**Mycobacterium tuberculosis** Complex Enhances Susceptibility of CD4 T Cells to HIV through a TLR2-Mediated Pathway

Seema M. Thayil¹, Ya-Chi Ho¹, Robert C. Bollinger¹, Joel N. Blankson¹, Robert F. Siliciano¹,², Petros C. Karakousis¹,³, Kathleen R. Page¹

¹Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States of America, ²Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States of America, ³Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States of America

**Abstract**

Among HIV-infected individuals, co-infection with *Mycobacterium tuberculosis* is associated with faster progression to AIDS. We investigated the hypothesis that *M. bovis* BCG and *M. tuberculosis* (Mtbc complex) could enhance susceptibility of CD4+ T cells to HIV infection. Peripheral blood mononuclear cells (PBMCs) collected from healthy donors were stimulated with *M. bovis* BCG, *M. tuberculosis* CDC1551 and *M. smegmatis* MC²¹⁵⁵, and stimulated CD4+ T cells were infected with R5- and X4-tropic single replication-competent pseudovirus. CD4+ T cells stimulated with Mtbc complex showed enhanced infection with R5- and X4-tropic HIV, compared to unstimulated cells or cells stimulated with *M. smegmatis* (p<0.01). Treatment with TLR2 siRNA reversed the increased susceptibility of CD4+ T cells with R5- and X4-tropic virus induced by Mtbc complex. These findings suggest that TB infection and/or BCG vaccination may be a risk factor for HIV acquisition.

**Introduction**

Chronic immune activation is central to the pathogenesis of HIV. Activation of CD4+ T cells, macrophages, and dendritic cells enhances and promotes viral replication [1,2,3]. In the primate model, natural SIV hosts that do not develop pathogenic immunodeficiency have low levels of generalized immune activation compared to pathogenic SIV infections [4,5]. In humans, systemic markers of immune activation are associated with higher HIV viral loads and faster progression to AIDS [6]. Variations in immune activation among HIV-infected individuals may result from several factors, such as host genetics, microbial translocation, malnutrition, and co-infection with other pathogens [7,8]. For example, HIV co-infection with pathogens that non-specifically activate host immunity, such as *P. falciparum*, helminths, HSV, and *M. tuberculosis*, has been associated with enhanced virological replication, which could lead to more efficient HIV transmission and faster progression to AIDS [7,9].

Among HIV-infected individuals, immune activation can also enhance HIV susceptibility following exposure to HIV. Non-ulcerative sexually transmitted diseases, such as asymptomatic incident HSV infections, increase the risk of HIV acquisition via local recruitment and activation of CD4+ T cells and macrophages, which increase the number of target cells for HIV entry [10,11]. In addition to local inflammation, some sexually transmitted infections, such as chronic asymptomatic HSV, are associated with systemic immune activation [12]. While the effect of systemic immune activation on HIV susceptibility has not been firmly established, there is evidence that factors other than local inflammation influence the immunologic milieu at mucosal sites of HIV exposure. For example, the genital tracts of Kenyan women have more activated CD4+ T cells compared to women from San Francisco, independent of genital co-infections or behavioral factors that may influence local genital inflammation [13]. In addition, systemic immunological profiles correlate with natural resistance to HIV, as low levels of peripheral T cell activation are found in individuals who remain uninfected despite frequent exposure to HIV [14,15]. The elevation of systemic immune activation markers found in HIV-uninfected Africans and Asians compared to Europeans has been attributed to chronic exposure to endemic infections, and may be one of the factors driving regional disparities in HIV rates around the world [16,17,18].

Among potential exposures in regions with high HIV rates, *M. tuberculosis* and *M. bovis* BCG (a live attenuated vaccine) are exceedingly common and elicit potent systemic immune responses that may influence HIV infectiousness. It is estimated that worldwide over 2 billion individuals have latent infection with *M. tuberculosis* and almost 10 million new cases of active tuberculosis (TB) occur each year. Despite controversy regarding the efficacy of the BCG vaccine, approximately 100 million doses are given to infants each year. The transcriptional signatures of
whole blood obtained from patients infected with *M. tuberculosis* reflect systemic immune activation in both patients with active TB and asymptomatic individuals with latent infection [19,20]. BCG vaccination can also activate systemic immune pathways associated with T cell activation which persist even after clearance of the bacteria, enhance immune responses to unrelated pathogens in infants, and modulate mucosal immunity [21,22,23,24]. Among HIV-infected individuals, co-infection with *M. tuberculosis* is associated with increased viral load and faster progression to AIDS [25].

