Virology 446 (2013) 77–85

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Kinetics of liver macrophages (Kupffer cells) in SIV-infected macaques

Muhammad H. Ahsan, Amy F. Gill, Xavier Alvarez, Andrew A. Lackner, Ronald S. Veazey*

Tulane National Primate Research Center, Tulane University School of Medicine, 18703 Three Rivers Road, Covington, LA 70433, USA

ARTICLE INFO

Article history:
Received 1 March 2013
Returned to author for revisions
3 April 2013
Accepted 22 July 2013
Available online 19 August 2013

Keywords:
Liver
SIV
HIV
T cell
Immunology
Mucosal Immunology

ABSTRACT

Since the liver drains antigens from the intestinal tract, and since the intestinal tract is a major site of viral replication, we examined the dynamics of liver macrophages (Kupffer cells) throughout SIV infection. Absolute numbers of Kupffer cells increased in the livers in acute infection, and in animals with AIDS. Significantly higher percentages of proliferating (BrdU+) Kupffer cells were detected in acute infection and in AIDS with similar trends in blood monocytes. Significantly higher percentages of apoptotic (AC3+) Kupffer cells were also found in acute and AIDS stages. However, productively infected cells were not detected in liver of 41/42 animals examined, despite abundant infected cells in gut and lymph nodes of all animals. Increased rates of Kupffer cell proliferation resulting in an increase in Kupffer cells without productive infection indicate SIV infection affects Kupffer cells, but the liver does not appear to be a major site of productive viral replication.

Introduction

Regardless of the route of transmission, the mucosal immune system in general, and the gastrointestinal system in particular, are central to the pathogenesis of HIV infection (Lackner et al., 2009). Several critical events in SIV/HIV pathogenesis including viral amplification, and CD4+ T cell destruction occur in the intestinal tract (Brenchley et al., 2004; Lackner et al., 2009; Mehandru et al., 2004; Veazey et al., 1998). Since the liver is the major draining organ for substances passing through the gut, it has a unique immunologic environment. The liver contains one of the largest populations of tissue macrophages (Crofton et al., 1978; Schmitt et al., 1990) and the specific distribution of liver macrophages (Kupffer cells) within the hepatic sinusoids allows them to be in close contact with circulating cells from blood. Housset et al., suggested infection of Kupffer cells (KC) may occur during primary HIV viremia (Housset et al., 1990a). Primary cultures of human KC have also been shown to be permissive for HIV infection (Schmitt et al., 1990). Moreover, HIV antigens or RNA have been detected in liver cells of HIV-infected individuals, but the percentage of virus positive cases, as well as the type and the number of virus-containing cells reported varies substantially (Cao et al., 1992; Hoda et al., 1991; Housset et al., 1990b, 1993). Further, studies by Hufert et al., (1993) suggested that KC isolated from livers of AIDS patients were only latently infected, and did not produce virus (Hufert et al., 1993). Thus whether infection of liver macrophages/Kupffer cells (KCs) occurs in vivo remains controversial (Hufert et al., 1993). Further, there is basically no information regarding the dynamics of lentivirus persistence or replication in the liver in HIV infection.

Lentiviruses can infect and replicate in non-dividing cells of monocyte/macrophage lineage (Verani et al., 2005). Macrophages contribute to innate immune responses to pathogens and are at the interface between innate and adaptive immunity. Thus, they play a central role in control of infection, either by secreting cytokines, directly destroying virus or infected cells, and/or activating either innate or adaptive immune responses. However, direct infection of macrophages with intracellular pathogens including HIV may impair their function and alter cytokine production, resulting in chronic inflammation and tissue damage. Unlike T cells, HIV infected macrophages appear resistant to the cytopathic effects of the virus, and the capacity of monocytes and macrophages to migrate to, and survive within tissues for years makes them potential major reservoirs for HIV-1 persistence (Herbein et al., 2002). Further, their role as APC and/or a source of chemoattractant cytokines for CD4 T cells may favor continual intercellular virus transmission. Therefore, monocytes/macrophages may play a dual role in HIV infection, contributing to both antiviral defenses and serving as targets for infection and persistence (Herbein et al., 2002).

The aim of this study was to examine and compare the kinetics of changes in absolute numbers of Kupffer cells in liver for comparison with monocytes in peripheral blood throughout the course of SIV infection. We also quantified the percentages of proliferating (BrdU+) and apoptotic (AC3+) Kupffer cells in liver by flow cytometry and performed in situ hybridization to quantify SIV-infected Kupffer cells throughout all stages of infection. Determining the kinetics of SIV infection and turnover of Kupffer cells in vivo may provide important information on the role of the liver in the pathogenesis of HIV infection.

* Correspondence to: Tulane National Primate Research Center, Division of Comparative Pathology, 18703 Three Rivers Road, Covington, LA 70433, USA. Fax: 1 985 871 6510.
E-mail address: rveazey@tulane.edu (R.S. Veazey).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.07.026

Results

Kupffer cells increase in the liver during SIV infection

Liver sections from all 42 infected and 8 uninfected macaques were stained with mouse anti-human Ham56 antibody, which labels tissue macrophages. Positive cells were manually counted and quantified per mm^2 of liver sections in a blinded fashion in 10 random fields for each section. Absolute numbers of Kupffer cells markedly and significantly increased in acutely infected macaques and in those with AIDS, with mean values of 471 ± 41.6 (13 DPI), 365 ± 102.7 (21 DPI) and 397 ± 47.2 (AIDS) vs 217 ± 45.0 (controls) Ham56+ cells/mm^2 liver ($p \leq 0.002$, 0.03 and 0.01 respectively) (Fig. 1B). Although there was a positive trend, numbers of Kupffer cells in the liver at 8 DPI were not significantly increased (262 ± 33.4). Interestingly the chronic, asymptomatic SIV-infected animals also did not show significant increases over controls, with means of 255 ± 24.9 in asymptomatic vs 217 ± 45.0 Ham56+ cells/mm^2 in controls (Fig. 1B). There were also no significant changes in percentages of CD68+ cells co-expressing CD163 or CD14 in liver and blood in response to infection (Fig. 1C–F).

