**Neisseria gonorrhoeae Enhances Infection of Dendritic Cells by HIV Type 1**

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Neisseria gonorrhoeae Enhances Infection of Dendritic Cells by HIV Type 1


Clinical studies indicate that Neisseria gonorrhoeae (gonococci (GC)) has the capacity to enhance HIV type 1 (HIV-1) infection. We studied whether GC enhances HIV infection of activated dendritic cells (DCs). The results show that GC can dramatically enhance HIV replication in human DCs during coinfection. The GC component responsible for HIV infection enhancement may be peptidoglycan, which activates TLR2. TLR2 involvement is suggested by bacterial lipoprotein, a TLR2-specific inducer, which stimulates a strong enhancement of HIV infection by human DCs. Moreover, participation of TLR2 is further implicated because GC is unable to stimulate expression of HIV in DCs of TLR2-deficient HIV-1-transgenic mice. These results provide one potential mechanism through which GC infection increases HIV replication in patients infected with both GC and HIV. The Journal of Immunology, 2005, 174: 7995–8002.

G onorhoea is one of the most frequently reported sexually transmitted diseases (STDs),4 with ~78 million new cases reported globally each year (1). Neisseria gonorrhoeae (gonococci (GC)) is also one of the leading etiological agents of pelvic inflammatory disease. GC can adhere to and penetrate mucosal cells and attain access to submucosal sites, where it can interact with host immune cells (2). Initial contact between GC and host tissues is thought to be mediated by neisserial type IV pili, and a tight secondary interaction can then be established by several GC components such as the phase-variable, colony opacity-associated (Opa) outer membrane proteins (3, 4).

Observational studies have suggested a strong association between acquisition of HIV type 1 (HIV-1) and other STDs, even after correcting for risky sexual behavior (5). STDs, such as syphilis, chancroid, or herpes viral infection, are thought to enhance HIV-1 transmission by disrupting mucosal integrity as a result of genital ulceration and inflammation. Biological studies of genital secretions from STD patients show that shedding of HIV-1 decreases after treatment of STD (6–9).

GC infection does not cause ulceration, but an increased HIV load is observed in semen from GC-infected adults (10). Mechanisms responsible for enhanced HIV replication are unclear but may relate to increased numbers of activated CD4+ T lymphocytes susceptible to HIV infection (11) and to high levels of proinflammatory cytokines capable of inducing HIV replication (12) and subsequent HIV infection of these cells.

Dendritic cells (DCs) have been associated with human HIV-1 infection since the virus was first identified. DCs can serve as a reservoir for HIV. Recent studies indicated that DCs, through DC-specific intercellular adhesion molecule-grabbing nonintegrin (DC-SIGN), act as a carrier for HIV-1 viruses for delivery to the target cells such as CD4 lymphocytes (13–15). The complicated and intriguing relationship between DCs and HIV has been reviewed (16). Several pathogens including Mycobacterium tuberculosis target DC-SIGN. The interaction of M. tuberculosis with DC-SIGN on DCs influences cellular functions (17, 18). M. tuberculosis may also stimulate HIV expression through a TLR2-dependent manner (19).

DCs express carinoembryonic Ag-related cell adhesion molecule 1 (CEACAM1) (20), which is the receptor for Opa proteins of neisserial strains (21–23). CEACAM1 is also an inhibitory receptor (24), whose functions, such as inhibition of T cell proliferation, are mediated through the ITIM (25). We hypothesized that GC may relate to increased numbers of activated CD4+ T lymphocytes (10). Mechanisms responsible for enhanced HIV replication are unclear but may relate to increased numbers of activated CD4+ T lymphocytes susceptible to HIV infection (11) and to high levels of proinflammatory cytokines capable of inducing HIV replication (12) and subsequent HIV infection of these cells.

Materials and Methods

Animals

HIV-1-transgenic mice deficient in TLR2 (HIV-Tg/TLR2−/−) were generated as previously described (19). These mice contain multiple copies of the complete proviral genome of HIV-1 strain NL4-3. Mice of both sexes between 6 and 12 wk old were used in all experiments.

