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

(Report from a Global HIV Vaccine Enterprise Working Group)

Robin Shattock*, Barton F. Haynes, Bali Pulendran, Jorge Flores and José Esparza on of a Working Group convened by the Global HIV Vaccine Enterprise

Robin Shattock is at St George's, University of London, London, United Kingdom. Barton Haynes is at the Human Vaccine Institute, Duke University School of Medicine, Durham, NC, United States. Bali Pulendran is at the Emory Vaccine Center & Department of Pathology, Emory University, Atlanta, GA, United States, Jorge Flores is at the Division of AIDS, National Institutes of Health, Bethesda, MD, United States. José Esparza is at the Bill & Melinda Gates Foundation, Seattle, WA, United States.

Other members of the Enterprise Working Group included: Alan Aderem, Lesley Bergmeir, James Bradac, Jason Brenchley, Heather Davis, Tom Hope, Pamela Kozlowski, Roger Le Grand, Thomas Lehner, Norman Letvin, Jay Levy, John Mascola, Julie McElrath, Philip Norris, Jo-Ann Passmore, Helen Quill, Robert Seder, Rafick-Pierre Sekaly, Barbara Shacklett, Georgia Tomaras, Ronald Veazey, Peter Wright

* To whom correspondence should be addresses. E-mail: Shattock@sgul.ac.uk

Introduction

The Global HIV Vaccine Enterprise convened a two-day workshop in June of 2007 to discuss improving defences at the portals of entry. The meeting was divided between discussion of innate responses to HIV in general and mucosal innate and adaptive responses against HIV at the portals of entry. Section I focuses on mucosal immunity, and should be read in context with the report on innate responses in Section II. The goals of this workshop were to identify key scientific issues that have emerged since the Enterprise Strategic Plan was first published in 2005, and to make recommendations that Enterprise stakeholders can use to plan new activities. The meeting was organized by Barton Haynes, Robin Shattock, Bali Pulendran, Jorge Flores and Jose Esparza and was attended by 27 scientists from the United States, Europe Canada, South Africa and Asia.

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. While sexual transmission accounts for over 90% of all instances of HIV-1 infection [1,2], the immediate events between exposure to infectious virus and establishment of infection are poorly understood. Mucosal transmission of HIV-1 infection is mediated by exposure to infectious virus and/or cells within mucosal secretions. It has been established in non-human primate studies that mucosal infection can occur following 30-60 min exposure to infectious virus, that localized infection is established within 16-72 hours and dissemination to draining lymph nodes is achieved within 24-72 hours [3,4], therefore the time for mucosal responses to impact on transmission events is critically short, and likely is within the first 14 days of transmission. While the risk of transmission is influenced by factors relating to the infected partner [2,5,6], transmission is dependent upon transfer of infectious virus across the mucosal epithelium providing access to sub-epithelial dendritic cells (DCs), macrophages and/or T-cells that express both CD4 and co-receptors CCR5 and CXCR4 [7,8]. Multiple mechanisms for mucosal HIV-1 transmission have been proposed including: direct HIV-1 infection of epithelial cells; transcytosis of HIV-1 through epithelial cells and/or specialized M cells; epithelial transmigration of HIV-1-infected donor cells; uptake of HIV-1 by intra-epithelial Langerhans and dendritic cells [9-15]; or entry via epithelial microabrasions or ulceration [2,5,6]. These events may be critically influenced: by viral genotype [15-19] and incorporation of host cell proteins [20-23]. However, none of these mechanisms, the receptors involved, nor their modulation by immune responses (adaptive and/or innate) have been fully defined in tissue and/or non-human primate or human clinical studies. A broad consensus from the meeting was that a preventative vaccine must effectively target the earliest events in the establishment HIV infection right after transmission (hours to days). It was recognized that adaptive memory responses may be to slow to combat such events, and that robust mucosal protection may require both, components of the innate response (active within minutes-hours) and adaptive effector immune response (humoral and/or T cell). Induction and maintenance of such responses is likely to require rational vaccine design based upon a fuller understanding of the correlates of mucosal protection against HIV infection. On the basis of the major roadblocks to advance the field, nine scientific priorities were identified that will bring the field closer to defining the correlates of

mucosal protection (adaptive and innate) and developing the enabling technology for 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 infection following exposure to HIV.

