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A significant productive *in vivo* infection of resting cells with simian immunodeficiency virus in a macaque with AIDS

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Abstract

Identifying the cells that can be infected with HIV *in vivo*, and thus potentially persist as latent reservoirs is of high priority. Here, we report the major infected cells in a chronically-SIV infected macaque that developed AIDS and encephalitis. Majority of the infected cells were detected as non-proliferating resting cells. SIV infected non-proliferating resting cells were found to be playing an important role in viral pathogenesis, persistence or reservoir formation.

Keywords

Infection; Macrophage; Reservoir; Resting cells; SIV; SIV-RNA; T-cells

CASE REPORT

Eradication of human immunodeficiency virus (HIV) in infected patients remains a major obstacle due to the establishment of a pool of either long-lived, productively-infected cells, or latently infected cells that harbor proviral DNA. It is currently believed that we may be able to cure HIV-1 infection if viral reservoirs are eliminated. The simian immunodeficiency virus (SIV) infected rhesus macaque (RM) model is a well-accepted model of AIDS and has been extremely useful in exploring viral reservoir where SIV infection causes escalating immune dysfunction by rapid and persistent depletion of both central and effector memory CD4+ T-cells, and perpetual immune activation and inflammation [1–7]. Here, we report an interesting case where SIV infected cells were in majority are nonproliferating cells that include T-cells, macrophages, and dendritic cells. These nonproliferating SIV+ cells may play a key role in the formation of viral persistence or establishment of reservoirs.

An 11.8-year-old, male Chinese rhesus macaque (RM, *Macaca mulatta*) was intravenously inoculated with 1 ml of plasma (194,981 viral RNA copies) from a $SIV_{MAC}239$ infected macaque. The animal was euthanized on day 316 after inoculation due to the end of the study. The macaque maintained a high plasma viral load (log_{10} 6.6 RNA copies/ml of plasma using bDNA assay from Siemens with detection limit of 125 copies/ml of plasma)

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Declaration of conflict interests

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and low peripheral blood CD4 counts (below 200 cells/μl of blood) at the end of the study. Fresh and formalin-fixed tissues were collected at necropsy. All experiments were approved by the Tulane Institutional Animal Care and Use Committee.

Grossly and microscopically, this macaque had typical SIV lesions, like emaciation, lymphoid depletion in peripheral lymph nodes (LN), lymphoid follicular hyperplasia, lymphoplasmic and multinucleated giant cell enterocolitis. Consistent with neurological symptoms, this animal also had SIV giant cell meningoencephalitis characterized by lymphoplasmacytic perivascular cuffing mixed with many foamy histiocytes and multinucleate giant cells. Cytomegaly, karyomegaly and occasionally intranuclear inclusion bodies were noted in multiple organs that indicated this animal had opportunistic CMV infection due to SIV induced immunodeficiency. It is one of the most common opportunistic viral infections in both SIV infected macaques and HIV infected patients [8].

Quantification of SIV RNA positive cells was performed by *in situ* hybridization using antisense SIV riboprobes (Lofstrand Labs, Gaithersburg, MD) comprising essentially the entire SIV genome as described previously [9–13]. Labeled cells were visualized using horseradish alkaline phosphatase-conjugated sheep antidigoxigenin antibodies. For objective quantification of the number of infected cells/mm^2 of tissue, computer assisted image analysis was used. Briefly, the number of infected cells/mm^2 was determined by using a Leica DMLB microscope with SPOT insight digital camera (Digital Instrument Inc, Sterling Heights, MI) interfaced to Image-Pro Plus (Media Cybernetics, Inc.) image analysis software. *In situ* hybridization demonstrated that midbrain had the highest number of SIV-RNA+ cells, followed by ileum, jejunum, inguinal LN, mesenteric LN, axillary LN, spleen and colon (Supplemental Fig. 1A). SIV RNA+ cells diffusely distributed throughout these organs (Supplemental Figs. 1B–E). However, SIV-RNA+ cells were undetectable in bone marrow and thymus suggesting that these organs play a minor role in viral replication or as a source of reservoir.

