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Animal Models for Microbicide Safety and Efficacy Testing

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Abstract

Purpose of review—The first several human clinical trials for HIV prevention resulted in failure, sometimes with disastrous results, as both vaccines and microbicides have occasionally demonstrated the potential to increase rates of HIV infections in some recipients. Recently however, both vaccines and microbicides have finally achieved some level of success in phase 3 human trials, demonstrating that protection from HIV-1 infection is at least possible.

Recent findings—Recent studies have shown that topically applied vaginal gels, and oral pre-exposure prophylaxis (PrEP) using single or combination antiretrovirals are indeed effective in preventing sexual HIV transmission in humans. Both the PrEP and topical efficacy results were predicted by nonhuman primate models, and several ongoing studies demonstrate both humanized mouse and NHP models of microbicide efficacy may reliably predict the success or failure of microbicide candidates in humans.

Summary—Now that we finally have positive correlations with prevention strategies and protection from HIV transmission, we can retrospectively validate animal models for their ability to predict these results, and hopefully use these models to better predict microbicide safety and efficacy in the future. Here we discuss the utility and relevance of animal models for safely and efficacy screening of microbicide candidates for advancing only the safest and most effective products to future human trials.

Keywords

Microbicide; nonhuman primate; mouse; humanized mice; vaginal transmission; mucosal transmission

Introduction

Advances in understanding the female reproductive tract, the immunology and pathogenesis of HIV, and the refinement of animal models are finally converging to provide a safe and effective path to topical microbicide development. Although we have made some success with antiretroviral (ART) pre-exposure prophylaxis (PrEP), we are a long way from licensing an inexpensive, topical microbicide that will actually have an impact on HIV transmission in endemic countries. Continued research in determining the early immune responses in human mucosal tissues responsible for HIV transmission is essential to this development, as topical applications of substances previously tested for safety *in vitro*, and sometimes even FDA-approved for use in mucosal tissues, resulted in mucosal irritation and recruitment of target cells after repeated use, and actually increased rates of HIV-1 transmission.

We now know that the early, innate immune response, characterized by a cytokine storm and recruitment of additional CD4⁺ T cells and antigen presenting cells, actually fuels early HIV replication in mucosal tissues [1], and HIV infection may even depend on this inflammatory response to complete transmission. Thus, infection-specific, and non-specific

inflammation, whether induced by a concurrent sexually transmitted disease (STD), an irritating topical microbicide or compound, or HIV antigens or virions themselves from prior or repeated exposures, may all facilitate, rather than prevent HIV transmission. This has been demonstrated in both microbicide [2,3] and vaccine studies [4,5]. This complex interaction of host defense and recruitment of target cells simply cannot be mimicked in *in vitro* or computer models, mandating the continued, and preferably, expanded use of relevant animal models for preclinical safety and efficacy screening of microbicides.

Comprehensive, recent reviews of current animal models for HIV/AIDS research [6,7] and of nonhuman primate (NHP) models for HIV infection for antiviral strategies [8] have been published. Detailed descriptions of the different types, and utility of transgenic and humanized mice [6,9], and differences in NHP host species [6-8], as well as the availability of new, mucosally relevant viral challenge stocks are also discussed in these reviews [6-8]. Therefore, we will focus this review primarily on the utility, relevance, and appropriate use of specific animal models for vaginal microbicide testing, which has evolved in parallel with our expanding knowledge of the early cellular and molecular events involved in vaginal HIV-1 transmission, which were in fact, mostly discovered in NHP models. Although rectal transmission is the focus of several ongoing microbicide investigations in macaques [10-12], much less is known regarding the early events involved in rectal transmission, partly due to technical limitations of studying the gut, so we focus here on what is known regarding the early events involved in vaginal transmission. However, studies that have compared intestinal and vaginal immune cells and responses in humans [13] and macaques [14] suggest these mucosal tissues have at least some parallels, so we may extrapolate the same mucosal responses to rectal transmission. We also discuss how specific differences in vaginal anatomy, physiology, and immunology may differ between animal models, and should be considered in the rational design of a standardized screening protocol for topical microbicides, *prior to* advancement of compounds to human clinical trials.

