Whole Body Positron Emission Tomography Imaging of Activated Lymphoid Tissues during Acute Simian–Human Immunodeficiency Virus 89.6PD Infection in Rhesus Macaques

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Mechanisms of acute retroviral pathogenesis have been examined during primary infection of rhesus macaques with simian–human immunodeficiency virus 89.6PD (SHIV89.6PD). During acute infection, between initial exposure and establishment of antigen-specific immune responses that stabilize the virus burden, rapid immune system changes influence the viral set-point and dictate subsequent steps in disease progression. In a previous study, we described specific patterns of lymphocyte activation during acute SHIV89.6PD infection. We now extend these studies to describe lymphoid tissue activation, using whole body positron emission tomography (PET) and the radioactive tracer 2-[18F]fluorodeoxyglucose (FDG). Within a few days after primary infection by intravenous, intrarectal, or intravaginal routes, PET–FDG imaging revealed a distinct pattern of lymphoid tissue activation centered on axillary, cervical, and mediastinum lymph nodes. Increased tissue FDG uptake preceded fulminant virus replication at these sites, suggesting that a diffusible factor of host or viral origin was responsible for lymphoid tissue changes. These data show that activation of lymphoid tissues in the upper body is an early response to virus infection and that diffusible mediators of activation might be important targets for vaccine or therapeutic intervention strategies. © 2000 Academic Press

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INTRODUCTION

Acute infection identifies the interval between human immunodeficiency virus type 1 transmission and the appearance of antigen-specific immune responses. Accompanying clinical sequelae comprise a common acute retroviral syndrome that is characterized by flu-like symptoms, including rash and night sweats (Clark et al., 1991; Tindall and Cooper, 1991). The severity and duration of acute retroviral syndrome have been linked to subsequent rates for disease progression (Pedersen et al., 1998). When virus-specific CD4+ T-cell helper and CD8+ T-cell cytotoxic responses arise early and at high levels, acute disease is less severe and the prognosis is more favorable (Musey et al., 1997; Rosenberg et al., 1997; Kalams and Walker, 1998). The virus burden fluctuates during primary human immunodeficiency virus (HIV) infection until viral RNA (vRNA) levels stabilize during the stage of persistent infection and become predictive for the time to AIDS (Henrard et al., 1995; Schacker et al., 1998). We believe that the earliest host–virus interactions act to limit the effectiveness of immune responses and thus dictate the viral set-point and subsequent disease progression (Wallace et al., 1999). Our study of acute retroviral pathogenesis seeks to characterize mechanisms unique to this interval and to identify early targets for therapeutic intervention.

We model the acute infection interval by using rhesus macaques inoculated with the chimeric simian–human immunodeficiency virus 89.6PD (SHIV89.6PD). Infection with this pathogenic virus depletes peripheral blood and lymphoid tissue CD4+ T cells by 1 month after inoculation, and these animals manifest high virus burden and declining B-cell numbers (Steger et al., 1998; Lu et al., 1998; Wallace et al., 1999) with correspondingly low levels of neutralizing antibodies (Crawford et al., 1999). Studies of the earliest events after mucosal inoculation, when virus was not yet detected in blood or peripheral lymphoid tissues, showed that T cells were already defective and had lost the lymphoproliferative response to mitogen (Steger et al., 1999; Wallace et al., 1999). The loss of mitogen responses is a marker for rapid progression (Wallace et al., unpublished observations) and reflects the fact that CD4+ T cells from acutely infected...
macaques were driven to apoptosis by phytohemagglutinin stimulation in vitro. An investigation of activation markers on these peripheral blood CD4+ T cells showed increased surface expression of CD25 (interleukin-2 receptor α chain), the early activation marker CD69, and HLA-DR. Together, these observations indicate that inappropriate cell signaling leading to activation-induced cell death may account for the hyporesponsiveness to mitogen in vitro and may contribute to the rapid depletion of CD4+ T cells in vivo. In the studies mentioned above, the patterns of cellular activation were defined mainly with purified cells from peripheral blood (Wallace et al., 1999). Our goals now are to discern the anatomical locations where lymphocyte activation is prominent, to show whether acute infection is associated with a defined pattern of tissue responses, and to compare these patterns for several routes of infection.

The use of whole body positron emission tomography (PET) imaging with radioactive 2-[18F]fluorodeoxyglucose (FDG) has been instrumental in identifying tumors or other foci of increased metabolic activity (Moon et al., 1998). In HIV-positive persons, PET scanning has helped to discriminate between central nervous system lymphoma and inflammatory toxoplasmosis lesions in the brain (Hoffman et al., 1993; O’Doherty et al., 1997). PET studies initiated to identify tumors showed that both benign lymph nodes (LNs) undergoing inflammation and granulomatous sites in persons with various diseases of infectious or other origin often have elevated FDG uptake, as reviewed by Shreve et al. (1999) and Coleman et al. (1999).

