

# Biology of Mucosally Transmitted Sexual Infection—Translating the Basic Science into Novel HIV Intervention: A Workshop Summary

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## Abstract

A group of over 200 international scientists came together on April 15 in Sydney, Australia just before the 2012 International Microbicides Conference as a part of a workshop to address the basic concepts and factors that modulate HIV infection at the mucosal surface. The meeting focused on defining the interaction between virus, prevailing host physiology, microbiota, and innate and adaptive immune responses and how they combine to impact the outcome at the moment of potential viral transmission. Speakers examined the biology of HIV entry during transmission, innate and natural antiviral mechanisms at the mucosa, microbicide efficacy, pharmacokinetic, and pharmacodynamics, animal models, and opportunities for combining HIV prevention strategies. Other viral infection models both *in vivo* and *in vitro* were considered for the insights they provided into HIV transmission events. The workshop raised important questions that we need to answer to further our basic understanding of host and viral factors influencing HIV transmission to inform the development of novel prevention strategies.

## Introduction

THE FIELD OF HIV TRANSMISSION has seen major advancements over the past 10 years. We have learned a significant amount about the mechanism of HIV transmission at mucosal sites using both *in vivo* animal models and *in vitro* systems. In addition, there have been advances in our understanding of the host mucosal immune system both for the innate and adaptive pathways. There have been exciting results of therapeutic trials utilizing topical antiviral strategies to prevent HIV transmission, but there have also been failures in the field. The field of HIV prevention has clearly advanced in the past 10 years but we still have a significant way to go. Our next advance in the field will be informed by basic scientific studies of the mechanism of mucosal HIV transmission. The workshop entitled “Biology of Mucosally Transmitted Sexual Infection—Translating the Basic Science into Novel HIV Interventions” was conceived with the idea that we can advance our HIV prevention agenda by more basic studies. The Workshop provided an international forum

for the interaction of scientists and clinicians from all aspects of HIV research in exploring our current knowledge of mucosal HIV biology.

## The Biology of HIV Entry During Transmission

The opening session focused on the anatomical, physiological, immunological, innate, and intrinsic barriers to infection at the sexual mucosae, and how virus has evolved to circumvent these obstacles. The opening presentation by Professor Tom Hope from Northwestern University highlighted the use of high-resolution microscopy for a comprehensive histological examination of the female reproductive tract following exposure of human explant cultures or of macaque tissues *in vivo* with photo-activated fluorescent GFP-labeled cell-free HIV. The analysis highlighted the differences in the mucosal epithelium of the vagina, ectocervix (stratified squamous), and endocervix (simple columnar) with the different expression levels of E-cadherins, desmogleins 1/2, and involucrin in the squamous epithelia. The tight junctions,

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adherans, and desmosomes serve as significant barriers in accessing the underlying interdigitating CD4<sup>+</sup> immune cells that include T-lymphocytes, dendritic cells, Langerhans cells, and macrophages by viral diffusion and/or transcytosis.<sup>1</sup> However, the layer of mucous that coats the epithelial surfaces was shown to form the greatest barrier to viral entry and reduction of mucous integrity following enzyme digestion dramatically increased penetration of virus into and through the epithelium.

Hope has moved past the “seeing-is-believing” maxim of many microscopy studies to rigorously enumerating viral entry by directly counting the number and depth of HIV virions in tissue. The mucous barrier had the best ability to slow virus penetration during the later luteal phase of the menstrual cycle in women using oral contraceptives. The mechanism of transmission that emerged from Hope’s work was entry of cell-free virus by diffusion and infection resulting from a chance encounter with a CD4<sup>+</sup> cell. Significantly, the mobility and concentration of target cells increased dramatically during local inflammation. Using simple mathematics Hope built upon these observations to calculate the chance of a male-to-female transmission by cell-free virus. While conceding that there were many assumptions, Hope concluded that the burden of HIV virions that penetrate sufficiently to encounter target cells in the real world was very small. Hope’s data favor transmission as an infrequent and random event. Simple things were shown to reduce the rate of HIV penetrative diffusion, such as cervical vaginal mucous, acid pH, Env protein, and Env-binding antibodies, whereas seminal plasma increased the rate of HIV diffusion and local inflammation greatly increased the chance of target cell infection.

