Understanding the Intersection of Young Age, Mucosal Injury, and HIV Susceptibility

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Abstract

Adolescent boys and girls are disproportionately affected in the current HIV epidemic. Numerous socio-behavioral studies have addressed the indirect drivers surrounding this vulnerability—for example, socioeconomic, geographical locale, and all forms of violence. However, the direct factors that may influence infection, such as the anatomical and physiological maturation of the anogenital tracts of adolescents or the trauma and wound-healing processes of injured mucosal tissue, are understudied and represent a gap within the HIV prevention field. This article reviews the epidemiology of HIV infection and violence in adolescents and the available basic science knowledge attending this research area. More importantly, this review highlights the most critical gaps that need to be addressed to design preventive interventions that are safe and effective for this population, which is key to ending the HIV pandemic.

Keywords: HIV, HIV/AIDS pathogenesis, HIV transmission, HIV prevention, mucosal

Introduction

ADOLESCENTS ARE A MAJOR POPULATION in the overall expansion of the AIDS pandemic, particularly in sub-Saharan Africa. Adolescents make up to one-third of all new HIV infections worldwide UNICEF,¹ with AIDS deaths in this population doubling between 2006 and 2012, despite a decline in overall AIDS-related deaths in other age groups.² Furthermore, there is growing evidence that violence in all of its forms (psychological, physical, and sexual) correlates with increased HIV susceptibility. Globally, one in four girls have experienced sexual violence, with 76% of those experiencing violence falling below the age of 18 years old (y.o.)³—thus creating a potential association between age-related HIV incidence and sexual violence.

The source of age-associated susceptibility to HIV acquisition is unknown but may reside in age-driven anatomical and physiological differences between adolescents and adults. In addition, high rates of violence may increase susceptibility through anatomical changes in the anogenital tracts that can potentially increase infection. Characterization

of the impact of these physiological, environmental, and biological factors that predispose adolescents to HIV infection remains a significant research gap.

The biological and physiological factors that influence HIV susceptibility are complex but can be separated into indirect and direct effects that can act as drivers of HIV infection and acquisition, as shown in Figure 1.^{4,5} Indirect drivers encompass a range of behavioral and structural factors, and include legal, economic, behavioral, and social factors—that is, poverty, career opportunities, level of education, witnessing or direct experience of violence, early sexual debut, multiple partners, gender inequality and norms, and use of alcohol and drugs. Direct drivers encompass the impact of sexual activity on the anogenital tract, such as injury and immunomodulatory effects of semen, as well as factors that define the development and biological function (e.g., reproduction and waste elimination).

In some cases, indirect and direct drivers can overlap in their impact on the anogenital tracts and overall susceptibility to HIV infection. Examples of direct drivers include sexually transmitted infections (STIs), pathogen-specific and

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FIG. 1. The complex spectrum of risk factors affecting HIV susceptibility.

nonspecific mucosal immune responses, and wound/injury/healing/resolution.

Several meetings have been convened to advance our understanding of the interaction of direct and indirect drivers and the intersection of age, violence, and trauma with HIV infection. The “Greentree I” was held in 2012 and identified three overarching gaps for the field: (1) the role of anogenital injury on HIV transmission, (2) the influence of sex and age-related anatomic characteristics on HIV transmission, and (3) the role of heterosexual anal intercourse in HIV transmission.^{6,7}

Following on this initial meeting, a high-level consultation (Greentree II) was also convened in 2015 by the STRIVE (Tackling the Structural Drivers of HIV) consortium to (1) clarify what is known about the epidemiological pathway linking violence and HIV, (2) identify shared risk factors, and (3) outline opportunities to act on synergies through common programming (L. Heise and C. Watts, pers. comm., September 2016). A more recent meeting entitled “Risks of young age and mucosal injury in HIV susceptibility” was convened by NIH in 2015 to gather experts who could inform and speak of the research needs regarding age, mucosal injury, and sexual trauma associated with HIV acquisition. The meeting engaged a multidisciplinary group of investigators to promote cross-fertilization of scientific knowledge and define the research priorities and opportunities to address the adolescent HIV pandemic.

This review builds on the outcome of these meetings and highlights what is known at the biological level about the role of HIV and trauma/violence on HIV acquisition and what still needs to be done to address the many outstanding questions in the field.

The Intersection of Youth, Age, Trauma, and Risk of Infection

The axis of adolescence, trauma, and enhanced HIV susceptibility is underappreciated within the HIV field; yet, it has significant implications for the epidemic.

