SHORT COMMUNICATION

HIV Interferes with *Mycobacterium tuberculosis* Antigen Presentation in Human Dendritic Cells

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HIV coinfection is the most prominent risk factor for progression of *Mycobacterium tuberculosis* (Mtb) infection into active tuberculosis (TB) disease. The mechanisms behind the increased transition from latent to active TB in coinfecting individuals have not been well elucidated at the cellular level. We hypothesized that HIV infection contributes to Mtb pathogenesis by interfering with the dendritic cell (DC)—mediated immune control. Mtb-antigen processing and presentation are key events in the immune response against TB. Human immature DCs coinfecting with HIV/Mtb had decreased expression of human leukocyte antigen D related and the costimulatory molecules CD40, CD80, and CD86. In addition, Mtb-infected DCs triggered a significant release of the proinflammatory cytokines IL-6, IL-1 β , and tumor necrosis factor- α , whereas coinfecting DCs did not. To assess the DC antigen presentation capacity, we measured interferon- γ from co-cultures of DCs and autologous Mtb antigen-specific CD4⁺ T cells. Interferon- γ release was significantly reduced when purified protein derivative— and Ag85B-specific CD4⁺ T cells had been activated with coinfecting DCs compared to Mtb-infected DCs, and this effect was attributed to Mtb antigen processing rather than peptide—major histocompatibility complex class II loading. Evaluating autophagy as a measure of vesicular processing and maturation further revealed that HIV efficiently blocks initiation of this pathway during coinfection. Overall, our results demonstrate that HIV impairs Mtb antigen presentation in DCs, thereby suppressing an important cell linking innate and adaptive immune response in TB. (*Am J Pathol* 2016, 186: 3083–3093; <http://dx.doi.org/10.1016/j.ajpath.2016.08.003>)

HIV and *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis (TB), are devastating coepidemics in many countries and represent a tremendous challenge in patient care, public health, and economic burden. The interaction between HIV and Mtb is synergistic, each facilitating the pathogenicity of the other.^{1,2} HIV affects the course of TB in several ways, including promoting progression into active TB, both in people with recently acquired infection and in those with latent infection. Both of these diseases affect the body's immune system, and coinfection prevents the body from properly fighting against these pathogens.³ Although the mechanisms involved are still largely unknown, they may include synergistic detrimental effects on the immune response, such as immune exhaustion.⁴

Dendritic cells (DCs) are critical mediators in the interaction between the adaptive and innate immune system and

are strategically located at sites of pathogen entry. The immune surveilling properties of DCs can be used by pathogens to facilitate infection and transmission.⁵ HIV makes use of DCs for infection and for efficient spread to CD4⁺ T cells.^{6,7} Although macrophages are the main target cells for Mtb, DCs also serve as an important intracellular niche and are readily infected.^{8–10} After phagocytosis and internalization of pathogens, DCs undergo a process of maturation¹¹ and increase their expression of surface molecules that facilitate antigen presentation, such as CD80, CD86, CD40, and major histocompatibility complex (MHC)

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class II.^{12–18} In addition, DCs secrete cytokines that promote the development of an appropriate immune response. During Mtb infection, DCs migrate into secondary lymphoid organs, where they present pathogen-derived peptide antigens on MHC class II molecules to naïve CD4⁺ T cells, initiating T-cell activation.^{10,19,20} Cells from HIV-seropositive asymptomatic individuals showed reduced T-cell proliferation attributed to a dysfunctional DC response appearing before any symptom and changes in T cells.²¹ Given the pivotal role for DCs in both these infections, it is important to investigate the effect of coinfection with HIV and Mtb on DC function.

An important mechanism involved in delivery of antigens to MHC class II molecules in DCs is autophagy.²² Autophagy plays key roles in immune defenses against invading bacterial and viral pathogens, by regulating antigen presentation, microorganism capture, and degradation.^{23–25} Disturbance in the autophagy pathway has been linked to increased infection and autoimmunity.^{26,27} The autophagy machinery is involved in delivering antigens to MHC class II,²⁸ and the antigen loading compartments for MHC class II receive continuous input from autophagosomes.²⁹ Autophagy in DCs is subsequently critical for an appropriate CD4⁺ T-cell response against infection.³⁰

The aim of the current investigation of HIV/Mtb coinfecting human DCs was to understand the mechanism behind HIV-accelerated Mtb disease progression on a cellular level. We found that HIV suppressed the Mtb-induced proinflammatory response, autophagy pathway activation, as well as up-regulation of MHC class II and costimulatory molecules on DCs, all contributing to reduced activation of Mtb-specific CD4⁺ T cells.