We reported previously that whole body PET of SIV-infected macaques injected with the FDG radiotracer highlighted activated lymphoid tissues and produced a map of the host responses to persistent virus infection during middle- and late-stage disease (Scharko et al., 1996). Here, we use PET scanning to look at lymphoid tissue activation during the interval of SHIV89.6PD acute infection of rhesus macaques. We are cautious that the rapid nature of SHIV89.6PD infection in rhesus macaques may not directly parallel the time course of pathogenic events of HIV infection in humans. However, we have established that virological and immunological changes during acute infection are determinative for disease progression rates (Steiger et al., 1998, 1999; Wallace et al., 1999), and we hypothesize that a similar relationship holds for HIV-1 infection in humans. Based on our imaging experiments, during acute infection, we noted remarkably similar patterns for LN activation after three different routes of SHIV89.6PD inoculation. Further, we observed that LN activation precedes fulminant virus replication in a key set of LNs in the upper body. These data show that lymphoid tissue activation precedes wide-scale virus dissemination or acute viremia, and tissue activation may be a key step in SHIV pathogenesis.

RESULTS

Six rhesus macaques were infected with SHIV89.6PD and used to study early host–virus interactions. Macaques 94074 and 94089 were inoculated intravenously (IV), macaques 94079 and 94077 were inoculated intrarectally (IR), and macaques 94069 and 92071 were inoculated intravaginally (IVAG). Animals were subjected to PET imaging to observe lymphoid tissue activation that occurred during the first few weeks after inoculation. Macaque 94074 was PET scanned on day 3 after inoculation and was euthanized on day 4. Macaque 94089 was scanned on days 3 and 7 after inoculation and was euthanized on day 8. Macaques 94079 and 94069 were scanned on day 7 after inoculation and were euthanized on day 8. Macaques 94077 and 92071 were scanned on days 7 and 14 after inoculation and were euthanized on day 15.

Euthanasia and necropsy were performed on the morning after the last PET scan, and lymphoid tissues were collected. In a previous publication (Wallace et al., 1999), we reported CD4+ and CD8+ T-cell subset data, a summary table of SHIV RNA in situ hybridization results, and mitogen responses for these samples. For the in situ hybridization, if no positive cells were detected in five high-powered fields, then the tissue samples were considered negative for SHIV RNA.

IV inoculated macaques

Macaque 94074 was euthanized 4 days after IV inoculation; vRNA was not detected with in situ hybridization in axillary, cervical, or inguinal LNs. The range of lymphocytes from axillary, cervical, inguinal, ileocecal, mesenteric, or iliac LNs that were CD4+ T cells was 36–54% with a mean of 47% (Wallace et al., 1999). These values are comparable to the percentage of CD4+ T cells present in LNs of healthy, uninfected macaques (Sopper et al., 1997; Schenkel et al., 1999). The pattern of FDG uptake on day 3 was visualized by PET imaging in macaque 94074 and was similar to that of an uninfected macaque, with greater areas of activity in the brain, heart, and bladder (normal distribution). A weaker thymus signal was also visible as a triangular shape above and to the right of the heart (Fig. 1A). FDG uptake in all of these organs is observed commonly in uninfected rhesus macaques, and FDG in the bladder was due to normal excretion and accumulation of the radiotracer despite urinary catheterization (Scharko et al., 1996). We used the day 3 time point because the kinetics for IV infection are more rapid than for mucosal inoculation. We did not observe substantial lymphoid tissue activation in this animal by day 3, possibly because the time point was too soon after inoculation.

Macaque 94089 was euthanized eight days after IV infection; vRNA was detected in axillary, ileocecal, mesenteric, and iliac LNs and spleen, as well as in the
intestine and thymus. By day 8, this animal lost 79% of circulating CD4\(^+\) T cells; blood and tissue lymphocytes were not responsive to mitogen stimulation (Wallace et al., 1999). Macaque 94079 was PET scanned on day 7 after IR inoculation and showed FDG uptake in the cervical and axillary LNs, in addition to lower-intensity FDG uptake in the hilar and mediastinum LN areas (Fig. 1D). These results suggest that one of the earliest markers associated with acute infection, the loss of T lymphocyte responsiveness to mitogen, is temporally correlated with upper body LN activation that was observed with PET imaging. Our observations parallel the finding of increased activation marker expression on peripheral blood CD4\(^+\) T cells from SHIV\(_{89.6PD}^{-}\) inoculated macaques during acute infection (Wallace et al., 1999). Tissue histopathology showed that the axillary, ileocecal, and inguinal LNs were well populated with lymphocytes and that the cortex regions contained active follicles with germinal centers (Figs. 2A–2C). These observations are consistent with normal LN architecture (Chan and Tsang, 1996). However, the increased FDG uptake in axillary and cervical LNs of macaque 94079 at 7 days after infection (Fig. 1D) may be an early indicator of incipient follicular hyperplasia, the first stage of immunodeficiency virus-associated lymphadenopathy (Racz et al., 1986).

