Immunity
Previews

HIF1α Allows Monocytes to Take a Breather during Sepsis

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How the immune system is negatively affected by sepsis is not fully understood. In this issue of *Immunity*, Shalova et al. (2015) show that during human sepsis monocytes upregulate hypoxia-inducible factor-α (HIF1-α) activity and acquire an immunosuppressive phenotype while retaining anti-bacterial and wound-healing properties.

Inflammation is a good thing. It removes infections and paves the way for tissue repair and the re-establishment of homeostasis. It's a delicate balance between pro- and anti-inflammatory signals followed by a sequence of pro-resolution events. However, inflammation can also be a very bad thing. During sepsis, for instance, an overexuberant and prolonged activation of the innate immune system results in a "cytokine storm" causing physiological shock and multiple organ failure. That notwithstanding, pharmacological interventions aimed at quelling these unbridled drivers of the innate response have proven largely ineffective in treating the critically ill. Instead, a predominantly anti-inflammatory period—the compensatory anti-inflammatory response syndrome (CARS)—is now regarded as the primary clinical concern. This phase is associated with microcirculatory dysfunction, coagulopathy, catabolic predominance, and bioenergetic failure leading to multi-organ failure (Fullerton and Singer, 2011). However, it is principally marked by vulnerability to hospital-acquired infection and repeated episodes of sepsis. Indeed, the immune component of CARS, described as immunoparalysis, anergy, or leukocyte re-programming, (Hotchkiss et al., 2009) might pose an equal if not a greater threat to the host than the initial "cytokine storm." The proposed mechanisms involved are complex. Loss of key effector cells especially via apoptosis, a shift in cytokines from a pro-inflammatory to an anti-inflammatory profile, alteration in cell-surface receptor expression, and multiple other alterations have been reported. Importantly, gene-expression profiling of the inflammatory response in the critically ill has demonstrated that although there is variability between different initiating stimuli (Tang et al., 2010), a consistent picture emerges regardless of etiology (Xiao et al., 2011) or pathogen (Tang et al., 2008). This indicates that a fundamental human transcriptomic response to severe inflammatory stress might exist across all forms of sepsis. Thus, these data indicate that the etiology of the innate immune response during sepsis episodes might not be as heterogeneous as previously suspected. This renders the regulation of inflammation an appealing therapeutic target in the critically ill. In this issue of *Immunity*, Shalova et al. (2015) have examined the impact of Gram negative-induced sepsis on monocyte-effector function. The overall aim is understand how a severe inflammatory insult negatively affects the innate immune system leading, in turn, to the poor clinical outcome that is typically associated with sepsis. These authors found that the phenotype of monocytes taken from patients within a few hours of infection shifted from a pro-inflammatory to an immune-suppressive phenotype in monocytes taken from patients who recovered. It is proposed that elevated hypoxia-inducible factor-α (HIF1-α) triggers IRAKM, a negative modulator of TLR signaling, leading to an endotoxin-tolerant monocyte that interestingly also acquires anti-bacterial and wound-healing properties.

Sepsis is a complex response to injury and infection, and trying to understand its regulatory pathways is no trivial undertaking. The pathogenesis that underpins its impact on organ dysfunction and its subsequent immune suppression predisposing to nosocomial infection, not the mention its dire long-term mortality, cannot be ascribed to one single cell, soluble mediator, receptor, or signaling factor. Nonetheless, in an attempt to understand the role of at least one of the key protagonists within the pantheon of sepsis, Shalova et al. carried out transcriptomic analysis on circulating monocyte populations from patients with active sepsis (within a few hours of a defined gram-negative infection) and compared this phenotype to monocytes from the same patients 4–12 weeks later, in which they termed "recovery phase." In cells taken from patients with active sepsis the usual pro-inflammatory suspects (nuclear factor-κB [NF-κB], interleukin-6 [IL-6], IL-1β, and chemokines CCL3, CCL5) emerged alongside elevated IL-10 and cell surface markers of immune dysfunction. As expected, a different picture was painted by monocytes taken from those patients who recovered from sepsis being more akin to the transcriptome of monocytes from healthy volunteers. Thereafter, a comparative transcriptomic profile of these respective monocyte populations was obtained after their stimulation with LPS ex vivo. "Recovery monocytes" displayed a range of cytokines and chemokines whereas "sepsis monocytes" were largely refractory to LPS in this regard. Moreover, sepsis monocytes displayed apparent reduced antigen presentation and downregulation of major histocompatibility complex-II (MHC-II) genes and co-stimulatory molecules; the hallmarks of cells experiencing endotoxin tolerance. The authors proposed that elevated HIF1-α and subsequent upregulation of

