The Role of Monocytes in the Progression of Sepsis

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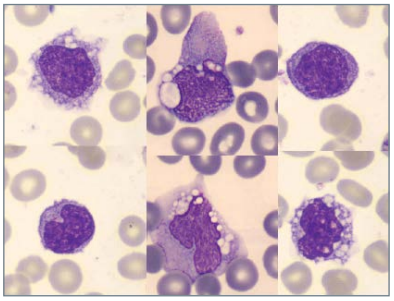
ABSTRACT

The increasing global burden of sepsis in health-care calls for better diagnostic tests that allow earlier detection of sepsis and infections that could lead to sepsis. The major problem for patients at risk for sepsis is an immunological imbalance. Cells of the innate immune system, such as monocytes and neutrophils, are the first-line of defense against infections. In the presence of sepsis, these cells produce a flood of inflammatory cytokines, causing widespread inflammation that can lead to death. Monocytes perform multiple immunological functions, and play a role in the development of sepsis-induced inflammation and immunosuppression. Monocyte subpopulations with different functions and morphologies vary in number over the course of the inflammatory response. The monocyte distribution width (MDW) is a novel cellular marker of monocyte anisocytosis that can add significant value to the white blood cell (WBC) count and help detect sepsis in patients entering the emergency department (ED).



Introduction:

Sepsis epidemiology and definitions

Sepsis is a major healthcare burden and, despite progress in diagnostic and treatment options, mortality from sepsis remains unacceptably high. The number of septic patients in the U.S., UK and EU is increasing.[1–4] Clearly, there is an unmet need for better diagnostic tests that can provide both the early detection of sepsis and the detection of severe infections that may progress to sepsis, if not diagnosed early enough. Global increases in sepsis frequency may be related to the aging population, as the incidence of sepsis is disproportionately increased in elderly adults, and age is an independent predictor of mortality.[5] Furthermore, immunosuppressive drugs, which are increasingly being used for diverse conditions, may result in more severe infections and increased sepsis frequency.[6]

The definition of sepsis has recently been changed from the previous Sepsis-2 definition of a systemic inflammatory response (SIRS) in the presence of an infection,[7] to the current Sepsis-3 definition of a life-threatening organ dysfunction caused by a dysregulated host response to infection.[8] The new Sepsis-3 definition reflects newfound understanding that the immune response in sepsis is more complex than previously thought, comprising both pro- and anti-inflammatory mechanisms.

Immune response in sepsis

It is now clear that the major problem for patients with sepsis, or at high risk of developing sepsis, is immunological imbalance, and dysregulation of the mechanisms of innate and adaptive immunity. Sepsis occurs when the immune system begins, in one way or another, to lose the battle against severe infection. After sepsis onset, the production of pro-inflammatory cytokines (IL-1β, IL-6, and tumor necrosis factor [TNFα]) by the cells of the innate immune system (neutrophils and monocytes) may result in a "cytokine storm" that produces overwhelming inflammation, which can lead to blood pressure collapse, coagulation abnormalities and, ultimately, organ failure and death. In the later stages of disease, patients who survive the cytokine storm may die from sepsis-related immunosuppression and an inability of the immune system to combat infection efficiently.[9] Inflammatory and immunosuppressive processes may overlap in sepsis,[10,11] further complicating the biology of this fatal condition whose mechanisms are still poorly understood by scientists. Figure 1 shows the current understanding of immune imbalance in sepsis.[12] While all immune cells are involved in the immune response in sepsis[13–16] (Figure 2), this document is mainly focused on changes in monocytes, with other cell populations discussed only briefly.

Under normal conditions, neutrophils usually stay in the circulation for only a few hours and undergo apoptosis within 24 hours of release from the bone marrow. In sepsis, the delay in neutrophil apoptosis,[17,18] combined with the increased neutrophil production in the bone marrow, results in neutrophilia. The function of these neutrophils, however, is impaired,[19] with decreased chemotactic activity,[20,21] decreased antibacterial function and increased production of anti-inflammatory cytokine interleukin 10 (IL-10).[22]

Sepsis also has a profound effect on all the main lymphocyte subpopulations:[14] CD4+ T-cells, CD8+ T-cells and B-cells undergo increased apoptosis; T-regulatory cells are more resistant to sepsis-induced apoptosis, leading to an increased proportion of T-regulatory cells and an immunosupressive phenotype. T-helper cell polarization from a pro-inflammatory Th1 phenotype towards an anti-inflammatory Th2 phenotype also contributes to increased immunosuppression in sepsis.

