Neisseria gonorrhoeae effectively blocks HIV-1 replication by eliciting a potent TLR9-dependent interferon-α response from plasmacytoid dendritic cells

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Summary

Clinical and epidemiological research provides evidence for a positive correlation between Neisseria gonorrhoeae infection and HIV transmission; however, mechanistic studies examining this relationship have yielded conflicting results. To explore this interaction, we exposed ex vivo cultured peripheral blood cells from acute HIV+ individuals to N. gonorrhoeae. Unexpectedly, we observed a profound inhibition in HIV-1 replication in the ex vivo cultures, and this was recapitulated when peripheral blood mononuclear cells (PBMCs) from healthy donors were co-infected with HIV-1 and N. gonorrhoeae. Next, we established that gonococcal-infected PBMCs liberated a soluble factor that effectively blocked HIV replication. Cytokine analyses and antibody blocking experiments revealed that the type I interferon, interferon-α (IFNα), was expressed upon exposure to N. gonorrhoeae and was responsible for the inhibition of HIV-1. Intracellular staining, TLR9-blocking and cell depletion-based studies demonstrated that the IFNα was elicited by plasmacytoid dendritic cells (pDCs) in a TLR9-dependent manner. The pDC response to N. gonorrhoeae was unexpected given pDCs more established role in innate defence against intracellular pathogens, suggesting this may be a bacterial immune evasion strategy. In the context of HIV, this overcomes the virus’s otherwise effective avoidance of the interferon response and represents a previously unrecognized intersection between these two sexually transmitted pathogens.

Introduction

As of 2007, approximately 33 million people worldwide were infected with the human immunodeficiency virus (HIV) and 2.7 million new infections were occurring annually (UNAIDS, 2008). Sexual intercourse is the main route of HIV transmission, yet the relative transmissibility by this route is low, with seroconversion occurring in as few as 1 in every 1000 episodes of intercourse with an infected partner (Galvin and Cohen, 2004). Efforts to stop the spread of this relatively non-infectious pathogen have sparked great interest into the external factors contributing to transmission. Several clinical and epidemiological studies have observed a positive correlation between co-infection with other sexually transmitted infections (STIs) and increased genital tract viral shedding and/or susceptibility to HIV-1 (Cohen, 1998; Fleming and Wasserheit, 1999; Galvin and Cohen, 2004).

Gonorrhoea is one of the most prevalent STIs, with an estimated 62 million new infections occurring globally per year (World Health Organization, 2001). It is a non-ulcerative STI, but symptomatic infection is characterized by localized inflammation and purulent urethral or cervical discharge (Salyers and Whitt, 2002). Neisseria gonorrhoeae, a Gram-negative bacterium, is the aetiological agent of gonorrhoea. The organism primarily infects the mucosal surfaces of the endocervix in women and the urethra in men, where it may either be associated with the massive infiltration of neutrophils that is the hallmark of gonorrhoea or persist in an ‘asymptomatic’ carrier state. Spread of the gonococci from the original site of infection can eventually lead to upper genital tract damage or disseminated infections (World Health Organization, 2001; Salyers and Whitt, 2002). The failure of asymptomatic...
individuals to seek treatment is one of the major factors contributing to the persistence of this organism within the population (Salyers and Whitt, 2002).

Until recently, gonococcal infection was presumed to facilitate the transmission of HIV. Simultaneous infection with gonorrhoea and HIV-1 had been associated with an increase in HIV-1 viraemia (Anzala et al., 2000; Nkengasong et al., 2001), and a decrease in CD4+ T lymphocyte counts (Anzala et al., 2000) and CD8+ T lymphocyte responses (Kaul et al., 2002). Symptomatic gonococcal infection has also been associated with more frequent HIV-1 DNA detection rates from urogenital tract swabs in both men and women (Moss et al., 1995; Ghys et al., 1997), as well as higher viral RNA concentrations in the seminal fluid of men (Cohen et al., 1997). Treatment of the gonococcal infection reversed these effects to a significant level in these patients. In terms of HIV transmission, gonorrhoea has been associated with a two- to fivefold increased rate of male to female HIV-1 transmission (Fleming and Wasserheit, 1999). Consistent with these observations, in vitro evidence suggesting a possible role for bacterial-induced human defensins in stimulating viral infectivity has recently been published (Klotman et al., 2008). However, when clinical studies designed to use treatment for various STIs as a preventive measure against HIV infection at the population level were performed, only one of three studies demonstrated a decrease in HIV acquisition (Korenromp et al., 2005). Moreover, a recent study found that women in a Kenyan commercial sex worker cohort in Africa who had gonorrhoea while, or just prior to, contracting HIV had stronger and broader virus-specific immune responses after HIV acquisition than those who were either not co-infected or were infected with other STIs (Sheung et al., 2008). This suggests that the link between gonorrhoea and HIV, or between HIV and STIs in general, may be more complicated than was previously believed.

The first in vitro study examining the effect of gonococcal infection on HIV replication demonstrated that N. gonorrhoeae directly stimulated HIV-1 long terminal repeat (LTR)-mediated transcription from a CD4+ T-cell line (Chen et al., 2003). Subsequent work established that gonococci can also stimulate HIV expression from monocyte-derived dendritic cells (Zhang et al., 2005) and primary CD4+ T cells (Ding et al., 2010) in vitro. However, in vitro treatment of HIV-infected primary macrophages with gonococcal lipo polysaccharide (LPS) can stimulate viral infectivity has recently been published (Sjolinder et al., 2008). This work, combined with recent in vivo data, reveals an unexpectedly complex interplay between the life cycle of these two important sexually transmitted pathogens.

Results

**Gonococcal infection inhibits ex vivo HIV-1 replication**

While exposure to N. gonorrhoeae can stimulate HIV expression by a purified infected T cell (Chen et al., 2003; Ding et al., 2010), we considered that this interaction cannot happen in isolation in vivo. Since both pathogens are human-restricted, we sought to develop an ex vivo infection protocol which could consider the effect of other cell types on N. gonorrhoeae-induced HIV expression. To this end, we isolated peripheral blood mononuclear cells (PBMCs) from HIV+ individuals and then exposed them to viable bacteria. While there is a paucity of HIV-infected cells in peripheral blood during chronic infection (Koot et al., 1996), the high titre of virus in acute patients provides a pool of in vivo-infected cells with a relatively high proportion of HIV-1-infected cells, thereby increasing the probability of detecting ex vivo HIV replication. As such, we selected volunteer donors at the acute stage of HIV infection to try and determine the effect of gonococci on HIV replication at an early stage of HIV disease and a high viral load. PBMCs were then depleted of CD8+ T lymphocytes by immunomagnetic selection to remove HIV-specific cytotoxic cells that would diminish detection of HIV during ex vivo culture, and our early studies confirmed that HIV replication was not apparent unless the CD8+ T cells were depleted (data not shown).

