

on than MEP or HSC subsets. The CMP and GMP subsets also displayed higher cell surface expression of a variety of endothelial antigens than MEP or HSC when cultured with endothelial culture medium and treated with VEGFs. CMP and GMP also adhered to and promoted capillary-like tube formation by human endothelial cells on Matrigel-coated dishes to a significantly greater extent than MEP or HSC. Furthermore, CMP and GMP infusion significantly improved recovery of blood perfusion into the experimentally injured limbs of immunodeficient mice compared with the effects of infused MEP or HSC [11]. As the CMPs express higher levels of the miR-16 family members, the results of Goretti and colleagues [9] in this issue would suggest that antagonism of these miRs may further improve the proangiogenic activity of these myeloid progenitor cells and provide some intriguing, novel hypotheses to test. As lack of sufficient EPCs for clinical therapies was one of the rationales for using miRs to enhance EPC proliferation in the present study, one may also wish to examine what is known about hematopoietic progenitor cell expansion. HSPCs are known to be robustly expanded ex vivo by a variety of hematopoietic growth factors and other morphogens in clinically relevant proto-

cols. With the use of these protocols, one could harvest the expanded myeloid progenitor cells and then augment further their proangiogenic activity by addition of reagents to modulate miR-16 family member expression prior to patient infusion. These new avenues of approach may enhance the field of vascular repair and regeneration, as investigators can isolate specific proangiogenic HSPCs (with or without miR treatment) and be assured that the cell product is more functionally homogenous than most of the EPC products that have been included in prior clinical trials [7].

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Editorial: Leukocytes in tularemia—so many cells, so little time

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Tularemia is a disease caused by the facultative intracellular, Gram-negative bacterium *Francisella tularensis* [1]. Infection ensues after the organism is inhaled into the lungs

or introduced into the skin by direct contact with an infected animal or the bite of an infected arthropod vector, and local bacterial replication is followed by dissemination to the liver and spleen. *F. tularensis* has been stockpiled as a bioweapon, and a distinguishing feature of the most highly virulent type A strains of this organism is the short

interval between infection at the onset of symptoms and death, which can ensue prior to development of an adaptive

Abbreviations: LVS=live vaccine strain, MMP-9=matrix metalloproteinase-9

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immune response. In mouse and non-human primate models of infection, moribund status is characterized by an accumulation of neutrophils, live bacteria, and extensive necrotic tissue damage. In contrast, infection with type B *F. tularensis* strains is less severe, and an attenuated type B strain (designated LVS) is often used as a model organism for tularemia research, as it can cause a range of diseases in mice depending on the dose administered. With the use of this strategy, as well as models in which type A *F. tularensis* infection is combined with antibiotics, it has been established that cell-mediated immunity, particularly T cells and IFN- γ , are essential for survival of primary infection and an effective anti-*F. tularensis* immune response [1, 2], whereas other correlates of effective immunity or vaccination are less well-defined.

Studies using mutant mice, inhibitors, and blocking reagents have shown that IL-12 and IFN- γ are critical for effective immunity and survival of mice infected with LVS or the type A *F. tularensis* strain Schu S4 [1, 2], and the pivotal role of IL-12p70 in IFN- γ induction and control of infection was confirmed by Melillo et al. [3] in this issue of the *Journal of Leukocyte Biology* using mice that lack the IL-12R subunit IL-12R β 2. Nevertheless, it is also clear that induction of an effective immune response is diminished and delayed by *F. tularensis* as part of its virulence strategy. Typically, DCs are the major source of IL-12, which is critical for T cell activation and IFN- γ production, and IL-12 synthesis is triggered by TLR or nucleotide-binding oligomerization-like receptor signaling, whereas IL-12p40 homodimers play a distinct role in stimulating DC maturation, activation, and migration to draining LNs. Unlike most Gram-negative bacteria, initial detection of *F. tularensis* is mediated, not by TLR4 and LPS but rather, by the interactions of bacterial lipoproteins with TLR2 [1]. Although NF- κ B is activated, synthesis and secretion of proinflammatory cytokines (including TNF- α , IL-6, IL-1 β , IFN- γ , and IL-12) by infected macrophages and DCs are diminished and delayed relative to control stimuli [1, 2] and may be undermined directly by rapid production of antiinflammatory cytokines, such as

TGF- β and IL-10 [4]. DC trafficking may also be impaired by the negative effects of IFN- β on IL-12p40 homodimer production [5].

Direct evidence that IL-12 and/or IL-23 are also of critical importance in *F. tularensis*-infected humans is indicated by the recent report of tularemia in a 61-year-old Canadian man being treated with ustekinumab [6]. Ustekinumab is a highly specific IL-12p40-blocking antibody that was developed for treatment of plaque psoriasis [7]. Although long-term studies are not yet in-hand, and specific effects of ustekinumab on tularemia severity and progression remain obscure, this clinical case, together with evidence of hepatitis B virus reactivation in another patient receiving ustekinumab [8], suggests that risk of infection with at least some bacterial and viral pathogens is enhanced by this immunomodulatory therapy.

To control *F. tularensis* replication in macrophages, IFN- γ must be present very early in infection [1]. Typically, NK cells are a prominent, initial source of this important cytokine. However, NK cells are depleted during infection with type A *F. tularensis* and have a limited role in IFN- γ production [2, 9]. Consequently, CD4- and CD8-positive T cells are essential sources of IFN- γ , but the delayed timing of the adaptive immune response undermines its efficacy and favors pathogen replication.