Monocytes also undergo multiple changes in sepsis, but before discussing these phenomena, it is important to discuss some basic information about the biology and classification of monocytes.

Monocytes' biology and classification

Monocytes are cells of the innate immune system, the body's first-line of defense against infection. Other cells of this system include neutrophils, basophils, eosinophils, mast cells, as well as certain types of lymphocytes such as γδ-T-cells and natural killer cells. The innate immune response develops during the first hours and days after pathogen invasion, and the majority of pathogens entering the human body usually are inactivated by this response and do not require adaptive mechanisms with lymphocyte involvement.

Myeloid precursors in the bone marrow differentiate into promonocytes and then into mature monocytes that enter the peripheral blood. These monocytes stay in the circulation

for one to three days, after which they migrate into tissues and organs, where they turn into macrophages and dendritic cells. Morphologically, monocytes are large cells measuring 10 to 18 µm in diameter, with convoluted nuclei and azurophilic granules in their cytoplasm.

Monocytes and dendritic cells perform multiple immunological functions that include phagocytosis, antigen presentation and cytokine production. The function of these cells is regulated by a number of cell surface receptors:

- CD14, the receptor for complexes of bacterial lipopolysaccharides and human serum proteins
- Receptors such as CD163 that scavenge membrane fragments and other components of damaged cells
- Multiple receptors for the Fc regions of IgG: CD64 (FcγR1, high-affinity receptor), CD32 (FcγR2, medium-affinity receptor) and CD16 (FcγR3, present only on subpopulations of so-called pro-inflammatory monocytes)
- Other receptors necessary for interaction with lymphocytes and receptors for cytokines

Three subpopulations of monocytes have been characterized in peripheral blood.[23–25] Classical monocytes make up the main monocyte population. Expressing high level CD14 and no CD16 (CD14++CD16-), they represent 80–90% of monocytes in peripheral blood. “Intermediate” monocytes expressing CD16 (CD14++CD16+) are normally found at low numbers, but increase with cytokine stimulation and inflammation. Nonclassical monocytes display decreased expression of CD14 and increased expression of CD16 (CD14+CD16++), and comprise 9%+/-5% of all monocytes, with an average count in healthy donors of approximately 45+/-22 cells/µL.[26]

In the literature, nonclassical monocytes are sometimes referred to as inflammatory or pro-inflammatory monocytes; however, published recommendations for the nomenclature of monocytes and dendritic cells in the blood clearly advocate avoiding functional terminology, “because this leads to confusion as the label ‘inflammatory’ has been used for different subpopulations in humans and mice.”[24] Also, “these terms may prematurely ascribe functional attributes to cells based on ex vivo studies while they largely remain to be functionally characterized in vivo.”[24] Subsets of nonclassical monocytes are expanded dramatically in several pathological conditions including sepsis,[26–28] HIV-1 infection,[29–33] diabetes,[34–35] tuberculosis[36] and other disease states.[37]

The recent detailed analysis performed by Mukherjee et al.[28] revealed the functions of monocyte subsets as follows: classical monocytes are phagocytic with no inflammatory attributes, nonclassical subtypes display inflammatory characteristics on activation and display properties for antigen presentation, and intermediate subtypes appear to have both phagocytic and inflammatory functions.[28] In 2017, research based on single-cell RNA sequencing discovered even more subtypes, describing six subpopulations of dendritic cells and four monocyte subpopulations.[39] This classification was based solely on transcriptional activity, and further studies will be needed to understand function and describe the phenotype of all cell subpopulations. Nonetheless, it is clear that morphologically similar cells that we call monocytes may actually have very different functions in human immunity.

Monocytes in sepsis

Monocytes, as cells of first-line defense against infection, are involved in the immune response from very early stages. Abundant literature exists on monocytes and the changes they undergo in sepsis.