Although the effect of TB on HIV pathogenesis has been characterized previously, the impact of systemic immune activation by mycobacterial infections on susceptibility to HIV infection among uninfected exposed individuals is not known. We hypothesized that immune activation by *M. tuberculosis* or *M. bovis* BCG, the two most common mycobacterial exposures worldwide, increases susceptibility to HIV infection. Using a single-cycle infection assay, we examined susceptibility to HIV infection in peripheral blood mononuclear cells (PBMC) stimulated with Mtb complex and identified immune pathways associated with enhanced susceptibility to HIV.

**Results**

*M. bovis* BCG and *M. Tuberculosis* (Mtb complex) Enhance Infection of CD4+ T Cells with R5-tropic and X4-tropic HIV

In order to assess whether exposure to mycobacteria affects HIV susceptibility *ex vivo*, we stimulated PBMC from healthy individuals to Mtb complex for 72 hours and subsequently infected the isolated CD4+ cells with an X4- or R5-tropic HIV pseudovirus. CD4+ cells exposed to Mtb complex had a significantly (p<0.005) higher HIV infectivity rate with X4-tropic (Fig. 1A) as well as R5-tropic virus (Fig. 1B) relative to that of unstimulated cells and to cells exposed to the nonpathogenic mycobacterium *M. smegmatis*, in which HIV infectivity was similar to that of unstimulated cells. The percentage of HIV-infected cells was comparable following stimulation with *M. bovis* BCG, a known immunomodulator, and with *M. tuberculosis* at the same MOI (Fig. 1C; p = 0.3).

**Exposure to Mtb Complex does not Alter Expression of CCR5 and CXCR4 Co-receptors in CD4+ T Cells**

To evaluate whether the enhanced infectivity observed in CD4+ cells exposed to Mtb complex was due to modulation of the co-receptors CCR5 and CXCR4, we measured the expression of CCR5 and CXCR4 in CD4+ cells exposed to mycobacteria. CCR5 receptors were expressed on 0–5% of CD4+ cells in all experimental and control groups (Fig. 2a). Mean fluorescence intensity values for CCR5 receptor were 366.4, 346.7, and 1403.3, and 403.4, 436.6, and 78.1, respectively. CXCR4 was observed to be expressed on 70–85% of CD4+ cells (Fig. 2b), but expression of this co-receptor did not vary among stimulated and unstimulated cells or between different groups of stimulated cells (p = 0.4). Mean fluorescence intensity values for CXCR4 receptor were 1406.4, 1328.2, 1540.0, 407.3, 1492.6, 346.7, and 1403.5, for unstimulated CD4+ cells, and those stimulated with *M. bovis* BCG, *M. tuberculosis*, and *M. smegmatis*, respectively. We concluded that differential expression of the entry co-receptors CXCR4 and CCR5 does not account for the increased susceptibility to HIV of cells exposed to Mtb complex.

**Increased Immune Activation Markers HLA DR and CD38 do not Explain the Increased Susceptibility to HIV Infection Associated with Mtb Complex Stimulation**

Next, we compared the expression of two different immune activation markers on the surface of CD4+ cells stimulated with different mycobacteria (Fig. 3a). As expected, CD38 expression was higher in stimulated cells compared to unstimulated cells (p<0.05), but there was no difference in CD38 expression between CD4+ cells exposed to *M. bovis* BCG, *M. tuberculosis*, or *M. smegmatis* (Fig. 3b). CD4+ cells exposed to *M. bovis* BCG and *M. tuberculosis* had diminished expression of HLA DR (p<0.05) when compared to cells exposed to *M. smegmatis* (Fig. 3C).

**Enhanced Expression of TLR2, but not of TLR4, in CD4+ T Cells Exposed to *M. bovis* BCG**

Since pathogenic mycobacteria are potent inducers of TLR [26], and TLR-mediated immune activation is associated with HIV replication [27], we evaluated the gene expression levels of TLR2, TLR4, and TLR9 in CD4+ cells exposed to *M. bovis* BCG and *M. smegmatis* by RT-PCR. Although TLR4 expression was higher and TLR9 expression lower in cells exposed to mycobacteria compared to unstimulated cells, there was no difference in expression of TLR4 or TLR9 between cells stimulated with *M. bovis* BCG or *M. smegmatis*. However, gene expression of TLR2 was approximately two-fold higher in cells exposed to *M. bovis* BCG than in unstimulated cells (Fig. 4a).

