REVIEW ARTICLE

Cervico-Vaginal Tissue *Ex Vivo* as a Model to Study Early Events in HIV-1 Infection

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Vaginal intercourse remains the most prevalent route of infection of women. In spite of many efforts, the detailed mechanisms of HIV-1 transmission in the female lower genital tract remain largely unknown. With all the obvious restrictions on studying these mechanisms in humans, their understanding depends on the development of adequate experimental models. Isolated cell cultures do not faithfully reproduce important aspects of cell–cell interactions in living tissues and tissue responses to pathogens. Explants and other types of *ex vivo* tissue models serve as a bridge between cell culture and tissues *in vivo*. Herein, we discuss various cervico-vaginal tissue models and their use in studying HIV vaginal transmission and consider future directions of such studies.

Introduction

Although vaginal intercourse is associated with a lower HIV-1 transmission probability per exposure than other routes of transmission, worldwide it remains the most prevalent route of infection.[1,2] In spite of many efforts, the reasons why male-to-female transmission is more efficient than female-to-male,[3] as well as the detailed mechanisms of HIV-1 transmission in the female lower genital tract, remain largely unknown.

Although it still remains unclear whether the source of infection is seminal cell-associated or cell-free virus, vaginal and cervical epithelia are obviously the primary sites where the HIV-1 virions in ejaculate and female genital tract cells first come together. The vagina and ectocervix are covered by the multiple cell layers of a stratified squamous epithelium, usually coated with keratin. It is difficult to imagine how viruses can go through these layers. Indeed, in *ex vivo* experiments[4] this multilayered epithelium was shown to be a reliable barrier against HIV-1 and small molecules.[5] So, why does this barrier fail so often? Possibly because epithelial layers can become ulcerated due to sexually transmitted infections and can be damaged by inflammations such as those caused by common infections like yeast (candidiasis)[6] and herpes simplex virus 2 (HSV-2).[7] Also, intercourse itself may damage these layers, as epithelial microabrasions were detected in 60% of women following consensual intercourse.[8,9] The damaged areas provide open access to the

sub-epithelial structures where HIV-1 cell targets predominantly reside.

In contrast to the vagina and ectocervix, the endocervix and the transformation zone (the squamo-columnar epithelial junction between the endo- and ectocervix) are covered by a single layer of columnar epithelium and are thus less protected. They are regarded as the primary sites for HIV-1 transmission during intercourse. The extension of the transformation zone is increased in young women with cervical ectopy,[10,11] probably easing HIV access to its target cells and making these women particularly vulnerable to HIV infection. Also, the effects of the phase of the menstrual cycle on the thickness of the vaginal epithelium and on the biological and physical properties of cervico-vaginal mucus seem to be relevant to the susceptibility to HIV-1 infection.

Potential HIV-1 cell targets beneath the cervico-vaginal epithelium are dendritic cells (DCs), macrophages, and CD4+ T lymphocytes. In *in vitro* experiments, DCs were shown to be able to capture HIV-1 virions, although they do not become productively infected.[12] These cells may protrude their dendrites to the surface of the epithelial layer and capture virus there, providing a path for the virus to penetrate intact epithelia as well as a potential transfer vehicle to the local lymph nodes. The role of macrophages in the first stages of HIV-1 transmission is still debated. In cervico-vaginal tissue macrophages seem to be able to support productive infection by HIV-1.[12] However, experiments on macaques demonstrated that CD4 lymphocytes, rather than macrophages, are the main producer of virus at the early stages of SIV vaginal transmission in these animals.[13]

Upon infecting the founder cell population, HIV then undergoes a local expansion and disseminates to the draining lymph node, thereby establishing an initial lymphoid tissue reservoir that will further spread infection to other organs and peripheral tissues (reviewed in Ref. 13). Thus, cells present in the sub-mucosa, whose first role is to fight any invading pathogens and orchestrate innate and adaptive immunity, are targeted by HIV-1 and thus facilitate its transmission and dissemination.[14]