Changes in CD68+ Kupffer cells in liver and CD68+ monocytes in peripheral blood

By flow cytometry, absolute numbers of CD68+ macrophages significantly increased in acutely infected macaques with a mean value of 26 (Control) vs 59 (acute) cells/mm^2 of liver tissue ($p \leq 0.0006$). Absolute numbers of CD68+ macrophages in the AIDS group also increased from 26 (control) to 40 (AIDS) cells/mm^2 of liver

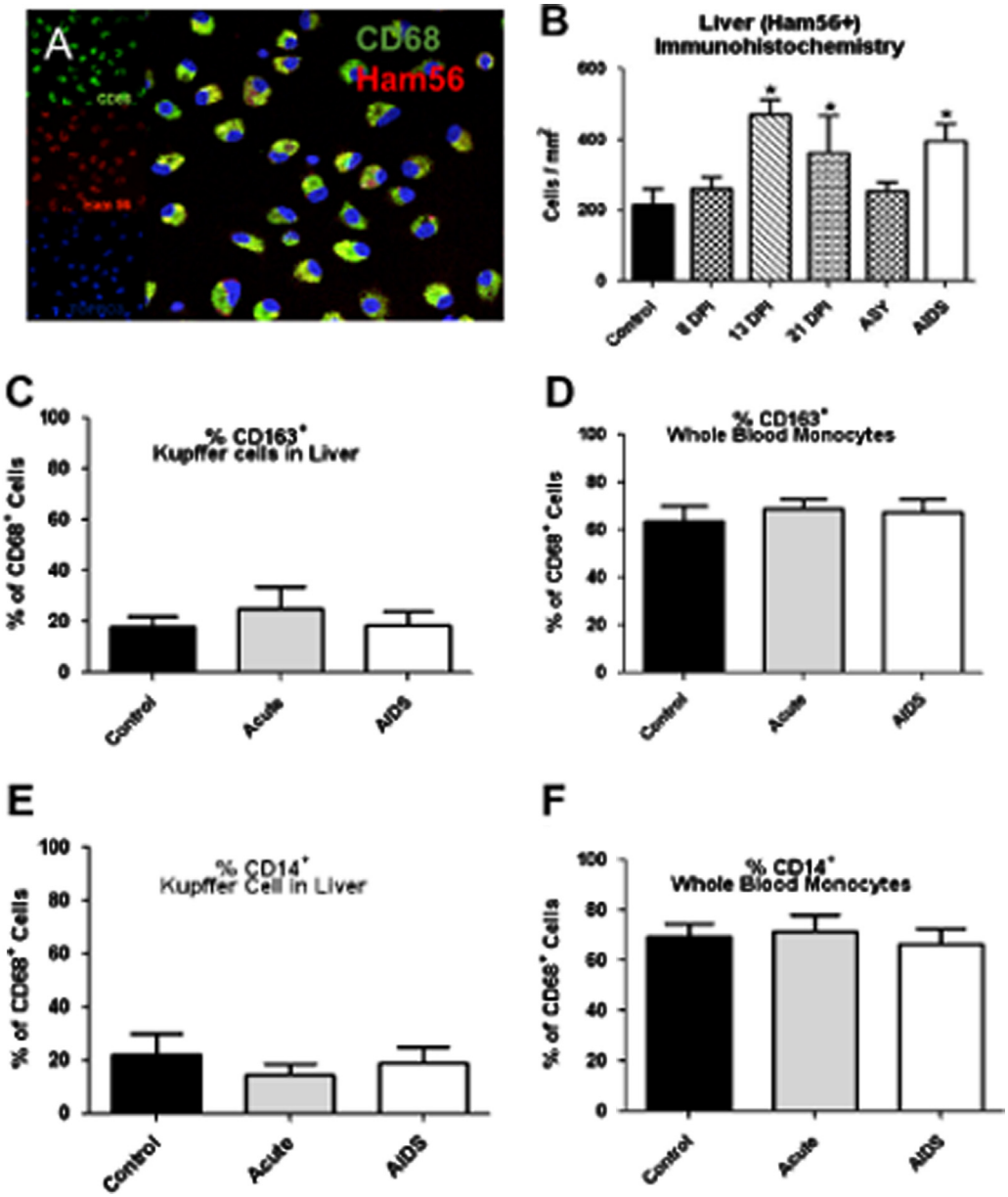

Fig. 1. (A): Triple-label confocal microscopy of liver macrophages (Kupffer cells) in vitro showing co-localization of CD68 (green) and Ham56 (red), indicating Kupffer cells co-express CD68 & Ham56 (CD68 with Alexa 488, green; Ham56 with Alexa 568, red; and cell nuclei with Topro3). (B): Absolute numbers of Ham56+ Kupffer cells per/mm^2 of liver in uninfected (control) and various stages of SIV infection as determined by immunohistochemistry for Ham56. Note significant increases in Ham56+ Kupffer cells per mm^2 are detected after early SIV infection and in macaques with AIDS. *Indicates significant differences from controls ($P < 0.05$). C–F: Percentages of CD68+ cells in the liver (C and E) and blood (D and F) co-expressing CD163 (C and D) or CD14 (E and F) in acute and chronic infection compared to controls. No significant differences in CD14 or CD163 expression were detected on CD68+ cells in liver or blood due to SIV infection (C–F).