Materials and Methods

Reagents

Purified protein derivative (PPD; culture filtrates from Mtb strain H37Rv) was obtained from the Statens Serum Institut (Copenhagen, Denmark). Recombinant human granulocyte-macrophage colony-stimulating factor and IL-4 were from Peprotech (Rocky Hill, NJ). Recombinant human IL-2 was from Miltenyi Biotec (Bergish Gladbach, Germany). Bafilomycin A1, poly IC, staphylococcal enterotoxin B, and human AB serum were from Sigma-Aldrich (St. Louis, MO). Ovalbumin was from InvivoGen (San Diego, CA). Monoclonal rabbit anti-human LC3B (D11), cGAS, STING, and phospho-tank-binding kinase 1 (TBK1) antibodies were from Cell Signaling Technology (Boston, MA), monoclonal anti-human p62 (SQSTM1) was from Santa Cruz Biotechnology (Dallas, TX), and monoclonal anti-human β -actin was from Sigma-Aldrich. Polyclonal goat anti-rabbit or anti-mouse immunoglobulins/horseradish peroxidase were from Dako Cytomation (Glostrup, Denmark). Cytometric Bead Array Flex sets, CD40 fluorescein isothiocyanate, CD14 phycoerythrin (PE), CD80 PE, CD83 PE, human leukocyte

antigen antigen D related (HLA-DR) PerCP, CCR7 AF647, CD1c PE, and isotype control antibodies were from BD Biosciences (San Jose, CA). CD3 AF647, CD4 Pacific Blue, CD8a PerCP, CD45RA AF700, CD45RO PE, and CD86 Pacific Blue were from Biolegend (San Diego, CA). TACS annexin V—fluorescein isothiocyanate kit was from R&D Systems (McKinley Place, MN). Human interferon (IFN)- β ELISA kit was from PBL Assay Science (Piscataway Township, NJ).

Peptides

Peptides representing Ag85B were synthesized as crude material on a small (1 mg) scale by A and A (San Diego, CA). Peptides were 15-mers overlapping by 10 amino acids spanning the entire protein, as well as one 15-mer identified as an epitope in previous studies.³¹ The 64 individual peptides were pooled to construct one peptide pool (Ag85B peptides).

Dendritic Cell Generation

Buffy coats were obtained from healthy individuals (from Linköping blood bank, Sweden) who had given written consent for research use of the donated blood in accordance with the Declaration of Helsinki and paragraph 4 of the Swedish law (2003:460) on Ethical Conduct in Human Research. DCs were propagated from peripheral blood mononuclear cells derived from buffy coats, as described previously,³² with some modifications using RPMI 1640 medium with 5% heat inactivated human AB serum, and supplemented with 20 ng/mL rh granulocyte-macrophage colony-stimulating factor and 5.5 ng/mL recombinant human IL-4 for 5 days. Flow cytometry was performed to confirm an immature DC phenotype (CD14⁺CD83^{lo}CD1c^{hi}), as shown in [Supplemental Figure S1](#).

Mycobacterium and HIV Strains, Treatments, and Infections

HIV-1BaL (lot p4238) was produced using chronically infected cultures of the ACVP/BCP cell line (number 204). Virus was purified and concentrated, as previously described,³³ and aliquots were frozen in liquid nitrogen vapor. HIV was opsonized by incubating equal volumes of HIV and human serum for 1 hour at 37°C (hereafter referred to as HIV), before infection. γ -Irradiated Mtb H37Rv was needle sheared and opsonized by incubating a single-cell suspension of Mtb with human serum (v/v) for 30 minutes at 37°C (referred to as Mtb). Immature DCs seeded at 3×10^5 /well in a 24-well plate were infected with HIV, 1 μ g/mL (p24 equivalent/mL), for 2 hours before infection with Mtb (multiplicity of infection, 5). DCs were also stimulated with poly IC (30 ng/mL) and lipopolysaccharide/IFN- γ (50 ng/mL lipopolysaccharide and 100 U/mL IFN- γ), both used as positive controls. After 48 hours, cell-free culture supernatants were saved and the cells were stained for surface marker expression.