As argued above, understanding the mechanisms of HIV infection across mucosal surfaces and the ability of immune responses to modulate these events is likely to be important for effective vaccine design and development. There was a clear consensus that for a preventative vaccine to extinguish HIV-1 following the transmission event, and achieve a viral reproductive ratio of less than 1 [24] , it must be able to target these very early events. One critical unanswered question is the relative role of cell free vs. infected cells in mucosal transmission [9], whether the relative importance of these varies by mucosal route and the relative impact of mucosal responses on these different pathways. A second knowledge gap relates to the different potential mechanism of viral transport across mucosal surfaces and their modulation by different aspects of the immune response [2,5,6,9-15]. 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, 9, 25, 26]. Attention should be paid to the difference in infectivity and protection between the stratified squamous epithelium of the ecto-cervico vaginal and foreskin epithelia in contrast to the columnar nature of the rectal and endo-cervical epithelium. Critical to evaluating the impact of vaccines on these initial events will be the development of better tools to track initial infection and dissemination, and the ability to cross reference different models of mucosal transmission.

Specific recommendations:

- *Develop tools for tracking virus and/or infected cell interaction with mucosal surfaces and subsequent spreading of infection within mucosal sites and dissemination to lymphoid tissue*
- *Determine the role of dendritic cells (DC) in the mucosa for dissemination of HIV.*
- *Cross reference and standardize cellular, tissue and non-human primate models of mucosal transmission*
- *Develop better and more relevant panels of HIV and SHIVs from transmitted sequences for human explant tissue and NHP studies*
- *Evaluate the impact of protective vaccines on initial events of transmission to determine the point at which the chain of events required to establish infection is aborted.*

2. Elucidation of acute mucosal sequellae that need to be prevented or subverted by HIV vaccines

Parallel studies of pathological events in acute infection in NHP and humans have generated important insights into the subversion and/or destruction of the mucosal immune system. This is most evident by the rapid depletion of CD4 T cells within the GALT during acute infection [27,28], although it is less apparent whether this is paralleled in other mucosal sites. However, it has become abundantly clear that once mucosal infection has occurred, mucosal immune

responses to infection are insufficient to prevent these events, what is less clear is whether they have any role in controlling mucosal replication, viral evolution, immune cell depletion [27-30] and in particular depletion of central memory CD4 T cells [31]. A number of studies have identified a paucity in the induction of robust HIV-specific mucosal IgA and IgG responses in GALT [32]. It is unclear whether this purely reflects the consequence of CD4 T cell depletion on localized humoral response or whether additional immunosuppressive mechanisms are mediated by CD4+CD24+ Treg cells, phosphatidylserine, apotoptic cells/microparticles or the PD1-PDL1/PDL2 pathway [33-36]. While important work has been carried out to identify pathogenic sequellae in acute infection, the underlying mechanisms driving these events are not fully understood. The critical relationship between immune cell depletion, and permeability of the GI tract to bacteria and bacterial products remains unclear [37,38], are these events solely the direct consequence of HIV pathology or is there a more dynamic interaction between immune cell depletion, intestinal permeability, cytokine induction, cell activation and epithelial integrity that serves to accelerate localized and systemic disease? Less still is known about the impact of immune response (innate and adaptive) in their imprinting and subsequent modulation of these events. Perhaps most critically for vaccine design, it is unclear whether mucosal immune responses can be primed in such a way as to overcome such pathogenic mechanisms and whether infection can ever be prevented or aborted after the initiation of these events or merely controlled.

Specific recommendations

- *Define why HIV fails to induce robust HIV-specific mucosal IgA and IgG responses*
- *Determine whether immunosuppressive mechanisms mediated by Treg cells and apoptotic pathways at mucosal surfaces prevent robust immune responses and/or promote viral replication?*
- *Develop a focused approach of parallel human and NHP studies of acute infection to further delineate common pathology of acute infection*
- *Define key differences in specific mucosal IgA and IgG responses and regulatory cytokines in acute infection and following vaccination with HIV antigens and control antigens*
- *Monitor mucosal immune depletion in multiple mucosal sites (GALT, BALT, GI)*
- *Characterize the relationship between immune depletion, intestinal permeability to bacteria and bacterial products, cytokine environment, activation status and viral quasispecies within different mucosal compartments*
- *Determine the mechanistic features that render the GI tract in acute HIV/SIV infection permeable to bacterial products*
- *Monitor mucosal immune responses, T-cell depletion and gut permeability in naïve NHP and vaccinated animals in response to rectal challenge.*