Already, the above brief and incomplete description of HIV-1 transmission through the mucosa of the female lower genital tract shows the complexity of this phenomenon. Understanding the mechanisms of HIV transmission in the female genital tract mucosa requires knowledge of the functions and interactions of all immune cells with each other and with the extracellular matrix in this tissue in vaginal intercourse. With all the obvious restrictions on studying these mechanisms in humans and the anatomical differences between the female genital tracts of humans and those of non-human primates, it is obvious that our progress in studying HIV-1 vaginal transmission greatly depends on the development of adequate experimental models. Although conventional cultures of cell lines or peripheral blood mononuclear cells have been useful in many areas of HIV research, their use is limited by the fact that they neither reproduce the morphology nor mimic the functions of living tissues, and therefore they lack the potential to predict tissue responses of real organisms. Explants or other types of *ex vivo* tissue models serve as a bridge between cell culture and the *in vivo* environment.

Meanwhile, most of our experimental knowledge of HIV infection in general comes from the study of cell lines and isolated primary cells. Although these experimental models continue to be important to the furtherance of our understanding of the mechanisms of HIV-1 transmission, they do not faithfully reflect an important aspect of the *in vivo* situation: the spatial distribution of cells and their native communication within the tissue cytoarchitecture. Yet, as indicated above, these aspects of tissues seem to be critical for 'gatekeeping' of HIV-1. Experimental systems consisting of cervico-vaginal tissue explants have been developed to overcome these problems. Although, like any *ex vivo* model, these systems have their own limitations (e.g., survival limited to 2–3 weeks, problems with reliable tissue polarization, donor-to-donor variability, etc.)[15], tissue explants seem to be the experimental model closest to *in vivo*, allowing HIV-1 transmission to be studied under controlled laboratory conditions. Below, we describe these models and how they are currently used to study male-to-female HIV-1 transmission.

Explant models and their application to HIV studies

The main tenets of three-dimensional (3D) tissue culture were laid down as early as 1912.[16] Later the development of synthetic culture media, culture vessels, and cell lines led in its turn to the development of various microscopic techniques adapted to the study of isolated cells. As a result such cells became a common object of study. Technical advances in

three-dimensional cell culture have not been introduced until more recently and started to be appreciated only in the last decade. However, over the decades many approaches have been pursued for developing three-dimensional cultures for biomedical research, including spontaneous cell aggregation/multicellular spheroids, microcarrier bead cultures, rotary cell culture systems, engineered scaffolds, gel/matrix systems, filter or mesh supported organ culture, and raft cultures.[17] Typically, tissue fidelity and function are best achieved by attempts to maintain tissue biopsies or explants *ex vivo*, rather than attempts to reconstruct tissue from individual cell types.

Although advantages and disadvantages exist for all organ culture explant models, for the study of multiple aspects of HIV pathogenesis our laboratory has favored the use of raft culture, in which blocks of human tissue are cultured on collagen sponges at the air–liquid interface. This *ex vivo* raft model of tissue culture is based on work of Joseph Leighton, an NIH investigator, who worked on this technique in the 1950s and 60s.[18,19] Leighton's three-dimensional histoculture method, which utilized a sponge matrix, was further developed in the 1980s by Hoffman[20] for anticancer drug studies. Our laboratory first benchmarked this method for HIV studies using cultures of human tonsils and lymph nodes.[21]

This model has many advantages, the first of which is the preservation of the tissue architecture for 2–3 weeks. Not only are all cell types retained in the tissues without a need for exogenous activation or stimulation, but key cell surface molecules relevant for HIV infection[22–25] are also maintained. Certain functions of the lymphoid tissue are also maintained *ex vivo*, including the ability to release a spectrum of cytokines similar to those released by *in vivo* tissue, and the ability of tissue challenged with recall antigens (tetanus or diphtheria toxoids) to respond by producing specific antibodies.[26]