U.S. epidemic

Of all new HIV infections per year in the United States, 21% are in adolescents, 80% of which are in males 13–19 y.o., with a predominance of young black men who have sex with men (MSM). In adolescent females, black teens are three to four times more likely to acquire HIV than white or Latino teens, with 84% of infections arising through heterosexual contact.⁸

Fifty percent of females in the United States have experienced some type of violence, with 20% reporting being raped. Of those experiencing rape, 40% are raped before the age of 18 y.o.⁸ Childhood abuse may also increase the chances of acquiring HIV. Retrospective studies have suggested that in HIV-positive adolescents (13–21 y.o.), 88% had experienced or witnessed violence, and 39% reported childhood sexual abuse—twice the national average. In the gay and bisexual male community, 32% of gay men and 21% of bisexual men have experienced sexual violence.^{9–11} In male and female 16–20 y.o., those experiencing intimate partner violence (IPV) are 50% more likely *not* to utilize condoms when practicing anal sex,^{9,12,13} establishing a link between violence and increased risky behavior.

Limited data exist for violence reporting in the transgender population; however, 28% are HIV positive, 78% have reported abuse, and 31% reported abuse from authority figures, such as teachers.⁴

International epidemic

As of 2012, 42% of new HIV infections were in adolescents (15–24 y.o.), with twice as many in adolescent females.² Outside of the United States, violence can be far more prevalent. Estimates range from 15% to 76% of women will or have experienced physical or sexual violence in their lifetime, with up to 50% of sexual assaults involving girls younger than 16 y.o. In addition, 130 million women and girls have experienced sexual assault/violence in the form of genital mutilation.⁵ A meta-analysis of 16 countries showed that IPV was significantly associated with HIV infection.¹⁴ The overall incidence of IPV was even higher if U.S. data were removed, supporting a greater magnitude of IPV and sexual violence for women and girls outside the United States—which through direct and indirect effects could predispose these women and girls to HIV infection. Thus, there is emerging epidemiological evidence that violence, in all its different forms, enhances the risk of HIV infection in adolescents. However, the role of age and violence in influencing HIV susceptibility at the basic biology level remains to be elucidated.

Biology of Age and Transmission

The intersection of age and violence is a global problem and exposes adolescents to HIV risks directly and indirectly. At the biological level (e.g., a direct driver), the anatomy and physiology of the adolescent anogenital tract are different from those of the adult. However, more is known about the adult female reproductive tract (FRT) than the anogenital tracts of adolescents. Still, our understanding of the physiology/immunology of the immature and maturing FRT and adolescent anal tract has significant gaps.

Adolescent FRT

The FRT of the adolescent is quite different than that of an adult female in which the adult tract is used to define a reproductively mature physiology and function. The transition from puberty and adolescence to adulthood is gradual and with the end goal of a mature adult reproductive tract, which is characterized by regular menstrual cycles and sexual reproduction. When considering the impact of maturation on the adolescent FRT and attainment of reproductive maturity, it is important to define and distinguish between puberty and adolescence. Biologically, puberty is the transition from reproductively immature to mature, traditionally occurring between the ages of 8 and 18 y.o.; seminal changes in the FRT sexual and reproductive maturity are shown in Figure 2.¹⁵ On average, this process takes between 3 and 5 years, where 98% of girls reach menarche (the beginning of menstrual function) at 14.5 years. Adolescence, however, is an age range used to define a period of time in human growth and development, specifically after childhood and before adulthood. This age range is commonly between 10 and 24 y.o., depending upon the defining source. This definition emphasizes anatomical/physiological maturation (also emphasized in the definition of puberty) as well as sociobehavioral development and maturity.

Puberty is driven, in part, by the hypothalamus–pituitary–ovary (HPO) axis, which controls FRT maturation and function. When the HPO is immature, there are greater fluctuations in estrogen levels than in adulthood. Further differences in adolescents include cervical ectopy, where the single columnar epithelium of the endocervix is exposed to the vaginal environment. Given its single epithelial layer, the endocervix may be more sensitive to trauma and infection than the multi-stratified epithelial layer of the ectocervix; yet, other FRT sites of infection remain to be completely elucidated.

