

TLR2-mediated neutrophil depletion exacerbates bacterial sepsis

Thierry Roger and Thierry Calandra¹

Infectious Diseases Service, Department of Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, CH-1011 Lausanne, Switzerland

The host antimicrobial defense response is initiated by the innate immune system, the most ancient of the 2 limbs of the immune system. The innate immune system is activated as soon as a pathogen crosses the host external defense barriers, lasts for a few hours, and is aimed at the elimination of the invading microorganism. Detection of microbial pathogens is first carried out by sentinel cells [macrophages and dendritic cells (DCs)] located in tissues that are in direct contact with the host's natural environment and are rapidly recruited to the site of infection (neutrophils). This process involves coordinated actions of soluble and cellular molecules comprising components of the complement system, acute phase proteins such as the lipopolysaccharide-binding protein, extracellular and intracellular pattern-recognition types of molecules including the Toll-like receptors (TLR), the nucleotide-binding oligomerization domain-like receptors, RIG-I-like helicases, C-type lectin, and scavenger receptors. Recognition of invasive pathogens by immune cells relies on their capacity to detect microbial molecular motifs (for example, endotoxin, peptidoglycan subcomponents, lipopeptides, glucans, mannans, flagellin, and nucleic acids) via pattern-recognition receptors or molecules (1, 2).

Granulocytes—neutrophils, also known as polymorphonuclear cells, are phagocytic cells that play a crucial role in the host defense mechanisms against bacterial infections, as illustrated by the increased susceptibility to infection and sepsis of individuals with congenital or acquired neutropenia or defects of neutrophil functions (3, 4). Neutrophils are produced and terminally differentiate in the bone marrow by a highly-regulated process (granulopoiesis) before being released into the blood stream where they account for ≈60% of all circulating leukocytes. The majority of mature neutrophils resides in the bone marrow and circulating neutrophils represent only 1–2% of the total neutrophil pool (lifespan of 6–10 h). The bone marrow provides a huge reserve of neutrophils that are rapidly mobilized during infection. The main hematopoietic cytokine regulating granulopoiesis is granulocyte colony-

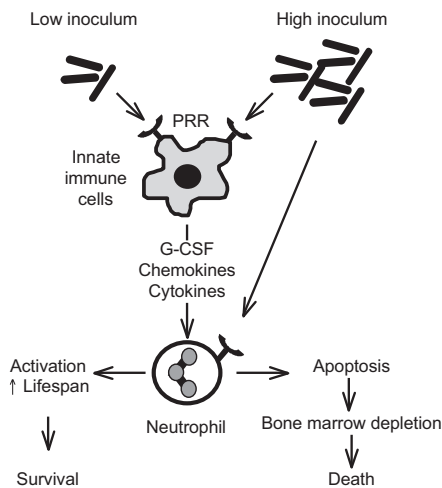


Fig. 1. Effect of a low or a high inoculum of *L. monocytogenes* on neutrophil. Bacteria interacts with pattern-recognition receptor (PRR) expressed by innate immune and bone marrow stromal cells to induce production of G-CSF, chemokines, and cytokines, resulting in granulopoiesis and neutrophil activation. Bacterial sepsis is known to augment the lifespan of neutrophils. Navarini et al. (7) showed that infection with a low *L. monocytogenes* inoculum augmented the lifespan of neutrophils, resulting in the clearance of bacterial infection and survival of mice. By contrast, infection with a high *L. monocytogenes* inoculum caused apoptosis of neutrophils, rapid bone marrow depletion, proliferation of bacteria in liver and spleen, dissemination into the blood stream, and death.

stimulating factor (G-CSF). Blood concentrations of G-CSF are very low in healthy individuals and increase markedly during infection to increase neutrophil number and enhance the host antimicrobial defense response. Recombinant G-CSF is used to prevent or reverse neutropenia in various clinical settings and as experimental adjunctive therapy for sepsis that yielded disappointing results (5, 6).

In this issue of PNAS, Navarini et al. (7) report the results of studies of the role of innate immune-induced neutrophil depletion in an experimental model of infection caused by *Listeria monocytogenes*, an opportunistic Gram-positive bacillus and food-borne pathogen causing serious infections in pregnant women and immunocompromised hosts associated with preterm deliveries, neonatal sepsis, and meningitis

(8). Whereas mice survived infection caused by a low *L. monocytogenes* inoculum, they rapidly succumbed from systemic infection when challenged with a high inoculum (Fig. 1). Analyses of cellular responses during the early phase of *L. monocytogenes* infection revealed striking differences in the kinetics of neutrophil counts that were associated with the outcome of infection.