CD8-depleted PBMCs were infected with gonococci at a multiplicity of infection (MOI) of one bacterium per cell and then cultured for 10 days. HIV-1 p24 levels were
PBMCs from acute HIV patients. Time-course and viability of co-infected cultures (Fig. 1A–C). This effect was not the result of bacterial-induced cytotoxicity since the number of viable cells detected after 10 days of culture in CD8+ T lymphocyte-depleted PBMCs from a variety of acute HIV+ patients left uninfected or infected with an moi of 1. Infections with N. gonorrhoeae expressing no bacterial adhesin (C) or the defined colony opacity-associated (Opa) proteins or pilus (D) were performed in the presence or absence of T-cell receptor cross-linking antibodies. OM# represents the coded internal identification number of individual donors. OM296 and OM348 were cultured in the presence of 30 U ml⁻¹ IL-2, whereas OM12, OM17, OM18, OM357 were not. Data in (D) are representative of the mean ± SD of a single experiment per donor, and are representative of the results of three different donors.

Fig. 1. HIV-1 replication in PBMCs from acute HIV+ patients is inhibited by N. gonorrhoeae infection. A and B. Time-course and viability of ex vivo HIV-1 replication in uninfected or N. gonorrhoeae (moi = 1) infected CD8+ T lymphocyte-depleted PBMCs from acute HIV+ patients in the absence (OM12) (A) or presence (OM348) (B) of IL-2 or T-cell receptor cross-linking antibodies as HIV replication stimulus. HIV-1 replication was measured by ELISA for p24 antigen in culture supernatants and the level of viable cells was calculated by trypan blue exclusion. C and D. Resultant ex vivo HIV-1 replication levels after 10 days of culture in CD8+ T lymphocyte-depleted PBMCs from a variety of acute HIV+ patients left uninfected or infected with an moi of 1. Infections with N. gonorrhoeae expressing no bacterial adhesin (C) or the defined colony opacity-associated (Opa) proteins or pilus (D) were performed in the presence or absence of T-cell receptor cross-linking antibodies. OM# represents the coded internal identification number of individual donors. OM296 and OM348 were cultured in the presence of 30 U ml⁻¹ IL-2, whereas OM12, OM17, OM18, OM357 were not. Data in (D) are representative of the mean ± SD of a single experiment per donor, and are representative of the results of three different donors.

quantified by ELISA 4 and 10 days after gonococcal infection. While PBMCs cultured ex vivo normally displayed HIV expression, those that had been infected with N. gonorrhoeae had no detectable virus (Fig. 1A and B, left panels). Since HIV replication occurs more readily in activated T lymphocytes (Lassen et al., 2004), we attempted to stimulate HIV expression in N. gonorrhoeae-infected cultures through the addition of IL-2 and/or immobilized anti-human T-cell receptor-specific CD3 and CD28 antibodies. While these agonists potently stimulated HIV production by the ex vivo cell cultures that were not exposed to N. gonorrhoeae, virus was still unapparent in co-infected cultures (Fig. 1A–C). This effect was not the result of bacterial-induced cytotoxicity since the number of viable cells detected after 10 days of ex vivo culture with N. gonorrhoeae was as high or higher than cells cultured in the absence of these bacteria (Fig. 1A and B, bar graphs). It was also irrespective of the particular
bacterial adhesins expressed by *N. gonorrhoeae* since neither colony opacity-associated (Opa) protein nor pilus expression affected the response (Fig. 1D). To ascertain whether the effect was unique to *N. gonorrhoeae*, we co-infected the cells with *Escherichia coli*. While *E. coli* reduced HIV expression, it caused substantial cytotoxicity (92 ± 13%) which could clearly explain this effect (data not shown).

**Gonococcal infection inhibits in vitro HIV-1 replication**

Given that gonococcal infection effectively blocked *ex vivo* HIV-1 replication, we considered that the inhibition of HIV infection could relate to HIV-associated immunosuppression in our volunteer donors. Therefore, we isolated PBMCs from healthy (HIV-uninfected) donors and recapitulated the experiment performed with *ex vivo* HIV+ PBMCs. These cells were infected with HIV-1 R5 strain BaL in the presence or absence of immobilized anti-human CD3 and CD28 antibodies, as we had done for samples from acute HIV+ patients. As seen with HIV+ *ex vivo* cultured cells, HIV-1 replication in CD8−-depleted PBMCs infected with HIV-1 *in vitro* was still potently inhibited upon *N. gonorrhoeae* infection (Fig. 2A). This inhibition was dose-dependent, especially in the presence of stimulatory anti-human CD3 and CD28 antibodies. Therefore, gonococcal infection could inhibit HIV-1 replication from mixed cell populations even under *in vitro* HIV infection conditions where cell infection rates are substantially higher than in acute HIV+ patients. There was no reduction in CCR5 expression upon exposure to *N. gonorrhoeae* (data not shown), suggesting that the effect occurred after receptor binding.

CD4+ T lymphocytes are considered to be one of the key target cells of HIV-1 virus. We have also previously shown that direct contact between *N. gonorrhoeae* and an immortalized CD4+ T-cell line stimulates HIV-1 transcription (Chen et al., 2003). Therefore, we aimed to determine whether this gonococcal-induced inhibition was evident when primary CD4+ T lymphocytes were not in the context of a mixed PBMC culture. We purified CD4+ T lymphocytes by immunomagnetic selection and infected these *in vitro* with HIV-1 R5 strain BaL in the presence or absence of immobilized anti-human CD3 and CD28 antibodies. *N. gonorrhoeae* co-infection did not significantly affect HIV-1 replication (Fig. 2B), indicating that *N. gonorrhoeae* was not effecting CD4+ T lymphocytes directly.

*N. gonorrhoeae* stimulate PBMCs to produce a soluble factor that inhibits HIV

We next investigated whether the release of soluble inhibitory factor(s) by the gonococci and/or bacterially infected PBMCs could be responsible for the observed inhibition of HIV replication. This was accomplished by isolating the cell-free supernatants of CD8+ T lymphocyte-depleted (HIV+) PBMCs that had been infected with gonococci, and then placing these on CD4+ T lymphocytes that were infected with virus. We used both the HIV-1 R5 strain BaL and a recombinant HIV-1 virus that was pseudotyped with the vesicular stomatitis virus glycoprotein (VSVg) in place of the HIV-1 envelope. Virions from this recombinant virus are able to infect a wide variety of cell types independent of CD4, CXCR4 or CCR5 expression, allowing us to infer whether a block in receptor engagement is responsible for observed effects. The replication-defective nature of pseudotyped particles restricts them to a single infection cycle, allowing us to discriminate between a substantial block in the first replication cycle versus a less potent inhibitory effect cumulating over multiple rounds of HIV-1 replication. Supernatants from PBMCs left unexposed to the bacteria or from cultures containing gonococci but no PBMCs were used as controls. Culture supernatants from *N. gonorrhoeae* alone did not inhibit HIV expression, whereas the supernatants from *N. gonorrhoeae*-infected PBMCs effectively inhibited HIV-1 expression in both of these systems (Fig. 3A and B). Combined, this implies that *N. gonorrhoeae* promotes PBMC expression of a soluble factor that effectively blocks the first round of HIV-1 replication, presumably after initial receptor binding.

