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Radical Host-Specific Therapies for TB

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Although proinflammatory cytokines such as TNF are critical for containment of tuberculosis, they can also exacerbate disease when produced at high levels. In this issue of *Cell*, Roca and Ramakrishnan demonstrate that high TNF production induces reactive oxygen species in infected macrophages, ultimately leading to macrophage necrosis and bacterial dissemination.

One-third of the human population is estimated to be infected with *Mycobacterium tuberculosis*; however, of those individuals, only 5% to 10% will develop active tuberculosis (TB) at some point during their lives. Historically, progression of latent to active TB infection has been attributed to a weakening of the immune system, resulting in an inability to control bacterial replication. This is perhaps best illustrated by humans coinfected with HIV, who are approximately 25 times more likely to develop TB disease compared to humans without HIV. However, it is becoming increasingly clear that the opposite might also be true and that in some cases TB susceptibility can be attributed to overactivity of the immune system. In this issue of *Cell*, Roca and Ramakrishnan (2013) illustrate how a critical mediator of the inflammatory response to TB, tumor necrosis factor (TNF), can increase both resistance and susceptibility to infection via the production of reactive oxygen species (ROS).

In their previous work, the Ramakrishnan laboratory identified a critical role for the enzyme leukotriene A4 hydrolase (LTA4H) in determining the susceptibility of zebrafish to infection with *Mycobacterium marinum*, a close relative of *M. tuberculosis* (Tobin et al., 2012). LTA4H is responsible for the production of the proinflammatory lipid leukotriene B4 (LTB_4), which can directly induce the transcription of TNF. In infected zebrafish with high LTA4H activity, increased TNF production was initially associated with reduced bacterial growth inside macrophages. However, this early growth restriction was rapidly followed by macrophage necrosis, which enabled bacterial replication in the permissive extracellular environment and resulted in higher overall bacterial burdens. In humans, differential LTA4H activity has also been implicated in the severity of TB infection, particularly in patients suffering from TB meningitis (Tobin et al., 2012). It was found that individuals homozygous for high *LTA4H* expression alleles had a significantly reduced survival compared to heterozygotes, strengthening the notion that high levels of inflammation can worsen the outcome of TB infection.

How can this increased susceptibility of TB meningitis patients with a proinflammatory *LTA4H* genotype be explained? Using their zebrafish model, Roca and Ramakrishnan set out to identify the source of macrophage death under LTA4H-high conditions. Because TNF is known to be a potent inducer of mitochondrial ROS production, they incubated zebrafish embryos with several ROS scavengers and found that this reversed the susceptibility of LTA4H-high animals (Roca and Ramakrishnan, 2013). These data suggested that excessive inflammation could be a driving factor for ROS production during mycobacterial infection. To test this further, the authors injected recombinant TNF, thereby mimicking the LTA4H-high state. Initially, this led to a reduction in bacterial growth, indicating that TNF-induced ROS can mediate antimicrobial activity inside macrophages. However, at later times this phenotype was reversed, at which point high TNF levels were associated with increased macrophage death and extracellular bacterial growth. It is interesting to point out that high TNF levels only increased ROS production in infected macrophages, raising the possibility that mycobacteria actively contribute to and perhaps benefit from this process. It has been observed previously that mycobacteria are capable of escaping from the phagosome into the cytosol by secreting the virulence factor ESAT6, a process that is rapidly followed by host cell necrosis (Simeone et al., 2012; van der Wel et al., 2007). It will be interesting to explore whether macrophage ROS production is involved in this escape-associated necrosis and, if so, to what extent mycobacterial factors are actively contributing to this.

Given that TNF-mediated ROS production can have both beneficial and detrimental effects for the host, could this be exploited to develop more effective antituberculosis therapies? Using gene-specific knockdown, Roca and Ramakrishnan identified that the macrophage death under high TNF conditions involved the kinases RIP1 and RIP3, indicating that the cells die via programmed necrosis or necroptosis. Following the induction of ROS, cells can undergo necroptosis via two distinct pathways. One involves the mitochondrial matrix protein cyclophilin D and the other the lipid ceramide. Knockdown of either the cyclophilin D or the ceramide pathway under high TNF conditions resulted in reduced bacterial burdens, while largely preventing macrophage necroptosis. Furthermore, combined inactivation of both pathways rendered the macrophages hyperresistant to infection, an effect that was mimicked by the orally available inhibitors alisporivir and desipramine that target the cyclophilin D and ceramide pathways, respectively. These findings illustrate that

