Commentary

Inflammation and the coagulation system in tuberculosis: Tissue Factor leads the dance

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Mycobacterium tuberculosis, the causative agent of tuberculosis, drives the formation of granulomas, structures in which both immune cells and the bacterial pathogen cohabit. The most abundant cells in granulomas are macrophages, which contribute as both cells with bactericidal activity and as targets for *M. tuberculosis* infection and proliferation during the entire course of infection. The mechanisms and factors involved in the regulation and control of macrophage microenvironment-specific polarization and plasticity are not well understood, as some granulomas are able to control bacteria growth and others fail to do so, permitting bacterial spread. In this issue of the *European Journal of Immunology,* Venkatasubramanian et al. [Eur. J. Immunol. 2016. 46: 464–479] show that mice lacking the tissue factor gene in myeloid cells have augmented *M. tuberculosis* growth and increased inflammation in the lungs. This suggests that tissue factor, an initiator of coagulation, is important for the generation of fibrin, which supports granuloma formation. This article demonstrates for the first time the involvement of tissue factor in inducing effective immunity against *M. tuberculosis*, and sheds new lights on the complex interplay between host inflammatory response, the coagulation system, and the control of *M. tuberculosis* infection.

See accompanying article by Venkatasubramanian et al.

Tuberculosis (TB) represents a global health problem: according to the World Health Organization Global Tuberculosis Report 2013, an estimated 8.6 million people developed TB and 1.3 million died from the disease in 2012 [1]. The pathogen responsible for this widespread and persistent infection is *Mycobacterium (M.) tuberculosis*, which resides in macrophages and induces the death of its host cells. Infection starts when inhaled bacilli are phagocytosed by alveolar macrophages. Once inside the phagocyte, *M. tuberculosis* regulates the activity of its phagosome by preventing its fusion with acidic, hydrolytically active lysosomes. In experimental hosts such as mice, this marks the start of a period of rapid division in which the bacteria grow exponentially inside macrophages until the emergence of an acquired immune response. This is concomitant with the development of the granuloma, which signifies immune-mediated containment of the infection. The granuloma can proceed either to localized sterilization of the infection and mineralization of the lesion, or to localized caseation and necrosis, which culminates in the release of infectious bacteria into the airways (reviewed in [2–4]).

Macrophages have been classified along the lines of their different activation states; M1 macrophages, for example, are polarized by Toll-like receptor (TLR) ligands and interferon (IFN) γ, while

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M2 macrophages are polarized by interleukin (IL) 4, IL-13, and IL-10 [4–8]. M1 macrophages are characterized by their expression of high levels of proinflammatory cytokines (e.g., TNF-α), high production of reactive nitrogen and oxygen intermediates, promotion of a Th1 response, and strong microbicidal and tumoricidal activity [5–9]. This type of macrophage is connected to resistance to infection with intracellular bacteria, and characterizes the early phases of infection with *M. tuberculosis* in mice [10]. Conversely, M2 macrophages are characterized by the expression of anti-inflammatory cytokines (e.g., IL-10) and the promotion of tissue healing and remodeling, as well as by immunoregulatory functions [7–9]. In vitro and in vivo experimental studies have both shown that these polarized macrophage phenotypes can be reversible [11–15]. It has been reported that a switch between pro- and anti-inflammatory cytokine profiles can be observed during the transition from acute to chronic infection, and that this may be a mechanism that provides protection against excessive inflammation. On the other hand, the switch in macrophage polarization toward the M2 phenotype has been shown to be a strategy to interfere with M1-associated killing in experimental tumor [12, 13] and infection models [16]. Therefore, a better understanding of macrophage differentiation programs could be useful to develop strategies to target and manipulate their functions in vivo.

In this issue of the *European Journal of Immunology*, Venkatasubramanian et al. [17] report that the expression of tissue factor (TF, a transmembrane glycoprotein involved in hemostasis by activating coagulation) in macrophages controls *M. tuberculosis* growth and dissemination, providing evidence that this molecule contributes to an effective immune response against this intracellular pathogen. TF (also known as Factor III, thromboplastin or CD142) is the principal initiator of the coagulation cascade (reviewed in [18]). It is a 47 kDa transmembrane glycoprotein receptor found on the surface of a variety of cells, including platelets, leukocytes, fibroblasts, and endothelial cells, as well as in the smooth muscle cells surrounding the vessel walls. TF has been shown to be expressed in response to injury (such as infections, inflammation, tumor growth, and atherosclerosis [18]), as well as to a number of different extracellular stimuli, including LPS, TFN-α, IL-1, IL-2, IL-6, IFN-γ, lipoprotein, FVIIa, plasmin, angiotensin II, and hypoxia [18]. TF plays many diverse roles, and in addition to promoting blood coagulation, it is involved in embryonic development, angiogenesis, tumor metastasis, cell adhesion/migration, inflammation, and innate immunity [18].

