Commentary

RAGE: Exacting a toll on the host in response to polymicrobial sepsis and *Listeria monocytogenes*

Raphael Clynes[1], Kevan Herold[2] and Ann Marie Schmidt[3]

[1]Department of Medicine and Microbiology, Columbia University Medical Center, 630 West 168th Street, New York, NY 10032, USA
[2]Department of Medicine and Immunobiology, Yale University School of Medicine, 10 Amistad Street, 131D, New Haven CT 06520, USA
[3]Division of Surgical Science, Department of Surgery, Columbia University Medical Center, 630 West 168th Street, P&S 17-401, New York, NY 10032, USA

Corresponding author: Ann Marie Schmidt, ams11@columbia.edu

Published: 28 December 2007
This article is online at http://ccforum.com/content/11/6/183

Critical Care 2007, **11:**183 (doi:10.1186/cc6193)

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Abstract

The receptor for advanced glycation endproducts (RAGE) has complex roles in the immune/inflammatory response. RAGE is expressed on monocytes/macrophages, T and B lymphocytes, and dendritic cells. Previous studies illustrated that homozygous *RAGE*$^{-/-}$ mice subjected to overwhelming bacterial sepsis displayed normal clearance of pathogenic bacteria and significantly increased survival. In this issue of *Critical Care*, Lutterloh and colleagues confirm these findings and provide evidence that blocking antibodies to RAGE afford similar protection in mice, even when administration of anti-RAGE is delayed by 24 hours. Furthermore, these authors illustrate that deletion of *RAGE* is remarkably protective in mice infected with the intracellular pathogen *Listeria monocytogenes.* In this Commentary, we consider these findings and propose possible mechanisms by which RAGE exacts a heavy toll on the host in response to polymicrobial sepsis and *L. monocytogenes.*

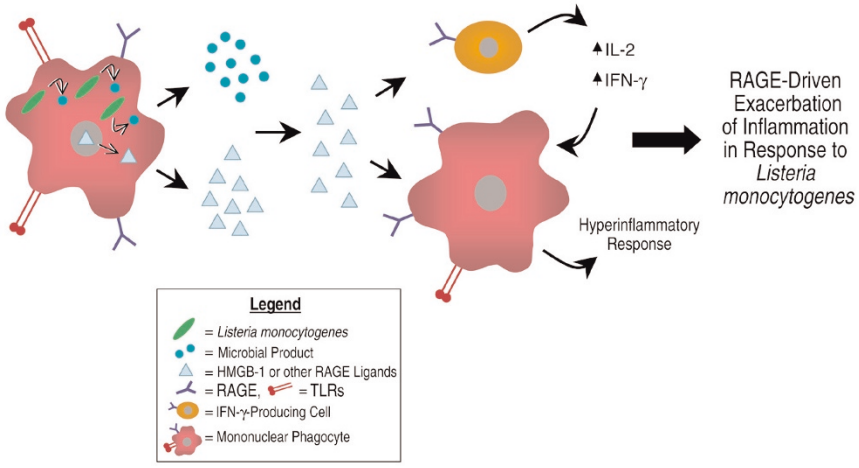
The receptor for advanced glycation endproducts (RAGE) plays central roles in the immune/inflammatory response. In this issue of *Critical Care*, Lutterloh and colleagues [1] tested roles for RAGE in two distinct models of severe infection in mice: (a) that induced by cecal ligation and puncture and (b) overwhelming infection with *Listeria monocytogenes*. RAGE is a multi-ligand receptor that binds fundamental regulatory molecules of inflammatory responses, the S100/calgranulins and high-mobility group box-1 (HMGB-1). The beneficial effects of ligand-RAGE blockade were observed in delayed-type hypersensitivity reactions, collagen-induced arthritis, experimental autoimmune encephalomyelitis, and alloimmunity, for example [2]. RAGE is expressed by multiple cell types implicated in the immune/inflammatory response, such as monocytes/macrophages, T and B lymphocytes, and dendritic cells. Ligand-RAGE interaction activates monocytes/macrophages, and recent studies provide compelling evidence for the role of RAGE in effective T-lymphocyte priming. *In vivo* and *in vitro*, T lymphocytes devoid of RAGE display markedly reduced proliferative and cytokine responses (interferon-γ and interleukin-2) to nominal or allo-antigen [3]. Lutterloh and colleagues show that RAGE is not essential for clearance of pathogenic bacteria in polymicrobial sepsis or to *L. monocytogenes*. Rather, they illustrate that deletion of *RAGE* enhances survival compared with wild-type mice. How may we explain these findings?

In confirming the results of others in *RAGE*$^{-/-}$ mice [4], these authors showed that homozygous or heterozygous deletion of the *RAGE* gene was strongly protective in mice subjected to cecal ligation and puncture. Furthermore, administration of blocking monoclonal antibodies to RAGE, even when delayed by 24 hours, afforded survival benefit in mice subjected to this procedure. These data provide compelling evidence that RAGE is not required for fundamental innate responses that clear bacteria. Rather, RAGE may mediate hyperinflammatory responses to the invading bacteria that are injurious to the host. Clues that this is a likely explanation have come from the novel findings of Lutterloh and colleagues in *L. monocytogenes*-challenged *RAGE*$^{-/-}$ mice.

