HIV-1-induced pulmonary oxidative and nitrosative stress: exacerbated response to endotoxin administration in HIV-1 transgenic mouse model

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THE LUNG IS A MAJOR TARGET of attack in human immunodeficiency virus (HIV) infection. Although the era of highly active antiretroviral therapy has proven to be vastly beneficial to HIV-1-seropositive individuals, lung disease still continues to be the leading cause of death among this population (2, 22). HIV-1 infection is detrimental to pulmonary function and is an important risk factor in the development of opportunistic lung diseases. Many of these effects are attributed to innate immune dysfunction. Indeed, HIV-1 has been shown to infect alveolar macrophages and cause impairment of phagocytosis and other innate immune functions such as cytokine production (27, 38). Such impairment of key immune functions can increase lung susceptibility to bacteria and mycobacteria pathogens, such as Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas, Mycobacterium tuberculosis, and Mycobacterium avium, which are frequently associated with HIV-related lung disease (5, 21, 29). Furthermore, impairment of key immune functions can create an imbalance between pro- and anti-inflammatory mechanisms that are crucial in the maintenance of proper lung function. The resulting acute lung injury is manifested by diffuse alveolar damage, increased capillary permeability, and thus interstitial and alveolar edema (36).

Although HIV-1 is able to contribute significantly to lung disease via immune dysfunction, the virus is not known to directly infect lung tissue to cause pulmonary complications. Kanmogne et al. (25) showed that lung microvascular endothelial cells are not a major reservoir of HIV or the primary route for HIV invasion. Although HIV-1 infection indirectly may cause lung disease via the host’s increased susceptibility to pathogens, it is also plausible that circulating (secreted) HIV-1 proteins may impact lung function via other mechanisms. For example, secreted HIV-1 proteins, such as Tat, Nef, Vpr, and gp120, are able to cause cellular damage via the induction of reactive oxygen species (14, 18, 19, 34, 39). However, the effect(s) of secreted HIV-1 proteins on lung cells independent of immune dysfunction is not clearly defined. It is plausible that these secreted HIV-1 proteins may be involved in HIV-associated pulmonary dysfunction via stimulation of reactive oxygen species production.

HIV-1-infected patients have systemically elevated levels of reactive oxygen species and are considered to be under oxidative stress (33). Oxidative stress is a general term used to describe the harmful condition that occurs when there is an excess of reactive oxygen species (or other free radicals), a decrease in antioxidant levels, or both. Heightened free radical production is associated with the progressive development of acquired immunodeficiency syndrome (AIDS) and, in particular, the viral replication, inflammatory response, immune dysfunction, weight loss, and decreased sensitivity to drug toxicities that attend this disease (33). HIV-1-infected individuals have drastically decreased oxidant defense mechanisms, such as superoxide dismutase, which detoxifies superoxide anions (41). Additionally, levels of systemic glutathione (GSH), a key antioxidant that is...
mandatory for proper lung function, are decreased in symptomatic AIDS patients compared with HIV patients without symptoms or healthy subjects (7). Decreased GSH in the lungs leads to a variety of abnormalities, including damage to the epithelial cells and fluid that lines the lungs, changes in barrier function of lung cells, and increased susceptibility to apoptosis, or cell death (5a, 5b). GSH is shown to protect mice from lethal sepsis via regulation of inflammation and potentiation of host defense mechanisms, such as neutrophil migration (42). Furthermore, GSH levels are significantly decreased in HIV-1 Tat transgenic mice, suggesting a Tat modulatory role during GSH biosynthesis (8).

Nitrosative stress is also seen in HIV-1 patients (43, 45) and can be very harmful to pulmonary function (3, 16, 28). This form of stress is created when reactive nitrogen species, radical nitrogen-based molecules that can act to facilitate nitrosylation reactions, exceed the system’s ability to neutralize and eliminate them. NO is one such reactive nitrogen species. At low concentrations, NO is able to rapidly exert its direct effects on various biological targets such as guanylate cyclase, cytochrome P-450, and hemoglobin (44), resulting in vasodilation and anti-inflammatory mediation. In contrast, elevated NO concentrations can exert harmful indirect effects through interactions with superoxide, forming a potent, highly reactive nitrogen species known as peroxynitrite (44). Peroxynitrite modifies tyrosine residues in certain proteins to form nitrotyrosine. The ensuing nitration of proteins can inactivate mitochondrial and cytosolic proteins and damage cellular constituents, resulting in nitrosative stress (45). Persistently high levels of inducible nitric oxide synthase, nitrite, and peroxynitrite are thought to contribute to the etiology of AIDS-related dementia, persistent immunosuppression, as well as Kaposi sarcoma (40, 45). As with reactive oxygen species, reactive nitrogen species are very damaging to biological molecules such as proteins, lipids, and DNA. Thus oxidation and nitrosylation/nitration reactions leading to altered protein structure result in oxidative and nitrosative/nitratve stresses, respectively, and can inhibit normal systemic function.