Ex vivo tissues support HIV replication without the artificial stimulation that is necessary for productive HIV infection in isolated lymphocytes. In this respect, tissues *ex vivo* are apparently similar to those *in vivo*. Also, these tissues support replication of other human viruses including human herpesvirus (HHV)-6,[27,28] HHV-7,[25] Human cytomegalovirus (HCMV or HHV-5),[29] HSV-2 (HHV-2),[30] vaccinia,[31] measles virus,[32,33] and West Nile virus[34] as well as non-viral human pathogens such as the bacterium *Borrelia burgdorferi*[35] and the parasite *Toxoplasma gondii*.[36] Some of these viral and non-viral pathogens are commonly associated with HIV-1 (HIV copathogens), thus allowing the simultaneous study of HIV copathogenesis.

Recently, this *ex vivo* tissue model was extended to include cultures of cervico-vaginal[4,37–40] and rectosigmoid tissues.[41–43] Cervico-vaginal, rectosigmoidal, and other *ex vivo* tissues can be used to evaluate potential microbicides in pre-clinical tests. As these tissues serve as the first gateway for HIV-1 sexual transmission, preserving the specific mucosal cell phenotypes and functions is critical for understanding early events in HIV-1 transmission and possible 'gatekeeping' mechanisms that select against CXCR4-utilizing HIV-1 variants.[92] Our recent work using this model is outlined below, as well as future directions of research.

Cervico-vaginal models to study HIV infection

Cervical explant cultures were first developed by Fink et al.[44] for the study of epithelium metaplasia *in vitro*. They consisted of large tissue explants ($5 \times 5 \times 2$ mm or $5 \times 10 \times 3$ mm) cultured on a thin slab of agarose-gelled serum-free Eagle's Basal Medium on top of a stainless steel supporting grid. This culture method was successfully adapted to the study of HSV-2 and HSV-1 infection *in vitro* and was shown to support the replication of these two human herpesviruses.[45] O'Brien et al.[46] modified this method for the study of the production of glycoprotein from normal and malignant cervical explants of smaller size (5 mm^3) cultured either fully immersed in serum-free culture medium or maintained at the air–liquid interface, supported only by a stainless steel grid mesh. On the basis of glycoprotein production, the authors concluded that the grid technique was superior to the immersion culture. It was this grid technique that was adapted to the study of HIV-1 infection in human cervical explants by Palacio et al.[38] In this method, cervical explants (3×3×2 mm) were infected by immersion in viral stocks, washed twice to remove un-adsorbed virus, and then supported on a stainless steel mesh at the air–liquid interface. Although no reverse transcriptase (RT) activity could be measured in the culture medium of explants exposed to X4 or R5 viruses, in R5-exposed tissues, viral antigens could be detected in cells that had the morphology of macrophages. These early results already illustrated all the

complexity of the study of HIV-1 replication in cervical explants, especially that the evaluation of actual HIV-1 replication, rather than viral adsorption or capture, requires sensitive assays and adequate controls.

The introduction of FCS-supplemented RPMI, cellular activators, and immersion cultures allowed the adaptation of explant cultures to 96-well plates.[47] Activation of explants with phytohemoagglutinin (PHA) and interleukin (IL)-2 was required for the replication of most of HIV-1 variants except for the lab-adapted R5 strain BaL. In contrast to the *in vivo* situation, where the first target cells were CD4+ T cells, in this *ex vivo* model the majority of viral antigen was detected in sub-epithelial cells expressing markers of macrophages. This infection of explants by soaking in cell-free virus suspension gives the virus full access to both epithelial and subepithelial cells, thus mimicking viral penetration through a damaged epithelium but not transmission through an intact epithelium.

