Anti-TNF immunotherapy and tuberculosis reactivation: another mechanism revealed

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Antibodies targeting TNF, an inflammatory cytokine, have revolutionized the treatment of some inflammatory diseases, including rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis, and Crohn disease, which are characterized by chronic inflammation. However, the use of anti-TNF agents also presents potential risks. For instance, administration of TNF-blocking drugs has been associated with an increased risk of tuberculosis (TB), a potentially lethal mycobacterial infection. The increased risk is thought to be mediated by a reduced efficacy of TB immune responses during treatment with anti-TNF drugs. In this review, we discuss the potential mechanisms by which anti-TNF agents might interfere with the control of TB, including the involvement of reactivation of latent TB. We also discuss how the use of anti-TNF drugs has influenced the treatment and prevention of TB, and the implications for TB control and the selection of TB preventive therapies in patients receiving anti-TNF agents.
phage pathogens by providing a local environment in which APCs and lymphocytes interact to suppress progressive growth of the pathogen. In the current issue of the JCI, Bruns et al. describe an additional mechanism for susceptibility to progressive TB in individuals treated with infliximab (3). Using PBMCs from tuberculin skin test–positive subjects, Bruns et al. identified granulysin-rich CD8+CD45RA−CCR7− effector memory T cells (CD8+ TEMRA cells) as a major subset of antmycobacterial effector cells and found that TEMRA cells are selectively depleted by infliximab therapy in patients with RA. Here we discuss the impact of these findings as they relate to the existing knowledge of CD8+ T cells and TNF in immunity to M. tuberculosis.

**CD8+ T cells and immunity to M. tuberculosis**

Cell-mediated immunity is critical for control of M. tuberculosis infection, and it has long been acknowledged that CD4+ T cells are important mediators of immunity to M. tuberculosis. More recently, M. tuberculosis–specific CD8+ T cells have been increasingly recognized, but their significance remains incompletely understood, especially in humans. CD8+ T cells are thought to limit mycobacterial growth directly through the killing of infected cells (4, 5), as well as indirectly through secretion of cytokines that promote activation of macrophages and chemokines that coordinate cell recruitment. Mice deficient in MHC class I molecules, and thus deficient in CD8+ T cells due to the absence of positive selection, have higher burdens of M. tuberculosis in the lungs and a modest decrease in survival (6). This is in contrast to CD4+ T cell–deficient mice, which display more rapidly progressive bacterial growth and a sharp decline in survival (6). These studies and others imply that CD8+ T cells are less crucial during the acute phase of M. tuberculosis infection, but are indispensable during the chronic phase of infection, and therefore may help prevent reactivation of TB (7, 8). When evaluating the contribution of CD8+ T cells to M. tuberculosis immunity, it is important to note that mice lack granulysin, a cytolytic granule protein that contributes to killing of M. tuberculosis by human CD8+ T cells (4). Therefore, murine studies may undervalue the importance of the antmycobacterial properties of CD8+ T cell subsets compared with their roles in human immunity. Human studies are more limited in number, but in vitro experiments provide evidence that CD8+ T cells can control M. tuberculosis through killing of infected macrophages and subsequent death of the bacteria, as well as by direct killing of mycobacteria by secreted granulysin (4, 5, 9). Bruns et al. (3) found in their assays that TEMRA cells stain for granulysin with high frequency and display the highest levels of both cytotoxicity and antmycobacterial activity compared with other T cell subsets.

**TNF blockade and progression of M. tuberculosis**

The authors go beyond defining TEMRA cells as potentially important antmycobacterial effector cells, to address the consequences of TNF blockade on these cells and on mycobacterial growth in cultured cells (3). In doing so, they reveal what we believe to be a novel potential mechanism for TNF neutralization to promote progression of TB. Previous studies in mice and in zebrafish embryos have revealed variable increases in bacillary load in the absence of TNF, ranging from small changes to multiple orders of magnitude (10–13). These observations indicated that TNF contributes directly to control of mycobacteria in granuloma macrophages (10) and may also function indirectly to suppress mycobacterial growth by modulating formation and/or maintenance...
of granulomas. Additional evidence suggests that TNF blockade may impair mycobacterial control as a result of effects on T lymphocytes. TNF blockade has previously been shown to induce production of Tregs via TGF-β in patients with RA (14, 15), and in mice infected with M. tuberculosis, depletion of Tregs leads to improved control of mycobacterial growth (16). Bruns et al. provide evidence that TNF blockade may also promote reactivation of TB through selective reduction of T<sub>EMRA</sub> cells (3). As a mechanism for their observation that T<sub>EMRA</sub> cells in peripheral blood are depleted following infliximab therapy, the authors demonstrate that T<sub>EMRA</sub> cells from RA patients possess high levels of membrane-bound (presumably transmembrane) TNF compared with healthy controls and are susceptible to in vitro complement-mediated lysis in the presence of infliximab. Additionally, PBMCs from patients receiving infliximab therapy displayed diminished capacity to limit mycobacterial growth in culture, an impairment that was rescued by the addition of autologous CD8<sup>+</sup> T<sub>EMRA</sub> cells, to a slightly lesser extent by CD8<sup>+</sup>CCR7<sup>-</sup>CD45RA<sup>-</sup> effector memory T cells, but not by circulating central memory CD8<sup>+</sup> T cells.