Hope’s approach did not visualize or measure the role of cell-associated virus in the inoculum. Stuart Turville from the Kirby Institute, addressed this question by illuminating cell-associated virus engineered to contain the small recognition peptide with a membrane-permeable biarsenical fluorescent dye (FLaSH) or green fluorescent protein (eGFP). This complementary imaging technique was used with cultured dendritic cells (DC) to reveal finger-like actin-rich membrane bridges called filopodia that are tipped with high concentrations of HIV particles. Dynamic imaging of HIV transmission from infected DC showed the prolific propulsion of immature virions upon the tip of more than 90% of filopodia that typically extend up to 10 to 20 nm and make as many as 800 DC to CD4 T cell contacts per hour. Upon engagement with a target T cell, multiple filopodia converge and tether budding HIV particles to the CD4 T cell membrane in a stable long-lasting synapse suited for viral transfer. Long viral filopodial formation was dependent on the formin diaphanous 2 (Diaph2), and required HIV Nef for greatest viral filopodia frequency.<sup>2</sup> Turville’s observations of powerful actin dynamics projecting HIV from infected DCs contrasted starkly with the inert diffusion of cell-free virus presented by Hope and showed that a biological inoculum containing cell-associated HIV might demonstrate DC to CD4 T cell interactions from HIV-capped long DC filopodia that increase the chances of transmission. However, the relative importance of cell-associated and cell-free HIV in transmission from sexual secretions still remains to be clarified. This latter concept was discussed several times at the conference and was addressed in the context of microbicide efficacy in animal models and will be discussed below in this context.

Florian Hladik from the Fred Hutchinson Cancer Research Center switched the focus to the CD4<sup>+</sup> target cell dynamics in the genital and rectal mucosa during transmission and measuring the impact of prophylactic intervention strategies in the mucosae.<sup>3</sup> He focused his thinking around understanding how the results from the 39% protective efficacy of 1% tenofovir achieved in the CAPRISA 004 trial could differ so starkly from the failure of a similar regimen to protect in the VOICE topical preexposure prophylaxis (PrEP) trial. He suggested that the mucosal immune system responded to microbicides and vaccines with a complex series of gene expression and inflammatory responses that could in part be protective or augmenting of the infection event. To illustrate this, he examined the identity and function of cellular markers induced in rectal tissue 7 days after treatment with a failed microbicide, 2% Nonoxynol 9 (N9), or the potentially successful 1% tenofovir compared to a gel control. The results showed a remarkable change in expression, up and down, of hundreds of genes to the two therapeutic compounds. The tenofovir arm had the larger number of gene expression changes and a large array of biological process genes showed altered expression and included many immune modulating proteins and strong inhibition of mitochondrial proteins such as polynucleotide phosphorylase, which mediates mitochondrial RNA import. There was little overlap between the gene sets identified following N9 treatment and its known toxicity induced many epithelial repair genes. These studies highlighted the need to examine mucosal tissue directly and the gene sets identified may eventually form a set of biomarkers that will help direct better prevention strategies.

Charles Wira, from Dartmouth Medical School, examined the impact of endocrine changes on immune cells of the female reproductive tract tissue imparted by estradiol during the menstrual cycle. He described a suppression of immune protection that created a 7- to 10-day “window of vulnerability” for viral infection that coincided with optimal conditions for fertilization and implantation.<sup>4</sup> He studied the effects of estradiol on human uterine epithelial cells (UEC) from hysterectomy patients cultured with CD4<sup>+</sup> T cells and monocyte-derived DCs from matched blood. He showed that estradiol acts both directly and indirectly to regulate immune functions in the female reproductive tract. Hormone-mediated effects include increased secretion from uterine epithelial cells of antibacterial, antiviral, and antifungal activities such as secretory leukocyte protease inhibitor (SLPI) and human B-defensin-2 (HBD2), but decreased expression of these molecules from primary vaginal epithelial cells. Estradiol reduced tight junction formation, altered secretion of cytokines, chemokines, and DC and CD4<sup>+</sup> T cell functions. Overall, the studies presented by Wira demonstrated that mucosal immune protection was precisely regulated by sex hormones that act both directly and indirectly via growth factors, cytokines, and chemokines throughout the female reproductive tract. He proposed that hormone contraceptives are probably also strongly bioactive for mucosal immune dynamics.