To permit study of the ability of intact cervical epithelia to transmit HIV to submucosal cells, explants have to be polarized in an Ussing chamber mechanically sealed with rubber O rings.[47] In such a system, the tightness of the seal is a critical issue and has to be constantly controlled, as monitored for example from the lack of permeability to a small tracer molecule, such as inulin. Such experiments demonstrated that the sealed intact epithelium in this experimental model was impervious to cell-free and to cell-associated HIV-1. This result was at variance with results obtained in a polarized and sealed cervical explants model, in which a rapid penetration of HIV through the epithelial layer was reported.[40] In the latter model, circular explants obtained by punch biopsies were cultured, epithelium pointing upward, on the upper chamber of a transwell and sealed with agarose. Production or transfer of virus was detected from the infection of indicator cell lines placed in the lower chamber, mimicking the basolateral submucosa. The differences between the two models sparked a series of discussions[4,48] in which Shattock et al.[4] suggested that the seal of the polarized explants in Gupta et al.'s study was not tight enough and may have let HIV-1 go through. As a result of this discussion, Gupta's group further refined its polarized culture system and introduced a more complex method to identify the infected cells in explant models. To avoid the loss of cell surface markers due to enzymatic digestion of the explants, these authors dissociated tissues using a mechanical disaggregation system.[49] Although these authors used a limited number of cellular markers to identify infected cells (CD4, CD45RO, and CD68), they concluded that HIV-1 RNA was first detectable in CD4 memory T cells. In spite of the use of the antiviral agents UC781 and Tenofovir ((*R*)-9-(2-phosphonomethoxypropyl)adenine,PMPA) in this study, their effect on the infection of cells residing within the cervical explant was not investigated, depriving the study of an important experimental control. The development of an explant enzymatic digestion protocol, which spares cell surface markers, together with the systematic use of anti-retrovirals, allowed a more precise characterization of cells infected within the explant.[39]

An alternative way to maintain cervico-vaginal explants is to culture them on rafts rather than immersed in culture medium.[50] Essentially, this is an adaptation to cervico-vaginal tissues of the collagen raft culture model initially developed for tonsils in our laboratory.[21] This adaptation consisted in immersing explants in viral stocks, transferring them on top of collagen sponges, and culturing them at the air–liquid interface. Under these conditions, HIV-1 $p24_{gag}$-expressing cells were detected in the exposed blocks. However, in this study,[50] HIV replication wasn't assessed by other methods such as the detection of late RT products, and anti-retrovirals weren't used to control for the mere adsorption of virus on tissue blocks.

Aside from their culture methods, the models described above differ by the way in which HIV-1 infection is detected. A productive infection in the immersion models, whether they involve explants containing epithelial and subepithelial tissues or epithelial sheets,[51] relies only on the infection and viral amplification by the cells which populate the explants. In the polarized models, HIV-1 infection does not rely on viral amplification by explant cells, but rather on the transfer of virus to indicator cell lines, a process that certainly explains why some authors have observed that frozen tissue explants performed as well as fresh tissues in transmitting HIV to indicator cell lines.[52] The group of C. Dezzutti[37] modified the method of culture in the transwell chamber and used explants not only as transmitters of HIV-1 but also as sites of viral replication. In this implementation, explants are cut with dermal biopsy punches (5-mm diameter) and inserted, with the epithelium oriented upward, into a smaller-diameter

hole cut into the transwell membrane insert. The epithelial surface of the explant is sealed with 2% agarose to maintain the tissue orientation, and in the basolateral chamber the stroma is cultured in DMEM supplemented with 10% human AB serum. The advantage of this model is that viral replication is evaluated directly without use of the indicators cells. However, activation with PHA and IL-2 was required to obtain reliable viral replication. This requirement is an impediment to the identification of HIV target cells as cellular activators modify the cell surface makeup of infected and non-infected cells.