Our hypothesis based on these data is that the oxidative and nitrosative stress environment created by HIV-1 proteins increases the deleterious effects of an opportunistic secondary infection in the absence of immune dysfunction. In this study, we have examined the impact of HIV-1 proteins on the response to endotoxin administration in a lipopolysaccharide (LPS)-treated HIV-1 transgenic mouse model. In HIV-1-infected patients, many HIV-1 proteins are secreted from infected cells and circulate to indirectly exert harmful effects. Here, we have used a model system that is best suited for examining these effects. Furthermore, this HIV-1 transgenic mouse model afforded us the ability to examine the effects of HIV-1 proteins independent of immune complications, as this established mouse line is nonreplicating and noninfectious (6, 12). We have examined biomarkers of oxidative and nitrosative/nitratve stress in the lung, such as lung edema, superoxide, NO metabolites, nitrotyrosine (a footprint of peroxynitrite), hydrogen peroxide, and GSH. Our results indicate that HIV-1 proteins increase oxidative/nitrosative stress and that this stress is further exacerbated with endotoxin treatment.

MATERIALS AND METHODS

Mice. The HIV-1 transgenic mice for this study came from established lines of an HIV provirus. The prototype FVB/N mouse, or Tg26, is a well-characterized line from the NL4-3A gag/pol AIDS transgenic line (1) (donated by Dr. Paul Klotman, Division of Nephrology, Mount Sinai Medical Center, New York, NY). The proviral DNA from this mouse line has a deleted gag and pol but intact Env and accessory genes. Transgenic Tg26 offspring develop congenital cataracts, nephropathy, cardiomyopathy, and skin lesions in hemizygotes and severe wasting in homozygotes (12). HIV-1 transgene expression has been detected at very high levels in skin, muscle, and tail, at intermediate levels in the intestines, and at low levels in kidney, lymph nodes, lung, and spleen (6). However, these mice are not immunosuppressed (1) (Table 1). Hemizygous mice were used for this study. The wild-type mice used in this study are from the FVB/n strain. Mice used in this study were males and females between 6 and 9 wk old. All studies were completed in compliance with protocols approved by the Department of Veterans Affairs Animal Care and Use Committee.

LPS injections. HIV-1 transgenic and wild-type mice were inoculated intraperitoneally with LPS (endotoxin) from Salmonella typhimurium (Sigma, St. Louis, MO) at 2 mg/kg weight. Control groups received intraperitoneal injections of phosphate-buffered saline (PBS) instead of endotoxin. Mice were euthanized at either 3 or 6 h after LPS injection.

Pulmonary edema analysis. Whole lungs (n = 5) were removed from the mice, placed in a tared aluminum weigh boat, and weighed (wet weight). Lungs were then desiccated overnight in a 70°C oven and weighed again (dry weight). The wet lung mass was divided by the dry lung mass to give the wet-to-dry ratio.

Superoxide analysis. Lung tissue (n = 4) was sectioned (30 μm), and superoxide production was visualized with dihydroethidium (Molecular Probes) staining. Dihydroethidium, a chemically reduced ethidium derivative, becomes oxidized to ethidium, then fluororesces red on DNA intercalation, and is visualized by fluorescence microscopy.