Professor Anthony Cunningham from the Westmead Millennium Institute completed this session with an examination of the role of Langerhan cells of the anogenital mucosae or skin during sexual transmission of HIV.<sup>5,6</sup> He addressed the controversy of whether epidermal Langerhan cells (eLCs) use the surface C-type lectin, langerin, to mediate the two routes

of HIV transfer to T cells more familiar from the better-studied monocyte-derived DC model (1) through binding and entry into deep tetraspanin vaginations of the plasma membrane for DC-SIGN in DC, and (2) by *de novo* replication. He presented evidence indicating that both these phases of uptake and transfer of HIV from purified primary eLCs (CD1a<sup>+</sup>, langerin<sup>+</sup>, mannose receptor- and DC-SIGN-low/negative) to T cells were mediated through HIV binding to langerin as a key step in both mechanisms. The most compelling data were the specific inhibition of HIV transmission from eLCs to T cells using antibody blocking langerin and dominant negative soluble recombinant langerin assembled into a trimeric conformation that has high affinity binding for the HIV Env gp140 trimer. These langerin-specific blocking approaches also inhibited the first phase of HIV transfer to T cells in the LC model cell line MUTZ3. Cunningham showed that langerin was expressed as a trimer on the surface of MUTZ 3 cells and the langerin trimer binds HIV more strongly than monomers. Cunningham proposed the possibility of using soluble recombinant langerin trimer or small molecule mimics, possibly in combination with CCR5 inhibitors, as a combination microbicide strategy.

### Innate and Natural Antiviral Mechanisms at the Mucosa

The cervicovaginal mucosa in women and anogenital mucosa in both sexes have evolved natural and innate immune barriers to viral infection that are important to understand in the context of developing microbicides for preventing HIV infection.

Deborah Anderson from Boston University described the structure of the vaginal stratum corneum, which is glycogen filled and has no tight junctions, and then its role as a potential barrier to pathogens, to the prevention of activation of innate immunity in the deeper layers of the epithelium and in facilitating neutralizing antibody activity.<sup>7,8</sup> She showed that very few immune cells are present in the healthy stratum corneum and, using artificially constructed epithelial layers, that the stratum corneum blocks the access of topical bacterial components (agonists) that bind to and activate toll-like receptors 1, 2, 3, and 5 on basal epithelial cells, thus preventing a continuous state of inflammation. However, if the stratum corneum is removed by scarification basal epithelial cells were activated to produce cytokines. Nevertheless, pathogens such as HIV can penetrate the vaginal stratum corneum, in particular because it has no tight junctions and they can remain infectious within the stratum corneum for a short period of time. Similarly, mononuclear cells act as carriers of HIV, especially those in semen, and can adhere to and penetrate the stratum corneum. Conversely, antibodies can also be absorbed in the stratum corneum and retain their activity after absorption. This was demonstrated by using fluorescently labeled antibodies to herpes simplex virus, which were retained for up to 12 h in the stratum corneum and reduced type 2 topical HSV-2 infection of vaginal epithelium. Similarly, the VRC01HIV neutralizing antibody was shown to be taken up into the stratum corneum and can block HIV infection (detected by the addition of the indicator TZM-bl cell line). Thus, cell-free and cell-associated pathogens including HIV can enter the stratum corneum but soluble immunological innate effectors (such as defensins and lysozyme) can be

concentrated and provide robust antiviral defenses in the stratum corneum. The implications for microbicides are that their concentration, activity, and retention within the stratum corneum need to be measured. Perhaps microbicides can be designed specifically to “fortify” the stratum corneum, for example, monoclonal antibodies.

Alan Landay from Rush University Medical Center reviewed the immune mechanisms of interactions between bacterial vaginosis (BV) and HIV infection in the vaginal mucosa. In particular, he reviewed the role of ligands for TLRs and short chain fatty acids (found in the cervicovaginal fluid of subjects with BV) and their effect on inflammatory cytokine production.<sup>9</sup> cervicovaginal lavage (CVL) fluid from BV-activated TLR2 rather than TLR4 on the surface of monocyte cells, indicating the dominance of gram-positive over gram-negative bacteria in these BV fluids. This led to induction of tumor necrosis factor (TNF)- $\alpha$  secretion by these monocyte cells, which in turn stimulate HIV production. Short chain fatty acids such as acetate, propionate, and butyrate are present in vaginal mucosal fluid. They bind to the receptor GPR43 on monocytes and neutrophils or diffuse directly through cell membranes. They enhance production of inflammatory cytokines such as interleukin 8, after stimulation of TLR2 on CD14<sup>+</sup> monocytes. Therefore, these events can lead to an increase in HIV replication in macrophages. He concluded by showing data that supported the rationale for blocking TLR ligands and short chain fatty acids to reduce the inflammatory environment in the vaginal mucosa, which is known to be stimulatory for HIV production.