1 Abbreviations used in this paper: STD, sexually transmitted disease; GC, gonococci; Opa, opacity-associated; HIV-1, HIV type 1; DC, dendritic cell; SIGN, specific intercellular adhesion molecule-grabbing nonintegrin; CEACAM, carinoembryonic Ag-related cell adhesion molecule; PGN, peptidoglycan; BLP, bacterial lipoprotein; Tg, transgenic, KO, knockout; MDDC, monocyte-derived DC; RT, reverse transcriptase.

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Cell lines
HeLa-CEACAM1 cells were constructed by transfecting HeLa cells with CEACAM1 cDNAs, and selected for surface Ag expression (22).

Bacterial strains, viral Abs, bacterial LPS, GC porin, and peptidoglycan (PGN)
Gonococcal strain MS11 was cultured and maintained as previously described (28). Filarus-negative gonococci with lacto-N-neotetraose (LNTO) phenotype (wild type) were used (29). The expression of Opa proteins in GC was routinely monitored by SDS-PAGE/Western blot with the Opa cross-reactive mAb 4B12 (30, 31). The limit of detection for the LPS contamination from GC PGN used in our experiments, each batch of differential centrifugation, ultrafiltration, and chromatography. To minimize and Immunology, Indiana University School of Medicine, Indianapolis, IN. This intact PGN was prepared using the trichloroacetic acid-SDS extraction procedure (34) with a combination of proteinase K treatment, differential centrifugation, ultrafiltration, and chromatography. To minimize contamination from GC PGN used in our experiments, each batch of the GC PGN used was analyzed by the Limulus Amebocyte Lysate Pyrochrome kit (Associates of Cape Cod) (35). The limit of detection for the Pyrochrome kit was 0.06 ng/ml endotoxin.

In general, bacterial PGN and LPS activate host cells through TLR2 (36, 37) and TLR4 (38, 39), respectively. We used a newly developed transgenic (Tg) mouse strain expressing complete DNA copies of the HIV-1 genome in a TLR2 knockout (KO) background (HIV-Tg/TLR2genic (Tg) mouse strain expressing complete DNA copies of the HIV-1 chromosome kit (Associates of Cape Cod) (35). The limit of detection for the LPS contamination from GC PGN used in our experiments, each batch of differential centrifugation, ultrafiltration, and chromatography. To minimize contamination from GC PGN used in our experiments, each batch of the GC PGN used was analyzed by the Limulus Amebocyte Lysate Pyrochrome kit (Associates of Cape Cod) (35). The limit of detection for the Pyrochrome kit was 0.06 ng/ml endotoxin.

Preparation of dendritic cells
PBMC were isolated fromuffy coats obtained from the Indiana Blood Center (Indianapolis, IN) by density gradient centrifugation over Ficoll-Paque Plus (1.077 g/ml; Pharmacia). Acquisition of the human blood was approved by the Institutional Review Board (IRB) and Study Committees at Indiana University School of Medicine (Clarian). Buffy coats were diluted 1/4 with PBS and loaded at a 1:1 (v:v) ratio on Ficoll and centrifuged without braking for 30 min. PBMC were washed four times with PBS, and monolayers were prepared from PBMC CD14 microbeads (Miltenyi Biotec) as previously described (40). To increase purity, cells were passed over a second CD14-microbead column. The final purity of the isolated monocytes was >98% as assessed by labeling with CD14-FITC Ab (Caltag Laboratories) and flow cytometric analysis. Purified CD14+ monocytes (5 × 10⁶ cells/ml) were cultured for 6 days to promote differentiation of immature monocyte-derived DCs (MDDCs) in culture medium consisting of RPMI 1640 (BioWhittaker), 10% heated inactivated PBS (HyClone), 100 U/ml penicillin, and 100 μg/ml streptomycin in the presence of 20 ng/ml recombinant human GM-CSF (Immunex) and 10 ng/ml recombinant human IL-4 (PeproTech). The DCs derived from these cultured monocytes display typical dendrites, promote activation of alloreactive T cells in mixed lymphocyte cultures, and express the DC phenotype of HLA-DR*, CD1a*, CD86*, CD40*, CD14* (40). Therefore, DCs described here are considered immature DCs. Upon LPS stimulation, these DCs express CD83 (41).

