Human Immunodeficiency Virus Type 1 Infection of Human Monocytes and Macrophages Does Not Alter Their Ability to Generate an Oxidative Burst

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Human immunodeficiency virus type 1 (HIV-1) infects mononuclear phagocytes, cells that may serve as a reservoir for viral persistence. Infection with HIV-1 leads to progressive compromise of the immune system, resulting in infections with opportunistic pathogens and eventual death. Experiments were designed to determine if in vitro HIV-1 infection of mononuclear phagocytes would diminish their oxidative capabilities, thus decreasing their antimicrobial effectiveness. Blood monocytes and peritoneal macrophages were obtained from uninfected donors and inoculated with a monocytotropic strain of HIV-1. Hydrogen peroxide production and reduction of nitroblue tetrazolium were measured after acute stimulation of cells with PMA or a phagocytic stimulus. Despite vigorous virus production, no difference was seen in oxidative burst between uninfected cells and infected cells or between monocyte-derived and peritoneal macrophages. In conclusion, reduced antimicrobial activity of HIV-infected mononuclear phagocytes is probably not secondary to decreased ability to generate reactive oxygen species.

Mononuclear phagocytes (including blood monocytes and tissue macrophages) are important components of the cellular immune system. Their stimulation triggers an oxidative burst that generates reactive oxygen species, such as hydrogen peroxide (H2O2), superoxide anions, and hydroxyl radicals, which may be toxic for microbes. Mononuclear phagocytes are especially important for control of intracellular pathogens such as Toxoplasma gondii, Listeria monocytogenes, and protozoa [1].

Infection with human immunodeficiency virus type 1 (HIV-1) leads to progressive compromise of the human immune system, resulting in infections with opportunistic pathogens and eventual death. CD4+ lymphocytes are the cells most commonly infected with HIV-1, and their numbers steadily decline after infection. Mononuclear phagocytes are also sites of HIV-1 replication and likely play a central role in viral persistence and distribution throughout the body [2-4]. HIV-1 infection of these cells may impair their ability to function normally. Decreased antimicrobial activity by HIV-1-infected mononuclear phagocytes has been reported in experiments using Cryptococcus neoformans, Leishmania major, and Candida pseudotropicalis [4]. Also, H2O2 alone or in combination with myeloperoxidase and a halide, as occurs during the respiratory burst of human neutrophils and monocytes/macrophages, will kill HIV-1 [5]. Dysfunction of HIV-1-infected mononuclear phagocytes might lead to uncontrolled growth of intracellular organisms, including HIV-1, in the HIV-1-infected host.

We hypothesized that HIV-1 infection of mononuclear phagocytes would reduce their ability to generate reactive oxygen species and thus contribute to their diminished antimicrobial effectiveness. Prior studies of oxidative capabilities...
of mononuclear phagocytes related to HIV infection have used cells from HIV-infected patients at various stages of their disease and in the setting of opportunistic infections. The results have been varied, showing decreased [6] and normal [7] oxidative burst by monocytes. To avoid the problems associated with the use of in vivo-infected cells, we designed in vitro experiments using human monocytes and peritoneal macrophages from normal donors. We infected these cells with a monocytotropic strain of HIV-1 and determined their ability to produce H2O2, an antimicrobial oxidant generated during the respiratory burst; we correlated this with another test of oxidative cellular function, reduction of nitroblue tetrazolium (NBT).

Materials and Methods

Sera, media, and reagents. Low-endotoxin-content Dulbecco’s modified Eagle medium (DMEM; HyClone, Logan, UT), formulated with lipopolysaccharide-free water and supplemented with penicillin (100 units/mL) and streptomycin (100 μg/mL), and autologous or pooled human serum were used for cell culture. Endotoxin content of culture materials was measured by the limulus amebocyte lysate assay and found to be ≤0.1 endotoxin unit. Recombinant human granulocyte-monocyte colony-stimulating factor (GM-CSF; Amgen, Thousand Oaks, CA) was used in half of the cell preparations. PMA (Sigma, St. Louis) was used as a soluble stimulus for H2O2 production and reduction of NBT. Serum-opsonized zymosan (Sigma) was used as a phagocytic stimulus of the oxidative burst.