Bronchoalveolar lavage. Bronchoalveolar lavage (BAL) fluid (BALF) was collected after a tracheal incision, 1-ml PBS injection, aspiration, and centrifugation at 12,000 relative centrifugal force for 5 min. For GSH analysis an equal amount of 10% perchloric acid was centrifuged to collect cells and then analyzed without filtration. BALF was also filtered through a 30-kDa filter to remove hemoglobin. BALF nitrate and nitrite analysis. Nitrate and nitrite levels were also quantified via chemiluminescence (Cayman Chemicals, Ann Arbor, MI) (4) for each group and sample type. Lung tissue was homogenized in PBS, centrifuged, and filtered through a 30-kDa molecular mass cutoff filter. Blood plasma was also filtered through a 30-kDa filter to remove hemoglobin. BALF was centrifuged to collect cells and then analyzed without filtration. All three sample types were assayed in accordance with the protocol supplied. Nitrate and nitrite levels were also quantified via chemiluminescence analysis (Sievers Instruments, Boulder, CO) (data not shown).

Nitrotyrosine analysis. Nitrotyrosine was visualized by immunohistochemistry (n = 4). A monoclonal anti-nitrotyrosine antibody (Zymed, San Francisco, CA) was used for detection in lung tissue sections. A standard immunohistochemical staining procedure for frozen sections was followed. First, 5-mm sections were prepared with a cryostat machine. Sections were then fixed in ice-cold acetone for 10 min, and endogenous peroxidase was blocked by immersing...
slides in a solution containing 0.3% hydrogen peroxide in 70% methanol-PBS for 30 min and then washing once with PBS. Next, sections were incubated for 10 min in 10% normal serum from the species in which the secondary antibody was raised (goat). Sections were then incubated in monoclonal anti-nitrotyrosine primary antibody at 1:50 dilution overnight at 4°C. The next day, the sections were washed three times with PBS and incubated in biotinylated goat anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) at 1:100 dilution for 30 min. The sections were washed three times again in PBS and then incubated in diaminobenzidine substrate until brown color was visualized. The slides were then put in water to stop the reaction. Tissue sections were counterstained with hematoxylin to view the nuclei. Negative control slides were prepared by utilizing mouse IgM primary antibody in place of mouse anti-nitrotyrosine primary antibody. All other steps in this protocol remained the same. Lung tissue nitrotyrosine levels were quantified via ELISA (Northwest Life Science Specialties, Vancouver, WA) as stated in the protocol (n = 4).

Hydrogen peroxide analysis. Lung tissue (n = 10) was isolated, and hydrogen peroxide was quantitated with the Amplex Red reagent (Molecular Probes), a highly sensitive and stable probe used as a fluorogenic substrate for horseradish peroxidase. Briefly, lung tissue was isolated and incubated at 37°C for 30 min in solution containing Amplex Red reagent, horseradish peroxidase, and a buffer solution. Supernatant was then collected, fluorescence was read at 560 nm, and concentrations were determined through extrapolation from a standard curve.

Cytokine analysis. Pro- and anti-inflammatory cytokines were measured in blood serum samples (n = 5). Samples were analyzed with a multiplex bead immunoassay (Luminex, Austin, TX), an instrument used to measure up to 100 different biological markers simultaneously and best described by Rojas et al. (36). Briefly, a multiplex kit (Biosource, Camarillo, CA) that detects granulocyte-macrophage colony-stimulating factor, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p40/p70, IFN-γ, and TNF-α in picograms per milliliter was used. The assay was performed in a filter-bottom ELISA plate. The plate was prewashed, and then samples were added with antibody-coated beads specific for the different cytokines and incubated in a shaker for 2 h at room temperature. After incubation, the plate was washed and biotinylated antibodies against the cytokines were added and incubated for 1 h. Streptavidin coupled to R-phycocerythrin was used for detection and read in a Luminex XYP platform. Data were analyzed with Masterplex 1.2 software from MiraiBio.

Statistical analysis. A Student t-test analysis was done for simple comparison of two groups. One-way ANOVA with Newman-Keuls posttest was used for comparison of multiple groups. Statistical significance was defined as P ≤ 0.05.

RESULTS

Lung edema. Because pulmonary edema is a key physiological effect of endotoxin-induced acute lung injury, lung tissue wet-to-dry ratios were measured to determine whether lungs from HIV-1 transgenic mice treated with LPS harbored more water than lungs from wild-type mice treated with LPS. Figure 1A shows that lungs from HIV-1 transgenic mice treated with LPS for 3 h had a significantly (P < 0.05) greater wet-to-dry ratio than all other groups analyzed. Furthermore, lungs from untreated HIV transgenic mice exhibited significantly (P < 0.01) greater wet-to-dry ratios compared with untreated FVB mice. At 6 h after LPS treatment, there were no significant differences in wet-to-dry ratios of HIV-1 transgenic and wild-type mice treated with LPS; however, lungs from untreated FVB mice still had significantly (P = 0.05) lower wet-to-dry ratios compared with the other groups (Fig. 1B).