These authors challenged mice with *L. monocytogenes* and report that *RAGE*$^{-/-}$ mice or *RAGE*$^{+/-}$ mice displayed an LD_{50} (median lethal dose) that was more than two orders of magnitude higher than that of wild-type mice. In BALB/c mice, administration of anti-RAGE antibody offered significant protection against listeriosis. Importantly, bacterial counts did not differ among *RAGE*$^{-/-}$ and antibody-treated mice compared with controls.

HMGB-1 = high-mobility group box-1; Myd88 = myeloid differentiation factor 88; RAGE = receptor for advanced glycation end products.

Figure 1



Proposed model of how the receptor for advanced glycation end products (RAGE) mediates tissue injury in listeriosis. Lutterloh and colleagues [1] show that in polymicrobial gut sepsis and in mice infected with *Listeria monocytogenes*, RAGE is not essential for host clearance of bacteria. In the case of listeriosis, macrophages release microbial products in the early response to infection. Although we do not have evidence that bacterial products directly bind RAGE, we predict that *Listeria*-infected macrophages release high-mobility group box-1 (HMGB-1) (or perhaps other RAGE ligands as well). HMGB-1 may stimulate interferon-γ (IFN-γ)-producing cells and thus mediate macrophage activation. Furthermore, HMGB-1 may directly activate macrophages via RAGE and/or Toll-like receptors (TLRs). Together, these processes synergize to stimulate hyperinflammatory responses that ultimately cause severe injury to the host. IL-2, interleukin-2.

As recently reviewed by Pamer [5], in the first few days of *Listeria* infection, the innate response is critical for early bacterial clearance and host survival. The adaptive response instead is required for controlling chronic, but not acute, infection since SCID (severe combined immunodeficiency disease) mice survive early listeriosis normally, but ultimately this infection is lethal due to long-term failure to clear the organism [6,7]. In the initial phase of infection, *Listeria* binds to splenic macrophages and is internalized; *Listeria* produces products that activate nuclear factor-kappa B and upregulate innate immune molecules such as CC-chemokine ligand CCL2 [5]. Infected macrophages then release microbial products and engage Toll-like receptors (TLRs). Via myeloid differentiation factor 88 (Myd88), these macrophages differentiate into TNF (tumor necrosis factor)- and iNOS (inducible nitric oxide synthase)-producing cells that directly promote bacterial killing. Innate immune responses are thoroughly essential for host survival to *Listeria* as mice deficient in *Myd88* are exquisitely vulnerable to this bacterium [8]. Interestingly, mice deficient in either *TLR-2* or *TLR-4* display relatively normal resistance to *Listeria* [8,9], suggesting that compensation by other *TLRs* may override the loss of a single *TLR* and permit macrophage activation and bacterial killing. Unlike the TLRs, RAGE is not likely to be activated directly by microbial products. However, RAGE ligands inducibly expressed upon macrophage activation may potentiate initial innate activation and the systemic inflammatory response.

It is well established that in listeriosis, production of interferon-γ presumably by natural killer cells or T lymphocytes is critical for macrophage activation and initial bacterial clearance as well as for promotion of long-term protective cellular immunity [10]. Herein lies an intriguing piece of the puzzle; *RAGE*$^{-/-}$ mice displayed strikingly decreased levels of interferon-γ compared with wild-type mice in listeriosis yet were significantly protected from the injurious effects of the microorganism. In addition to revealing that production of this cytokine is not absolutely linked to the initial clearance of *L. monocytogenes*, these findings are consistent with the notion that the RAGE-induced proinflammatory state may be deleterious in the early stages of infection yet may ultimately promote protective adaptive Th1 cellular immunity. Thus, we may predict that deletion of *RAGE* or RAGE blockade suppresses interferon-γ-propagated macrophage activation and the hyperinflammation response that injures the host. However, since RAGE is critical for the Th1 adaptive response [3], it will be important to address the effect of *RAGE* deficiency or blockade on long-term anti-*Listeria* immunity. Note that Lutterloh and colleagues examined interferon-γ levels at 48 hours post-infection and in plasma only, not tissue. Thus, it remains possible that levels of this cytokine in *RAGE*$^{-/-}$ mice might have been different at distinct sites or time points in the infection.

How may RAGE signaling pathways be specifically recruited in listeriosis? Adaptive RAGE-dependent mechanisms may contribute to production of interferon-γ. What, then, overrides the otherwise protective effects of interferon-γ production? We propose that stimulated macrophages, in addition to releasing microbial products in the early response to infection

[11], release the RAGE ligand HMGB-1 [12,13]. HMGB-1, which may engage TLR-2 and TLR-4 [14], as well as RAGE, might activate signaling systems that stimulate hyperinflammatory responses (Figure 1).

In conclusion, in polymicrobial sepsis and in response to *L. monocytogenes*, genetic deletion of *RAGE* is remarkably protective. RAGE is not directly required for bacterial clearance, but the compelling experiments of Lutterloh and colleagues confirm that RAGE action mediates tissue damage initiated in response to overwhelming bacterial infection. Future studies must dissect the precise mechanisms by which RAGE exacts a heavy toll on the host during efforts to combat pathogenic bacteria.

Competing interests

AMS receives research support from TransTech Pharma, Inc. and is a member of their Scientific Advisory Board.

Acknowledgments

The authors gratefully acknowledge the support of the Juvenile Diabetes Research Foundation and the US Public Health Service.

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