Cell preparation. Human peripheral blood monocytes were prepared as previously described [8]. The cells were plated at a concentration of 3 × 10^5/well in 200 μL of DMEM with 10% serum. In each experiment, half the cells were treated with GM-CSF (500 units/mL) and half were left untreated. The GM-CSF dose was based on preliminary experiments that showed this to be the lowest concentration (testing 0-10,000 units/mL) that prolonged the ability of cells to produce H2O2. Peritoneal macrophages were obtained from women undergoing laparoscopic examination and were purified as described [9]. These were seeded at a concentration of 2 × 10^5 cells/well in 200 μL of DMEM with 10% serum and 250 units/mL GM-CSF. Resulting monolayers of monocyte-derived macrophages and tissue macrophages were ~90% and 98% mononuclear phagocytes, respectively, as assessed by morphology and nonspecific esterase cytochemistry [9].

HIV strains. HIV-1Bal, a monocytotropic strain, was used to produce active cellular infection [2]. HIV-1mb, a lymphocytotropic strain, and heat-inactivated HIV-1Bal (56°C for 2 h) were used in some experiments as negative controls. Stock strains of HIV-1Bal and HIV-1mb were produced in CEM cells and titrated on T cells and mononuclear phagocytes. Cells were inoculated at an MOI of ~0.01.

Assays. Adherent cells in wells representative of each treatment variable were washed, lysed with 1% Triton X and assayed for total protein content using the Pierce (Rockford, IL) BCA protein assay reagent (incubation for 30 min at 65°C). Reverse transcriptase (RT) activity was measured as described [10]. Hydrogen peroxide was measured using a microassay based on the horseradish peroxidase-dependent oxidation of phenol red [9]. Reduction of NBT as a second test of oxidative metabolism [11] was assessed in selected experiments and correlated microscopically with viral cytopathologic effect (CPE).

Experimental design. Blood monocytes from 9 normal donors and peritoneal macrophages from 3 donors were used. The donors were healthy adults without known HIV disease or risk factors for HIV infection. Cells were inoculated with virus (or, for controls) within 24 h of their isolation. Every 3-4 days, 0.1 mL of medium was removed from each well, and the same volume of fresh medium, serum, and GM-CSF was replaced.

 Supernatants for RT assays and lysates for protein assays were collected on the day of the peroxide and NBT measurements and stored at -70°C. Cells were washed twice with PBS before the peroxide and NBT assays or collection of lysates for protein; 200 nM PMA (a soluble inducer of the oxidative burst) or 1 mg/mL serum-opsonized zymosan (a phagocytic stimulant of the oxidative burst made up of cell walls of Saccharomyces cerevisiae) was added for acute stimulation, and the plates were incubated at 37°C for 2 h (peroxide assay) or 1 h (NBT assay). The peroxide assay was stopped by the addition of 0.1 N NaOH, and the plates were read within 15 min using a microtiter plate reader (Titertek: Flow Laboratories, McLean, VA) at an absorbance of 600 nm. Standards in triplicate were used with each assay, and the amount of peroxide produced was calculated from a standard curve. The NBT assay was done in situ with adherent phagocytes in the microtiter wells. After incubation with NBT, the cells were washed with PBS, fixed with 100% methanol, and counterstained with safranin for microscopic evaluation.

Statistics. Student’s paired or unpaired t tests were used to compare peroxide production in infected and uninfected cells.

Results

We have shown in prior work that 80%-100% of mononuclear phagocytes under the conditions used here are infected and express HIV-1 p24 antigen 14 days after viral inoculation [10, 12]. In agreement with this, the monocyte-derived and peritoneal macrophages inoculated with the monocytotropic strain of HIV-1 displayed marked cytopathology manifested by multinucleated giant cells and vigorous virus production as evidenced by supernatant media with high levels of RT activity. The RT assay was done using [32P]dTTP, quantified in a scintillation counter, and reported as counts per minute. RT activity 14 days after HIV-1Bal inoculation was 1786 ± 314 cpm (mean ± SE) for monocyte-derived macrophages and 1364 ± 418 for peritoneal macrophages; inoculation with HIV-1mb resulted in RT activity of 180 ± 35 cpm, and negative controls had 90 ± 4 cpm. These values are from cells treated with GM-CSF, which enhanced viral replication, as noted by comparing RT activity in monocytes without GM-CSF (670 ± 140, HIV-1Bal) and as has been previously reported [4]. Uninoculated cells and those inocu-
Table 1. Peroxide production on day 14 after inoculation of mononuclear phagocytes with HIV-1.