**IFNα is the N. gonorrhoeae infection-induced soluble factor that effectively blocks HIV replication**

A broad screen of cytokines and chemokines was performed by quantitative PCR and ELISA to identify potential soluble inhibitory factors expressed when PBMCs were exposed to the gonococci (data not shown). This survey revealed that the type I interferon, IFNα, was consistently increased in a dose-dependent manner upon gonococcal infection of PBMCs (Fig. 3C). While past work suggests that HIV can suppress IFN responses (Martinelli et al., 2007), a similar level of IFNα was produced in cells co-infected with gonococci and HIV-1 R5 BaL (Fig. 3D), indicating that the virus does not influence the gonococcal effect. While IFNα can be produced from a variety of cell types after the initial production of IFNβ, we were unable to detect IFNβ by ELISA or qPCR (data not shown). Since gonococcal lipo-oligosaccharide has been shown to stimulate the production of IFNβ from primary human macrophages (Liu et al., 2006), we considered that subdetectable levels of IFNβ from macrophages could be responsible for initiating the production of the IFNα we were detecting. Therefore, we depleted CD14+ cells prior to bacterial infection, and examined the effect on the production of IFNα from
Fig. 2. Gonococcal infection inhibits HIV-1 replication by in vitro infected PBMCs but not purified CD4<sup>+</sup> T lymphocytes. Resultant HIV-1 replication levels after 7 days of culture of HIV and N. gonorrhoeae co-infected (A) CD8<sup>+</sup> T lymphocyte-depleted PBMCs or (B) purified CD4<sup>+</sup> T lymphocytes. Cells were infected with HIV-1 R5 strain BaL just prior to co-infection with the indicated moi of N. gonorrhoeae. In vitro HIV-1 replication occurred in the presence or absence of T-cell receptor cross-linking antibodies as HIV-1 replication stimulus and was measured by HIV-1 ELISA for p24 antigen. The top two panels of each grouping represent the mean ± SD of the actual p24 antigen levels of triplicate wells of one experiment to represent viral load differences in the presence or absence of HIV-1 replication stimulus. In contrast, the bottom two panels of each grouping represent the mean ± SEM of a minimum of three separate experiments with the amount of HIV-1 replication expressed as the percentage of p24 antigen found in bacterial-uninfected samples. All bacterial infected samples in CD8<sup>+</sup> T lymphocyte-depleted PBMCs were significantly different from bacterial uninfected samples with \( P < 0.0001 \). No significant difference between bacterial-infected or uninfected samples in purified CD4<sup>+</sup> T lymphocyte was observed except in the bacterial-infected sample at an moi of 100 in the presence of T-cell receptor cross-linking antibodies, which reached a significance level of \( P = 0.0081 \).
gonococcal-infected PBMCs. Under these conditions, not only was IFNα production not inhibited, it was significantly increased in CD8+ and CD14+ double-depleted PBMCs (Fig. 3E) indicating that monocytes/macrophages did not initiate the IFNα production.

Next, we examined whether the induction of IFNα explained the gonococci-dependent inhibition of HIV-1 replication. This was accomplished by exposing the gonococcal-induced inhibitory supernatants to polyclonal antibodies capable of neutralizing either IFNβ or multiple types of human IFNα before applying the supernatants to cells infected with the recombinant HIV-1 virus or the replication-competent BaL. We observed that the IFNα antiseras reversed the inhibition mediated by the gonococcal infection-induced supernatants, while neither the IFNβ-specific nor the control sera had any significant effect (Fig. 4A and B). In the pseudotyped HIV system, the reversal was complete, with no statistically significant difference between the uninfected cell supernatant and the anti-IFNα-treated gonococcal-infected cell supernatant (Fig. 4A). In the BaL HIV-infected system, the neutralizing IFNα antibody caused a 10-fold increase in viral expression (Fig. 4B). While this was not complete reversal, since there was a persistent difference between uninfected cell supernatant and anti-IFNα-treated gonococcal-infected cell supernatant, this was presumably due to the difficulty

Fig. 3. *N. gonorrhoeae* infection of CD8+-depleted PBMCs results in the production of a soluble anti-HIV-1 factor coincident with the production of IFNα and irrespective of CD14+ cell presence.

A and B. Cell-free supernatants of CD8+ T lymphocyte-depleted HIV−PBMCs infected with an moi of one *N. gonorrhoeae* per cell were placed on purified CD4+ T lymphocytes infected with (A) receptor unrestricted pseudotyped HIV-1 (VSVg-NL4-3-ΔE) or (B) HIV-1 R5 strain BaL. Comparison controls of bacterial uninfected cells, whole bacteria-infected cells, and cultured supernatants from cultured media in the presence or absence of bacteria or from uninfected CD8+ T lymphocyte-depleted HIV−PBMCs were included. HIV-1 replication was quantified (A) 72 h or (B) 7 days post-HIV-1 infection by HIV-1 ELISA for p24 antigen. Data are representative of the mean ± SEM of a minimum of three separate experiments with the amount of HIV-1 replication expressed as the percentage of p24 antigen found in bacterial-uninfected samples.

C–E. (C and D) The level of IFNα from CD8+ T lymphocyte-depleted HIV−PBMCs infected with indicated moi of *N. gonorrhoeae* alone or co-infected with HIV-1 R5 strain BaL or (E) from single CD8+ T lymphocyte-depleted or double CD8+ T lymphocyte and CD14+-depleted HIV−PBMCs infected with an moi of 1 of *N. gonorrhoeae*. Exactly 5 μg ml−1 CpG DNA (ODN2216) was used as a positive control for IFNα production in (C). Supernatants were collected 24 h post-bacterial infection and IFNα levels were measured by ELISA. Data are representative of the mean ± SEM IFNα levels from a minimum of three separate donors. Data points present on the x-axis of IFNα ELISA graphs had undetectable levels of IFNα. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
in completely blocking all viral- and bacterial-induced IFNα-mediated responses when continual cycles of HIV replication can occur.

Gonococcal infection induces the production of IFNα by pDCs in a TLR9-dependent manner

Finally, we sought to determine which cell type and what signalling pathway was involved in the production of IFNα in response to N. gonorrhoeae infection. We performed intracellular staining (ICS) for IFNα with co-staining for a variety of cell markers, including T lymphocytes, B lymphocytes, NK cells, monocytes and pDCs. pDCs were the only apparent source of gonococcal infection-induced IFNα (Fig. 5A), and depletion of pDCs prior to N. gonorrhoeae infection confirmed that they were the source of all detectable IFNα (Fig. 5C). While the induced IFNα was consistently sufficient to block HIV expression, the kinetics and absolute amount of IFNα detected varied between experiments, due to either intrinsic differences between volunteer donors and/or sample preparation-dependent effects (Fig. 5B). Considering the recent demonstration that murine pDC inflammatory responses to N. meningitidis are attributable to TLR9 (Sjolinder et al., 2008), we considered that TLR9 may be contributing to the human pDC response to N. gonorrhoeae. As such, we utilized an inhibitory oligonucleotide molecule derived from human telomeric sequence known to block CpG interactions with TLR9 (Gursel et al., 2003) and found a complete inhibition of IFNα production, indicating that the pDC response was fully attributable to TLR9 signalling (Fig. 5D).

Discussion

The prevalence of N. gonorrhoeae and its reported effect on HIV-1 expression imply that co-infections involving these two pathogens can have a dramatic effect on the spread of HIV. Due to the lack of relevant animal models for either N. gonorrhoeae or HIV-1, in vivo co-infection studies are not possible. Studies aiming to delve deeper to explain clinical correlates have employed immortalized cell lines or single primary cell types in isolation. These models do not allow one to appreciate the interplay between the different cell types that exist in vivo. Our ex vivo model reflects the level and frequency of ex vivo HIV-1+ cells and peripheral blood cell types; while HIV+ individuals have relatively low numbers of HIV+ cells (Koot et al., 1996), those cells that are expressing the virus were infected in a natural context. In fact, because the effects revealed in this study involve communication between leucocyte types, they have not been apparent in cell lines or purified cell systems.

