



PERSPECTIVE

The two faces of vaccine-induced immune response: protection or increased risk of HIV infection?!

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Since the first HIV vaccine trial in 1986 a variety of vaccination strategies have been developed and tested for immunogenicity in clinical phase I safety trials. Nevertheless, only six vaccines have been approved for phase IIb/III efficacy trials with only one, the Thai RV144 trial, showing modest efficacy in reducing the infection rate (reviewed in Esparza J, 2013). A major obstacle in HIV vaccine development is the absence of clear correlates of protection, although the Thai trial revealed some correlation of non-neutralizing antibodies specific for the V1/V2 region of the envelope (env) protein and a reduced rate of infection (Haynes B F, et al., 2012). Generally, an effective HIV vaccine is considered to induce one of the following scenarios; it could either lead to sterile immunity by preventing the initial HIV infection through broadly-neutralizing antibodies or restrict the spread of the virus by early mucosal immune mechanisms including Fc-mediated effector functions (e.g. ADCC) or cell-mediated immunity (e.g. HIV-specific cytotoxic T-cells). The latter was also the goal of the STEP trial enrolled in 2004 and halted in 2007 due to the absence of vaccine efficacy (Buchbinder S P, et al., 2008). In this study, a trivalent adenoviral vector vaccine encoding HIV Gag, Pol and Nef were used to induce high numbers of HIV-specific T-cells, which in similar approaches demonstrated the potential to control SIV or SHIV

infections in non-human primate models. Beside the disappointing vaccine efficacy, the STEP trial provided evidence that immune responses induced by an HIV vaccine based on an adenoviral vector may be detrimental. There was an increased risk of acquiring an HIV infection for vaccinated volunteers who had pre-existing antibodies to the adenoviral vector and/or were uncircumcised (Duerr A, et al., 2012).

Since the higher rate of infection was associated with adenovirus-specific immune responses, it was hypothesized that anamnestic adenovirus-specific CD4⁺ T lymphocyte responses were responsible for the enhancement of HIV-1 acquisition in adenovirus-seropositive subjects (Buchbinder S P, et al., 2008, Sekaly R P, 2008). Although subsequent studies provided evidence against this hypothesis (Masek-Hammerman K, et al., 2010, O'Brien K L, et al., 2009), it is still of debate if a general immune activation in the mucosa at the time of HIV acquisition could facilitate the establishment of a systemic infection. Indeed, increased cellular susceptibility to *in vitro* HIV infection was shown to be associated with elevated activated CD4⁺ T-cells and viral production was preferentially established in activated CD4⁺ T-cells (Zhang Z, et al., 1999; Card C M, et al., 2012). Furthermore, it is known that quiescent CD4⁺ T-cells can get infected by HIV but very inefficiently; defects are observed at the

early stages of infection (reviewed in Vatakis D N, et al., 2010).

A fact that supports the correlation of mucosal immune activation and the risk of HIV infection is the 2-3 fold increased risk of HIV infection in HSV-2 infected woman and men (Freeman E E, et al., 2006). In a non-human primate model of HSV-2 and HIV co-infection, innate as well as adaptive immune responses coincided with the increased HIV infection rate (Crostarosa F, et al., 2009). Furthermore, rhesus macaques vaccinated with live-attenuated Rev-Independent Nef⁻ SIV two weeks prior to the onset of challenges with pathogenic SIVsmE660 were more susceptible to infections compared to monkeys with much longer time intervals between live-virus exposure and SIV challenges (Byrareddy S N, et al., 2013). Gene-expression analyses in acutely vaccinated macaques revealed up-regulation of proteins involved in innate immune signaling, like chemokines (e.g. CCL3) and chemokine receptors (CCR5), which supports the idea of vaccine-induced non-specific recruitment of HIV-target cells to the mucosal surface. Since gp120 of HIV could trigger chemokine signaling by CXCR4 engagement and actin cytoskeleton reorganization which facilitates HIV infection in resting CD4⁺ T-cells (Yoder A, et al., 2008), it might be also worthy to explore structural env proteins lacking these signaling domains as vaccine antigen.

Nevertheless, as mentioned above, subsequent analyses of the results from the STEP trial provide evidence against the re-activation of adenovirus-specific CD4⁺ T-cells being responsible for the enhanced risk of infection (Masek-Hammerman K, et al., 2010, O'Brien K L, et al., 2009). This suggests rather an alteration of the vaccine-induced HIV-specific cellular response than a non-specific immune activation which could facilitate the systemic spread of the early HIV infection (discussed in Überla K, 2008).

This is further supported by a non-human primate study modeling the STEP study, in which macaques infected with adenovirus prior to adenoviral vector immunization against SIV had a higher risk of SIV infection. Since the trend to enhanced susceptibility to infection was not observed in macaques infected with adenovirus prior to immunization with a control adenoviral vector, SIV-specific immune responses seem to be responsible for enhanced susceptibility (Qureshi H, et al., 2012). Previously, it had been reported that vaccination with a recombinant Varicella-Zoster Virus expressing SIV Env enhanced SIV replication and faster progression to AIDS in non-human primates, which correlated with SIV-specific CD4⁺ T-cell responses early after infection (Staprans S I, et al., 2004). Since the authors performed a single intravenous challenge with a high-dose of SIV, they could not specify if the SIV-specific immune response enhanced the risk of SIV acquisition or increased viral loads once infection was established.

