

# Improving Defences at the Portal of HIV Entry: Mucosal and Innate Immunity

A summary report from a Global HIV Vaccine Enterprise Working Group

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The workshop on Improving Defences at the Portal of Entry: Mucosal and Innate Immunity was held in June of 2007 in Durham, North Carolina, United States, with the goal of identifying key scientific issues in mucosal (section I) and innate (section II) immunity, as it pertains to immune protection against HIV infection, that have emerged since the Enterprise Strategic Plan was first published in 2005 [1].

Defining the earliest events in mucosally transmitted HIV-1 infection is of central importance for characterizing the precise virus–host interactions that must be altered by vaccine-induced immune responses. Mucosal transmission of HIV-1 infection is mediated by exposure to infectious virus and/or cells within mucosal secretions, can occur within minutes, is established within hours, and can be disseminated to draining lymph nodes within days (reviewed in [2,3]). Transmission itself is dependent upon transfer of infectious virus across the mucosal epithelium, providing access to subepithelial dendritic cells (DCs), macrophages, and/or T cells that express both CD4 and coreceptors CCR5 and CXCR4 [4,5]. Multiple mechanisms for mucosal HIV-1 transmission have been proposed (reviewed in [6]), however, none of these mechanisms, the receptors involved, nor their modulation by immune responses

(adaptive and/or innate) have been fully defined. A broad consensus from the meeting was that a preventative vaccine must effectively target the earliest events in the establishment of HIV infection. It was recognized that adaptive memory responses may be too slow to counteract such events and that robust mucosal protection may require components of both the innate response (active within minutes or hours) and adaptive effector immune response (humoral and/or T cell, active within days). On the basis of the major roadblocks to advances in the field, nine scientific priorities were identified to facilitate characterization of the correlates of mucosal protection (adaptive and innate) and to harness and develop the technology to enable an effective HIV-1 vaccine.

## Section I: Roadblocks to Inducing Protective Adaptive Immunity at Mucosal Surfaces

### 1. Definition of the sequence of events required to establish mucosal infection.

Understanding the mechanisms of HIV infection across mucosal surfaces is likely to be important for effective vaccine design and development. One critical set of unanswered questions is the relative role of cell-free versus infected cells in mucosal transmission [3,6], including whether the relative importance of these roles varies by mucosal route and the impact of mucosal responses on these different pathways. A second knowledge gap relates to the different potential mechanisms of viral transport across mucosal surfaces and their modulation by different aspects of the immune response [3,6]. Furthermore, there is still debate as to the identity, frequency, location, and role of the primary targets of infection, and the primary targets may vary depending on the type of mucosal epithelium present [3,6–8]. Priority

should be given to understanding these issues as they pertain to vaccine development. Critical to such an approach will be the development of new tools to track initial HIV mucosal infection and dissemination, the availability of a wider panel of HIV-1 and R5 simian-human immunodeficiency virus isolates from transmitted viruses, and the ability to cross-reference human and nonhuman primate (NHP) models of mucosal transmission.

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**Abbreviations:** DC, dendritic cell; IgA, immunoglobulin A; IgG, immunoglobulin G; NHP, nonhuman primate; NK, natural killer; PRR, pattern recognition receptor; TLR, Toll-like receptor

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The Policy Forum allows health policy makers around the world to discuss challenges and opportunities for improving health care in their societies.

**2. Elucidation of acute mucosal sequelae that need to be prevented by HIV vaccines.** Parallel studies of pathological events in acute infection in NHPs and humans have generated important insights into the subversion and/or destruction of the mucosal immune system. This destruction is most evident in the rapid depletion of CD4 T cells within the gut-associated lymphoid tissue during acute infection [9,10]. However, it has become abundantly clear that once mucosal infection has occurred, immune responses to infection are insufficient to prevent these events; what is less clear is whether they have any role in controlling mucosal viral replication, viral evolution, and immune cell depletion [9,11]. A number of studies have identified a paucity in the induction of robust HIV-specific mucosal immunoglobulin A (IgA) and IgG responses in gut-associated lymphoid tissue [12], and definition of the mechanisms leading to reduced responses represent an important priority. It is unclear whether this reflects the consequence of CD4 T cell depletion on localized humoral response, or whether additional immunosuppressive mechanisms are mediated by apoptotic cell products, regulatory T cells, or other pathways [13,14]. Another priority is to define the relationship between immune cell depletion, intestinal permeability to bacteria and bacterial products, cytokine induction, cell activation, and epithelial integrity that may serve to accelerate localized disease and systemic immune activation. Comparative studies of specific cellular and humoral mucosal responses and regulatory cytokines in acute infection and following vaccination may now provide new insight into how to prevent or ameliorate early mucosal pathogenic events.