Hiller et al.[5] developed a new model of tissue polarization to study the permeability of cervico-vaginal tissue. This model is based on the use of a Franz cell[5], in which the seal does not depend on agarose but rather on a mechanical seal assured by a rubber O ring and a pinch clamp. In this model, Hiller et al. established that the permeability of the explants was decreased from the ectocervix to the endocervix, reflecting the thickness and nature of the epithelia, and confirmed that fresh and frozen tissue had equivalent permeability properties. This model was also used to evaluate cervical permeability to a new galenic formulation of PSC-RANTES,[53] and, together with the polarized explants, was used to study the penetration and anti-HIV activity of a gel formulation of tenofovir.[54]

In spite of their important advantages, none of the studies utilizing either tissue polarization or immersion/raft protocols addressed the nature of the infected cells. Analysis of these cells requires polychromatic flow cytometry in the absence of cell activation. In an attempt to improve these models, we have developed an explant culture model built on the collagen sponge raft culture system (Fig. 1). In this model, small blocks of cervical tissue mucosa are infected by immersion in viral stock for 2 hr, washed extensively, and cultured on top of collagen sponge gel in RPMI supplemented with 10% FCS in the absence of cellular activation. To account for tissue variation due to sampling, at least 16 explants are used per experimental condition. Donor-matched explant cultures containing potent antiviral agents are used to control for viral adsorption and desorption during culture.

To apply the power of polychromatic flow cytometry to characterize the cells infected in explants, one needs to prepare a single-cell suspension of tissue cells. Although mechanical digestion has been used by other groups,[49] it is our experience that such a treatment results in poor cell recovery because of cell death during the processing. Instead, we have developed an enzymatic digestion protocol, which spares most of the cell surface markers studied (Fig. 1). This method of cell isolation, together with the explant culture method, are described in details

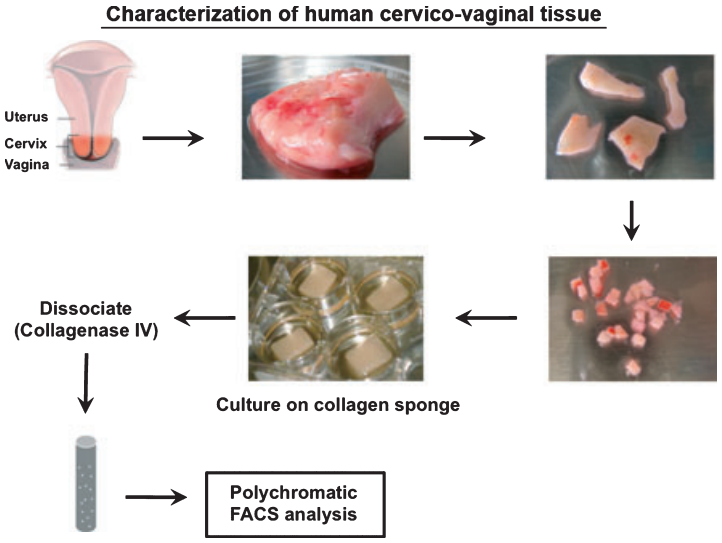

Fig. 1 Explants of cervico-vaginal tissue. Cervico-vaginal tissue is dissected into small blocks and cultured on top of collagen rafts at the air–medium interface. Under this protocol, cervico-vaginal explants support productive infection of HIV-1. Tissue blocks are dissociated into single cells that preserve their surface antigens and can be analyzed with polychromatic flow cytometry.

elsewhere.[17] Briefly, it is extremely important for this methodology to titer the lot of enzymes used for the enzymatic digestion of the tissues. It is advisable to perform this step on PBMCs or tonsillar tissue, for which mechanical cell release is efficient and not traumatic, allowing a comparison of digested, undigested, and mechanically released cells. Unfortunately, this procedure should be performed for each lot of the crude collagenase IV preparations, which are contaminated with neutral proteases to various degrees. However, this step can be skipped if the highly purified proteases, Liberases (Roche, Indianapolis, IN, USA), which are collagenases I and II spiked with purified neutral proteases, are used to digest the tissues. Because of their quasi lot-to-lot uniformity, these enzyme mixtures do not need to be titered. In our hands, digestion of cervical explants with Liberase DL (Collagenase I and II spiked with low amounts of dispase) solutions in the vicinity of 15 μg/mL is optimal (J.C. Grivel, unpublished data). The inclusion of FCS in the digestion medium seems to protect certain cell surface markers such as CD8 from excessive digestion (J.C. Grivel, unpublished data).