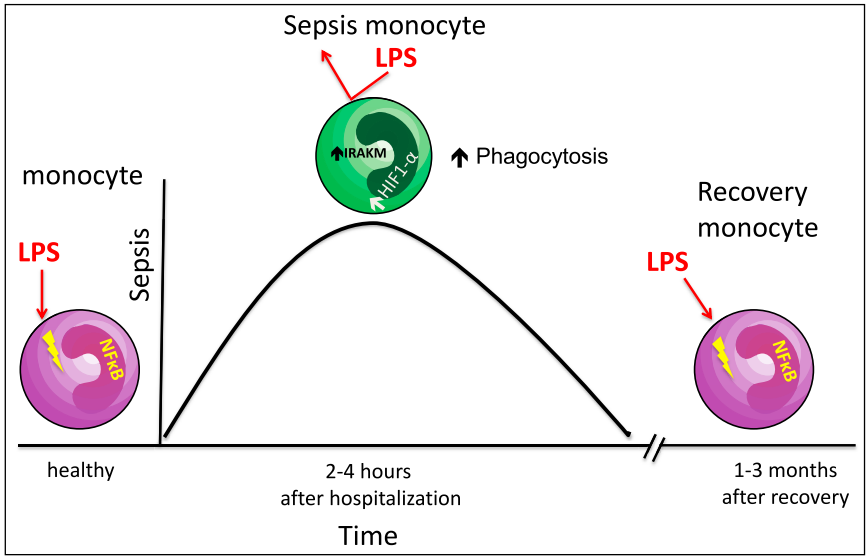

Figure 1. Sepsis-Conditioned Monocyte Plasticity
As a consequence of a Gram-negative bacteria-induced sepsis in adult humans, monocytes upregulate HIF1-α that, in turn, mediates functional reprograming of monocytes. These monocytes acquire an immunosuppressive phenotype compared to monocytes from healthy volunteers or monocytes from patients who have recovered from sepsis stimulated with endotoxin in vitro. It is proposed that HIF1α acts as a regulator of IRAKM, a negative modulator of TLR signaling, leading to an endotoxin-tolerant monocyte.

the negative regulator of TLR signaling IRAKM, mediated this immune anergy in sepsis circulating monocytes, see Figure 1. However, one must be careful not to over-interpret these findings as complete immune paralysis, because the authors also observed an elevation in genes associated with metabolism and phagocytosis in sepsis monocytes stimulated with LPS compared to recovery monocytes. For instance, monocytes from patients experiencing sepsis exhibited apparent increased bacterial phagocytosis and increased expression of the antimicrobial peptide HAMP. Moreover, despite expressing matrix metallopeptidase 9 (MMP9), MMP19, and vascular endothelial growth factor (VEGF), sepsis monocytes stimulated re-epithelialization of wounded fibroblasts in an ex vivo assay of wound healing compared to monocytes from patients who survived sepsis. These findings belie the notion that monocytes from the height of sepsis lack host defense capabilities. Rather, it suggests a form of "homeostatic-to-inflammation" reprogramming, a type of reconfiguration commensurate with its inflammatory environment rather than global suppression.

Shalova et al. shed light on the transcriptome of circulating monocytes taken from humans with sepsis and identified the HIF1α-IRAKM axis as a potential culprit in mediating endotoxin tolerance; a factor that might be responsible for the long term clinical sequelae typical of sepsis. These experiments used conventional adherence assays to isolate monocytes for analysis. Circulating monocytes in the healthy adult are composed of classical monocytes ($CD14^{hi}CD16^{-}$), double-positive monocytes ($CD14^{+}CD16^{+}$), and non-classical monocytes ($CD14^{dim}CD16^{hi}$) (Ziegler-Heitbrock et al., 2010), with distinct monocyte subsets most likely undertaking different effector functions during pathology. A number of studies have demonstrated that the natural equilibrium of these subsets is altered during chronic and acute inflammation including sepsis, liver fibrosis, and rheumatoid arthritis favoring increased $CD14^{+}CD16^{+}$ cells. Whether this is a bona fide quantitative increase in these inflammatory monocytes or simply a sequestration of classical monocytes thereby altering the relative ratio of these cells to one another remains to be verified. Moreover, it's not known whether this ratio reverts back to that experienced during steady state when inflammation resolves. Therefore, the transcriptome signature reported by Shalova and colleagues unlocks new avenues for monocyte research during sepsis. However, the septic phenotype reported needs to be ascribed to a particular monocyte subset or subsets. This would also inform on putative sepsis-induced shifts in monocyte populations during and after sepsis; an undoubtedly invaluable piece of information.

