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Editorial

Editorial: **Tregs and BCG—dangerous liaisons in TB**

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Following the discovery that a population of T cells with suppressive activity, termed Tregs, functions to maintain self-tolerance and prevent autoimmune diseases, it has been firmly established that Tregs can also control immune responses to pathogens [1]. During infection, Tregs appear to play a pivotal role in preventing immunopathology and limiting collateral damage to the host caused by immune responses to the pathogen. However, Tregs can also be exploited by the pathogen to subvert protective immune responses and thereby, prolong their survival in the host. This is a particular feature of pathogens that causes chronic infection, including many parasites, viruses, and bacteria, such as Mtb. In many of these infections, a failure to clear the infection can be attributed directly to potent Treg responses, and depletion or inactivation of Tregs in mouse models can enhance pathogen clearance.

Infection with Treg-inducing pathogens has also been associated with bystander suppression of immune responses to other pathogens but also, to allergens and self-antigens, exacerbating the coinfection or attenuating experimental allergy and autoimmunity [2, 3]. Indeed, this has provided experimental evidence in support of the hygiene hypothesis, which arose from the observations that a lower incidence of allergy/asthma and autoimmunity in rural communities in developing countries has been associated with a higher incidence of infections. The immunomodulatory effects of pathogens are mediated in a number of ways, including interference with signaling pathways that mediated maturation or proinflammatory cytokine production in innate cells, the enhancement of anti-inflammatory cytokines, and the induction or conversion of Tregs. $CD25^+$ $Foxp3^+$ natural Tregs and adaptive pathogen-specific Tregs are especially prominent in chronic infections that are associated with immune suppression.

Mtb is a good example of a bacterium that causes chronic infection, where Tregs have been implicated in pathogen persistence and protection against immune-mediated pathology. The possible consequences of exposure to Mtb include immune-mediated eradication, the development of latent infection involving immune-mediated containment of the bacillus in granulomas, or progression to active disease with associated pathology. Tregs are enhanced in patients with active disease, where they impair immune responses, especially IFN-γ production by protective Th1 cells. Tregs are prominent in TB granulomas and in TB pleural effusions. $Foxp3^+$ Tregs are significantly higher in the peripheral blood of patients with active compared with latent TB. Tregs may also render latent TB patients susceptible to progression to reactivation disease. Ex vivo depletion of Tregs in human PBMCs from patients with active disease enhances IFN-γ production in response to Mtb antigens. In vivo depletion of $CD25^+$ cells in mice was also shown to enhance IFN-γ production but did not affect bacterial burden or pathology [4]. However, transfer of $CD25^+$ T cells from Mtb-infected mice enhanced bacterial load in recipient mice [5]. On the other hand, the Tregs have also been shown to attenuate immune-mediated tissue injury.

Although still widely used for vaccination against TB, BCG is a poorly effective vaccine, especially in developing countries. Indeed, there is evidence of exacerbated disease in certain individuals vaccinated with BCG. The poor efficacy of the BCG vaccine has been attributed to a number of factors, including a failure to induce appropriate protective immune responses. However, it has also been suggested that prior exposure to environmental mycobacteria may reduce the efficacy of the vaccine; BCG vaccination is less effective in developing countries with a higher prevalence of environmental mycobacteria. In a recent paper demonstrating that antigen-specific Tregs impair immunity to Mtb by suppressing recruitment of effector T cells to the lung, the authors speculate that exposure to environmental mycobacteria may induce Mtb-specific Tregs, which cross-react with BCG antigens and impede vaccine-induced immunity against Mtb [5]. In this issue of the *Journal of Leukocyte Biology*, Ho et al. [6] provide evidence to support the hypothesis that Tregs induced by environmental mycobacteria do indeed constrain immunity induced by BCG.

Abbreviations: BCG=bacilli Calmette-Guérin, $Foxp3^+$=forkhead box $p3^+$, Mtb=*Mycobacterium tuberculosis*, TB=tuberculosis, Treg=regulatory T cell

