HIV Vaccine Trial Exploits a Dual and Central Role for Innate Immunity

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Limited understanding of correlates of protection from HIV transmission hinders development of an efficacious vaccine.

D. J. M. Lewis and colleagues (*J. Virol.* 88:11648–11657, 2014, doi:10.1128/JVI.01621-14) now report that vaginal immunization with an HIVgp140 vaccine linked to the 70-kDa heat shock protein downregulated the human immunodeficiency virus (HIV) coreceptor CCR5 (chemokine [C-C motif] receptor 5) and increased expression of the HIV resistance factor APOBEC3G (apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G), in women. These effects correlated with HIV suppression *ex vivo*. Thus, vaccine-induced innate responses not only facilitate adaptive immunity—they may prove to be critical for preventing HIV transmission.

Traditionally, vaccines are designed to induce adaptive immunity to provide long-term memory immune responses that can be recalled upon exposure to a pathogen. Consistent with this paradigm, human immunodeficiency virus (HIV) vaccine development has mostly focused on innovative ways to induce stronger and better virus-specific antibody and T cell responses. To date, clinical trials of candidate vaccines have been largely disappointing with the exception of the recent RV144 Thai trial that was designed to induce both antibody and T cell responses, which resulted in limited (approximately 30%) protective efficacy (1–3). However, protection appeared to be short-lived in vaccinated individuals, and the immune mechanisms are still not fully understood. Thus, classical notions that vaccine-induced protection against HIV will be achieved via induction of strong adaptive immune responses have failed thus far. New vaccine approaches that challenge this dogma and tap into an even wider range of immune defenses, beyond antibody and T cell responses, particularly at mucosal surfaces, are now needed.

Innate immunity is classically thought to play a crucial, but mostly supporting or adjuvant role in vaccine induction of adaptive immune responses. As our understanding of the link between innate and adaptive immunity expands, so have efforts to exploit this link to develop better adjuvants that can augment or tailor the type or quality of the adaptive immune response induced by HIV vaccines (4). Since innate responses also have potential to mediate immunopathogenic effects (5–7), crucial to this effort is selective induction of specific types of innate responses that can enhance protection without enhancing infection. Innate responses induced by these new approaches are largely intended to augment adaptive immune effector mechanisms of protection but are generally thought to contribute little or no direct role in protection because innate immunity is transient and believed to induce no memory, thus exerting its effects only within a short window after vaccination. As a result, the elusive search for immune correlates of protection that can be harnessed for vaccination against HIV tends to focus mostly on adaptive immune correlates that can induce long-term immunity. A paper by Lewis and colleagues in this issue (8) provides the first proof-of-concept trial in humans for an alternative concept whereby an HIV vaccine could induce specific innate immunity factors that not only enhance the devel-

opment of virus-specific adaptive immunity but could also contribute directly to protection by increasing the host's resistance to HIV infection.

In an effort to exploit innate immunity in a rationally designed HIV vaccine, Lewis and colleagues developed a vaccine consisting of HIV-1 gp140 linked to the chaperone 70-kDa heat shock protein (HSP70) for mucosal (i.e., vaginal or rectal) administration. This platform was built on previous findings which showed that HSP70 binds CD40 receptor, chemokine (C-C motif) receptor 5 (CCR5), and the Toll-like receptor 4 (TLR4) (9). Further, it is a potent stimulator of CC chemokines and APOBEC3G (apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G), an endogenous restriction factor that inhibits HIV type 1 (HIV-1) and simian immunodeficiency virus (SIV) (9, 10). A key question that was addressed in this clinical study is whether vaginal immunization with this vaccine could increase expression of innate and adaptive immune responses in humans. The vaccine stimulated a significant increase of chemokine (C-C motif) ligand 3 (CCL-3) (macrophage inflammatory protein 1 α [MIP-1 α]), CCL-4 (MIP-1 β), and CCL-5 (RANTES [regulated upon activation, normal T cell expressed and secreted]) chemokines in plasma or culture supernatants of peripheral blood mononuclear cells (PBMCs) compared to baseline levels measured prior to immunization. These chemokines bind CCR5, the primary coreceptor for transmission of HIV, and can block and/or downregulate its expression. Consistent with this possibility, this study found a significant inverse correlation between CCR5 expression on CD4⁺ T cells and CCL-5 and CCL-3. The increase in CC chemokines, and corresponding suppression of CCR5 expression, correlated with