Establishment of Mtb Ag-Specific CD4 T-Cell Lines

Peripheral blood mononuclear cells from the same donors used for DC generation were used to purify untouched CD4⁺ naïve T cells using the human naïve CD4⁺ T-cell isolation kit, according to manufacturer instructions (Stem Cell Technologies, Grenoble, France). The sorted cells were >90% pure naïve CD4⁺ T cells (CD3⁺CD4⁺CD45RA⁺CD45RO⁻). The generated DCs were γ -irradiated (25 Gy) before coincubation with CD4⁺ T cells for priming. CD4⁺ T cells were cultured with autologous γ -irradiated DCs at a 4:1 ratio, and PPD or Ag85B (10 μ g/mL), the major secretory and highly immunogenic protein of Mtb,^{34,35} was used to generate Mtb antigen (Ag)-specific CD4⁺ T cells. Cells were supplemented with IL-2 (20 IU/mL) at the onset of culture and replenished once a week. The antigen specificity was evaluated 3 to 4 weeks later. For that, the CD4⁺ T cells and thawed autologous DCs were co-cultured at a 5:1 ratio along with PPD or Ag85B (10 μ g/mL) or staphylococcal enterotoxin B (1 μ g/mL; positive control) or ovalbumin (10 μ g/mL; background), analyzing the IFN- γ levels after 72 hours.

Cytokine Analysis

Cytokines in the culture supernatants were determined by cytometric bead array analysis, performed according to the manufacturer's protocol (BD Biosciences). Detection of cytokines was performed on a Gallios (Beckman Coulter, Brea, CA) flow cytometer, and cytokine concentrations were analyzed using Kaluza software version 1.3 (Beckman Coulter).

Surface Staining and Flow Cytometry Analysis of Infected DCs

Infected DCs were stained for 30 minutes with a mixture of HLA-DR PerCP, CD40 fluorescein isothiocyanate, CD80 PE, CD86 Pacific Blue, and CCR7 AF647, or single stained with CD83 PE, or appropriate isotype controls. Cells were fixed with 4% paraformaldehyde for 20 minutes, and surface marker detection was performed on a Gallios flow cytometer and analyzed using Kaluza software version 1.3.

Annexin V Apoptosis Assay

Infected DCs were washed and stained with fluorescein isothiocyanate—annexin V, according to manufacturer's protocol, using propidium iodide counterstaining to detect necrotic cells. As positive control, DCs were either exposed to UV light for 10 minutes (240 J/second/m²) and incubated for 24 hours, or treated with staurosporine (2.5 μ mol/L) for 24 hours. Stained cells were fixed with 4% paraformaldehyde and detected on a Gallios flow cytometer and analyzed using Kaluza software version 1.3.

Antigen Presentation Assay

DCs were thawed, seeded (2×10^4 /well), and infected with HIV for 2 hours before infection with Mtb (multiplicity of

infection, 5). After 24 hours, supernatants were removed and DCs were co-cultured with autologous PPD- or Ag85B-specific CD4⁺ T cells (1×10^5 /well). At the time of co-culture, the fresh medium was supplemented with 10 μ mol/L of azidothymidine to prevent HIV replication. Cell-free supernatants were assayed for IFN- γ after 72 hours of co-culture.

To discriminate between antigen processing and MHC class II loading, DCs were pulsed with the Ag85B peptide pool that does not require processing for presentation. For this, DCs were infected with HIV for 24 hours, and pulsed with Ag85B peptides (2 μ g/mL) during the last 2 hours of incubation. Thereafter, DCs were washed four times, to remove any peptides not bound by MHC, co-cultured with Mtb antigen-specific CD4⁺ T cells (1:4; DC/T ratio), supplemented with azidothymidine. IFN- γ in cell-free supernatants was assayed after 48 and 72 hours of co-culture.