To further phenotype these SIV infected cells, tissues were processed for *in situ* hybridization for SIV mRNA and immunohistochemistry for CD3 (Rabbit anti-human polyclonal, Dako), Ham56 (clone Ham56, IgM kappa, Dako), dendritic cell specific ICAM-3 grabbing nonintegrin (DC-SIGN, clone DCN46, IgG2b kappa, BD Biosciences), Mac387 (clone MAC387, IgG1 kappa, Dako), and Ki67 (clone MIB-1, IgG1 kappa, Dako) by multilabel confocal microscopy. In brief, formalin-fixed, paraffin-embedded tissue sections were stained first with anti-sense SIV riboprobes. SIV mRNA positive cells were detected by a 2-hydroxy-3-naphthoic acid-2 phenylanilide phosphate (HNPP) fluorescence detection kit (Roche Diagnostic Corporation, USA). Sections were then co-labeled with any one of the unconjugated primary antibodies (CD3, Mac387, Ham56, DC-SIGN and Ki67) and then with secondary antibodies conjugated to either Alexa 488 or Alexa 633 (Life Technologies, USA). Nuclear staining was performed with anti-nuclear ToPro3 antibodies (Life Technologies). After staining, slides were washed, and labeled tissue sections were mounted using Prolong Gold antifade medium (Life Technologies) and imaged using a TCS SP2 confocal laser scanning microscope (Leica, Germany) [14–17]. Negative control slides were incorporated in each experiment either by omitting the primary antibody or using isotype IgG1 and IgG (H+L) controls [14–16]. The midbrain contained multiple multi-

nucleated giant cells (MNGC) infected with SIV with occasional CD3+ T-cells around these MNGC (Supplemental Fig. 2A). In both jejunum and ileum, SIV infected CD3+ T-cells were also detected (Supplemental Fig. 3B–C). Consistent with previous findings [18, 19] SIV infected monocytes/histiocytes (Mac387+) and/or Ham56+ macrophages were also detected in midbrain, jejunum and ileum demonstrating these cells may act as reservoirs for the virus (Fig. 1 and Supplemental Figs. 2D–F). Finally, DC-SIGN (a marker of dendritic cells) positive cells were also found in midbrain, ileum and mesenteric LN tissues (Fig. 1 and Supplemental Figs. 2G–H). A few DC-SIGN+ cells distributed in the lamina propria of the ileum were positive for SIV. Similarly, DC-SIGN+ cells distributed along the barrier of the germinal center and T-cell zone of the follicle were also positive for SIV indicating that dendritic cells may also play an important role as a viral reservoir. The data consistent with previous findings [10, 18, 20] suggest that CD3+, Mac387+, Ham56+, and DC-SIGN+ cells were all contributing to the marked viral infection and replication in these tissues.

Total $Ki67^{pos}$ proliferating cells in various tissues were quantified (Table 1). Interestingly the colon (491 ± 54 cells/mm^2) had the highest number of proliferating cells compared to other tissues examined. However, interestingly, a significant higher numbers of SIV positive cells were nonproliferating, resting ($Ki67^{neg}$) cells in midbrain, mesenteric LN, and jejunum (Fig. 1, Table 1). Representative confocal images of midbrain, ileum, mesenteric LN and jejunum tissues (Fig. 1 and Supplemental Figs. 2I–J) suggested that majority of the SIV infected cells in this particular animal were nonproliferating ($Ki67^{neg}$) cells.