A recent study on the dynamics of monocyte subpopulations in peripheral blood at the onset of infection has demonstrated a decrease in the number of peripheral blood monocytes during the early stages of lipopolysaccharide (LPS)-induced acute inflammation in humans. This loss may be due to the migration of monocytes from the blood into tissues, where they differentiate into macrophages and dendritic cells, or it may reflect an increase in monocytes residing in the marginal pool or rolling on the vessel walls.[40] For all three subpopulations of monocytes, the number of cells was decreased at one to two hours after LPS injection. This decrease was followed by a return to the baseline count, but with differences in timing for the three monocyte subsets. This difference in timing means that the early stages of infection, before the appearance of any clinical symptoms, are characterized by differences in the proportions of monocyte subpopulations relative to baseline pre-infection proportions.

Functional changes in monocytes and, in parallel, changes in their cellular morphology, have been demonstrated in the past for a human THP-1 monocytic cell line infected with viable *C. pneumonia* bacteria.[41] The differentiation of infected cells into macrophages was accompanied by a change to an amoeboid or diffused morphology as assessed by microscopy after Giemsa staining.

Multiple studies have demonstrated the importance of HLA-DR expression on monocytes as a prognostic marker in septic patients. A decreased level of HLA-DR expression on monocytes has been found to be a negative prognostic indicator[42–44] and may be used to evaluate the functional activity of the immune system.[45,46] Decreased HLA-DR, as a marker of monocyte anergy, correlates with decreased antigen presentation capacity and decreased pro-inflammatory cytokine release. This has been analyzed mainly by flow cytometry, but, recently, new methods based on real-time PCR have emerged.[47,48]

Another monocyte marker, CD16, plays an important role in orchestrating the response of monocytes to Gram-negative sepsis. It has been demonstrated that CD16 on human monocytes is a key regulator of the TRIF-dependent TLR4 signaling pathway, and this pathway is preferentially activated in the CD16+ monocyte subset.[49] Recent publications suggest the variability of monocyte properties in sepsis. Detailed analysis of gene expression in patient monocytes during sepsis and after recovery demonstrated plasticity of monocytes in the course of disease.[50] The significant up-regulation of pro-inflammatory cytokines (IL-1b, IL-6) and chemokines (CCL3 and CCL5) has been demonstrated in sepsis monocytes compared to monocytes after recovery. Transcriptional factor NF-kB, a central transcriptional regulator of the inflammatory response, was also activated in sepsis monocytes, supporting their involvement in severe inflammation. At the same time, anti-inflammatory cytokine IL-10 was found to be up-regulated in sepsis monocytes. These studies

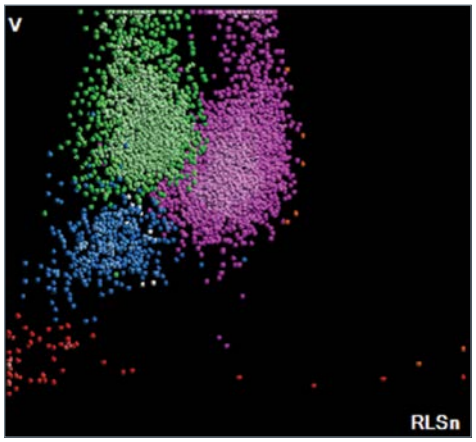
once again highlight the diversity of monocytes' function in sepsis pathogenesis, and their key role in disease progression, with the possible polarization from a pro-inflammatory state to an immunosuppressive state.

More recently, Crouser et al. demonstrated that the morphological variability that occurs during monocyte activation in the early inflammatory response can be captured by measuring the monocyte distribution width (MDW), an indicator of monocyte anisocytosis. Investigators showed that MDW could be a novel cellular marker that may help detect sepsis early in patients admitted to the emergency department (ED).[51] Multiple morphometric characteristics of monocytes were obtained using a DxH 800 cellular analysis system, which employs physical measurement of cell volume, conductivity and multiple angles of laser scatter to classify leukocytes into five sub-populations and detect the presence of abnormal cells. This study showed that anisocytosis of circulating monocytes provides significant added value to WBC count for the detection of sepsis in the ED population.

Conclusion

In summary, monocytes are a very heterogeneous population of cells that differ in phenotype, size, nuclear morphology, gene profile and function.[52] In sepsis, this diversity is even more pronounced due to functional changes of monocyte subsets, and is accompanied by a variation in monocyte morphology.