**3. Tools for measuring mucosal immune responses: Assay development, standardization, and validation.** To date, the techniques for evaluating mucosal immune responses have been primarily based on assays established for the evaluation of systemic responses, where sample volume and cell numbers are not rate limiting. In NHP studies, intestinal responses are currently assessed by extracting T and B cells from mucosal biopsies. However, this technique poses a

significant hurdle for human studies, which is further complicated when studying the female genital tract, where it is not possible to obtain multiple biopsies and yields from cytobrush samples are rate limiting. Furthermore, current approaches to evaluate mucosal cellular responses require fresh samples, providing a logistical challenge for large-scale vaccine trials. While significant information is known about specific homing receptors for small intestine, skin, and lymph nodes [15,16], specific addressins for colorectal and genital tissue have yet to be identified. Thus identification of specific homing markers for these sites would enable monitoring of mucosal immune response via analysis of cells trafficking through the systemic circulation.

The lack of an accessible, reliable, and sensitive method for assessing mucosal cellular responses was highlighted as a significant bottleneck in the ability to determine the mucosal correlates of protection and/or viral control. As a consequence, little is known about the quality, quantity, and duration of mucosal T cell responses following infection or vaccination and their relation to systemic T cell response. Sampling and storage methods for acquiring mucosal T cells require further optimization and standardization to facilitate cross-comparison in different studies. Furthermore, an emphasis on the development of new technology using broad systems approaches that include multiparametric cytokine analysis and genomics [17,18] to enable single cell evaluation of mucosal T cell responses would provide significant gains in the ability to assess these responses.

Assessment of mucosal antibodies will benefit from use of high-sensitivity ELISA technology [19]. Additional advances are being realized, with the use of multiparameter Luminex assays to evaluate responses to a wide range of antigens using small sample volumes [20], surface plasmon resonance to evaluate kinetics and avidity of binding [21], and resonant acoustic profiling to detect antibody binding to whole virions [22]. However, these gains in technology have not been matched with optimization and standardization of mucosal sampling, processing, and storage techniques to detect mucosal vaccine humoral-induced responses.

Of particular concern was the wide variability in reported detection of mucosal IgA responses to HIV [12], resulting from the influence of mucosal secretions, immune complexes, and IgG competition in different assay platforms. It was recommended that an emphasis be placed on optimizing assays for the detection of HIV-specific antibody isotypes in different mucosal secretions and the establishment of validated biomarkers and assay controls.

**4. Defining the role of the common mucosal system in protection.** There is much debate over the role of the common mucosal system in evoking protective immune responses. While established in mice, the commonalities of the mucosal immune system should be determined through parallel studies in humans and NHPs. The central dogma that protection is best primed by mucosal vaccination has not been fully validated. Indeed, protection against mucosal challenge has been demonstrated in NHP studies with parenteral vaccines (at least with homologous virus) and live attenuated vaccines [23]. Determination of whether such systemic immunization induces protection at mucosal surfaces, or whether more robust protection might be achieved with mucosal priming and/or boosting [24] was recognized as an important priority. New tools for tracking virus and infected cells [25–28] may now facilitate NHP studies designed to characterize which events in mucosal transmission of simian-human immunodeficiency virus/simian immunodeficiency virus infection need to be altered in vaccinated animals to provide protection.

