Published in final edited form as:

Curr HIV Res. 2012 April ; 10(3): 211–217.

Modulation of HIV Transmission by *Neisseria gonorrhoeae*: Molecular and Immunological Aspects

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Abstract

Neisseria gonorrhoeae (GC), a major cause of pelvic inflammatory disease, can facilitate HIV transmission. In response to GC infection, genital epithelial cells can produce cytokines, chemokines and defensins to modulate HIV infection and infectivity. GC can also induce the production of cytokines and chemokines in monocytes and modulate T cell activation. *In vivo*, an increase in the number of endocervical CD4⁺ T cells has been found in GC-infected women. Additionally, GC appears to modulate HIV-specific immune responses in HIV-exposed sex workers. Interestingly, *in vitro*, GC exhibits HIV enhancing or inhibitory effects depending on the HIV target cells. This review summarizes molecular and immunological aspects of the modulation of HIV infection and transmission by GC. Future studies using a multi-cellular system or in animal models will offer insight into the mechanisms by which GC increases HIV transmission.

Keywords

Neisseria gonorrhoea; GC-HIV co-infection; HIV transmission

INTRODUCTION

Sexual transmission is the most common route (~85%) of HIV infection, and women account for nearly half of HIV-infected individuals worldwide and more than 70% in sub-Saharan Africa [1, 2]. Although the spread of HIV is not efficient, occurring in only about one of every 1,000 episodes of sexual intercourse with an infected person, epidemiologic and clinical studies strongly indicate that the presence of other ulcerative and non-ulcerative

sexually transmitted infections (STIs) increase the likelihood of HIV transmission [3-8]. In addition, a significant increase in the prevalence of STIs in China, Eastern Europe and Russia correlates with a rapid increase in new HIV infections. Women are more susceptible to HIV infection because of a higher prevalence of STIs [1, 2]. Sexually transmitted pathogens including herpes simplex virus (HSV), human papillomavirus, *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), and *Trichomonas vaginalis* are associated with increased HIV shedding in genital secretions [3, 9, 10], and treatment of STIs decreases this shedding [3, 11-13]. STIs not only increase HIV shedding in HIV-infected adults, but they also increase susceptibility to HIV infection in those who are HIV-negative [4, 5, 11]. Unlike HSV2, GC infection does not cause ulceration, and semen from GC-infected men has an increased HIV load when compared to infected men after receiving antimicrobial therapy [6]. Several epidemiological studies demonstrate that GC infection is a risk factor for HIV transmission [3, 11, 14, 15]. However, studies in men who have sex with men (MSM) report conflicting data on the role of GC in increasing rectal HIV transmission [16, 17]. This review will primarily focus on the role of GC in HIV transmission at the cellular and molecular levels. Understanding the mechanisms by which GC increases HIV transmission may offer insights into novel approaches to prevent HIV transmission.

GONOCOCCAL INFECTION

Gonorrhea, caused by the gram-negative diplococcus *Neisseria gonorrhoeae*, is one of the oldest recorded human diseases (reviewed in [18]). Today, GC infection remains a major global health problem with more than 60 million new cases diagnosed worldwide each year [19]. Men with gonorrhea frequently have acute urethritis, an inflammatory response mediated by GC infection [20], with only a small percentage of infected men remaining asymptomatic [18]. Gonorrhea is the most frequently identified cause of proctitis in men who have sex with men [21]. In contrast, 50-80% of women with lower genital tract GC infection are asymptomatic [22, 23], and 45% of women with GC cervicitis will develop an ascending infection which is a prerequisite for pelvic inflammatory disease in 10-20% of female infections [18, 24].

Infection of the genital mucosa by GC involves attachment to and invasion of epithelial cells [25-27]. GC infection of epithelial cells involves the phase-variable expression of several adhesins, including pili, opacity-associated (Opa) proteins, lactosyl lipooligosaccharide (LOS), and PorB porin protein. Of these, the roles of pili and Opa are the most well-characterized [28, 29]. These bacterial components can induce production of cytokines through activation of toll-like receptor 2 (TLR2) and TLR4 (Fig. 1). The effect of GC infection on induction of cytokines and chemokines *in vitro* and *in vivo* is summarized in Table 1.

