Bleomycin treatment causes enhancement of virus replication in the lungs of SHIV-infected macaques

Navneet Kaur Dhillon, David Pinson, Sukhbir Dhillon, Ossama Tawfik, Marsha Danley, Marilyn Davis, Olga Nemon, Matthew Mayo, Anil Kumar, Yi-Jou Tsai, Amit Kumar, and Shilpa Buch

1Department of Molecular and Integrative Physiology, 2Department of Pathology and Laboratory Medicine, 3Department of Microbiology, Immunology, and Molecular Genetics, 4Center for Biostatistics and Advanced Informatics, and 5Cardiovascular Disease, University of Kansas Medical Center, and 6University of Missouri Kansas City, Kansas City, Kansas

Submitted 4 August 2006; accepted in final form 6 January 2007

Am J Physiol Lung Cell Mol Physiol 292: L1233–L1240, 2007. First published January 12, 2007; doi:10.1152/ajplung.00293.2006.—Pneumonia is a major complication of human immunodeficiency virus (HIV) infection and occurs subsequent to depletion of CD4+ T cells by the virus when the host has been rendered immunologically incapable of resisting infections caused by opportunistic pathogens (OP) (2). The disease may be caused either by primary infection of the lung by HIV-1, as seen in pediatric acquired immune deficiency syndrome (AIDS) patients, or as a consequence of OPs in adult immunosuppressed patients. Pathological changes in the lungs are underpinned by the effects of virus infection in the macrophage population that results in further recruitment of monocytes from the blood and viral cytopathic effects in macrophages including development of multinucleated giant cells. These changes are accompanied by additional characteristic pathological effects induced by opportunistic infections such as Pneumocystis carinii, cytomegalovirus, or Mycobacterium tuberculosis. The infections by these OPs are associated with a plethora of cytokine and chemokine changes (1, 22, 23, 25), and these no doubt have a significant effect on expression of the virus.

Simian immunodeficiency virus (SIV) and simian HIV (SHIV) infections in macaques have reproduced the pathogenesis of HIV infection in macaques and have been used as models to explore mechanisms of pathogenesis, treatment strategies, and vaccine development against HIV (4, 11, 17, 20). Pneumonia is a rare complication of the disease in macaques, even in animals that have been rendered severely immunodeficient by the virus. The precise reasons for lack of predictability of the syndrome are not clear, but it may be related to the very clean environments in which macaques are housed in research institutions. Indeed, our experience has been that macaques can survive for months to years with very low CD4+ T cell counts and not develop pneumonia. These macaques more often develop syndromes of diarrhea and wasting disease. Given this observation, we hypothesized that development of pulmonary AIDS may require a local triggering agent that induces the virus replication. In an earlier report, we had investigated the plausibility of this two-hit mechanism using Schistosoma mansoni eggs injected intratracheally into SHIV-infected macaques as the second hit agent. The eggs induced granulomas and a strong T helper type 2 (Th2) response. Macrophages comprising the granulomas developed highly productive infection with the virus (8). The dramatic effect of host factors on replication of the virus was identified in our subsequent studies. In previous reports, we (26, 33) have shown that monocyte chemotactic protein-1 (MCP-1), CXC chemokine ligand 10 (CXCL10), interleukin-4 (IL-4), and platelet-derived growth factor (PDGF) caused enhancement of viral replication in macrophages, and these cytokines were found in abundance in inflamed virus-infected lungs of macaques (8, 11, 33).