The natural response to sublethal listeriosis was characterized by a robust and transient infiltration of liver and spleen by neutrophils associated with bacterial clearance. Bone marrow neutrophil counts were reduced during the first 3 days of a sublethal infection because of cell egress into blood and migration to infected organs. This was followed by a rapid return to subnormal counts caused by stimulation of granulopoiesis by increased G-CSF concentrations. In contrast, challenge with a high bacterial inoculum led to a massive depletion of bone marrow neutrophils within 3 days after the onset of infection and a rapid disappearance of neutrophil liver and spleen infiltrates despite the presence of large numbers of bacteria and death (Fig. 1). Immuno-depletion of granulocytes with antigranulocyte receptor-1 (Gr-1) antibodies was also shown to enhance the susceptibility to listeriosis, confirming the critical role played by neutrophils in the outcome of infection.

What was the cause of neutrophil depletion in the bone marrow during the course of *L. monocytogenes* infection? Navarini et al. (7) convincingly showed that neutrophil apoptosis was massively augmented between days 1 and 3 in mice challenged with a high, but not a low, bacterial inoculum. Interestingly, mortality was increased by the injection of heat-killed *L. monocytogenes* during the course of an otherwise nonlethal infection. This led Navarini et al. to explore the role of bacterial cell wall components in this process. Pam₂Cys, a synthetic lipopeptide analogous to the

Author contributions: T.R. and T.C. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 7107.

¹To whom correspondence should be addressed. E-mail: thierry.calandra@chuv.ch.

lipopeptides of Gram-positive bacteria and potent TLR2 activator of innate immune cells, was found to increase apoptosis of bone marrow neutrophils, bacterial counts in liver and spleen, and mortality. An intriguing observation was the dichotomy between a lack of deleterious effects of TLR2 deficiency on the outcome of listeria sublethal infection and the detrimental effects of high amounts of Pam₂Cys lipopeptide. Of note, activation of the TLR2 pathway has been recently reported to inhibit CXCR2 chemokine receptor expression by neutrophils, thereby impairing their migration to the site of infection and increasing mortality in a model of cecal ligation and puncture polymicrobial sepsis (9). Thus, depletion of bone marrow neutrophil reserve via increased apoptosis and inhibition of the migratory capacity of neutrophils are 2 mechanisms whereby TLR2 agonists may play a detrimental role during the course of sepsis. In addition to listeriosis, activation of the TLR2 signaling pathway impaired host defenses against sepsis induced by extracellular Gram-positive pyogenic bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*) and intracellular Gram-negative bacteria (*Salmonella typhimurium*). The deleterious effect of an overwhelming activation of the TLR pathway was also not limited to TLR2 agonist. Indeed, lipopolysaccharide (LPS or endotoxin), a powerful proinflammatory component of the outer membrane of Gram-negative bacteria and TLR4 agonist, also increased the rate of neutrophil apoptosis and bone marrow neutrophil depletion. These observations are another illustration of how microbial products impact natural defenses against infection.

The control of neutrophil apoptosis during infection is far more complex than just a question of life or death. On one hand, proinflammatory mediators and microbial compounds produced or released during infection have been reported to activate and extend the lifespan of neutrophils, which is a key feature of host defenses. On the other hand, a high number of activated and

proinflammatory neutrophils may contribute to tissue injury and death. The data by Navarini et al. (7) also suggest the possibility that endogenous danger signals released in response to tissue damage (i.e., necrosis) induced by neutrophils may act as TLR2 agonists also contributing to bone-marrow neutrophil depletion. Activation of an apoptotic program is critical for terminating the potentially harmful facet of the host antimicrobial defensive response. Ingestion and destruction of apoptotic neutrophils by macrophages and DCs at a site of inflammation or in the bone marrow is a major clearance mechanism of dying cells. Although that process serves to resolve inflammation and protect tissue

Activation of the TLR2 signaling pathway impaired host defenses against sepsis.

injury mediated by the release of toxic products by senescent neutrophils, it also promotes an immunosuppressive environment for surviving immune cells. Uptake of apoptotic cells by macrophages and DCs skew the pattern of cytokines toward the release of antiinflammatory cytokines and does not stimulate the expression of costimulatory molecules by DCs. As a consequence, T cells that come into contact with DCs do not receive appropriate signals for their activation and expansion, becoming anergic or undergoing apoptosis (10, 11). Therefore, massive apoptosis of neutrophils during bacterial infection may impair host defenses through at least 2 mechanisms: first, it depletes critical effector cells involved in the recognition and destruction of the invading microorganisms; second, it favors the establishment of an immunosuppressive state that may impact morbidity and mortality in septic patients.