Autophagy Assay and Western Blot Analysis

DCs were plated and infected with HIV; after 1 hour, some cells were treated with bafilomycin (100 nmol/L). Bafilomycin was used to monitor the complete generation of LC3BII and SQSTM1 (ie, to study formation of autophagosomes without their subsequent degradation or flux). DCs were infected with Mtb after 2 hours of HIV infection. After 6 hours (or 24 hours for cGAS, STING, and pTBK1) of coinfection, DCs were washed with phosphate-buffered saline, lysed in 2 $\times$ Laemmli sample buffer, and boiled for 10 minutes. Lysates were separated by 8% to 16% SDS-PAGE and electrotransferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% milk powder in phosphate-buffered saline Tween-20 (0.075%) and incubated with primary antibodies recognizing LC3 (1:5000), SQSTM1 (1:2000), cGAS (1:1000), STING (1:1000), pTBK1 (1:1000), and β -actin (1:10,000) as a loading control. The specific proteins were detected with a commercial ECL kit (Amersham, Buckinghamshire, UK). Densitometric band intensity analyses were performed with ImageJ (NIH, Bethesda, MD; <https://imagej.nih.gov/ij>).

Statistical Analysis

Statistical analyses were performed with GraphPad prism software version 5.0f (Graphpad Software Inc., La Jolla, CA), using one-way repeated measures analysis of variance with Bonferroni correction. $P < 0.05$ was considered significant.

Results

HIV Coinfection Down-Regulates Mtb-Induced MHC Class II and Costimulatory Molecules on DCs

Surface expression of MHC class II/HLA-DR, costimulatory molecules, and CCR7 on DCs was analyzed to assess whether HIV affected Mtb-induced up-regulation of

Figure 1 HIV coinfection reduces Mtb-induced major histocompatibility complex class II and costimulatory molecule expression on DCs. DCs were either uninfected (unstim) or infected with HIV, Mtb, or coinfecting (HIV + Mtb), or stimulated with poly IC or lipopolysaccharide (LPS)/interferon (IFN)- γ as positive controls. After 48 hours, DCs were stained with antibodies against CD40, CD80, CD83, CD86, human leukocyte antigen antigen D related (HLA-DR), and CCR7, and surface marker expression was analyzed by flow cytometry. The bar graphs present data as percentage marker positive DCs (A), and fold increase (compared to isotype) in the total surface expression of the markers (B). In the cell viability assay (C), DCs were either uninfected (unstim) or infected and incubated as in A and B, irradiated with UV or treated with staurosporine (used as positive apoptosis controls). DCs were subjected to annexin V/propidium iodide (PI) staining and analyzed by flow cytometry. Less than 3% of infected DCs were annexin V⁺PI⁺, and are included in the data shown as percentage annexin-V positive DCs. Data are expressed as means $\pm$ SEM (A–C). $n = 8$ independent experiments (A and B); $n = 4$ independent experiments (C). * $P < 0.05$, **** $P < 0.0001$. MFI, median fluorescence intensity.

these factors. At 48 hours after Mtb infection, DCs had significantly increased expression of CD40, CD80, CD83, CD86, and HLA-DR, to that in unstimulated DCs (Figure 1, A and B, and Supplemental Figure S2). HIV single infection did not affect surface marker expression compared to that of unstimulated DCs (Figure 1, A and B). However, HIV coinfection significantly suppressed the percentage of HLA-DR–positive DCs (Figure 1A), and the total surface expression of the Mtb-induced CD40, CD80, CD86, and HLA-DR (Figure 1B). Poly IC and lipopolysaccharide/IFN- γ up-regulated all markers to comparable levels as that for Mtb infection alone, with the exception of CCR7, which was not significantly elevated by any infection. No changes in the viability of DCs were noticed in coinfecting and single infected DCs (Figure 1C).