The critical need for animal testing of topical HIV microbicide candidates

Perhaps no field of research more than the HIV microbicide field has demonstrated such a valid case for continued, and even expanded preclinical testing of microbicide candidates in relevant animal models. A series of failed human clinical trials taught us that thoroughly examining the safety of compounds repeatedly applied to the vagina, and using a relevant animal model that faithfully mimics the human female reproductive tract (FRT) is necessary for selecting candidates for future trials. Despite showing safety and efficacy in a series of *in vitro* assays, the first few topical microbicide trials in humans failed, and two of them with disastrous consequences. Two candidates (nonoxynol-9 and cellulose sulfate gels) resulted in more HIV infections in the treatment groups compared to placebos [3]. Both compounds had failed to show toxicity in cell or tissue cultures, and N-9 was even tested in monkeys, which demonstrated efficacy in preventing against a single vaginal challenge. In hindsight, it became evident that substances that attack HIV envelope lipid membranes also affect mucosal epithelial cells, since the HIV lipid envelope is derived from host cells. Thus, repeated use of detergents/surfactants (like N-9), or polyanions that damage cells, lipid bilayers, or that alter the mucosal environment can result in irritation, local recruitment of viral target cells, and increased susceptibility to HIV transmission *in vivo*, which could not have been predicted in cell or tissue culture models. However this irritation could have been predicted, and subsequently has been demonstrated, in appropriately designed NHP experiments [8].

In vitro systems lack the intact mucosal barrier system of a living animal model. The intact mucosa is comprised of several layers of epithelial cells, coated by a variably thick layer of mucus, containing antimicrobials and antibodies that may affect HIV-1 transmission rates

[15]. Further, the epithelial cells and resident dendritic cells co-express a variety of receptors that trigger rapid immune responses from the underlying resident immunoregulatory cells, which then initiate dynamic cell to cell interactions resulting in a local cytokine storm, which elicits and recruits additional target cells from the bloodstream (Figure 1)[1]. Thus, *in vitro* models simply lack the potential for detecting inflammation elicited by compounds or infection. The complicated interactions of HIV with cervicovaginal mucus, vaginal epithelial integrity/TLR receptor expression, resident T, NK, B cell and dendritic cells, and recruited cells in inflammation after repeated exposure to candidate microbicides make it even more critical that we utilize an animal model that recapitulates all of these events as closely as possible, which makes the NHP model of vaginal transmission still the best model for vaginal microbicide testing (see below).

In summary, the vast and complex interactions of HIV with the multiple layers of the mucosal immune system cannot be mimicked in any meaningful way in any cell or tissue culture system in existence at this time. Similarly, these interactions cannot be reproduced in any relevant way by computer models or simulators, mainly because we simply lack the relevant data to plug into the transmission equations. For example, both vaginal and rectal HIV transmission is apparently highly inefficient, with a transmission rate estimated to be less than 1 in 900 vaginal HIV exposures [16]. Since we do not know the reason(s) why mucosal transmission is so inefficient, and since relevant human samples simply cannot be obtained at the precise times needed to track successful HIV exposure/transmission, we may never prove the mucosal mechanisms involved in natural human HIV transmission. Thus, relevant animal models for addressing specific questions of HIV-1 transmission and vaginal susceptibility are essential for deciphering these early events, which are undoubtedly critical for the development of an effective microbicide or vaccine.

Mice, sheep, goats, horses, cats, etc., all have their own natural lentivirus infections that predominantly infect macrophages (a characteristic of lentiviruses) but none of these have the specific cell tropism to directly infect, and replicate in mucosal CD4+CCR5+ T cells, which is an early, major feature of HIV infection in humans and SIV infection in susceptible nonhuman primate hosts. Further, we now know HIV and SIV infections occurs as the result of infection with a single transmitted founder virus (TFV) which invariably use CD4 and CCR5 for attachment and entry of host cells, and infected macrophages are not detected until weeks after viral exposure [17-19] Thus, optimally models for HIV transmission should use TFV or at least R5 tropic HIV-1 or SIV/SHIVs for mucosal challenges. However, CD4 and CCR5 receptors in primates differ from those of lower animals, and only human and NHP cells are susceptible to infection with HIV-1.