Adherence assays
DCs and HeLa cells were suspended in RPMI 1640 with 2% FCS at a concentration of 4 × 10⁶/ml. Cell suspension (0.5 ml) was added to coverslips in 24-well plates and incubated alone or in the presence of 50 μl of bacterial suspensions at a concentration of 4 × 10⁶ CFU/ml. From this presentation, the ratio of bacteria to cells is 2:1, based on our previous publications (22, 42, 43). These DCs were allowed to incubate for 2.5 h at 37°C in the presence of 5% CO₂. The resulting DC monolayers were washed twice with PBS using a cytopsin, and fixed with 2% paraformaldehyde in PBS containing Giemsa stain. The number of cell-associated bacteria (adherent and internalized) per DC was determined microscopically by counting bacteria associated with 100 cells on the coverslips.

DC activation assay
DCs plus or minus GC were incubated for 2.5 h at 37°C in the presence of 5% CO₂ as described above, then an equal volume of RPMI 1640, containing 18% of FCS, 200 μg/ml gentamicin, 200 U/ml penicillin, and 200 μg/ml streptomycin, was added to each well to kill the GC followed by incubation at 37°C with 5% CO₂ for 24 or 48 h. The activation state of DCs was assessed by staining these human cells with Abs against CD83 (BD Pharmingen) and MHC class I (the L243, which recognizes the HLA-DR αβ dimer). The Abs were gifts from Dr. J. Blum (Department of Microbiology and Immunology, Indiana University School of Medicine) and were previously described (44). LPS from E. coli was used at 1 μg/ml and served as a positive control for DC activation.

HIV infection and detection of reverse transcriptase (RT)
Human DCs were cocultured with or without GC as described above. HIV at concentration of 0.25 multiplicity of infection (an equivalent of 2000 cpm RT activity) was then added to each well containing DCs alone or DCs incubated with GC in the presence of 8 μg/ml Polybrene and then incubated for 3 h. Unbound HIV was removed by washing the DCs three times with RPMI 1640. Human DCs were cultured in RPMI 1640 with 10% FCS for 3 days. After centrifugation of these DCs for 5 min at 1200 rpm, cell media were collected and cell pellets discarded. Resulting media were centrifuged for 1 h at 12,000 rpm to precipitate viral particles, followed by resuspension of these samples in 10 μl of dissociation buffer (0.2% Triton X-100, 20% glycerol, 0.05 M Tris at pH 7.5, 0.01 M DTT, and 0.25 M KC1). HIV in these samples were lysed by three rounds of freezing on dry ice, and thawing at 37°C in a water bath. HIV loads were obtained by measuring RT enzyme activity as described (45, 46). A total of 10 μl of viral lysates were mixed with 40 μl of master buffer consisting of 33 μl of RT assay medium, 1 μl of [3H]dThdTP, 5 μl of poly(A)+-dT, and 1 μl of 0.1 M EGTA and incubated at 37°C for 1 h. RT assay medium consists of 0.0625 M Tris at pH 7.5, 0.01 M DTT, 0.01 M MgCl₂ and 0.5% Triton X-100. The mixtures were placed on DE81 filter circles and washed twice with 2× SSC to stop the reaction and wash off unincorporated [3H]dThdTP. After rinsing two times with 100% ethanol, filters were dried and radioactivity was counted in a scintillation counter.