Navarini et al. (7) provide evidence in favor of the existence of a novel mechanism whereby activation of the TLR pathways (i.e., TLR2 and TLR4) by microbial products results in a potentially deleterious counterregulation of the neutrophil lifespan in the absence of concomitant antibiotic therapy. Challenging this view, a recent publication (12) suggested that phagocytosis of infected apoptotic neutrophils by DCs preferentially induce a Th17 cell response promoting inflammation.

As is the case with novel findings, the results of the studies by Navarini et al. (7) trigger several obvious questions that might be the topic of further investigations. What is the nature of the molecule released by *L. monocytogenes* inducing neutrophil apoptosis? Does this molecule act directly or indirectly on neutrophils? What are the mechanisms whereby microbial product-induced and TLR2-mediated effects lead to apoptosis of bone marrow neutrophils? Are these effects mediated by proinflammatory or antiinflammatory mediators? Exuberant apoptosis of lymphocytes and DCs, but not neutrophils, has been detected in postmortem studies of patients who died of severe sepsis or septic shock (10). We might therefore speculate that inhibition of apoptosis rather than stimulation of granulopoiesis with G-CSF (an unsuccessful adjunctive therapy of sepsis) might be a better treatment option for septic patients as suggested by the work of Hotchkiss and Nicholson (10). This hypothesis is supported by experimental data demonstrating that inhibition of the apoptotic pathway improved survival in preclinical models of sepsis (10). Taken together with recent evidence supporting anti-TLR treatment strategies for Gram-negative sepsis (13), the data by Navarini et al. (7) provide strong conceptual support to TLR-targeted ongoing sepsis therapy also as a means of modulating neutrophil lifespan.

ACKNOWLEDGMENTS. This work was supported by Swiss National Science Foundation Grants 3100-118266.07 (to T.C.) and 310000-114073 (to T.R.), the Leenaards Foundation, and the Santos-Suarez Foundation for Medical Research.

- Beutler B, et al. (2006) Genetic analysis of host resistance: Toll-like receptor signaling and immunity at large. *Annu Rev Immunol* 24:353–389.
- Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S (2008) Host innate immune receptors and beyond: Making sense of microbial infections. *Cell Host Microbe* 3:352–363.
- Lekstrom-Himes JA, Gallin JI (2000) Immunodeficiency diseases caused by defects in phagocytes. *N Engl J Med* 343:1703–1714.
- Marchetti O, Calandra T (2004) *Infections in the Neutropenic Patient in Infectious Diseases*, eds Cohen J, Powderly WG (Mosby, London), pp 1077–1092.
- Brown KA, et al. (2006) Neutrophils in development of multiple organ failure in sepsis. *Lancet* 368:157–169.
- Kaushansky K (2006) Lineage-specific hematopoietic growth factors. *N Engl J Med* 354:2034–2045.
- Navarini AA, et al. (2009) Innate immune-induced depletion of bone marrow neutrophils aggravates systemic bacterial infections. *Proc Natl Acad Sci USA* 106:7107–7112.
- Pamer EG (2004) Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol* 4:812–823.
- Alves-Filho JC, et al. (2009) Regulation of chemokine receptor by Toll-like receptor 2 is critical to neutrophil migration and resistance to polymicrobial sepsis. *Proc Natl Acad Sci USA* 106:4018–4023.
- Hotchkiss RS, Nicholson DW (2006) Apoptosis and caspases regulate death and inflammation in sepsis. *Nat Rev Immunol* 6:813–822.
- Rittirsch D, Flierl MA, Ward PA (2008) Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* 8:776–787.
- Torchinsky MB, Garaude J, Martin AP, Blander JM (2009) Innate immune recognition of infected apoptotic cells directs T(H)17 cell differentiation. *Nature* 458:78–82.
- Roger T, et al. (2009) Protection from lethal gram-negative bacterial sepsis by targeting Toll-like receptor 4. *Proc Natl Acad Sci USA* 106:2348–2352.