We now know that differences in host restriction factors such as TRIM5 α that interferes with capsid uncoating, APOBEC3G that interferes with replication (which is inhibited by vif) differ among primate species (reviewed in [6]) and even between individuals within species, likely due to co-evolution of primates with lentiviruses for millenia. There is evidence in prosimians and chimpanzees that SIV has been evolving in African primates for millions of years [20,21]. Humans have lost the inherent ability to combat this particular CD4+CCR5+ T cell tropic retrovirus (HIV-1) apparently due to an ancient infection with a related, yet different retrovirus. Primates, including humans, have co-evolved with lentiviruses for millennia, and the battle between this particularly lethal strain of T cell tropic virus, and its various extant nonhuman primate hosts, has likely changed the evolution of both virus and host in a constant struggle to co-exist for millions of years [22]. Unfortunately for us, this particular T cell tropic SIV/HIV recombination was a relative newcomer to the human race, and possible diversions the human immune system made to compensate to archaic retroviruses have left us vulnerable to HIV-1, which if untreated, it essentially devastates the human immune system, starting with the mucosal immune system.

The same regulatory CD4⁺ T cells that govern essentially all adaptive immune responses lie in mucosal tissues, and serve as “fuel” for HIV transmission, amplification, and persistence in susceptible, naïve human hosts. Very closely related strains of CCR5 tropic SIV, which are endemic in African monkeys, induce an almost identical CD4⁺CCR5⁺ T cell depletion and AIDS in Asian macaques, as they, like us, are defenseless and naïve hosts for these CD4/CCR5 tropic lentiviruses.

Due to remarkably close similarities in CD4 and CCR5 receptors on T cells from primates, most monkey CD4⁺CCR5⁺ T cells can be infected with HIV-1; however HIV-1 does not successfully replicate in monkey cells, due to differences in host restriction factors which prevent downstream steps in viral replication (reviewed in [6]). Therefore, to mimic HIV-1 infection of mucosal surfaces, and for proper testing of microbicides to prevent vaginal and rectal transmission, we must either use genetically engineered, immunodeficient “humanized” mouse containing primary human fetal lymphoid tissues, which can be challenged with relevant HIV-1 strains, or else NHP models challenged with SIV or SHIVs. Fortunately, we now have a plethora of genetically engineered SHIVs that mimic the envelope proteins of HIV-1 TFH, or the different clades of HIV. Since the envelope can be manipulated to express relevant human env proteins, while still maintaining a replication competent SIV backbone, these SHIVs have added a new dimension of relevance to NHP models for mucosal prevention studies.

Humanized mouse models

In the last few years, there have been marked advances in the development of humanized murine models for HIV-1 research [9]. The most frequently used and relevant models for HIV involve immunodeficient mice transplanted with human fetal cells and tissues to create “humanized” mice that are susceptible to HIV-1 infection. The development and current availability of the different transgenic/humanized mouse models has recently been reviewed in detail [6]. Basically, the most promising models used lack the ability to generate T and B cells (scid-hu), and/or non-obese diabetic (NOD) mice crossed with scid-hu mice, conferring additional defects that render NK cells dysfunctional, and/or further genetic mutations (Il2rg, Rag2, etc.) that render the murine immune system even more defective. These mice are then surgically implanted with human fetal tissue transplants in an attempt to repopulating the murine immune system with human cells. For example, the “BLT mouse” consists of a NOD/scid mouse transplanted with human fetal bone marrow, liver, and thymus (BLT) tissue [23]. This model is particularly encouraging for microbicide testing, as it is one of the few murine models in which engrafted human CD4⁺ T cells appear to repopulate the mucosal tissues [24,25].

The advantage of such genetically altered and physically manipulated mice is obvious; we can use them to test actual HIV-1 strains, including TFH, and also, the virus is clearly infecting human cells, and not murine cells, thus removing any doubt regarding the relevance of the challenge virus or infected cells. Further, since the BLT model has human mucosal target cells, it is generally considered more relevant for mucosal challenge models, and for microbicide efficacy screening [26,27]. Thus, there are distinct advantages of using rodent models, especially for preliminary efficacy screening, as they are less expensive than NHP models, and they utilize HIV-1 and human cells for transmission. However, and particularly for microbicide testing, it is concerning that we are discarding key regulatory roles of innate immune responses, including interactions between vaginal epithelial cells and infiltrating human lymphocytes, the lack of a bacterial or vaginal flora to interact with, and the co-dependence of specific cytokines and other inflammatory mediators that are likely mismatched in this artificial model. Nonetheless, a number of studies are examining the

mucosal immune responses and effects of microbicides to validate the humanized mouse models [9,26].