HIV Coinfection of DCs Reduces Mtb-Induced Proinflammatory Cytokine Production

Next, we analyzed the cytokine levels in cell-free culture supernatant collected at 48 hours after infection. During Mtb single infection, there was a significant release of the proinflammatory cytokines IL-6, IL-1 β , and tumor necrosis factor (TNF)- α , whereas HIV and Mtb coinfecting DCs did not mount this response (Figure 2, A–C). In contrast, the level of IL-12p40 was increased in coinfecting DCs, whereas the level of biologically active IL-12 (IL-12p70) was not (Figure 2, D and E). There was a nonsignificant increase in IL-10 in both Mtb single infected and coinfecting DCs (Figure 2F). When analyzing the type I IFN molecules IFN- α , IFN- β , and IFN- γ –inducible protein 10 (IP-10; CXCL10), only background

Figure 2 HIV-coinfected DCs reduce Mtb-induced secretion of the proinflammatory cytokines IL-6, IL-1 β , and tumor necrosis factor (TNF)- α . DCs were either uninfected (unstim) or infected with HIV, Mtb, or coinfecting (HIV + Mtb), or stimulated with poly IC or lipopolysaccharide (LPS)/interferon (IFN)- γ as positive controls. Cell-free culture supernatants were collected after 48 hours and analyzed for IL-6 (A), IL-1 β (B), TNF- α (C), IL-12p70 (D), IL-12p40 (E), IL-10 (F), IFN- γ -inducible protein 10 (IP-10; G), IFN- β (H), IFN- α (I), and IFN- γ (J). Supernatants were from the same experiments as presented in Figure 1, A and B. Data are expressed as means $\pm$ SEM (A–J). $n = 8$ independent experiments (A–J). * $P < 0.05$, ** $P < 0.01$.

levels of IFN- α and IFN- β were detected, with no changes observed between HIV coinfecting and Mtb infected DCs (Figure 2, H and I). IP-10, which is a surrogate cytokine for type I IFNs, showed no significant increase in coinfecting or Mtb single infected DCs (Figure 2G). IFN- γ , included as a control cytokine to rule out contamination of T cells, was negligible, and confirms that all released cytokines were a consequence of direct stimulation/infection of DCs (Figure 2J).

HIV Interferes with DC Mtb Ag Presentation to Mtb Ag-Specific CD4 $^{+}$ T Cells

Because HIV coinfection led to a down-regulation of HLA-DR and costimulatory molecules on DCs, together with a reduction in several Mtb-induced proinflammatory cytokines, we generated Mtb-specific CD4 $^{+}$ T cells to investigate whether these alterations caused by HIV could also affect Mtb antigen presentation by DCs. HIV-coinfected DCs co-cultured with autologous PPD- or Ag85B-specific CD4 $^{+}$ T cells significantly reduced the Mtb-induced

IFN- γ secretion by the Mtb Ag-specific CD4 $^{+}$ T cells ($P < 0.01$ for both) (Figure 3, A and B). Staphylococcal enterotoxin B, which was used as a positive control, markedly induced IFN- γ , whereas the negative control ovalbumin did not (Figure 3, A and B). To ascertain the role of DCs in Mtb antigen presentation leading to IFN- γ production, the direct effect of Mtb on CD4 $^{+}$ T cells was evaluated, showing no induction of IFN- γ in absence of DCs (Figure 3, A and B). IFN- γ production therefore constitutes a reliable read-out for Mtb antigen presentation by DCs. Similar to the HIV/Mtb coinfecting DCs, PPD stimulation of HIV-infected DCs also exhibited defective antigen presentation when co-cultured with PPD-specific ($P < 0.05$) and Ag85B-specific (nonsignificant) CD4 $^{+}$ T cells.

To determine whether HIV affects DC Mtb antigen presentation at the level of antigen processing/degradation or MHC class II loading, HIV-infected DCs were pulsed with Ag85B peptides. There was no difference in T-cell activation when HIV infected and uninfected DCs were peptides pulsed and co-cultured for 48 hours (data not shown) or 72 hours (Figure 3C), indicating that HIV might affect

