



# How Does Voluntary Medical Male Circumcision Reduce HIV Risk?

Jessica L. Prodger<sup>1</sup> · Ronald M. Galiwango<sup>2</sup> · Aaron A. R. Tobian<sup>3</sup> · Daniel Park<sup>4</sup> · Cindy M. Liu<sup>4</sup> · Rupert Kaul<sup>5,6</sup> 

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## Abstract

**Purpose of Review** Voluntary medical male circumcision (VMMC) is a surgical procedure that reduces HIV acquisition risk by almost two-thirds. However, global implementation is lagging, in part due to VMMC hesitancy. A better understanding of the mechanism(s) by which this procedure protects against HIV may increase acceptance of VMMC as an HIV risk reduction approach among health care providers and their clients.

**Recent Findings** HIV acquisition in the uncircumcised penis occurs preferentially across the inner foreskin tissues, due to increased susceptibility that is linked to elevated inflammatory cytokine levels in the sub-preputial space and an increased tissue density of HIV-susceptible CD4+ T cells. Inflammation can be caused by sexually transmitted infections, but is more commonly induced by specific anaerobic components of the penile microbiome. Circumcision protects by both directly removing the susceptible tissues of the inner foreskin, and by inducing a less inflammatory residual penile microbiome.

**Summary** VMMC reduces HIV susceptibility by removing susceptible penile tissues, and also through impacts on the penile immune and microbial milieu. Understanding these mechanisms may not only increase VMMC acceptability and reinvigorate global VMMC programs, but may also lead to non-surgical HIV prevention approaches focused on penile immunology and/or microbiota.

**Keywords** Penile circumcision · HIV susceptibility · Mucosal immunology · Microbiome

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Jessica L. Prodger and Ronald M. Galiwango contributed equally.

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✉ Rupert Kaul  
rupert.kaul@utoronto.ca

- <sup>1</sup> Departments of Microbiology and Immunology and Epidemiology and Biostatistics, Schulich School of Medicine and Dentistry, Western University, London, ON N6A 5C1, Canada
- <sup>2</sup> Rakai Health Sciences Program, Kalisizo, Uganda
- <sup>3</sup> Department of Pathology, Johns Hopkins University School of Medicine, Johns Hopkins University, Baltimore, MD 21205, USA
- <sup>4</sup> Department of Environmental and Occupational Health, Milken Institute School of Public Health, George Washington University, Washington, DC 20052, USA
- <sup>5</sup> Departments of Medicine and Immunology, University of Toronto, Medical Sciences Building Rm. 6356, 1 King's College Circle, Toronto, ON M5S 1A8, Canada
- <sup>6</sup> University Health Network, Toronto, ON, Canada

## Introduction

Clinical trials performed over a decade ago demonstrated that voluntary medical male circumcision (VMMC) reduces the risk of heterosexual HIV-1 (HIV) acquisition by approximately 60% [1–3]. As a result, this one-time surgical intervention has been rolled out as a key prevention tool by UNAIDS in 15 high HIV prevalence countries with low baseline rates of penile circumcision, with the goal of performing 25 million procedures by 2020 [4]. Disruption to these surgical programs by the COVID-19 pandemic was partly to blame for the fact that this target was missed by 7 million [5], but another important barrier is that many uncircumcised men have been hesitant or unwilling to undergo VMMC for a variety of personal and cultural reasons [6]. Therefore, it is important to develop a better understanding of the mechanism(s) by which VMMC protects against HIV acquisition, since these remain controversial and relatively understudied. Understanding these mechanisms may not only increase VMMC acceptability and reinvigorate global VMMC programs, but in theory might also lead to non-surgical HIV prevention approaches.

Regardless of the tissue site of virus acquisition, a central determinant of HIV transmission risk is the level of HIV RNA in the genital or rectal secretions of a person's sexual partner [7]; this in turn varies with the clinical stage of HIV infection [8], and most profoundly with effective virus suppression on antiretroviral therapy (ART) [9, 10]. However, since penile circumcision does not alter HIV levels in the genital tract or the probability that a person living with HIV transmits the virus to their partner [11], this is not a focus of the current review. Rather, the focus will be on biological parameters specific to the penis that profoundly alter the probability of HIV acquisition after penile exposure to HIV during insertive penile sex, and how these parameters are impacted by VMMC.