| Cell type               | Stimulant | No virus | HIV-1 
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<tr>
<td>Monocyte-derived macrophages</td>
<td>PMA</td>
<td>2.3 ± 0.9</td>
<td>3.3 ± 1.2</td>
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<tr>
<td></td>
<td>Zymosan</td>
<td>9.3 ± 1.8</td>
<td>8.8 ± 2.3</td>
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<tr>
<td></td>
<td>No PMA</td>
<td>0.1 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>Peritoneal macrophages</td>
<td>PMA</td>
<td>4.4 ± 2.9</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>No PMA</td>
<td>0</td>
<td>0</td>
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NOTE. ND = not done. All values are nmol peroxide/mg protein, mean ± SE, from cells treated with granulocyte-monocyte colony-stimulating factor. Assays were done in triplicate. Peritoneal macrophage results are from 3 donors; PMA-stimulated monocyte-derived macrophage results are from 9 and zymosan-stimulated from 2 donors.

Discussion

Studies of mononuclear phagocyte function using cells from patients with HIV-1 infection have yielded varying results, probably because of the complex nature of these patients’ illnesses and the inability to control for endogenous sources of immune activation [6]. Therefore, we designed in vitro experiments to determine the direct effect of HIV-1 infection on one aspect, oxidative metabolism, by using cultured monocytes and peritoneal macrophages from uninfected donors. We specifically tested the effect on tissue macrophages because these cells are the most likely reservoir for HIV-1 infection in tissues [2–4] and are important in local cellular immunity. We used the monocytotropic strain of HIV-1, HIV-1 

![Figure 1](https://academic.oup.com/jid/article-abstract/168/2/459/921833)
demonstrated that HIV-infected mononuclear phagocytes maintain their ability to generate a respiratory burst when treated acutely with either the soluble stimulant PMA or the phagocytic stimulus zymosan. Therefore, despite obvious virus-mediated changes (multinucleated giant cell formation and production of HIV RT), the cells functioned normally in their abilities to generate H₂O₂ and reduce NBT.

On the basis of our in vitro experiments, we think that HIV-1 infection does not depress the ability of mononuclear phagocytes to produce hydrogen peroxide and that this is an unlikely cause of defective antimicrobial function. This does not preclude the possibility that other, non-oxygen-dependent antimicrobial mechanisms could be altered by HIV infection or that other functions, such as tumor cell killing, might be impaired. It is likely that dysfunctional cellular immunity is related to decreased lymphokine stimulation as CD4 lymphocytes decline during HIV disease progression. Evidence of this comes from Murray et al. [13], who have shown that peripheral blood mononuclear cells (i.e., combined lymphocytes and monocytes) from patients infected with HIV-1 produce less interferon-γ than did control cells; however, when these cells were stimulated in vitro with physiologic doses of interferon-γ, they produced normal amounts of hydrogen peroxide and they exhibited normal inhibition of two organisms, T. gondii (oxygen-dependent) and Chlamydia psittaci (oxygen-independent) [14].

The demonstration of normal H₂O₂ production in mononuclear phagocytes is important. Immune modulators such as interferon-γ may have a myriad of effects in clinical trials and, in accordance with our results and those of others [14], may result in increased antimicrobial effectiveness by stimulating tissue macrophages. In addition to serving as an antimicrobial effector molecule, H₂O₂ could also serve to increase HIV-1 expression in infected cells. Investigators have noted that cellular H₂O₂ produced in response to PMA or cytokines activates the cellular transcription factor NF-xB [15]. Activated NF-xB moves from the cytoplasm to the nucleus and induces transcription of HIV mRNA. Thus, an infected cell maintains its ability to promote its own (autocrine) or a neighboring cell’s (paracrine) expression of HIV.

In conclusion, we showed that human mononuclear phagocytes infected with HIV-1 can produce H₂O₂ and reduce NBT normally. The cause of reduced antimicrobial activity of HIV-infected mononuclear phagocytes remains unknown, but defective capacity to generate reactive oxygen species is probably not the explanation.

Acknowledgments

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References