Gilda Tachedjian from the Burnet Institute reviewed the role of L-lactic acid as a natural microbicide in the female genital tract. First, she emphasized that the decrease in lactobacilli in BV is associated with an increase in the vaginal pH to greater than 4.5 and BV is a risk factor for HIV and other STIs.<sup>10,11</sup> DL-Lactic acid is produced by lactobacilli and maintains the low vaginal pH for antimicrobial activity.<sup>12</sup> In particular, it inactivates 18 BV-associated microbes.<sup>13</sup> In vaginal fluid, bacteria associated with BV can be suppressed with lactic acid but not hydrogen peroxide. HIV is also trapped in L-lactic acid acidified cervicovaginal mucus gel.<sup>14</sup> HIV is trapped by acidic but not neutralized human cervicovaginal mucus. L-Lactic acid was found to be more potent than D- and DL-lactic acid in inactivating HIV at a low pH (4.0). This appears to be not just an effect of pH: L-lactic acid is more rapid and potent in inactivating HIV than low pH alone (provided by acetic acid or hydrochloric acid). Furthermore, L-lactic acid inactivates different HIV subtypes, X4 and R5 HIV strains, patient isolates, and also HIV-2. The HIV-inactivating effect is retained in the presence of 75% seminal plasma and 50% cervicovaginal fluid. Indeed, it is lactic acid rather than lactate anion that inactivates HIV-1. The stereochemical-dependent activity suggests that L-lactic acid acts on proteins. Thus, L-lactic acid is a microbial defense factor as well as a potential natural microbicide. Its mechanism of action is under active investigation.

Paul Hertzog from Monash University presented interesting data on a new type of interferon, interferon epsilon (IFN- $\epsilon$ ), in controlling sexually transmitted infections in the female reproductive tract, which is thus a new component of innate immunity in this site.<sup>15,16</sup> IFN- $\epsilon$  is a Type-1 interferon, grouped together with IFN- $\alpha$  (13 subtypes) and IFN- $\beta$  (1 subtype), and is approximately 30% different from IFN- $\alpha$  and

IFN- $\beta$ , respectively. Its genetic locus is on chromosome 9. It probably has a three-dimensional structure similar to the other Type 1 interferons and is able to induce typical interferon-regulated genes via interferon receptors 1 and 2 but is not induced by the TLR pathways. Perhaps the most striking feature is that IFN- $\epsilon$  is constitutively expressed in the female reproductive tract in both mice and humans. It is also regulated hormonally, increasing in estrus and in pregnancy. In particular, it is increased during the proliferative phase of the human endometrium and is reduced in postmenopausal uterine endometrium. Its unique distribution and regulation may be because it has evolved a unique promoter that is not stimulated via the TLR pathway, but is necessary for reproductive fertility. Professor Hertzog and his colleagues have generated an IFN- $\epsilon$  genetic knockout mouse that was then used to demonstrate that they are more susceptible to infection with HSV-2 and murine *Chlamydia* (*C. muridarum*). Furthermore, add back experiments with recombinant IFN- $\epsilon$  showed reduction of *Chlamydia* infection. Thus, hormonal regulation of IFN- $\epsilon$  may be involved in susceptibility to sexually transmitted infections.

Dr. Charani Ranasinghe from the John Curtin School continued the theme on IFN- $\epsilon$ .<sup>17</sup> She presented her studies using IFN- $\epsilon$  incorporated into recombinant viral vectors, as potential mucosal adjuvants for enhancing mucosal HIV vaccines. For these studies IFN- $\epsilon$  in recombinant vaccinia and fowlpox viral vectors was inoculated into mice and systemic T cell responses were studied in the spleen and mucosal T cell responses were studied in the lung and intestine (Peyer's patches). Using these tools, Dr. Ranasinghe showed that IFN- $\epsilon$  can induce enhanced lung and gut-specific CD8 lymphocytes, suggesting it plays a role in mucosal immunity at these sites. These CD8 lymphocytes expressed IFN- $\gamma$  and cytotoxicity markers and were associated with more rapid viral clearance from the lung. These studies suggested that IFN- $\epsilon$  incorporated into hybrid vectors may act to enhance such adaptive immune responses in lung, gut, and perhaps in genital mucosa (thus enhancing endogenous secretion) to reduce mucosal infections such as TB (lung), HIV, or other sexually transmitted diseases.