The extent of Schistosoma egg-induced inflammation in the lungs was limited to areas of egg deposition where granulomas developed only after prolonged infection. We used the macaque model to explore a hypothesis that the disease is a two-stage process, the first stage being establishment of the viral infection in the lung and the second amplification of virus replication by host factors induced by chemical agents or opportunistic pathogens in the lung. Bleomycin, a chemical known to induce diffuse alveolar damage and pulmonary fibrosis with accumulation of macrophages and a rich T helper type 2 (Th2) cytokine environment, was inoculated intratracheally into five of eight SHIV 89.6P-infected macaques and into one uninfected macaque. There was enhanced production of the chemokine, monocyte chemotactic protein-1 (MCP-1), that had previously been shown without bleomycin treatment served as untreated virus controls. Although none of the animals became clinically ill, bleomycin induced classical host responses in the lungs of all the treated, virus-infected macaques. There was enhanced production of the chemokine, monocyte chemotactic protein-1 (MCP-1), that had previously been shown to cause enhanced replication of the virus. Four of the five treated animals developed more productive SHIV infection in the lungs compared with the infected untreated animals. Enhanced virus replication was found primarily in infiltrating macrophages. Enhanced replication of the virus in the lungs was associated with host factors induced by the drug and supported the hypothesis for a two-stage process of pulmonary pathogenesis.

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developed. In the current study, we therefore sought to determine whether a chemical compound, such as bleomycin, could provide the second hit signal and cause a more diffuse effect that would potentiate replication of the virus throughout the lungs as seen in HIV-1 pneumonias. Bleomycin induces an acute form of interstitial pneumonia with extensive parenchymal destruction leading to pulmonary fibrosis (6, 9, 29, 35). This is usually accompanied by enhanced expression of MCP-1 (30, 35), a chemokine that is known to promote recruitment of monocytes to the lungs and also cause enhancement of replication of the virus in macrophages differentiation from the recruited monocytes.

To determine whether bleomycin-induced changes in the lungs would enhance the replication of SHIV in lungs of infected macaques, we treated five infected and one uninfected macaques with the drug intratracheally and followed the course of the infection by determining viral RNA content in plasma and in lung tissue after the animals had been euthanized. Bleomycin caused significant parenchymal damage with fibroplasia and histiocytic inflammation in all treated animals, but in the animals infected with the virus (SHIV-BL), it caused additional infiltration and activation of macrophages in the tissue. These cells are the major targets of the virus in the lungs. Virus burdens in the blood remained unchanged by the treatment, but virus concentrations in the lungs were enhanced in drug-treated, infected animals, and this was associated with pronounced replication of the virus in the macrophage population.

MATERIALS AND METHODS

Animals. The animals used in this study were maintained at the University of Kansas Medical Center Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited animal facility. The work with macaques was approved by the Kansas University Institutional Animal Care and Use Committee. Eight rhesus macaques were inoculated intratracheally with 2 ml of Hanks’ balanced salt solution containing SHIV 89.6P [10⁵ 50% tissue culture infective dose (TCID₅₀)/ml]. One month later, five of the macaques (13–523, 13–559, RRP5, RQP5, and RQQ5) were inoculated intratracheally with a solution of bleomycin using a dose of 1 mg/kg. The other three (13–508, 13–548, and PHP) were inoculated intravenously with 2 ml of Hanks’ balanced salt solution containing SHIV 89.6P [10⁵ 50% tissue culture infective dose (TCID₅₀)/ml].

Table 1. Virus inoculation, plasma/lung viral RNA, and lung pathology in SHIV-infected rhesus macaques with and without bleomycin treatment

