

Pathogenic Molecular Profile Is Established In Primary SIV Infection In The Oral Mucosa: Effects of Early Intervention with Combinatorial ART

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Abstract

Background:

Progression to AIDS in HIV infected patients is characterized by the emergence of opportunistic secondary infections and immunosuppression that result from a devastating loss of CD4+ helper T cell function. Previous investigations have established that CD4+ T cell depletion is much more rapid and dramatic at mucosal sites where viral replication is high than in peripheral blood. Accordingly, mucosal surfaces with more direct exposure to pathogens from the outside environment, such as those in the lung, nasal, and oral cavities are often plagued by secondary infections. Among these compartments, the oral mucosa is particularly susceptible, being directly exposed to microorganisms from dietary and air-borne sources. Chronic secondary infections in the oral cavity can originate from a variety of fungal, bacterial, viral, and eukaryotic organisms and lead to debilitating complications such as wasting and malignancies. While much research has been rightfully focused on effective treatment strategies to combat simultaneous opportunistic microbial invasions, the molecular signature of host response and mechanisms that correlate with early pathological events in the oral mucosa remain poorly understood. To improve our understanding of the link between oral pathogenesis and manifestation of susceptibility to secondary infection and disease progression, we have investigated changes in T cell homeostasis, morphology, and host gene expression patterns in the oropharyngeal compartment during the course of SIVmac251 infection and ART in rhesus macaques.

Materials and Methods:

Juvenile, colony bred male rhesus macaques from the California National Primate Research Center were intravenously infected with SIVmac251. Animals receiving anti-retroviral therapy began receiving PMPA and FTC within 10 weeks of infection. Oropharyngeal tissue samples were obtained from healthy uninfected controls and SIV infected animals (primary and chronic stage). Oropharyngeal morphology was assessed following H & E staining of 6µm formalin fixed paraffin embedded tissue sections. CD4+ and CD8+ T cell subsets were measured in oropharynx tissue samples by flow cytometry. Fluorescence-based immunohistochemistry was also utilized to directly assess the quantities of CD4+ and CD8+ T cells in oropharynx fresh frozen tissue sections. Host and SIV specific gene expression was monitored by DNA microarray analysis. Briefly, total RNA was extracted from fresh frozen oropharyngeal tissue using the RNeasy lysis and reagents, and mRNA was amplified, labeled with biotin, and hybridized to newly available Affymetrix rhesus macaque specific GeneChips. Statistical analysis of the microarray data was performed utilizing model-based algorithms in dChip. Genes were considered to be differentially expressed when the mRNA level changed 50% or more (1.5-fold) with 95% statistical confidence (p-value < 0.05).

Results and Conclusions:

We found that SIV was actively replicating in the oropharynx in the primary acute stage of infection as well as in chronic stage in ART naïve animals (Figure 3). In the presence of anti-retroviral therapy (ART), viral replication was suppressed in the oral mucosa, coinciding with some repopulation of CD4+ T cells (Figure 2) and decreased expression of cytotoxicity and inflammatory response associated genes (Figure 5). In the absence of ART, genes mediating cytotoxicity, humoral responses, stress and inflammatory responses and innate immunity predominated. Although the induction of innate response factors was reduced somewhat in the presence of ART, anti-microbial response genes remained up regulated. Intriguingly, genes regulating tissue and muscle growth and muscular functions were dramatically down regulated in primary stage infection as well as in chronically infected untreated animals (Figure 8), including Wnt signaling proteins required to maintain epithelial growth and maintenance (Figure 9). Administration of ART appeared to lead to resolution of gene expression patterns toward more normalized levels and an up regulation in growth associated transcription.

We conclude that SIV is rapidly disseminated to mucosal sites, including the oropharynx, early in primary acute infection and that ongoing viral replication and hyper-activated host immune responses also rapidly lead to pathological tissue damage and disruption of function in that compartment. Thus, the level of local viral replication is likely to be the predominant factor in the manifestation of pathogenesis. As we have observed in previous studies of the small intestinal mucosa in our lab, early intervention with ART may provide significant benefits in maintaining the integrity of mucosal structure and function, and thus improve the efficacy of the host's own natural anti-viral processes throughout the course of infection.

Animals Utilized and Clinical Status

Animal	Status	Weeks P.I.	ART	Viral RNA Copies/ml Plasma	CD4+ T Cells in WB	CD8+ T Cells in WB	CD4+CD8+ Ratio
30327	UI	N/A	None	N/A	1425	648	2.2
34249	UI	N/A	None	N/A	695	371	1.87
33655	SIVmac251+	6	None	6.1E+03	1251	1813	0.69
35182	SIVmac251+	7	None	9.6E+03	435	680	0.64
31353	SIVmac251+	30	None	8.5E+05	741	1006	0.74
34540	SIVmac251+	34	20 wks UD	UD	379	308	1.23
34548	SIVmac251+	38	29 wks UD	UD	503	443	1.13

Figure 1. Anatomy of the Oral Cavity and Clinical Status of Animals. A. A general diagram of major regions in the oral cavity including the oropharynx, the mucosal tissue utilized for this study. B. Infection and antiretroviral treatment status, and T cell subpopulations are shown for the animals used in the study. Two primary stage (33655 and 35182) and 2 ART and 3 chronic stage (31353 no ART; 34540 and 34548 +ART) animals were investigated with respect to healthy uninfected controls.