Macaque 94077, which was euthanized on day 15 after IR infection, was scanned on both days 7 and 14. On day 7, macaque 94077 had high FDG uptake in cervical, axillary, and mediastinum LNs (Fig. 1E). Five distinct foci of FDG uptake appeared in the abdominal cavity and may represent active gut associated lymphoid tissues or abdominal LNs but had much lower signal intensity than the upper body LN sites. On day 14, when macaque 94077 was scanned a second time, FDG uptake in the cervical and axillary LNs was decreased compared with day 7, although these signals were still clearly visible (Fig. 1F). By 15 days after infection, explosive follicular hyperplasia of LNs (Racz et al., 1986) was apparent with numerous and sometimes irregularly shaped secondary follicles. Many of the follicles had mantle zone alterations, some of which aberrantly protruded into the germinal centers (Figs. 2E–2G). In situ hybridization for vRNA using day-15 tissue samples from macaque 94077 demonstrated widespread dissemination of virus. For example, the axillary and inguinal LNs from macaque 94077 showed dispersed and distinct hybridization signals throughout the paracortical region and cortex that were consistent with infection of individual cells (Figs. 2E and 2G). In situ hybridization of ileocecal LNs showed regions of intense staining inside some of the germinal centers (Fig. 2F). The distribution of viral RNA in the thymus of macaque 94077 was restricted to the medulla, with a noticeable absence of infection in the cortex (Fig. 2H). The range of LN lymphocytes that were CD4\(^+\) T cells...
was 9–15% with a mean of 12% (below normal levels); lymphocytes isolated from LNs of macaque 94077 were not responsive to mitogen, and this macaque had lost 96% of circulating CD4$^+$ T cells by day 15 after infection (Wallace et al., 1999).

IVAG inoculated macaques

Macaque 94069, which was euthanized 8 days after IVAG infection, was scanned on day 7 and looked very similar to the day-7 IR infected macaques in showing significantly elevated FDG uptake in the cervical and axillary LNs, along with distinct signals for mediastinum and hilar LN (Fig. 1G). vRNA was not detected in axillary, cervical, ileocecal, or inguinal LNs but was present in mesenteric LN and cecum. The range of CD4$^+$ LN lymphocytes was 39–60% with a mean of 47%, which is similar to healthy, uninfected macaques. However, this macaque had lost 39% of circulating CD4$^+$ T cells by day 8 after infection due to a sharp drop in peripheral blood lymphocyte count. Lymphocytes from macaque 94069 at day 8 were not responsive to mitogen (Wallace et al., 1999). These findings show again that LN activation measured by PET–FDG is associated with lymphocyte nonresponsiveness to mitogenic stimulation measured in vitro. In macaque 94069 at day 8 after IVAG inoculation, all regions of axillary, inguinal, cervical, and mesenteric LNs were appropriately populated with lymphocytes and contained follicles with prominent germinal centers but were not dramatically hyperplastic (data not shown). Together, these observations again suggest that activation of LNs as visualized by PET–FDG may be predictive for the ensuing follicular hyperplasia and abundant virus replication.

Macaque 92071, which was euthanized 15 days after IVAG inoculation, was imaged on days 7 and 14. Similar to the other day-7 mucosally infected macaques, macaque 92071 showed intense signals in the cervical and axillary LNs along with activity in mediastinum and hilar LN areas (Fig. 1H). At day 14, macaque 92071 still had very strong cervical and axillary LN signals (Fig. 1). In situ hybridization for macaque 92071 demonstrated widespread dissemination of virus in all tissues examined 15 days after infection. The range of CD4$^+$ LN lymphocytes was 2–5% with a mean of 4%. The remaining LN lymphocytes from macaque 92071 were not responsive to mitogen (Wallace et al., 1999). This macaque had lost 94% of circulating CD4$^+$ T cells by day 15 after infection. The intensity of PET–FDG signals in cervical and axillary LNs despite the near-complete depletion of CD4$^+$ T cells suggests either that a small number of cells can give rise to intense signals or that cells other than CD4$^+$ T cells also take up FDG in these activated LNs. In a previous study, we reported that a majority of FDG uptake in activated LN mononuclear cells occurred in B-lymphocytes (Scharko et al., 1996).

