Reduction of HIV-1 Load in Semen During Follow-up Study of RV144 Vaccine Trial Boosts Interest for Novel Correlates of Immune Protection in Genital Mucosa

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(See the major article by Rerks-Ngarm et al on pages 1195–205.)

The design and production of effective vaccines against human immunodeficiency virus type 1 (HIV-1) are persisting major challenges for the HIV-1 scientific community. It also remains unclear which immune correlates should be considered as part of a successful HIV-1 vaccine and in which tissues these correlates should be measured. The discussion, which has been ongoing for the 30 years since HIV was isolated, is whether a potentially successful HIV-1 vaccine should lead to sterilizing immunity against the virus or whether a vaccine would be considered successful if the clinical syndromes linked to the infection were milder or delayed.

The RV144 trial conducted in Thailand, using the combination of AIDSVAX B/E gp120 vaccine and the CD4+ T-cell-stimulating ALVAC canarypox vaccine, demonstrated an estimated efficacy of 31.2% for protection against acquisition of HIV-1 [1]. Analyses conducted to define RV144 vaccine immune correlates of protection suggested that serum antibodies specific for the V1/V2 regions of the gp120 HIV-1 protein were associated with protection against HIV-1 acquisition [2]. On the contrary, high concentrations of HIV-1 Env-specific immunoglobulin (Ig) A in plasma were directly correlated with infection risk [2]. Hence, these in vivo findings obtained from a human vaccination study clearly illustrate that certain HIV-1–specific antibodies may not be beneficial in preventing HIV-1 acquisition [3], warranting further studies to map the specificity and property of antibodies protecting from, or enhancing, HIV-1 infection and of antibodies that may mitigate the effects of protective antibodies.

In the study by Rerks-Ngarm and collaborators in the present issue of The Journal of Infectious Diseases [4], the investigators extended the virologic, immunologic, and clinical course studies of volunteers (receiving either placebo [n = 65] or HIV-1 vaccine [n = 49]) who acquired HIV-1 infection during the RV144 trial, with the aim of determining whether vaccination influenced disease progression linked to HIV-1 infection. High concentrations of specific plasma antibodies are often correlates of protection against acquisition of pathogens for most licensed vaccines [5, 6], and in the RV144 trial the fact that the presence of serum V1/V2 antibodies in vaccinated subjects protected from infection points in the same direction [2]. Whereas the role of humoral immunity on disease progression in HIV-1 infection can be questioned [7–9], control of viremia has often been associated with functional CD8+ T cells [10]. Nevertheless, because passive transfer of broadly reactive HIV-1 neutralizing antibodies can protect macaques against simian-human immunodeficiency virus infection [11–19], the induction of HIV-1 neutralizing antibodies through HIV-1 vaccination remains a highly desirable goal [20].

In the extended follow-up RV152 study reported by Rerks-Ngarm et al, 61 different end points were studied, including both humoral and cellular responses, during a follow-up of 66 months after the estimated time of HIV-1 acquisition. Unfortunately, the ALVAC-HIV and AIDSVAX vaccination did not significantly affect the clinical course of HIV-1 disease, even when the follow-up was extended to 66 months. However, a tendency for lower viral load in plasma and higher blood CD4+ T-cell counts appearing late, at 60 and 66 months after infection, was reported. It will be important to follow the analyses of the cellular immune responses that developed in the
vaccine recipients (compared with the placebo group), because these analyses may shed light on immune parameters desirable to elicit when designing the next generation of HIV-1 vaccines.

The present publication by Rerks-Ngarm et al provides an additional piece of information needed for successful HIV-1 vaccination, by showing the presence of lower viral loads in mucosal samples from vaccine recipients, particularly semen samples (from earlier than 6 months to later than 12 months after infection). Semen and cervicovaginal mucosal samples for the measurement of viral load were collected at the time of enrollment in the RV152 study, hence around the time of HIV-1 acquisition. The findings highlight the importance of analyzing mucosal responses as potential correlates of protection from HIV-1 acquisition during the course of HIV-1 vaccine testing. Although it is conceivable that collection of mucosal samples may be challenging in phase III vaccine efficacy trials, particularly for cervicovaginal mucosal samples, such collection must be undertaken to evaluate parameters of vaccination responses that may take place only in the genital mucosal tissue (Figure 1).