Immunologically, the maturing FRT exhibits far more variability in antibody production and expression (IgG and IgA) and type I cytokine levels than adult FRT tissues. These

fluctuations correlate with the onset of menarche and establishment of a stable menstrual cycle.¹⁶ Although these data give some insight into the differences between the adolescent and adult FRT and the changes that occur during reproductive maturation, there are still significant gaps in our understanding of the overall maturation process, integration, and role of individual biological and physiological changes with their impact on susceptibility to HIV infection (Fig. 2).¹⁵

Microbiome of the adolescent FRT

Microbial colonization of the FRT begins at birth. Analysis of prepuberty communities shows significant bacterial diversity when compared with communities associated with adulthood. Prepuberty communities are composed of a greater variety and diversity of aerobes, anaerobes, and enteric bacteria. Between the ages 8 and 13 y.o., with the transition from premenarche (before onset of menstruation) to postmenarche (after onset of menstruation), bacterial communities begin in the majority of U.S. women to shift to the predominately *Lactobacillus* sp. found in the majority of reproductive-age women and girls.¹⁷ This shift is probably related to increases in estrogen levels and intracellular production of glycogen, which favors outgrowth of bacterial species that utilize glycogen as an energy source. Although the established paradigm for reproductive maturity and microbiome composition is that the microbiome will be predominated by H₂O₂-producing *Lactobacillus* sp., this is not always the case.

The Females Rising through Education, Support, and Health (FRESH) study (<http://ragoninstitute.org/international/fresh>) has longitudinally followed 18–23 y.o. women in South Africa for 12 months. In this study, increased levels of genital inflammation were observed that were not associated with concomitant STIs. The microbial communities in these women are characterized by low *Lactobacillus* abundance and overall high bacterial diversity. The low *Lactobacillus* content and

FIG. 2. Transition and events during puberty and reproductive maturation of the female reproductive tract. Although secondary sexual characteristics begin at approximately age 8 y.o. (childhood), they continue through the ages of adolescence. In some cases, full reproductive and sexual maturation is not complete until the age of 18 y.o., the lagging end of adolescence.¹⁵ Adapted with permission from presentation by Dr. Amanda Kallen at the “Risks of young age and mucosal injury in HIV susceptibility” meeting.

increased diversity are closely associated with the observed genital inflammation.

In the same study, lower prevalence of *Lactobacillus* species in bacterial communities was associated with increased HIV acquisition. Although genital inflammation defined by increased type 1 cytokine production appeared to be associated with increased community diversity, the composition of these communities appears to directly correlate with the level of immune activation.¹⁸ A correlation with *Lactobacillus* sp. dominance was also noted. *Lactobacillus crispatus*-dominated communities had none to minimal increases in proinflammatory cytokines and trended toward decreased HIV acquisition, whereas *Lactobacillus iners*-dominated communities were associated with increased interleukin (IL)-8 levels both *in vivo* and *in vitro*.¹⁹ Although the FRESH study has suggested significant correlation between microbiome diversity and inflammatory status that may translate to increased susceptibility to HIV infection, overall, there is a paucity of data with regard to the microbiome in adolescents, and several outstanding questions still need to be addressed. We still do not know the answers to the following questions: (1) How does the cervicovaginal microbiome develop in sub-Saharan women? (2) What are the mechanisms that lead to genital inflammation? (3) What are the nonbacterial microbial components of the genital microbiome? and, most importantly, (4) How do violence and sexual intercourse alter the genital microbiome and potentially affect biological risk of HIV acquisition?

Adolescent gastrointestinal tract

The adult and infant gastrointestinal (GI) tracts have been the subject of extensive research; however, little is known about the development of GI tracts of children and, in particular, adolescents and its development. Age-related differences have been shown in the plasma and intracellular concentration of antiretroviral drugs in HIV-infected children, adolescents, and adults after oral dosing—further suggesting differences that might impact the efficacy and safety of prevention strategies.²⁰ Thus, for adolescents, it can be inferred that the transition from infant to mature adult will result in an adolescent GI tract phenotype that reflects the transition from infant to adult. Whether this is an incremental transition or whether there are unique identifiable stages of the GI tract, maturation in the adolescent is unknown.

Biopsy-challenge models have been utilized by the HIV prevention field as a preliminary indicator of pharmacokinetics/pharmacodynamics. Specifically, vaginal, cervical, and rectal tissue is treated with an antiretroviral agent and challenged with virus.²¹ Interestingly, in studies in which adolescent GI biopsies are obtained and challenged with HIV *ex vivo*, delayed infectability/virus-replication capacity, as compared with adults, has been observed—suggesting that there are unique features and conditions during the adolescent transition to adult that impact HIV susceptibility.²²

Although the adolescent gut microbiota composition (number of major Taxa present) can be similar to adults' composition, the bacterial community composition is very different.²³ It is unknown whether this difference is significant in terms of HIV susceptibility or inflammation, as seen in the FRT for the FRESH study. Although we are beginning to answer these questions, progress continues to suffer from

nonvalidated methodologies and assays to measure GI tract HIV susceptibility and the factors that influence it. Normative ranges for mucosal indicators, such as GI tract signatures/phenotypes indicative of risk for infection, are still not robust enough to allow comparisons between adults and adolescents. This is further complicated by a lack of information on the impact of rectal intercourse, sexual injury, and trauma on normative values in adults and adolescents.