Measurement of HIV p24 proteins in mouse DCs
Expression of p24 was measured either in wild-type HIV transgenic mice (HIV-Tg/TLR2KO or TLR2-KO HIV Tg mice (HIV-Tg/TLR2–/–) after stimulation of cells with GC, porin, or PGN. Splenic DCs were partially purified as described (47). Briefly, spleens harvested from mice were digested with Liberase CI (Roche Biochemicals). Low-density leukocytes were obtained after centrifugation in a BSA gradient and CD11c⁺ cell fractions were further purified using magnetic bead-conjugated Ab (Miltenyi Biotec). After washing in PBS, cells were resuspended in RPMI 1640 with 10% FCS at 1 × 10⁶/ml in 96-well plates (Corning) and GC, porin, PGN, LPS, or BLP (EMC Microcollections) were added as indicated for each experiment. After a 2-day culture at 37°C, supernatants were harvested and p24 was measured by ELISA ( Coulter) in quadruplicate (49).

All experiments were performed in triplicate and SE bar is indicated. Statistical significance was determined using the Student t test.

Results
Association of GC with DCs promotes up-regulation of CD83 and MHC class I molecules
Human CEACAM1, which is also expressed on mouse DCs (20), is a receptor for Opa proteins and promotes binding and phagocytosis of GC by host cells (21–23). Therefore, we tested the adherence of GC to human DCs. HeLa and HeLa-CEACAM1 cell lines were, respectively, used as negative and positive cell controls to evaluate adherence between GC and DCs. GC efficiently bound to human DCs regardless of Opa expression (Fig. 1A). After incubation at 37°C for 4 h, 80% of GC, which can cross-reactive mAb 4B12 (30, 31). Only Opa-expressing GC, which can interact with CEACAM1 (CD66a), was used in this study. The HIV strain used was NL4-3. Bacterial LPS isolated from Salmonella typhimurium, Vibrio cholerae, Yersinia pseudotuberculosis, and Pseudomonas aeruginosa, were purchased from Sigma-Aldrich. Bacterial lipoprotein (BLP) is a synthetic lipoprotein S-[2,3-bis(palmitoyloxy)-(2-RS)-pro-
Thus, we evaluated whether binding of GC to DCs influenced expression of these surface molecules. DCs were challenged with Opa− and Opa+ GC for 2.5 h and then extracellular bacteria were killed by adding antibiotics. Expression of CD83, class I, and class II molecules was examined after 48 h. LPS from E. coli was used as a positive control for DC activation. Fig. 1B showed that CD83 and MHC class I Ags were up-regulated by GC in the absence or presence of Opa+ expression. MHC class II molecule expression, which is high in DCs, was not affected by GC infection (data not shown).

Direct HIV-1 infection of DCs treated with GC

The above data demonstrated that GC activates DCs. Activated DCs can activate and result in the proliferation of naïve T cells in preparations of human PBMC (PBMCs). HIV replicates better in activated PBMCs than in resting PBMCs. Thus, we determined whether GC-activated DCs would lead to enhanced HIV-1 replication in PBMCs mediated by activated DCs. DCs were treated with GC and Opa protein expression for 2.5 h, and GC was then killed with antibiotics. GC-treated DCs were allowed to grow overnight, and then freshly isolated PBMCs were added at a ratio of 1:10 (DCs to PBMC). The mixed cells were cocultured for an additional 48 h, and challenged with HIV-1 NL4-3 viruses (Fig. 2A). PBMCs treated with GC alone (Fig. 2B) and DCs treated with GC alone (Fig. 2C) were included as controls. HIV infection was analyzed by RT activity. Treatment of DCs with GC, followed by coculture with PBMCs, resulted in robust RT activity, while DCs treated without GC in the presence of PBMCs had little RT activity (Fig. 2A). No increase in RT activity was detected in PBMCs treated with GC alone (Fig. 2B). Very surprisingly, a comparable level of HIV infection was noted in DCs treated with GC in the absence of PBMCs, but not in DCs without treatment with GC (Fig. 2C). To confirm that GC can enhance HIV infection in DCs, the GC dose challenge experiment was performed. As shown in Fig. 2D, a dose-dependent effect of GC on HIV replication in DCs was observed (p < 0.001). These results suggest that GC treatment activates DCs, and leads to direct infection of these cells by HIV-1. It should be noted that capacity of DCs to transmit HIV and to promote its replication in unstimulated autologous PBMC or T cells has been well-documented. However, the negative result on Fig. 2B, remains to be explained, and may result from different settings in the present study.

