by providing rich intracellular niches, rich in lipids and other carbon-related sources, in the context of MP-Mac cells and MP-MNGCs (Figure 1).

In addition, these cells seem to be an opportune source of IL-10, an important anti-inflammatory cytokine long-established as critical in the immune-evasion strategy by multiple pathogens, including *M. tuberculosis*. Collectively, this timely study unveils a novel cellular component, the platelets, as a catalyst of the transformation that takes place in the macrophage population in the context of tuberculosis.

In the near future, we envision that animal models will be used to demonstrate the in vivo role of platelets during *M. tuberculosis* infection. On the one hand, studies of specific platelet inhibitors may reveal their actual role in promoting immunity against *M. tuberculosis*, as is the case for other pathogens for which platelets play a detrimental role in terms of directing antimicrobial defense, enhancing the innate immune effectors, and coordinating the adaptive immune response [3, 13]. On the other hand, a similar approach using platelet inhibitors may determine the role of platelets in driving the transformation of the macrophage population and may indicate whether this switch contributes to *M. tuberculosis* pathogenesis or protects against the immunopathology deriving from infection. Indeed, future studies will further dissect the actual mechanism by which platelets drive monocyte differentiation toward an...
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Immunosuppressive macrophage program, including the role of the platelet cargo such as δ granules (containing nucleotides, bioactive amines, and bioactive ions), α granules (enclosing adhesion molecules, platelet-derived microbicidal proteins, and kinocidins), and λ granules (composed of proteases and glycosidases; Figure 1) [3]. Finally, future perspectives in vaccine development and therapy should be developed using the platelet (and/or their cargo) as vehicles to modulate the immune response against M. tuberculosis.

Regardless of what the future will bring, what is certain is that the current study by Feng and colleagues has brought us a step closer to understanding and solving the enigma of how the macrophage

Figure 1. Contribution of platelets to the establishment of suppressive macrophage program with potential consequences for granuloma formation and function in tuberculosis. The macrophage population within the tuberculous granuloma undergoes various specialized transformations: acquisition of an epithelioid phenotype characterized by tightly interdigitated cell membranes that link adjacent cells, fusion into multinucleated giant cells, or differentiation into foamy cells with a high content of intracellular lipids. Although none of these specialized transformations in the granuloma macrophage population are well understood, the report by Feng et al sheds light into how platelets enable these transformations to take place from the monocyte stage (top). Presumably, the phagocytosis of platelets by monocytes recently recruited to the site of infection would trigger a differentiation program toward an epithelioid-like macrophage (MP-Mac); while not proven, the platelet-derived granule content could be responsible for this effect. Subsequent challenge of MP-Mac with Mycobacterium tuberculosis then reveals critical features of this cell; that is, high rate of phagocytosis of mycobacteria accompanied by robust secretion of anti-inflammatory IL-10. This cytokine in turn, along with further exposure to platelet-derived granule content, may continue the activation of the MP-Mac into foamy platelet/monocyte-derived multinucleated giant cell (MP-MNGC). In the context of a tuberculous granuloma (bottom), Feng et al correlate the expression of platelet-derived markers (eg, CD42b and PDPN), and MP-Mac markers (eg, NTSE and ARG1), in both foam and multinucleated giant cells. In the tuberculous granulomas, the role of macrophage polarization is an emerging concept in the formation and function of these structures [11]. Based on these new findings, we propose that platelets may change the tuberculosis granuloma environment during the late stages of M. tuberculosis infection, distinguished by the M2 Macrophage polarization driven by the high expression of transcription factors (eg, PPARγ, STAT6) antagonistic for type-1 inflammation, and characterized by a cell-surface receptor repertoire promoting tissue repair activities (eg, IL-4) and the formation of foamy cells (eg, CD36). Indeed, the formation of foam and multinucleated giant cells, whose presence is noted to be most frequently at the rim and center of mature tuberculosis granulomas, seems likely to favor the intracellular resilience of M. tuberculosis. Abbreviations: IL, interleukin; MP-Mac, monocyte/platelet-derived macrophage; MP-MNGC, platelet/monocyte-differentiated macrophage multinucleated giant cell.
population undergoes profound changes in response to *M. tuberculosis* and is likely to open up new research areas in platelet-dependent immunological functions in infection and disease.

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