LOS, which interacts with TLR4 [30], has been shown to induce the secretion of TNF- α , IL-1 β , IL-6, and IL-8 in primary urethral epithelial cells [26]. In experimental gonococcal infection of men, the levels of the same four cytokines have been found to be elevated in both the urine and the plasma after gonococcal challenge [20]. Studies of cytokine induction in response to GC infection in women have not been consistent [18]; IL-1, IL-6, and IL-8 levels do not increase in genital tract secretions from women with GC cervicitis [31], but are

elevated in immortalized human cervical and vaginal epithelial cells [27]. In primary human monocytes, phosphoryl and acyl moieties in the lipid A portion of LOS activate NF- β B through the TLR4-MD2 signaling pathway and induce a broad range of cytokines including TNF- β , IL-1 β , IL-6, IL-8, IL-10, IL-12, IL-18, RANTES, MIP-1 α , MIP- α , and MCP-1 [32, 33]. For IL-1 β and IL-18, the induction of expression proceeds through NLRP3 (nucleotide-binding domain, leucine-rich repeat 3) activation and inflammasome formation, which is dependent on activation of the cysteine-proteinase cathepsin B [33]. Activation of NLRP3-mediated inflammatory response pathways also promotes NLRP3-dependent monocytic cell death *via* pyro necrosis in response to GC infection. LOS activates both MyD88 and TRIF-dependent pathways through NF- κ B and IRF-3, respectively. Furthermore, it plays a role in induction of cell surface CD80 on monocytes through IRF1 [32]. In addition to induction of pro-inflammatory cytokines, an increase in the number of endocervical CD4+ T cells is found in GC-infected women [34]. Thus, GC infection may enhance HIV infection at the genital mucosa by activating immune cells and increasing the numbers of HIV target cells.

Opa proteins from various GC strains can bind to heparan sulfate proteoglycans (HSPG), including syndecan, vitronectin and fibronectin (reviewed in [29]), as well as CEACAM1 (carcinoembryonic antigen-related cellular adhesion molecule; CD66) [35]. Interestingly, the binding of GC expressing various Opa proteins enhance or inhibit primary CD4+ T cell proliferation [36]. For example, the binding of GC expressing Opa₅₀ to HSPG promote proliferation of primary CD4+ T cells in response to IL-2 and anti-CD3 antibody (Ab) or anti-CD3/CD28 Ab stimulation, whereas the binding of GC expressing Opa₅₂ to CEACAM1 suppresses proliferation of CD4+ T cells activated in response to T cell receptor activation. HSPG plays an important role in HIV attachment [37], although it remains to be determined whether the binding of GC to HSPG modulates HIV attachment. Similarly, down-regulation of CD4+ T cell proliferation by the binding of GC to CEACAM1 may impact HIV infection of activated CD4+ T cells.

HIV TRANSMISSION AT FEMALE GENITAL MUCOSA

After vaginal challenge with SIV, viral RNA can be detected in the endocervix and the transformation zone (the junction between endocervix and ectocervix) within 3-4 days (reviewed in [3, 38]). CD4+ T cells are the principal target in acute SIV and HIV infection and are crucial for the establishment and dissemination of HIV infection in mucosal tissues [39]. While both activated and resting CD4+ T cells are the initial target cells for SIV and HIV, resting CD4+ T cells are the major and earliest target cells during sexual transmission in the SIV rhesus macaque models [40]. In early SIV infection, over 60% of infected CD4+ T cells are memory cells that express low levels of CCR5 [41]. In early HIV infection, the majority of HIV-infected CD4+ T cells (viral RNA+ cells) do not have early and other activation markers such as CD25, CD71, CD30, CD38, and CD134, indicating that HIV can propagate in resting CD4+ T cells in addition to activated CD4+ T cells [40, 42]. In addition to CD4+ T cells, dendritic cells (DCs) and macrophages are also involved in HIV amplification and dissemination (reviewed in [43]). Several molecular mechanisms of HIV transmission at the vaginal mucosa have been proposed based on *in vitro* studies (reviewed in [44, 45]). HIV can infect CD4+ T cells in the cervical lumen, cross the mucosal epithelium *via* transcytosis, and can be captured by subepithelial DCs and Langerhans cells

and transferred to CD4⁺ T cells. Of note is that Langerhans cells can capture HIV through Langerin but do not transmit HIV efficiently compared to DCs utilizing DC-SIGN to capture HIV with subsequent transfer to CD4⁺ T cells [46]. This suggests a protective role of Langerhans cells against mucosal HIV transmission. Once it has crossed the mucosa, HIV is then disseminated and amplified in the lymph nodes where many activated CD4⁺ T cells reside.