DISCUSSION

Before our studies of the macaque model for AIDS (Scharko et al., 1996), whole body PET scanning has not been used to study lymphoid tissue activation in response to viral infection. Previously, we had used PET imaging in a well-established SIV infection model to evaluate lymphoid tissue activation patterns during persistent infection (Scharko et al., 1996). Here we used the same imaging technology to elucidate patterns of tissue activation in our acute infection model that uses SHIV in macaques.

We had used whole body PET imaging to study lymphoid tissue activation in SIV-infected rhesus macaques, based on the idea that lymphocyte activation in tissues was both a normal response to virus and necessary to support productive virus replication (Scharko et al., 1996). PET images of SIV-infected macaques showed disease-associated lymphoid tissue activation that was not
PET imaging has been used successfully to localize tumors and foci of bacterial or fungal infection in HIV-positive persons (O’Doherty et al., 1997). To date, there are no published studies of PET imaging in acutely HIV-infected persons; however, two cases studied in our center indicated a very similar pattern of FDG uptake in axillary and cervical LNs (Pauza et al., unpublished observations). Our macaque studies show that upper body LNs were critically involved in acute infection, regardless of the route for inoculation. Localized activation of lymphoid tissues was correlated with in vitro susceptibility to activation-induced cell death, and tissue activation preceded fulminant virus replication. These phenomena suggest that the initial wave of lymphoid tissue activation increases host susceptibility to virus and promotes dissemination to all peripheral lymphoid tissues. Activation was most rapid after IV infection, suggesting that access to the blood compartment is a critical step in acute pathogenesis.

The identification of specific LNs and their roles in acute SHIV pathogenesis raises important questions about the unique properties of individual sites within the secondary lymphoid tissues. Although it is common to interpret LN function only in terms of “draining” one anatomical region and to assume that all LNs are functionally equivalent, there are important examples of locally unique properties. Transgenic mice deficient for expression of the CXCR5 (BLR1) chemokine receptor lacked inguinal LNs and had few intact Peyer’s patch structures, despite the normal appearance of many other secondary lymphoid tissues, including axillary, mesenteric, and popliteal LNs (Förster et al., 1996). Diseases of the lymphoid system are also often localized, with the important examples of Hodgkin’s disease, which frequently presents as enlarged upper body LNs, or Burkitt’s lymphoma, which arises more commonly in the abdomen. These examples and the PET–FDG data presented here suggest that the early patterns and kinetics of lymphoid tissue involvement are crucial to acute pathogenesis. The mechanisms for activation of specific tissues, possibly involving regulation of unique chemokines and/or their receptors, should constitute new targets for therapeutic intervention during acute HIV infection.
MATERIALS AND METHODS

Animals and viral infections

Six captive bred juvenile rhesus macaques (Macaca mulatta) were housed at the Wisconsin Regional Primate Research Center (WRPRC) and used in these studies. The WRPRC is accredited by the American Academy of Laboratory Animal Care. All animal research protocols were approved by the Institutional Animal Care and Use Committee. Macaques were immobilized with ketamine hydrochloride (10 mg/kg body wt) before all virus inoculations and blood collections. General Medical Laboratories (Madison, WI) performed automated complete blood cell counts (CBCs) on all samples. Two macaques were infected IV in the saphenous vein with 25 tissue culture infectious doses (TCID) SHIV89.6PD (provided by Dr. Yichen Lu, Virus Research Institute, Boston, MA), two macaques were infected IR with 2500 TCID, and two macaques were infected IVAG with 25,000 TCID.

PET imaging

PET imaging of SHIV89.6PD-infected rhesus macaques was performed as described previously (Scharko et al., 1996). IV infected animals were imaged on day 3 or 7 after infection, and mucosally infected animals were imaged on day 7 or 14 after infection.

Tissue collection and processing

To investigate virus dissemination, lymphocyte subset distribution, and immune function of blood and tissue lymphocytes during acute infection, animals were sacrificed within 15 days after inoculation. The IV infected animals were euthanized on day 4 or 8 after infection. One animal from each of the mucosally infected groups was euthanized on day 8 or 15 after infection. After the administration of ketamine hydrochloride, blood was drawn and the animals were euthanized by the IV administration of 0.1 ml/kg body wt pentobarbital sodium and phenytoin sodium (Beuthanasia; Schering-Plough Animal Health, Kenilworth, NJ). At necropsy, LNs, spleen, thymus, ileum, cecum, and rectum were harvested, among other tissues. Paraffin-embedded tissue blocks used for histopathology and in situ hybridization were prepared from portions of these tissues.

Localization of viral RNA by in situ hybridization

In situ hybridization for vRNA used a pool of digoxigenin-labeled RNA probes generated by Sp6 or T7 polymerase transcription from the entire genome of both SIVmac239 and HIV-1,pho. The detailed method was described previously (Hirsch et al., 1995; Wallace et al., 1999).

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