The first application of this new method of cervical explant culture and analysis was reported by Saba et al.[39] This report established that the majority of cervical T cells are effector memory cells. Moreover, as *in vivo*, in spite of the broad expression of CXC chemokine receptor 4 (CXCR4), explants supported R5 rather than X4 HIV-1 replication. Because this model does not involve the use of cellular activators, Saba et al. were able to characterize cells in the infected explants: R5 HIV-1 infection occurred preferentially in activated CD38+ CD4 T cells and was followed by activation of bystander CD4 T cells.

Although each of these models has its intrinsic limitations, they have all proven to be valuable tools for studying several aspects of HIV-1 infection in the lower female genital tract. These models[37,40,47] have been widely used as platforms to test potential microbicides[37,53–60] and viral fitness.[61] Obviously, none of these models fully reproduces the complex phenomenon of HIV-1 transmission, and a compound that is efficient in an *ex vivo* system may fail in a future clinical trial. However, if a microbicide candidate does not demonstrate its efficiency in an *ex vivo* tissue model, it is probably not worth developing further.

Cervico-vaginal explant models suffer from the limitations inherent in any explant models: the tissue is disconnected from the body and its supply of cells, especially of immune cells, which may be recruited to the genital tract upon infection by HIV (or other pathogens) and fuel the initial infection *in vivo*. However, this model is fully adequate for the study and characterization of the very early steps of the infection.

Cervico-vaginal explant model: coinfection and cytokines

A cervico-vaginal explant model allows the study, under controlled laboratory conditions, of another important aspect of HIV transmission, namely HIV genital copathogens and their interactions with HIV-1. This is an important issue, as genital copathogens, by triggering the host immune response, can greatly affect the probability of HIV-1 transmission/acquisition.[62] Interactions of HIV-1 with other microbes in tissues in general, and in the female genital tract in particular, are largely mediated by cytokines. Indeed, a complex network of cytokines regulates and links the innate and adaptive immune responses to human pathogens. Tissues infected with different microbes change their cytokine spectra, upregulating or downregulating some of them. In tissues infected with several microbes, modulation of the production of a specific cytokine may result in the facilitation or, in some cases, in the suppression of the replication of another microbe. This is of particular interest if one of the coinfecting microbes is HIV-1.[63–66] Besides cytokine-mediated interactions, copathogens may affect sexual transmission of HIV-1 by the recruitment of HIV-1 target cells or by direct interaction with HIV-1.[62]

All these types of inter-microbial interactions were identified in studies with various *in vitro* models. In particular, studies on vaginal punch-biopsy explants demonstrated that *Neisseria gonorrhea* and *Candida albicans*, two common genital copathogens, are potent inducers of TNF-α.[62] This cytokine increases HIV-1 transcription via the NF-kB pathway in infected T cells and macrophages.[67] Therefore, TNF-α upregulation may amplify HIV-1 genital shedding in *Neisseria*- and/or *Candida*-coinfected individuals, facilitating the transmission of HIV-1 infection. Furthermore, the TNF-α-mediated activation of Langerhans cells allows HIV-1 productive infection in these cells, contributing to the increased susceptibility to HIV-1 of individuals infected with *Neisseria gonorrhea* and/or *Candida albicans*.[68]

Another common microbial disease, which may, via cytokines, affect HIV-1 in coinfected individuals

is bacterial vaginosis, the most common vaginal infectious condition. Bacterial vaginosis has been reported to be associated with increased IL-8 expression and enhanced HIV-1 genital shedding.[69] These two reported effects of bacterial vaginosis may be related to each other: studies in cervico-vaginal explants showed that IL-8 affects HIV-1 replication and that the pattern of this effect depends on the timing of the IL-8 exposure and the explant culture system adopted. Although the replication of HIV-1 was increased at 24 hr post-infection in cervical punch biopsies pretreated with IL-8,[70] this cytokine reduced HIV-1 transcription in ectocervical tissue explants after 5 days of culture.[71]