Superoxide analysis. To determine whether reactive oxygen species are present in the lungs at higher concentrations after endotoxin administration in HIV-1 transgenic mice, lung tissue superoxide was qualitatively examined by dihydroethidium staining. The excitation and detection conditions used were identical for all images. Figure 2 shows lung tissue from wild-type (Fig. 2A) and HIV-1 transgenic (Fig. 2B) mice untreated with LPS. There are no distinct differences in the intensity of staining in these tissues. However, at 3 h after LPS injection, lung tissue from HIV-1 transgenic mice (Fig. 2D) shows a greater staining intensity compared with wild-type mouse lung tissue (Fig. 2C). At 6 h after LPS injection, the signal intensity decreased back to what is seen in untreated tissues (results not shown).

Nitrate and nitrite analysis. NO metabolites were quantified as a measure of NO levels, which is a marker of nitrosative stress. The results in Fig. 3 show that BALF (Fig. 3A), lung tissue (Fig. 3B), and blood plasma (Fig. 3C) from untreated HIV transgenic mice have significantly (P < 0.05) greater nitrate and nitrite levels than BALF, lung tissue, and blood plasma from untreated wild-type mice. In addition, 3 h of LPS treatment induced significantly (P < 0.05) greater nitrate and nitrite levels in HIV transgenic BALF and lung tissue and greater blood plasma nitrate and nitrite levels compared with LPS-treated wild-type mice. After 6 h of LPS treatment, nitrate and nitrite levels in all sample types of the HIV-1 transgenic mice slightly decreased to levels similar to those in wild-type mice, with no significant
differences between the two groups (results not shown). NO metabolites were also quantified via chemiluminescence analysis, and these data were highly correlative to the above findings (results not shown).

Nitrotyrosine analysis. Nitrotyrosine is a footprint of peroxynitrite, which is the product of superoxide anion and NO. To determine the amount of lung peroxynitrite produced after LPS administration, nitrotyrosine was visualized via immunohistochemistry. The results show that HIV-1 transgenic mice treated with LPS for 3 h had greater staining (nitrotyrosine) intensity than FVB mice treated with LPS for 3 h (Fig. 4, B and D). At 6 h after LPS treatment and with no LPS treatment (control), there were no apparent differences in nitrotyrosine staining intensity between HIV-1 transgenic and FVB mice (data not shown). Nitrotyrosine was quantitated via ELISA (Northwest Life Science Specialties). Nitrotyrosine levels were significantly ($P < 0.05$) greater in HIV-1 transgenic mice treated with LPS (Fig. 4E).

Hydrogen peroxide analysis. To determine whether reactive oxygen species are present in the lungs at higher concentrations after endotoxin administration in an HIV-1 transgenic mouse model, hydrogen peroxide levels were quantitated. Hydrogen peroxide levels, measured by Amplex Red reagent, were significantly ($P < 0.001$) elevated for HIV-1 transgenic mice treated with LPS for 3 h compared with all other groups (Fig. 5A). At 6 h, hydrogen peroxide levels dropped to relatively equal amounts for all groups (Fig. 5B).

GSH analysis. GSH, a key antioxidant involved in maintaining proper lung function, was measured to determine pulmonary oxidative/nitrosative stress susceptibility after endotoxin administration in an HIV-1 transgenic mouse model. Figure 6 shows that LPS-treated HIV-1 transgenic mouse lung lavage fluid collected at 3 h had significantly ($P < 0.01$) decreased levels of GSH compared with all other groups. However, lavage fluid collected at 6 h showed relatively equal amounts of GSH but slightly decreased from 3 h. Interestingly, blood plasma GSH levels showed no significant differences among the groups analyzed (data not shown).

Cytokine analysis. The immunological response, before and after LPS treatment, was examined to verify the nonimmunocompromised characteristic of HIV-1 transgenic mice. Several pro- and anti-inflammatory cytokines in blood serum were quantified. Table 1 shows that, without LPS treatment, cytokine levels of HIV-1 transgenic and wild-type mice are low and similar in concentration. After LPS treatment, cytokine levels are significantly elevated in both wild-type and transgenic mice ($P < 0.001$).