TF expression on monocytes and endothelial cells has been shown to occur during several different bacterial infections in which TF plays a dual role—on the one side it contributes to infection-associated mortality and inflammation, as in the case of *Toxoplasma* and *Listeria* infection of mice, while on other side it plays a protective role by limiting pathogen burden and dissemination, as in the case of *Yersinia* and *Escherichia coli* infection of mice and rats, respectively [19–22].

A previous study has evaluated the role of TF in the *M. tuberculosis* induced inflammatory response, using transgenic mice that express either very low or normal levels of human TF [23]. It was shown that *M. tuberculosis* infection does not increase TF expression in the lungs, although the macrophages in the granulomatous lung lesions showed increased levels of TF expression [21]. However, there were no significant differences in pro-inflammatory cytokines among wild-type and TF-transgenic mice and mycobacterial burden and dissemination into organs of mice were essentially similar in all tested mice. This indicates that TF does not contribute to the overall defense against *M. tuberculosis* challenge [21].

Venkatasubramanian et al. [17] instead have used mice with selective inactivation of the TF gene in myeloid cells to study the role of TF in *M. tuberculosis* infection. They found that TF expression by myeloid cells contributes to the protective immune response against *M. tuberculosis*. In particular, TF deficiency in myeloid cells is associated with enhanced IL-10 production, reduced inducible nitric oxide synthase (iNOS) expression and enhanced arginase 1 (Arg1) expression, increased MMP-2 and MMP-9-mediated inflammation, and decreased fibrin deposition in the lungs during *M. tuberculosis* infection [17] See Figure 1. Taken together, these responses are associated with increased *M. tuberculosis* replication in the lungs.

One of the hallmark features of TB pathology is the development of granulomas, which contain not only the infection but also the host inflammatory response. Granulomas are aggregates of organized immune cells consisting of blood-derived cells, such as giant-multinucleated cells, lymphocytes, and fibroblasts and finally, at a later stage, they become surrounded by fibrosis, which has the role of demarcating and containing the infection. The granuloma has an innate inflammatory basis but Th1 cells play a key role in its formation and maintenance. According to a recent review [24], three distinct types of granulomas, each correlating with a different stage of *M. tuberculosis* infection, have been described in humans. Solid granuloma, characterized by low bacterial growth and metabolic activity of *M. tuberculosis*, is characteristic of latent *M. tuberculosis* infection [24]. In the transition to active TB disease, solid granulomas develop central necrosis, together with the transformation of *M. tuberculosis* into a metabolically active and highly replicative pathogen [24]. Liquefaction of the necrotic granuloma leads to the formation of caseous granuloma and cavities with tissue damage [24]. In patients with active TB infection, the three forms of granuloma co-exist, although necrotic and caseous granulomas predominate [24]. In a working granuloma, both innate and adaptive immune responses are organized to contain the infection; however, knowledge on the specific factors that facilitate the formation of functional granulomas capable of eliminating or containing *M. tuberculosis* is limited. Since TF is key element in the generation of fibrin, which in turn plays an important role in granuloma formation, it is possible that during *M. tuberculosis* infection, TF expression on the surface of activated macrophages may contribute to the containment of mycobacterial infection through fibrin generation.