Based upon previous studies with purified cells and/or immortalized cell lines, we expected that N. gonorrhoeae co-infection would stimulate HIV expression. Instead, we observed a profound inhibition of HIV-1 replication during co-infection, even at an moi of only one gonococcus per cell in both ex vivo cultured and in vitro co-infected...
Gonococcal suppression of HIV

**Fig. 5.** Plasmacytoid dendritic cells produce IFNα in response to *N. gonorrhoeae.*

A. Flow cytometric histogram overlay of the level of intracellular IFNα in BDCA-2+ cells infected 16 h prior with indicated moi of whole *N. gonorrhoeae* in mixed CD8+ T lymphocyte-depleted HIV+ PBMC culture from donor SG-02.

B. Time-course of the percentage of BDCA-2+ cells positive for IFNα after infection with indicated moi of whole *N. gonorrhoeae* in mixed CD8+ T lymphocyte-depleted HIV+ PBMC. Each graph represents an individual donor. SG# represents the coded internal identification number of individual donors.

C. Level of IFNα liberated from cells either sorted to remove BDCA-2+ cells prior to bacterial infection or left unsorted and infected with bacteria for a period of 24 h. Data points present on the x-axis had undetectable levels of IFNα.

D. Level of IFNα liberated from cells infected with bacteria or a positive control CpG oligonucleotide in the presence or absence of an inhibitory oligonucleotide that blocks CpG and TLR9 interactions (iCpG). The comparative dose of iCpG was normalized by molarity to the amount of stimulatory CpG given in the control. IFNα levels were measured by ELISA. Data are representative of the mean ± SEM from a minimum of three separate donors. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

systems. This inhibition occurred irrespective of *N. gonorrhoeae* Opa protein or plius expression, suggesting that these adhesins do not influence this effect. While culture supernatants from *N. gonorrhoeae* itself did not inhibit HIV-1 replication, supernatants from PBMCs isolated from healthy (HIV-) individuals and then exposed to the gonococci did. This suggested that *N. gonorrhoeae* stimulated the production of a soluble HIV-inhibitory factor from one or more PBMC cell types. As a first step to define a possible mechanism of action, we monitored viral expression by cells co-infected with *N. gonorrhoeae* and VSVg-pseudotyped HIV-1. This recombinant virus allowed us to determine if the soluble factor was acting to alter HIV-1 receptor and/or co-receptor-mediated processes since the pseudotyped virus bypasses these stages in the HIV-1 life cycle. Since gonococcal infection caused ~80% reduction in expression of the pseudotyped virus, we deduce that the block likely occurs after receptor binding. *N. gonorrhoeae*-infected PBMC culture supernatants also effectively blocked replication-competent HIV-1 BaL expression in purified CD4+ T cells, establishing that the soluble PBMC-derived factor could inhibit HIV replication in the absence of viable bacteria.

A screen for *N. gonorrhoeae*-induced factors with potential to suppress T-cell activation and/or dampen HIV-1 replication unexpectedly revealed a substantial type I interferon IFNα expression in response to the gonococci. In most cell types, innate immune signalling can lead to the production of IFNβ. This binds to the cells’ type I interferon receptor, stimulating the production of the transcription factor, IRF7, which drives IFNα gene expression and effectively creates an autocrine loop of type I interferon production (reviewed in Mogensen et al., 1999; Sato et al., 2001; Taniguchi et al., 2001). Gonococcal lipoooligosaccharide has previously been shown to induce IFNβ in purified primary macrophages (Liu et al., 2006), yet we could not detect any IFNβ production by either RT-PCR or ELISA. To clarify the role of IFNβ and demonstrate a causal role for IFNα in the gonococcal-induced inhibition of HIV-1 replication, we exposed the culture supernatants from gonococcal-infected PBMCs to antibodies that block these two cytokines individually. While the IFNα-neutralizing antibodies effectively eliminated the HIV-inhibitory activity of the supernatant, the IFNβ-specific antibodies had no effect. Moreover, the magnetic depletion of CD14-expressing cells prior to gonococcal infection actually increased the amount of IFNα produced in response to gonococcal infection, suggesting that any monocyte/macrophage-derived IFNβ (Liu et al., 2006) was not responsible for the appearance of IFNα in our mixed PBMC system.

IFNα has well-documented anti-viral effects including the ability to inhibit host cell transcription and cell proliferation and/or induce apoptosis of the infected cell (Stark et al., 1998). It has been shown to specifically inhibit the early stages of HIV-1 replication, possibly by decreasing co-receptor expression (Shirazi and Pitha, 1992; 1998) and inhibiting viral particle release (Ho et al., 1985; Ferrie et al., 1991; Meylan et al., 1993; Baca-Regen et al., 1994; Coccia et al., 1994; Okumura et al., 2006; Neil et al., 2008). To identify the cell type(s) producing IFNα, we performed ICS for IFNα after gonococcal infection and then identified the IFNα-expressing cell type using surface markers. pDCs were the only cell type expressing IFNα in the *N. gonorrhoeae*-infected samples, and depletion of this cell type prior to *N. gonorrhoeae* infection decreased the levels of IFNα to undetectable levels. pDCs are considered powerhouses for IFNα production since they are the only cell type known to constitutively expresses IRF7 at high levels in the absence of IFNβ (Izaguirre et al., 2003), thereby explaining our ability to detect IFNα without IFNβ. pDCs are normally present in the genital tract and a further influx occurs upon infection with the sexually transmitted intracellular pathogens herpes simplex virus 2 (HSV2) (Lund et al., 2006) and Chlamydia (Agrawal et al., 2009; Moniz et al., 2009). While pDCs are integral to HSV2 control, their contribution to chlamydial immunity remains undefined (Agrawal et al., 2009; Moniz et al., 2009). It seems reasonable to assume that pDCs may also be recruited during the gonococcal-induced inflammatory response (Levine et al., 1998), testing this premise awaits future in vivo study.

In contrast to the broad number of microbial-associated molecular signals detected by the variety of TLRs expressed by other dendritic cell types, pDCs only
express the viral RNA-specific TLR7 and bacterial CpG-containing DNA-specific TLR9 (Jarrossay et al., 2001; Kadowaki et al., 2001). This, paired with recent demonstration that the closely related N. meningitidis elicits TLR9-dependent inflammatory responses in a variety of cell types (Mogensen et al., 2006; Magnusson et al., 2007), including murine pDCs (Spolinder et al., 2008), suggested that gonococcal DNA may also contribute to the IFNα response by human pDCs. Considering the breadth of innate immune agonists expressed by bacteria, it is remarkable that the HIV-inhibitory IFNα response elicited by N. gonorrhoeae was specifically attributable to TLR9. However, in contrast to macrophage and conventional dendritic cell responses, pDCs isolated from TLR9-deficient mice have a complete defect in type I interferon and inflammatory cytokine responses to N. meningitidis (Spolinder et al., 2008). While the parallel between the pathogenic Neisseria suggests that pDC activation through TLR9 is a true immune consequence of neisserial infection, the benefit of IFNα production during infection with an extracellular microorganism is not intuitive. Type I interferons have been shown to play a role in linking the innate immune system to the adaptive immune response through a variety of effects on dendritic cells, NK cells, T cells and B cells (reviewed in Rothenfusser et al., 2002; Bogdan et al., 2004). Type I interferon production in response to extracellular pathogens may therefore serve as an early innate mechanism to stimulate adaptive immune cell types. However, it must also be considered that the relatively uncharacteristic induction of type I interferon by the gonococci could serve as an immune evasion strategy to polarize the immune system towards a non-protective immune response.