Unlike normal mammals, genetically altered/immunodeficient mice must be kept in sterile environments, as their mucosal and systemic immune systems are not guided, nor dependent on the external microflora for normal development and homeostasis. We now know that the complex interplay between the mucosal microbiome and host mucosal immunity are tightly interwoven, and this may play a key role in the early events in HIV-1 transmission or early immune responses that facilitate transmission. Moreover, we also now know vaginal epithelial cells are major components of the earliest immune response to foreign antigens encountered in the vagina. Vaginal epithelial cells express numerous toll-like receptors (TLR) that recognize pathogen-associated molecular patterns on bacteria and viruses [15], and it is increasingly apparent that natural exposure to antigens including SIV/HIV result in rapid, marked, severe vaginal inflammation, possibly mediated through this pathway (Figures 1-2). The ensuing inflammation leads to a local cytokine storm which recruits additional target cells, which also become infected, thus, expanding the initial foci of infection, until the lesion reaches a critical mass, which seeds the systemic circulation, and the intestine, in which infection becomes irreversibly established [1,28,29]. An effective microbicide must thus contain infection to the site of exposure, before this critical mass is reached. However, testing microbicides in an animal model in which the interaction of host epithelia, cytokines/chemokines/and other innate immune factors are defective, or essentially deleted, remains suspect. Thus, the disadvantage of murine models is that their natural immune system is so dramatically altered that it may complicate the early inflammatory events currently thought to be important for HIV transmission. Although the early events in HIV transmission are being pursued in NHP models, it is not clear whether vaginal infection can even be accomplished without this pre-existing inflammation. Finally, there are major differences between the rodent and primate female reproductive tract including the absence of menstrual cycles in rodents (rodents have estrous cycles), which may be associated with differences in vaginal transmissibility. Nonetheless, the development of humanized mouse models has accelerated the pace of microbicide screening, but the interactions of HIV and mucosal innate immune responses needs to be further explored in this model.

Nonhuman primate models

Studies of the early events in SIV/SHIV transmission show that *in vivo*, TFV infect CD4+CCR5+ T cells in the vaginal mucosa, resulting in a cytokine storm [29], marked recruitment of additional CD4+ lymphocytes, resulting in the establishment of an expanding nidus of infection (Figures 1-2) that finally spills over to the circulation and intestinal tract, in which most of the hosts CD4+CCR5+ T cells reside [1,30]. Intestinal infection results in both massive somatic CD4 depletion 11-21 days after infection (depending on route and viral virulence) and importantly, an exponential burst of viral replication (peak viremia). This viral amplification results in the production and dissemination of numerous viral mutants already resistant to the lagging mucosal and systemic immune response. Thus, infection must be stopped in mucosal tissues, before dissemination to the intestine. It is also increasingly clear that early inflammatory responses in the vagina and possibly rectum play a key role in establishing infection. Thus, it is important to use a model that shares similarities in the structure and function of the FRT, as well as one that recapitulates the early mucosal immune responses to viral exposure, as these are apparently fundamentally involved in transmission.

In addition, the human FRT is a very dynamic system, with dramatic changes occurring in the vaginal epithelium, mucus production, microflora, pH, and immune responses, which in women, are all highly regulated by hormonal fluctuations [15]. NHP have similar

reproductive cycles as women, including menstrual cycles, which may increase a woman's susceptibility to HIV transmission, and may prove to be the best predictor of microbicide safety and efficacy in women. The role of hormones and associated risk factors on vaginal HIV transmission rates have been debated for decades, but a recent study in Africa demonstrated nearly a 2-fold increase in HIV acquisition rates in women taking injectable progestin-based contraceptives (Depo-Provera)[31]. This confirmed our observations made years ago in the NHP model, demonstrating that progesterone markedly increases vaginal transmission rates of SIV and SHIV in macaques [32] most likely due to thinning of the vaginal and ectocervical epithelium [12,13]. Since thinning of the vaginal epithelium may result in higher rates of transmission and increased local inflammation, we have been using this progesterone model as a more stringent test for the safety and efficacy of a microbicide [33-37].

Hormone-induced vaginal epithelial thinning, which brings luminal antigens in closer proximity to underlying target cells in the vagina may partially account for this increased susceptibility to HIV infection. However, hormonal influences associated with menstrual cycles, pregnancy, menopause, etc., may also affect the production of antimicrobials and/or early mucosal inflammatory responses to exposure, which could influence HIV transmission rates [15]. In support of this, studies of rhesus [38] and pigtail [39] macaques have shown that macaques are more susceptible to vaginal SIV/SHIV transmission during the luteal phase of the menstrual cycle, which could also have profound implications for HIV transmission to women. Further, we have found that the vaginal epithelium of pigtail macaques is less keratinized, and more similar to that of women than rhesus macaques, which is likely why pigtails are more susceptible to multiple low dose vaginal transmission of SIV/SHIV [7,39].