Figure 3 HIV-coinfected DCs show reduced Mtb antigen presentation to purified protein derivative (PPD)– and Ag85B-specific CD4⁺ T cells when bacteria are intact. DCs were left uninfected (unstim) or infected with either HIV or Mtb, or coinfecting. Stimulation (stim) with PPD and staphylococcal enterotoxin B (SEB) served as positive controls and ovalbumin (Ova) as background. After 24 hours of infection, DCs were co-cultured with autologous PPD-specific (A) or Ag85B-specific (B) CD4⁺ T cells (DC/T-cell ratio was 1:5). In some wells, antigen-specific CD4⁺ T cells were incubated with Mtb in absence of DCs (T cells + Mtb; negative control). Cell-free culture supernatants were collected 72 hours later, and assayed for interferon (IFN)–γ. C: HIV does not affect antigen presentation stimulated by 15-mer Ag85B peptides in nonactivated DCs. DCs were left uninfected, infected with HIV, or stimulated with SEB or Ova for 24 hours, and pulsed with Ag85B peptides the last 2 hours, as indicated. DCs were extensively washed to remove peptides, and then co-cultured with autologous Mtb antigen–specific CD4⁺ T cells. Cell-free culture supernatants were collected and assayed as in A and B. D: Ag85B peptides do not activate DCs. DCs were either unstimulated (unstim) or stimulated with Ag85B peptides for 2 hours, then washed extensively, and cultured for 48 hours. DCs were analyzed for the total surface expression of CD80, CD83, CD86, and human leukocyte antigen D related (HLA-DR), in the same manner as explained for Figure 1B. Data are expressed as means ± SEM (A–D). *n* = 4 independent experiments (A and B); *n* = 3 independent experiments (C and D). **P* < 0.05, ***P* < 0.01.

processing of intact Mtb/Mtb antigens. However, dissimilar to the Mtb-induced expression of HLA-DR and costimulatory molecules (Figure 1B), DCs remain unaffected by Ag85B peptide stimulation, showing a resting DC phenotype with CD80, CD83, CD86, and HLA-DR expression similar to that of unstimulated DCs (Figure 3D).

HIV Efficiently Blocks Autophagy during Coinfection with Mtb

As autophagy has been identified as a pathway by which cytoplasmic and nuclear antigens are delivered to MHC class II molecule–containing compartments for subsequent presentation to CD4⁺ T cells,³⁶ we hypothesized that HIV coinfection blocks Mtb antigen processing by manipulating the autophagy pathway in DCs. In the absence of the

autophagy maturation/flux inhibitor bafilomycin, Mtb single-infected DCs accumulated significant amounts of LC3BII protein compared to that in uninfected control. With bafilomycin pretreatment, on the other hand, there was no difference in LC3BII (or SQSTM1) protein levels between uninfected and Mtb-infected DCs, supporting previous findings^{37,38} and indicating that Mtb can obstruct autophagy by limiting maturation/degradation of formed autophagosomes. Compared to Mtb single infection, HIV coinfection showed a 55% reduced expression of LC3BII and 30% reduced SQSTM1, that with bafilomycin treatment still exhibited a 48% decrease in LC3BII (*P* < 0.01) and 38% decrease in SQSTM1 (*P* < 0.0001) (Figure 4, A–C). This indicates that HIV inhibits the initial autophagosome formation in Mtb-infected DCs, which attributes the reduced presentation and T-cell activation shown in Figure 3, A and

Figure 4 HIV blocks the generation of the autophagy proteins LC3BII and SQSTM1 in Mtb-infected and uninfected DCs. DCs were left uninfected (unstim), infected with HIV or Mtb, or coinfecting (HIV + Mtb). DCs were also treated with bafilomycin (Baf) 1 hour after HIV infection. After 6 hours of coinfection, the cellular lysates were subjected to Western blot using anti-LC3 and anti-SQSTM1, and anti-β-actin antibody as loading control. **A:** Representative Western blot images of LC3BII, SQSTM1, and β-actin. Densitometry analysis of LC3BII (**B**) and SQSTM1 (**C**), with or without bafilomycin normalized by β-actin. For analysis of cGAS, STING, and phosphorylated tank-binding kinase 1 (pTBK1), DCs were left uninfected (unstim) or infected as in **A–C**, and after 24 hours of coinfection, the cellular lysates were subjected to Western blot using anti-cGAS, anti-STING, anti-phospho TBK1 (pTBK1), and anti-β-actin as loading control. Representative Western blot images and densitometry analysis of cGAS (**D**), STING (**E**), and pTBK1 (**F**) normalized by β-actin. Data are expressed as means ± SEM (**B–F**). $n = 4$ independent experiments (**B** and **C**); $n = 6$ independent experiments (**D–F**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

B, to this impaired processing of Mtb antigens. The bafilomycin data further show that HIV single infection also has the capacity to decrease non-pathogen-induced LC3BII and SQSTM1 ($P < 0.001$) (Figure 4, A–C), indicating that uninfected DCs have a high turnover of autophagic proteins, which is in line with DCs being highly specialized antigen-presenting cells.

