NOTES

Heightened Cytotoxic Responses and Impaired Biogenesis Contribute to Early Pathogenesis in the Oral Mucosa of Simian Immunodeficiency Virus-Infected Rhesus Macaques

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Simian immunodeficiency virus (SIV) infection disseminated into the oropharyngeal tissues of rhesus macaques 6 weeks following intravenous inoculation. Severe local CD4+ T-cell depletion coincided with increases in NK cell and proinflammatory biomarkers and the disruption of growth-associated gene transcription, demonstrating the rapid establishment of pathogenesis in the oral mucosa.

Previous studies of oral mucosal responses to simian immunodeficiency virus (SIV) infection have generally focused on analyses of infections that were initiated in the oral cavity (1, 8, 12). While evidence suggests that the mechanisms of the immune responses and the interaction between lymphocytes and the oral epithelium are impaired as a result of immunodeficiency virus infection (2), the characteristics of oral pathogenesis that result from systemic infection remain largely unknown. We have addressed some of these questions through the comparative analysis of oropharyngeal tissues from three healthy SIV-negative rhesus macaques (animals 30327, 34816, and 34249) and two SIV-infected animals (animals 33655 and 35182) 6 weeks after intravenous inoculation with 100 50% tissue culture infective doses of SIVmac251. Measurement of SIV replication by reverse transcription (RT-PCR) (5) showed that plasma viral loads peaked at about 10^7 to 10^8 RNA copies/ml at 2 weeks postinfection (p.i.) and remained relatively high at 6 weeks p.i. (Fig. 1A). Through comparisons to standardized curves generated from known SIV copy numbers, we determined that the viral loads in oral mucosal tissues were between 10^5 and 10^6 SIV copies/μg of total RNA (Fig. 1B). Immunohistochemical analysis (9, 15) showed SIV p27 expression in both macrophages and CD4+ T cells that were localized to lymphoid areas in the oral mucosa (Fig. 1C). Thus, an actively replicating viral reservoir was established in the oropharynx within 6 weeks of intravenous SIV inoculation.

To evaluate potential alterations in oral mucosal T-cell homeostasis, we determined changes in the levels of CD4+ and CD8+ T-cell subsets in SIV-infected animals compared to those in healthy uninfected controls utilizing the flow cytometry methods described in our previous studies (5, 23). While CD4+ T cells represented 45.4% and 66.6% of the total T-cell population in the oropharynges of healthy controls (animals 34816 and 34249, respectively), their numbers appeared to be greatly reduced in SIV-infected animals (16.1% and 12.4%, respectively) (Fig. 2). As observed in studies of other mucosal compartments, CD4+ T-cell depletion coincided with a proportional increase in the percentage of CD8+ T cells (10, 17, 19, 20). Similar to reports of SIV-infected gut lymphoid tissue (7, 18, 21, 22), we found by flow cytometry increased levels of expression of activation (CD69) and proliferation (Ki67) biomarkers on the oral mucosal CD4+ T cells that had not yet been depleted from the infected animals (data not shown). Collectively, these data suggest that a massive disruption in local T-cell homeostasis is associated with the rapid dissemination of SIV into oropharyngeal tissues.

Mechanisms of host response to SIV in oral mucosa. To increase our understanding of the mechanisms of the host response and elucidate the biomarkers of pathogenesis in the oral mucosa, we compared the gene expression profiles in the oropharynges of healthy control and SIV-infected animals utilizing previously reported methods (5, 6). Briefly, the mean transcription levels in the oropharyngeal tissues of healthy uninfected animals (animals 30327, 34816, and 34249) were compared to the corresponding transcription levels in the SIV-infected animals (animals 33655 and 35182). Genes whose transcription levels were altered by at least 1.5-fold (up or down) in infected animals (P ≤ 0.05 by the unpaired Student t test) were considered for further evaluation (Fig. 3A). Using these criteria, we determined that 280 genes were upmodulated and that 430 were downmodulated in the oropharynx as a result of SIV infection. The pathways and processes that were statistically overrepresented in the filtered gene set were identified (Fig. 3B).

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The levels of transcription of genes associated with innate immunity, inflammation, cell adhesion, and response to wounding were increased in the oropharyngeal tissues of SIV-infected animals (Fig. 3B). The innate response category included gamma interferon and genes associated with interferon induction, antiviral molecules (e.g., Trim5α), and several NK cell biomarkers (Fig. 4). While many genes regulating NK cell and CD8+ T-cell functions overlap (e.g., granzymes and perforin), the added detection of increases in the levels of expression of NKG2-C, NKG7, and KIR3DH suggest that both of these cell types may have contributed to ongoing cytolytic activity in the oropharynges of the SIV-infected animals. These data are consistent with those reported previously (3, 4, 11, 13, 14, 16) and highlight a potentially important role for NK cells in anti-SIV responses within the oral mucosa. Taken together, evidence of increased inflammation, elevated cytolytic activity, and the death of SIV-infected cells also suggested considerable potential for bystander tissue damage, a conclusion supported by the upregulation of genes mediating the response to wounding (Fig. 4).

FIG. 1. SIVmac251 viral loads in plasma were determined at 2, 4, and 6 weeks p.i. (A) and those in the oropharynx were determined at 6 weeks p.i. (B) by RT-PCR. (C) Immunohistochemistry-based detection of SIV p27 in macrophages and T cells within oropharyngeal tissues at 6 weeks p.i.

FIG. 2. Depletion of CD4+ T cells and expansion of CD8+ T cells in the oral mucosa of rhesus macaques 6 weeks following intravenous infection with SIV. SIV+, SIV infected.
Downregulation of genes mediating growth in oropharyngeal tissue. In contrast to the increased levels of expression of genes controlling defense responses and inflammation, genes mediating biogenesis pathways, transcriptional regulation, cytoskeletal organization, tight junction formation, and Wnt pathway signaling were expressed at considerably lower levels in the SIV-infected animals than in the healthy uninfected control animals (Fig. 3B). More than two dozen genes associated with biogenesis were transcriptionally repressed in the SIV-infected animals. These included genes involved in B- and T-cell differentiation (c-Maf-inducing protein, Kruppel-like factor 6), cytoskeletal development (actin, tubulin) epithelial development (decorin, calmodulin-like 5), muscle development (myosins, troponins), and neuronal development (BAIAP2, neurotrimin). A number of genes involved in Wnt signaling were also downmodulated, highlighted by strong (more than fivefold) reductions in the levels of expression of casein kinase 1AI, PPP2R5E, DAAM1, Y-linked transducin, and TCF-4 (Fig. 5).

The cumulative loss of transcription of genes regulating biogenesis may underscore a reduced capacity for oropharyngeal tissue regeneration during SIV infection.
an inability to repair early damage to the oral mucosa could set the stage for continuing deterioration throughout the course of infection and may ultimately contribute to an impaired efficacy of the host response to challenge from secondary opportunistic pathogens. It is important to note, however, that our findings are based on analyses with a small number of animals and should therefore be considered preliminary until larger groups of animals can be evaluated.

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