Assessments of antibody responses in cervicovaginal specimens have been performed in certain human papillomavirus (HPV) vaccine studies; so far, these studies were not powered to define immune correlates of protection against HPV acquisition but, nevertheless, showed significant induction of HPV-specific antibodies in the mucosa of vaccine recipients [21, 22]. The results presented by Rerks-Ngarm et al in the current issue suggest that it may prove valuable to analyze mucosal samples in upcoming HIV-1 vaccine trials, provided that enough samples can be collected to perform the studies. The conduct of such studies entails careful standardization of the sampling procedures, including type of collection device, precise anatomical location of sampling, other detection of confounding sexually transmitted infections or vaginosis, and time in ovulation cycle [23, 24]. The mucosal samples need to be processed in a standardized way before storage; whether or not a protease inhibitor is added to the specimens may interfere with the assays and can affect the outcome of the analysis. There is also the issue of normalization of mucosal secretion specimens in order to adjust for differences in collected volumes, as discussed by Jespers and collaborators [24]; however, all of these issues can be overcome for the purpose of acquiring knowledge important for

Figure 1. Reduction of viral load in the semen of RV144 vaccinees may be due to specific actions of various components of the immunologic system (eg, human immunodeficiency virus type 1 [HIV-1]–specific CD8+ T cells or HIV-1–specific immunoglobulin [Ig] G and secretory IgA). Nonspecific mechanisms, such as trapping of HIV-1 particles in mucin network or down-regulation of CD4 receptors on T cells by unknown mechanisms present in the semen, may also contribute to reduced levels of viral load in the semen.
HIV-1 vaccination. Assessments that can be made in cervicovaginal samples include measurement of viral load; analyses of HIV-1–specific antibodies; limited cellular immune responses; and measurement of secreted factors, such as cytokines and innate restriction factors.

The observations by Rerks-Ngarm and collaborators boost interest in the identification of mucosal immune response(s) governing the viral load reduction in semen early after HIV-1 infection. Techniques are available to analyze both humoral [25] and cellular immune responses in semen [26, 27], and further lessons can be learned from infertility studies in both humans [28, 29] and animals [30]. Despite the observation that high concentrations of HIV-1 Env–specific IgA in plasma were directly correlated with infection risk [2], it is possible that IgA may play a role in controlling the level of viral replication in genital fluids; IgA present in genital fluid has profoundly different biologic effects than the IgA present in serum, with the former being a dimeric secretory IgA molecule and the latter a monomeric form.

Semen, similarly to other mucosal secretions, also contains secreted factors that can restrict HIV-1 infection. Interestingly, it was shown that the semen protects CD4+ target cells from HIV-1 infection by causing a decline in CD4+ surface expression on T cells [31], although the molecular mechanisms responsible for this effect remain to be identified (Figure 1). The mucin, MUC6, from seminal plasma, was shown to bind to DC-SIGN, preventing HIV-1 capture and transfer to CD4+ T cells [32]. Semen, however, may also contain enhancers of HIV-1 infection; as an example, a small molecule peptide named semen-derived enhancer of virus infection, has the capacity to enhance direct infection of CD4+ T cells or transfer of virus by cells expressing DC-SIGN to CD4+ T cells [33]. It is possible that differential expression of factors protecting from, or enhancing, HIV-1 infection in the genital compartment may be influenced on vaccination, either directly or indirectly, by the presence of antibodies or cellular immune responses.

The reduction of viral load in the semen of vaccinated individuals as compared to the placebo group, although statistically significant, is not high. Caution should, therefore, be used when interpreting the relevance of these findings in support of the RV144 vaccine efficacy, as indicated by the authors (…). It should be determined whether the virus titers declined in semen as a result of a specific immunologic effect of vaccination or whether other mechanisms may account for the reduced HIV-1 levels in semen. It could be asked whether the methods used for collection of semen specimens for viral load determination were adequate to exclude the possibility that the virus may be trapped in complexes with specific antibodies (eg, HIV-1 dimeric IgA) or trapped in mucin formations (Figure 1). To measure the impact of the reduction in viral load in the semen of RV144 vaccinees on dynamics of HIV-1 spread to the population (eg, by computational biology), it would also be important to assess whether the numbers presented by Rerks-Ngarm and collaborators may affect HIV-1 spread through sexual intercourse in areas highly endemic for HIV-1.

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**References**