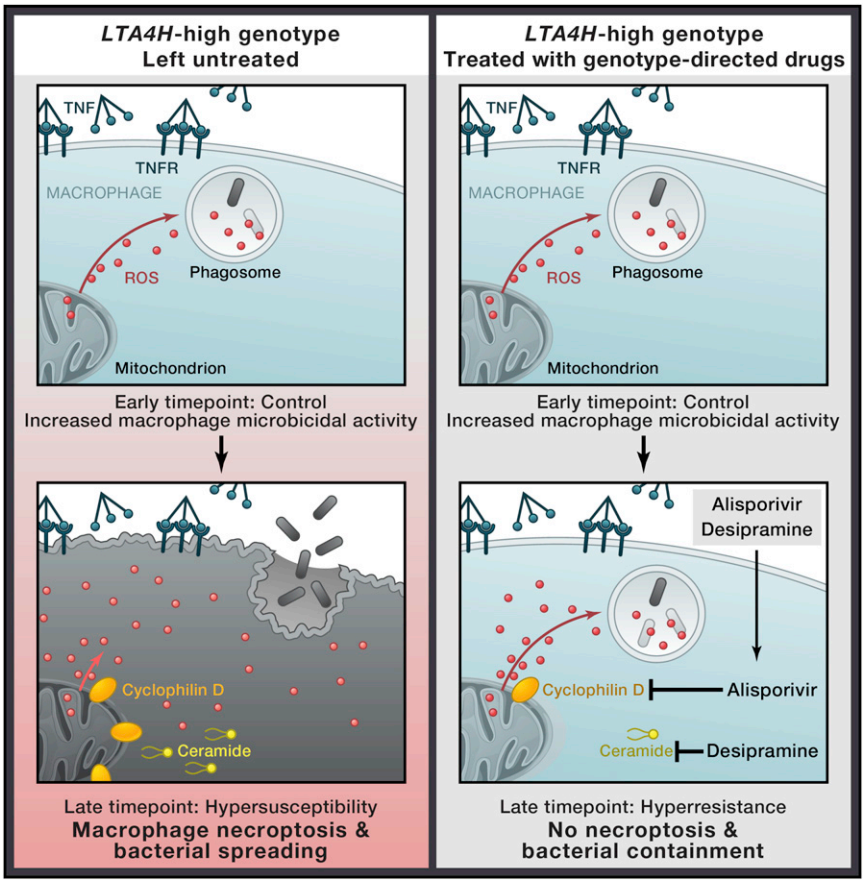

Figure 1. Increasing TB Treatment Efficacy Using Host-Specific Therapies

In individuals with an *LTA4H*-high genotype, mycobacterial infection is initially controlled by TNF-mediated ROS production. If infected individuals are left untreated, consistently high ROS production can lead to macrophage necroptosis, resulting in release of bacteria into the permissive extracellular environment and bacterial dissemination (left). However, if these individuals are treated using specific genotype-directed drugs such as alisporivir and desipramine, macrophage necroptosis can be blocked while ROS-mediated antimicrobial activity can be maintained, thereby resulting in intracellular bacterial containment and a lower overall bacterial burden (right).

TNF-mediated ROS production functions as a double-edged sword; it lowers the number of bacteria per macrophage, but it also induces macrophage necroptosis, leading to enhanced extracellular bacterial replication. However, by using specific necroptosis inhibitors, it might be possible to devise novel antituberculosis therapies that preserve ROS-dependent antimicrobial activity without facilitating bacterial dissemination (Figure 1). Such specific therapies could substantially benefit people with *LTA4H*-high genotypes, whereas they presumably wouldn't affect those with an *LTA4H*-low genotype. This contrasts to more general anti-inflammatory therapies like glucocorticoids, which might harm individuals that suffer from TB disease as a result of low inflammation.

What other factors might contribute to the increased TB susceptibility of humans with an *LTA4H*-high genotype? Next to inducing TNF transcription, LTB_4 also functions as a chemoattractant for granulocytes, macrophages, and effector T cells (Serhan and Prescott, 2000; Tager et al., 2003). These recruited cells could contribute substantially to the overall inflammation in humans with an *LTA4H*-high genotype, in part via the overproduction of TNF. Given that high TNF levels can have detrimental effects on TB susceptibility, this raises an important question regarding vaccine design because most vaccine candidates are screened for the capacity to induce multifunctional T cells capable of producing cytokines such as TNF (Derrick et al., 2011; Seder et al., 2008). Although multifunctional T cells might be associated with the highest degree of protection, it is not clear whether the tested cytokines like TNF are actually involved in this. In fact, TNF appears dispensable for the protection of Th1 cells against TB, at least in mice (Gallegos et al., 2011). Furthermore, in settings of necrotic granulomas, TNF blockade can actually enhance bacterial clearance (Skerry et al., 2012). Therefore, these findings together with the new data by Roca and Ramakrishnan make a strong case that vaccine development should not just focus on inducing inflammation but rather on inducing the right type of inflammation. If successful, the combination of such vaccines with genotype-directed drug therapies promises to bring us one step closer toward eliminating TB in the coming decades.

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