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Research Article

Chlamydia trachomatis Enhances HIV Infection of Non-Activated PBMCs

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Abstract

Sexual contact is the most common route of HIV transmission, and the concurrent presence of sexually transmitted infections (STIs) such as *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (gonococcus, GC) is known to increase the HIV risk. Antibiotic treatment decreases the incidence of STIs but not HIV. CT and GC activate Toll-like receptors (TLRs) 2 and 4, which act as sensors of microbial infection are critical for initiating immune responses to control infection. We have previously shown that GC enhances HIV infection of primary resting CD4+ T cells through activation of TLR2 but not TLR4. In this study, we determined the effect of live and fixed CT and different species of lactobacilli including *L. jensenii* and *L. reuteri* on HIV infection of freshly isolated PBMCs. We found that pretreatment of freshly isolated PBMCs with fresh or fixed CT, but not *lactobacilli*, promoted HIV infection of freshly isolated CD4+ T cells. Together with our previous reports, we concluded that STIs such as CT and GC but not commensal bacteria like lactobacilli enhanced HIV infection, possibly through immune activation. Importantly, the enhancement effect of fixed CT on HIV infection may explain the failure of antibiotic treatments to reduce the HIV incidence. Combined strategies to inhibit STI growth and STI-mediated mucosal immune activation should be considered for HIV prevention in the settings of STIs.

Keywords: *Chlamydia trachomatis; Sexually Transmitted Infections (STIs); HIV Infection*

Introduction

Sexual transmission is the main route of HIV infection, and sexually transmitted infections (STIs) including *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (gonococcus, GC) increase the likelihood of HIV transmission [1-4]. Antimicrobial therapies for bacteria or herpes simplex virus 2 reduce the incidence of STIs but not HIV [5,6]. The contribution of STIs to the increase in HIV transmission is likely to be multifaceted (reviewed in [7,8], understanding how STIs enhance HIV infection is vital to the development of new strategies to reduce the spread of HIV. CT and GC are known to activate Toll-like receptors (TLRs) 2 and 4 [9-11] that detect microbial infection and are critical for the initiation of innate inflammatory and adaptive immune responses [12-14]. Induction of mucosal innate immune response

by non-ulcerative STIs such as CT or GC may lead to enhanced HIV transmission. Indeed, we have previously shown that GC promotes HIV infection of primary resting CD4+ T cells through activation of TLR2 but not TLR4 [15]. In this study, we compared the effect of live and fixed CT on HIV infection of freshly isolated PBMCs. *Lactobacillus jensenii* and *L. reuteri*, commensal bacteria in the vagina and gut, respectively, were included as a comparison. Our data showed that live and fixed CT but not lactobacilli promoted HIV infection of freshly isolated PBMCs, suggesting that strategies targeting STI-mediated immune activation in addition to antimicrobial therapies are needed for HIV prevention.

Materials and Methods

Reagents

Recombinant human interleukin 2 (IL-2) was purchased from R&D systems (Minneapolis, MN). Peptidoglycan (PGN), Histopaque®-1077, RPMI-1640 medium and CsA were from Sigma-Aldrich (St Louis, MO).

Pre-treatment of PBMCs with bacteria, and HIV-1 infection assay

Peripheral blood mononuclear cells (PBMCs) from normal healthy blood donors were isolated over a Histopaque gradient. Cells were cultured in RPMI-1640 media with 10% FBS in the presence of IL-2 (50 IU/ml). Replication-defective pseudotyped HIV-1_{VSV-G} luciferase reporter viruses were produced as described previously [16,17]. Live or fixed extracellular, infectious elementary bodies of Chlamydia trachomatis serovar D (D/ UW3/Cx) were kindly provided by Dr. Alison J. Quayle at Louisiana State University Health Sciences Center, New Orleans, Louisiana. Lactobacilli were cultured in MRS broth (Difco) at 37°C in 5% CO_2 as described previously [18]. Bacterial cell density was adjusted to an OD_{600} nm equal to 0.1, corresponding to 10^8 colony forming units/ml bacteria. Fixed bacteria were prepared by using 1% paraformaldehyde in PBS as described previously [15].

Freshly isolated PBMCs (1×10^6 cells per sample) were treated with bacteria in the presence of IL-2 for 2 days. As a control, PBMCs were treated with PGN (20 µg/ml) as described previously [15]. Cells were washed and then infected with pseudotyped CCR5-using HIV-1_{JR-FL} luciferase reporter virus at 37°C for 2h. After washing off unbound viruses, infected cells were cultured in the presence of IL-2 at 37°C for 3 days. Cells were then lysed with passive cell lysis buffer (Promega, Madison WI) and luciferase activity (in relative light units [RLUs]) was measured on a Glomax 20/20 luminometer (Promega, Madison WI).