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Inoculum</th>
<th>Bleomycin, Yes/No</th>
<th>Time of Kill Relative to Bleomycin Exposure</th>
<th>Plasma Viral Burden at Necropsy, Copy number/ml</th>
<th>Lung Viral Burden at Necropsy (gag/HPRT)</th>
<th>Histological Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-508</td>
<td>SHIV 89.6</td>
<td>No</td>
<td>3 wk</td>
<td>34,158</td>
<td>0.13</td>
<td>No inflammation</td>
</tr>
<tr>
<td>13-548</td>
<td>SHIV 89.6</td>
<td>No</td>
<td>3 wk</td>
<td>47,248</td>
<td>0.17</td>
<td>99% normal; two microscopic foci of histiocytic inflammation</td>
</tr>
<tr>
<td>PHP</td>
<td>SHIV 89.6</td>
<td>No</td>
<td>Undetectable</td>
<td>180,669</td>
<td>1.57</td>
<td>Histiocytic interstitial pneumonia with some fibrosis</td>
</tr>
<tr>
<td>13-523</td>
<td>SHIV 89.6</td>
<td>Yes</td>
<td>3 wk</td>
<td>19,280</td>
<td>3.28</td>
<td>Patchy interstitial pneumonia with limited alveolar proteinosis; rare syncytial giant cells</td>
</tr>
<tr>
<td>13-559</td>
<td>SHIV 89.6</td>
<td>Yes</td>
<td>4 wk</td>
<td>46,134,395</td>
<td>358</td>
<td>Pneumocystis carinii pneumonia; diffuse interstitial disease</td>
</tr>
<tr>
<td>RRP5</td>
<td>SHIV 89.6</td>
<td>Yes</td>
<td>2 wk</td>
<td>1,039</td>
<td>0.12</td>
<td>Interstitial pneumonia with rare syncytial giant cells</td>
</tr>
<tr>
<td>RQP5</td>
<td>SHIV 89.6</td>
<td>Yes</td>
<td>4 wk</td>
<td>4,627</td>
<td>4</td>
<td>Patchy interstitial pneumonia with fibroplasia and rare syncytial giant cells</td>
</tr>
<tr>
<td>RZs5</td>
<td>SHIV 89.6</td>
<td>No</td>
<td>4 wk</td>
<td>None</td>
<td>None</td>
<td>Histiocytic interstitial pneumonia with fibroplasia</td>
</tr>
</tbody>
</table>

SHIV, simian-human immunodeficiency virus.
Fig. 1. Histopathological changes in the lungs of simian-human immunodeficiency virus (SHIV)-infected macaques with or without bleomycin (BL) treatment. Representative staining of paraffin-embedded lung sections from SHIV (A, C, E, G, and I) and SHIV-BL (B, D, F, H, and J) macaques for hematoxylin and eosin (H&E) (A and B) and with an antibody specific for proliferation marker Ki67 (C and D), cytokeratin (E and F), and vimentin (G and H). Fibrotic lesions containing collagen (blue trichrome staining) were abundant in the lungs from SHIV-BL macaques (J) compared with SHIV-control macaques (I). Images shown were captured at ×200 magnification, except H&E, which was at ×100.
aminobenzidine hydrogen peroxide was used creating a brown reaction product.

**Quantification of immunohistochemistry.** Positive immunohistochemical reactions were defined as dark brown reaction on the cell membrane CCR2, cytoplasmic staining for HAM 56, and positive nuclear staining for Ki67. Staining parameters were evaluated at ×100, and areas of high density immunostaining were chosen for image analysis. Once the immunostaining was completed, the slides were placed on the Clarient Automated Cellular Imaging System (ACIS) (San Juan Capistrano, CA) for quantitating the tissue staining. The system consists of an automated microscope, a three-chip Sony progressive scan camera, a computer, and Windows NT v4.0 workstation software interface. Each slide was scanned by the robotic microscope. The ACIS system captures images from each slide and quantifies staining intensity within a selected region and presents a numerical score. The system specifically quantitates immunostaining intensity and percent positivity. The ACIS system recognized 256 levels of immunohistochemical staining intensity and converted these into fractional scores for the selected individual areas. An average score for all selected areas was then calculated. For proliferation index (PI) of Ki67, the percentage of nuclei with immunopositivity was determined using the PI program of the ACIS system. For HAM 56 and CCR2, the ACIS system was used for automated counts as well. Twenty to 35 areas with the highest staining intensity were selected for quantitative from each slide. An average score for all selected areas was then calculated.

**Monocyte-derived macrophages.** These cells were derived from peripheral blood of uninfected macaques. Mononuclear cells were purified from heparinized blood by centrifugation through Ficoll-Hypaque gradients. The cells were then cultivated in RPMI supplemented with 20% FBS and macrophage colony-stimulating factor (M-CSF; 5 U/ml) and granulocyte-macrophage CSF (GM-CSF; 100 U/ml). Monocytes in the cell preparations differentiated to become adherent macrophages (MDMs) in culture vessels. Approximately 7 days after initiation of the cultures, the MDMs were used for specific experiments.