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FIG 1 Hypothetical schematic of how the HIV-1 gp140 vaccine linked to heat shock protein 70, administered intravaginally, may protect from HIV infection.

lower HIV production in an *ex vivo* HIV infectivity assay. Surprisingly, the reduced CCR5 expression following immunization also significantly correlated with induction of a strong virus-specific CD4⁺ T cell proliferative response, a finding that suggests a novel link between the effects of the vaccine on innate responses that downregulated CCR5 and the development of adaptive immunity that will require further investigation. An intriguing finding is that the vaccine also significantly increased the expression of APOBEC3G in PBMCs. HIV has an accessory gene (*vif*) to counteract this restriction factor (11), but a vaccine that induces a sufficient increase in APOBEC3G expression might theoretically overcome the effects of HIV *vif*, potentially leading to enhanced resistance to HIV infection. Indeed, the authors report a significant inverse correlation between the increase they observed in APOBEC3G expression postvaccination and lower HIV infectivity *ex vivo*. On the basis of these findings, the authors propose that the vaccine affords a dual innate mechanism for direct inhibition of HIV-1 first by increasing CC chemokines that would reduce HIV infection via downregulation of CCR5 expression on CD4⁺ T cells, and then any virus that managed to enter CD4⁺ T cells would be further inhibited by upregulated APOBEC3G. At the same time, the effects of the vaccine on these innate factors correlated with an increase in virus-specific T cell responses that can contribute to containment of viral replication. Figure 1 summarizes the mechanisms by which the authors postulate this HIVgp140-HSP70 vaccine protects from HIV infection after intravaginal inoculation.

The concept of using vaccines to harness innate immunity that contributes to protection or viral suppression is not new. For example, vaccines that induce HIV-specific antibodies capable of mediating antibody-dependent cellular viral inhibition (ADCVI) have been shown to correlate with reduced infectivity (3, 12, 13). In addition, innate immunity has been found to be essential in vaccination against yellow fever (14, 15). Furthermore, cells that play a role in innate immunity, such as dendritic cells, have long been targeted to promote adaptive immunity after vaccination

(16, 17). However, the primary distinction in this vaccine approach is the induction of innate immune mechanisms that could offer early, immediate protection against HIV. Recent studies focused on designing vaccines to induce broadly neutralizing antibody (bNAb) suggest that following vaccination, development of high-affinity B cells that promote maturation of a broadly neutralizing antibody response may require numerous sequential immunizations or time to evolve over several months after vaccination to reach full potency (18, 19). In this regard, it may be imperative that vaccines are designed to induce innate responses that can provide more-immediate protection against early and acute infection while allowing the adaptive response to fully develop. Of note, the vaccine here failed to induce a significant antibody response in either the blood or mucosa, suggesting the dual innate responses elicited by this vaccine may potentially work best with T cell-based vaccines.

In typical vaccination strategies, little attention has been given to the very specific microenvironment of the mucosal tissue where infection occurs. Current systemic vaccination platforms do not account for the wide diversity of microbial communities in mucosal tissues, such as the vagina and rectum, or the roles of these bacterial species and associated immune responses in protection from HIV transmission. However, it is well established that alterations in the mucosal microbiome affect HIV transmission, as demonstrated by the consistent correlation with increased risk of HIV transmission and bacterial vaginosis (20–23). Thus, considering microbial factors as vaccine adjuvants, such as HSP70 here, is commended and should be continued. However, including microbiome analysis prior to and following vaccination would be beneficial to these studies and other vaccination strategies. Indeed, given the known important role of HSP70 and the microbiome, together with these studies demonstrating that HSP70 can alter immunity systemically, these studies highlight the importance of studying the microbiome in the context of mucosal vaccination strategies.

