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Mycobacterial Infections and the Inflammatory Seesaw

Charles A. Scanga[1,2] and JoAnne L. Flynn[1,2,*]
[1]Center for Vaccine Research
[2]Department of Microbiology and Molecular Genetics
University of Pittsburgh School of Medicine, W1144 Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA 15261, USA
*Correspondence: joanne@pitt.edu
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Eicosanoids can have either proinflammatory effects or anti-inflammatory effects. Tobin and colleagues use a forward genetic screen in zebrafish to identify a key eicosanoid enzyme, leukotriene A_4 hydrolase (LTA_4H), that controls susceptibility to mycobacterial infection. They also demonstrate that polymorphisms in *LTA_4H* are associated with susceptibility to mycobacteria in humans.

Infection with *Mycobacterium tuberculosis* poses a complex challenge to the host. A vigorous Th1 response must be generated by a proinflammatory cascade that activates macrophages to a mycobactericidal state and results in the formation of granulomas—organized masses of macrophages, lymphocytes, and other cell types such as neutrophils and fibroblasts. However, these granulomas are also responsible for much of the pathophysiology of tuberculosis (TB) (Dannenberg, 2006). It is essential that the infected host controls this inflammatory reaction with an opposing anti-inflammatory response. Cytokines such as IL-10 and TGF-β, and cells such as regulatory T cells likely play roles in this counterbalance but seem not to be the sole mediators (Flynn and Chan, 2001). It may also be that each infected person relies on a different balance of factors to maintain control of infection as well as limit pathology. The need for both pro- and anti-inflammatory factors in ultimate control of mycobacterial infections makes it difficult to define a factor as "good" or "bad" in the immune response against TB, adding to the complexity of studying host responses against this infection.

Recently, a role for eicosanoids in mediating the balance between pro- and anti-inflammatory forces in TB has emerged. Eicosanoids are lipid mediators derived from arachidonic acid and include the proinflammatory leukotrienes as well as the anti-inflammatory lipoxins. Leukotrienes are generated by oxidation of arachidonic acid by 5-lipoxygenase (5-LO) to produce leukotriene A_4 (LTA_4). Neutrophils and monocytes express LTA_4 hydrolase (LTA_4H) that converts LTA_4 to LTB_4, a powerful proinflammatory agent. Lipoxins are a family of anti-inflammatory eicosanoids that also are generated via a 5-LO-dependent pathway, and they downregulate proinflammatory responses by several mechanisms such as inhibiting neutrophil migration and downregulating IL-12 production (Serhan, 2007).

Insights into the important role played by 5-LO-derived eicosanoids in TB have come from studies using an inhibitor of 5-LO (Peres et al., 2007) or using mice lacking 5-LO (Bafica et al., 2005). In a recent issue of *Cell*, Tobin and colleagues used a forward genetic screen in zebrafish to identify another component of the eicosanoid pathway, LTA_4H, as important in host control of mycobacteria (Tobin et al., 2010). In this elegant study, the authors sought to identify loci involved in host defense against mycobacteria by screening mutagenized zebrafish larvae for phenotypes that differed in their ability to control the replication of *M. marinum*. This model has several advantages: zebrafish are genetically well characterized and manipulable; mycobacterial burden and granuloma formation can be directly observed by microscope; and pharmacologic probes can be administered easily by soaking the fish in drug solutions. However, one must extrapolate carefully the findings from this model since zebrafish larvae possess innate immunity but have not yet developed an adaptive immune system and since *M. marinum* differs significantly from its more pathogenic relative, *M. tuberculosis*. Nonetheless, results from this model have been confirmed in other model systems and even in humans (Clay et al., 2008; Flynn et al., 1995; Keane, 2005).

Tobin and colleagues identified three classes of mutants that were hypersusceptible to *M. marinum* (Tobin et al., 2010). The largest class induced less *tnf* expression and exhibited accelerated granuloma formation and subsequent dissolution following infection. The mutations in this class were mapped to the *lta4h* locus, and its hypersusceptible phenotype was recapitulated when zebrafish embryos were treated with a morpholino targeting *lta4h* expression. One might presume that the *lta4h* mutants exhibited reduced resistance to *M. marinum* due to the loss of LTA_4H function and decreased production of proinflammatory LTB_4. However, the addition of exogenous LTB_4 did not reverse the phenotype suggesting that the enhanced susceptibility to mycobacteria was not a result of defective LTB_4 production but rather a shift toward an anti-inflammatory state due to increased conversion of LTA_4 to lipoxinA_4 (LXA_4) by the actions of 12- and 15-LO. Indeed, LTB_4-induced neutrophil migration was reduced in both *lta4h* mutant larvae as well as in wild-type larvae treated with an LXA_4 epimer, and chemical inhibition of 15-LO in *lta4h* mutants returned this neutrophil migration to normal. Administration of exogenous LXA_4 to wild-type larvae infected with *M. marinum* recapitulated the decreased *tnf* expression and increased bacterial burden that was observed in infected *lta4h* mutants. The study would have been strengthened if the authors had measured eicosanoids in *lta4* mutants infected with *M. marinum* to show the LTB_4