**Flow cytometry.** MDMs were inoculated with the virus at a multiplicity of 1 and used for flow cytometric assays 5–10 days later. Flow cytometry was used to assess expression of CCR2, the receptor for MCP-1, in uninfected or SHIV 89.6P-infected MDMs. Cultures were determined in the lung samples. TRIzol reagent was used to extract RNA from lung tissues. Gag mRNA was determined using the TaqMan probe and primers as described above for the plasma viral RNA determinations during 44 cycles of RT-PCR (ABI). As a measure of cellular mRNA levels, the hypoxanthine phosphoribosyltransferase (HPRT) mRNA copy numbers in the lung RNA samples were also determined by a real-time RT-PCR TaqMan assay over 40 cycles (ABI). mRNA numbers based on the use of constant standards were determined in the gag mRNA assays, and since the amplification efficiencies of the gag and HPRT targets can be considered essentially equal [differences in the slopes (ΔS) of the standard curves was within 0.2], the gag mRNA levels were normalized to cellular HPRT mRNA number.

**MCP-1 ELISA in lung protein extracts.** An MCP-1 ELISA kit (R&D Systems) was used to determine the MCP-1 protein levels in the lung samples. TRIZol reagent was used to extract protein from lung tissues according to the manufacturer’s recommendations. Briefly, pieces of rapidly frozen lung tissue were homogenized in 1 ml of TRIZol, followed by protein precipitation after the RNA, and DNA was removed from the samples. After extensive washing in a solution containing 0.3 mol/l guanidine hydrochloride in 95% ethanol, the protein pellet was redissolved in either PBS or 1% SDS. Protein concentration was determined by using the bicinchoninic acid assay (Bio-Rad, Hercules, CA).

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Fig. 2. Immunohistochemistry on a representative lung section from a SHIV-BL macaque with macrophage-specific antibody HAM 56 (C) and with antiviral p27-specific antibody (B) at ×200 and ×400 magnification, respectively. Note the virus-induced multinucleated giant cells in B. Macrophage staining at ×200 magnification of a representative lung section from a virus control animal that did not receive bleomycin is shown in A.
Statistical analyses. Comparisons of lung viral burden, plasma viral burden, and MCP-1 between bleomycin-treated or untreated SHIV-infected animals were conducted using the Wilcoxon rank sum test. Exact two-sided *P* values were calculated using SAS v9.1 software (SAS Institute, Cary, NC). A type I error rate of 5% was used to determine statistical significance.

RESULTS

Virological parameters. None of the animals developed any clinical signs of disease. All assays were conducted on peripheral blood collected during the infection and on lung tissue samples collected at the time of necropsy of the animals. With the exception of macaque PHP, for which plasma viral load was undetectable, all remaining animals became productively infected with virus, as evidenced by the presence of viral RNA in plasma. There was no statistically significant difference in viral RNA concentrations in plasma between the virus control (SHIV) group and the SHIV-bleomycin (SHIV-BL) group of animals (*P* = 0.7857) (Table 1).

Histopathological parameters. Lung tissue samples from the BL-control animal and the entire SHIV-BL group showed evidence of patchy acute interstitial pneumonia with fibrinous exudates and organizing fibrosis (Table 1). Minimal histiocytic inflammation was noted in tissues from the animals treated with bleomycin alone or SHIV alone. In fact, lungs from the SHIV group (13-508, 13-548, and PHP) were without any significant histological abnormalities (Fig. 1A). In contrast,
lung tissue from SHIV-BL group (13–523, 13–559, RRP5, RQ5, and RQ5P) showed intense histiocytic inflammation in addition to the interstitial pneumonia and fibroplasias (Fig. 1B, Table 1). Additionally, one showed evidence of *P. carinii* pneumonia (RRP5), and two showed syncytial giant cells classically noted in SHIV-infected animals (13–559 and RQ5P) (Table 1).

