Localization of Productively Infected Cells in the Spleen and Peyer's Patches of Rhesus Macaques During Acute Infection with SIVmac239 Δ nef-Enhanced Green Fluorescent Protein

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FIG. 1. Localization of enhanced green fluorescent protein (EGFP⁺) cells in spleen (**a**, **c**, **d**) and Peyer's patches (**b**). EGFP⁺ cells (green) were present in lymphoid nodules in spleen (**a**, **c**, **d**) and Peyer's patches (**b**) labeled with PNA (**a**, **b**: red) and Ki67 (**c**, **d**: red) in rhesus macaques infected with SIVmac239 Δ nef-EGFP. Blue: cell nuclei.

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IMPROVED KNOWLEDGE of the early targets of productive simian immunodeficiency virus (SIV) infection in the context of tissue architecture could provide important information for our understanding of AIDS pathogenesis and vaccine development. While immunohistochemistry and *in situ* hybridization have been used to detect SIV *in situ*, in this article we describe results using an SIV engineered to express green fluorescent protein (GFP). SIVmac239 Δ nef-enhanced green fluorescent protein (EGFP) has been shown to productively infect cells of the same phenotype as wild-type SIVmac239.¹

As shown in Fig. 1, large numbers of productively infected cells expressing GFP can be seen in spleen and Peyer's patches of rhesus macaques 7 days after infection with SIVmac239 Δ nef-EGFP.

In the spleen and Peyer's patches, most of these productively infected cells were localized to secondary lymphoid follicles. The infected cells were mostly distributed at the edge of germinal centers. As shown in Fig. 1a and b, most of the infected cells (green) were colocalized in the peanut agglutinin (PNA)⁺ area, which is indicative of germinal centers.²

Since most of the cells in germinal centers should be undergoing proliferation or differentiation, cells were labeled for Ki67 antibody to further delineate the germinal centers. As shown in Fig. 1c and d, the infected cells (green) were distributed in an area positive for Ki67 (red), which confirmed the above results. Of note, although rare, a few infected cells expressing Ki67 were observed (arrowhead).

Germinal centers are the areas in which antigen-specific B lymphocytes proliferate, interact with T helper cells (mostly Tfh) and follicular dendritic cells, and finally differentiate into effector cells.² As shown above, during acute infection with SIVmac239 Δ nef-EGFP we find numerous infected cells within germinal centers that could be Tfh cells. Infection and loss of Tfh cells would be expected to have negative impacts on humoral immunity. Infection of Tfh cells might also alter

the function of these cells, which might result in abnormal differentiation due to lack of normal T cell help.

In infections with wild-type SIV, large numbers of infected cells are detected in the interfollicular areas with lesser numbers in organized lymphoid nodules.³ In contrast, with SIVmac239 Δ nef, which is attenuated, infection of lymphoid follicles and particularly germinal centers is more prominent. While SIVmac239 Δ nef-EGFP is attenuated it is likely that the same cell types in germinal centers are early targets for virulent SIV infection of rhesus macaques and HIV-1 infection of humans.

Author Disclosure Statement

No competing financial interests exist.

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