**5. Characterization of protective mucosal antibody responses.** While there is general agreement that a protective vaccine will require the induction of a humoral response, questions remain about the characteristics of such a response that will provide protection. The contribution of local versus systemic antibodies to mucosal protection remains an area of debate. Nevertheless, passive infusion studies have demonstrated protection against mucosal challenge [29,30]. This approach could be further utilized to establish the role of mucosal antibodies (and the relative importance of

different isotypes) in NHP mucosal challenge studies. It also remains unclear whether the induction of neutralizing antibodies is the only response contributing to robust protection, or whether other functional characteristics of non-neutralizing antibodies have equal or additional importance [30–32]. This question may be key in focusing vaccine development efforts.

NHP challenge studies following passive infusion of antibodies that demonstrate distinct functional characteristics could further define the humoral correlates of protection and provide cross-validation of relevant *in vitro* assays. Therefore it was recommended that an emphasis be placed on understanding the contribution to mucosal protection of functional activities of antibodies including: complement fixation, inhibition of epithelial transcytosis, blockade of cell–cell transmission across infectious synapses, and antibody-dependent cell-mediated cytotoxicity. At present, there is no certainty as to which of the many different functional antibody assays might correlate with mucosal protection, and thus these must be tested in parallel with NHP and human protection studies, providing a way forward to understanding the humoral correlates of protection.

**6. Definition of the role of T cell responses in eliciting mucosal protection.** Should the hurdles to studying mucosal T cell responses be overcome (see Priority 3), there are several strategically important questions about the role of mucosal T cell responses that could enhance the design of protective vaccines against mucosal HIV transmission. Comparison of systemic and mucosal responses in infected individuals would define whether there was any compartmentalization of T cell responses that would require differences in prime/boosting by vaccines. Definition of the correlates of protection and/or nonprogression in elite controllers (NHPs and humans) may provide new insight into the role of T cell responses in protection/control of HIV infection. Furthermore, NHP studies could assess the relative contribution of specific mucosal memory versus effector cell numbers to the duration of protection. In addition,

study of durable low-level localized infection (replication competent vectors, attenuated virus) could provide greater understanding of factors influencing the durability of mucosal T cell immunity.

## **Section II: Roadblocks to Harnessing the Innate Immune System to Stimulate Protective Immunity against HIV**

The “innate” immune response is an evolutionarily ancient system of host defence, which occurs within minutes or hours of pathogen entry or vaccination. A fundamental property of the innate immune system is its ability to “sense” or recognize microbial or viral stimuli, and to elicit rapid-acting defence mechanisms [33–35]. The innate immune system consists of a network of interacting cell types including DCs, macrophages, epithelial cells, endothelial cells, natural killer (NK) cells, NK T cells, mast cells, and gamma-delta T cells, which play a fundamental role in “sensing” microbes or viruses and launching innate defence mechanisms against them [33–35]. Among these cells, DCs play a preeminent role, not only in directly sensing the presence of pathogens, but also in orchestrating the interactions between the other innate immune cell types and facilitating the elicitation of antiviral defences such as secretion of type I interferons and defensins [36]. In addition to their roles in sensing pathogens and orchestrating innate immune defences, DCs also play a critical part in translating innate immunity into adaptive immunity [36–38]. Understanding the impact of innate immunity on the regulation of adaptive immunity, and harnessing such knowledge to induce optimal immunity to HIV, was recognized as an area of the highest importance.

**7. Harnessing dendritic cells, Toll-like receptors, and non-Toll-like receptors in HIV vaccine development.** The innate immune system is able to sense components of viruses, bacteria, parasites, and fungi through the expression of so-called pattern recognition receptors (PRRs), which are expressed by DCs and other cells of the innate immune system [33–35]. Toll-like receptors (TLRs) represent the most studied family of PRRs. However, growing evidence suggests that other non-TLR families

of innate receptors, such as C-type lectin-like receptors [37], NOD-like receptors [39], and RIG-I-like receptors [40], also play critical roles in innate sensing of pathogens and induction of inflammatory responses. There are several different subpopulations of DCs that differ in their surface phenotype, function, and immune stimulatory potentials [36,37]. Emerging evidence suggests that the nature of the DC subtype, as well as the particular TLRs and/or non-TLRs triggered, play critical roles in modulating the strength, quality, and persistence of adaptive immune responses [37]. Thus specific ligands that stimulate DCs via TLRs or non-TLRs may represent novel adjuvants for vaccines against HIV [41]. Towards this end, a fundamental challenge is to understand the mechanisms by which DCs and PRRs regulate adaptive immune responses. In this context, the application of high-throughput technologies to evaluate changes in gene and protein expression and kinase profiles in response to TLRs and non-TLRs is likely to yield significant gains. Such an approach will offer an understanding of the signalling networks in the innate immune system that regulate the adaptive immune response, and this is likely to provide new insights into how to tune the adaptive immune response [18].