HIV Transmission: Modeling Infection and Susceptibility

To dissect the role of direct drivers at HIV susceptible sites, *in vivo* models are required, and recent developments in imaging technologies have allowed the identification of the early events in simian immunodeficiency virus (SIV) infection in nonhuman primates (NHPs).²⁴ In contrast to previous assumptions about where virus infection may be established within the FRT, infected cells representing initial infection sites have been identified in all tissues of the FRT from the labia to the ovaries.²⁴ These results represent the first indication that virus infection is not restricted to just the cervicovaginal region, but that the entire FRT including the external genitalia may be potential sites for infection. These results are of particular importance since studies have shown that the external FRT and, in particular, the labia minora and fossa navicularis are the most common sites of trauma and injury in adolescents after consensual or nonconsensual intercourse.²⁵

Furthermore, imaging studies have suggested that although the FRT is populated by a diverse range of cell types, which can be infected *in vitro* with SIV and HIV, in the NHPs the “first” infected cell type may be T cells with a Th17 phenotype. Th17 cells have been implicated in maintaining epithelial and mucosal integrity through the production of IL-17 and IL-22²⁴ and have been identified in the cervix and ectocervix of the human FRT.²⁶ Thus, the potential role of Th17 cells in mucosal integrity and their emerging identification as the first cells infected represent significant advances in our understanding of early HIV infection and provide a basis for understanding the factors that may influence susceptibility to infection. However, these studies have not been extended to juvenile animals nor to a central role for Th17 cells as the first target for virus infection established in humans. Thus, although this information seems to be a tantalizing platform upon which to begin to understand adolescent susceptibility and the roles of injury and sexual violence, substantial scientific gaps remain before we can apply this information to the adolescent HIV pandemic.

Effect of Hormones on HIV Acquisition

Reproductive hormones

In NHPs (pigtail and rhesus macaques) and humans, a window of vulnerability to infection has been hypothesized that hormonal changes during the secretory phase of the menstrual cycle alter multiple immunological parameters (innate and adaptive immunity) in the lower and upper FRT to increase HIV infection potential.²⁷ This hypothesis has been supported by the finding that pigtail and rhesus macaques are more susceptible in the late luteal and menstrual phase,²⁸ when estrogen levels are reduced and progesterone levels are higher.²⁹ In other studies, *ex vivo* HIV incubation

with human cervical explants showed that productive infection does not occur in tissues from the proliferative phase of the menstrual cycle (E₂ dominated) but only from the secretory phase.³⁰

Indeed, vaginal treatment of NHPs with estrogen reduces SIV transmission,³¹ and the use of DMPA (high-dose progesterone 40 mg/kg) to thin the epithelium is a standard method to obtain more reproducible viral infection in NHP challenge studies. In humans, the window of vulnerability was first proposed in 2008²⁷ and hypothesized that 7–10 days postovulation (secretory phase) susceptibility to infection will be increased, similar to NHPs. In recent studies,^{32,33} both the high level of endogenous progesterone during the secretory phase of the menstrual cycle and the use of the injectable progestin-only contraceptives are associated with higher levels of HIV target cells in the cervix, providing a biological explanation for the reported increase in acquisition risk.³⁴

Emerging data suggest that endogenous hormones can influence HIV target infection *in vitro*³⁵ as well as modify the metabolism of Tenofovir, an antiretroviral drug used in HIV prevention, to its active phosphorylated form.³² Therefore, an emerging body of data points to a critical role of endogenous and exogenous hormones in HIV susceptibility. As already noted, the transition through menarche to stable adult menstrual cycle is characterized by fluctuations in estrogen, suggesting that for adolescents, hormonal status could be a significant determinant—along with other physiological factors for determining susceptibility to infection.