Histology data in TF-deficient mice [17] have shown increased inflammation with elevated numbers of small granulomas containing elevated numbers of IL-10-positive foamy M2 macrophages [17]. Whether or not these IL-10-positive foamy macrophages contain *M. tuberculosis* bacilli and express pro-inflammatory cytokines
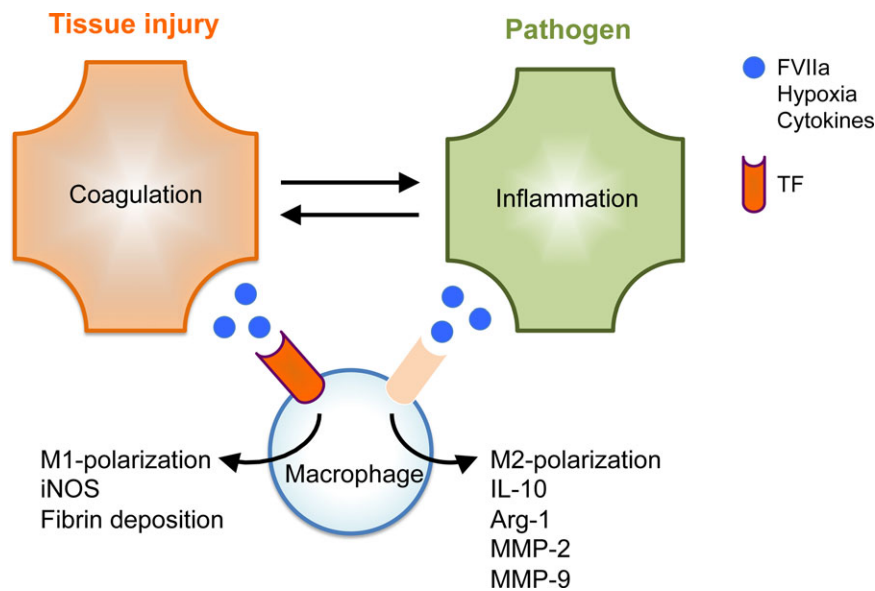
Figure 1. TF is expressed in response to injury (left), as well as to a number of different extracellular stimuli, including LPS, TNF-α, IL-1, IL-2, IL-6, IFN-γ, lipoprotein, FVIIa, plasmin, angiotensin II, and hypoxia. TF is an initiator of the coagulation cascade, and is involved in fibrin generation and deposition, which is important in granuloma formation. TF expression by myeloid cells has also been shown to contribute to the protective immune response against *M. tuberculosis*. In particular, TF deficiency in myeloid cells (right) is associated with enhanced IL-10 production, reduced iNOS expression and enhanced arginase 1 (Arg1) expression, increased MMP-2- and MMP-9-mediated inflammation and decreased fibrin deposition in lungs. Overall, this drives inflammation and damage, and is ultimately associated with increased *M. tuberculosis* replication (right).

has not been investigated in the study of Venkatasubramania et al. [17]. Thus, the development of an exaggerated host inflammatory response in the presence of increased levels of IL-10, clearly indicates that TF deficiency promotes an inappropriate (M2 type) innate response, supporting the concept that one of the major roles of the granuloma is to localize and contain not only the bacteria but also the inflammatory response to the bacteria itself. Indeed, if immune cells are not tightly controlled within the lungs, this could lead to excessive inflammation, as previously demonstrated by ourselves in mice lacking inhibitory receptors for pro-inflammatory cytokines (such as Tir8, [25]) and chemokines (such as D6, [26]). Thus, rigorous control of granuloma organization is likely necessary to prevent immunopathology. Therefore, TF may favor resolution of chronic inflammatory responses and overall provide a fine mechanism for the control of the balance between protective immune responses and immunopathology, possibly inhibiting some signaling pathways to prevent the polarization toward M2 macrophages during infection. It should be stressed that TB is a chronic disease, but experiments in this study were performed at a single time point of 30 days [17], which reflects the participation of innate immune responses and early stages of adaptive response to *M. tuberculosis*, making it impossible to clearly identify the role of TF in the long-term control of *M. tuberculosis* infection and disease.

In conclusion, Venkatasubramanian et al. [17] provide evidence that TF expressed by myeloid cells contributes to protective immunity against *M. tuberculosis* infection. This may have implications for both vaccine and chemotherapy programs. The progression of TB seems to be determined locally, independently of the nature of the systemic immune response. Therefore, to be maximally effective, vaccines need to limit bacterial growth before granuloma formation, because once formed the granuloma protects *M. tuberculosis* and prevents bacterial clearance. The data also indicate that there might be avenues for manipulating the host tissue response to infection that could interfere with the tissue state needed to support persistent organisms.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

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Abbreviations: **TB**: tuberculosis · **M. tuberculosis**: *Mycobacterium tuberculosis*

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