## HIV Acquisition in the Uncircumcised Penis

In order to understand how VMMC protects against HIV, it is first important to review what we know about HIV acquisition in penile tissues. The foreskin is a continuous sheet of skin whose surface extends from the coronal sulcus to the shaft of the penis, folding back on itself in the non-erect state to form a hood that covers the glans penis. In the non-erect state, the surface of the proximal half of the foreskin (the outer foreskin) is exposed to air, while the distal half (the inner foreskin) lies against the glans enclosing an anaerobic sub-preputial space [12]. Both the inner and outer foreskin tissues are removed during VMMC, both contain CD4+ T cell and dendritic cell subsets that are susceptible to HIV [13•, 14], and both are susceptible to HIV challenge *ex vivo*, although in explant studies the inner foreskin is more easily infected [15–17]. In uncircumcised Ugandan men, a higher foreskin surface area (including both inner and outer) was associated with an increased risk of HIV acquisition [18], suggesting that at least part of the protection afforded by VMMC is simple stoichiometry, mediated through a reduction in the surface area of HIV-susceptible tissue exposed to HIV during sex. While it was initially thought that the inner foreskin was more susceptible to HIV due to a thinner keratin layer (stratum corneum), subsequent blinded studies found no or very minimal differences in keratin thickness between the two sites [19–23]. However, there may be important physical differences, including increased wetness of the inner foreskin, that may enhance micro-abrasions during sex [22, 24]. Therefore, the observation that the inner foreskin is more HIV-susceptible *ex vivo* may instead relate to the substantial differences that exist in the immunology and microbiome of the inner and outer foreskin [13•], which will be discussed in more detail later in this review.

Given that a circumcised man can also acquire HIV through insertive sex, the foreskin cannot be the only penile tissue site of HIV acquisition. The penile urethra has the features of an HIV-susceptible mucosa [25••, 26–28], and is exposed to genital/rectal secretions (and hence to HIV virions)

during sex with an HIV-infected partner who is not taking suppressive antiretroviral treatment (ART). Therefore, the distal urethra has been proposed as the site of most penile HIV acquisition in circumcised men, and of some HIV acquisition in uncircumcised men [28]. Indeed, since the foreskin overhangs the urethra of a non-erect penis, this means that VMMC would be expected to alter the urethral immune and microbial microenvironments, and it has been suggested that this could mediate some or all of the protection afforded by this procedure [28]. However, while a recent study did demonstrate that VMMC had effects on the urethral microbiome, these effects were much less than those in the coronal sulcus and were not accompanied by changes in urethral immunology [25••]. Therefore, it seems more likely that VMMC primarily protects through effects on the foreskin and coronal sulcus.

## Clinical Parameters Associated with HIV Acquisition

Although the most consistent clinical correlate of penile HIV acquisition in both observational studies and interventional trials is the presence of a foreskin, a number of additional clinical and behavioral parameters can alter HIV susceptibility.

Sexually transmitted infections (STIs) are consistently associated with increased HIV acquisition risk, both bacterial STIs such as gonorrhea and syphilis [29] and viral STIs such as *Herpes simplex virus type 2* (HSV-2) [30] and human papilloma virus (HPV) [31]. However, the direction of causation in these associations has not been defined. While all these STIs alter penile immunology in a way that could plausibly increase risk, as discussed below, they are also linked to other higher-risk sexual practices and to increased levels of genital HIV RNA shedding in a co-infected sexual partner who is living with HIV (reviewed in [32]). Importantly, in addition to reducing HIV risk, VMMC reduced the subsequent incidence of HSV-2, HPV, genital ulcer disease (GUD), and syphilis in randomized clinical trials [33–36], as well as the transmission of HPV, *Trichomonas vaginalis*, and bacterial vaginosis to female partners [37, 38]. However, the prevention of these STIs is thought to play a modest role in the ability of VMMC to protect against HIV acquisition [39, 40], and modelling suggests that the fraction of HIV prevention that is attributable to reduced STIs is unlikely to exceed 10–20% [41].

There is no evidence that enhanced penile hygiene reduces HIV risk, and in fact there was a trend to increased HIV acquisition among men who practice postcoital washing [42].

## Penile Immunology and HIV Susceptibility

To date, prospective cohort studies of penile immunology and the immune associations of HIV acquisition have only been performed in uncircumcised men [12]. In general, the

immune correlates of penile HIV susceptibility demonstrate considerable overlap with those in the female genital tract, where mucosal immune activation and inflammation have been strongly and consistently linked to HIV risk [43–46]. Compared to the blood, the foreskin is enriched for dendritic cells, activated CD4+ T cells expressing the HIV co-receptor CCR5, and the Th17 CD4+ T cell subset [47, 48]. These cell populations are preferential targets for SIV/HIV in the rectum and female genital tract [49–52], and their density is much higher within the more HIV-susceptible tissues of the inner foreskin [13•, 16, 17, 53]. In addition, a reduced relative abundance of foreskin Th17 cells is seen in men who are Highly Exposed to HIV but remain SeroNegative (HESN) [54]. Further evidence that the density of these foreskin cell subsets serves as a common pathway for penile HIV acquisition comes from their association with STIs; asymptomatic HSV-2 infection is associated with an increased foreskin density of CCR5/CD4+ T cells [55, 56], HPV with increased foreskin Langerhans cells [57], and *Chlamydia trachomatis* infection with an increased density of both these cell subsets [21].

