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Previews

"The Very Pulse of the Machine": The Tuberculous Granuloma in Motion

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What is happening inside the tuberculous granuloma? In this issue of *Immunity*, Egen et al. (2008) present live images of tuberculous granulomas of the mouse, demonstrating the influx and incessant wandering of T lymphocytes.

Ilya Metchnikov published his observations of macrophages in action 116 years ago, 10 years after Robert Koch had established *Mycobacterium tuberculosis* (Mtb) as the causative agent of tuberculosis (TB). These findings have since become linked by the appreciation that mycobacteria are inveterate macrophage pathogens, having evolved to circumvent and even exploit these key immune effector cells (Clay et al., 2007). Direct observation of immune cells in action has since become far more detailed, thanks to the invention of new microscope technology and the software necessary to explore image data in three and even four dimensions (Bajenoff and Germain, 2007). During the same time frame, tuberculosis has been under more or less continuous study, but despite advances in antibiotic therapy, remains as intractable a global health problem as ever with the advent of increasingly drug-resistant Mtb strains. Setting aside the obvious public health, social, and economic failures to control TB, its continued status as the "Captain of all Men of Death" stems from the inexplicable persistence of Mtb in the face of an apparently solid immune response and vaccination with *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG), the world's most widely used yet relatively ineffective vaccine. An emerging body of work utilizing the power of in vivo imaging to crack the secrets of TB has begun to shed light on the arguable center of the mystery: the granuloma (Davis et al., 2002; Volkman et al., 2004). The study in this issue of *Immunity* by Egen et al. (2008) extends these earlier studies to offer insights into the granuloma after adaptive immunity has come into play.

What then are granulomas? These complex organized immunological structures are comprised of differentiated, interdigitated macrophages (the so-called epithelioid cells) that are subsequently joined by other immune cells such as T and B lymphocytes and NK cells (Adams, 1976). Granulomas form in humans in response to a variety of persistent stimuli, be they pathogens (e.g., *Mycobacteria* and *Brucella*) or foreign bodies, as well as in certain mystery diseases such as sarcoidosis. Tuberculosis is by far the most prevalent cause of human granulomas world-wide, such that the pathologist's finding of granulomas promptly sets off a search for signs of tuberculosis. Tuberculous infection is initiated in humans by airborne Mtb within cough droplets that gain access to the deepest alveoli of a victim's lungs, where the bacterium is phagocytosed by alveolar macrophages and dendritic cells (Dannenberg, 1993). Frequently failing to kill their new cargo, these cells serve as effective transporters of the bacteria from the airway into deeper tissues (Clay et al., 2007), where they soon aggregate into granulomas. Live imaging studies in the transparent developing zebrafish infected with *Mycobacterium marinum*, a close genetic relative of Mtb, have shown that granulomas can form as a result of mycobacterial interactions with innate immunity alone (Davis et al., 2002). Adaptive immune elements then come into play but mysteriously even the resultant bolstered response can fail to eradicate these organisms, suggesting that mycobacteria may counter or even usurp a full range of host defenses (Flynn, 2006; Cosma et al., 2004). For years it has been thought that tuberculous granulomas, like foreign body granulomas, at least serve as an encircling barrier to "wall off" material that cannot be destroyed—a view that this study along with other recent ones shows is simplistic (Cosma et al., 2004; Egen et al., 2008; Volkman et al., 2004).

Granuloma formation and maintenance is an area ripe for in vivo imaging studies. First, it is not clear exactly how infected macrophages give rise to granulomas. The initial inoculum of Mtb can be exceedingly small, perhaps even fewer than 10 organisms, so that infection of a single macrophage might well be sufficient to establish infection. How would single macrophages so lightly infected give rise to granulomas that contain hundreds of immune cells and many more bacteria? Do single infected macrophages simply attract more macrophages to achieve a critical mass of infectable cells? What induces these infected macrophages to become "granulomagenic"? The mycobacterial RD1 secretion system, a virulence determinant, is required to induce granuloma formation, but the mode (e.g., cell migration, infection, or adhesion) and mechanism are not clear (Volkman et al., 2004). Moreover, the source of new macrophages (local versus systemic, tissue versus blood-borne) is not clear. The picture becomes increasingly murky as the granuloma matures and other immune cell types appear in the granuloma. When and in what numbers do they appear? Is their order and number critical to the maturation of the lesion, or to the outcome of infection? Are granulomas permanent structures, or do they come and go in different locations during the years in which the average infected human is thought to harbor infection? Finally, which, if any, of these steps are susceptible to intervention? Visual evidence, and especially live, time-lapsed evidence,