Morphological variability is just the tip of the iceberg of the underlying biological heterogeneity, and may be an important early marker of sepsis or severe infections with a high risk of progressing to sepsis. A recent publication from Crouser,[51] together with previous research on sepsis using cellular morphometric parameters gathered using a DxH 800 analyzer,[53–56] may build the foundation for practical usage of MDW in combination with currently used sepsis markers (WBC, PCT, CRP, IL-6) for early sepsis screening and diagnosis, leading to early initiation of appropriate therapy.



VCS Diff 1 plot from patient with sepsis

Figure 1. Immune dysregulation in sepsis

From: Delano MJ, Ward PA. “Sepsis-induced immune dysfunction: can immune therapies reduce mortality?” J Clin Invest, 2016, vol. 126, no. 1, pp. 23–31.

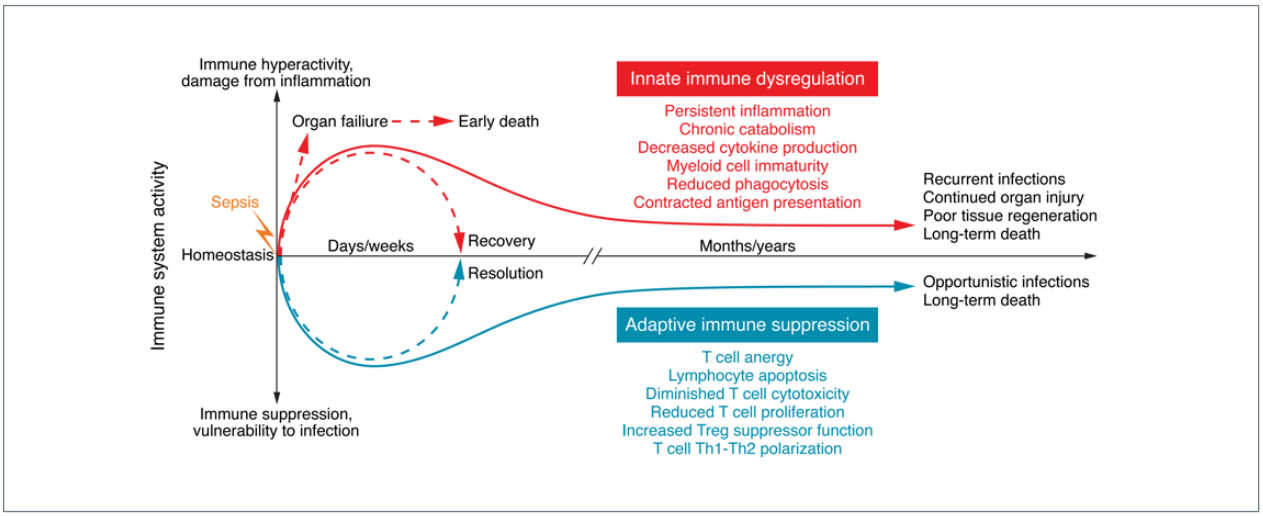
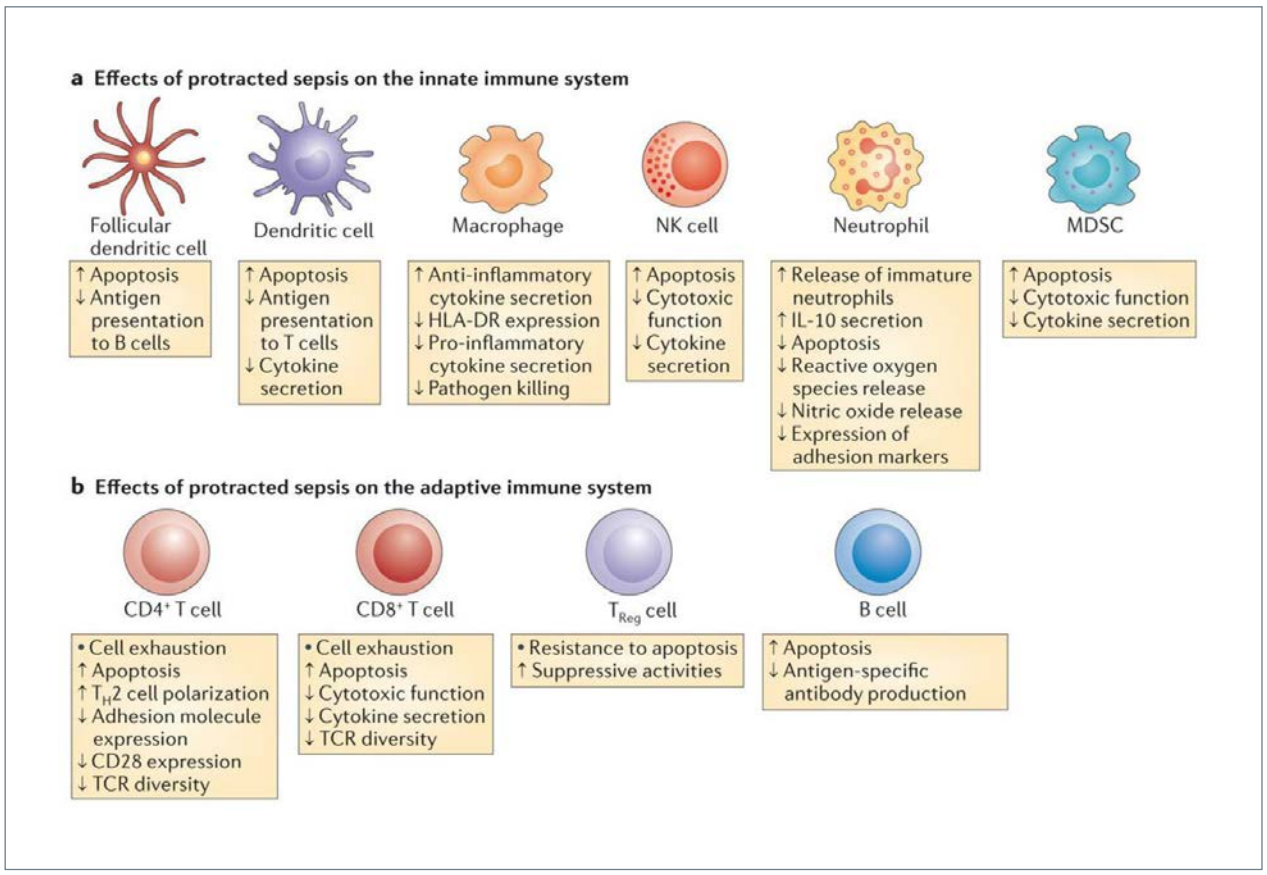