An equally important issue is the targeted delivery of antigen plus adjuvant to the antigen-presenting cell so as to optimize immunity and minimize systemic toxicities. Therefore the development of delivery systems that facilitate local or mucosal delivery of specific ligands for TLRs and non-TLRs may be a key step in the advancement of such novel adjuvants [42].

Finally, there is a growing realization that many of our best empirically derived vaccines and adjuvants mediate their efficacy by activating specific innate immune receptors. For example, the highly effective yellow fever vaccine 17D signals via at least four different TLRs, as well as RIG-I-like receptors, to elicit a broad spectrum of T cell responses [43]. This example suggests that the immune response generated by a live attenuated vaccine can be effectively mimicked by adjuvants composed of the appropriate TLR and/or non-

TLR ligands. Furthermore, recent work suggests that some adjuvants can induce robust adaptive immunity in a TLR-independent manner, perhaps through other receptors in the innate immune system [44]. Therefore, understanding the precise roles played by TLRs and other non-TLRs, in the induction and regulation of adaptive immune responses is critical for the design of optimally effective vaccines against HIV.

**8. Understanding the role of natural antiviral factors and innate immune cells in mediating the interface between innate and adaptive immunity in HIV.** Although much attention has been focused on antigen-presenting cells, it is now clear that other innate components, including antiviral cytokines and cells such as NK cells, NK T cells, and gamma-delta T cells, play fundamental roles in mediating innate immune responses. Their function in inducing and in regulating adaptive immunity against HIV are beginning to be understood [45–47], and have yet to be exploited in vaccine design against HIV. Furthermore, the potential roles of innate B-1 and marginal zone B cells in mediating rapid induction of neutralizing antibodies against HIV remains an area of interest that may provide additional insight. Understanding the role of innate immunity in the induction and imprinting of adaptive immune responses was identified as an important knowledge gap in our current understanding, and it was recognized that advances in this area might facilitate the effective manipulation of innate immunity to induce optimally effective adaptive immunity against HIV.

**9. Understanding the role of innate immunity in early HIV infection.** There is at present little knowledge about the early innate immune events that occur in response to mucosal HIV infection, and their potential influence on the ensuing adaptive immune response and disease progression. This issue was identified as an important gap in current understanding, and it was widely recognized that advances in this area might facilitate the rationale and design of interventions in acute infections. For example, intracellular innate antiviral factors such as APOBEC3G may be up-regulated by cellular activation [48]. It is unclear

whether APOBEC3G or other innate factors can be harnessed to modulate HIV infection in the first few days after exposure to the virus. Therefore further study of how innate antiviral factors can curtail early events in HIV transmission and development of vaccine approaches that can induce and maintain such responses may provide new leads.

## Summary, Discussion, and Final Recommendations

In summary, the potential importance of innate and specific mucosal immunity in protection against HIV transmission is only now beginning to emerge. Furthermore, recent safety concerns over the prematurely halted phase IIb STEP trial of the Merck adenovirus-5 vectored vaccine [49] emphasize that studies of innate and adaptive mucosal immunity may be equally important in determining any potential enhancement of infection following vaccination. Considerable gains could now be made with the development of new technology to monitor the earliest events in mucosal infection and the application of a focused approach to understanding the contribution of localized immune responses in prevention and/or potential enhancement of localized mucosal HIV infection. It was recognized that acceleration of work in this area would most likely be met by the establishment of validated and standardized mucosal assay platforms that could facilitate cross-comparative NHP and human studies, coupled with the development of innovative vaccine strategies specifically targeted at inducing and maintaining protective mucosal immune responses at the portal of HIV entry. ■

## Supporting Information

**Text S1.** More detailed version of the article  
Found at doi:10.1371/journal.pmed.0050081.sd001 (151 KB DOC)

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