Results and Discussions

To determine whether CT increased the susceptibility of PBMCs to HIV, we treated freshly isolated PBMCs with live and fixed CT at MOI (multiplicity of infection) of 10 in the presence of IL-2 for 2 days before HIV infection. Live and fixed *L. jensenii* and *L. reuteri* at MOI of 1 and 10 were included as a comparison. PGNs were included as a positive control as we have previously shown that pretreatment of primary CD4+ T cells with PGNs enhances HIV infection [15]. As shown in figure 1, there was a low level of HIV infection of non-activated PBMCs. In agreement with our previous finding, the degree of HIV infection of PGN-treated PBMCs was higher than that of untreated cells (10-fold). Live CT slightly, but not significantly, increased HIV infection. Fixed CT significantly increased HIV infection by 6-fold compared to untreated PBMCs. *L. jensenii* and *L. reuteri* did not promote HIV infection regardless whether live or fixed bacteria were used. In fact, lactobacilli suppressed the low level of baseline HIV infection.

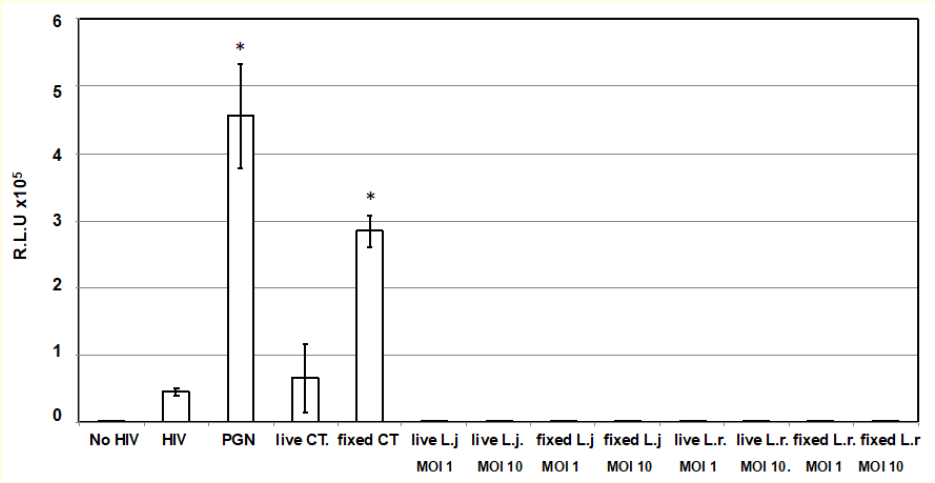

Figure 1: *Chlamydia trachomatis but not Lactobacillus species enhance HIV infection of non-activated PBMCs.*

Freshly isolated PBMCs were pretreated with live or fixed CT, L. jensenii (L.j) or L. reuteri (L.r) at MOI 1 or 10 in the presence of IL-2 for 2 days. Cells were treated with PGN (20 μg/ml) as a positive control. PBMCs without bacterial pretreatment or without HIV infection were included. PBMCs were then infected with pseudotyped HIV-1JR-FL luciferase reporter virus and HIV infection was determined by measuring luciferase activity at day 3 after infection. The difference between samples with treatment of PGN or fixed CT vs samples without treatment is significant ($p < 0.05$).*

We have previously shown that GC and TLR2 signal activate primary CD4+ T cells [15,19]. Since primary CD4+ T cells did not respond to TLR4, the enhanced HIV infection by fixed CT is likely mediated through TLR2. The differential effect of live and fixed CT on HIV infection may be due to CT-induced immune responses from other cell types such as monocytes that may have an indirect effect on CD4+ T cells. Studies using primary CD4+ T cells alone or stimulation of specific immune cells with CT followed by co-culture systems will provide insights into the effect of CT on HIV infection in the mixed cell-type culture.

The ability of lactobacilli to induce TLR activation has been in consistent. Some reports indicated that lactobacilli are involved in TLR2, 4, 5, and 9 [20,21] but others show little activation or suppression of TLR4-medated response. For example, in Caco-2 cells, *Lactobacillus rhamnosus* GG down-regulated the enterotoxigenic *Escherichia coli*-mediated induction of IL-1β and TNF-α [22]. Further investigation is needed to confirm the suppressive effect of lactobacilli on HIV infection in both non-activated and activated PBMCs and to determine the underlying mechanism.

CT increases HIV transmission through several mechanisms. CD4+ T cells are recruited in the endocervix in women with CT [23]. CT infection of endocervical cells facilitates HIV crossing of mucosal barrier and subsequent infection [24,25]. CT also modulates mucosal immune milieu including antimicrobial peptides including defensins 5 and 6, which promote HIV infectivity [26]. Our result showed that CT promoted HIV infection of PBMCs. Interestingly, similar to our previous finding indicating that GC products increased HIV infection via TLR2 activation [15], fixed CT promoted HIV infection of freshly isolated PBMCs. Our results may explain why antibiotics reduce the incidence of bacterial STIs but not HIV [6] as dead bacteria can activate TLR signaling.

Conclusion

In conclusion, we found that live and fixed CT but not lactobacilli enhanced HIV infection. Together with our previous findings using GC and GC products that activate TLR2 to promote HIV infection, blocking STI-mediated immune activation in addition to the antibiotic treatment may offer a better strategy to prevent HIV spread in women with asymptomatic STIs.