Figure 2. Impact of sepsis on innate and adaptive immunity

From: Richard S. Hotchkiss, Guillaume Monneret, and Didier Payen. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013 December; 13(12): 862–874. doi:10.1038/nri3552.



a) Sepsis has diverse and profound effects on all cellular elements comprising the innate immune system. Sepsis rapidly triggers extensive apoptosis in dendritic cells, monocytes and immature macrophages, natural killer (NK) cells and myeloid-derived suppressor cells (MDSCs). Conversely, sepsis delays neutrophil apoptosis, a result thought to be secondary to the mechanisms of neutrophil activation. After initial mobilization and activation of neutrophils, subsequent neutrophils that are released from bone marrow have lower bactericidal functions and decreased cytokine production. Recent data show that a subset of neutrophils release large amounts of the immunosuppressive cytokine interleukin-10 (IL-10). Decreased HLA-DR expression on antigen presenting cells including monocyte/macrophages and dendritic cells is a hallmark of sepsis, which may impair the optimal presentation of microbial antigens to T cells.

b) Sepsis causes massive loss of CD4+ and CD8+ T cells as well as B cells. T regulatory (TReg) cells are more resistant to sepsis-induced apoptosis, and, consequently, there is an increased percentage of TReg cells in the circulation relative to the other lymphocyte subsets. This contributes on a more immunosuppressive phenotype. Surviving CD4+ and CD8+ T cells have either a shift from a pro-inflammatory Th1 cell to an anti-inflammatory Th2 cell phenotype, or develop an “exhaustive” phenotype, characterized by increased programmed cell death-1 expression and reduced cytokine secretion. CD4+ T cells have decreased expression of CD28 and reduced T cell receptor (TCR) diversity, which both likely contributing to the impaired anti-microbial response to invading pathogens.

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