Microbicides: still a long road to success

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The development of efficient microbicides, the topically applied compounds that protect uninfected individuals from acquiring HIV-1, is a promising strategy to contain HIV-1 epidemics. Such microbicides should of course possess anti-HIV-1 activity, but they should also act against other genital pathogens, which facilitate HIV-1 transmission. The new trend in microbicide strategy is to use drugs currently used in HIV-1 therapy. The success of this strategy is mixed so far and is impaired by our limited knowledge of the basic mechanisms of HIV-1 transmission as well as by the inadequacy of the systems in which microbicides are tested in preclinical studies.

Microbicides: definition and sites of action

To date, the majority of newly HIV-1-infected individuals worldwide acquire HIV-1 through sexual transmission when the virus crosses mucosal surfaces. Therefore, an understanding of the mechanisms of HIV-1 mucosal transmission is crucial to the development of means to prevent it. Recently, the diminution of the HIV-1 load of an infected partner to levels at which transmission becomes inefficient was demonstrated to be a successful preventive strategy [1]. However, this strategy leaves prevention measures in the hands of the infected donor, while it is necessary to give power also to the uninfected partners, especially to women. Microbicides as well as oral pre-exposure prophylaxis (PrEP) and preventive vaccines, if developed, provide such power.

Microbicides are compounds that are applied topically aiming at protecting individuals from acquiring pathogenic microbes, in particular HIV-1. Despite the semantics of a word that includes 'cide' (the suffix originates from Latin *caedere* 'to kill'), the above definition of microbicide does not require actual 'killing' of a microbe but includes compounds that may act through other mechanisms, e.g., by blocking viral entry or suppressing initial steps of the viral reproductive cycle (Figure 1).

Mucosal sites crucial for HIV-1 transmission to which microbicides should be applied are cervicovaginal, penile, and rectal mucosa. Here, we predominantly limit ourselves to the discussion of microbicides that aim at preventing male-to-female HIV-1 transmission via the female genital tract. However, rectal microbicides should also remain a main focus of interest as unprotected receptive anal sex, which is practiced by both men and women, is associated with the highest probability of sexual HIV-1 transmission [2,3].

HIV-1 transmission through cervicovaginal mucosa

HIV-1 male-to-female cervicovaginal transmission is a complex phenomenon, and despite many efforts its basic mechanisms are still poorly understood. It is believed that the cervicovaginal mucosa constitutes a strong natural barrier against HIV-1 and other pathogens [4]. Although HIV-1 may enter through transcytosis (better studied in the gut) [5] or be carried through the mucosa by epithelial Langerhans cells [6], experimental data suggest that HIV-1 penetrates the female lower genital tract epithelial layer inefficiently unless the tract is damaged by lesions of various natures (Figure 1) [7]. Unfortunately, lesions in the female genital tract are common and some of them can result from sexual intercourse [8].

Furthermore, the vulnerability of the lower female genital tract to HIV-1 is heterogeneous in space and time: whereas the vagina and ectocervix, the forefront barriers against the virus, are composed of multiple layers of stratified squamous epithelium, the endocervix is composed of a single epithelial monolayer [9]. It is believed that the transition area between the ecto- and endocervix is one of the most common sites for HIV-1 transmission (see [10]). Moreover, in different phases of the menstrual cycle the thickness of the epithelium varies. Elevated levels of progesterone during the luteal phase lead to thinning of epithelia, increasing organ susceptibility to HIV-1 [11].

Also, various genital pathogens, including bacteria and herpes simplex virus type 2 (HSV-2), cause inflammation and facilitate infection by thinning and disrupting the multilayered lining, recruiting a pool of target cells for local HIV-1 expansion and interfering with innate antimicrobial activity (see [12]). Therefore, the ideal microbicide should not only be active against HIV-1 but also against HIV-1 copathogens, most importantly HSV-2. A better understanding of the initial steps of HIV-1 mucosal infection and the role of other genital pathogens in HIV-1 transmission will help to identify when HIV-1 is most vulnerable to potential microbicides as it enters the host [13].

Microbicides: past failures and a current success

Microbicide development began more than 20 years ago with the intention of developing a spermicidal vaginal gel active against sexually transmitted infections, including HIV-1. Since then, microbicide compounds have been formulated not only as gels but also as creams, vaginal rings, tablets, foams, films, and suppositories [14].