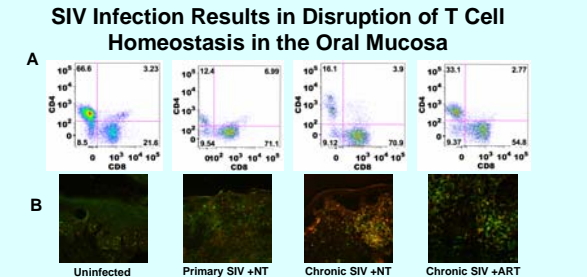


Figure 2. Severe disruption of T cell homeostasis is initiated in primary SIV infection. To determine the extent of disruption in T cell homeostasis in the oral mucosa resulting from SIV infection, CD4+ and CD8+ T cell levels were measured and compared between healthy uninfected controls utilizing flow cytometry (A) and fluorescence-based immunohistochemistry (B). As observed in studies of other mucosal compartments, CD4+ T cells were severely depleted during primary stage and were not replaced in the absence of ART. Although ART led to improvement in peripheral CD4+ T cell populations, 20+ weeks of therapy did not result in complete restoration of T cell homeostasis in the oral mucosa.

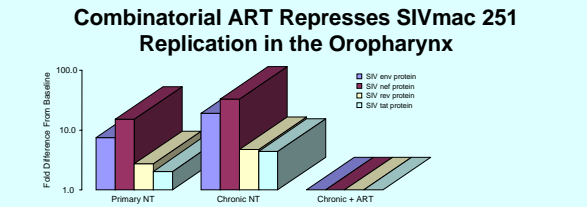


Figure 3. SIV is disseminated and actively replicates in the oral mucosa in primary stage infection. SIVmac251 transcription was detected by DNA microarray analysis utilizing SIV specific probes on the Affymetrix rhesus macaque GeneChip. SIV expression was detected in both primary and chronic stage animals not receiving anti-retroviral therapy (ART), while in contrast, no SIV transcription was detected in animals receiving ART.

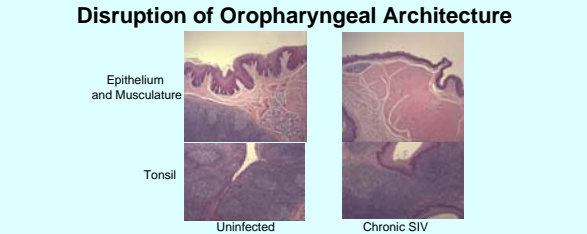


Figure 4. Morphological changes indicate that SIV infection results in severe atrophy of the oral mucosa. Changes in the structure and organization of the oropharyngeal cavity were examined to provide further evidence of SIV induced pathologies. In comparison to the structure observed in healthy uninfected tissue, the gross morphology as well as internal morphology and organization were markedly deteriorated in chronic stage infection, including a loss of B cell germinal centers and transition zones, suggesting impaired mucosal and epithelial function.

Transcriptome-based Analysis of Pathways Regulated in the Oropharynx by SIV Infection and ART

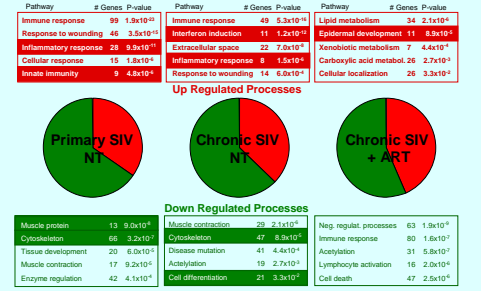


Figure 5. Pathways induced and repressed in the oral mucosa during primary and chronic SIV infection. To determine how changes in T cell homeostasis and disruption of tissue morphology affect overall function of the oropharynx, we evaluated changes in gene expression patterns by microarray analysis. As expected, pathway analysis indicated that immune and inflammatory responses predominated the local microenvironment in animals not receiving ART. In contrast, animals receiving therapy up regulated genes involved in development, metabolism and localization, indicating increased repair and maintenance of the oropharynx was associated with repression of viral replication. In the absence of therapy, a clear pattern of down regulation of genes involved in growth and development and muscle function was also observed, suggesting that chronic immune activation and inflammation may lead to unresolved tissue damage and loss of organ function.