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Bibliography

1. Cohen MS and Miller WC. "Sexually transmitted diseases and human immunodeficiency virus infection: cause, effect, or both?" *International Journal of Infectious Diseases* 3.1 (1998): 1-4.
2. Plummer FA., *et al*. "Cofactors in male-female sexual transmission of human immunodeficiency virus type 1". *Journal of Infectious Diseases* 163.2 (1991): 233-239.
3. Cameron DW., *et al*. "Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men". *Lancet* 2.8660 (1989): 403-407.
4. Ghys PD., *et al*. "The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire". *AIDS* 11.12 (1997): F85-F93.
5. Celum C., *et al*. "Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial". *Lancet* 371.9630 (2008): 2109-2119.
6. Kaul R., *et al*. "Monthly antibiotic chemoprophylaxis and incidence of sexually transmitted infections and HIV-1 infection in Kenyan sex workers: a randomized controlled trial". *Journal of the American Medical Association* 291.21 (2004): 2555-2562.
7. Galvin SR and Cohen MS. "The role of sexually transmitted diseases in HIV transmission". *Nature Reviews Microbiology* 2.1 (2004): 33-42.
8. Plummer FA. "Heterosexual transmission of human immunodeficiency virus type 1 (HIV): interactions of conventional sexually transmitted diseases, hormonal contraception and HIV-1". *AIDS Research and Human Retroviruses* 14.1 (1998): S5-S10.
9. Prebeck S., *et al*. "The Gram-negative bacterium Chlamydia trachomatis L2 stimulates tumor necrosis factor secretion by innate immune cells independently of its endotoxin". *Microbes and Infection* 5.6 (2003): 463-470.
10. Singleton TE., *et al*. "Neisserial porin-induced dendritic cell activation is MyD88 and TLR2 dependent". *Journal of Immunology* 174.6 (2005): 3545-3550.
11. Ding J., *et al*. "Neisseria gonorrhoeae enhances HIV-1 infection of primary resting CD4+ T cells through TLR2 activation". *Journal of Immunology* 184.6 (2010): 2814-2824.
12. Lee MS and Kim YJ. "Signaling pathways downstream of pattern-recognition receptors and their cross talk". *Annual Review of Biochemistry* 76 (2007): 447-480.

13. Palm NW and Medzhitov R. "Pattern recognition receptors and control of adaptive immunity". *Immunological Reviews* 227.1 (2009): 221-233.

14. Kawai T and Akira S. "The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors". *Nature Immunology* 11.5 (2010): 373-384.

15. Ding J., *et al*. "Neisseria gonorrhoeae Enhances HIV-1 Infection of Primary Resting CD4+ T Cells through TLR2 Activation". *Journal of Immunology* 184.6 (2010): 2814-2824.

16. Connor RI., *et al*. "Change in coreceptor use coreceptor use correlates with disease progression in HIV-1--infected individuals". *Journal of Experimental Medicine* 185.4 (1997): 621-628.

17. Chen BK., *et al*. "Distinct modes of human immunodeficiency virus type 1 proviral latency revealed by superinfection of nonproductively infected cell lines with recombinant luciferase-encoding viruses". *Journal of Virology* 68.2 (1994): 654-660.

18. Chang TL., *et al*. "Inhibition of HIV infectivity by a natural human isolate of Lactobacillus jensenii engineered to express functional two-domain CD4". *Proceedings of the National Academy of Sciences of the United States of America* 100.20 (2003): 11672-11677.

19. Ding J and Chang TL. "TLR2 activation enhances HIV nuclear import and infection through T cell activation-independent and -dependent pathways". *Journal of Immunology* 188.3 (2012): 992-1001.

20. Wells J. "Immunomodulatory mechanisms of lactobacilli". *Microbial Cell Factories* 10.1 (2011): S17.

21. Neville BA., *et al*. "Characterization of Pro-Inflammatory Flagellin Proteins Produced by Lactobacillus ruminis and Related Motile Lactobacilli". *PloS one* 7.7 (2012): e0040592.

22. Roselli M., *et al*. "The novel porcine Lactobacillus sobrius strain protects intestinal cells from enterotoxigenic Escherichia coli K88 infection and prevents membrane barrier damage". *Journal of Nutrition* 137.12 (2007): 2709-2716.

23. Ficarra M., *et al*. "A distinct cellular profile is seen in the human endocervix during Chlamydia trachomatis infection". *American Journal of Reproductive Immunology* 60.5 (2008): 415-425.

24. Buckner LR., *et al*. "Chlamydia trachomatis Infection of Endocervical Epithelial Cells Enhances Early HIV Transmission Events". *PloS one* 11.1 (2016): e0146663.

25. Schust DJ., *et al*. "Potential mechanisms for increased HIV-1 transmission across the endocervical epithelium during C. trachomatis infection". *Current HIV Research* 10.3 (2012): 218-227.

26. Rapista A., *et al*. "Human defensins 5 and 6 enhance HIV-1 infectivity through promoting HIV attachment". *Retrovirology* 8.1 (2011): 45.

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