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Using cell-transfer experiments, Ho et al. [6] demonstrate that intranasal administration of a heat-killed form of an environmental mycobacteria, *Mycobacterium chelonae*, sensitizes mice to generate excessive Treg responses after challenge with BCG. The *M. chelonae*-sensitized mice had less inflammatory infiltrate in the lungs after BCG challenge and more IL-10 production. When compared with $CD25^+$ T cells from naïve mice, the Tregs from *M. chelonae*-sensitized mice were more effective at suppressing proliferation and IFN-γ production by effector T cells in an in vitro suppression assay. The study provides clear evidence that environmental mycobacteria can promote expansion or recruitment of Tregs, which affects the immune response to BCG. It remains to be established whether this modified immune response will affect bacterial burden following Mtb challenge. It will also be necessary to extend these findings to studies about the effect of nasal or oral infection with different strains of environmental mycobacteria on the protective immune response induced with BCG. Finally, the antigen specificity of the phenomenon needs to be addressed. Is the suppression bystander, or is it mediated by activation of antigen-specific Tregs that are cross-reactive between environmental mycobacteria and BCG? Bystander suppression has been demonstrated for other pathogens [2], and if this is the case, it is more likely to be short-lived, unless there is constant re-exposure to the environmental mycobacteria. Indeed, there is evidence of bystander suppression induced by Mtb in humans; in vitro depletion of $CD25^+CD39^+$ Tregs, which secrete TGF-β and are highly suppressive, enhances IFN-γ production in response to Mtb antigens but also to an unrelated pathogen, CMV [7]. However, it has also been shown that adoptive transfer of Mtb-specific $CD25^+$ Tregs suppresses immune responses and exacerbates the course of Mtb infection following adoptive transfer in mice, whereas Tregs that did not recognize the Mtb were not suppressive [5]. If suppression is mediated by antigen-specific Tregs that cross-react between environmental mycobacteria and BCG, the problem may be even greater, as pre-existing, environmental mycobacteria-induced Tregs could be re-activated by cross-reactive antigens following exposure to BCG several months or years after initial exposure. These may suppress the development of