Quantitative immunohistochemical studies revealed an increase in proliferative activity and Ki67 count in the SHIV-BL group (Figs. 1D and 4) compared with the SHIV group (Figs. 1C and 4). Additionally, there was a noticeable increase in epithelial cells highlighted by cytokeratin staining (Fig. 1F) and mesenchymal cells highlighted by vimentin (Fig. 1H) in the SHIV-BL group. SHIV-infected animals, on the other hand, showed flat alveolar epithelial cells and a few fibroblasts in the interstitium (Fig. 1, E and G). High deposits of collagen were also observed in the SHIV-BL group of animals (Fig. 1J) compared with SHIV-group (Fig. 1I).

We performed immunocytochemical studies using macrophage-specific marker HAM 56 to determine whether macrophages were a prominent feature following bleomycin treatment. Examination of tissue from the bleomycin control animal showed only minimal numbers of macrophages in the tissue (data not shown). However, there was intense infiltration of macrophages throughout the lung parenchyma in the SHIV-BL group of animals (Figs. 2A and 4). Staining of sections with antibodies to viral p27 showed that viral antigen was associated exclusively with the macrophages in the lung sections (Fig. 2B).

Examination of lung tissues from the three SHIV-group animals showed that the lungs were histologically normal (Fig. 1A) with very few positive macrophages (Figs. 2C and 4). Sections of these tissues reacted with antibodies to viral p27 yielded negative results (data not shown).

**SHIV viral load in the plasma and in the lung parenchyma of bleomycin-treated, virus-infected macaques.** As shown in Table 1, real-time RT-PCR indicated that bleomycin treatment had no effect on viremia titers. Only two out of the five SHIV-BL group animals (RRP5 and 13–523) had higher plasma viral load compared with the plasma viral loads of virus-infected, untreated animals. Moreover, there was no statistically significant difference in plasma viral burden between treated and untreated groups of animals (*P* = 0.7857). On the contrary, three of the five SHIV-BL animals had less viral RNA in plasma than infected control macaques. However, assessment of viral RNA in lung tissue samples showed that four of the five SHIV-BL animals had higher viral loads in the lungs than the virus control animals (Table 1). Overall, there was no statistically significant difference in lung viral burden between treated and untreated animals (*P* = 0.2500). One SHIV-BL animal, RQ5P, had lower viral production in the lung tissue, and this correlated with milder histological abnormalities in the lungs. Virus was also detected in the lung tissues of PHP macaque even though it had undetectable viral load in plasma. Thus, although there was no effect of bleomycin in promoting virus replication in the peripheral blood, the drug was able to increase viral load in the lungs.

**MCP-1/CCR2 expression in the lungs of SHIV-infected macaques treated with bleomycin.** Since MCP-1, also termed macrophage chemotactic and activating factor (MCAF/CCL2), and its receptor, CCR2, are reported to contribute to progressive fibrosis (16, 24, 27, 39), and since MCP-1 also plays an important role in promoting virus replication in infected MDMs and in macaques with SHIV-associated pneumonia (15, 32, 33), we determined whether levels of MCP-1 in lung homogenates and the immunohistochemical expression of its receptor, CCR2, in lung sections were elevated in the SHIV-BL animals. The study showed that there was a statistically significant difference in MCP-1 in lung homogenates between the two groups of animals: treated with bleomycin and untreated with bleomycin (*P* = 0.0357; Fig. 3A).

Many of the cells in the lungs of SHIV-BL group also stained positively with antibodies to CCR2, the receptor for MCP-1 (Fig. 3B). There was a corresponding increase in CCR2 expression in the lungs of SHIV-BL macaques (Figs. 3B, C and 4) compared with CCR2 expression in SHIV-control macaques (Figs. 3B, A and 4). These changes were ascribed to the effect of bleomycin since BL-control group animal also showed these changes in lung tissue (Fig. 3B). Since lungs of SHIV-BL macaques demonstrated enhanced virus replication, the effect of virus infection on CCR2 expression was further explored in MDMs isolated from healthy macaques and infected with virus. We inoculated MDMs with SHIV 89.6P and, after 5 days, tested the cells for CCR2 expression using fluorescence-activated cell sorting (FACS). In Fig. 3C, a representative of three independent experiments, we found that 60% cells were positive for CCR2 in infected compared with 40% in uninfected cultures.