The increased target cell density and HIV susceptibility in the inner foreskin is strongly associated with the higher levels of inflammatory cytokines and chemoattractant chemokines that are present in the adjacent sub-preputial space, compared to the surface of the outer foreskin [13•, 58••]. In keeping with the clinical relevance of these findings, increased prepuce levels of the chemoattractant cytokines IL-8 and CXCL9 (MIG) were directly linked to HIV acquisition in a prospective cohort study of uncircumcised Ugandan men [58••, 59], along with prepuce levels of soluble innate immune molecules such as neutrophil-derived  $\alpha$ -defensins [60]. Interestingly, condomless penile-vaginal sex itself is associated with an immediate increase in prepuce cytokine levels that persists for up to 48 h. The degree of change of cytokines on the penis reflects the concentration of cytokines in the cervico-vaginal secretions of a man's female partner [61], demonstrating that direct passive transfer of these immunological mediators may occur from a sexual partner. However, whether frequent coitus or the time frame after sex during which their levels are elevated on penile tissues (generally < 72 h) is sufficient for them to induce epithelial damage or to recruit HIV target cells to penile tissues is not currently known.

The tissues of the penile urethra are also highly HIV susceptible and can serve as a virus reservoir after HIV infection [26, 27, 62]. However, while it is assumed that the distal urethra is the main tissue site for penile HIV acquisition among circumcised men, VMMC does not reduce urethral inflammation and was actually associated with increases in urethral levels of the chemoattractant cytokine IL-8 and of soluble E-cadherin, a biomarker of epithelial disruption [25••]. Therefore, it does not seem likely that the protective effects of VMMC are mediated through the urethra.

## The Genital Microbiome as a Mediator of Penile Immunology

Overall, there is a strong association of inflammatory cytokine levels in the sub-preputial space with the tissue density of foreskin target cells and in vivo HIV acquisition [12]. What factors are driving this inflammation, and what is the impact of VMMC? STIs are certainly inflammatory, and while at the population level most HIV is acquired in the absence of classical STIs, asymptomatic HSV-2 infection prevalence exceeds 50% in many parts of SSA [63] and is associated with increased HIV target cell density in the foreskin [55, 56]. However, it is now becoming clear that the penile microbiome is diverse, and that the composition of this microbiome is a key driver of local inflammation. The low oxygen tension in the sub-preputial space means that the microbiome is dominated by anaerobes such as *Prevotella*, *Porphyromonas*, *Dialister*, and *Finegoldia* spp., with a dramatic reduction in their coronal sulcus density after VMMC when oxygen tension rises [64]. While the density of these many anaerobes tends to be co-associated, foreskin immunology and HIV risk is most tightly linked to the density of six specific Bacteria Associated with Seroconversion, Immunology, and Cells (BASIC) species, namely *Peptostreptococcus anaerobius*, *Prevotella bivia*, *Prevotella disiens*, and three *Dialister* spp. [58••]. Among uncircumcised men with high levels of BASIC bacteria on the coronal sulcus, their inner foreskin CCR5/CD4+ T cell density is increased up to sixfold compared with their own outer foreskin, or indeed with the inner foreskin of other men with a similar density of non-BASIC bacteria [58••]. A causal role for these associations is being explored in a randomized trial of application of the topical antimicrobials metronidazole and clindamycin to the uncircumcised penis [65], each of which has broad activity against genital anaerobes. Antimicrobial application demonstrated a reduction in penile inflammation (IL-1 $\beta$  levels) and an increase in the epithelial integrity of the inner foreskin [66].

## Impact of Circumcision on the Microbial and Immune Parameters Linked to HIV Risk

VMMC induces biological changes in penile tissues that reduce the per-exposure risk of HIV acquisition, without reducing the behavioral risks of exposure to HIV. As described above, there are clear immune and microbial drivers of HIV acquisition in the uncircumcised penis, and the broad impact of VMMC on these biological parameters is likely to be the mechanism for HIV protection.

