Immunophenotype of Simian Immunodeficiency Virus-Infected Cells in the Spleen of a Rhesus Monkey

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FIG. 1. SIV RNA⁺ cells in spleen include CD3⁺ T cells, p55⁺ dendritic cells, and CD68⁺ macrophages. SIV RNA⁺ cells (blue) were found in the T cell zone of the spleen with a few vRNA⁺ cells at the edges of follicles (bottom panel). While the majority of SIV RNA-positive cells were CD3⁺ T lymphocytes (red, upper middle), a few p55⁺ dendritic cells (green, upper left) and CD68⁺ macrophage (yellow, upper right) were also SIV RNA positive. *In situ* hybridization (ISH) and immunohistochemistry (IHC) were performed consecutively on the same section of spleen from a chronically SIV-infected rhesus macaque.

GIVEN THE EFFECTIVENESS of available antiviral drugs at blocking HIV replication to undetectable levels, it is reasonable to attempt to eradicate the infection. To design strategies to eliminate HIV, a complete understanding of all the cell types that can serve as reservoirs of HIV infection is needed. Simian immunodeficiency virus (SIV) infection of the rhesus monkey recapitulates the key features of HIV pathogenesis and disease. Since it is possible to rapidly collect and process tissues from infected animals, SIV nucleic acids and proteins are preserved, facilitating detection of infected cells.

Here we used a method that combines a very sensitive *in situ* hybridization (ISH) and immunohistochemistry (IHC) to immunophenotype SIV RNA⁺ cells in the spleen of an SIV-infected monkey. We demonstrate, as previously reported,^{1–3} that T lymphocytes, dendritic cells, and macrophages are target cells of SIV infection. All three types of infected cells were detected in a single section of spleen from a chronically infected rhesus macaque. This result emphasizes that efforts to eradicate HIV must involve eliminating infected dendritic cells, macrophages, and T cells in lymphoid tissues.

Spleen was collected from an SIVmac25-infected monkey at necropsy, then fixed in 4% paraformaldehyde, and sections (4 μ m) were made from a paraffin embedded block. First, SIV RNA was detected by the optimized RNAscope 2.0 High Definition (HD)-BROWN Assay.⁴ Then, anti-CD3, anti-fascin (p55), and anti-CD68 antibodies were used to identify T lymphocytes, dendritic cells, and macrophages, respectively. DAPI was used for nuclei staining. DAB was applied as chromogen of the RNA. Alexa fluor 488, 568, and 647 were used to show p55-positive dendritic cell, CD3-positive T lymphocytes, and CD68-positive macrophages, respectively. An image of the DAB-stained slide was captured under bright light field, and then the image was digitally manipulated so that the DAB appears cyan and the background is black for overlay with other fluorescence images.

Alexa fluor 647 was firstly captured into a black and white image and pseudocolored yellow.

SIV RNA⁺ cells were common in the spleen of this chronically infected animal. While most of the SIV RNA⁺ cells were CD3⁺ T cells, located in the T cell zone of the spleen with fewer in the follicles, SIV RNA⁺ fascin⁺ dendritic cells and SIV RNA⁺ CD68⁺ macrophages were also found in sections of the spleen. In addition, considerable variation in the number of vRNA copies was observed among these infected cells (Fig. 1).

Author Disclosure Statement

No competing financial interests exist.

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