3. Development of better tools for measuring mucosal immune responses: assay development, standardization and validation

As already discussed, understanding the role of mucosal immunity in HIV transmission and prevention is likely to be key to the rational development of HIV vaccines. However, to date the techniques for evaluating mucosal immune responses (in humans and NHP) have been primarily based on assays established for the evaluation of systemic responses where sample volume and

cell numbers are not rate limiting. The technological hurdles are different for mucosal T cell and humoral responses and these will be discussed separately. However, one common research need is to identify novel mucosal specific homing markers to enable monitoring of mucosal immune responses via analysis of cells trafficking through the systemic circulation. While significant information is known about lymphocyte homing to the small intestine, skin and lymph nodes [39,40] and a number of lymphocyte homing receptors have been identified, including apha4 beta7 for GALT, etc [41,42], these lack sufficient specificity in humans for other mucosal sites (colorectum, male and female genital tract).Thus identification of specific homing markers for these sites would enable monitoring of immune mucosal immune response via analysis of cells trafficking through the systemic circulation.

While there is a substantial body of literature describing antigen specific T cells responses to HIV in peripheral blood [43, 44], far less is known about mucosal T cell responses. This reflects the lack of an accessible, reliable, and sensitive method for assessing mucosal cellular responses and represents 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. Analysis of mucosal T-Cell responses (both vaginal and colorectal) in humans faces a number challenges. Optimal sampling methods for acquiring mucosal T cells have not been standardized and typically involve the collection of biopsies for colorectal responses, semen for male genital responses and cervical cytobrush samples for female genital responses. Transport and storage conditions for mucosal T cell samples have not been validated with the majority of investigations requiring the use of fresh samples, reducing their applicability to multiple trial sites. Furthermore, the number of cells for analysis is rate limiting when compared to established PBMC assays. Indeed mucosal assays need to be able to be performed on 10^4 T-cells of which >1% may be responsive to HIV antigens, severely limiting the ability of current technology to assess the breadth (ELISPOT) [45] and quality (multiparametric cytokine analysis) [46] of mucosal responses. Polyclonal expansion of mucosal T cells has been successfully used to facilitate analysis of mucosal memory T cell responses [47] and may provide some gains in sensitivity, however it was recognised the development of new technology including microfluidics and tetramer technology [48,49] could optimize single cell evaluation of mucosal T cell responses

In contrast much has been done to increase the sensitivity of antibody binding assays using high sensitivity ELISA technology [50]. Additional advances are being realised, with multiparameter luminex assays able to evaluate responses to a wide range of antigens using small sample volumes [51], the use of Surface Plasmon Resonance [52] to evaluate kinetics and avidity of binding, and Resonant Acoustic Profiling able to detect antibody binding to whole virions [53,54]. 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 [32], the influence of mucosal secretions, immune complexes and IgG competition on different assay platforms.

Specific recommendations

- *Identification of novel mucosal (colorectal, genital) specific homing markers*
- *Define optimal methods to acquire, transport and store mucosal samples*

- *Establish/validated biomarkers for sample standardization and assay controls that will facilitate cross comparison between NHP and human vaccine studies*
- *Optimize detection of HIV specific antibody isotypes in different mucosal samples (colorectal, cervicovaginal, semen)*
- *Maximize the efficiency of polyclonal expansion of mucosal T cells to facilitate mucosal assessment.*
- *Establish novel platform technology for single cell evaluation of mucosal T cell responses*

4. Defining the role of the common mucosal system in protection

The central dogma that protective anti-HIV-1 immune reponses 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 [42]. It is unclear whether such systemic immunization induces protection at mucosal surfaces, or whether more robust protection might be achieved with mucosal priming and/or boosting [55]. New tools for tracking virus and infected cells [56-59] may now allow studies to determine the point of protection in vaccinated animals. The unique contribution of NHP studies to addressing these questions was clearly recognized, but this was underscored with an emphasis for parallel immunogenicity studies in humans. Again the requirement for cross comparison between trials (and the tools to facilitate this) was seen as paramount.