Stress response hormones

Stress can be in the form of biological, environmental, cognitive, and behavioral as well as major adverse events in life. Stressors, or the type of stress encountered, can (1) be in the form of a single component, (2) result from multiple components, or (3) occur as a result of sequential or simultaneous stressors over time. Stress results in the induction and release of several hormones including epinephrine, adrenocorticotrophic hormone (ACTH), also known as corticotropin, and glucocorticoids such as cortisol act through the hypothalamic–pituitary–adrenal (HPA) axis, also known as the stress axis. If the stressor is chronic, HPA activation can result in systemic changes such as chronic immune system dysfunction. Because adolescence is a time of substantial environmental, social, and physiological shifts, multiple stressors may manifest, with different types and magnitudes of stressors occurring at different stages in the adolescent's life. Animal studies have shown that prepubescent animals display greater and more protracted hormonal stress responses than adult animals; however, the basal ACTH and glucocorticoid levels will stabilize as maturation progresses, resulting in fairly stable levels in adolescence.

There are many scientific gaps that need to be filled to address the overarching question of to what extent does physiological stress mediate the relationship between abuse (physical, sexual, and psychological) and HIV acquisition for both adults and adolescents. We need to elucidate (1) the contribution of cumulative violence to chronic stress and subsequent HIV risk, (2) the interaction of stress with systemic and local immune function that may affect HIV risk, (3) the effect of anti-inflammatory hormones and immunomodulatory hormones on systemic inflammation among

abused girls who suffer from chronic stress, and (4) the effect of stress because of violence/abuse on the adolescent anatomy and physiology. Elucidating these interactions are complicated by the lack of reliable biomarkers to measure and categorize stress.³⁶

Although cortisol production has been used as a potential biomarker, it is not all-inclusive or bioindicative of physiological changes that can accompany acute or chronic stress. It also does not factor in sociostructural stressors, which may not result in increased cortisol production. Although we have begun to obtain an appreciation of the potential role of stress/stressors in HIV susceptibility, there are still significant gaps in our understanding of the relationship between stress-related biological changes, normal maturation, and their role in HIV susceptibility.

Mucosal Trauma

An additional factor to consider when addressing potential susceptibility of adolescents to HIV infection is the role of anogenital trauma. The high rate of sexual violence that adolescents experience can manifest as anogenital injuries of various magnitudes—from bruising, to scrapes, to lesions, and tears of the reproductive mucosa. Although it is recognized that sexual trauma can result in mucosal injury, widely accepted clinical definitions of anogenital injury have not been developed. In addition, methods currently used to measure trauma are not generally designed to accurately capture mucosal injury for (1) consensual versus nonconsensual intercourse, (2) frequency at which injuries occur, (3) cumulative impact of injuries, (4) number of injuries sustained per event and their location, and (5) type and severity.

The lack of validated injury scales to assess the intensity and impact of injuries has been a major source of acknowledged variation in data collected on injury after both consensual and forced sex, resulting in a range of conclusions on the impact of injury in epidemiological and forensic medicine on potential health and susceptibility to disease.^{37,38} Even with the mentioned caveats, estimates are that between 50% and 90% of rape cases result in genital injury detected by multiple methods, including visual examination, colposcopy with digital image capture, and contrast staining of genital tissues. When genital trauma of adolescents is compared with that of adults, there is more trauma detected over a greater surface area of the lower reproductive system.³⁹

FRT Trauma

A recent study demonstrated that 55% of women experienced at least one anogenital injury during consensual sex, with the most common site of injury being the external genitalia—for example, injuries of the labia majora, labia minora, periurethral area, perineum, posterior fourchette, and fossa navicularis. The prevalence of consensual, external genitalia injury was influenced by the phase of the menstrual cycle and the use of hormonal contraceptives. In addition, it is important to note that genital injury, such as microabrasions, can manifest as late as 72 h postcoitus,⁴⁰ complicating injury assessments. Thus, despite the lack of procedures for consistent measurement and categorization of genital injury, current evidence shows that adolescents and reproductively mature women may experience genital injury throughout the FRT—whether this is the result of sexual assault or consensual

sex—and that age and hormonal status can impact the degree and frequency of injury. Creation of new tools that take into account skin color, hormonal status, and type of sex will be critical to standardize assessments of the anatomical and physiological changes that occur in the FRT mucosal environment after trauma and their contribution to HIV susceptibility.

GI Trauma

HIV infection risk associated with receptive anal intercourse (RAI) is 18-fold higher than with vaginal intercourse.⁴¹ RAI is not just a practice of concern for MSM, but it also impacts heterosexual couples who practice RAI routinely or episodically. Heterosexual RAI practice is often in the context of gender-power relationships or used as a pregnancy prevention method. Between 13% and 22.5% of women who have been sexually assaulted report forced anal penetration^{42,43} and sometimes both anal and vaginal penetration during the same sex act. Therefore, injury in the anus and rectum after both consensual and nonconsensual intercourse is a problem for adolescents of both sexes.