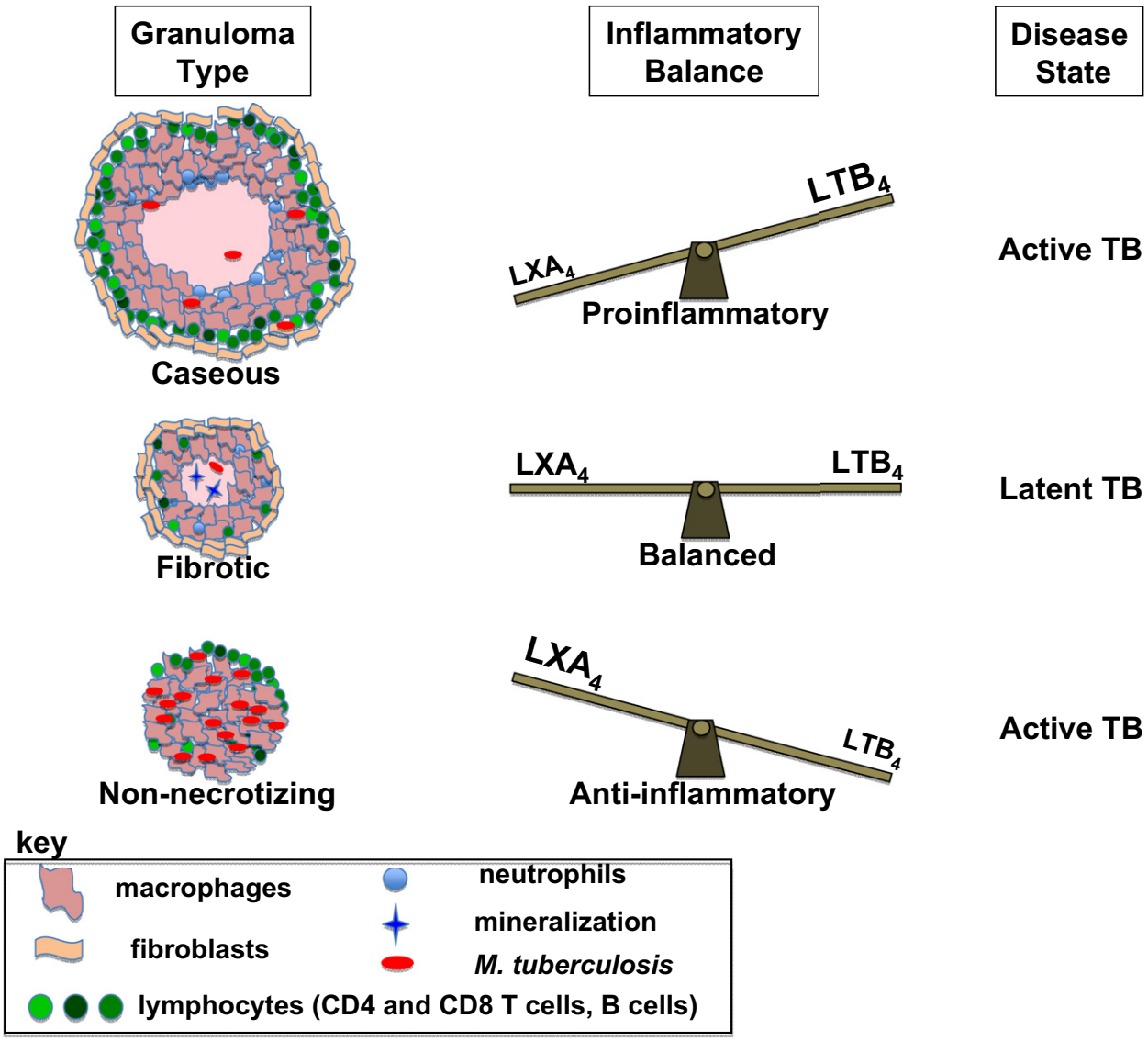

Figure 1. Model Depicting the Balance between Proinflammatory and Anti-inflammatory Responses in Tuberculosis
Infection by *M. tuberculosis* can result in different disease states depending predominantly on the host response. When a proinflammatory response predominates, excessive granuloma formation and abundant necrosis occur, resulting in active tuberculosis. In the other extreme, when the anti-inflammatory response predominates, granulomas are poorly formed, macrophages are less likely to be activated, and bacterial replication is not well controlled, again resulting in active tuberculosis. When pro- and anti-inflammatory forces are optimally balanced, granulomas are well structured and controlled with activated macrophages capable of restricting bacterial proliferation, resulting in latent tuberculosis. Recent evidence, including that published in a recent issue of *Cell* (Tobin et al., 2010), highlights the important role for eicosanoids in balancing these forces: leukotriene B_4 (LTB_4) induces a proinflammatory response while lipoxin A_4 (LXA_4) induces an anti-inflammatory response. Thus, eicosanoids join cytokines and regulatory T cells as immune modulators that can determine tuberculosis outcome.

levels and to confirm the presumed increase in LXA_4 production. Nonetheless, this article by Tobin et al. makes a strong case for LTA_4H as an important component in determining the outcome of mycobacterial infection.

