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Original article

Increased intracellular growth of *Mycobacterium avium* in HIV-1 exposed monocyte-derived dendritic cells

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Abstract

Dendritic cells (DC) are the most potent antigen-presenting cells, and form a link between the innate and adaptive immune system. They sample the periphery of the body for antigens and present them to T cells to elicit a proper immune response. It has been shown that dendritic cells phagocytose mycobacteria, but there have been conflicting reports as to whether the bacteria are capable of intracellular replication in DCs. *Mycobacterium avium* is a facultative intracellular bacterium, part of the Mycobacterium avium complex (MAC) of mycobacteria and are commonly seen as opportunistic pathogens in patients infected by Human immunodeficiency virus type 1 (HIV-1). To clarify the issue of whether DCs are capable of controlling the intracellular growth of *M. avium* and whether this control is lost upon HIV-1 exposure, we investigated the intracellular replication of *M. avium* in monocyte-derived dendritic cells and compared it to bacterial growth in dendritic cultures exposed to HIV-1 for 24 h. Our results show that exposure of DCs to HIV-1 promotes or facilitates the intracellular growth of *M. avium*.

Keywords: Immunology; Immunity; Innate; HIV; *Mycobacterium avium*; Dendritic cells

1. Introduction

Infection with Human immunodeficiency virus type 1 (HIV-1) impairs immunological control of bacterial infections. *Mycobacterium avium* is one of the most common opportunistic infections in Acquired immunodeficiency syndrome (AIDS) patients. Prior to the introduction of highly active antiretroviral treatment, more than 40% of patients developed a *M. avium* complex (MAC) bacteremia two years after the AIDS diagnosis [1] and a disseminated MAC infection was found in as much as 50% of autopsied AIDS patients [2]. This has predominantly been attributed to the impairment of the adaptive part of the immune system in HIV-1 infected individuals due to the loss of $CD4^+$ T cells, as the susceptibility to opportunistic infections, including *M. avium* infection, is correlated with a decline in $CD4^+$ T cells [3]. However, several reports indicate that HIV-1 may also influence the innate immune system and have a detrimental effect on the mononuclear phagocytic cells including monocytes, macrophages and dendritic cells (reviewed in [4,5]).

Dendritic cells (DCs) are the most potent antigen-presenting cells and have a wide distribution throughout body tissues. In humans, they comprise three distinct subsets; Langerhans cells, interstitial myeloid DCs, and lymphoid DCs. Dendritic cells exposed to HIV-1 are known to transmit a vigorous infection to $CD4^+$ T cells [6], pointing to an important role for DCs during the natural course of HIV-1 infection. However, few studies have investigated the intracellular growth of bacteria in DCs. As far as mycobacteria are concerned, *M. avium* has been shown by Mohaghegpour et al. [7]

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to be phagocytosed by, and to grow in monocyte-derived DCs. In another study, Tailleux et al. [8] report that monocyte-derived DCs constrained the intracellular growth of *Mycobacterium tuberculosis*.

The aim of the present study was to investigate the intracellular growth of *M. avium* in monocyte-derived DCs. In particular, we wanted to examine if the growth of *M. avium* was modulated by exposure of the dendritic cells to HIV-1.

2. Materials and methods

2.1. Isolation of primary monocytes and in vitro differentiation to monocyte-derived dendritic cells

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh buffy coats (The Blood Bank at Bergen Hospital Trust, Haukeland University Hospital, Bergen) by centrifugation on a Lymfoprep (Axis-Shield PoC AS, Norway) density gradient, according to the manufacturers instructions. The PBMCs were added to 225 cm^2 flasks at a concentration of $1{,}5 \times 10^8$ cells in 35 ml medium (RPMI1640 + 10% FCS + Penicillin–Streptomycin), and allowed to adhere for 2 h. Subsequently, adherent cells were washed with PBS at room temperature to remove non-adherent cells. Monocyte-derived dendritic cells (moDCs) were generated by culturing of adherent cells in 15 ml RPMI medium supplemented with 10% FCS, IL-4 (120 ng/ml) (ImmunoTools GmbH, Germany) and GM-CSF (600 ng/ml) (ImmunoTools GmbH, Germany) at 37 °C in 5% CO_2. After three days, half the medium was replaced with medium containing fresh cytokines. After six days of culture, non-adherent cells were harvested. A sufficient number of cells were withheld for flow cytometric analysis. Remaining cells were resuspended at a concentration of 3×10^5/ml (7,5 ml in 225 cm^2 tissue culture flasks) in fresh medium containing cytokines for use in experiments.