**DISCUSSION**

Pulmonary disease is an important complication of HIV infection but difficult to reproduce experimentally in animal models because reproduction of the syndrome seems to require a two-hit phenomenon: first, establishment of the infection, and, second, presence of a factor that would promote replication of the virus mainly by inducing histiocytic responses. OPs do not proliferate until the host has lost the ability to mount cell-mediated immune responses. Previous studies (10, 28) have suggested that Th2-inducing parasite infestations enhance lentiviral production at local sites. Work in our labo-
Bleomycin historically induces free radical injury (21, 38) followed by elaboration of profibrotic cytokines that include TGF-β (3), MCP-1 (24, 27, 39), PDGF (40), and the Th2 cytokine IL-4 (12, 14). The drug induces early histiocytic interstitial inflammation followed by fibroplasia and fibrosis. Our hypothesis was that the histiocytic inflammation that included production of MCP-1 by the fibroblasts would promote viral replication. The data indicated that the drug-induced inflammation provided an appropriate environment for support of local production of the virus. Lungs of SHIV-BL animals contained severalfold higher virus levels than untreated, infected controls except for one macaque (RQP5). The increased virus burden in the lungs of SHIV-BL animals compared with the untreated, infected controls was not due to 1) increased T cell infiltration (data not shown) or 2) increased blood in inflamed lungs since all the blood was washed out with lactated Ringer solution before sampling of tissues. We thus surmise that the increased numbers of virus-positive multinucleated giant cells, one of the key cells for productive HIV-1 replication, are the major contributors of increased virus load in the lungs of SHIV-BL animals. The fact that histiocytic changes were more intense in the SHIV-BL compared with the BL-control macaque suggested that once the virus infection became established in the transient macrophage population induced by the drug, the viral infection became self-perpetuating. One of the self-perpetuating factors could have been MCP-1 since we had shown previously that virus infection in macrophages resulted in enhanced production of MCP-1.

Although pneumonia is a frequent complication of HIV-1 infection, the pathogenesis of the syndrome is poorly understood. Even though HIV-1 infection in and depletion of CD4+ T cells can be reproduced in the macaque model of the infection, pneumonia with productive viral replication in the lung is an infrequent complication in SIV/SHIV-infected macaques. We have found that many of our macaques failed to develop pneumonia despite having extremely high viral RNA burdens in plasma and very low CD4+ T cell counts. We surmised that the exceptionally clean environment in which research macaques are maintained provided few opportunities for OPs to cause superinfections. Intersitial pneumonias with few opportunistic agents are uncommon. Our present study showed that pneumonia with enhanced virus replication in macrophages of lungs, strikingly similar to the human system, could be reproduced in virus-infected macaques treated with chemical compound bleomycin. The fact that MCP-1 could be produced by both fibroblasts and macrophages in the bleomycin-treated animals and that many of the cells also expressed CCR2, the receptor for MCP-1, provided the basis for activation of the cells since it is well known that binding of MCP-1 to its receptor usually results in activation (5, 31). The effects of bleomycin in recruiting monocytes to the lung where they developed into activated histiocytes provided the ideal environment for the virus to flourish. The reproducibility of the human disease now provides a model system to explore therapeutic strategies that could be aimed either at control of the virus or control of host responses.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants MH-62969-01, RR-016443, MH-068212, MH-072355, and DA-020392-01.

REFERENCES

15. Huffnagle GB, Strieter RM, Staniford TJ, McDonald RA, Burdick MD, Kunkel SL, Toews GB. The role of monocyte chemoattractant protein-1...