GC facilitates HIV infection of DCs but not of monocytes

To ascertain that the enhanced HIV infection is due to GC-mediated activation of DCs, and not other cells such as monocytes, freshly isolated monocytes were challenged with GC and HIV following the same procedures as in Fig. 2. A parallel culture of monocytes with the same number of cells was induced to differentiate into DCs. After 6 days, these DCs were challenged with GC and the same lot of HIV. Monocytes and DCs were from the same donors in these experiments. As shown in Fig. 3, only GC-treated DCs supported HIV infection (p < 0.001). GC treatment did not influence HIV infection of monocytes. This result also ruled out the possibility that enhancement of HIV infection of DCs was a result of nonspecific or unknown effects such as interaction of HIV with GC.

GC-treated DCs do not up-regulate expression of CD4, CCR5, CXCR4, or DC-SIGN

To determine whether expression of four major HIV receptors, CD4, CCR5, CXCR4, and DC-SIGN, is changed in GC-treated DCs, expression of these receptors was monitored by flow cytometry after challenging with GC. As shown in Fig. 4, the expression of CD4, CCR5, CXCR4, and DC-SIGN did not change. This result indicates that the enhanced DC infection with HIV in the presence of GC is not due to up-regulation of HIV receptors.

Gotschlich, J. Klena, and T. Chen. DC-SIGN (CD209) is a receptor for Neisseria gonorrhoeae. Submitted for publication.
of GC may not be due to the increased expression of these HIV receptors, which are pivotal for facilitating entry of HIV.

Enhancement of HIV infections by DCs occurs mainly in postentry

The expression of four major receptors for HIV undergoes no up-regulation in response to GC adherence, suggesting that the enhancement induced by GC was possibly due to transcriptional up-regulation of HIV gene products within the infected DCs and occurs after HIV enter DCs. To test this hypothesis, DCs were infected with HIV first for 3 h, and then the same amount of GC was added. As a positive control, the parallel samples of DCs were challenged with GC first and then infected with HIV. It should be noted that the DCs used for either infecting with HIV or GC first were from the same donors, and HIV virus was from the same batch. As shown in Fig. 5, the same pattern of enhancement of HIV was exhibited regardless of the order of incubation, demonstrating that enhancement of HIV replication mainly occurs postentry ($p < 0.005$). However, DCs infected with HIV before the addition of GC resulted in a decrease in HIV replication when compared with DCs infected with GC first ($p < 0.01$), suggesting that enhanced viral entry played a role in the enhanced HIV infection mediated by GC, in addition to enhanced replication in already HIV-infected cells.

BLP and GC PGN play a role in the enhancement of DC infection by HIV-1

GC express many surface components, such as Opa, pili, porin, lipoproteins, LPS, and PGN, that can interact with cells of the immune system. GC Opa and pili were probably not involved in the enhancement of HIV infection in DCs described above, as we only used pilus-negative strains and the effect was independent of Opa expression (Figs. 1 and 2). Neisserial porin and PGN, as well as the synthetic lipoprotein BLP, have been shown to activate host cells through TLR2 (19, 33, 36, 37). Thus, we tested the ability of GC PGN and porin, as well as paraformaldehyde-fixed GC and the synthetic BLP to stimulate HIV infection.

E. coli LPS may increase HIV-1 transmission of DCs (15), although some reports contradict this assertion (50, 51). To determine whether LPS plays a role in HIV-1 infection of DCs, LPS (1 μg/ml) from E. coli and GC that activate DCs (Fig. 1B) were also tested for their effects on HIV infection of DCs. Live GC was included as a positive control in these experiments.