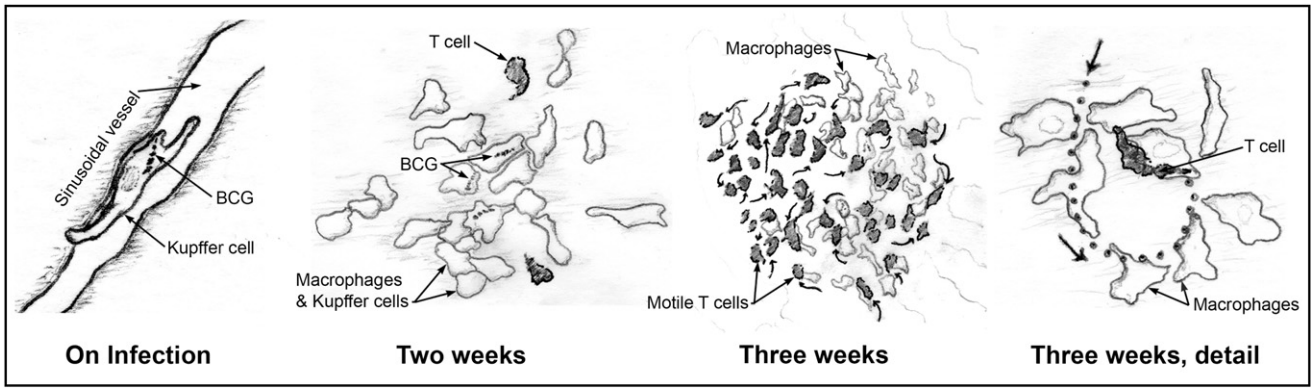

Figure 1. Stages of BCG Granuloma Formation in the Mouse Liver
Upon infection, blood-borne bacteria are phagocytosed by resident Kupffer cells. At 2 weeks after infection, infected Kupffer cells have attracted local resident macrophages and monocyte-derived macrophages. Arriving T lymphocytes are also seen. By 3 weeks after infection, T cells are plentiful among the macrophages. T cells migrate in constant contact with a "scaffold" of macrophages.

will be key to asking and answering these questions.

The transparent zebrafish embryo has provided new insights into the early innate immune phases of macrophage migration and granuloma formation (Clay et al., 2007; Davis et al., 2002; Volkman et al., 2004). For the adaptive immune phase of granuloma formation, however, the mouse with its plentiful immunological reagents and strains has distinct advantages as a model organism. In an impressive technical display, Egen et al. (2008) have used the mouse to produce the first live images of mycobacterial infection in a mammalian host. Although the lung is generally the subject of study in the mouse model of tuberculosis, its constant movement, practically a condition for physiologic normalcy, makes it unsuitable for time-lapsed imaging under the microscope. To overcome this problem, these authors used intravenous infection to initiate granulomas in the liver. Also, Mtb work requires dedicated biosafety level 3 (BSL3) facilities, so these authors have resorted to using a large inoculum of the attenuated BCG vaccine strain (requiring only BSL2 containment) to produce a persistent infection with granulomas numerous enough to be found under the microscope without difficulty.

In a series of intricately clever manipulations of transgenic mice and 3D time-lapse microscopy, Egen et al. (2008) demonstrate that blood-borne BCG is rapidly taken up by Kupffer cells, the resident macrophages of the liver (Figure 1). Both the infected cells and the invading bacteria appear to survive well over time, and after several days, aggregates consisting of the original infected Kupffer cells, recruited Kupffer cells, and monocyte-derived macrophages are visible. These myeloid cells are not very motile, although their membranes do appear to be in constant flux. The precise details of this phase must be interpreted with caution, because BCG is attenuated and lacks the RD1 determinant that has been found to enhance macrophage aggregation into granulomas (Volkman et al., 2004). Consistent with its attenuation, there is little or no net bacterial growth in the first 3 weeks of infection, a period when pathogenic mycobacteria grow logarithmically in their hosts, exemplified by Mtb in mice (Flynn, 2006).

To probe granuloma dynamics further, the authors blocked tumor necrosis factor-α (TNF-α) in established granulomas, and within 4 days found smaller lesions with reduced macrophage numbers. Immunofluorescence histology reveals that TNF-α blockade does not alter the number of infected macrophages in the lesion. The selective loss of uninfected macrophages has several possible explanations: the treatment could lead to a reduced migration of new uninfected macrophages to the lesion, or could differentially affect the retention or survival of infected versus uninfected macrophages. These possible mechanisms and their effects on pathogenesis can be explored further with this and other in vivo models.

It is the movement and behavior of T lymphocytes in granulomas that provide the most intriguing findings of this study. These cells, which arrive at the granulomas within days (Figure 1), are in constant motion throughout the lesion. Their motion is such that each lymphocyte appears to wander through the entire granuloma, likely making direct contact with most of its macrophages. This finding begs the question of how antigen-specific T lymphocytes would behave. Would they make more prolonged contacts with macrophages expressing the relevant antigen? The mechanics of the interaction between arriving T cells and the macrophage matrix are of particular interest. Activated T lymphocytes readily enter lesions but they appear restrained from leaving so that they accumulate therein. Egen et al. (2008) propose that this retention of T cells is due to the macrophages acting as a scaffold upon which the lymphocytes crawl, rather than to a physical barrier to departure (Figure 1). This model is similar to that proposed by this same group for T cells migrating within lymph nodes (Bajenoff and Germain, 2007). It has been suggested that the granuloma may serve as a form of tertiary lymphoid organ (Ulrichs et al., 2004), and these new data add to that discussion. After TNF blockade, T lymphocytes are still found in granulomas in reduced numbers. This reduction may be due to the overall reduction in granuloma size after anti-TNF treatment.

The emerging picture of the granuloma as a tight accumulation of macrophages that serves to support T cell migration and contact—with infected cells, with each other, with some unseen cell type—is a revealing one indeed. Observations of early, innate immune granuloma formation have suggested a highly dynamic

lesion, with constant innate cell motility (Davis et al., 2002). Egen et al. (2008) similarly find an adaptive immune-stage granuloma in constant motion. The ultimate contribution of all this motion and action to pathogenesis will be known only after further study; however, this report adds convincingly to the argument that the granuloma cannot be thought of as simply a barricade to contain mycobacteria, even after adaptive immunity is established.

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