**Implications for future study**

As with any provocative results, the findings reported by Bruns et al. (3) raise several questions, with implications for understanding immunity to TB and with practical application to clinical medicine. First, do infliximab and etanercept differ in their ability to deplete T<sub>EMRA</sub> cells? Infliximab, a monoclonal antibody to TNF, and etanercept, a soluble TNF receptor, are both TNF-neutralizing agents used in the treatment of RA and other inflammatory conditions. For unclear reasons, infliximab causes significantly more infectious complications, including reactivation of M. tuberculosis, than does etanercept; this has been attributed by some to the differential ability of these agents to bind membrane TNF. Several studies have found that apoptosis ensues upon binding of infliximab to membrane TNF on T cells (17), monocytes (18), and cells transfected to express membrane TNF (19, 20), which may lead to a reduction in the number of antimycobacterial effector cells and/or to dissolution of granulomas. In contrast, etanercept does not cause apoptosis of cells that express membrane TNF (17, 19, 20). Additionally, cell lines transfected with membrane TNF have been used to evaluate complement-dependent cytotoxicity (CDC) and antibody-dependent, cell-mediated cytotoxicity (ADCC); while both etanercept and infliximab lead to ADCC, only infliximab induces CDC (20, 21). Consistent with these results, Bruns et al. (3) revealed that T<sub>EMRA</sub> cells are susceptible to CDC in the presence of infliximab (Figure 1). If etanercept does not cause CDC of T<sub>EMRA</sub> cells, this outcome would suggest that the differential risk of reactivation of TB with these agents may be caused, at least in part, by relative differences in depletion of T<sub>EMRA</sub> cells. Likewise, if etanercept does not deplete T<sub>EMRA</sub> cells to the same extent as does infliximab, this could at least partially explain the therapeutic differences observed with these 2 drugs. While both drugs are equally efficacious for the treatment of RA, infliximab is superior to etanercept in the treatment of other chronic inflammatory diseases, especially Crohn disease (22). If selective reduction of T<sub>EMRA</sub> cells does not occur following etanercept therapy, this finding could provide insight into differences in the immunopathology of these inflammatory conditions. In contrast, if etanercept does deplete T<sub>EMRA</sub> cells to the same extent as does infliximab, it would weaken the case that T<sub>EMRA</sub> cells are an essential element of protective immunity against TB.

Second, how long does infliximab-mediated depletion of T<sub>EMRA</sub> cells persist? The frequency of progressive TB in people treated with infliximab is highest in the first 90 days after initiating the therapy (23), but the studies by Bruns et al. were limited to a single time point 2 weeks after initiating therapy (3). Additional studies of the duration of depletion of T<sub>EMRA</sub> cells after anti-TNF therapy should shed further light on the roles of these cells in protection against TB.

Third, what are the antigens recognized by T<sub>EMRA</sub> cells, and what determines differential expression of CD8<sup>+</sup> effector cells into T<sub>EMRA</sub> cells rather than CD45RA<sup>-</sup> effector memory T cells? Answers to these questions could provide guidance in the design of improved TB vaccines by delineating optimal antigens and adjutants and might also provide insight into the mechanisms of differential susceptibility and resistance to TB in populations and in individuals. In addition, they may identify Ag-specific T<sub>EMRA</sub> cells as potential surrogate markers of vaccine efficacy and thereby provide improved predictive information in the design and evaluation of clinical trials of novel TB vaccines.

Finally, while the findings reported by Bruns et al. clearly demonstrate that T<sub>EMRA</sub> cells are targets of the anti-TNF action of infliximab (3), they do not provide proof that TNF has an essential function in the development, maintenance, or effector functions of this interesting subset of CD8<sup>+</sup> T cells. If membrane TNF is simply a bystander, then future development of agents that block TNF activities without depleting T<sub>EMRA</sub> cells may provide for safer therapy of chronic inflammatory diseases.