The majority of injuries occur in the anus and rectum for both consensual and forced RAI. Although the two sites are quite different histologically, the presence of HIV susceptible cells and cytokine profiles conducive to infection is similar at both sites in adult *ex vivo* models and has demonstrated similar HIV infectability. The anal epithelium is composed of stratified squamous epithelium, whereas the rectum is composed of a single layer of columnar epithelium. The submucosa of both has a sheath of muscle that sets the tonicity of anal and rectal tissues, resulting in active closure of the sphincter and control of excretory processes. Because of the excretory function of the GI tract, the anus and rectum are subjected to recurring daily trauma by excretory processes. Because of the tonicity of the anus and rectum, injury during consensual sex can occur at a high frequency, although this effect appears to be compensated for by a high rate of injury resolution.

Unlike vaginal injury, there have not been substantial efforts to quantify trauma and injury in the anus and rectum of either males or females. As already noted, the tonicity of both the anus and rectum, although a critical physiological factor, can also be a liability in the context of sexual injury. Relaxation of the sphincter muscles and/or preintercourse preparation can be an essential factor in reducing injury. However, some practices, although facilitating RAI, may not completely reduce the potential for tissue injury.

Those practicing RAI often use lubricants, which may reduce friction-based injuries but may cause irritation to the epithelium because of their osmolality⁴⁴ or toxic excipients. In addition, abnormalities and inflammation of anal and rectal tissues from hemorrhoids, fistulas, abscesses, and/or STIs can increase the potential for anal/rectal injuries/trauma during both consensual and nonconsensual sex. The lack of defined measurements for anal and rectal injury, coupled with physiological factors that can make penile penetration difficult and/or likely to aggravate an existing condition, represents a significant gap in our understanding of the impact of RAI in both heterosexual and homosexual adolescents on HIV and STI susceptibility. Further studies should focus on defining and determining the degree of trauma through consensual and forced RAI and on examining existing models,

such as biopsy-challenge models, or animal models for their potential to be used to mimic trauma at these sites.

Wound Healing

Wound healing is a complex process that involves closure of the wound, accompanied by tissue remodeling to restore the tissue to or near to its preinjury state. Although many observations have been made regarding wound closure in anogenital tissues, there are still significant gaps in our understanding of anogenital mucosal wound/injury/healing. In the field of mucosal immunology and tissue repair, there are two common fallacies that should be dispelled: (1) that mucosal immunity is like systemic immunity and (2) that mucosal tissue injury repair is identical to skin/dermal wound/injury repair. Evidence of this dichotomy between dermal and mucosal wound healing is clearly seen when examining oral mucosal wound healing, whereby oral mucosa healing is slowed by the types of inflammatory states usually associated with dermal wound healing and resolution. Another salient feature of mucosal wound repair is that it appears, at least for injury closure, to be more rapid than skin. After GI and cervicovaginal biopsies, wound closure (expansion or movement of local epithelial cells to cover the wound site) occurs in a very short time—from a few hours to days. Resolution of the anogenital tissue damage is less well characterized but ultimately under the control of the immunological and inflammatory state of the tissue.

Mucosal immunity is generally characterized by secretory IgA, an abundance of regulatory T cells (Tregs), phagocytes to trap pathogens, and high levels of adhesion molecules providing a tight barrier to the external environment. However, not all mucosal sites are similar. The predominant antibody in the FRT is IgG (not IgA), and in the stratified squamous epithelium of the lower FRT, tight junctions are primarily present between basal epithelial cells. Their absence in the superficial epithelium results in weakly joined cells and may allow pathogens such as HIV to penetrate the epithelial layer.¹⁶ Immunological status may then determine the response to incoming pathogens and immune altering signals created/exposed when cells are injured.

In skin healing, increased type I inflammation increases healing time and the potential for scar tissue formation, which is not often seen after anogenital injury repair.^{45–48} Although anogenital healing is not fully understood, there are a number of factors that *a priori* will impact healing and wound resolution, which might be relevant to injury and HIV susceptibility in adolescents. They include (1) age—young adults' wounds heal more rapidly than older adults' wounds, with postmenopausal women's wounds being the slowest (Fig. 3B)⁴⁹; (2) gender—in general, women heal wounds more slowly than men (Fig. 3A)⁵⁰; and (3) stress—which may slow healing because of hyperinflammation, or further influenced by drug usage (e.g., corticosteroids). Hormones can also play a role in wound healing because higher testosterone levels in men are associated with faster healing; however, wound closure may be independent of sex hormones.⁴⁹