Most studies of tularemia have focused on mononuclear phagocytes as vehicles for bacterial replication and dissemination from the site of infection to the liver and spleen. Nevertheless, a distinguishing feature of *F. tularensis* is its ability to productively infect many types of cells, including DCs, neutrophils, and epithelial cells, as well as monocytes and macrophages. Neutrophils can account for up to one-half of the infected cells in the lung, and there is strong evidence that PMNs contribute directly to disease progression rather than effective host defense. N-Acetyl Pro-Gly-Pro, a potent neutrophil chemoattractant that is generated when collagen in the ECM is cleaved by MMP-9, is essential for PMN recruitment to *F. tularensis*-infected tissues, and neutrophil accumulation correlates directly with the severity of tissue destruction [4, 10].

In keeping with this, neutrophilia is associated with increased susceptibility to this infection [1, 3]. Conversely, neutrophil recruitment to the lung is diminished profoundly in MMP-9 null mice, and these animals are able to survive infection with type A *F. tularensis* strains as well as LVS [10]. At the single-cell level, *F. tularensis* impairs neutrophil oxidative host defense via effects on NADPH oxidase assembly and activity and as in other cell types, escapes the phagosome to replicate in the cytosol [11, 12]. At the same time, *F. tularensis* profoundly prolongs neutrophil lifespan via effects on the intrinsic and extrinsic apoptotic pathways [12]. As defects in PMN turnover and clearance are emblematic of an ineffective and dysregulated inflammatory response that undermines control of infection and increases the risk of tissue damage by neutrophil progression to secondary necrosis [13], these data suggest a mechanism to account for the role of neutrophils in tularemia pathogenesis.

An important role for the IL-23/Th17 pathway and IL-17A in recruiting neutrophils to sites of infection for phagocytosis and killing of extracellular bacteria and fungi is established. Typically, IL-17A synergizes with TNF- α , IL-1 β , and IL-6 to enhance neutrophil activation and killing capacity. Recent data indicate that IL-17A is present relatively early during *F. tularensis* infection, even though accumulation of most proinflammatory cytokines is diminished and delayed for several days [1, 4]. It is attractive to predict that modulation of the cytokine milieu in this manner may synergize with *F. tularensis* virulence factors to drive neutrophil accumulation, while simultaneously undermining their killing capacity.

It is also clear that neutrophils play an important and previously unappreciated role in regulation of innate and adaptive immunity that is mediated by direct interactions with other leukocytes and by secretion of cytokines and lipid mediators [14]. In this manner, neutrophils influence the function of NK cells and B- and T-lymphocytes, as well as macrophages and DCs. For example, PMNs can directly affect production of IL-12p70 and IFN- γ by DCs and NK cells, respectively, and influence NK cell

survival, while also acting via secreted cytokines to affect the function of various T cell subsets. Thus, the regulatory properties of neutrophils extend well beyond the ability of apoptotic cells to reprogram macrophages toward a pro-resolution phenotype. Whether neutrophils play an immunoregulatory role during tularemia remains to be determined. Nevertheless, this idea is attractive, as it is consistent with the phenotype of MMP-9 null mice, as inhibition of neutrophil influx markedly diminishes morbidity and mortality without significantly altering bacterial burden in the lung, liver, or spleen [10]. Recent data also show that PMNs contribute to cytokine synthesis and secretion in the lungs of *F. tularensis*-infected mice [4]. Of note, enhanced liver pathology and neutrophilia are characteristic features of IL-12R β 2 null mice infected with LVS [3], but whether PMNs directly contribute to the enhanced hepatotoxicity is unclear.

Altogether, the data suggest that the infection of multiple leukocyte types by *F. tularensis* is an important aspect of virulence, with each cell type playing a distinct role in disease progression. Thus, whereas macrophages are major sites of *F. tularensis* replication, bacteria-induced defects in DC activation and migration undermine development of adaptive immunity to this organism. On the other hand, profound neutrophil accumulation, together with defects in cell activation and turnover, plays a central role in dysregulation of the inflammatory response, tissue destruction, and death. Recent studies have also substantially advanced our understanding of protective and detrimental aspects of the cytokine response. These data are summarized in **Fig. 1**. A major challenge in future studies will be to define whether and how direct interactions between leukocytes and their secreted products regulate tularemia progression to the benefit or detriment of the host.

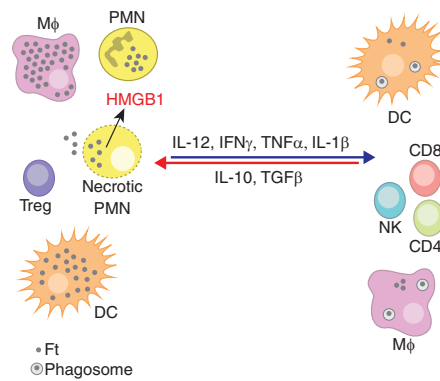


Figure 1. Protective and detrimental responses to *F. tularensis*. The opposing effects of pro- and anti-inflammatory cytokines on the nature of the inflammatory infiltrate and bacterial growth in the cytosol of macrophages (M ϕ), DCs, and PMNs are shown. High levels of IL-12, IFN- γ , and other proinflammatory cytokines control *F. tularensis* (Ft) replication in macrophages and DCs at the level of phagosome escape and replication in the cytosol. *F. tularensis* grows less well in neutrophils, but delayed apoptosis of these cells favors their progression to secondary necrosis. Release of toxic cell contents and alarmins, including high-mobility group box 1 (HMGB1), from dying PMNs amplifies tissue destruction. Treg, Regulatory T cell.

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