In contrast to still unclear role of interactions between pDCs and bacterial pathogens, the role of pDCs in HIV-1 infection is an area of intense research due to the observation that the levels of pDCs correlate negatively with plasma viral load (Donaghy et al., 2001; Soumelis et al., 2001; Finke et al., 2004) and positively with both peripheral blood CD4+ T lymphocyte counts (Donaghy et al., 2001; Barron et al., 2003; Finke et al., 2004) and lymphoproliferative responses against HIV-1 p24 (Barron et al., 2003). Individuals with chronic HIV infection have lower pDC levels than uninfected controls, whereas long-term HIV non-progressors, who remain asymptomatic for long periods without HIV therapy, have higher pDC levels than uninfected controls (Soumelis et al., 2001). HIV-1 appears to actively circumvent pDC-mediated IFNα production as a method of immune evasion (Martinelli et al., 2007); however, the virus also stimulates the TRAIL-mediated cytotoxic activity in pDCs (Hardy et al., 2007) suggesting a direct protective role in addition to cytokine production. Considering that we observed that the level of IFNα was the same whether cells were infected with N. gonorrhoeae alone or were co-infected with N. gonorrhoeae and HIV, it is enticing to consider that gonococcal stimulation of pDCs may circumvent the normal HIV-mediated block on cytokine production and/or provide sufficient stimulus to surpass the higher activation threshold for IFNα release.

Finally, while other bacteria may elicit an IFNα response from pDCs, it is important to consider that the gonococci are unique in that their biological niche overlaps with the primary site of HIV exposure in both the male and female urogenital tracts. Indeed, gonococci clearly affect HIV transmission. For instance, while N. gonorrhoeae infection tends to correlate with increased HIV transmission and shedding in population-based studies (Cohen et al., 1997; Fleming and Wasserheit, 1999; Galvin and Cohen, 2004), it appears that exposure to gonococci during HIV acquisition may enhance adaptive immune responses to the virus (Sheung et al., 2008). Given the impact of IFNα on the adaptive immunity, including the recruitment of potential target cells to the co-infected site, our present findings may explain this effect. When taken together, the studies to date suggest that N. gonorrhoeae stimulates HIV-1 replication by upregulating host cellular activation pathways that lead through NF-κB in the infected CD4+ T cell (Chen et al., 2003; Ding et al., 2010), while simultaneously stimulating anti-viral immunity by eliciting a potent IFNα response by pDCs; the cumulative result of these opposing effects will ultimately determine the outcome in a co-infected individual. Therapeutic intervention in a manner that will tip the scale between these two distinct processes would thereby allow us to abrogate the synergistic effects of these two important human pathogens.

**Experimental procedures**

**Study participants**

The volunteer blood donors involved with this study were either healthy (HIV-uninfected) or were infected with HIV-1 but still within the acute phase of infection (infected for < 1 year) and not receiving any anti-retroviral medications. Informed consent was obtained from participants in accordance with the guidelines for conduct of clinical research at the University of Toronto and St. Michael’s Hospital, Toronto, Ontario, Canada. All investigational protocols were approved by the University of Toronto and/or St. Michael’s Hospital institutional review boards.

**Primary cell preparation**

PBMCs were isolated by leukopheresis (Spectra apheresis system; Gambro BCT) from the volunteers. The HIV+ donors had a mean viral load of 160,000 copies ml⁻¹ (range 1600–440,000) and mean CD4+ count of 726 cells μl⁻¹ (range 555–1312). Buffy coats were collected using Ficoll-Paque Plus (Amersham Biosciences) following the manufacturer’s instructions, and frozen.
at −80°C in 90% (vol/vol) heat-inactivated fetal calf serum (FCS) (HyClone) and 10% (vol/vol) dimethyl sulfoxide (DMSO) (Sigma-Aldrich) for subsequent experimentation. When needed, PBMCs were thawed and depleted of either CD8+ T lymphocytes alone, or both CD8+ T lymphocytes and CD14+ cells, or negatively selected for CD4+ T lymphocytes using the EasySep immumomagnetic-based cell purification system (StemCell Technologies) according to the manufacturer’s instructions.

HIV-1 p24 antigen ELISAs

The levels of HIV-1 present in culture supernatants were quantified by ELISA for p24 antigen using high sensitivity kits purchased from ZeptoMetrix Corporation when the levels of p24 were within the range of 7.8–313 pg ml−1 or from Biological Products Laboratory (Frederick Cancer and Research Development Centre) when the levels of p24 were above 313 pg ml−1. All ELISAs were performed according to manufacturer directions. ELISA plates were read at 450 nm on a VersaMax microplate reader using Softmax Pro 5.0 software (Molecular Devices).

Bacterial strains

The N. gonorrhoeae MS11-derived strains used in this study (Kupsch et al., 1993) were generously provided by T.F. Meyer (Max-Planck-Institut für Infektionsbiologie, Berlin, Germany). The Opa+ strain (N302) expresses neither the Opa proteins nor the pilus adhesins, and has a deletion in the opaC locus encoding this strain’s only heparan sulfate proteoglycan (HSPG) receptor-specific Opa variant. N303 expresses the HSPG-binding Opa60 variant and N313 expresses a carcinoembryonic antigen-related cell adhesion molecule (CEACAM)-specific Opa37 variant (Kupsch et al., 1993). Gonococci were grown at 37°C in a 5% CO2 humidified incubator from frozen stocks on GC agar (BBL – Becton Dickinson Microbiology Systems) supplemented with 1% (vol/vol) IsoVitaleX enrichment (BBL). Opa expression and variant type was monitored with a back-ill binocular microscope and confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%). Resolved proteins were subjected to immunoblot analysis using the Opa cross-specific monoclonal antibody 4B12/C11 (Achtman et al., 1988) which was generously provided by M. Achtman (Max-Planck-Institut für Infektionsbiologie, Berlin, Germany).