DISCUSSION

In this study, we have investigated the role of oxidative and nitrosative stress in potentiating HIV-associated pulmonary complications. We have shown that when HIV-1 transgenic mice are treated with endotoxin (LPS), biomarkers of oxidative and nitrosative stress are more prevalent compared with wild-type mice treated with endotoxin and control mice. It is important to note that we were able to confirm enhanced oxidative and nitrosative stress via several different mechanisms in our animal model. We began by assessing lung edema in our experimental groups. At the outset, HIV-1 transgenic mice untreated with LPS had significantly greater lung edema than wild-type mice untreated with LPS. This is a clear indication that the lungs of HIV-1 transgenic mice, before LPS administration, may contain a physiologically different
Next, we sectioned lung tissue from our experimental groups to examine in situ biomarkers of oxidative/nitrosative stress. Studies have shown that regulation of superoxide in the alveolar interstitium and extracellular space of the lungs is of significant importance for proper lung function (4). We found that lung superoxide, 3 h after LPS treatment, was more prevalent in tissue collected from HIV-1 transgenic mice compared with wild-type mice. We also examined nitrate and nitrite levels in lung tissue, blood plasma, and BALF as markers for nitrosative stress. In all samples analyzed, untreated HIV-1 transgenic mice had significantly greater levels of NO metabolites than untreated wild-type mice. Furthermore, after LPS treatment, HIV-1 transgenic mice had significantly elevated BALF, lung tissue, and plasma NO metabolites compared with wild-type mice. Studies show that increased concentrations of NO and superoxide can combine to form peroxynitrite, which is a very potent source of nitrosative stress (28, 35, 44). Because superoxide was also in higher abundance in the lungs of HIV-1 transgenic mice after LPS administration, we determined the levels of nitrotyrosine, a footprint of peroxynitrite. Nitrotyrosine accumulation and quantification were significantly greater in the lungs of HIV-1 transgenic mice than wild-type mice 3 h after LPS administration. We speculate that HIV-1 transgenic mice, both untreated and treated with LPS, have exacerbated lung NO production in an attempt to combat inflammation and disease. However, this compensatory mechanism is, perhaps, detrimental to the lung via increases in nitrosative/nitritative stress levels, thus altering lung function.

We also determined that lung tissue hydrogen peroxide levels were significantly elevated in HIV-1 transgenic mice treated with LPS compared with wild-type mice treated with LPS and controls. In addition, levels of GSH, an antioxidant crucial for proper lung function, were significantly lower in transgenic mice treated with LPS than in wild-type mice treated with LPS. The increase in hydrogen peroxide levels in the lungs of HIV-1 transgenic mice mentioned above may be attributed to this makeup compared with wild-type mice, predisposing HIV-1 transgenic mice to pulmonary complications. Furthermore, lung edema was significantly greater 3 h after LPS treatment in HIV-1 transgenic mice than in wild-type mice or untreated groups. Our data show that with LPS administration, lung edema can be further enhanced or exacerbated within the lungs of HIV-1 transgenic mice.

Table 1. Pro- and anti-inflammatory cytokines from mouse serum samples after LPS treatment compared with untreated controls

