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COMMENTARY

Trained Immunity and Susceptibility to HIV

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ABSTRACT In this issue of *Clinical and Vaccine Immunology*, K. Jensen et al. (Clin Vaccine Immunol 24:e00360-16, 2017, https://doi.org/10.1128/CVI.00360-16) describe a dual-purpose attenuated *Mycobacterium tuberculosis*-simian immunodeficiency virus vaccine (AMTB-SIV). Interestingly, immunized infant macaques required fewer oral exposures to SIV to become infected relative to nonimmunized animals. The authors hypothesized that augmented susceptibility to SIV was due to activation of $CD4^+$ T cells through trained immunity. This commentary explores the possible relationship between trained immunity, enhanced CD4 T cell responses, and increased susceptibility to human immunodeficiency virus (HIV).

KEYWORDS BCG, HIV, trained immunity, tuberculosis, vaccine

The failure of the adenovirus type 5 (Ad5) human immunodeficiency virus type 1 (HIV-1) vaccine used in the Step phase 2b study to provide protection against HIV was a great disappointment (1). The vaccine (Ad5 HIV-1 Gag/Pol/Nef), which was designed to elicit cell-mediated immunity, neither prevented infection nor reduced viral loads in vaccinees. Not only did the vaccine fail to protect, there were actually more cases of HIV in the vaccine arm of the study than in the placebo group. Initial analysis of the data revealed a higher incidence of HIV infection among subjects with preexisting Ad5 antibodies relative to the placebo group. There was no difference, however, in HIV infection rates between the placebo group and vaccinees with no preexisting Ad5-specific antibodies, leading some to speculate that the vaccine led to induction and expansion of Ad5-specific $CD4^+$ T cells which served as targets for HIV replication following infection. However, subsequent analysis of samples from subjects with differing levels of preexisting Ad5 neutralizing antibody did not show a positive correlation between baseline Ad5-specific antibody levels and Ad5-specific $CD4^+$ T cell responses in the volunteers (2). The results of a second trial of the vaccine, the HVTN 503/Phambili study, also showed increased HIV-1 infection rates in vaccinated subjects relative to the placebo group, irrespective of preexisting Ad5 serostatus (3, 4). Of the 3,000 enrolled in the study, 63 vaccinees acquired HIV-1 infections compared with 37 in the placebo group. The natural fallout from these studies was an understandable reservation against future use of adenovirus-based HIV vaccines and a desire to uncover an immunological basis for this surprising outcome so that safer HIV vaccines and trials could be designed in the future.

Interestingly, subsequent analysis of stored samples from the Step study showed that preexisting herpes simplex virus 2 (HSV-2) infection correlated with an increased risk of infection with HIV-1, but not viral load or early progression of disease (5). Other investigators, attempting to uncover immune correlates that might predict increased risk of infection, analyzed peripheral blood mononuclear cell (PBMC) samples from the study with or without stimulation with Gag, Pol, and Nef peptides by flow cytometry or enzyme-linked immunosorbent spot assay (ELISpot) analysis. They discovered that while HIV-specific immune responses were not associated with risk of HIV-1 infection, increased mock responses (increased gamma interferon [IFN-γ] secretion without

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antigen stimulation) correlated with increased risk of HIV-1 among vaccinees but not among the placebo group (6). Moreover, uncircumcised men and men seropositive for Ad5 possessed the highest frequencies of nonspecific IFN-γ-producing cells which appeared to be $CD4^+$ T cells. These authors concluded that the observed nonspecific IFN-γ responses may be due to previous viral infections, including Ad5, and the results supported the idea that the enhanced risk of HIV infection in the Step trial was associated with $CD4^+$ T cell responses.

The concept of trained immunity, also referred to as innate immune memory, was first proposed by Netea et al. (7) and is defined as an enhanced, nonspecific, T cell-independent secondary response. Thus, following a primary infection, the enhanced secondary response can be against infection with the same or a different microorganism. Features of trained immunity include the involvement of cells of the innate immune response (NK cells and myeloid cells, i.e., T cell independent) and their pattern recognition receptors (PRRs), a heightened, yet nonspecific response to a secondary infection, relatively short-lived time frame lasting weeks rather than years, and mediation through epigenetic reprogramming (8–10).