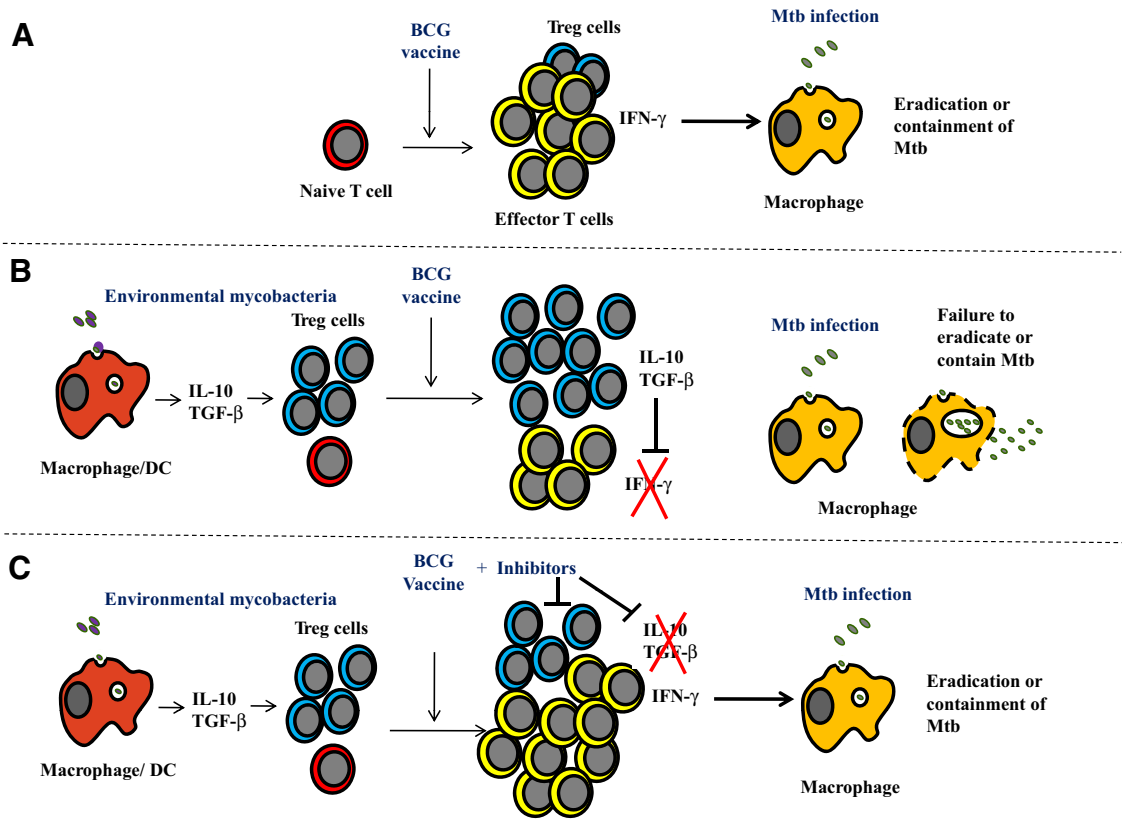

Figure 1. Suppression of regulatory cells induced by environmental mycobacteria has the capacity to enhance the efficacy of the BCG vaccine. (A) Immunization with the BCG vaccine in a proportion of healthy children in developed countries induces protective effector T cells that secrete IFN-γ, which activates macrophages to kill intracellular bacteria following exposure to Mtb. (B) Exposure to environmental mycobacteria, prevalent in developing countries, induces innate IL-10 and TGF-β production by macrophages and DCs that enhances the induction of Tregs. Following vaccination with BCG, cross-reactive Tregs are expanded and secrete high levels of IL-10 and TGF-β, which suppress the induction of protective effector T cells and IFN-γ production, resulting in a failure to contain the bacillus following exposure to Mtb. (C) Vaccination with BCG in the presence of inhibitors of Tregs or with neutralization of IL-10 and TGF-β has the potential to attenuate the suppressive effects of sensitization with environmental mycobacteria, allowing the development of protective effector T cell responses following exposure to Mtb.

Th1 responses and may thus compromise protective immunity induced by vaccination with BCG.

The findings of Ho et al. [6] have considerable implications for immunity to Mtb in humans and are complemented by some but not all clinical studies with environmental mycobacteria. It has been demonstrated that children from Malawi have higher IFN-γ responses to environmental mycobacteria than children in the United Kingdom, reflecting the higher prevalence of environmental mycobacteria in many developing countries [8]. Following BCG vaccination, the IFN-γ response in the United Kingdom cohort was enhanced to the level found in those from Malawi. Furthermore, the IFN-γ was induced in response to in vitro stimulation with a range of strains of environmental mycobacteria. Although the study provided evidence of T cell cross-reactivity between antigens of BCG and environmental mycobacteria, it also found that the initial response to environmental mycobacteria did not affect the subsequent response to BCG. Conversely, another study showed that infection with the environmental mycobacteria strain *Mycobacterium ulcerans* generated Th2 responses that cross-react with BCG, whereas individuals that had not been infected previously mounted Th1 responses [9], providing evidence that infection with certain environmental mycobacteria may influence the response to BCG in humans. The work of Ho et al. in mice is consistent with this observation in humans but suggests that Tregs, rather than Th2 cells induced by environmental mycobacteria, can suppress inflammatory responses to BCG. The suppression mediated by Tregs is more consistent with other literature about the suppressive role of Tregs versus Th2 cells and provides a plausible explanation for the poorer efficacy of the BCG vaccines in developing countries. If verified in humans, the study opens up the possibility of developing improved TB vaccines based on manipulating immune responses by removing or suppressing Tregs during immunization (**Fig. 1**).

There is growing evidence that Tregs may constrain the development of vaccine-induced immunity, especially against chronic infections but also against tumors. Indeed, strategies of depletion of Tregs or the use of adjuvant formulations that circumvent the induction of Tregs have already been shown to improve the efficacy of prophylactic vaccines against infection and therapeutic vaccines against tumors in mouse models [10]. Indeed Treg depletion has been examined in experimental studies with the BCG vaccine. Depletion of $CD25^+$ cells in mice prior to immunization with BCG enhanced IFN-γ production but only modestly enhanced protection against Mtb challenge [11]. It is possible that the effect might be more dramatic in the context of prior exposure to environmental mycobacteria, where the Tregs have already been induced. However, it is also possible that $CD25^+$ T cells may not be the best target for attenuation, as other populations of Tregs and indeed, innate cells with regulatory activity may account for suppression induced by environmental mycobacteria. It is of interest that in the study by Ho et al. [6], DCs were a major source of IL-10. Furthermore, it has been reported that suppression of allergic responses in mice by exposure to killed *Mycobacterium vaccae* is reversed by neutralization of IL-10 and TGF-β [3]. Consequently, strategies that transiently attenuate the induction of innate and T cell-derived, anti-inflammatory cytokines, including IL-10 and TGF-β, may have greater promise in enhancing the efficacy of BCG (Fig. 1). Finally, one of the problems with BCG is that it too promotes Tregs. Therefore, what is really needed to contain the spread of Mtb is a new generation subunit vaccine with an adjuvant formulation that selectively promotes effector cells but not Tregs.

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