HIV Does Not Affect Autophagy via the cGAS-STING Pathway in Coinfected DCs

Because HIV during coinfection was found to suppress autophagosome formation (Figure 4, A–C), and type I IFNs were not significantly expressed (Figure 2), we decided to dissect the cGAS-STING pathway. This is the major type I

IFN activating pathway, that upstream can mediate enforced autophagy through phosphorylation of TBK1.³⁹ We found that Mtb induced cGAS and pTBK1 to a greater extent than HIV monoinfection, and that the expression levels of these molecules were comparable in coinfecting and Mtb single-infected DCs. Although coinfection improved expression of cGAS minutely compared to that in Mtb, this trend did not proceed downstream to TBK1 phosphorylation (Figure 4, D–F). Therefore, the suppression in autophagy by HIV during coinfection could not be attributed to negative modulation of upstream activation of the cGAS-STING pathway.

Discussion

In the present study, we show that HIV infection contributes to Mtb pathogenesis by interfering with the DC-mediated immune activation. DCs are one of the first cells encountering invading pathogens, on which they mature and migrate to the regional lymphoid tissue, where they develop into more efficient antigen-presenting cells by up-regulating costimulatory and other surface molecules.¹¹ Costimulatory molecules on DCs play a decisive role in shaping the intensity and nature of the immune response, where they are critical for the priming of naïve T cells, and controlling infections. Thus, modulation of these molecules by pathogens, such as HIV, Mtb, and *Leishmania*, could help them to survive in the host.⁴⁰

Herein, we show that HIV/Mtb coinfecting DCs have suppressed up-regulation of HLA-DR and the costimulatory molecules CD40, CD80, and CD86. Similar to 4-1BBL, CD40 belongs to the TNF superfamily of molecules, with costimulatory properties that on DCs play an important role in T-cell activation and expansion.⁴¹ The expression of the activation marker CD83 and of the maturation marker CCR7 was not significantly different comparing coinfecting DCs and those infected by Mtb alone. Similar to our results, Hertoghs et al⁴² have shown that HIV-1 infection does not induce DC maturation. In addition, Mazurek et al⁴³ have previously found that DCs, when exposed to soluble factors derived from mycobacteria-infected cells, up-regulated costimulatory and MHC class II molecules. In our study, monoinfection with HIV-1 did not cause changes in the basal level of the surface molecules, but was responsible for suppressing the Mtb-induced costimulatory and HLA-DR molecules on DCs, thereby dampening the immune response and promoting Mtb pathogenesis.

Compared to the minute levels of biologically active IL-12p70, there was a large excess of IL-12p40 released from coinfecting DCs, which through its antagonistic properties⁴⁴ could constitute a pathogenic strategy induced by HIV in coinfecting DCs. Similar to our findings that HIV reduces production of TNF- α , Saukkonen et al⁴⁵ reported that alveolar macrophages from HIV-infected individuals, compared to HIV-negative individuals, produce less TNF- α

when infected with Mtb *in vitro*. Decreased TNF- α production on Mtb infection of peripheral blood mononuclear cells from HIV/Mtb-coinfecting individuals has also been shown by Hertoghe et al,⁴⁶ and more recently it was shown that HIV impairs the TNF-mediated macrophage apoptotic response to Mtb, thus facilitating bacterial survival.⁴⁷ Herein, we report that in addition to its effect on TNF- α production, HIV also reduces production of IL-1 β and IL-6 in Mtb-infected DCs. Both IL-1 β and IL-6 have a critical role in the protective immunity against TB,^{48,49} and IL-6-deficient mice have been reported to be highly susceptible to Mtb infection.⁴⁹ Our study supports the idea that downmodulating Mtb-induced proinflammatory cytokine release by DCs is a strategy used by HIV during coinfection to restrict the delivery of DC-derived signals required for inducing an optimal T helper cell response. Compared to unopsonized HIV (free HIV), opsonized HIV (which we have used for our study) has been shown to decrease the induction of type I IFNs and inflammatory factors, such as IL-1 β , IL-6, and TNF- α , and this is an important immune evasion mechanism used by HIV to establish infection in a silent manner.³² We have used opsonized HIV and Mtb because pathogens are normally opsonized by body fluids, as well as in tissues, before infection.^{50–52}

