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Commentary

The Software and Hardware of Macrophages: A Diversity of Options

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Macrophages play important immune and homeostatic roles that depend on the ability to receive and interpret specific signals from environmental stimuli. Here we describe the different activation states these cells can exhibit in response to signals and how these states affect and can be affected by bacterial pathogens.

More than a century ago, Elie Metchnikoff first identified specific cells within an animal with the ability to internalize particles from their environment (Metchnikoff, 1893; Tauber, 2003). These cells came to be called phagocytes, and the process of internalization was termed phagocytosis. In the intervening century, it has become clear that animals contain several distinct populations of phagocytes. Macrophages are the most ancient of these cell types and yet, functionally, are among the most enigmatic. As macrophages respond to specific stimuli and become activated, they engage different cell-biological programs, which may be thought of as macrophage "software packages." In this piece we discuss the diversity of macrophage states: how the macrophage interaction with microbes defines the choice of activation program, how microbes may subvert these choices to promote their survival, and how the recent appreciation for distinct developmental origins among macrophages raises the possibility of further sources of diversity ("hardware") in macrophage function.

Macrophage Function and Activation States

Macrophages are professional phagocytes found throughout the animal kingdom (Buchmann, 2014; Tauber, 2003). Their basic job is to react to microbial infections or tissue damage by phagocytosing and eliminating intruding microbes and debris from necrotic or apoptotic host cells. This is accomplished by internalization of encountered particles in a vesicle called the phagosome, which is progressively transformed by a series of vesicle fusion and modification events resulting in a compartment that is acidified, oxidative, and high in proteases. This vesicle fuses with the lysosome to form the phagolysosome, where further breakdown and clearance of internalized particles is completed. Macrophages are found in most, if not all, tissues, and in many organs they form an evenly distributed network (Chorro et al., 2009; Nimmerjahn et al., 2005). Microbe detection by cell-surface receptors on macrophages initiates the expression and secretion of inflammatory molecules such as cytokines and chemokines, which in turn activate and recruit additional immune cell types—for example, neutrophils and T cells in vertebrates—to fight the infection. In noninfectious contexts, macrophages phagocytose cell corpses and perform homeostatic support functions in tissues; in these cases, macrophages normally do not produce high levels of inflammatory signals (Davies et al., 2013). Inappropriate generation of inflammatory signals in this context can be a source of pathology. The physiological effects of macrophage activity are thus dependent on the nature of the encountered stimulus.

In recent years, many specific stimulus-dependent activation states have been described (Figure 1). The best-characterized activation state—often called "classical" activation—is typically driven by detection of bacteria, for example via Toll-like receptor 4 (TLR4), expressed on macrophages, binding to lipopolysaccharide and other bacterial molecular moieties. The cytokine interferon-γ (IFN-γ) also promotes the classical state in macrophages. Classically activated macrophages produce pro-inflammatory cytokines, in particular interleukin-1 (IL-1), IL-12, and TNF-α, and exhibit a distinct phagocytic program. They express phagocytic receptors for bacteria, and the phagocytosed bacterial particles are degraded primarily by an oxidative burst, in which the phagolysosome becomes enriched in superoxide, peroxide, and nitric oxide (Canton, 2014). Macrophages showing indications of this classical activation are often referred to as M1 macrophages.

This state contrasts with various forms of "alternative" activation. Alternative activation, like classical activation, can be driven by invading organisms, most commonly by helminths and other macropathogens, or by a wide variety of cytokines, including IL-4, IL-13, IL-10, and IL-6 (Martinez et al., 2009). Compared with classically activated macrophages, alternatively activated macrophages produce fewer or no pro-inflammatory signals. Their repertoire of phagocytic receptors is biased toward receptors for apoptotic cells, and they rely on low pH and protease activity, rather than oxidative conditions, to degrade phagocytosed particles. Macrophages with an alternative activation pattern are often described as M2 macrophages in the literature. These cells massively proliferate and expand in infected tissues, recruit other immune cells (T cells, B cells), and appear to drive a transcriptional program to maintain tissue and repair damage, rather than playing a major role in the killing of pathogens (Davies et al., 2013; Jenkins et al., 2011). Thus, the particular activation state is determined by signals derived from the host (e.g., cytokines), from microbes, or from both (Figure 1).

Importantly, while it is easy to produce cells in vitro that represent the simple extremes of these activation states,

