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Reply to Hu et al: Could there be detrimental effects of statin adjunctive TB therapy on immune responses?

To The Editor—We thank Hu et al [1] for bringing attention to the potential deleterious immunological effects of statins, particularly on Bacillus Calmette-Guérin (BCG)-induced trained immunity and intravesical BCG therapy for bladder cancer [2]. Work over the past decade has overturned the simplistic dichotomous view of mammalian immune responses as innate, involving cells such as macrophages and natural killer cells, which are rapid, nonspecific and lack immunological memory, and adaptive, which are specific but slow, protecting the host against reinfection with the same organism through the clonal expansion of specialized memory T and B lymphocytes. Nonspecific innate immune memory responses previously described in plants and invertebrates were recently also recognized in mammals and termed "trained immunity", which is induced after a primary infection or vaccination and confers protection against the same microorganism or another (cross-protection) through mechanisms independent of classic adaptive responses [3]. Various organisms, vaccines, and biomolecules can confer trained immunity, such as the widely used tuberculosis (TB) vaccine BCG, the fungal cell wall polysaccharide β-glucan, and oxidized low-density lipoprotein (ox-LDL). This process seems to depend on epigenetic reprogramming during the transition from monocytes to macrophages [4].

Bacillus Calmette-Guérin vaccination is protective against childhood TB, including TB meningitis, but, because of waning immunity, has limited protection against pulmonary and other forms of TB in adults [5]. It is interesting to note that BCG vaccination reduces infant mortality due to infections other than TB in West Africa and reduces infant pneumonia-related deaths by 50% in Brazil [3]. Bacillus Calmette-Guérin vaccination protected severe combined immunodeficiency mice from disseminated *Candida albicans* infection through T- and B-lymphocyte–independent mechanisms, and circulating monocytes of BCG-vaccinated healthy humans showed increased release of tumor necrosis factor (TNF)α and interleukin (IL)-1β in response to unrelated bacterial and fungal pathogens; these training effects were induced through the NOD2 receptor and mediated by increased histone 3 lysine 4 trimethylation (H3K4) [6]. Indeed, studies in healthy volunteers vaccinated with BCG showed that TNFα and IL-1β release from peripheral blood mononuclear cells stimulated with mycobacteria or unrelated pathogens remain elevated for several months postvaccination, although, consistent with the known waning immunity of BCG, these effects were less pronounced 1 year after vaccination [7].

Trained immunity is associated with induction of specific cellular metabolic pathways, including glycolysis and glutamine metabolism. In particular, the

metabolite mevalonate of the cholesterol synthesis pathway (but not cholesterol synthesis per se) is required for induction of trained immunity via activation of insulin like growth factor-1 receptor and mammalian target of rapamycin and histone modifications in inflammatory pathways, and fluvastatin pre-exposure of monocytes downregulated the increased cytokine production and epigenetic reprogramming induced by BCG, β-glucan, or ox-LDL [8]. As highlighted by Hu et al, this finding raises the possibility that statins may reverse the beneficial effects of BCG vaccination on trained immunity. However, Bekkering et al [9] reported that in 25 patients with familial hypercholesterolemia, 3 months of statin therapy did not significantly alter cytokine expression or epigenetic reprogramming of monocytes, potentially explaining the persistently elevated cardiovascular risk in these patients despite statin therapy. In light of these findings, as well as the loss of BCG-induced protection against TB by adolescence [5] and waning trained immunity reported 1 year post-BCG vaccination [7], we think it is highly unlikely to observe reversal of BCG-induced trained immunity among adult TB patients receiving statin adjunctive therapy.

Previous studies have shown that statin use is associated with worse outcomes among patients with superficial bladder cancer receiving intravesical BCG instillation [10]. Because pharmacologic or genetic inhibition of autophagy has been shown to block epigenetic reprogramming of monocytes by BCG at the level of H3K4 trimethylation, and mutations in the autophagy pathway are associated with worse outcomes after BCG immunotherapy in patients with bladder cancer [11], it has been postulated that the effects of statin on survival of these patients might be explained by interfering with BCG-induced trained immunity [8]. However, this hypothesis is countered by the observation that statins induce autophagy in *Mycobacterium tuberculosis*-infected macrophages [12]. The potential roles of mevalonate accumulation and trained immunity in the therapeutic effects of intravesical BCG therapy remain to be determined, especially given the myriad immunomodulatory properties of statins.

In summary, we do not believe that statin adjunctive therapy of adults with TB will adversely impact trained immunity engendered by BCG vaccination given at birth. However, we agree that further preclinical and clinical studies should be performed to characterize the potential effects of statins and other host-directed therapies for TB on innate and adaptive immune responses, as well as on trained immunity.

Notes

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