**Increased Susceptibility of CD4+ T Cells Exposed to Mtb Complex is Mediated through a TLR2-dependent Mechanism**

In order to determine whether higher expression of TLR2 results in increased HIV susceptibility of *M. bovis* BCG-exposed cells, we silenced TLR2 expression in cells prior to stimulation with *M. bovis* BCG. Relative to *M. bovis* BCG-stimulated CD4+ cells treated with scrambled siRNA, transfection of CD4+ cells with TLR2 siRNA prior to *M. bovis* BCG stimulation decreased infection with R5 virus from 6.2±0.7% to 3.6±1.0% (p = 0.005; Fig. 4b), and with X4 virus from 10.7±0.8% to 7.0±1.0% (p = 0.001; data not shown). Flow cytometry confirmed that prior treatment with TLR2 siRNA reduced TLR2 expression in CD4+ cells stimulated with Mtb complex relative to that of similarly stimulated cells pre-treated with scrambled siRNA with *M. smegmatis* (p<0.005; Figs. 4c and 4d). Therefore, we conclude that the increased susceptibility of CD4+ T cells to HIV infection following Mtb complex exposure is at least partly mediated by TLR2.

**Discussion**

In this study, we demonstrate that Mtb complex, but not the nonpathogenic *M. smegmatis*, enhances HIV infection of CD4+ T cells through a TLR2-dependent mechanism. While increased HIV replication has been previously associated with Mtb, this is the first study demonstrating that mycobacterial infections increase CD4 cell susceptibility to HIV infection. Given the magnitude of the TB epidemic worldwide, these findings could have significant public health implications.

We evaluated various immunological pathways that could be associated with increased HIV susceptibility in T cells exposed to *M. tuberculosis* and *M. bovis* BCG, such as increased expression of entry co-receptors (CCR5 and CXCR4), upregulation of immune activation markers HLA DR and CD38, or mediation through TLR pathways known to be modulated by *M. tuberculosis* and
M. bovis BCG (TLR2, TLR4 and TLR9) [28,29,30]. We found that TLR2 was significantly upregulated in T cells exposed to M. bovis BCG and that blockade of TLR2 expression attenuated the increased susceptibility to HIV. The role of TLR2 in enhanced HIV susceptibility is consistent with previous studies of HIV and TB pathogenesis. Both M. tuberculosis and M. bovis BCG are potent inducers of TLR2 [28,29,30,31]. In HIV transgenic mouse spleens, TLR2 signaling enhances HIV replication through trans-activation of HIV long terminal repeats [27,32]. Signaling through TLR2 has been shown to trigger HIV replication in a variety of human cell lines, including latently infected mast cells and dendritic cells [33,34]. Co-infection with certain bacteria that stimulate TLR2 can lead to HIV reactivation. For example, periodontal pathogens cause HIV reactivation in a monocyte model of HIV latency via TLR2 activation [35]. HIV transgenic mice infected with M. tuberculosis have enhanced viral production, but this effect is not observed in TLR2-deficient HIV mice [36]. Dendritic cells obtained from HIV-infected individuals co-infected with M. tuberculosis have increased expression of TLR2 and TLR4, which may promote HIV replication and contribute to the faster

Figure 1. M. tuberculosis complex increases susceptibility to HIV infectivity. The percentage of unstimulated CD4+ cells and CD4+ cells stimulated with PHA (50 μg/ml), M. bovis BCG (Copenhagen), M. tuberculosis (CDC1551), M. smegmatis (MC²155) from individual subjects infected with X4-tropic pseudovirus (A) or R5-tropic pseudovirus (B). Results from individual donors are color-coded. Statistical analysis was performed using student T test (** p < 0.005). Flow cytometry analysis of the representative CD4+ cells after infection with GFP-expressing pseudovirus (C). doi:10.1371/journal.pone.0041093.g001
disease progression observed in patients with opportunistic infections [37].