<table>
<thead>
<tr>
<th></th>
<th>No LPS</th>
<th>HIV Tg No LPS</th>
<th>FVB 3-h LPS</th>
<th>HIV Tg 3-h LPS</th>
<th>FVB 6 h</th>
<th>HIV Tg 6-h LPS</th>
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<tr>
<td>GM-CSF</td>
<td>10.9 ± 1.2</td>
<td>21.1 ± 0.9</td>
<td>284.6 ± 2.3*</td>
<td>188.1 ± 0.9†</td>
<td>690.1 ± 2.2*</td>
<td>577.8 ± 4.5†</td>
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<tr>
<td>IL-12</td>
<td>10.1 ± 0.2</td>
<td>7.0 ± 0.8</td>
<td>122.4 ± 1.7*</td>
<td>101.3 ± 1.7</td>
<td>184.2 ± 1.9*</td>
<td>172.8 ± 2.2†</td>
</tr>
<tr>
<td>IL-6</td>
<td>161.0 ± 1.3</td>
<td>111.0 ± 3.0</td>
<td>541.0 ± 12.6*</td>
<td>161.2 ± 2.6†</td>
<td>547.1 ± 2.3*</td>
<td>393.1 ± 5.7†</td>
</tr>
<tr>
<td>IL-10</td>
<td>101.2 ± 4.0</td>
<td>86.4 ± 2.2</td>
<td>324.1 ± 5.0*</td>
<td>111.2 ± 3.7†</td>
<td>378.8 ± 8.1*</td>
<td>208.0 ± 1.7†</td>
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<tr>
<td>IL-4</td>
<td>15.8 ± 2.0</td>
<td>10.3 ± 0.8</td>
<td>71.4 ± 3.5*</td>
<td>72.8 ± 3.5†</td>
<td>68.9 ± 3.5*</td>
<td>78.1 ± 4.1†</td>
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<tr>
<td>IL-2</td>
<td>11.3 ± 1.6</td>
<td>12.4 ± 1.9</td>
<td>79.5 ± 3.3*</td>
<td>61.5 ± 4.6†</td>
<td>81.8 ± 6.8*</td>
<td>78.9 ± 4.3†</td>
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<tr>
<td>IL-1β</td>
<td>121.1 ± 4.7</td>
<td>101.8 ± 3.4</td>
<td>378.2 ± 3.4*</td>
<td>352.0 ± 7.7†</td>
<td>400.1 ± 4.2*</td>
<td>395.2 ± 4.4†</td>
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<tr>
<td>TNF-α</td>
<td>102.3 ± 4.5</td>
<td>111.1 ± 5.1</td>
<td>252.1 ± 6.0*</td>
<td>231.2 ± 5.1†</td>
<td>403.1 ± 11.5*</td>
<td>428.1 ± 16.2†</td>
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Results (in pg/ml) are means ± SD (n = 5 for each group). Without lipopolysaccharide (LPS) treatment, there was no significant difference of cytokine levels between HIV-1 transgenic (HIV Tg) and wild-type (FVB) mice. FVB mice 3 and 6 h after LPS treatment produced significantly (*P < 0.001) greater levels of all cytokines compared with untreated FVB mice. HIV Tg mice 3 and 6 h after LPS treatment produced significantly (†P < 0.001) greater levels of all cytokines compared with untreated HIV Tg mice. GM-CSF, granulocyte-macrophage colony-stimulating factor.
decrease in GSH levels, because GSH is utilized in the metabolism of hydrogen peroxide. This metabolic reaction occurs when GSH is converted to its oxidized form (known as GSSG) via an enzyme called glutathione peroxidase, changing hydrogen peroxide into water. Studies have speculated that HIV-1 may contain a human glutathione peroxidase homologue (9, 32, 46). It is thought that during replication and through competitive inhibition, HIV-1 is able to deplete the host of glutathione peroxidase and the basic components of this enzyme such as selenium, cysteine, glutamine, and tryptophan, leaving the host with depleted GSH levels and many symptoms of AIDS (9, 32, 46). Nevertheless, glutathione peroxidase deficiency has been well documented in HIV-infected patients (11, 15). Studies have also shown that LPS in vitro can cause a decrease in GSH, and it is thought to also do so via the inhibition of glutathione peroxidase (37). It is important to note that the significant difference in lung edema observed between HIV-1 transgenic mice treated with LPS and wild-type mice treated with LPS mentioned above did not cause a dilutional change in GSH, because GSH-to-GSSG ratios were dissimilar to GSH-to-total pool ratios from all groups analyzed (data not shown).

In HIV-infected patients, immune dysfunction has been linked to increased oxidative stress and decreased GSH levels (20, 31, 33). In vitro studies have shown that oxidative stress and depleted GSH levels lead to altered lymphocyte function and impaired proliferation response (20, 31, 33). It is speculated that the transport of cysteine, an integral component of GSH synthesis, into T cells is inhibited (possibly through mediation of the cysteine-glutamate transporter system) and this decreases intracellular GSH and creates diminishing resistance to intracellular oxidative stress (13). Furthermore, cell culture experiments show that GSH replacement, via a variety of GSH precursors, restores the proliferative response of T cells that are infected with HIV-1 (20, 23, 30).