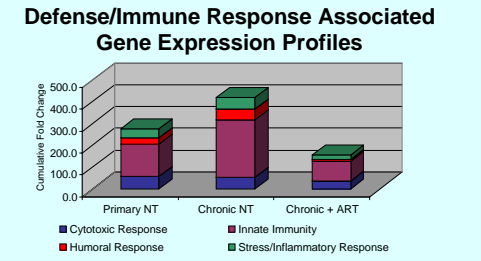


Figure 6. Induction of both innate and adaptive immune responses in the oral mucosa is initiated in primary stage SIV infection. Gene associated with cytotoxic response, stress and inflammation, humoral response, and innate immunity were all up regulated in the oropharynx beginning in primary SIV infection and were further increased in chronic stage. Innate responses remain up regulated in animals receiving ART while cell-mediated, humoral and inflammatory responses are substantially decreased in response to therapy. Fold changes are in comparison to healthy uninfected controls.

Imbalance in Cytotoxic and B-Cell Mediated Responses is Initiated in Primary SIV Infection

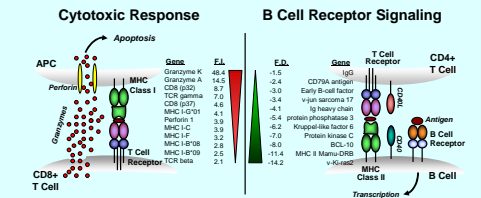


Figure 7. Loss of B cell receptor signaling in the oral mucosa during primary stage SIV infection. In contrast to up regulation of genes involved in cytotoxic response, genes controlling B cell differentiation and function were markedly down regulated in the oral mucosa beginning in primary stage infection. These findings may explain, in part, the loss of development in B cell germinal centers observed in chronically infected untreated animals (Figure 4) Data depicts mean fold changes in primary stage animals in comparison to healthy uninfected controls.

Genes Regulating Growth and Muscle Function are Down Regulated Early in Primary Stage SIV Infection

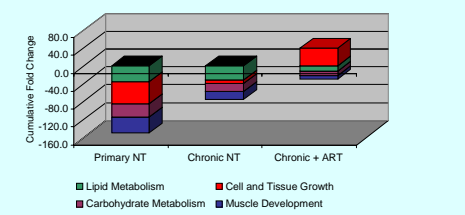


Figure 8. Genes involved in muscle growth and function are down regulated during primary stage infection. Microarray analysis indicated that multiple genes involved in tissue and muscle growth, and muscle function were down regulated beginning in primary SIV infection. Repression of this set of genes continued in chronic stage infection, but was relieved in animals that received ART, including up regulation of growth associated transcription. These data provide evidence that initiation of ART in primary stage improve the ability of the host to repair mucosal tissue damage occurring in initial response to SIV.

Wnt Signaling in Oral Mucosa is Down Regulated in Primary SIV Infection

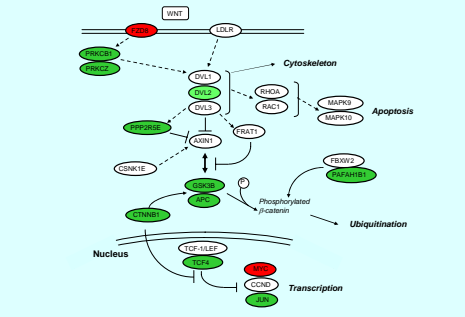


Figure 9. Loss of Wnt signaling within the oral mucosa in primary SIV infection. Changes in expression of genes associated with Wnt signaling that mediates epithelial cell growth and development were determined and used to color-code a pathway diagram. The comprehensive down regulation of genes may suggest that, in the oral mucosa, epithelial repair and maintenance is impaired very early in immunodeficiency virus infection, setting the stage for continued pathogenesis and deterioration of epithelial structure and function as well as susceptibility to secondary infection. Fold changes indicated are in comparison to healthy uninfected controls.

Future Directions

Future studies will focus on monitoring expression of targeted sets of genes identified in the current study by the microarray analysis. With the objective of determining key biomarkers and understanding the molecular mechanisms associated with disease progression versus viral suppression and long-term survival, we will utilize immunohistochemical analysis of specific oropharyngeal cell subtypes, including CD8+ and CD4+ T cells and epithelial cells. Laser capture microdissection (LCM) and magnetic bead-based protocols will be utilized to isolate these specific cell types for downstream analysis. We will compare changes in T cell subset distribution, tissue pathology, and gene expression patterns in long term non-progressing animals (LTNP) to the phenotypes observed in primary and chronic stage infection to determine the molecular correlates of protection. Additionally, we will carefully investigate the relationship between the manifestation of molecular and morphological oral pathologies to the development of susceptibility to secondary infection in SIV infected macaques and in HIV infected humans.