In addition to VMMC reducing the surface area of HIV-susceptible penile tissues and thereby directly reducing HIV risk, the removal of the foreskin alters the microenvironment overlying the glans and coronal sulcus by exposing these tissues to the air, with a 60-fold reduction in anaerobic taxa overall

and a particularly profound reduction in *Prevotella bivia*, the bacterial species that is most strongly associated with inner foreskin inflammation and HIV risk [25••]. Skin-associated aerobic taxa, such as *Corynebacterium*, are associated with less inflammation in uncircumcised men and their enrichment in the penile microbiome relative to BASIC species is associated with a reduced risk of HIV acquisition [43]. While the absolute abundance of *Corynebacterium* does not increase in the coronal sulcus post-VMMC, due to a reduction in anaerobes, they subsequently make up a much higher proportion of the penile microbiome. In keeping with this shift to a less inflammatory microbiome, levels of IL-8 in the coronal sulcus fall by approximately tenfold after VMMC [25••, 59], with progressive reductions in CS levels of IL-8 for at least two years after VMMC [59]. There is also progressive enhancement of epithelial integrity, indicated by falling levels of soluble E-cadherin shed into the sub-preputial space [25••]. However, the impact of VMMC on the density of HIV target cells in penile tissues has not been defined for practical reasons.

Interestingly, the dramatic increase in coronal sulcus inflammatory cytokines that is seen after condomless penile-vaginal sex does not vary in magnitude or duration based on circumcision status, implying that the protective effects of VMMC cannot be attributed to modulation of these sex-induced effects. Neither does VMMC reduce inflammation in the urethra, where there is a modest reduction in anaerobes and total bacterial load after VMMC, but no decrease in inflammatory cytokines and an increase in biomarkers of epithelial disruption [25••].

## VMMC, Socio-behavioral Factors, and HIV Transmission

While biology has been the main focus of this review, it is important to consider whether VMMC might lower HIV risk by reducing the probability of penile exposure to HIV in the

first place, either by reducing the frequency of condomless insertive penile sex or the probability that a sexual partner has untreated HIV infection. The three randomized clinical trials (RCTs) that demonstrated the efficacy of VMMC in reducing HIV incidence all assessed (and controlled for in their analyses) the frequency of condomless sex; one found increased numbers of sexual partners among men who had been randomized to immediate vs. delayed penile circumcision [1], while the other two found no difference [2, 3]. Furthermore, a recent meta-analysis found no impact of VMMC on partner numbers or condom use among almost 100,000 men [67•]. Therefore, although we are unaware of studies that compare the seroprevalence of HIV among new sexual partners of circumcised vs. uncircumcised men, it seems unlikely that the HIV protection afforded by VMMC relates to differences in the probability of sexual HIV exposure.

In addition, it is important to emphasize that VMMC can only protect against penile HIV acquisition and will not alter HIV acquisition risk after virus exposure at other mucosal sites. This means that, although HIV incidence is high among men who have sex with other men (MSM) in the same high HIV prevalence countries targeted by VMMC programs [68], since the per-act risk of HIV acquisition is approximately tenfold higher after a rectal vs. a penile HIV exposure [69], VMMC provides a much lower degree of protection (approximately a 23% reduction overall) against HIV acquisition in MSM [70].

## Implications for HIV Prevention

Overall, the beneficial effects of VMMC on HIV prevention are clear, with protection mediated through several overlapping biological mechanisms (summarized in Table 1). HIV acquisition in the uncircumcised penis occurs preferentially in the tissues of the inner foreskin,

**Table 1** The effect of penile circumcision on immune and microbial correlates of penile HIV acquisition

Parameter enhancing HIV risk	Penile tissue site	Reduced by VMMC
Density of HIV target cells	Foreskin	Yes (tissues removed)
	Coronal sulcus	Unknown
	Urethra	Unknown
Sexually transmitted infections	Foreskin	Yes (HPV, HSV-2, syphilis)
	Urethra	No (gonorrhea, chlamydia)
Density of inflammatory anaerobes	Coronal sulcus	Yes
	Urethra	Yes
Immune activation	Coronal sulcus	Yes
	Urethra	No
Epithelial disruption	Coronal sulcus	Yes
	Urethra	No
Dendritic cell maturation	Coronal sulcus	Unknown
	Urethra	Unknown

where an anaerobic environment promotes a microbiome that is enriched for the pro-inflammatory BASIC species, with subsequent recruitment of HIV targets such as Th17 cells and disruption of epithelial integrity. VMMC not only directly removes foreskin tissues with a high density of HIV target cells, reducing the surface area of susceptible tissues, but also promotes an aerobic micro-environment with a less inflammatory penile microbiome and decreased biological susceptibility in the context of HIV exposure during sex. A better understanding of these mechanisms may increase acceptability of VMMC as an HIV prevention tool by both health care providers and their clients, reinvigorating global VMMC programs. In addition, defining the determinants of penile microbiome composition in uncircumcised men and how the BASIC species elicit inflammation and epithelial disruption may enable the development of non-surgical prevention modalities for men who wish to remain uncircumcised, such as antimicrobials and/or immune modulators.

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## Declarations

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- Of importance
- Of major importance

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