Location, location, location—Unraveling the nuances of innate immune regulation*

Multicellular organisms have evolved in a complicated world. Potential threats are ubiquitous, and the capacity to mount a rapid and effective response to infection or tissue injury is a prerequisite for survival. Yet, threats are relative, and what is threatening in one context may bring benefit beneficial in another. Thus, a key challenge for innate host immune defenses is to respond when needed, but at other times, to remain quiescent in response to the same stimulus.

The normal gastrointestinal tract, for example, harbors an extraordinarily diverse array of microbial species (1), along with gram quantities of endotoxin—a microbial product whose systemic toxicity is experienced at nanogram doses. This flora is necessary for normal intestinal development and for optimal maturation of the immune system. Yet, a mechanical breech in the physical barrier of the gut can produce a devastating infection that triggers a massive innate host response. A healthy immune system must ignore commensal microorganisms when they are within the lumen of the gut, but respond aggressively when they enter the body (2). It must be oblivious to a foreign antigen when that antigen is a foodstuff, but mount a specific response to it when it is a virus or other intestinal pathogen. Furthermore, it must be capable of turning off an activated response so that bystander damage is limited, and the processes of tissue repair can proceed. A recognition that the immune system is capable of such discrimination and nuanced responsiveness, and can become tolerant to stimuli that hold the potential to bring harm, is leading to a refinement of classical views of the immune system, and so providing valuable new insights into a spectrum of diseases characterized by dysregulation of normal tolerogenic mechanisms (3, 4).

Multiple mechanisms underlie the capacity of the immune system to respond selectively and differentially to a single stimulus. Among the cellular elements of the innate immune system, macrophages play a particularly important role in immune regulation—recognizing pathogen invasion, releasing chemoattractants that recruit neutrophils and cytokines that modulate the local environment to optimize host defense mechanisms, and interacting with cells of the adaptive immune system to promote a specific antibody- or cell-mediated immune response. It has recently been appreciated that these diverse functions are facilitated by the presence of distinct subpopulations of macrophages and by the capacity of macrophages to become polarized toward specific functional phenotypes known as M1 and M2 macrophages (5). M1 macrophages become activated by interferon-γ or microbial products and produce inflammatory cytokines and reactive intermediates of oxygen and nitrogen. M2 macrophages, however, are activated by interleukin-4 and interleukin-13, produce anti-inflammatory mediators such as the interleukin-1 receptor antagonist, and promote tissue repair and remodeling (6), and become tolerant to stimulation by endotoxin (7).

In this issue of *Critical Care Medicine*, Philippart and coauthors (8) from the Institut Pasteur report experimental studies that shed further light on the subtleties of macrophage function following exposure to a prototypical inflammatory stimulus, endotoxin. Using a variety of genetically modified mouse strains, they show that whereas circulating monocytes or macrophages harvested from the peritoneal cavity can be tolerized to endotoxin, alveolar macrophages cannot. They further show that resistance to tolerization in alveolar macrophages is dependent on interferon-γ from B cells interacting with the macrophage interferon-γ receptor and is further prevented when interleukin-18—also known as interferon-γ–inducing factor—is genetically deficient. In contrast to peritoneal macrophages, therefore, alveolar macrophages maintain an M1 phenotype, even in the presence of repeated exposure to endotoxin. Factors present in the microenvironment of the lung and, in particular, interferon-γ appear to be responsible. The finding that local B cells may be the source of the interferon-γ that blocks polarization to an M2 phenotype is particularly noteworthy. Their work raises two key questions of interpretation: why might this distinct property of alveolar cells arise, and what does it potentially mean in the context of disease pathogenesis? While animal studies can provide compelling information on *in vivo* biology, they are limited in their potential to support an interpretation of that biology. Nonetheless, because the findings are convincing, and this is simply an invited editorial commentary, let me make some suggestions.

Alveolar macrophages serve an important role on the front line of pulmonary defenses. They are continually exposed to potentially harmful forces in the air the animal breathes—bacterial, viruses, dust, etc. While much of this is filtered out by upper airway defenses, low level ongoing exposure is inevitable and the delicate architecture of the alveolus favors entry into the host. Tolerization under these circumstances would be distinctly maladaptive, for it would prevent a robust local response to a recurring threat. Indeed it has been reported that, in contrast to administration of endotoxin into the upper airways, the administration of endotoxin directly into the alveoli incites a potent response including neutrophil recruitment and increased systemic levels of interleukin-6 (9). Systemic challenge, whether within the circulation or the peritoneal cavity, occurs infrequently

*See also p. 2987.

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and usually as a single large event; tolerization under these circumstances would serve to limit ongoing damage and promote tissue repair. Macrophage polarization to either an M1 nontolerizing or an M2-tolerant phenotype is plausibly linked to prior symbiotic exposure to microorganisms, and it would be instructive to evaluate the tolerogenic capacity of alveolar and peritoneal macrophages harvested from mice raised under strict germ-free conditions. The phenomenon of oral tolerance—a state of nonresponsiveness to enterally delivered antigen—requires the presence of an intact flora, and germ-free mice can be rendered tolerant by exposure to endotoxin (10).

If the capacity of the alveolar macrophage to resist tolerization has adaptive benefits in health, what is the consequence in disease? The lung of the critically ill patient is repetitively exposed to stimuli that can evoke macrophage activation, including injury from overdistension (11), bacteria from the upper airways and endotracheal tube, and endotoxin in the inhaled gases (12). The failure of tolerization may contribute to sustained local inflammation and the development of acute respiratory distress syndrome. The lung is particularly susceptible to acute inflammatory injury in critical illness, and acute respiratory distress syndrome is a common and prominent manifestation, while even in the setting of massive bacterial contamination in peritonitis, the normal biologic response is containment and activation of coagulation creating an abscess, rather than diffuse ongoing inflammatory injury. Endotoxin is present in cigarette smoke (13), and chronic exposure to this potent inflammatory stimulus may contribute to the changes of chronic lung disease.

Finally, the identification of a dynamic process, through which tissue macrophages can modify their functional phenotype between an aggressive proinflammatory state and a tolerant one, raises the possibility that macrophage polarization might be amenable to therapeutic manipulation. Epigenetic alterations in heterochromatin formation have been shown to silence inflammatory gene expression during endotoxin tolerance (14, 15), pointing to novel targets for future intervention.

A model of innate immunity that dichotomizes responses as pro- or anti-inflammatory and that seeks to modulate these by inhibiting or stimulating immunity has proven to be of limited utility as we seek effective means to modulate the host response in critical illness. A more nuanced view that addresses context, location, resolution, and the dynamic and changing nature of that response is needed.

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