The high rate of anogenital injury because of both consensual and nonconsensual sex makes consideration of injury and repair of injury potentially important factors in determining susceptibility to HIV infection. Wound healing

FIG. 3. Mucosal wound healing by gender and age. Mucosal wound closure occurs more rapidly in males than in females (A), $*P < 0.05$ and $^{\dagger}P < 0.01$.⁵⁰ Furthermore, mucosal wound closure occurs more rapidly in young women than in pre- and postmenopausal women (B) $*P < 0.05$ and $^{\dagger}P < 0.01$,⁴⁹ suggesting that both gender- and age-related, direct drivers play a role in wound healing. (A) Adapted with permission from page 1196 in Engeland *et al.*⁵⁰ Copyright 2006 by the American Medical Association. (B) Adapted with permission from page 632 in Engeland *et al.*⁴⁹ Copyright 2008 by Elsevier, Inc.

appears (at least for skin) to be a dynamic process, with site of injury, age, sex, and stress playing roles in the rate of repair and resolution. Other factors such as the local inflammatory and immune environment appear to be modifiers of the repair process. However, there are still many gaps in our understanding of how these many factors integrate to maintain tissue integrity and constitute a barrier to HIV entry and infection in both adults and adolescents.

In Vivo Models of Age and Trauma

A significant challenge to integrating our knowledge of anogenital injury and the factors involved in its initiation and resolution is the lack of suitable animal models. This challenge is made more significant when we consider the need to study mucosal tissues from the anogenital tracts of adolescents or from those experiencing trauma in any age group. Although all animal models have their limitations, they can play a central role in refining questions/hypotheses on the relationship between age, trauma, and HIV susceptibility. The NHPs model using either the SIV or genetically engineered SIV with critical HIV genes, simian human immunodeficiency virus (SHIV), is the most used model for animal infection studies. The SIV/SHIV infection models have many similarities with HIV infection in terms of virus transmission and progression to disease depending upon the type of virus, species chosen, and how the model is used. An additional advantage for these models is the availability of a variety of reagents and methods for mucosal tissue analysis.

The NHPs model can also be used to study chronic stress responses. Dominance hierarchies are naturally occurring within NHP populations, and subordinate rank is an established chronic stressor. Thus, NHP infection models are a potential starting place to begin to incorporate hypotheses and experimental designs, which explore the role of age and trauma in HIV susceptibility. Toward this end, a coital model in pigtail macaques has been developed.⁵¹ In this model, sexual activity of the monkeys can be monitored and documented. Although sex-related injury can be detected, it does

not appear to occur at a rate comparable with that of humans, and the intensity and severity of the injuries vary significantly. Thus, a significant gap in animal model development is the ability to create a defined injury (intensity, location, and frequency) that can then be used to explore the various factors and conditions that could impact injury and HIV susceptibility.

Although not a model of HIV transmission and infection, sheep have recently been proposed as a potentially advantageous model for studying age, trauma, and wound healing. Recent research has begun to explore the sheep anogenital mucosa and develop reproducible methods to create defined injuries using chemicals, which have been shown to damage the anogenital mucosa in animal studies and clinical trials.⁵² The size of the cervical vaginal canal, body mass, vaginal squamous epithelium, ovulatory cycle, and some immunological features of mucosal inflammation in sheep are similar to those of humans. These features—coupled with affordability, accessibility, and ease of visual and histologic examination of the anogenital tract—render this model potentially very valuable. A number of high-resolution imaging techniques, such as high-resolution optical coherence tomography and confocal microscopy/endomicroscopy, have been developed and can be used *in vivo* to track and quantify injuries.⁵² Despite these clear advantages, there are limitations to the sheep model because they have a seasonal estrous cycle and a higher vaginal pH than humans. However, the coupling of noninvasive imaging technology with the ability to control the severity, location, and frequency of injury will enable the designing of wound repair and resolution studies required to map wound resolution and the relationship of tissue trauma to susceptibility to infection.

The Way Forward

HIV incidence rates in male and female adolescents in sub-Saharan Africa are staggering. In South Africa alone, more than 2,300 girls between the ages of 15 and 24 y.o. acquire HIV every single week. Preventing HIV infection in this age group is a must if we aim to control the HIV epidemic and create an AIDS-free generation.