Recently, during the analysis of the efficacy of a novel complementary prime-boost immunization in non-human primates, we obtained evidence that the risk of immunodeficiency virus infection may increase with vaccine-induced immune response (Tenbusch M, et al., 2012b). In this study, we boosted a cohort of rhesus macaques, which had been

previously primed with DC-targeting DNA vaccines (Tenbusch M, et al., 2012a), with SIV virus like particles (VLPs) and analyzed the protective capacity in a repeated low-dose challenge experiment. Antibodies specific to SIV Gag and SIV Env were induced in all animals, but, consistent with a poor neutralizing activity at the time of challenge, vaccinated monkeys were not protected from acquisition of infection. Strikingly, in the absence of strong HIV-specific CD8⁺ T-cells the magnitude of the vaccine-induced, SIV-specific IFN- γ -secreting cells detected by ELISpot analyses correlates with the susceptibility to acquisition of SIV infection. Important to mention, the higher risk of infection did not correlate with high viral loads after the establishment of the SIV infection (Tenbusch M, et al., 2012b). This indicates that one has to differentiate between correlates of protection from acquisition and correlates of early control of viral replication. In this regard, Barouch et al. reported on protection against acquisition of neutralizing-resistant SIV infection in vaccinated monkeys which correlates solely with the amount of non-neutralizing antibodies against the envelope protein. In contrast, a variety of immunological parameters, i.a. ADCC activity and the magnitude and breadth of gag-specific ELISpot responses, correlated with the virological control after infection (Barouch D H, et al., 2012).

Considering the fact that HIV infects preferentially HIV-specific CD4⁺ target cells (Douek DC, et al., 2002), our results support the hypothesis that vaccine-induced HIV-specific CD4⁺ T-cells could facilitate the establishment of a systemic infection. In the absence of protective HIV-specific cytotoxic T-cells, the re-activation of HIV-specific CD4⁺ T-cells by HIV-presenting DCs could lead to clonal expansion of potential HIV target cells supporting the spread of the virus, e.g. by migration of these cells

to the lymph node. Furthermore the secretion of IFN- γ might promote further recruitment of immune cells to the site of infection via IFN- γ inducible chemokine production (e.g. IP-10) in the mucosa.

It should be also noted that not all virus-specific CD4⁺ T-cell responses seem to be detrimental. During acute HIV infection, virus-specific T cells with cytolytic activity are associated with better control of viremia (Soghoian D Z, et al., 2012). It might be even possible that the same virus-specific CD4⁺ T-cells might increase susceptibility to acquisition of infection if present at the time of exposure and contribute to control of virus replication once infection has been established.

Thus, the efficacy of HIV vaccines may not only depend on the strength of protective immune responses induced, but also on the magnitude of vaccine-induced immune mechanisms increasing the susceptibility to infection. It is essential to explore innovative immune modulation strategies to translate this consideration into a HIV prevention approach.

One perspective concept suggests that inducing immune quiescence at the site of HIV exposure will reduce the number of activated target cells, thereby preventing infection or limiting it to small foci of infected resting target cells. This concept is based on findings in a cohort of HIV-exposed seronegative (HESN) people in Pumwani, which demonstrated a lower general immune activation status in the mucosal compartment (rev. in Card C M, et al., 2013). It might be achieved either by topical administration of anti-inflammatory compounds (Li Q, et al., 2009) or by induction of regulatory cells. CD4⁺ T regulatory cells (Tregs) are known to suppress activation of antigen-specific CD4⁺ effector T-cells. However, CD4⁺ Tregs can be targets of HIV infection themselves (Moreno-Fernandez M E, et al., 2009) and, therefore, activation and expansion of CD4⁺ Tregs might

counteract the protective effect of limiting effector CD4⁺ T-cell activation. In this regard, JM Andrieu and colleagues made an interesting observation in their non-human primate study. They reported on a tolerogenic vaccine inducing MHC-Ib/E-restricted CD8⁺ regulatory T cells that suppressed SIV-harboring CD4⁺ T-cell activation and *ex vivo* SIV replication without inducing SIV-specific antibodies or cytotoxic T lymphocytes. Remarkably, 15 out of 16 vaccinated macaques that were intrarectally challenged with SIVmac239 or heterologous strain SIVB670, were protected from infection (Lu W, et al., 2012). However, the induction of immune quiescence is not equivalent to the induction of anergy (rev in Card C M, et al., 2013) and active virus-specific immune mechanisms are still needed to clear the free virus or/and infected resting target cells.

Since the follow-up studies of the Thai trial and the non-human primate study of Barouch and colleagues revealed that non-neutralizing env-specific antibodies are the best correlate of protection against a lentiviral infection (Haynes B F, et al., 2012, Barouch D H, et al., 2012), an effective HIV vaccine should ideally induce broadly-reactive antibodies, but at the same time avoid stimulation of HIV-specific T helper cell responses at the time of HIV acquisition. Therefore it might be a potential approach to first induce HIV-specific antibodies by vaccination with structural env proteins followed by topical treatment with suitable microbicides containing anti-inflammatory mediators to inhibit excessive mucosal T-cell activation.

Alternatively, inducing antibodies

to the HIV envelope without prior activation of HIV-specific CD4⁺ T-cells would circumvent the enhanced risk of infection. An interesting approach was recently described in a mouse model, where a specific blockade of a retinoic acid producing enzyme (ALDH1a2) in dendritic cells during vaccination inhibits the activation of potential $\alpha 4\beta 7^{\text{hi}}$ CD4⁺ target cells but not the induction of systemic and mucosal anti-HIV antibody and cytotoxic T-cell responses (Zhu W, et al., 2013).

Since it was demonstrated that the maintenance of env-specific antibodies is largely T-cell independent (Nabi G, et al., 2012), it might be also possible to develop a method to induce these antibodies independent of HIV-specific CD4⁺ T-cells.

Nevertheless, the unique characteristic of HIV to infect activated CD4⁺ T-cells at the mucosal surface makes it necessary to develop alternative vaccination strategies which differ from the classical approaches used for the already licensed vaccines against other viruses.

FOOTNOTES

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