Besides bacterial and fungal copathogens, several viruses also target the male and female genital tracts. Among them, HSV-2 is a common genital copathogen that establishes with HIV-1 a vicious circle in which each virus facilitates replication, shedding, and acquisition of the other.[72] The intimate mechanisms of interaction of HSV-2 and HIV-1 remain to be studied, and obviously such studies require an adequate experimental model. In our opinion, cervico-vaginal tissues *ex vivo* can serve as such a model. Recent data on drug-mediated HSV-2/HIV-1 interactions, in particular, on the anti-HIV-1 activity of the anti-herpetic drug acyclovir[30,73] and on the surprising protective effect of a tenofovir-based anti-HIV-1 microbicide gel on HSV-2 acquisition,[74] further emphasize the need for a cervico-vaginal explant model to investigate interactions between HSV-2 and HIV-1 in coinfected tissues.

Human cytomegalovirus is another common HIV-1 viral copathogen. HIV-1/HCMV-coinfected individuals frequently shed HCMV in semen and in cervico-vaginal secretions along with HIV-1.[75,76] An adequate investigation of interactions of HCMV with HIV-1 in the context of human tissues also requires an explant model. A study of HIV/HCMV coinfection in a human cervical explant model found that HIV infection enhances HCMV replication, possibly by altering cytokine production.[77] Similarly, in tonsillar tissues HIV-1 increased HCMV production, probably by inducing the activation of cell targets.[29]

Cervico-vaginal explant models not only allow the study of the basic mechanisms of HIV-1 transmission and pathogenesis as well as the role of other microbes in these processes, but can also be used to test various antimicrobials in pre-clinical tests. Also, they allow the study of natural antimicrobial factors that, in female genital tract secretions, inhibit bacteria, fungi, and viruses. Indeed, studies in polarized epithelial explants of human fallopian tubes, uterus, vagina, and cervix have shown that epithelial cells secrete natural microbicides capable of suppressing different sexually transmitted pathogens including HIV-1 but not commensal microbes like *Lactobacillus*.[78] In particular, these studies suggest that the constitutive production of several cytokines may inhibit HIV-1 infection by preventing viral entry, inhibiting NF-kB activation, or inactivating HIV-1 virions by other yet undefined mechanisms.[78,79] Importantly, in contrast with HIV-1 and other genital co-pathogens, commensal bacteria (*Lactobacillus*) are either adapted or naturally resistant to such innate antimicrobial responses.[78] Thus, mimicking of these compounds may lead to the development of a drug that inhibits HIV-1 without changing the vaginal commensal flora.

Direct interaction of HIV-1 and coinfecting sexual pathogens can also affect their transmission and the course of diseases: HIV-1 Tat not only increases the expression of HPV-16 E6 and E7 oncogenes in human keratinocytes, but also enhances the proliferative capacity of these cells *in vitro*.[80] Similarly, HSV-encoded protein [infected cell protein (ICP)-O, ICP4, ICP-27, and US11] can increase HIV-1 replication by trans-activating HIV-1 LTR.[81–83]

If HIV-1 penetrates the natural defense barriers and establishes infection, its replication is capable of reprogramming the cytokines production of the genital tract for its own benefit. Experiments on ectocervical and endometrial human tissue explants have revealed that HIV-1 infection upregulates IL-6, which promotes HIV-1 replication by transcriptional and post-transcriptional mechanisms.[84,85] Moreover, virus-triggered modulation of cytokine production (i.e., of IL-6, IL-8, GRO-α, TNF-α) can lead to activation of HIV-1 target cells, thus enhancing their susceptibility to productive HIV-1 infection.[68,77] This phenomenon too can now be studied under controlled laboratory conditions in cervico-vaginal explants: it was recently reported that in this system, HIV-1 activates uninfected (bystander) cells, thus supplying new potential cell targets for itself.[39]

In conclusion, human cervico-vaginal tissue models have revealed new mechanisms of interaction between HIV-1 and genital copathogens. The use of this and similar tissue models will allow the decipherment of novel key aspects of HIV-1 transmission and acquisition.