Many reports in the literature describing the discovery of a novel disease susceptibility determinant in animal models include the hopeful caveat that the discovery may also hold true in humans, as well. However, Tobin and colleagues extended their identification of a direct role for LTA_4H in determining the outcome of mycobacterial infection in zebrafish by examining whether *LTA_4H* polymorphisms were associated with susceptibility to mycobacteria in humans (Tobin et al., 2010). Two separate cohorts were studied: (1) persons with TB in Vietnam and (2) persons with leprosy in Nepal. Several single nucleotide polymorphisms (SNPs) at the *LTA_4H* locus were identified and heterozygosity was associated with protection from pulmonary and meningeal TB and with lower incidence of the more severe form of leprosy. Therefore, in this manuscript the authors both report the discovery of a role for LTA_4H in zebrafish larvae infected with *M. marinum* and

establish a link between this key enzyme in the eicosanoid pathway and the susceptibility of humans to two diverse mycobacterial diseases.

The precise mechanism by which these effects are mediated remains obscure. In the zebrafish, mutation of *lta4h* results in hypersusceptibility to *M. marinum*, presumably because LTA_4 can no longer be converted to the proinflammatory eicosanoid LTB_4 and is instead shunted to form the anti-inflammatory eicosanoid LXA_4. In this scenario, the inflammatory balance is shifted too far toward an anti-inflammatory state (Figure 1). In marked contrast, *LTA_4H* polymorphisms in humans are strongly associated with protection from diseases caused by *M. tuberculosis* and *M. leprae*. SNPs in the *LTA_4H* locus have previously been reported and there was found to be a positive correlation between *LTA_4H* polymorphism and the amount of LTB_4 produced in vitro by granulocytes from the affected individuals (Helgadottir et al., 2006). If the heterozygosity at *LTA_4H* described by Tobin and colleagues is also associated with increased LTB_4 production in vivo resulting in enhanced protection from TB or leprosy, this contravenes the conclusions from the zebrafish model where *lta_4h* mutations are hypothesized to cause lower LTB_4 levels. Measurement of LTB_4 and LXA_4 concentrations in sera from the human subjects and in the mutant zebrafish infected with *M. marinum* certainly would help clarify this issue. Nonetheless, this report highlights two features of LTA_4H in mycobacterial diseases: (1) Perturbations of the eicosanoid axis in animal models as well as humans can affect the outcome of mycobacterial infections, and (2) the interplay between proinflammatory leukotrienes and anti-inflammatory lipoxins is complex. Additional studies clearly are warranted to more fully elucidate the roles of eicosanoids in mediating the optimal balance between proinflammatory and anti-inflammatory effects for a given mycobacterial infection (Figure 1).

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The HIV-1 Tat Team Gets Bigger

Andrew P. Rice[1,*]
[1]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA
*Correspondence: arice@bcm.edu
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Productive transcription of the HIV-1 genome involves RNA polymerase II working in concert with the viral protein Tat and its team of cofactors in a highly orchestrated process. Now, Pagans and colleagues report that the lysine methyltransferase Set7/9-KMT7 associates with Tat to stimulate RNA polymerase II elongation of the integrated provirus. Set7/9-KMT7 also methylates Tat, and this enhances Tat function.

A defining step in the replication cycle of HIV-1 and all retroviruses is reverse transcription of the viral genomic RNA into cDNA and integration of the proviral genome into a host cell chromosome. After integration, RNA polymerase II (RNAP II) is recruited to the promoter located in the 5′ long terminal repeat (LTR) sequences of the provirus, and the viral genome is transcribed back into RNA. Spliced RNA serves as viral mRNA, and unspliced RNA serves as mRNA or is packaged into viral particles that assemble at the plasma membrane. Mechanisms involved in RNAP II transcription of the HIV-1 provirus have been actively studied, both because of their general interest and because this essential step in the viral life cycle has the potential to be targeted by antiviral drugs. It has become clear in recent years that productive transcription of the HIV-1 genome involves RNAP II working in concert with a viral protein known as Tat and its team of cofactors in a highly orchestrated process. A publication from Pagans and colleagues (Pagans et al., 2010) in this issue of *Cell Host & Microbe* identifies a new teammate of Tat that functions in an early