In addition to promoting HIV replication in various models of HIV infection, TLR2 has also been implicated in enhancing susceptibility to HIV infection [38]. TLR2 stimulation of Langherhan cells, which are the initial targets of HIV infection following sexual exposure, results in enhanced infection of these cells and subsequent trans-infection of T cells [39]. TLR2 signaling also enhances infection of quiescent naïve and memory CD4+ T cells, which are normally relatively resistant to HIV infection [40]. N. gonorrhea, a sexually transmitted pathogen strongly associated with increased HIV risk, enhanced HIV infection of resting CD4+ cells through TLR2 activation [41]. Similar to the mycobacterial effect we observed, the enhanced susceptibility of CD4+ T cells to HIV following exposure to N. gonorrhea was reduced upon TLR2 blockade. Recently, Ding and Chang have shown that TLR2 activation promotes HIV infection and nuclear import in resting CD4+ T cells through both T cell activation-dependent and -independent mechanisms [42].

Although previous clinical studies have shown that infection with M. tuberculosis or vaccination with M. bovis BCG systemically activate TLR2-mediated pathways, more work is needed to determine whether these responses lead to enhanced HIV susceptibility in humans as suggested by our ex vivo model [30,43]. Potential implications of our findings could be significant, especially for regions with a high prevalence of TB and HIV. Our findings may also have implications for TB vaccine development, since current strategies involve genetically modifying the BCG vaccine, developing adjuvants to strengthen protective responses, or using other attenuated mycobacterial species as potential vaccines [28], all of which may activate TLR2-mediated pathways.

There are several important limitations of our model, which require further study to assess the clinical and public health implications of our findings. Direct infection of PBMCs with M. tuberculosis complex in our ex vivo model most likely overestimates the level of antigenic stimulation resulting from natural infection in the lungs or intradermal vaccination. Further work is...
needed to determine whether pulmonary TB infection or intradermal BCG vaccination modulates immune responses at sites of HIV exposure, such as the genital and gastrointestinal mucosa, where local flora may drive mucosal immune responses. Although in the current study we have not elucidated the cell type responsible for CD4+ T cell activation, the potential role of different antigen-presenting cells, including macrophages and dendritic cells, deserves further study. One hypothesis is that TLR2-stimulated dendritic cells encountering \textit{M. tuberculosis} or \textit{M. bovis} BCG traffic to lymphoid organs and mucosa-associated lymphoid tissue, where they prime and activate CD4+ T cells. Recent data in a murine model shows dynamic trafficking patterns in dendritic cells exposed to a chronic \textit{M. bovis} BCG granuloma [44]. Despite very low antigenic availability following BCG vaccination, CD4+ T cell priming still was observed as a result of dendritic cell migration from the site of chronic granuloma formation to systemic sites and lymphoid organs. Furthermore, upregulation of TLR2 has been reported in dendritic cells from patients co-infected with TB and HIV [37,45].

While our findings in an \textit{ex vivo} model of co-infection likely overestimate the impact of dual infection on actual HIV risk, they provide insights about mechanisms of HIV susceptibility that could guide future studies using clinical samples from patients with TB or BCG vaccination. Given the magnitude of the TB epidemic worldwide, even a modest level of enhanced susceptibility to HIV due to \textit{M. tuberculosis} infection or BCG vaccination could have a significant impact at a population level. These results provide initial support for the intriguing possibility that pathogenic mycobacterial infections could partially explain variations in HIV susceptibility worldwide.

**Materials and Methods**

**Ethics Statement**

Written informed consent was obtained from all subjects prior to entry into the study, which was approved by the Johns Hopkins University School of Medicine Institutional Review Board.
Subjects

Ten HIV-seronegative healthy individuals (ages 23–54) without prior history of BCG vaccination or TB infection were recruited at Johns Hopkins University School of Medicine. All subjects were confirmed to have negative tuberculin skin tests prior to entry into the study.

Cell Isolation and Culture

Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteers and antigen stimulation of cells was performed by incubating cells with whole bacteria or antigen for 48–72 hours at 37°C with 5% CO₂. For infectivity assays, unstimulated cells were used as negative control and PHA (50 μg/ml) stimulated cells were used as positive control to ensure that cells were responsive to in vitro stimulation.

Bacterial Strains and Mycobacterial Infection

*M. bovis* BCG (Copenhagen), *M. tuberculosis* CDC1551, and *M. smegmatis* MC²155 strains procured from ATCC were used for stimulation of PBMC at an MOI of 1:4. The bacterial strains were grown to mid-logarithmic phase in 7H9 broth (Difco, Sparks MD) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC, Difco), glycerol, 0.05% Tween 80 at 37°C on a shaker. At the time of stimulation, bacteria were pelleted and resuspended in complete RPMI and co-cultured with isolated PBMC in complete RPMI supplemented with IL-2 at 37°C for 24–48 hours.