GC INFECTION MODULATES HIV-SPECIFIC IMMUNE RESPONSES

During chronic HIV infection, GC infection has been associated with a transient increase in plasma viremia and in plasma type 2 cytokines such as IL-4 and IL-10, and a decrease in CD4⁺ T cell counts [47]. An increase in plasma viral load and enhanced activation of circulating CD4⁺ T cells are found in HIV-infected sex workers with gonococcal cervicitis [48]. Gonococcal cervicitis is also associated with reduced systemic CD8⁺ T cell IFN- γ responses in HIV-infected as well as in highly-exposed, persistently seronegative female sex workers [49]. Taken together, GC cervicitis is associated with enhanced Th2 responses but reduced Th1 responses. Conflicting results were found in a study designed to address the immune responses in women with mucosal GC co-infection during HIV acquisition by monthly screening for STIs in high risk, HIV seronegative Kenyan female sex workers [50]. GC co-infection during HIV acquisition was associated with an increased breadth and magnitude of systemic HIV-specific CD8⁺ T cell responses using IFN- γ and MIP-1 β as cytokine biomarkers, although there was no difference in the HIV viral load set point. This association was not found in women with other genital infections including *C. trachomatis*, *T. vaginalis* and bacterial vaginosis prior to HIV acquisition [50].

IN VITRO EFFECT OF GC INFECTION OF HIV TARGET CELLS

Depending on the target cells, GC can promote or inhibit HIV infection *in vitro*. The effect of GC on HIV infection is summarized in Table 2.

CD4⁺ T Cells

GC can active the HIV long-terminal repeat (LTR) in a transformed Jurkat CD4⁺ T cell line [51]. We have previously shown that GC exposure promotes replication of both CXCR4 (X4)- and CCR5 (R5)-dependent HIV-1 strains in primary resting CD4⁺ T cells [52]. While the HIV enhancing effect of GC is not dependent on the presence of Opa and pili, these bacterial components modulate the degree of HIV enhancement in primary resting CD4⁺ T cells. GC and TLR2 agonists, such as peptidoglycan (PGN), Pam₃CSK₄, and Pam₃C-Lip, a GC-derived synthetic lipopeptide, but not TLR4 agonists including LPS or GC LOS promoted HIV infection of primary resting CD4⁺ T cells after viral entry. Pretreatment of CD4⁺ T cells with PGN and GC also promoted HIV infection. PGN and GC exposure activate CD4⁺ T cells and induce cell-surface expression of HIV co-receptors, CCR5 and CXCR4. IL-2 is required for the HIV enhancing effect of TLR2 in primary CD4⁺ T cells. Anti-TLR2 Ab can neutralize the HIV enhancing effect of GC, indicating that GC enhances HIV infection of primary CD4⁺ T cells through TLR2 activation. Analysis of HIV DNA products revealed that GC exposure and TLR2 activation promote HIV nuclear import. Taken together, the results of our study demonstrate that GC exposure increases the

susceptibility of primary resting CD4⁺ T cells to HIV as well as enhances HIV infection in HIV-exposed CD4⁺ T cells. It is noteworthy that the effect of TLR2 activation on HIV infection is more pronounced in primary resting CD4⁺ T cells, the major HIV/SIV target cells during vaginal transmission [40, 42], than in activated CD4⁺ T cells [52]. Additionally, TLR2 activation promotes HIV infection of both naïve and memory CD4⁺ T cells [53]. Analysis of the molecular mechanism by which TLR2-mediated enhancement of HIV infection of resting CD4⁺ T cells revealed that TLR2 activation promotes HIV nuclear import and infection through a T cell function dependent and independent manner [54]