Replication-competent HIV-1 virus generation, titreing and infection

HIV-1 R5 virus BaL was generated in PBMCs pre-activated with 2 μg ml−1 PHA-L (Sigma-Aldrich) and 30 U ml−1 IL-2 (BD biosciences) in complete RPMI 1640 [10% (vol/vol) FCS, 1% (vol/vol) GlutaMax-1 (Gibco-Invitrogen)] for 48 h prior to HIV-1 infection. Half of the culture medium was removed and replaced with fresh medium containing 30 μl ml−1 IL-2 every 3–4 days. At peak levels of viral replication, as determined by ELISA for HIV-1 p24 antigen, virus was harvested by filtering through a 0.45 μm filter and stored in aliquots at −80°C. The level of infective HIV-1 R5 virus was measured with titreing by limiting dilution into PBMCs that were pre-activated as described for HIV-1 BaL stock generation. Supernatants were analysed 10 days post-infection by ELISA for HIV-1 p24 antigen and TCID50 ml−1 values were calculated using the Reed-Muench method (Koup et al., 2001). Primary CD8+ T lymphocyte-depleted PBMCs or purified CD4+ T lymphocytes were infected with an moi of 10−4 Bal in a total volume of 100 μl complete RPMI 1640 per 106 cells at 37°C for 2 h. Subsequently, cells were washed with cold RPMI 1640 medium containing 2% FCS (vol/vol) three times and resuspended to a concentration of 2 × 106 cells ml−1 in complete RPMI 1640 containing 30 U ml−1 IL-2 in preparation for bacterial infection.

Pseudotyped HIV-1 virus generation and infection

Replication-incompetent pseudotyped HIV-1 expressing the vesicular stomatitis virus glycoprotein as its envelope protein was generated as previously described with modifications (Zhou et al., 2005). 293T cells were co-transfected with pVSVG (BD biosciences) and pNL4-3-ΔE-EGFP (courtesy of Robert Siliciano, Johns Hopkins University School of Medicine, Baltimore, Maryland) using FuGENE 6 (Roche Applied Science) according to manufacturer’s instructions. Forty-eight hours post-transfection, VSVg-pseudotyped HIV-1 virus was isolated, clarified by centrifugation at 450 g for 10 min, and exposed to 20 U ml−1 benzonase (Sigma-Aldrich) for 30 min. Viral supernatant was then filtered through a 0.45 μm filter and concentrated by ultracentrifugation at 112 000 g for 2 h and 25 min at 4°C over a 20% sucrose cushion (20% sucrose (wt/vol) prepared in TNE buffer [20 mM Tris (pH 8.0), 150 mM NaCl and 2 mM EDTA]). The pellets were resuspended in a minimal volume of complete RPMI 1640, filtered through a 0.22 μm filter and frozen at −80°C in aliquots. The level of HIV-1 p24 in the concentrated sample was measured by ELISA. Purified CD4+ T lymphocytes were resuspended in 125 ng ml−1 VSVg-pseudotyped HIV-1 in a total volume of 100 μl per 106 cells and infected by spinoculation for 2 h at 1800 g at room temperature. Subsequently, cells were washed three times with cold RPMI 1640 medium containing 2% FCS (vol/vol) and resuspended to a concentration of 2 × 106 cells ml−1 in complete RPMI 1640 [10% (vol/vol) FCS, 1% (vol/vol) GlutaMax-1] in preparation for bacterial infection.

Gonococcal infection

CD8+ T lymphocyte-depleted PBMCs, or purified CD4+ T lymphocytes infected with virus in vivo from HIV+ donors or in vitro, as described, were either exposed to moi of gonococci ranging from 1 to 100 bacteria per cell or to a single moi of 1 for 3 h at 37°C in a 5% CO2 humidified incubator followed by the addition of 100 μg ml−1 gentamicin (Bioshop Canada) to prevent bacterial overgrowth. Cells were returned to the incubator for an additional 69 h when infected with VSVg-pseudotyped HIV-1, 7 days when infected in vitro with HIV-1, or 10 days when isolated from HIV+ patients. Incubations greater than 3 days received exchange of half of the culture medium every 3–4 days. Supernatants were collected by centrifugation at 450 g for 10 min at the end of each assay or during any medium changes, and frozen at −80°C till analysed for HIV-1 p24 antigen by ELISA. Upon completion of full incubation, cell viability was monitored by trypan blue exclusion using trypan blue solution (Sigma-Aldrich). All in vitro HIV-1-infected cells were cultured in medium containing 30 μl ml−1 IL-2. Cells from HIV+ patients were cultured in medium containing
uninfected or exposed to 5 µg ml⁻¹ CpGA (ODN2216, Invivogen) as negative and positive controls for IFNα production respectively. PBMCs were incubated for a total of 8, 12, 16 and/or 20 h at 37% in a 5% CO₂ incubator and 3 µg ml⁻¹ Brefeldin A (eBioscience) was added 6 h prior to the end of the incubation to block golgi transport. Subsequently, cells were collected and surface-expressed Fc receptors were blocked with human FcR blocking reagent (Milenyi Biotec) according to manufacturer directions. PBMCs were stained live for surface expressed human BDCA-2 (PE-BDCA-2, Miltenyi Biotec), CD3 (PerCp-CD3, BD Biosciences or APC-CD3, eBiosciences), CD4 (PerCp-CD4, BD Biosciences), CD8 (PE-CD8, BD Biosciences), CD14 (PE-CD14, eBiosciences or PerCp-CD14, BD Biosciences), CD19 (APC-CD19, eBiosciences) and/or CD56 (PE-CD56 and APC-CD56, BD Biosciences) in PBS containing 2% FBS (vol/vol) according to manufacturer directions. Cells were then fixed in Cytofix/Cytoperm™ (BD Biosciences) and internal Fc receptors were again blocked with human FcR blocking reagent. Finally, cells were stained for intracellular IFNα (FITC-IFNα2, ChromaProbe) using 0.2 µg per 10⁶ cells in Perm/Wash™ buffer (BD Biosciences) according to manufacturer directions. Approximately 1 000 000–2 000 000 live gated events were collected on a FACS Canto II flow cytometer using CellQuest software (BD Biosciences) and analysed using FlowJo software (TreeStar).

pDC depletion

CD8⁺ T lymphocyte-depleted PBMCs were stained live for BDCA-2, as described above, and sorted to remove all BDCA-2⁺ cells on a FACS Aria™ cell sorter (BD Biosciences) using FACS Diva™ software (BD Biosciences). Unsorted cells were maintained in PBS containing 2% FBS (vol/vol) on ice until the sorted sample was processed. Subsequently, both sorted and unsorted cells were infected with gonococci at moi of 1 and 10, incubated in the presence of 50 µg ml⁻¹ gentamicin, 50 IU ml⁻¹ penicillin and 50 µg ml⁻¹ streptomycin (pen/strep, Gibco), 5 µg ml⁻¹ Amphotericin B (Life Technologies) to preclude the growth of contaminating microorganisms after the sorting process. Culture supernatants were collected 24 h post-infection and analysed by ELISA for IFNα as described above.

TLR9 blocking assays

CD8⁺ T lymphocyte-depleted PBMCs were infected with whole bacteria or stimulatory CpGA as described above in the presence or absence of the TLR9-blocking CpG (ODN TTAGGG, Invivo- gen). Blocking CpG (iCpG) was titrated into the sample by molar ratio to the amount of stimulatory CpG, given as the positive control, using concentrations 10, 1 or 0.1 times the amount of CpGA. IFNα concentration was quantified 24 h post-bacterial infection or CpG exposure as described above.

Statistical analysis

Ex vivo experiments were performed in triplicate only once per donor due to cell availability restrictions, but were performed with multiple donors per observed effect. These data are presented as the mean ± the standard deviation of the experiment. In vitro experiments were all performed on at least three separate
occasions and are represented as the standard error of the mean. Inhibitory supernatants were generated in one donor and applied to cells from allogeneic donors to remove donor-specific effects. Statistical significance comparisons between two samples were calculated using the paired two-tailed Student’s t-test. Statistical significance comparisons between multiple samples were calculated using a one-way ANOVA using the Dunnett’s post-test and comparing to uninfected samples. All significance calculations were performed in Prism 5.0 software (GraphPad). Significant differences are represented by comparison (*) with the following legend: * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. Significance levels of P ≤ 0.05 were considered significant.