Perspectives

In spite of the fact that important results have already been obtained with the use of cervico-vaginal tissues *ex vivo*, the use of these models promises new important data in the future. Here, we mention only a few of them.

Microbicides

As the use of topical microbicide has recently proved its clinical efficacy,[74,86] the system of cervico-vaginal tissue can now be used for preclinical screening and development of new potential microbicides and other anti-viral components. The advantage of the tissue explant system is that, under controlled laboratory conditions, it can provide information regarding different aspects and consequences of microbicide-tissue interactions: the efficacy of the drug against HIV-1 variants of different clades, its toxicity profile, whether it changes cell contacts, how it affects viral evolution, etc. Also, using polychromatic flow cytometry and a multiplex cytokine assay, we can evaluate the risk of inflammation, anticipate the mucosal response to the drug, and avoid further testing of compounds that may be potentially harmful. It is conceivable that the increased risk of HIV transmission caused by cellulose sulfate and nonoxynol-9 which was revealed in clinical trials[87] would have been revealed in the tissue model prior to such trials.[88] In general, the model of cervico-vaginal explants is now considered to be among the standard test-models for potential microbicides.[88,89]

Early Virus

In order to develop an effective HIV-1 prevention measure, it is necessary to understand whether the particular HIV-1 variants that transmit infection have particular characteristics that distinguish them from the bulk of HIV-1 variants. Recently, HIV-1 variants present in the very early stages of the infection have been isolated.[90] These variants were identified by means of single genome amplification, and phylogenetic analysis coupled with mathematical modeling showed that, in 80% of cases, these early viruses coalesce to one common ancestor (the transmitted/founder variant, T/F).[90,91] It has been suggested that the HIV-1 variants present in semen pass through an extreme genetic bottleneck that selects a particular T/F variant. The phenomenon of the selection of particular HIV-1 variants has been known since the early studies of the epidemic, when it became clear that CXCR4-utilizing HIV-1 are not capable of transmitting infection, whereas CCR5-utilizing HIV-1 are. It is probable that, at least in heterosexual transmission, the mucosal barrier imposes a selective pressure in favor of those transmitted viruses.[92] The recent discovery that among R5 viruses there is also a genetic bottleneck, which results in the establishment of the infection by one or a few variants, raises the question of the properties of the transmitted viruses. This is why cervico-vaginal explants may represent an adequate model to study the early events in HIV infection and reveal such properties, if they exist. In general, in this model it will be possible to investigate whether the T/F viruses differ by their ability to infect cervico-vaginal tissue *ex vivo* from those that are present in semen but are not transmitted or from the viruses present in the chronic phase of HIV-1 disease. The results of these experiments will have important implications for mucosal immunization and microbicide strategies.

Non-HIV Pathogens

The system of cervico-vaginal tissue *ex vivo* can be used to study non-HIV pathogens as well. Earlier tonsillar explants have been used to study tissue pathogenesis of various microbes. For those of them that are transmitted sexually, cervico-vaginal explants may be a more adequate model. Their contribution to HIV-1 infection can be studied in this model as well. Indeed, several ulcerative and non-ulcerative sexually transmitted diseases, including syphilis, Candidiasis, HSV-2, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and HPV, have been implicated as risk factors for HIV transmission/acquisition.[93] Also, in HIV-infected individuals progressive loss of CD4 T cells is associated with an increased prevalence of HPV.[94]

We think that the *ex vivo* cervico-vaginal model will help us to decipher mechanisms by which coinfecting microbes increase the probability of HIV-1 transmission/acquisition and by which HIV affects copathogens. Recently, several microbes have been identified that suppress HIV-1 infection *ex vivo* and *in vivo*, rather than enhance it.[62,63] Understanding the mechanisms of their effects on HIV and mimicking these effects may help in the development of new anti-HIV-strategies.

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