Recent experimental evidence for trained immunity included studies of mice without functional T cells that were infected with a low, sublethal dose of *Candida albicans* followed by a lethal challenge 7 days later. The "trained" mice survived significantly longer than controls and had lower CFU in the kidneys, though not statistically significantly different (11). *Candida albicans* or β-glucan, a component of the fungal cell wall, induced dectin-1-dependent enhanced cytokine production from monocytes which was mediated through epigenetic changes. These changes included histone trimethylation at the promoters of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and IL-18 and of PRRs such as dectin-1 and Toll-like receptor 4 (TLR4). Such epigenetic reprogramming or "training" of monocytes is thought to lead to enhanced and more-durable nonspecific protective responses.

Immunization with *Mycobacterium bovis* BCG has been shown to provide variable protective efficacy against pulmonary tuberculosis in humans; however, early observations suggested that BCG vaccination conferred an overall beneficial impact on childhood survival (12). More recently, epidemiologic studies have shown that BCG vaccination was associated with a 45% decrease in infant mortality in Guinea-Bissau and Benin, which transcended its impact on reducing disseminated childhood tuberculosis (TB) (13, 14). Moreover, studies in Brazil demonstrated that BCG immunization reduced the risk of pneumonia-related deaths by 50% (15), and an observational study in Guinea-Bissau concluded that the presence of a BCG scar in children significantly reduced the risk of death from malaria (14). Animal studies have shown that BCG confers partial protection against unrelated pathogens, including *Babesia microti*, *Plasmodium berghei*, *Toxoplasma gondii*, and *Trypanosoma cruzi*, and diseases such as influenza and malaria (16). Furthermore, for several decades, intravesical BCG therapy has been the treatment of choice for several forms of bladder cancer (16).

Netea and colleagues (7, 8) have proposed that the nonspecific protective benefits provided by BCG immunization may be mediated through trained immunity. Interestingly, immunization of neonates with BCG enhanced Th1 responses to other vaccines given later in infancy (17). Experimental support for the trained immunity hypothesis was provided by Kleinnijenhuis et al. where PBMCs from humans, shortly after immunization with BCG, were stimulated with *Mycobacterium tuberculosis* or unrelated microorganisms (18). Following incubation with *M. tuberculosis*, *Staphylococcus aureus*, or *Candida albicans*, an enhanced release of cytokines (IFN-γ, TNF-α, or IL-1β) and expression of CD14, CD11b, and TLR4 were observed from circulating monocytes relative to cells from the same volunteers obtained prior to immunization. Mechanistic studies showed that trimethylation of histone H3 at lysine 4 was significantly increased at the TNF-α and IL-6 promoters in stimulated monocytes collected after BCG immunization relative to preimmunization levels up to 3 months following vaccination. Further, monocytes isolated from NOD2-deficient, but not dectin-1-deficient, human subjects failed to exhibit enhanced release of TNF-α following exposure to BCG in

culture. To demonstrate trained immunity in the context of BCG-mediated protection against an unrelated pathogen (i.e., T cell independent and nonspecific), SCID mice were immunized with BCG and subsequently infected with *C. albicans*. All the immunized mice survived 1 month after challenge compared with 30% of the control mice, and kidney CFU were significantly reduced in the vaccinated mice relative to naive control mice.

In another study, monocytes obtained from volunteers up to 1 year following BCG immunization were found to have significantly enhanced expression of CD14, TLR4, CD11b, and mannose receptors relative to baseline levels (19). Intriguingly, following stimulation of PBMCs with either sonicated *M. tuberculosis* or heat-killed *C. albicans* or *S. aureus*, elevated Th1 (IFN-γ) and Th17 (IL-17 and IL-22) responses were observed up to 1 year after vaccination relative to prevaccination levels. Heat-killed *C. albicans* or lipopolysaccharide (LPS) also stimulated enhanced TNF-α and IL-1β production from PBMCs from these individuals for up to 3 months after BCG immunization, but these responses began to decline by 1 year after vaccination. Importantly, these results suggest that trained innate responses may also augment adaptive immune responses.

Despite the protective benefit of BCG immunization against disseminated forms of TB and the overall survival advantage, BCG vaccination is contraindicated in the HIV-infected population due to potential disseminated BCG infection in these immunocompromised people. Furthermore, an *ex vivo* study suggested that BCG may also increase susceptibility to HIV infection. Thayil et al. showed that stimulation of PBMCs from healthy human subjects with *M. tuberculosis* or BCG, but not *Mycobacterium smegmatis*, enhanced susceptibility of $CD4^+$ T cells to HIV infection, which was shown to be TLR2 dependent (20). It is known that HIV preferentially infects activated $CD4^+$ T cells, and interestingly, the greatest prognosticator of progression to AIDS, even more than viral loads, is T cell activation (21).