While transformed cell lines frequently serve as useful tools to understand various aspects of HIV infection, it is crucial to validate *in vitro* studies using primary cells as transformed cell lines have altered signaling pathways. For example, IFN responsiveness differs between primary cells and transformed cell lines such as the Jurkat T cell line or the monocytic U1 cell line, which are often used in HIV research. Dobson-Belarie *et al.* demonstrated the differential response of primary and immortalized CD4⁺ T cells to GC-induced cytokines from PBMCs and their subsequent impact on HIV infection [55]. Conditioned media from GC-exposed PBMCs promote X4 HIV infection of Jurkat CD4⁺ T cells, but inhibit X4 HIV infection of primary CD4⁺ T cells. Cytokine analyses of conditioned media from GC-exposed PBMCs compared to unexposed control cells demonstrate that GC exposure induces TNF α , IL-6, IL-1 β , and IFN α , but not IL-8 and IL-17. TNF α stimulation significantly increases HIV p24 production in Jurkat cells, but has much less impact on HIV infection of unstimulated primary CD4⁺ T cells, whereas IFN α strongly inhibits HIV replication in TCR-activated CD4⁺ T cells, but has no effect on infection of Jurkat cells. The lack of the IFN α signaling in Jurkat cells results in an HIV enhancing effect of conditioned media from GC-exposed PBMCs rather than the HIV inhibitory effect found in primary CD4⁺ T cells [55].

Dendritic Cells

GC promotes activation of monocyte-derived dendritic cells (MDDCs) and this activation is Opa independent [56]. GC exposure enhances both X4 and R5 HIV infection of MDDCs, but not monocytes. GC exposure does not modulate expression on the cell surface of CD4, CCR5, CXCR4 or DC-SIGN, which are HIV receptors on DCs. Analysis of specific bacterial components involved in the HIV enhancing effect of GC revealed that bacterial lipoprotein and peptidoglycan, but not GC LOS or *E. coli* LPS, promote HIV infection of MDDC suggesting a role for TLR2 activation (Fig. 1). The involvement of TLR2 activation in GC-mediated enhancement of HIV infection of MDDCs was confirmed using cells from TLR2-deficient HIV-1 transgenic mice [56].

Thibault *et al.* demonstrated that TLR2 and TLR4 activation exhibits contrasting effects on HIV infection of MDDCs and subsequent HIV transfer to CD4⁺ T cells [57]. In agreement with the report by Zhang *et al.* [56], TLR2 agonism promotes HIV infection of MDDCs. However, TLR4 activation inhibits HIV production in MDDCs. Delineation of underlying mechanisms indicates that, while both TLR2 and TLR4 activation increases the binding of DCs to HIV, TLR2 activation promotes production of HIV early reversed transcribed DNA products. In contrast, TLR4 activation induces type I IFN production (Fig. 1) and blocks the

step of reverse transcription in MDDCs [57]. Together with the report by Zhang *et al.* [56], it is interesting that TLR2 signaling is dominant over TLR4 signaling with respect to the effect of GC on HIV infection of MDDCs.

GC induces TNF α production in skin and vaginal biopsies and activation of TLR2 but not TLR4 consistently induces TNF α production in skin biopsies [58]. TNF α and TLR2 activation enhance HIV transmission *ex vivo* by increasing the susceptibility of Langerhans cells to HIV. Although this report demonstrates GC-mediated TNF α induction in explant models, the effect of GC on HIV transmission *ex vivo* as well as the role of TNF α and TLR activation in modulation of HIV transmission in explants in the setting of GC infection has not been defined.

GC exposure blocks HIV production in CD8-depleted PBMCs from HIV-infected individuals as well as *in vitro* HIV-infected PBMCs [59]. Anti-IFN α Ab blocks the HIV inhibitory effect of conditioned media from GC-exposed PBMCs depleted of CD8+ T cells. GC exposure induces IFN α production in plasmacytoid dendritic cells (pDCs) through TLR9 activation [59]. An elegant study by Li *et al.* demonstrates recruitment of pDCs and induction of IFN α and MIP-3 α in the endocervical epithelium after vaginal challenge with SIV in a macaque model [60]. These signals in combination with innate immune signals and inflammatory cells lead to recruitment of CD4+ T cells for HIV dissemination. Additionally, TLR7 and TLR9 agonists do not prevent vaginal transmission of SIV when intravaginally applied to rhesus macaques [61]. These agonists induce mucosal IFN production and recruitment of immune cells but increase plasma viral load. Thus, while pDCs block HIV replication *in vitro*, the role of pDCs in GC-mediated modulation of HIV transmission *in vivo* remains to be determined.