Fig. 4. Immunohistochemistry staining of nitrotyrosine in lung tissue 3 h after LPS administration (magnification ×200; n = 4). A and C: lung tissue negative control IgM staining for FVB and HIV-1 Tg mice, respectively. B and D: FVB and HIV-1 TG mouse nitrotyrosine staining, respectively, which show a more intensive staining (brownish color) in the HIV-1 Tg lung tissue compared with FVB lung tissue 3 h after LPS treatment. E: nitrotyrosine concentrations for each group (n = 4). These results show a statistically significant (P < 0.01) nitrotyrosine increase for HIV Tg mice 3 h after LPS treatment and coincide with the immunohistochemistry staining.
In contrast to HIV-1-infected patients, the HIV-1 mouse model used in this study is not immunocompromised. We chose to use this model to examine the effects of secreted HIV-1 proteins independent of immune system complications. We verified immune function of our animal model by examining cytokine profiles. We have shown similarity in blood serum cytokine levels of HIV-1 transgenic mice and wild-type mice before LPS treatment. Furthermore, after LPS challenge, both HIV-1 transgenic and wild-type mice are able to elicit an immune response, as indicated by the increase in cytokine levels. In particular, key proinflammatory cytokines, such as TNF-α and IL-1β, are especially important in inducing an immune response to LPS treatment. TNF-α and IL-1β levels after LPS treatment are similarly elevated in HIV-1 transgenic mice compared with wild-type mice. In all, these cytokine levels are consistent with published data from wild-type mice after LPS treatment (36). This suggests that HIV-1 transgenic mice are able to immunologically respond to LPS and that the lung dysfunction in this model is perhaps due to nonimmunologic pathways as shown in our experiments.

HIV-1 transgene expression has been detected in lung tissue of the HIV-1 transgenic mouse (6), yet the expressing cell type is not known. In addition, individual HIV-1 proteins have not been localized in lung tissue. Perhaps circulating HIV-1 proteins may play a role in lung injury in our mouse model as well as in HIV-1 patients.

HIV-1 proteins, such as Tat, Nef, Vpr, and gp120, have been implicated in the initiation and/or intensification of oxidative stress. HIV-1 Tat is shown to induce hydrogen peroxide production in T cells via interference of T-cell receptor signaling (18). In this case, HIV-1 Tat-induced hydrogen peroxide generation is shown to cause massive T-cell apoptosis (18). Furthermore, HIV-1 Tat is shown to lower the total and reduced GSH concentration, in various cell lines and in HIV-1 Tat transgenic mice, through the inhibition of GSH biosynthesis (8). HIV-1 Tat is also shown to downregulate manganese superoxide dismutase activity (14) and enhance the activity of the redox-sensitive transcription factor nuclear factor-κB, resulting in cellular apoptosis (10). HIV-1 Nef is thought to interfere with GSH biosynthesis and regulate nuclear factor-κB activity to increase HIV-1 replication (39). HIV-1 Vpr is shown to increase mitochondrial permeability and ultimately cell death (19). HIV-1 gp120 is shown to significantly decrease intracellular GSH through the inactivation of key enzymes involved in GSH biosynthesis and is shown to increase malondialdehyde (34), a highly reactive by-product of polyunsaturated fatty acid peroxidation. Furthermore, gp120 is shown to induce apoptosis of lung endothelial cells via a downregulation in antiapoptotic genes and proteins (26).

We speculate that the lung oxidative/nitrosative stress seen in HIV-1 transgenic mice treated with LPS is independent of immune dysfunction, as indicated by similar changes in cytokine levels before and after LPS exposure. We have shown that HIV-1 proteins are able to increase pulmonary dysfunction after endotoxin administration. This increase is mediated through an acute phase of oxidative and nitrosative stress, as indicated by the biomarkers that were studied here. These findings are novel in that, to date, lung redox stress has not been established in an HIV-1 transgenic model. Moreover, the theory that LPS-exacerbated HIV-1-associated pulmonary redox stress is independent of immune dysfunction is also a novel concept. Therefore, we surmise that the oxidative/nitrosative stress observed in this study is not only due to an imbalance between prooxidants and antioxidants but also involves a disruption of redox signaling independent of immune dysfunction. Our data suggest that HIV-1 is perhaps working to cause pulmonary dysfunction via two separate mechanisms: impairment of immunity from pathogens and mediation of redox stress. Reactive oxygen species and reactive nitrogen species may therefore play a prominent role in the exacerbation of HIV-1-associated pulmonary complications independent of immune dysfunction.
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