Majority of the SIV infected cells were documented as resting memory CD4+ T-cells during acute stage of SIV infection [21, 22]. The presence of increased number of SIV-infected resting-cells suggested these cells may serve as a potential source of viral reservoirs and replications. It may be possible that these cells were initially infected while they were activated (expressed reservoir), yet later became quiescent or “resting” cells that had persistent virus (latent reservoir). Overall, we presented an interesting case where the nonproliferating resting cells could be the reservoir of SIV or HIV in AIDS patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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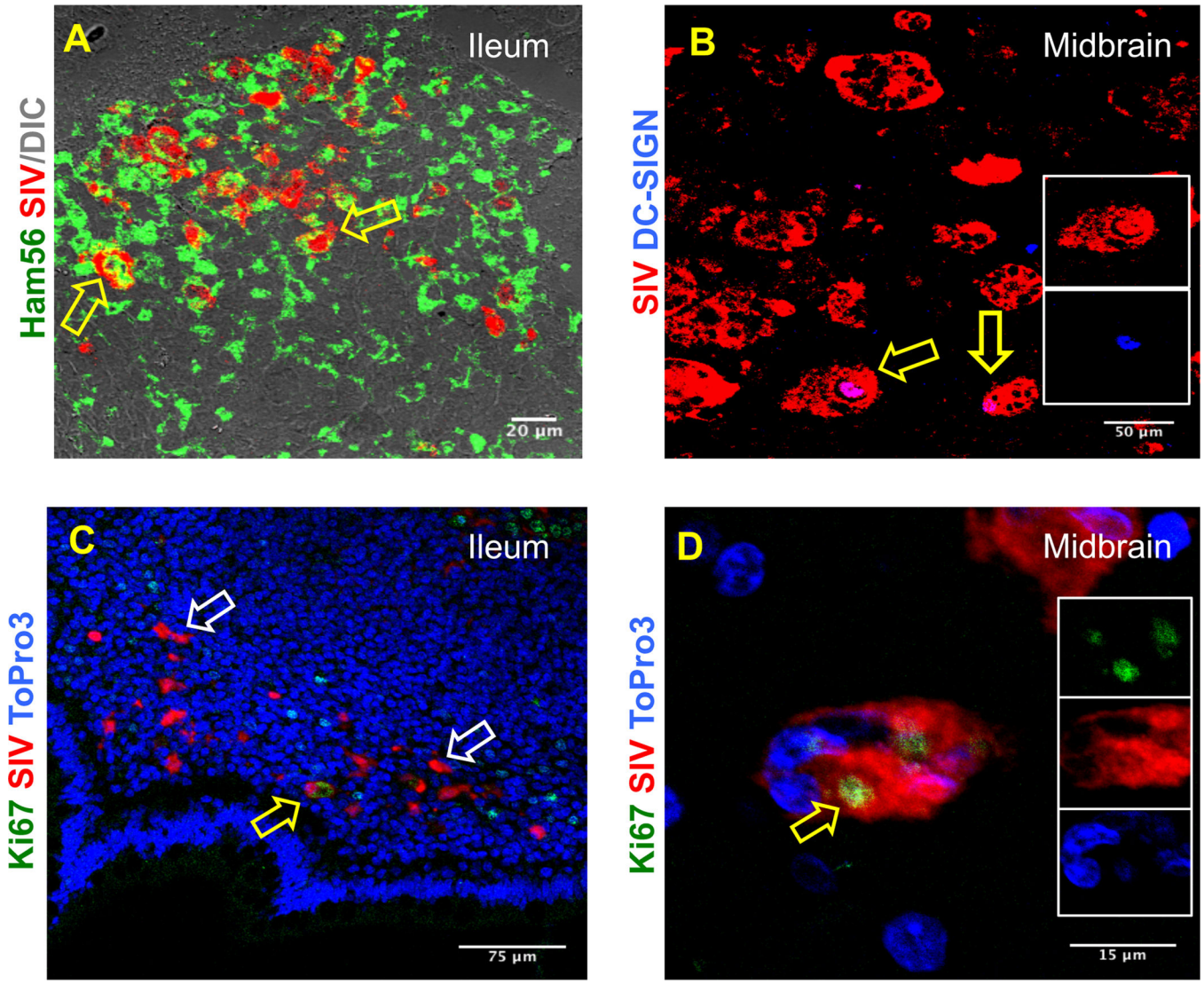

Figure 1.

SIV RNA positive cells were detected by multilabel confocal microscopy in ileum and midbrain. Ham56+ macrophages (**A**) and DC-SIGN positive dendritic cells (**B**) are shown in ileum and midbrain tissues respectively. Colocalization of Ham56 and SIV positive cells were shown in yellow. Distribution of SIV infected cells in proliferating ($Ki67^{pos}$) and resting ($Ki67^{neg}$) cells were shown in ileum (**C**) and midbrain (**D**) tissues by multilabel confocal microscopy. SIV positive cells in resting cells are indicated by white arrows. Yellow arrows represent the double positive cells for the respective markers in each panel. Inserts in figures B and D show DC-SIGN positive and Ki67 positive SIV infected cells respectively.

Table 1

Quantification of total Ki67^{pos}and SIV RNA positive proliferating (Ki67^{pos}) and resting (Ki67^{neg}) cells in different tissues.

Tissue	Ki67^{pos} (mean ± SEM)$^{\#}$	SIV^{pos}Ki67^{pos} (mean ± SEM)$^{\#}$	SIV^{pos}Ki67^{neg} (mean ± SEM)$^{\#}$	Significance between SIV^{pos}Ki67^{pos} and SIV^{pos}Ki67^{neg} cells/mm^2
Midbrain	11 ± 4	5 ± 2	58 ± 8	P<0.0001
Mesenteric LN	265 ± 58	3 ± 2	13 ± 3	P<0.001
Jejunum	191 ± 44	2 ± 1	28 ± 6	P<0.0001
Ileum	459 ± 93	15 ± 4	36 ± 14	NS
Colon	491 ± 54	0.7 ± 0.5	1.4 ± 0.8	NS

$^{\#}$cells/mm^2; Twenty fields (40× magnification) in each slide were counted by two different individuals

SEM: standard error of mean
"pos" and "neg" denote positive and negative respectively
NS: not significant (using two-tailed paired t test, α=0.05)