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References

Achtman, M., Neibert, M., Crowe, B.A., Strittmatter, W., Kusecek, B., Weyse, E., et al. (1988) Purification and characterization of eight class 5 outer membrane protein variants from a clone of Neisseria meningitidis serogroup A. J Exp Med 168: 507–525.

Agrawal, T., Vats, V., Wallace, P.K., Singh, A., Salhan, S., and Mittal, A. (2009) Recruitment of myeloid and plasmacytoid dendritic cells in cervical mucosa during Chlamydia trachomatis infection. Clin Microbiol Infect 15: 50–59.

Anzala, A.O., Simonsen, J.N., Kimani, J., Ball, T.B., Nagelkerke, N.J., Rutherford, J., et al. (2000) Acute sexually transmitted infections increase human immunodeficiency virus type 1 plasma viremia, increase plasma type 2 cytokines, and decrease CD4 cell counts. J Infect Dis 182: 459–466.

Baca-Regen, L., Heinzinger, N., Stevenson, M., and Gendelman, H.E. (1994) Alpha interferon-induced antiretroviral activities: restriction of viral nucleic acid synthesis and progeny virion production in human immunodeficiency virus type 1-infected monocytes. J Virol 68: 7559–7565.

Barron, M.A., Blyweis, N., Palmer, B.E., MaWhinney, S., and Wilson, C.C. (2003) Influence of plasma viremia on defects in number and immunophenotype of blood dendritic cell subsets in human immunodeficiency virus 1-infected individuals. J Infect Dis 187: 26–37.

Bogdan, C., Mattner, J., and Schleicher, U. (2004) The role of type I interferons in non-viral infections. ImmunoL Rev 202: 33–48.

Chen, A., Boulton, I.C., Pongoski, J., Cochrane, A., and Gray-Owen, S.D. (2003) Induction of HIV-1 LTR-mediated transcription by Neisseria gonorrhoeae. AIDS 17: 625–628.

Coccia, E.M., Krust, B., and Hovanessian, A.G. (1994) Specific inhibition of viral protein synthesis in HIV-infected cells in response to interferon treatment. J Biol Chem 269: 23087–23094.

Cohen, M.S. (1998) Sexually transmitted diseases enhance HIV transmission: no longer a hypothesis. Lancet 351 (Suppl. 3): 5–7.

Cohen, M.S., Hoffman, I.F., Royce, R.A., Kazembe, P., Dyer, J.R., Daly, C.C., et al. (1997) Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. Lancet 349: 1868–1873.

Ding, J., Rapista, A., Teleshova, N., Mosoyan, G., Jarvis, G.A., Klotman, M.E., et al. (2010) Neisseria gonorrhoeae enhances HIV-1 infection of primary resting CD4+ T cells through TLR2 activation. J Immunol 184: 2814–2824.

Donaghy, H., Pozniak, A., Gazzard, B., Qazi, N., Gilmour, J., Gotch, F., et al. (2001) Loss of blood CD11c(+) myeloid and CD11c(−) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load. Blood 98: 2574–2576.

Fernie, B.F., Poli, G., and Fauci, A.S. (1991) Alpha interferon suppresses virion but not soluble human immunodeficiency virus antigen production in chronically infected T-lymphocytic cells. J Virol 65: 3968–3971.

Finke, J.S., Shodell, M., Shah, K., Siegal, F.P., and Steinman, R.M. (2004) Dendritic cell numbers in the blood of HIV-1 infected patients before and after changes in antiretroviral therapy. J Clin Immunol 24: 647–652.

Fleming, D.T., and Wasserheit, J.N. (1999) From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect 75: 3–17.

Galvin, S.R., and Cohen, M.S. (2004) The role of sexually transmitted diseases in HIV transmission. Nat Rev Microbiol 2: 33–42.

Ghys, P.D., Fransen, K., Diallo, M.O., Ettingue-Traore, V., Couilbaly, I.M., Yeboue, K.M., et al. (1997) The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d’Ivoire. AIDS 11: F85–F93.

Gursel, I., Gursel, M., Yamada, H., Ishii, K.J., Takeshita, F., and Klinman, D.M. (2003) Repetitive elements in mammalian telomeres suppress bacterial DNA-induced immune activation. J Immunol 171: 1393–1400.

Hardy, A.W., Graham, D.R., Shearer, G.M., and Herbeuval, J.P. (2007) HIV turns plasmacytoid dendritic cells (pDC) into TRAIL-expressing killer pDC and down-regulates HIV coreceptors by Toll-like receptor 7-induced IFN-alpha. Proc Natl Acad Sci USA 104: 17453–17458.

Ho, D.D., Hartshorn, K.L., Rota, T.R., Andrews, C.A., Kaplan, J.C., Schooley, R.T., et al. (1985) Recombinant human interferon alfa-A suppresses HTLV-III replication in vitro. Lancet 1: 602–604.

Izaguirre, A., Barnes, B.J., Amrute, S., Yeow, W.S., Megiugorac, N., Dai, J., et al. (2003) Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. J Leukoc Biol 74: 1125–1138.

Jarrossay, D., Napolitani, G., Colonna, M., Stalluto, F., and
Lanzavecchia, A. (2001) Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur J Immunol* **31**: 3388–3393.

Kadowaki, N., Ho, S., Antonenko, S., Malefyt, R.W., Kastelein, R.A., Bazan, F., et al. (2001) Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* **194**: 863–869.

Kaul, R., Rowland-Jones, S.L., Gillespie, G., Kimani, J., Dong, T., Kiama, P., et al. (2002) Gonococcal cervicitis is associated with reduced systemic CD8+ T cell responses in human immunodeficiency virus type 1-infected and exposed, uninfected sex workers. *J Infect Dis* **185**: 1525–1529.

Klotman, M.E., Rapista, A., Teleshova, N., Micsenyi, A., Korenromp, E.L., White, R.G., Orroth, K.K., Bakker, R., Kaul, R., Rowland-Jones, S.L., Gillespie, G., Kimani, J., Dong, T., Kiama, P., et al. (2001) *Neisseria gonorrhoeae*-induced human defensins 5 and 6 increase HIV infectivity: role in enhanced transmission. *J Immunol* **180**: 6186–6185.

Koot, M., Wout, A.B., Kootstra, N.A., de Goede, R.E., Tersmette, M., and Schuitmaker, H. (1996) Relation between changes in cellular load, evolution of viral phenotype, and the clonal composition of virus populations in the course of human immunodeficiency virus type 1 infection. *J Infect Dis* **173**: 349–354.

Korenromp, E.L., White, R.G., Orroth, K.K., Bakker, R., Kamali, A., Serwadda, D., et al. (2005) Determinants of the impact of sexually transmitted infection treatment on prevention of HIV infection: a synthesis of evidence from the Mwanza, Rakai, and Masaka intervention trials. *J Infect Dis* **191** (Suppl. 1): S168–S178.