As shown in Fig. 6, only a very marginal increase of HIV infection was noted after LPS stimulation in comparison to whole GC ($p > 0.05$). LPS from S. typhimurium, V. cholerae, Y. pseudotuberculosis, and Pseudomonas aeruginosa also did not stimulate the enhancement (data not shown). However, fixed GC and purified GC PGN enhanced HIV-1 infection to similar extents, while porin showed no effect on HIV infection of human DCs (Fig. 6). It should be noted that the enhancement by PGN and dead GC was lower than enhancement by live GC. Notably, BLP stimulated the highest HIV infection by human DCs in a dose-dependent manner ($p < 0.001$). These data showed that enhancement was associated
with GC components that can activate TLR2, such as PGN. Although the relevant bacterial products have not been identified, a role for PGN is implicated. It is possible that a combination of several components on GC, such as PGN, porin, and H.8 lipoprotein contributes to the final effects.

**TLR2 involvement in HIV expression induced by GC**

Because PGN, porin, and BLP are ligands for TLR2 (33, 36, 37), we investigated whether TLR2 was involved in GC-enhanced HIV-1 infection. We took advantage of a newly developed HIV-Tg/TLR2/H11002/H11002 mouse, which contains the full-length HIV-1 proviral genome integrated with TLR2 KO (19). DCs were isolated from wild-type and TLR2-KO-HIV mice, and challenged with dead GC, GC porin, and PGN. HIV-1 expression was analyzed by measuring viral core Ag p24 production in the culture supernatants. Bacterial LPS and BLP were also included as controls in these experiments, as they are known to activate TLR4 (38, 39) and TLR2 (52), respectively. Fig. 7 showed that GC, porin, and BLP stimulated TLR2-dependent expression of HIV in DCs, \( p < 0.005 \), whereas the effect of PGN was only partially dependent on TLR2 (Fig. 7). Taken together, these data suggested that GC-induced expression of HIV depended mainly on TLR2 in mouse DCs, but other receptors may be involved as well.

**R5 virus is also infectious GC-treated DCs**

Sexually transmitted HIV is, in most cases, a virus that uses CCR5, but not CXCR4, as coreceptor (R5 viruses) to infect and replicate in target cells. Therefore, R5 viruses are more relevant than CXCR4-using viruses in the context of HIV sexual transmission. We have originally chosen the NL4-3 virus because the HIV Tg mouse model used in the present work is integrated this strain. In the following study, both X4 (NL4-3) and R5 (YU-2) viruses were used to infect DCs treated with GC and BLP to repeat the experiments in Fig. 2 and 6. As shown in Fig. 8, the DCs after treatment of either GC or BLP are more susceptible to both forms of HIV infection. Very Interestingly, R5 virus appears to be more infectious to GC or BLP-treated DCs than the X4 virus.

**Discussion**

Clinical studies indicate that gonorrhea can significantly amplify the concentration of HIV-1 in the semen of AIDS patients (10), and possibly facilitate infection of HIV (6, 8). In this study, we demonstrated that GC can directly enhance HIV infection as well as HIV replication in MDDCs. Several possible mechanisms could account for this enhancement of HIV infection in DCs.

Studies recently indicated that DCs, acting as carriers, capture HIV virions and transfer them to target cells such as T cells (13–15). It is possible that GC might simply facilitate HIV delivery or susceptibility of T cells (50, 53), which were contaminated in the DC preparation. However, our results in Fig. 2 do not support this scenario, in that GC stimulated similar levels of DC infection by HIV with or without the presence of PBMC present. Also, the monocyte cultures were over 98% CD14, ensuring very few contaminating cells. Further, only DCs (MDDCs) could be stimulated by GC to enhance HIV infection because the same batch of fresh monocytes from the same donor did not show the same effects (Fig. 3). It should be noted that the number of cells did not increase with the addition of the cytokines or GC (data not shown). Moreover, HIV infection of DCs was not enhanced with PMA treatment (data not shown). Cellular activation by PMA has been shown to enhance HIV infection of other host cells. Thus, GC might induce...
whether TLR2 is involved. Second, this HIV-Tg mouse and its
mice follows: first, these mice present a means to determine