In general, this session indicated the recent increase in interest in innate immunity of the genital mucosa, which has opened up many new avenues of research of direct relevance to the development of microbicides and mucosal vaccines.

### **Microbicide Efficacy: Pharmacokinetics, Pharmacodynamics, and Animal Models**

With respect to HIV microbicides we do not suffer from a lack of potential candidates. The mixed successes of microbicide trials in the past has brought with it knowledge of not only how to streamline clinical trials, but also what potential studies can be used as surrogates to select which (of many) products actually make it to clinical trials. Within this symposia, several experts in their respective fields presented valuable models that will help streamline microbicide candidate selection.

Victor Garcia from the University of North Carolina presented on the use of a novel mouse model in microbicide testing. While murine models of HIV infection in the past have utilized the now well-known thy/liv SCID-hu mouse, their applicability to microbicide research has been limited due to

the lack of human target cells populating the genital tract. Garcia presented the various aspects of the Bone Marrow Liver Thymic (BLT) HIV mouse model that he pioneered for use in HIV research and its potential applicability to HIV microbicide research. The greatest advantage of the BLT mouse was the seeding of human hematopoietic cells throughout the female genital tract and their ability to first be infected across the genital mucosa and second the ability to establish plasma viral loads similar in nature to that observed in HIV and SIV infections.<sup>18</sup> Yet how does this model fit with microbicide preclinical testing? Garcia presented several study designs with regard to testing the efficacy of microbicide formulations in this new animal model. The first demonstration of the model retrospectively tested tenofovir formulations of similar nature to the now concluded CAPRISA clinical trial.<sup>19</sup> By comparing study designs in the nonhuman primate (NHP) animal models and the outcome of the CAPRISA trial, the BLT murine model predicted protection as well as that in the NHP.

In addition to developing and using this new mouse model in key experiments to demonstrate microbicide efficacy after vaginal challenge, Garcia and colleagues utilized this model to address the important question of how transmission across the female genital tract actually occurs. Hypotheses of cell-free viral transmission across the genital tract are controversial. If we consider male-to-female transmission there are three key factors that are presently driving debate. First is the absolute numbers of virions in seminal fluid, second is HIV inhibitory factors, and third is the role of seminal amyloid fibrils in potentially enhancing cell-free viral transmission (the two latter recently reviewed).<sup>20-22</sup> Yet it is still unclear what role infected leukocytes have in the transmission process. Given this new mouse model Garcia and colleagues tested the capacity of an infected CD4 T cell for its ability to transmit virus across the vaginal mucosa and also the capacity of a tenofovir topical microbicide to block it. Not only did cell-associated virus result in HIV transmission in BLT mice, but the use of the same tenofovir-containing microbicide that blocked cell-free infection could not block HIV transmission in this scenario. If this also translates to the situation in humans, this last observation imparts two very key messages: first, microbicides need to be more effective in the face of cell-associated inocula and second, cell-associated inocula may play a role during transmission.

For an alternative murine model, Ramesh Akkina from Colorado State University described a model for evaluating the pharmacokinetics (PK) and pharmacodynamics (PD) of oral antiretroviral (ARV)-based PrEP that was distinct from the above-mentioned Garcia BLT model.<sup>23</sup> In the Akkina model, humanized Rag2<sup>-/-</sup> $\gamma$ c<sup>-/-</sup> mice (Rag-hu) are created by intrahepatic injection of irradiated mice with CD34<sup>+</sup> hematopoietic stem cells from cord blood. Sustained long-term multilineage human hematopoiesis is achieved in the blood, the vaginal and rectal mucosa, and the large intestine. Rag-hu can be intraperitoneally, vaginally, or rectally infected with R5- and X4-tropic HIV-1 strains demonstrating sustained and prolonged viral RNA levels in plasma. The efficacy of oral PrEP with either raltegravir or maraviroc was demonstrated in this model as measured by lack of plasma viral RNA and preservation of CD4 T cells (CD4:CD3 T cell ratio) compared to nontreated infected mice. Furthermore, vaginally applied maraviroc gel (CCR5 inhibitor) was effective in preventing

HIV-1 transmission in this model. PK/PD studies performed with oral tenofovir showed decreased levels of tenofovir and tenofovir diphosphate in the vaginal compartment compared to the rectum, which are similar to findings seen in humans. Thus, both models demonstrate considerable promise for the study of the efficacy and PK/PD of oral and topical PrEP candidates, especially with regard to the flexibility of numbers in experimental designs. NHPs have served as the gold standard in HIV microbicide testing and also HIV transmission studies.