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CaMKII and a failing strategy for growth in heart

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Patients with systolic left ventricular dysfunction die progressively from congestive heart failure or die suddenly from cardiac arrhythmias. Myocardial hypertrophy is an early event in most forms of heart failure, but the majority of patients with myocardial hypertrophy do not develop heart failure. Developing improved therapies for targeting the cell signaling pathways that enable this deadly transition from early myocardial insult to heart failure and sudden death is a key goal for improving public health. In this issue of the JCI, Ling and colleagues provide new evidence that activation of the multifunctional Ca\(^{2+}\)/calmodulin–dependent kinase IIδ is a decisive step on the path to heart failure in mice (see the related article beginning on page 1230).

The heart failure epidemic
Heart failure is a leading cause of death in the developed world, and the incidence of heart failure continues to rise despite impressive improvements in treating coronary artery disease and hypertension. Heart failure patients encounter 2 main problems: reduced cardiac pumping that is inadequate to meet metabolic demands, and electrical instability that causes arrhythmias and sudden death. Heart failure is a clinical syndrome, but most heart failure in the developed world is caused by reduced left ventricular systolic function as a result of hypertension and myocardial infarction. Antihypertensive medications have resulted in a reduction in the number of patients with myocardial hypertrophy (1), and improved treatment of myocardial infarction patients has drastically reduced the acute and 5-year mortality of this common form of structural heart disease (2). Unfortunately, the incidence of heart failure has increased, despite reducing the burden of hypertension and the lethality of myocardial infarction (2). Improved understanding of myocardial biology favoring conversion of early forms of structural heart disease into the advanced syndrome of heart failure and sudden death is needed to devise more effective approaches to preventing and treating heart failure.

Why is CaMKII important in myocardial disease?
Many signaling molecules are implicated in structural heart disease. However, few, if any, molecules appear to be as central to pathological response mechanisms in heart as Ca\(^{2+}\)/calmodulin–dependent kinase II (CaMKII). CaMKII expression and activity are increased in failing human myocardium and in many animal models of cardiac hypertrophy and heart failure. CaMKII overexpression in mouse myocardium causes hypertrophy, failure, and sudden death (3), while CaMKII inhibition suppresses these phenotypes (4, 5). Pathological stress induces a cardiomyocyte milieu of dysregulated and prolonged cytoplasmic Ca\(^{2+}\) transients (6) and increased oxidative stress (7). CaMKII is an ideal nodal molecule for transducing Ca\(^{2+}\) and redox signals—fundamental upstream signals common to most forms of structural heart disease—into downstream events, such as apoptosis (8), hypertrophy (3), and proarrhythmic electrical remodeling (3, 4), that lead to the clinical phenotypes of congestive heart failure and sudden death (Figure 1A). Finally, CaMKII itself amplifies the disruption of intracellular Ca\(^{2+}\) homeostasis in heart failure by increasing the opening probability of voltage-gated Ca\(^{2+}\) channels (9) and ryanodine receptors, the latter being the major mediators of Ca\(^{2+}\)–induced Ca\(^{2+}\) release from intracellular sarcoplasmic reticulum Ca\(^{2+}\) stores in cardiac myocytes (3).

CaMKII is initially activated by binding to calciﬁed calmodulin (Ca\(^{2+}\)/CaM). Ca\(^{2+}\)/CaM binding reorders the structure of the CaMKII molecule by disinhibiting the catalytic domain and exposing the regulatory domain (Figure 1B). The CaMKII regulatory domain is the target for activity-sustaining modifications that bring about the transition of CaMKII into a Ca\(^{2+}\)/CaM–autonomous enzyme. Ca\(^{2+}\)/CaM–activated CaMKII monomers are susceptible to autophosphorylation (10) at threonine 286/287 (the specific number is isoform dependent) and oxidation of paired methionines 281 and 282 (8). CaMKII auto phosphorylation and oxidation prevent...

Conflict of interest: Mark E. Anderson is named as the inventor on patents issued and pending regarding the treatment of heart failure and arrhythmias by CaMKII inhibition.

Nonstandard abbreviations used: Ca\(^{2+}\)/CaM, calcified calmodulin; CaMKII, Ca\(^{2+}\)/calmodulin–dependent kinase II; HDAC, class II histone deacetylase; MEF2, myocyte enhancer factor 2.

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