There are a number of caveats in this study that could limit the

potential for this approach as an effective HIV vaccine. Although there is evidence in rhesus macaques that *ex vivo* viral inhibition predicts *in vivo* viral control (24, 25), a correlation has yet to be established in humans, which can be achieved only by testing the vaccine for efficacy in humans. However, importantly, the authors found no serious side effects from this vaccine, which was the primary goal of this study, and thus these studies may move forward into additional clinical trials. The ability of the proposed dual innate mechanism to provide durable protection is also unknown, although expression of APOBEC3G following immunization with this vaccine in rhesus macaques was found to persist for up to 20 weeks (9). Supporting the role of long-term innate immune responses, recent studies have demonstrated that cells that play a role in innate immunity, such as natural killer (NK) cells, that are classically thought to have only innate activity, have memory responses as well (26, 27). Conversely, adaptive cells such as CD8⁺ T cells can exert innate properties (28), demonstrating the plasticity of innate and adaptive immune responses that we currently do not fully understand.

A crucial question is whether the effects on innate responses induced by this vaccine in PBMCs would be similarly induced in the mucosa where they could have a greater impact in blocking/inhibiting HIV infection and dissemination at the initial mucosal site of exposure. Indeed, given that mucosal sites, namely, the vagina and rectum, are the primary sites of transmission, understanding the local vaccine responses and long-term memory in these compartments is crucial. Thus, experiments with the non-human primate models for AIDS are needed to provide a more in-depth analysis of the effects of this vaccine on innate responses and protection from transmission. In addition, studies to determine whether this approach induces innate and/or adaptive immunity in rectal mucosa will also be critical to protect both mucosal surfaces. Furthermore, the authors here focused on chemokines and APOBEC expression; however, measuring the frequency of cells that play a role in innate immunity and functionality of these cells at mucosal sites will be essential in future studies. Indeed, cells that play a role in innate immunity, such as neutrophils, dendritic cells, and monocytes, are crucial in inducing and maintaining adaptive immunity, as well as contributing to the microenvironment in mucosal tissues. Another caveat is whether chemokines such as CCL-3, CCL-4, and CCL-5 will actually enhance or decrease HIV transmission at mucosal sites, which is controversial. Indeed, previous studies suggest that these chemokines protect from HIV infection *in vitro* (29, 30). However, increases in these chemokines and other chemokines and cytokines in the vagina have been associated with increased HIV transmission (31, 32). In addition, increased activation of CD4⁺ T cells in mucosal tissues after vaccination has been associated with increased transmission of SIV in nonhuman primate studies (33). Thus, whether the increased proliferation of T cells that the authors demonstrated in PBMCs extends to mucosal sites will need to be investigated. Whereas the induction of chemokines and CD4⁺ T cell proliferation in the vagina by this vaccination strategy in theory should be protective, these processes could also potentially result in increased infectivity, and must be thoroughly investigated prior to administering this vaccine to subjects at high risk for HIV infection.

This is the first clinical trial demonstrating the potential for a vaccine to induce an innate protective immune mechanism by immunizing via a mucosal route. Classical vaccine approaches

typically target long-lived adaptive immune responses, such as T cell and antibody memory responses, which have had only limited efficacy in clinical trials. The novel approach of Lewis and colleagues to target alternative mucosal innate immune responses by linking HSP70 to HIV-1 gp140 and inoculating women intravaginally is an exciting and novel avenue for HIV vaccination research. Indeed, the authors' results demonstrating decreased expression of CCR5, increased HIV restriction factor APOBEC3G, increased proliferation of HIV-specific CD4⁺ T cells, and increased expression of chemokines which are also CCR5 ligands, including CCL-3, CCL-4, and CCL-5, induce excitement that a novel alternative HIV vaccination strategy such as this could potentially be protective. Although significant protection in humans or in a rigorous challenge model in the nonhuman primate model for AIDS has yet to be established with this vaccine strategy, the findings from this study are pivotal in demonstrating the potential role for innate immunity induced by vaccines as not only a strategy to enhance the adaptive immune response but also to contribute directly to protection from HIV infection. In addition, the results reported in this paper demonstrate that it will be important to measure the effects of current and future vaccination strategies in human trials on innate responses alongside traditional antibody and T cell responses. Overall, these studies provide optimism that the next generation of vaccines designed to induce strong innate and adaptive immunity at mucosal sites could move us closer to an efficacious vaccine for HIV.

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