Ronald Veazey from the Tulane National Primate Research Center thoroughly highlighted the complexities of this model and key components that need to be considered with specific reference to microbicide study design. The importance of NHP HIV research is the fact that NHPs have an anatomy and reproductive physiology comparable to that of humans.<sup>24</sup> Added to this is the similar immune system and the nearest relative to HIV, simian immune-deficiency virus (SIV), can readily infect animals after vaginal challenge.

Yet which NHP do you use? Rhesus macaque (*Macaca mulatta*) models have served as the animal of choice for U.S.-based national primate facilities, as there is a significant supply from Chinese macaque colonies. While *mulatta* may indeed be easier to source for larger microbicide studies, pigtailed macaques (*Macaca nemestrina*) are sometimes often preferred in HIV/SIV research studies. The major underlying reason for this preference is the fact they have menstrual cycles similar to humans and also can be readily infected with SIV or SHIV (SIV chimeras expressing either HIV envelope or reverse transcriptase) using inocula that approximates that present in seminal fluid.<sup>25,26</sup> Thus, Veazey presented the physiological differences of *mulatta* versus *nemestrina* and how they influence animal study design. Histological observations of *nemestrina* vaginal tissues reveal a closer similarity of epithelial thickness to that of humans during menses. In conclusion, while *Macaca nemestrina* may be a closer model to humans when considering reproductive physiology, the counterargument for *Macaca mulatta* was presented indicating that following Depo-Provera treatment (progesterone), the animal model is histologically similar to humans during menses.

Yet with all the queries and different experimental designs, the NHP model has brought the field a better understanding of which microbicides have the potential to work and how we should deliver them. The early work by Veazey and colleagues was seminal in demonstrating that HIV neutralizing antibodies, fusion inhibitors, and CCR5 small molecule inhibitors all had the potential to work in the context of a topical microbicide gel.<sup>27-29</sup> The key aspect of this cumulative work is summarized by the dose studies of the CCR5 inhibitor PSC-RANTES, which clearly demonstrated that protection *in vivo* by a microbicide gel requires several orders of magnitude more than that observed *in vitro*.<sup>28</sup>

Although both murine and NHP animal models have become more applicable for HIV microbicide testing and HIV transmission studies, there are still significant precedents in both HIV research and elsewhere in the form of failed clinical trials that have made investigators aware of the shortcomings in animal research. Yet in the defense of animal model researchers, it is indeed difficult to recapitulate what happens in the real world, including issues such as coitus and adherence. While adherence is not an issue in animal research, it is

refreshing to see the pioneering work of Patton and colleagues, who are now introducing coitus in their NHP study designs.<sup>30</sup> Although the abovementioned criteria are known issues in translating animal studies through to humans, there may be more fundamental differences of which we are not aware.

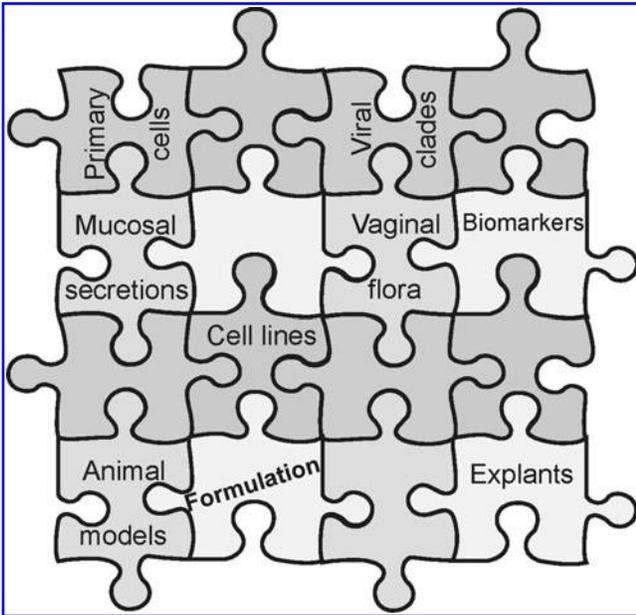
To cover the closest mimic of HIV transmission, Charlene Dezzutti from Magee-Women's Research Institute, University of Pittsburgh, presented the advantages and drawbacks of using human tissue explants in microbicide research.<sup>31</sup> Human tissue explants are derived from anogenital mucosal surfaces of humans either as a consequence of surgery (e.g., corrective surgery that results in mucosal tissue being excised) or through the collection of mucosal punch biopsies. It is apparent immediately that not only is human tissue being used, but it is also derived from the sites of HIV transmission with the relevant target cells present in the tissue. For study designs using mucosal tissue the immediate question posed is how to present virus to this tissue? Should studies be designed to reflect how the virus would initially interact with the tissue (i.e., in a polarized manner) or simply expose the whole tissue to the virus (unpolarized)? While for obvious reasons researchers elect for the polarized tissue models, this does limit the number of replicates for this tissue. The arguments for the nonpolarized model is the increased number of replicates and the fact that virus may, under certain conditions (traumatic), interact with mucosal tissue in a nonpolarized manner during transmission. Yet researchers using explant tissue for microbicide research have really made use of what is available to them. For instance, human mucosal explants can be initially used for toxicity testing, determination of drug permeability, and efficacy studies.