Specific recommendations

- *Establish a broad paradigm of the commonalities of the mucosal immune system through parallel studies in humans and NHP (BALT, GALT, genito-rectal associated lymphoid tissues).*
- *Define the potential of mucosal immunization in different prime boost strategies to optimize protective mucosal responses*
- *Determine the role of mucosal immunity in protection afforded by parenteral vaccines (tracking of infectious events, determine differences by route of challenge)*
- *Determine whether protection afforded by parenteral vaccines can be boosted by mucosal immunization.*
- *Establish the role of mucosal antibodies (passive infusion studies, topical application of IgG, sIgA etc, neutralizing/non-neutralizing)in prevention of mucosal transmission*

5. Characterization of protective mucosal antibody responses

While there is general agreement that a protective vaccine will require the induction of a humoral response, a large number of questions remain about the characteristics of such a response that will provide protection. The contribution of mucosal vs. systemic antibodies to mucosal protection remains an area of debate. It is unclear how much spill over of systemic antibodies there is into mucosal compartments, whether this could be sufficient for protection, if it is changed by sexual arousal, and whether luminal antibodies are required. Nevertheless, passive infusion studies have demonstrated protection against mucosal challenge [60,61]

It 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 may have equal or additional importance [62,63]. 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 of functional activities of antibodies including: complement fixation, inhibition of epithelial transcytosis, blockade of cell-cell transmission across infectious synapses (in particular those between dendritic cells and T cells), and ADCC to mucosal protection. 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.

Specific recommendations

- *Determine the correlates of protective antibody responses. Are mucosal antibodies necessary for protection or will antibodies of systemic origin suffice?*
- *Define the role of antibody isotype in mucosal protection (combination of passive infusion NHP studies and in vitro functional assays)*
- *Define the kinetics of protective antibodies- what is the time frame in which they have to work – hours, days? Can this be elicited by memory responses?*
- *Determine the concentration of Ab needed at mucosal sites for an effective initial response.*
- *Define the different characteristics of protective humoral responses against HIV transmission mediated by cell free virus and infected cells*
- *Characterize the role of immune complexes in viral transmission and their impact of vaccine induced responses*

6. Definition of the role of T-cell responses in eliciting mucosal protection

As discussed above, there are a number of technological hurdles to studying mucosal T cell responses (see Priority 3). Should these hurdles be overcome, 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 non-progression in elite controllers (NHP and humans), using broad systems approaches that includes multiparametric cytokine analysis and genomics may provide new insight into the role of T cell responses in protection/control of HIV infection and may explain why elite controllers have virus in their semen but not in their blood [43]. Furthermore, NHP studies could assess the relative contribution of specific mucosal memory vs. effector cell numbers and the duration of protection. Ex-vivo challenge studies of mucosal tissue might be developed as a tool for bridging studies between NHP and human studies. It was recognised that study of durable low-level localized infection (replication competent vectors, attenuated virus) could provide additional insight into the type of vaccine that will most likely stimulate mucosal T cell immunity. Furthermore, valuable lesions could be drawn through the study of other vaccines including: live versus inactivated polio; live versus inactivated flu; mucosal versus systemic delivery of measles vaccine; replication competent versus defective adenovirus; and exploration of response to vectors as a predictor of vaccine response [64].

Specific recommendations

- *In depth comparison of mucosal and systemic T cell responses in acute and chronic infection of humans (frequency and functionality)*
- *Comparison of mucosal and systemic T cell responses in protected NHP studies (frequency and functionality)*
- *Definition of the correlates of non-progression in elite controllers(NHP and human studies)*
- *Define any correlation between mucosal vs. systemic T cell responses (effector/memory ratio, specificity, functionality) with protection in NHP studies and their potential role in duration of protection/viral control*
- *Characterize the role of durable low-level infection (replication competent vectors, attenuated virus) in inducing T cell response, and determine if it induces compartmentalized mucosal immunity at the site of exposure.*
- *Explore the use of ex-vivo mucosal tissue challenge model as a tool for bridging studies between NHP and human immunogenicity studies.*

Section II: Roadblocks to harnessing the innate immune system to stimulate protective immunity against HIV

Research done over the past decade has placed innate immunity at the center of immune regulation. The "innate" immune response is an evolutionarily ancient system of host defense, 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 [65-67]. The innate immune system consists of a network of interacting cell types including dendritic cells (DCs), macrophages, epithelial cells, endothelial cells, NK cells, NK T cells, mast cells, gamma-delta T cells which play a fundamental role in "sensing" microbes or viruses, and launching innate defence mechanisms against them [65-67]. Amongst these cells, DCs play a pre-eminent 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 anti-viral defences such secretion of type I interferons and defensins [68]. In addition to their roles in sensing pathogens and orchestrating innate immune defences, DCs also play a critical role in translating innate immunity into adaptive immunity [68, 69]. 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.