Macrophages

GC LOS from various strains as well as *E. coli* LPS trigger TLR4 activation (Fig. 1) and inhibit HIV infection of monocyte-derived macrophages (MDMs) [62]. Neutralization Ab against IFN β but not IFN α blocks the anti-HIV activity of GC LOS and *E. coli* LPS, indicating that IFN β is a mediator for GC-mediated HIV suppression in MDMs [62]. Our unpublished data indicated that GC, containing bacterial components that activate TLR2 and TLR4, inhibited HIV infection of MDMs. The TLR2 agonist, Pam₃CSK₄, had no effect on HIV infection, whereas TLR4 activation by LPS blocked HIV infection of MDMs, which explains the dominant effect of TLR4 over TLR2 in the effect of GC on HIV infection of MDMs. The specific role played by macrophages in mucosal HIV transmission in the setting of GC infection remains to be determined.

ROLE OF DEFENSINS IN GC-MEDIATED ENHANCED HIV INFECTIVITY

Mammalian defensins are abundant cysteine-rich antimicrobial peptides important for innate host defense [63]. The fact that defensin levels are frequently elevated in humans in response to mucosal infection [64-66] suggests that they may also modulate sexual transmission of HIV. Although several human defensins, including human neutrophil peptides (HNPs) 1-4 from neutrophils and human beta defensins (HBDs) 2-3 from epithelial cells, exhibit anti-HIV-1 activity [67, 68], other defensins, such as HBD1, display little anti-

HIV activity [69, 70], and paneth cell defensins (human alpha defensin (HD)5 and HD6) enhance HIV infectivity [71], indicating the specificity of defensins.

In addition to their role as antimicrobial peptides, defensins play an important role in modulating immune function. Both HNPs and HBDs display chemotactic activity for T cells, monocytes and immature DCs and can induce cytokine production in monocytes and epithelial cells [72]. Murine β -defensin-2 can recruit bone-marrow-derived immature DCs through CCR6 and can induce DC maturation through TLR4 [73]. HBD3 activates antigen presenting cells (DCs and monocytes) *via* TLR1/2 [74]. Defensins are frequently induced by pro-inflammatory cytokines or TLR activation [63, 68]. Conversely, defensins can induce cytokines and chemokines. HNPs upregulate the expression of CC-chemokines and IL-8 in macrophages and epithelial cells, respectively [75, 76]. HBD2, known to be inducible in response to bacterial infection and pro-inflammatory cytokines [63, 72] can up-regulate IL-6, IL-8, IL-10, MCP-1, IL-1 β , MIP-1 β and RANTES in PBMCs [77]. HD5 can induce IL-8 [78] that enhances HIV infection in cervical tissues [79]. While defensins exhibit anti-HIV activity, their immunomodulator role to induce inflammation and to recruit HIV target cells may facilitate HIV dissemination and amplification.

HD5 and HD6 are human α -defensins that play a role in mucosal immunity [63, 80-82]. HD5 and HD6 are constitutively expressed in intestinal Paneth cells, and HD5 is found in the epithelium of the vagina, endocervix and ectocervix [83-85]. Induction of HD5 has been shown to control pathogen invasion in the male urethra during CT and GC infection [64]. However, unlike HNPs and HBDs, which inhibit HIV infection in a single cell type system, we have shown that HD5 and HD6 enhance HIV entry and contribute to GC-mediated enhancement of HIV infectivity *in vitro* [71]. HD5 and HD6 significantly enhanced infectivity of HIV-1 R5 strains [71], which are preferentially transmitted during mucosal transmission of HIV. Studies on the molecular mechanism of the HIV enhancing effect of HD5 and HD6 indicate that defensins enhance HIV infection through promoting HIV attachment [86]. HD5 but not HD6 competes with heparan for binding to HIV. Importantly, these defensins block *in vitro* anti-HIV activity of polyanionic microbicides, which have failed to protect women against HIV infection, and to interfere with anti-HIV activity of HIV entry and fusion inhibitors under specific conditions [86, 87]. Taken together, in response to GC infection, cervicovaginal epithelial cells may secrete high levels of HD5 and HD6 into the lumen to control bacterial infection. However induction of defensins results in promoting HIV infection. HD5 is normally present at a concentration of 1-50 μ g/ml in vaginal fluid and is induced in women with bacterial vaginosis [88] and in men with GC and CT. Although there is no report with respect to the concentrations of mucosal defensins in GC-infected women, HD5 and HD6 at 10 μ g/ml are sufficient to enhance HIV infection *in vitro* and are most likely biologically relevant in women with STIs.