Dezzutti and colleagues have even taken this further to cover the efficacy of microbicides on viruses that are resistant to therapy, including efficacy studies using HIV envelope genotypes that are known transmitting viruses. Thus, in a manner very similar to the murine models, it is apparent that multiple questions can be asked with the use of a limited source of human tissue. While explants are indeed promising and consist solely of human tissue, like any model there are limitations. These limitations can be 4-fold: first, they are expensive and often difficult to obtain, second they are independent of hormonal control, third they are unable to regenerate or repair, and finally they lack vascularization and thus a renewed supply of leukocytes/HIV targets.

It is apparent from the presentations that covered each model that no individual approach is perfect. Thus, it is only appropriate that in the final slide of this important session Charlene Dezzutti presented each model as a jigsaw puzzle representative of the preclinical landscape (Fig. 1). It is also important to note that this picture is fluid and the more we learn from HIV transmission studies and microbicide clinical trials in the future, the better we will be able to find the best candidate microbicide for future clinical trials.

### Opportunities for Combination HIV Prevention

At the M2012 closing session Gina Brown from the NIH Office of AIDS Research and Stephen Becker from the Bill & Melinda Gates Foundation, representing the major funders of Microbicides 2012, announced that there will be a single HIV Prevention meeting in 2014, merging the existing



**FIG. 1.** Jigsaw puzzle of HIV prevention models. Kindly provided by Charlene Dezzutti.

Microbicides and Vaccines meetings. One of the rationales is to promote cross-fertilization in the HIV prevention field where common areas of investigation exist, for example, in understanding the basic science of HIV infection at the genital tract mucosae and host immune responses that can either mitigate or promote HIV infection. Another area of relevance is combining biomedical HIV prevention modalities. In this regard, strategies to combine a vaccine, to elicit humoral or cell-mediated immunity against HIV, with PrEP were presented by Robin Shattock from Imperial College London and Cecelia Cheng-Mayer from the Aaron Diamond AIDS Research Center. In an alternative strategy, Damian Purcell from the University of Melbourne presented studies on a passive immunity-based approach, which could be combined with an ARV as a topical microbicide.

Robin Shattock described the rationale for combining ARV PrEP with a vaccine (VaccPrEP) for both antiviral and immunological protection against HIV. Advantages of this combination approach include potential increased efficacy, protection during the immunization period, reducing infectious challenge and the primary foci of infection, increasing the eclipse phase prior to systemic dissemination providing extended opportunity for an adaptive immune response boosting local immunity to viral antigen, broadening localized immunity through protected exposure to prevalent virus, converting a high-risk challenge for a vaccine with low efficacy (i.e., RV144) to a low-risk challenge, providing coverage between potential revaccination regimens as immunity wanes, and providing immunological coverage of intermittent PrEP adherence, breakthrough virus, and resistance evolution.

The strategy described by Shattock is to coformulate a microbicide with a vaccine candidate comprising HIV envelope gp140 immunogen to elicit antibody production at the genital mucosa and systemically. This strategy presents several challenges including the stability of the antigen in low pH microbicide formulations compatible with the female genital

tract, the stability of the antigen in the presence of bodily secretions, and the ability of the antigen to elicit a mucosal humoral response in humans. HIV-1 (CN54) clade C trimeric envelope (gp140) formulated in a Carbopol mucoadhesive gel was able to elicit IgG responses in the serum and female genital tract in the rabbit in the absence of adjuvant (which could upregulate activation of HIV target cells and subsequent infection) following repeated exposures. The vaccine-induced serum antibodies were able to neutralize the infectivity of a pseudovirus carrying a heterologous clade C envelope. Unfortunately, this strategy did not lead to the induction of local or systemic immune responses in healthy women, which is consistent with the lower genital tract being a relatively inefficient immunological inductive site.<sup>32</sup> However, mucosal immunization with nonadjuvanted CN54 gp140 in Carbopol gel was able to boost serum and mucosal antibody responses in macaques primed intramuscularly with CN54 delivered in the AS01 adjuvant.<sup>33</sup> Antibody responses after intravaginal immunization with trimeric HIV-1 CN54 clade C gp140 in Carbopol gel are augmented by systemic priming or boosting with an adjuvanted formulation.