FIGURE 8. DCs exposed to GC or BLP are also sensitive to infection
with R5 virus. DCs follows the same treatments with GC or BLP as
described in Figs. 2 and 6, and challenged with both R5 (YU-2) and X4
(NL-4). Again, the DCs without treatment of GC or BLP were used as
controls.

unique derivative, Tg/TLR2−/−, have already contributed to in-
vestigation of coinfecting pathogens on virus expression (19, 55).
Third, the NL4-3 virus that is integrated in these mice is the same
virus used in our other experiments. In short, these models allowed
us to demonstrate a role for TLR2 in the coinfection.

Our results in both human and mouse cells indicate that en-
hancement of HIV expression by GC involves TLR2. However,
GC porin, which stimulated a classical TLR2-dependent expres-
sion of HIV in mouse cells, did not enhance HIV infection in
human cells. This may affect the sensitivities of the mouse sys-
tems. Also, GC PGN, which usually activates TLR2 (36, 37), only
partially stimulated TLR2-dependent expression of HIV in mouse
cells. Recent studies showed that PGN stimulated mouse DCs
through both TLR2 and TLR4 (56), suggesting that PGN might not
activate TLR2 exclusively. This may explain why GC PGN
induced HIV expression in a TLR2-independent manner. It is also
possible that chemokine expression induced by porin and PGN is
different between mice and humans, which may also influence the
expression of HIV (57). Therefore, the differences between human
and mouse DCs are not surprising.

The disadvantage of applying this mouse model is that neither
GC nor HIV is infectious in mice. These mice seemingly do not
produce real virus, but produce viral proteins. This is a model for
activation of HIV expression and these transgenic mice do not
present the complete virus cycle, although virus could be measured
when cocultured with human cells (58). This is the main reason for
detection of HIV loads in the mouse model by using p24 expres-
sion rather than HIV RT activity. However, data from these mod-
els are useful in interpreting human cell data. Human DCs can be
activated by bacterial LPS and GC (Fig. 1B), but whole neisserial
bacteria activate host cells mainly through TLR2 (33, 35, 59–62)
to stimulate TLR2-dependent HIV expression. This rationale is
supported by the fact that BLP, a synthetic TLR2 ligand, stimu-
lates specific TLR2-dependent HIV expression in the mouse
model, and induces the strongest enhancement of HIV replication
in human DCs. The relationship among TLRs, coinfection, and
HIV has been recently reviewed (63).

Are the effects of HIV enhancement in DCs GC-specific? It has
been reported that with respect to the effects of individual STDs on
HIV load, only genital ulcers and gonococcal infection are asso-
ciated with higher viral load. Chlamydia, which is very close to
GC in terms of infectious properties, does not enhance HIV load
(64). We did not examine the specificity of GC to HIV infection of
DCs. Thus, it is possible that other bacteria or bacterial compo-
nents may also be active in these effects.

We propose the following model. When GC invades the host, it
binds to DCs, effectively delivering the PGN and possibly other
GC components, to DCs to activate the TLR2 signal pathway

FIGURE 7. TLR2 is necessary for HIV-1 induction by dead GC and
porin. Spleen cells from HIV-Tg/TLR2−/+ or HIV-Tg/TLR2−/− mice
were left untreated or exposed to LPS (TLR4, 100 ng/ml; BLP (TLR2, 100
ng/ml), and GC products as indicated. *, p < 0.005, compared HIV-Tg/
TLR2−/+ with HIV-Tg/TLR2−/−.
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Disclosures

The authors have no financial conflict of interest.

References

Enhancement of HIV-1 infection of DCs by GC