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 [65-67]. 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 (CLRs) [70], NOD-like receptors (NLRs)[71] and RIG-I-like receptors (RLRs)[72] also play critical roles in innate sensing of pathogens, and induction of inflammatory responses. Furthermore, the

chemokine receptor CCR5 has been shown to recognize HIV, *M.tuberculosis*, *Toxoplasma gondii* and microbial HSP70, and stimulate the maturation of dendritic cells. The importance of such non-TLRs in regulation of adaptive immunity is only beginning to be understood. There are several different sub-populations of DCs that differ in their surface phenotype, function, and immune stimulatory potentials [68, 69]. 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 [69]. Thus DCs and PRRs represent attractive targets for enhancing HIV-specific immunity in vaccination. 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 new insights on how to tune the adaptive immune response [73].

In the near term, understanding the precise roles played by TLRs and non TLRs, in the induction and regulation of adaptive immune responses, is critical for the design of optimally effective vaccines against HIV. Thus specific ligands which stimulate DCs via TLRs or non TLRs may represent novel adjuvants for vaccines against HIV [74]. However an important issue is to ensure 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, formulations, nanoparticles that facilitate the local or mucosal delivery of specific ligands for TLRs and non-TLRs may be a key step in the advancement of such novel adjuvants [75].

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, one of the most successful vaccines which has been administered to over half a billion people globally, signals via at least 4 different TLRs, as well as RIG-I like receptors, to elicit a broad spectrum of T cell responses [76]. This 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 [77]. 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.

7. Harnessing TLRs and non-TLRs in HIV vaccine development

There was consensus that there should be enhanced efforts to understand how dendritic cell subsets, TLRs, and other innate immune receptors (non-TLRs) all represent potential targets, which can be manipulated to induce effective HIV-specific immunity.

Specific recommendations:

- *Determine how innate immune activation controls the strength and quality of adaptive*

immune responses.

- *Apply systems biological approaches to understanding the complex gene and protein regulatory networks stimulated by adjuvants, and their impact on adaptive immunity*
- *Determine how to exploit TLRs, non-TLRs and antigen presenting cells (APCs) to induce protective immune responses, systemically and at mucosal surfaces.*
- *Develop novel and safe adjuvants stimulate TLRs and/or non-TLRs.*
- *Develop delivery systems, formulations, nanoparticles that facilitate the local or mucosal delivery of specific ligands for TLRs and non-TLRs. There is a growing belief that delivery of multiple TLR ligands might result in synergistic activation of DCs and a consequent enhancement of the adaptive immune response.*
- *Determine how successful vaccines and adjuvants activate the innate immune system, with a view to exploiting such knowledge in the generation of new vaccines against HIV.*
- *Use systems biological approaches to identify signatures of early innate immune activation that can predict the immunogenicity of vaccines.*

8. Understanding the role of natural anti-HIV factors and innate immune cells (e.g., NK cells, NK-T cells, gamma-delta T cells, B-1 B cells, marginal zone B cells) in mediating the interface between innate and adaptive immunity in HIV.

Although much attention has focused on antigen-presenting cells, it is now clear that other innate activities including antiviral cytokines and cells such as NK cells, NK T cells, 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 [78-80], 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 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.

Specific Recommendations

- *Determine if NK, NK-T, gamma/delta T cells have biologically relevant roles in control of HIV-1 during the transmission event.*
- *Determine if B-1 B cells and marginal zone cells can be induced to rapidly produce protective antibodies in response to AHI by previous vaccination.*

9. Understanding the role of innate immunity in early HIV infection

There is presently 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 design of interventions in acute infections. Intracellular innate antiviral factors such as APOBEC3CG can be upregulated and maintained so it may play an important role in prevention of HIV infection in the first few days after exposure to the virus [81]. 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.

Specific recommendations

- *Understand the roles of DC subsets, TLRs and non-TLRs in mediating innate and adaptive responses to HIV in early infection.*
- *Role of other innate immune cells – NK, macrophages, marginal zone, B-1 B cells in mediating innate and adaptive immunity to HIV in early infection*
- *Understand the role of innate antiviral cytokines in curtailing early HIV infection*
- *Understand the role of innate intracellular antiviral factors in curtailing early HIV infection*

Summary, Discussion and Final Recommendations

In summary, there was a general agreement that understanding the role of both innate and mucosal immunity in protection against mucosal HIV transmission was still in its infancy and may represent a significant bottleneck to development of a preventative HIV vaccine. Furthermore, recent safety concerns over the prematurely halted phase IIb STEP trial of the Merck adenovirus-5 vectored vaccine [82] 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 these areas would most likely to 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.

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