Single-cycle HIV-1 Infection Assay

After stimulation, the cells were washed with fresh RPMI and CD4+ cells were isolated from stimulated PBMC by negative selection using a CD4+ T cell isolation kit (Miltenyi biotech; GBMII) according to the manufacturer’s instructions. Isolated CD4+ cells were infected with a single-cycle replication-competent, green fluorescent protein (GFP)-expressing proviral construct pseudotyped with CCR5-tropic or CXCR4-tropic envelope (NL43-deltaEnvGFP), as previously described [46]. Briefly, infected CD4+ cells were spinoculated for 2 hrs with the virus. The infected cells were incubated at 37°C for 72 hrs. Fluorescence emitted by the cells carrying GFP-expressing virus was quantified using Cellquest Pro software (Beckton Dickinson) to calculate percent infectivity.

Immunofluorescence Staining and Flow Cytometry Analysis

Stimulated CD4+ cells were washed and then stained with directly conjugated antibodies to cell surface markers, including CD4-FITC, CD3-PE, CD38-PE, HLA DR-FITC, CD195-PE, and CD184-FITC (Becton Dickinson, San Jose, CA) and TLR-2 directly conjugated antibodies to cell surface markers, including TLR-2 (Santa Cruz Biotechnology, Inc.) following the manufacturer’s instructions. Paired samples were subsequently subject to either immunofluorescence staining with TLR-2 antibody or virus infectivity assays, as described above.

Quantitative Reverse-transcription PCR

Total RNA was isolated from 10⁶ to 10⁷ CD4+ stimulated cells using RNeasy Mini Kit (Qiagen). Fluorescently-labeled cDNA was generated using oligo(dT) primers and SuperScript III (Invitrogen), using fluorescent dyes Cy3 and Cy5 (Amersham). cDNA corresponding to each transcript was subjected to 34 cycles of PCR for quantification using the primers listed in Table S1. The cycle threshold value (Cₚ) obtained for each gene of interest (GOI) was normalized with that of GAPDH, a housekeeping gene, in order to obtain the normalized cycle threshold (nCₚ = (GOI Cₚ) – (HKG Cₚ)). The change in cycle threshold (ΔCₚ) was calculated using the following formula: ΔCₚ = Cₚcontrol – Cₚsample, where C represents unstimulated cells (negative control) and S represents cells stimulated with mycobacteria or phytohemagglutinin (PHA; positive control). Prior to reverse transcription, control and mutant RNA (10 ng) were treated with RNase-free DNase (Invitrogen) and subjected to 36 cycles of PCR to assure that all DNA had been removed, as assessed by ethidium bromide-stained agarose gel analysis.

siRNA Transfection

Transfection of monocytes was performed using negative control scrambled small interfering RNA (siRNA) or siRNA targeting TLR-2 (Santa Cruz Biotechnology, Inc.) following the manufacturer’s instructions. Briefly, cells were seeded at 2×10⁶ cells per well in a 6 well plate with reduced siRNA transfection reagent (Santa Cruz Biotechnology Inc.) for 5–8 hours at 37°C with 5% CO₂. The media were then replaced and the cells were allowed to rest overnight, followed by stimulation with individual strains of mycobacteria. Paired samples were subsequently subject to either immunofluorescence staining with TLR 2 antibody or virus infectivity assays, as described above.

Statistical Analysis

Statistical significance was determined by students T Test. P-value of <.05 was considered statistically significant.

Supporting Information

Table S1 Primers used for RT-PCR studies.

DOCX

Acknowledgments

We are grateful to all participants in this study.

Author Contributions

Conceived and designed the experiments: KRP PCK RFS JNB RCB. Performed the experiments: SMT Y-CH KRP. Analyzed the data: SMT KRP PCK. Contributed reagents/materials/analysis tools: PCK RFS JNB RCB. Wrote the paper: KRP PCK SMT.