Koup, R.A., Ho, D.D., Poli, G., and Fauci, A.S. (2001) Isolation and quantitation of HIV in peripheral blood. *Curr Protoc Immunol* Chapter 12: Unit 12.2.

Kupsch, E.-M., Knepper, B., Kuroki, T., Heuer, I., and Meyer, T.F. (1993) Variable opacity (Opa) outer membrane proteins account for the cell tropisms displayed by *Neisseria gonorrhoeae* for human leukocytes and epithelial cells. *EMBO J* **12**: 641–650.

Lassen, K., Han, Y., Zhou, Y., Siliciano, J., and Siliciano, R.F. (2004) The multifactorial nature of HIV-1 latency. *Trends Mol Med* **10**: 525–531.

Levine, W.C., Pope, V., Bhoomkar, A., Tambe, P., Lewis, J.S., Zaidi, A.A., et al. (1998) Increase in endocervical CD4 lymphocytes among women with nonulcerative sexually transmitted diseases. *J Infect Dis* **177**: 167–174.

Liu, X., Mosoiyan, A., Li-Yun, C.T., Zerhouni-Layachi, B., Snyder, A., Jarvis, G.A., et al. (2006) Gonococcal lipooligosaccharide suppresses HIV infection in human primary macrophages through induction of innate immunity. *J Infect Dis* **194**: 751–759.

Lund, J.M., Linehan, M.M., Iijima, N., and Iwasaki, A. (2006) Cutting edge: plasmacytoid dendritic cells provide innate immune protection against mucosal viral infection in situ. *J Immunol* **177**: 7510–7514.

Magnusson, M., Tobes, R., Sancho, J., and Pareja, E. (2007) Cutting edge: natural DNA repetitive extragenic sequences from gram-negative pathogens strongly stimulate TLR9. *J Immunol* **179**: 31–35.

Martinelli, E., Cicala, C., Van, R.D., Goode, D.J., Macleod, K., Arthos, J., et al. (2007) HIV-1 gp120 inhibits TLR9-mediated activation and IFN-(alpha) secretion in plasmacytoid dendritic cells. *Proc Natl Acad Sci USA* **104**: 3396–3401.

Meylan, P.R., Guatelli, J.C., Munis, J.R., Richman, D.D., and Kornbluth, R.S. (1993) Mechanisms for the inhibition of HIV replication by interferons-alpha, -beta, and -gamma in primary human macrophages. *Virology* **193**: 138–148.

Mogensen, K.E., Lewerenz, M., Reboul, J., Luftfalla, G., and Uze, G. (1999) The type I interferon receptor: structure, function, and evolution of a family business. *J Interferon Cytokine Res* **19**: 1069–1098.

Mogensen, T.H., Paludan, S.R., Kilian, M., and Ostergaard, L. (2006) Live *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* activate the inflammatory response through Toll-like receptors 2, 4, and 9 in species-specific patterns. *J Leukoc Biol* **80**: 267–277.

Moniz, R.J., Chan, A.M., and Kelly, K.A. (2009) Identification of dendritic cell subsets responding to genital infection by *Chlamydia muridarum*. *FEMS Immunol Med Microbiol* **55**: 226–236.

Moss, G.B., Overbaugh, J., Welch, M., Reilly, M., Bwayo, J., Plummer, F.A., et al. (1995) Human immunodeficiency virus DNA in urethral secretions in men: association with gonococcal urethritis and CD4 cell depletion. *J Infect Dis* **172**: 1469–1474.

Neil, S.J., Zang, T., and Bieniasz, P.D. (2008) Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* **451**: 425–430.

Nkengasong, J.N., Kentens, L., Ghys, P.D., Koblavi-Deme, S., Bile, C., Kalou, M., et al. (2001) Human immunodeficiency virus Type 1 (HIV-1) plasma virus load and markers of immune activation among HIV-infected female sex workers with sexually transmitted diseases in Abidjan, Cote d’Ivoire. *J Infect Dis* **183**: 1405–1408.

Okumura, A., Lu, G., Pitha-Rowe, I., and Pitha, P.M. (2006) Innate antiviral response targets HIV-1 release by the induction of ubiquitin-like protein ISG15. *Proc Natl Acad Sci USA* **103**: 1440–1445.

Rothentusser, S., Tuma, E., Endres, S., and Hartmann, G. (2002) Plasmacytoid dendritic cells: the key to CpG. *Hum Immunol* **63**: 1111–1119.

Salyers, A.A., and Whitt, D.D. (2002) *Neisseria* species. In *Bacterial Pathogenesis, A Molecular Approach*. Washington, DC: ASM Press, pp. 437–451.

Sato, M., Taniguchi, T., and Tanaka, N. (2001) The interferon system and interferon regulatory factor transcription factors – studies from gene knockout mice. *Cytokine Growth Factor Rev* **12**: 133–142.

Sheung, A., Rebbapragada, A., Shin, L.Y., Dobson-Belaire, W., Kimani, J., Ngugi, E., et al. (2008) Mucosal *Neisseria gonorrhoeae* coinfection during HIV acquisition is associated with enhanced systemic HIV-specific CD8 T-cell responses. *AIDS* **22**: 1729–1737.

Shirazi, Y., and Pitha, P.M. (1992) Alpha interferon inhibits early stages of the human immunodeficiency virus type 1 replication cycle. *J Virol* **66**: 1321–1328.

Shirazi, Y., and Pitha, P.M. (1998) Interferon downregulates CXCR4 (fusin) gene expression in peripheral blood mononuclear cells. *J Hum Virol* **1**: 69–76.

© 2010 Blackwell Publishing Ltd, *Cellular Microbiology*, 12, 1703–1717.
Simard, S., Maurais, E., Gilbert, C., and Tremblay, M.J. (2008) LPS reduces HIV-1 replication in primary human macrophages partly through an endogenous production of type I interferons. *Clin Immunol* **127**: 198–205.

Sjolinder, H., Mogensen, T.H., Kilian, M., Jonsson, A.B., and Paludan, S.R. (2008) Important role for Toll-like receptor 9 in host defense against meningococcal sepsis. *Infect Immun* **76**: 5421–5428.

Soumelis, V., Scott, I., Gheyas, F., Bouhour, D., Cozon, G., Cotte, L., et al. (2001) Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood* **98**: 906–912.

Stark, G.R., Kerr, I.M., Williams, B.R., Silverman, R.H., and Schreiber, R.D. (1998) How cells respond to interferons. *Annu Rev Biochem* **67**: 227–264.

Taniguchi, T., Ogasawara, K., Takaoka, A., and Tanaka, N. (2001) IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol* **19**: 623–655.

UNAIDS (2008) *2008 Report on the Global AIDS Epidemic*. Geneva: UNAIDS.

World Health Organization (2001) *Global Prevalence and Incidence of Selected Curable Sexually Transmitted Infections: Overview and Estimates*. Geneva: World Health Organization.

Zhang, J., Li, G., Bafica, A., Pantelic, M., Zhang, P., Broxmeyer, H., et al. (2005) *Neisseria gonorrhoeae* enhances infection of dendritic cells by HIV type 1. *J Immunol* **174**: 7995–8002.

Zhou, Y., Zhang, H., Siliciano, J.D., and Siliciano, R.F. (2005) Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4+ T cells. *J Virol* **79**: 2199–2210.