Shattock also presented novel delivery strategies for gp140 including a tablet and vaginal ring devices comprising a microbicide and gp140 loaded in rod-inserts in the ring where sustained release of the antigen was observed in both formats. Furthermore, a strategy comprising intramuscular prime followed by boost from the vaginal ring demonstrated anti-gp140 antibody responses in serum and the mucosa. Provocatively, tenofovir gel-protected rectal challenge in macaques leads to T cell priming providing another rationale for combining vaccines with PrEP.<sup>34</sup> Shattock showed that the VaccPrEP concept, comprising intranasal and intramuscular immunization of macaques with gp140 followed by viral challenge in the presence of 1% tenofovir gel, resulted in systemic and vaginal humoral responses and enhanced protection against infection in the combination group compared to each modality alone. Shattock closed his presentation by discussing the potential challenges in conducting combination trials requiring increased sample size, trial complexity, and cost to evaluate placebo vs. PrEP vs. vaccine vs. Vacc-PrEP, although there is potential for an adaptive clinical design.

Cecelia Cheng-Mayer presented her studies of vaccine PrEP combinations using a partially effective T cell-based vaccine (SIV gag-pol DNA prime/rAD5 boost with no envelope and limited number of DNA prime boosts to attenuate vaccine efficacy) in combination with a suboptimal dose of a zinc finger inhibitor (0.1% SAMT-247) topical microbicide. Rhesus macaques were subjected to repeated vaginal SHIV challenge in the absence of Depo-Provera treatment normally used to thin the vaginal epithelium. The rationale for this combination approach is that a topical microbicide that inactivates HIV-1 and reduces the inoculum dose will slow infection and potentiate the efficacy of a moderately effective T cell-based vaccine. The Cheng-Mayer combined vaccine and PrEP approach demonstrated a significantly longer infection free state in the combination treated macaques than in the naive and untreated monkeys.<sup>35</sup> However, study limitations include the use of genetically matched immunogen and challenge virus, the assumption that susceptibilities to infection for all animals within each experimental group were equal, and that SHIV T cell immunity was analyzed using

peripheral blood cells, which may not accurately reflect mucosal events. Nevertheless, a recently published study<sup>36</sup> using maraviroc topical PrEP and a T cell-based adenovirus vectored vaccine supports the notion that vaccines and microbicides may provide better protection against viral infection when used together compared to separately.

An alternative to using antigens to elicit humoral or cell-mediated immune responses against HIV-1 is to directly deliver anti-HIV antibodies in a gel, which could potentially be combined with an ARV. In the approach presented by Damian Purcell, large-scale neutralizing antibody and antibody-dependent cellular cytotoxicity (ADCC)-promoting antibody can be produced in bovine first milk, known as colostrum, representing a cost-effective source of large quantities of IgG antibodies. Cows were vaccinated either when pregnant or prior to and during pregnancy with either purified clade B or equal amounts of clade A, B, and C envelope (trimix) gp140 oligomers. Colostrum-derived polyclonal IgG showed broad cross-clade binding and neutralization in cows vaccinated with HIV-1 Env gp140 oligomers in a long duration regimen.<sup>37</sup> Bovine IgG with strong binding to gp140 had robust ADCC function and could mediate HIV-specific killing by human immune cells.<sup>38</sup> HIV neutralization and FcγR-mediated cell functions have the potential to provide a rapid and potent response against both cell-free HIV-1 virus and HIV-infected cells, which might be particularly important in Ab-mediated microbicides for prevention of HIV transmission. In addition, colostrum IgG can be easily produced in large scale at a fraction of the cost of conventionally produced monoclonal neutralizing and/or ADCC-mediating Ab.

This session brought together the concept that combined methods (vaccine and microbicide) for HIV prevention will be the future for this field. This focus provides an even stronger impetus for developing a better understanding of the basic mechanism of mucosal HIV transmission in order to move novel therapeutic strategies forward.

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