References

1. Brenchley JM, Price DA, Douek DC (2006) HIV disease: fallout from a mucosal catastrophe? Nat Immunol 7: 235–239.
2. Lama J, Planche Y (2007) Host factors influencing susceptibility to HIV infection and AIDS progression. Retrovirology 4: 52.
3. Moir S, Chuan TW, Fauzi AS (2011) Pathogenic mechanisms of HIV disease. Annu Rev Pathol 6: 223–248.
4. Bosinger SE, Sodora DL, Silvestri G (2011) Generalized immune activation and innate immune responses in simian immunodeficiency virus infection. Curr Opin HIV AIDS 6: 411–418.
5. Sodora DL, Allan JS, Apreti C, Brenchley JM, Douek DC, et al. (2009) Toward an AIDS vaccine: lessons from natural simian immunodeficiency virus infections of African nonhuman primate hosts. Nat Med 15: 861–865.
6. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, et al. (1999) Shorter survival in advanced human immunodeficiency virus type-1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis 179: 839–470.
7. Barnabas RV, Webb EL, Weiss HA, Wasserheit JN (2011) The role of coinfections in HIV epidemic trajectory and positive prevention: a systematic review and meta-analysis. AIDS 25: 1539–1573.
8. Kaul R, Cohen CR, Chege D, Yi TJ, Tharao W, et al. (2011) Biological factors that may contribute to regional and racial disparities in HIV prevalence. Am J Reprod Immunol 65: 317–324.
9. Waison JL, Herrin BR, John-Stewart G (2009) Deworming helminth co-infected individuals for delaying HIV disease progression. Cochrane Database Syst Rev: CD006419.
10. Heng MC, Heng SY, Allen SG (1994) Co-infection and synergy of human immunodeficiency virus-1 and herpes simplex virus-1. Lancet 343: 253–258.

11. Reynolds SJ, Rabidu AR, Shepherd ME, Zenilman JM, Besokmeyer RS, et al. (2003) Recent herpes simplex virus type 2 infection and the risk of human immunodeficiency virus type 1 acquisition in India. J Infect Dis 187: 1513–1521.

12. Sheth PM, Sunderji S, Shin LY, Rebbapragada A, Huibner S, et al. (2008) Coinfection with herpes simplex virus type 2 is associated with reduced HIV-specific T cell responses and systemic immune activation. J Infect Dis 197: 1394–1401.

13. Cohn CR, Moscicki AB, Scott ME, Shiboski S, et al. (2010) Increased levels of immune activation in the genital tract of healthy young women from sub-Saharan Africa. AIDS 24: 2069–2074.

14. McLaren PJ, Bell TB, Wachholtz C, Jaeo W, Kelvin DJ, et al. (2010) HIV-exposed seronegative commercial sex workers show a quiescent phenotype in the CD4+ T cell compartment and reduced expression of HIV-dependent host factors. J Infect Dis 202 Suppl 3: S299–344.

15. Pancino G, Sarz-Clutton A, Scott-Algara D, Paul P (2010) Natural resistance to HIV infection: lessons learned from HIV-exposed uninfected individuals. J Infect Dis 202 Suppl 3: S345–350.

16. Kalinovich A, Borkow G, Weisman Z, Tsimani A, Stein M, et al. (2001) Increased CCR5 and CXCR4 expression in Ethiopians living in Israel: environmental and constitutive factors. Clin Immunol 100: 107–117.

17. Koesters SA, Matu I, Kiama P, Amala O, Embree J, et al. (2004) Elevation of immune activation in kenyan women is associated with alterations in immune function: implications for vaccine development. J Clin Immunol 24: 762–769.

18. WHO (2011) TB Data 2011. Available: www.who.int/tb/data. Accessed 2012 June 25.

19. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, et al. (2010) An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466: 973–977.

20. Jacobsen M, Repsilber D, Gutschmidt A, Neher A, Feldmann K, et al. (2007) Candidate biomarkers for discrimination between infection and disease caused by Mycobacterium tuberculosis. J Mol Med (Berl) 85: 613–621.

21. Alexandroff AB, Jackson AM, O’Donnell MA, James K (1999) BCG immunotherapy of bladder cancer: 20 years on. Lancet 353: 1689–1694.

22. Croix DA, Capuano S, 3rd, Simpson L, Fallert BA, Fuller CL, et al. (2000) Increased CCR5 and CXCR4 expression in Ethiopians living in Israel: environmental and constitutive factors. Clin Immunol 100: 107–117.