Antigen processing and presentation is a key event in the immune response to control pathogens. Compared to Mtb-infected DCs, we found that HIV-coinfecting DCs have reduced capacity to induce Mtb Ag-specific CD4⁺ T cells to secrete IFN- γ . The ability of DCs to stimulate CD4⁺ T cells was impaired, probably as a result of the defective expression of costimulatory molecules, MHC class II/HLA-DR, and the altered cytokine profile observed during HIV coinfection. In addition, HIV infection of DCs suppressed the PPD-induced IFN- γ response in both PPD- and Ag85B-specific CD4⁺ T cells. The functional phenotypic changes in DCs could explain why HIV-infected individuals in TB endemic areas have reduced tuberculin skin test reactivity despite having similar proportion of CD4⁺, CD8⁺, and CD1a⁺ cells at the tuberculin infusion site compared to HIV-1-uninfected persons.⁵³ Antigen processing and presentation involves fragmentation of proteins, loading of the peptides onto MHC molecules, and expression of these peptide-MHC complexes on the cell surface for recognition by T-cell receptors.⁵⁴ Although HIV suppressed activation of Mtb antigen-specific CD4⁺ T cells by DCs stimulated by whole antigens or Mtb, the activation of Mtb antigen-specific CD4⁺ T cells by Ag85B-peptide pulsed DCs was not altered by HIV DC infection. The level of costimulatory molecules on the DCs remained unaffected by Ag85B peptide stimulation. Our data therefore indicate that HIV does not affect peptide–MHC class II loading and presentation in nonactivated DCs, and that HIV in coinfecting DCs mainly interrupts the processing stage of antigen presentation. In line with this, it has also been reported that maturation-defective DCs in advanced stages of HIV disease show suboptimal antigen presentation.⁵⁵ This effect

may be mediated by specific HIV proteins, where Nef together with Vpu affects CD1d,⁵⁶ and Nef alone has been shown to affect CD1a antigen presentation in DCs.⁵⁷ In addition to HIV's effect on antigen presentation, it was recently reported that Mtb uses host proteins (specifically kinesin-2) to limit antigen presentation, by reducing the accumulation and availability of bacterial antigens in DCs.⁵⁸ Our observation suggests that reduction in Mtb antigen processing and further presentation by the DCs could be an immune evasion mechanism used by HIV that as a consequence further exacerbates Mtb pathogenesis.

Autophagy is a major mechanism for elimination of intracellular Mtb and inhibition of HIV replication.⁵⁹ It was recently reported that Vif and Env proteins of HIV can inhibit autophagy; Vif was able to inhibit the early steps of autophagy,⁶⁰ whereas the Env protein of HIV rapidly down-regulated autophagy in DCs through activation of mammalian target of rapamycin, thereby protecting the virus from autophagy-mediated degradation.⁶¹ As autophagy is an important mechanism involved in processing and delivery of antigens to MHC class II molecules,²² we hypothesized that HIV affects the Mtb antigen presentation by suppressing autophagy. Our results show that Mtb blocks autophagic flux/degradation in formed autophagosomes, and that HIV blocks the initial formation of autophagosomes. This additional obstruction in autophagy by HIV in coinfecting DCs would greatly impede any chances of Mtb processing leading to presentation of bacterial antigens.

The findings reported in this study reveal new insights into the interactions between DCs and the pathogens HIV and Mtb during coinfection and their impact on the immune responses. Our results suggest that HIV interferes with the expression of costimulatory molecules, and autophagy induction in DCs, all leading to an impaired Mtb antigen processing with reduced MHC class II presentation and subsequent dampening of CD4 T-cell responsiveness. These mechanisms would account for the accelerated pathogenesis during HIV/Mtb coinfection, and should be taken into consideration when developing more appropriate therapeutic and preventive regimens for HIV⁺ TB patients.

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Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2016.08.003>.

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