In summary, Shalova et al. have taken the bold step of trying to understand the phenotype of monocyte during sepsis with a view of developing better management protocols and novel treatment regimen for sepsis patients; an area of medicine that has experienced considerable pharmacological failure. An explanation for this failure is frustratingly unclear, particularly given that a fundamental human transcriptomic response to severe inflammatory stress (Xiao et al., 2011) might exist across all forms of sepsis and now between human and rodents (Seok et al., 2013; Shay et al., 2015; Takao and Miyakawa, 2015). Certainly, there are many differences between rodents and humans, including ratios of peripheral blood leukocyte populations, while humans might have comorbidities and be prescribed complex drug cocktails. Moreover, with rodents, tissues can be sampled at specific time points after defined stimulation, whereas samples from humans are most likely derived from patients at various stages of disease progression driven by a less well-defined stimulus. Armed with data from Shalova et al., the next step should be a transcriptomic analysis on monocyte subsets and other innate cells including the indispensable neutrophil, from controlled individuals before, during and after experimentally-induced endotoxemia. Aligning this transcriptomic profile with clinical signs of disease status might provide a window of drug intervention that would have being otherwise obscured by the asynchronous nature of sepsis in the human population. This will provide a treatment regime aimed at targeting pathogenic effector molecules as well as biochemical and molecular pathways that drive sepsis and in particular those responsible for CARS. Results from these experiments might also inform on the abysmal long-term survival rates of patients who experience severe sepsis (5-year mortality rate of >70%). These studies will surely determine how these signatures could be harnessed to ensure a more positive outcome of systemic inflammation.

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Infected Cells Call Their Killers to the Scene of the Crime

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Effector CD8$^+$ T cells scan tissues to locate and kill infected host cells. In this issue of *Immunity*, Hickman et al. (2015) show that the exploration is not random: infected monocytes attract their assassins by secreting chemokines, which accelerates clearance of epicutaneous vaccinia virus infection.

During viral infections, effector CD8$^+$ T cells patrol sites of inflammation and scan host cells for evidence of infection. Virally infected cells announce their infected state to these effector CD8$^+$ T cells by presenting pathogen-derived peptides on their surface major histocompatibility complex (MHC) class I molecules, and this requires contact between both cells. Recognition by CD8$^+$ T cells triggers elimination of the infected host cell, which takes the virus down with it. The amazing thing about this process is the vast number of host cells that might need to be surveyed. How do CD8$^+$ T cells find infected targets among millions of host cells? A number of observed behaviors might increase the efficiency of this process. In cell-dense compartments, such as the epidermis, CD8$^+$ T cells can adopt dendritic morphologies to increase the number of cell contacts (Ariotti et al., 2012). Effector T cells are highly motile within many infected nonlymphoid tissues (Mueller, 2013). Expression of the inflammatory chemokine CXCL10, which is one of two CXCR3 ligands in C57BL/6 mice (CXCL9 is the other), has also been shown to increase the rate of effector CD8$^+$ T cell motility in the brain during chronic *Toxoplasma gondii* infection (Harris et al., 2012). In this study, effector CD8$^+$ T cells have been shown to exhibit specialized migration patterns known as Lèvy walks, which are characterized by short steps within a small area and occasional longer runs. Lèvy walks have been proposed to increase the foraging efficiency of marine predators, insects, and human hunter-gatherers when food is sparse (Raichlen et al., 2014; Viswanathan et al., 1999). Lèvy walk behavior, although still fundamentally random, is believed to allow CD8$^+$ T cells to find rare infected targets with more than an order of magnitude more efficiency than random Brownian motion walks (Harris et al., 2012). A critical question is whether CD8$^+$ T cells searching within nonlymphoid sites of infection are limited to random migration behavior or whether they might also be specifically directed toward infected cells in some contexts.

Hickman et al. explored this question after epicutaneously infecting mice with vaccinia virus, which replicates in both epidermal keratinocytes and dermal inflammatory monocytes (Hickman et al., 2013). They previously reported that Ly6G$^+$ innate immune cells are important for clearance of virus from keratinocytic foci, whereas infected inflammatory monocytes are targeted principally by CD8$^+$ T cells (Hickman et al., 2013). In the current study, they investigated the hypothesis that effector CD8$^+$ T cells within the dermis migrate toward chemokine-mediated cries for help emanating from infected cells.

After vaccinia virus infection, CD8$^+$ T cells became activated and migrated to the infected ear. Transcriptional profiling revealed that compared to uninfected controls, infected inflammatory monocytes showed upregulation of chemokines CXCL9 and CXCL10. The chemokine receptor CXCR3 is expressed on subsets of effector and memory CD8$^+$ T cells and has been implicated in T cell priming, effector differentiation, and migration. Hickman et al. found that CXCR3-deficient mice exhibited a greater number of