Due to their close relation of NHP to humans, the similarity of the FRT, availability of tissues, and the ability to directly inoculate animals with specific pathogenic viruses, at precise times and doses of exposure, most of the major discoveries of vaginal and rectal HIV transmission, pathogenesis, immunology, etc. have been made in NHP models, sometimes decades in advance of confirmation in humans. For example, the recent success in finally obtaining U.S. Food and Drug Administration (FDA) approval for use of oral tenofovir disoproxil fumarate plus emtricitabine (TDF/FTC) as a pre-exposure prophylaxis (PrEP) among sexually active adults at risk for HIV infection was predicted in the NHP model over 15 years ago by Tsai and Black et al. [40,41]. Further, the success of topical tenofovir gels for prevention of vaginal HIV-1 transmission in humans [42] was first predicted in macaques [10,43]. Interestingly, now that human studies have finally confirmed the efficacy of oral tenofovir for prevention as PrEP [44], and tenofovir in topical gels [42], there has been a marked increase in animal testing of tenofovir to thoroughly validate the predictive nature of the models, and examine the tissue biodistribution (PK), and continued analysis of tenofovir safety and efficacy with repeated use [11,12,45-48]. Hopefully, now that successful human trials have finally been conducted, and the animal models have been fully validated, NHP models will be better utilized to *predict* rather than confirm human trials.

Despite the success with tenofovir in human trials, PrEP with potentially toxic antiretrovirals may not be the safest option for HIV prevention in uninfected populations. NHP models show that at least in higher doses, tenofovir has the potential for marked renal toxicity, which may not be detectable with routine blood tests [49]. In addition, in one cohort of patients on oral tenofovir disoproxil fumarate and emtricitabine, there were significant signs of toxicity reported in the study participants including nausea, vomiting, and other concerning side effects [50]. Moreover, topical tenofovir results in marked increases in RANTES and TNF- α , both known promoters of cell recruitment and inflammation [18]. The latter should be a concern for vaginal and rectal applications, and poses the question of

whether local tissue inflammation may occur in mucosal tissues with repeated dosing. For example, if repeated dosing results in inflammation, and subsequent doses are missed, could rates of HIV transmission increase in patients who are only partially compliant with topical antivirals? Finally, there is the potential for development of drug resistance with orally administered prophylactics, if suboptimal levels are achieved in circulation, and resistance mutations develop frequently in patients on tenofovir.

Thus, the quest for safer, topically applied microbicides should continue, as well as increased formulations with sustained delivery systems to mitigate issues of non-compliance. In macaques, we have shown that topically applied microbicide gels or vaginal rings result in minimal uptake of drugs into the systemic circulation [51,52], mitigating any possible effects of systemic toxicity or viral resistance. Further, fusion inhibitors prevent cellular infection, which may also mitigate the inflammatory response compared to a “microbicide” that acts post entry, and permits infection of CD4+CCR5+ T cells in tissues. Thus, our lab and others have focused primarily on testing topical viral “fusion inhibitors” in NHP models for prevention of HIV. Using the Depo-provera treated macaque model, we and others have shown that various fusion inhibitors including those that interfere either with gp120/CD4 binding [33,53,54] or CCR5 attachment [35-37,55,56] can completely prevent vaginal SHIV transmission. Not only does this prove that molecular fusion inhibitors, which prevent infection of cells, may be effective as topical microbicides, it also tells us that CD4 and CCR5 binding are both essential for successful vaginal HIV-1 transmission, providing further clues to the mechanisms involved in HIV transmission.