SUMMARY

GC appears to facilitate HIV infection/transmission through multiple mechanisms. GC infection results in cytokine/chemokine production by epithelial cells and immune cells, recruitment of CD4⁺ T cells and other immune cells, and induction of defensins that can enhance HIV infectivity. The mechanism by which GC increases the risk of HIV

transmission *in vivo* is not well-defined in part due to the lack of ideal animal models to study HIV-GC co-infection as both GC and HIV primarily infect humans. Although rhesus macaques have been widely used for SIV transmission studies and can be used to investigate GC pathogenesis, co-infection studies have not been reported in this model. While results of studies on the effect of GC on HIV infection using specific immune cells may be important, it is important to gain insights into the impact of GC on HIV infection in a multi-cellular system such as tissue explants or in animal models. Monthly antibiotic chemoprophylaxis reduced the incidence of STIs but did not reduce the risk of HIV acquisition in Kenyan sex workers [89], highlighting the complexity of STI and HIV co-infection. Future studies on the mechanism of GC-HIV co-infection will have direct implications for HIV prevention.

Acknowledgments

This work was supported by the U.S. Department of Veterans Affairs, Office of Biomedical Laboratory Research and Development, and by National Institutes of Health Grants AI063927 (GAJ) and AI A1081559 (TLC) from the National Institute of Allergy and Infectious Diseases.

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Fig. (1). GC induces cytokine production through TLR2 and TLR4 signaling pathways
 Formation of the TLR4-MD2-LOS complex can activate MyD88-dependent or TRIF-dependent pathways. The GC bacterial components, Porin and lipoprotein, induce NF-κB activation and cytokine production through MyD88-dependent pathways.

Table 1

Effect of GC on Induction of Cytokines and Chemokines

Targets	Induction of Cytokine/Chemokine	References
Men with GC infection	TNF- α , IL-1 β , IL-6, IL-8	[20]
Women with GC cervicitis	No increase in IL-1, IL-6, and IL-8	[31]
Urethral epithelial cells	TNF- α , IL-1 β , IL-6, IL-8	[26]
Cervical, vaginal epithelial cells	IL-1, IL-6, IL-8	[27]
Monocytes	TNF- α , IL-1 β , IL-6, IL-8, IL-10, IL-12, IL-18, RANTES, MIP-1 α , MIP-1 β , and MCP-1	[32,33]

Table 2Effect of GC on HIV Infection *In Vitro*

Target Cells or Tissues	HIV Effect	Mechanism(s)	Reference
Transformed CD4 Jurkat cells	enhance	activates HIV LTR promoter	[51]
Primary CD4+ T cells	enhance	Induces expression of cell surface HIV co-receptors, activates T cells, and promotes HIV nuclear import after viral entry <i>via</i> TLR2 activation	[52]
PBMCs	inhibit	Induces IFN α production <i>via</i> pDCs	[59]
MDDCs	enhance	Promotes HIV infection <i>via</i> TLR2 activation	[56]
MDMs	inhibit	Induces IFN β production	[62]
Skin, vaginal biopsies	NA	Induces TNF α production	[58]

LTR: long-terminal repeat; PBMCs: peripheral blood mononuclear cells; pDCs: plasmacytoid dendritic cells; MDDCs: monocyte-derived dendritic cells; MDMs: monocyte-derived macrophages; NA: not available.