Consistent with this idea, Jensen and colleagues hypothesize that vaccine-induced trained immunity led to increased susceptibility of infant macaques to SIV infection through enhanced activation of $CD4^+$ T cells (22). They used a replication-deficient *M. tuberculosis* strain (*panCD*, *leuCD*, and *secA2* deletion mutant) expressing the SIV Gag and Env antigens (attenuated *M. tuberculosis*-simian immunodeficiency virus vaccine [AMTB-SIV]) as a dual-purpose SIV-*M. tuberculosis* vaccine. The authors examined the efficacy of their vaccine using an infant SIV repeated oral challenge model to replicate breast milk transmission of HIV in humans. In a previous study (using AMTB-SIV boosted with a modified vaccinia Ankara [MVA]-SIV construct), they noted that more than 80% of the immunized infants became infected following one or two oral SIV infections compared to 50% of the control group (23). Although this difference did not reach statistical significance, very similar results were reported in the current study with 79% of the AMTB-SIV-immunized animals becoming infected after two oral SIV exposures compared to 47% for control animals. Furthermore, for BCG-immunized animals, 88% became infected after two exposures. Again, however, the differences did not reach statistical significance. These results indicate that the apparent enhanced susceptibility to SIV was irrespective of the vaccine's specificity (not SIV specific), suggesting a role for trained immunity.

Using stored frozen samples from their previous study (plasma, lymph nodes, mucosal tissue, and PBMCs), Jensen et al. (22) measured activation marker expression and cytokine production in both myeloid (monocytes and myeloid dendritic cells [mDCs]) and $CD4^+$ T cells and provided evidence for enhanced activation in the animals immunized with BCG or AMTB-SIV relative to naive controls. Peripheral blood monocytes/macrophages and $CD4^+$ T cells from nonchallenged, immunized infant macaques expressed significantly elevated levels of the activation markers CCR5 (chemokine [C-C motif] receptor 5) and CD69 relative to nonimmunized animals at 16 to 18 weeks following immunization. The Ki67 and PD-1 activation markers were also significantly elevated on $CD4^+$ T cells found in PBMCs and colon sample from the AMTB-SIV groups at this time point. Moreover, elevated myeloid cell activation states as indicated by increased levels of plasma macrophage chemoattractant 1 (MCP-1) as well as IL-12^+

$CD14^+$ and IL-12^+ mDC cell frequencies were evident at 9 weeks postimmunization, the time of SIV challenge. Activation states of these cell populations before challenge appeared to be more pronounced in the BCG-immunized animals, although it depended on the tissue examined. Additionally, the authors demonstrated that both BCG and AMTB-SIV vaccines could activate myeloid cells from human adult PBMCs following *ex vivo* stimulation.

At the start of SIV challenge, significantly higher frequencies of $CD4^+$ T cells from PBMCs from immunized animals produced cytokines (IL-2, IFN-γ, and TNF-α) relative to naive control samples without *ex vivo* stimulation. Jensen et al. (22) point out that these frequencies exceeded the antigen-specific $CD4^+$ T cell responses (both SIV and TB) observed in their previous studies; thus, the comparatively elevated activation states of the $CD4^+$ T cell populations seen in this study appear to be unrelated to vaccine specificity. As indicated above, some of the highest responses after challenge were observed in the BCG-immunized animals.

This paper (22) adds to the growing body of evidence that some infections may enhance susceptibility to HIV infection and suggests the involvement of trained immunity though more mechanistic studies are needed. For example, the authors did not demonstrate AMTB-SIV-mediated epigenetic changes that would promote enhanced activation of myeloid cells, nor was a role for NOD2 implicated. Would stimulation of PBMCs from the AMTB-SIV-vaccinated animals with an unrelated pathogen lead to similar levels of activation and would some level of protection be observed in immunized animals? Would *ex vivo* exposure of cells to AMTB-SIV enhance infection of $CD4^+$ T cells with HIV? More work is clearly warranted, since studies that improve our understanding of immune mechanisms that lead to enhanced susceptibility to HIV infection would help in the design of safer HIV vaccines.

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