23. Underhill DM, Ozinsky A, Smith KD, Aderem A (1999) Toll-like receptor-2 trans-activation and HIV replication in HIV-1 transgenic mouse spleen cells: implications of simultaneous activation of TLRs on HIV replication. J Immunol 188: 992–1001.

24. Thibault S, Fromentin R, Tardif MR, Tremblay MJ (2009) TLR2 and TLR4 triggering exerts contrasting effects with regard to HIV-1 infection of dendritic cells and subsequent virus transfer to CD4+ T cells. Retrovirology 6: 42.

25. Gonzalez OA, Li M, Ebersole JL, Huang CB (2010) HIV-1 reactivation induced by the periodontal pathogens Fusobacterium nucleatum and Porphyromonas gingivalis involves Toll-like receptor 2 [corrected] and 9 activation in monocytes/macrophages. Clin Vaccine Immunol 17: 1417–1427.

26. Balica A, Scanga CA, Schito ML, Heny S, Sher A (2003) Cutting edge: in vivo induction of integrated HIV-1 expression by mycobacteria is critically dependent on Toll-like receptor 2 expression. J Immunol 171: 1123–1127.

27. Hernandez JC, Arteaga J, Paul S, Kumar A, Latz E, et al. (2011) Up-regulation of TLR2 and TLR4 in dendritic cells in response to HIV type 1 and coinfection with opportunistic pathogens. AIDS Res Hum Retroviruses 27: 1099–1109.

28. Ogawa Y, Kawamura T, Kimura T, Ito M, Blanchev A, et al. (2009) Gram-positive bacteria enhance HIV-1 susceptibility in Langerhans cells, but not in dendritic cells, via Toll-like receptor activation. Blood 113: 5157–5166.

29. Thibault S, Tardif MR, Barat C, Tremblay MJ (2007) TLR2 signaling renders quiescent naive and memory CD4+ T cells more susceptible to productive infection with X4 and R5 HIV-type 1. J Immunol 179: 4357–4366.

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

31. Dung J, Chang TL (2012) TLR2 activation enhances HIV nuclear import and infection through T cell activation-independent and -dependent pathways. J Immunol 188: 992–1001.

32. Chang JS, Huggett JF, Dheka K, Kim LU, Zumla A, et al. (2006) Mycobacterium tuberculosis induces selective up-regulation of TLRs in the mononuclear leukocytes of patients with active pulmonary tuberculosis. J Immunol 176: 3010–3018.

33. Schreiber HA, Harding JS, Hunt O, Altamirano CJ, Huleberg FD, et al. (2011) Inflammatory dendritic cells migrate in and out of transplanted chronic mycobacterial granulomas in mice. J Clin Invest 121: 3902–3913.

34. Tam DB, Lin A, Yong YK, Ponnampalamaran S, Omar S, et al. (2011) TLR2-induced cytokine responses may characterize HIV-infected patients experiencing mycobacterial immune restoration disease. AIDS 25: 1455–1460.

35. Zhang H, Zhou Y, Alcock C, Kiefer T, Monie D, et al. (2004) Novel single-cell-specific transduction in vivo of cells and tissues by Mycobacterium tuberculosis: a role for Toll-like receptors. Nat Rev Microbiol 4: 5159–5164.

36. Ogawa Y, Kawamura T, Kimura T, Ito M, Blanchev A, et al. (2009) Gram-positive bacteria enhance HIV-1 susceptibility in Langerhans cells, but not in dendritic cells, via Toll-like receptor activation. Blood 113: 5157–5166.

37. Hernandez JC, Arteaga J, Paul S, Kumar A, Latz E, et al. (2011) Up-regulation of TLR2 and TLR4 in dendritic cells in response to HIV type 1 and coinfection with opportunistic pathogens. AIDS Res Hum Retroviruses 27: 1099–1109.

38. Balica A, Scanga CA, Schito ML, Heny S, Sher A (2003) Cutting edge: in vivo induction of integrated HIV-1 expression by mycobacteria is critically dependent on Toll-like receptor 2 expression. J Immunol 171: 1123–1127.

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

40. Dung J, Chang TL (2012) TLR2 activation enhances HIV nuclear import and infection through T cell activation-independent and -dependent pathways. J Immunol 188: 992–1001.

41. Dung J, Chang TL (2012) TLR2 activation enhances HIV nuclear import and infection through T cell activation-independent and -dependent pathways. J Immunol 188: 992–1001.