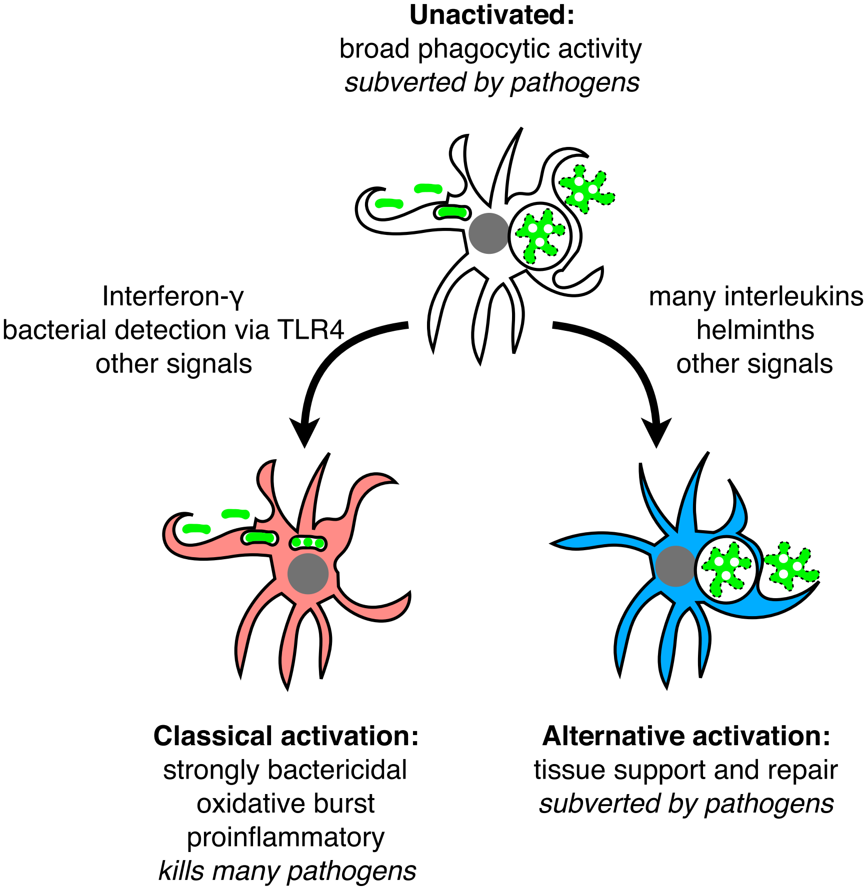

Figure 1. Heterogeneity in Activation States of Macrophages
Classical and alternative activation states are characterized by distinct phagocytic preferences (microbes or apoptotic cells) and distinct physiological roles (proinflammatory or tissue repair). Classically activated macrophages (red) can kill many intracellular pathogens that are able to survive and proliferate inside unactivated (white) or alternatively activated (blue) cells.

macrophages in vivo encounter a variety of signals, each of which can shift the activation program toward one extreme or the other, resulting in a combinatorial diversity of intermediate macrophage activation states. In addition to this, there is some variation in the states of alternative activation generated by different signals even in the in vitro context. Thus, macrophages can employ a range of “software packages” in response to different environmental signals.

Evolutionary Conservation of Macrophage Activation Programs

Macrophage activation states have been explored primarily in mice and humans. Our understanding of these processes in other animals is limited. In fish, classical and alternative activation states and the corresponding signals that drive them are present, implying an evolutionary origin more ancient than the radiation of jawed vertebrates (Buchmann, 2014). In *Drosophila*, however, the number and character of macrophage activation states is probably different (*Drosophila* macrophages are generally called plasmatocytes, but for simplicity we will refer to them here as macrophages). For example, the ability to drive classical activation in mammals depends largely on IFN-γ signaling via STAT1, while alternative activation is associated with various cytokines signaling via STAT3, STAT5, or STAT6. The *Drosophila* genome, by contrast, contains only one STAT, thus limiting the number of activation programs that can be triggered by STAT-activating cytokines. It is known that the expression of at least one such cytokine is induced in adult *Drosophila* macrophages by bacterial infection, but the effect of this signal on macrophage bactericidal activity is unclear (Agaisse et al., 2003). It remains to be seen whether alternate sources of heterogeneity in phagocyte programming exist in *Drosophila*.

Subversion of Macrophage Activation Programs by Pathogenic Bacteria

One of the major physiological roles of macrophages is to engulf, and hopefully kill, invading microbes. The activation states described thus far are sufficient to deal with most bacteria that a typical macrophage might encounter. However, the bacterial world is one of almost unimaginable diversity. For every mechanism the host develops to deal with bacteria in general, there will be specific bacteria that find effective countermeasures. Bacteria that can circumvent host defenses are, in general, pathogens. Therefore, if we are interested in the biology of infectious disease, we need to understand the ways that host defenses *don’t* work and the ways bacteria evade or subvert them.

Many pathogens are able to evade effective immune responses and thus cause disease because they have evolved the ability to subvert cellular function and manipulate the intracellular environment to allow survival and proliferation inside the macrophage. Essentially, these bacteria bring their own software. Many of these organisms have come to depend on their ability to enter and thrive within a phagocyte, which affords these bacteria a sheltered niche, safe from most other aspects of the immune system. *Mycobacterium tuberculosis*, the causative agent of human tuberculosis, is one example of a bacterium that is able to exploit the macrophage as a sheltered environment. In fact, *M. tuberculosis* is dependent on its ability to persist and proliferate inside macrophages in order to cause disease (Canton, 2014). When *M. tuberculosis* is phagocytosed by a non-activated macrophage, it is able to rapidly alter the identity of the phagocytic vesicle. By secreting a battery of effector proteins and shedding bioactive lipids, the bacterium inhibits the accumulation of phosphatidylinositol 3-phosphate (PI3P) on the surface of its vesicle, thereby blocking its maturation into the phagolysosome. It also recruits the host protein coronin-1 to the vesicle surface, again preventing downstream vesicle fusion and