2.2. Flow cytometry

The generation of immature moDCs was verified by flow cytometric analysis. Cells were washed in PBS, stained with fluorescein isotiocyanate (FITC) conjugated CD14 (AbD Serotec, Düsseldorf, Germany) and CD86 (AbD Serotec, Düsseldorf, Germany) and R-phycoerythrin (R-PE) conjugated CD1a (HI149; BD Biosciences Pharmingen, San Jose, CA), DC-SIGN (R&D systems, Minneapolis, MN) and HLA-DR (AbD Serotec, Düsseldorf, Germany), and analyzed on a BD FACS Calibur instrument (BD Biosciences, Franklin Lakes, NJ, USA).

Surface expression of Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4) was also analyzed by flow cytometry. HIV-1 exposed and unexposed dendritic cells were fixed in 4% paraformaldehyde, labelled with receptor specific antibodies, monoclonal mouse anti-human TLR2.1 (InvivoGen, Toulouse, France) and monoclonal mouse anti-human TLR4 (HTA125; InvivoGen), and secondarily stained with R-PE conjugated polyclonal goat anti-mouse $F(ab')_2$ fragments (R0480; DakoCytomation, Glostrup, Denmark). Cells stained only with the secondary antibody were used to define the negative cell population on a histogram plot.

2.3. Exposure to HIV-1 BaL

The HIV-1 BaL isolate was grown by repeated passages in primary monocyte-derived macrophage cultures and the viral titer in the supernatants harvested from these macrophages were determined by p24 ELISA (Vironostika, BioMerieux) and aliquots stored at −80 °C for later use in experiments. Dendritic cells (4×10^5 MoDCs/ml, 7,5 ml in 225 cm^2 tissue culture flasks) were either exposed to HIV-1 BaL at a viral titer corresponding to 5 ng/ml of p24 in cell culture medium or exposed to cell culture medium only. Cells exposed to HIV-1 were incubated for 24 h with HIV-1 at 37 °C in 5% CO_2 prior to *M. avium* infection.

2.4. Infection with M. avium

Frozen aliquots of *M. avium avium* serovar 4, human isolate S72/89 from the blood of an AIDS patient (kindly provided by Dr. Sven Hoffner, Dept. of Bacteriology/TB Section, Swedish Center for Infectious Disease Control, Solna, Sweden) with predetermined colony forming unit counts (CFU) were stored at −80 °C until use, when bacterial suspensions were rapidly thawed and vortexed. MoDCs, either unexposed or exposed to HIV-1, were infected at a multiplicity of infection of 5:1 bacteria to cell ratio. Of note, the DCs were not washed before *M. avium* infection. Thus, in double exposed cultures the HIV-1 virons remained in the supernatant also during the subsequent 24 h incubation with *M. avium*. After the incubation, extracellular bacteria along with the virus in HIV-1 exposed cultures were removed by washing three times with PBS and low speed centrifugation (800 rpm/10 min). The cells were finally resuspended in medium containing cytokines (4×10^5 MoDCs/ml, 1 ml per well) and were plated on 24-well plates (Nunc, Thermo Fischer Scientific, Denmark).

Cells were harvested from wells at specified time points after mycobacterial infection (1, 4 and 7 days). Cells were spun down and supernatants frozen at −80 °C. The pelleted cells were lysed in double distilled water for 30 min and then vortexed. Appropriate dilutions of the lysates were seeded on oleic acid albumin dextrose complex (OADC) enriched Middlebrook 7H10 agar plates (Difco/BD) in triplicate. The remaining lysates were stored at −80 °C in cryotubes containing 250 µl of acid washed glass beads ≤106 µm in size (Sigma–Aldrich, Norway) for later DNA extraction and use in quantitative real-time PCR (qPCR). All experiments were performed in triplicate. A total of 4 experiments with cells from 4 independent donors were performed.

In preliminary experiments performed to control for possible interference from extracellular growth of *M. avium*, wells with RPMI medium only were treated in the same way as dendritic cells infected with *M. avium* with respect to infectious dose and washing protocol after infection. CFU counts from such wells were less than 1% of the corresponding wells with cells.