Early microbicides were based on nonspecific surfactants, polyanions, and vaginal milieu protectors. The history
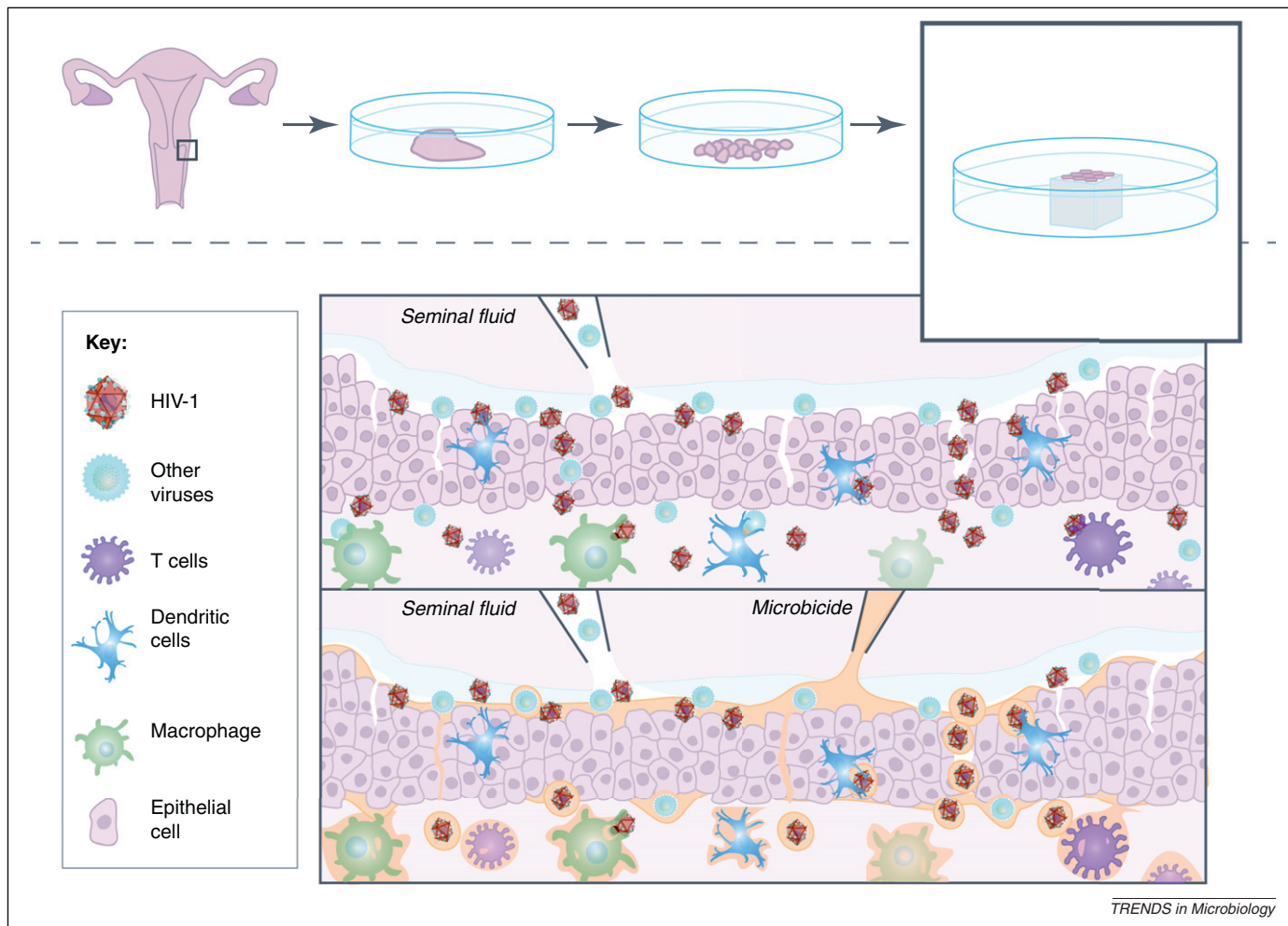
Figure 1. Use of human cervicovaginal tissue *ex vivo* as a microbicide testing platform. Upper panel: human tissue explants cultured *ex vivo* serve as a model for HIV-1 transmission. Briefly, human cervicovaginal tissues obtained from surgery are dissected into tissue blocks, which are cultured at the liquid–air interface. Transmissions of HIV-1 and HIV-1 copathogens are simulated by applying viral suspensions in seminal fluid. This model simulates some of the *in vivo* mechanisms by which HIV-1 penetrates cervicovaginal mucosa and infects cell targets. Lower panel: a human cervicovaginal tissue system complemented with seminal fluid is used as a platform to test microbicides. Microbicides may prevent HIV-1 transmission by inactivating pathogens, preventing viral entry, and suppressing HIV-1 infection of target cells.

of early surfactant-based microbicides was stained with disappointments, as nonoxynol-9 (N9), successfully tested *in vitro* [15] and in animals [16], increased HIV-1 suscepti-bility in the Phase III clinical trial [17]. Also, a trend towards an increase of infection rates among participants was ob-served in a clinical trial of another surfactant, Savvy (C31G) [18,19]. Two polyanion-based microbicides (VivaGel[TM] and dextrin sulfate) are currently in Phase I/Phase II clinical trials [20–22]. Other polyanion-based microbicides (PRO2000, cellulose sulfate, carraguard), which prevent HIV-1 from binding to and entering target cells, although active *in vitro* [23,24], repeatedly and disappointingly failed to prevent HIV-1 acquisition in the Phase III trials [25–28].

Thus, the problems with these microbicides were not identified in preclinical studies, indicating that the systems in which they were tested do not faithfully reflect important aspects of the *in vivo* situation. For example, although nontoxic, some of these compounds may subtly disrupt cell–cell contacts in the epithelial layer, allowing the virus to go through [29]. Such effects could hardly be observed in single cell cultures, which poorly reflect *in vivo* cell–cell interactions [30]. The problem of tissue damage becomes even more important in the case of rectal microbicides, as in

contrast to the vagina, the rectal mucosa consists of a single layer columnar epithelium.

The failure of the first generations of microbicides in clinical trials indicates that compounds that do not damage tissues should be used to prevent HIV-1 transmission.

Therefore, instead of nonspecific compounds, highly potent and specific anti-HIV-1 antivirals such as nucleo-side reverse transcriptase inhibitors (NRTIs), as well as inhibitors of HIV-1 integrase and protease that are cur-rently successfully used in therapy, have recently been proposed as microbicides [31,32]. In particular, the NRTI tenofovir formulated as a 1% gel was tested in CAPRISA 004, a double-blinded randomized controlled clinical trial for prevention of HIV-1 acquisition in South African wom-en [33]. In CAPRISA 004, 1% tenofovir gel reduced HIV-1 acquisition by an estimated 39% overall, and by 54% in women who used the gel 80% or more of the time [33].

PrEP: topical or oral?

The CAPRISA 004 findings raised enthusiasm in the sci-entific community, as they came as a proof-of-concept for the feasibility of preventing HIV-1 acquisition with an antiviral formulation delivered topically [33]. However,