Only two HIV vaccines have been taken through efficacy trials so far. In the first HIV vaccine efficacy trial started ten years ago, recombinant gp120 protein, the CD4-binding subunit of the HIV envelope, was used as vaccine antigen [1]. The vaccine neither prevented HIV acquisition nor reduced the viral load in those acquiring HIV infection. Although the vaccine was able to induce antibodies to gp120, these did not neutralize field isolates of HIV. Differences in the conformation between the monomeric gp120 subunit of the vaccine and the functionally active trimeric envelope spike on the surface of virus particles, HIV diversity, as well as various antibody escape mechanisms of the HIV envelope (reviewed in [2]), have been proposed to explain the inefficacy of the antibody-based gp120 vaccine. Given the difficulties of antibody-based HIV prevention strategies, the second HIV efficacy trial, the STEP study, tested whether the second arm of the adaptive immune response, cytotoxic T cells, would be able to provide protection. To induce cytotoxic T cell responses, replication-deficient adenoaviral vectors transferring the gag, pol, and nef genes of HIV were used. Since all the three vaccine antigens used in this study are intracellular proteins that are usually not expressed on the surface of HIV-infected cells or HIV particles, vaccine-induced HIV-specific antibodies should not be able to contribute to protection. Thus, the study was specifically designed to explore the efficacy of HIV-specific cytotoxic T cells. A total of 3,000 volunteers with a high risk of acquiring HIV infection were either immunized three times intramuscularly with replication-deficient adenoaviral vectors transferring the gag, pol, and nef genes of HIV, or received a placebo. As observed in non-human primate studies and previous phase I clinical trials, the adenoaviral vector vaccine induced substantial HIV-specific cytotoxic T cell responses in most of the vaccinees [3]. However, at a planned interim analysis, 19 individuals in the vaccine arm and 11 individuals of the placebo arm acquired HIV infection during a follow-up of approximately 620 person years in both groups [4]. Incidences of 3.07 and 1.77 per 100 volunteers in the vaccine and placebo group, respectively, indicate that there was no beneficial effect of the vaccine on HIV acquisition.

The HIV virus particle transmitted to an individual cannot be targeted by the vaccinees’ cytotoxic T cells, because they require presentation of HIV-derived peptides on autologous MHC-I molecules. When looking at the different stages in the establishment of HIV infection after mucosal exposure (Figure 1), the earliest stage cytotoxic T cells could exert their beneficial effect is the killing of the first HIV-infected cell, presumably in the lamina propria of the exposed mucosa. However, given the low density of T cells in this compartment, it seems highly unlikely that an HIV-specific cytotoxic T cell encounters this single HIV-infected cell. Rather, it can be assumed that additional replication cycles and local spread of the virus or virus-infected cells to the draining lymph nodes occur prior to encounter with HIV-specific T cells. Subsequent activation and expansion of the HIV-specific T cells might be too slow to prevent further spread of the virus. Thus, rather than preventing HIV infection, the benefit of the cytotoxic T cells might be the reduction of viral load. However, the interim analysis of the STEP study also failed to provide any evidence for lower viral loads in the vaccine group [4]. Therefore, neither non-neutralizing gp120-specific antibodies nor HIV-specific cytotoxic T cells induced by the adenoaviral vector vaccine were sufficient to provide protection.

The incidence of HIV infections in the vaccine group seemed to be higher than in the placebo group. This raised the critical question of whether vaccination actually enhances the frequency of HIV acquisition. Post-hoc analyses of different subgroups indicated that the incidence of HIV infections in volunteers with pre-existing humoral immunity to adenovirus prior to immunization was 2.3-fold (95% confidence interval 1.1 to 4.7) higher than in the respective placebo subgroup [4]. In contrast, there was no difference in the incidence of HIV infection between the vaccine and placebo groups in the absence of pre-existing antibodies to adenovirus. An initial comparison of the distribution of risk factors in the vaccine and placebo subgroups with high levels of pre-existing adenoviral immunity revealed a good match of baseline variables such as location, race, age, risk behavior, circumcision, and history of sexual transmitted diseases [5]. If this holds up for other confounding factors, the enhanced incidence of HIV infections in this vaccinated subgroup might have severe implications for all subsequent HIV vaccine trials, as well as for vaccine and gene therapy trials using adenoaviral vectors. It is therefore important to explore how such a vaccine could increase the susceptibility to HIV infection. Theoretically, this could be either due to an excessive adenovirus-specific immune reaction or due to the induction of detrimental HIV-specific immune responses.

Injection of the adenoaviral vector particle can induce an immediate innate response leading to increased inflammatory cytokine levels in the blood, which...
intraepithelial DC, macrophages, or CD4+ mucosa through breaks or by transport on dendritic cells (DC), transcytosis, or infection of the lamina propria of the exposed mucosa or in its draining lymph node. In the absence of effective antiviral effector mechanisms, these activated HIV-specific “enhancer” T cells could favour early spread of the HIV infection. Whether this is only a risk associated with adenoviral vector vaccines encoding HIV antigens or is also shared by other HIV vaccines is unclear. However, two characteristics of the vaccine used in the STEP study suggest a note of caution to premature generalization. First, the enhancement of HIV infection is only detected in the presence of pre-existing adenoviral immunity. Thus, the injected adenoviral vector particle might trigger regulatory events, which also suppress or modulate as a bystander effect the HIV-specific T cell responses induced by the adenoviral vector-encoded HIV vaccine antigens. Second, the absence of an Env component might delay the sensing of the HIV infection by antibodies (even non-neutralizing ones) at an early infection stage, thereby preventing timely recruitment of vaccine-induced effector mechanisms to the early replication sites of HIV. As neither humoral nor cellular immune responses alone seem to be sufficient for protection from HIV, vaccines inducing both effector arms need to be evaluated. It seems too early to dispense with the potential of adenoviral vector-based HIV vaccines since they are among the most efficient inducers of cytotoxic T cell responses in humans and a large number of animal models.

Whatever the precise mechanism is that led to enhanced acquisition of HIV infection in the STEP study vaccine group with pre-existing adenovirus immunity, it most likely acts at a stage subsequent to infection of the first cell in the recipient individual. The barrier function of the mucosal epithelium should not be affected by the intramuscular vaccination. The number of HIV-susceptible cells in the lamina propria prior to HIV exposure should not be enhanced above the pre-existing background level given the continuous exposure to antigens and frequent infections with all kinds of pathogens and commensals. Since all the vaccine antigens return to baseline levels within 3 to 7 days [6]. However, since the increased HIV incidence in the vaccine subgroup with pre-existing adenoviral immunity seems to persist for at least a year [4], the immediate innate response is unlikely to be responsible for the observed increase in susceptibility to HIV infection in the vaccine subgroup. Injection of the adenoviral particle into individuals with pre-existing adenoviral immunity also raises a recall response, including activation of adenovirus-specific CD4+ T cells. These activated CD4+ T cells could serve as additional target cells for HIV in the lamina propria and therefore enhance the risk of HIV infection. However, given the vast amount of antigens and infections humans are continuously exposed to, it seems highly unlikely that intramuscular injection of the adenoviral particle can notably raise the number of susceptible CD4+ T cells in the rectal or male genital mucosa above their pre-existing back-
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