Conclusion

With respect to animal research, it has been said that “mice lie, and monkeys exaggerate”. However, animal models actually do neither, as animal experiments simply provide scientific information that are subject to human interpretation, and are limited by the experimental design, which is often based on preconceived opinions. For example, the protective effects of a candidate adenoviral vaccine tested in monkeys did not predict the failure of the same vaccine regimen in humans in the MERCK STEP trial. In hindsight we now know the viral challenge used in the NHP studies was a CXCR4 using SHIV, which does not reflect the transmission or pathogenesis of HIV or SIV. Subsequent studies using similar vaccines, yet challenged with pathogenic R5 tropic SIVmac showed the vaccines do not protect monkeys from infection either. Similarly, the protection of macaques treated with N-9 and challenged once did not predict the failure and increased rates of HIV transmission in humans who repeatedly used N-9. However, had the studies been designed the same way, the results in macaques would have been the same, as it has been subsequently shown that repeated mucosal N-9 applications in NHP also result in inflammation and tissue damage. Animal models are extremely useful for testing microbicide safety and efficacy, because unlike human trials, animals may be directly exposed to infectious doses of pathogenic viruses, resulting in very small numbers of animals required to significantly tackle key questions. Sometimes, human clinical trials “lie or exaggerate” as data from human clinical trials are often contradictory or unclear. For example, the Carraguard microbicide study, which did not demonstrate efficacy in protection, still taught us much about human compliance and reporting. This study used gel applicators which had a groove that could be tested for the presence of vaginal mucus, which revealed that only 42% of the empty applicators had actually been used, despite self-reporting from the women stating that they used the applicator 96% of the time. Moreover, although a study of oral tenofovir for PrEP in one African cohort of men and women showed significant protection from HIV infection [44], another study of oral tenofovir in a different African cohort failed to show protection, which the authors concluded was due to lack of compliance and/or adherence to the protocol [50]. Thus, compliance is a major issue

with microbicide testing in humans, which is largely why there are such intense efforts to develop sustained delivery systems, such as vaginal rings [51,57-62]. This may explain why animal studies can demonstrate almost complete protection with a microbicide, whereas rates of protection may be much lower in human trials.

Due to their similarities to humans, nonhuman primate models for microbicides and vaccines remain the best option for safety and efficacy testing. Although transgenic, humanized mice are certainly useful, and somewhat less expensive for efficacy screening, the vast differences in the immune responses, particularly in the FRT, combined with a T cell/macrophage trophic HIV that selectively targets primate immune systems, make such immunologically deficient mice questionable for predicting safety and continued efficacy.

Despite the success with tenofovir as PrEP, continued screening and testing for a safe, and inexpensive topical microbicide should continue in relevant animal models. There are too many microbicide candidates currently in clinical development for all of them to be tested in human trials. Therefore, standards or benchmarks need to be developed for advancing only the most promising candidates to clinical trials. Furthermore, with the tragic failure of nonoxynol-9 and cellulose sulfate, rational testing protocols and standard criteria for multiple dosing experiments should be developed for routine screening and selection of candidates. Unfortunately, there is no current consensus as to what kinds of testing a microbicide candidate must pass before such decisions are made. However, we now have at least partially effective compounds, and candidate compounds now have a bar to be compared against. In addition, compounds with greater efficacy, and less potential for toxicity should be pursued, such as fusion inhibitors, which do not permit infection of cells, and may potentially prevent some of the downstream inflammatory signaling associated with tenofovir, or antivirals that permit cellular infection. Now that animal models are being validated as predictive of success in human trials, we hope that expansion, refinement and standardization of animal model testing will accelerate the development and advancement of an effective microbicide that will eventually make a difference in stemming the tide of the HIV epidemic.

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Key points

1. Successful human trials of antiretrovirals administered as a topical mucosal gel, or as oral PrEP have shown efficacy in preventing HIV infections.
2. HIV-1 mucosal transmission may depend on inflammation, recruitment of target cells, and local expansion of the foci of infection for successful transmission, which cannot be duplicated in in vitro models.
3. Repeated use of compounds that elicit mucosal inflammation, or repeated exposure to SIV/HIV alone may elicit mucosal inflammation, which must be considered in the design and safety testing of microbicides.
4. Microbicide testing in humanized mouse models of HIV-1 infection, and nonhuman primate models of SIV/SHIV transmission, are predictive of microbicide efficacy in humans.

Figure 1.

Histology of the vagina of a rhesus macaque 48 hrs after vaginal SIV exposure (A) and 10 days after SIV infection (B). Note a small foci of inflammation in the interface between the epithelium and lamina propria is detected within 48 hrs of vaginal challenge (arrow). After 10 days of infection, there is marked, diffuse infiltration of lymphocytes and macrophages into the vaginal epithelium consistent with severe inflammation.

Figure 2.

Three color immunohistochemistry of the same animal in Fig 2B; infected for 10 days with SIVmac. Note marked infiltration of CD3+ T cells into the epithelium (green) and many are infected with SIV as shown by immunohistochemistry for p28 (gag – red). Macrophages (stained with mAb Ham56) are shown in blue. Note all infected cells at this stage are T cells, and no infected macrophages are detected, consistent with infection from a CD4+CCR5+ founder virus.