However, to date, few studies have specifically targeted adolescents—especially those who are younger than 18 y.o.. Part of the reason for this is the erroneous assumption that adult and adolescent biology, physiology, and behavior result in similar outcomes in terms of HIV susceptibility and the ethical and regulatory complexities related to adolescent participation in clinical research. Recent results from the ASPIRE trial [MTN-020, Phase III trial of dapivirine, a non-nucleoside reverse transcriptase inhibitor 30-day intravaginal ring (IVR)] illustrate the impact of these assumptions and issues. The IVR was found to reduce the risk of acquiring HIV in women of ages 21 y.o. and older by 56% in a modified intent-to-treat analysis; however, the IVR lacked efficacy in women of ages 18–21 y.o. Concomitantly, the International Partnerships for Microbicide (IPM) Ring Study (IPM-027, Phase III trial with the same IVR) demonstrated an overall 37% reduction in HIV acquisition for women older than 21 y.o.—yet, only 15% for women younger than 21 y.o. Whether this lack of protection is the result of imperfect adherence, behavior, biology, or a combination of all these different factors remains an open question.

The very limited knowledge available regarding the nexus of age, injury, and HIV acquisition calls for renewed research efforts to better understand this critical area of HIV research. Three major overarching gaps (Table 1) need to be addressed: (1) the exploration of the anatomical, physiological, and immunological features of the immature and maturing anogenital tracts of adolescents; (2) a better understanding of the contribution of genital, anal, and oral injuries sustained from

sexual intercourse (consensual and forced) to HIV transmission; and (3) the development of *in vitro*, *ex vivo*, and *in vivo* human and animal models to study adolescent/juvenile stress, trauma, and wound healing. Each of the overarching gaps can be broken down into priority actions and research is needed to address the overarching objectives (Table 1).

However, conducting biological studies in adolescents can be difficult. Federal regulations (www.ecfr.gov/cgi-bin/textidx?tpl=/ecfrbrowse/Title45/45cfr46_main_02.tpl) controlling clinical studies without specific benefit to individuals within vulnerable populations can limit the scope of what research is considered feasible in adolescents. This is further complicated by a broad and diverse range of state laws, which further control what an adolescent may be able to self-consent to or require parental consent to perform. Furthermore, Institutional Review Boards (IRBs) for clinical studies can add additional constraints to assure the welfare, safety, and well-being of human participants in clinical research. Similarly, Institutional Animal Care and Use Committees can add requirements for the ethical use of juvenile animals in research.

Clinical research involving adolescents must address a number of challenges, such as (1) the role of parental oversight and consent for the participant, (2) IRBs' inexperience with adolescent research and guidelines, and (3) the lack of sufficient training for research staff working with adolescents. Adolescents' ability to make autonomous decisions about participation in clinical research has also been cited as an important obstacle; however, there is no evidence that older minors are different than young adults in their understanding and ability to make decisions in this regard. Despite these challenges, given the disproportionate impact of the HIV epidemic on adolescents, we should make every effort to enhance their inclusion in current and future HIV prevention studies and trials.

To address the research gaps and inclusion concerns, challenges to cross-disciplinary research collaborations involving clinicians, behaviorists, social scientists, and basic scientists must be addressed and resolved. Addressing the research actions and gaps identified in Table 1 will require not only increasing the number of research collaborations but also the breadth of disciplines involved in the research, as we evaluate singly and in concert the many and complex factors (both direct and indirect) that determine susceptibility to HIV infection in boys and girls.

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TABLE 1. GAPS AND RESEARCH PRIORITIES FOR UNDERSTANDING THE INTERSECTION OF HIV AND VIOLENCE IN ADOLESCENTS

GAP area	Actions and research needed
Age, trauma, and the HIV epidemic	<p>Education of local IRBs in clinical research practices and laws regarding research in vulnerable populations.</p> <p>Establish best practices for recruiting, treating, and maintaining adolescents in clinical studies.</p> <p>Improvement of sampling methods and assay standardization in clinical research.</p> <p>Optimization of clinical protocols to include vulnerable populations and data gathering.</p> <p>Implementation of effective HIV and violence prevention programs for vulnerable populations.</p>
Biology of the age, trauma, and HIV	<p>Basic science studies of the mucosal environment of the adolescent and wounded anogenital tracts.</p> <p>Development of new biomarkers for stress in clinical studies.</p> <p>Mucosal trauma and wound-healing studies specifically in the anogenital tracts.</p>
Models to understand age and sexual trauma	<p>Inclusion of both consensual and nonconsensual sex in injury studies.</p> <p>Establishment of <i>in vivo</i> and <i>in vitro</i> models for age and trauma studies.</p>

IRB, Institutional Review Board.

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