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NOTES

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

Michael D. George,* David Verhoeven, Sumathi Sankaran, Tiffany Glavan, Elizabeth Reay, and Satya Dandekar

Department of Medical Microbiology, School of Medicine, University of California, Davis, California 95616

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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 SIV_{mac251}. Measurement of SIV replication by reverse transcription (RT-PCR) (5) showed that plasma viral loads peaked at about 10^5 to 10^6 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 10^3 and 10^4 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 ($P \leq 0.05$ 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).

* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, University of California, Davis, Room 3318 Tupper Hall, One Shields Ave., Davis, CA 95616. Phone: (530) 752-7207. Fax: (530) 754-7242. E-mail: mdgeorge@ucdavis.edu.

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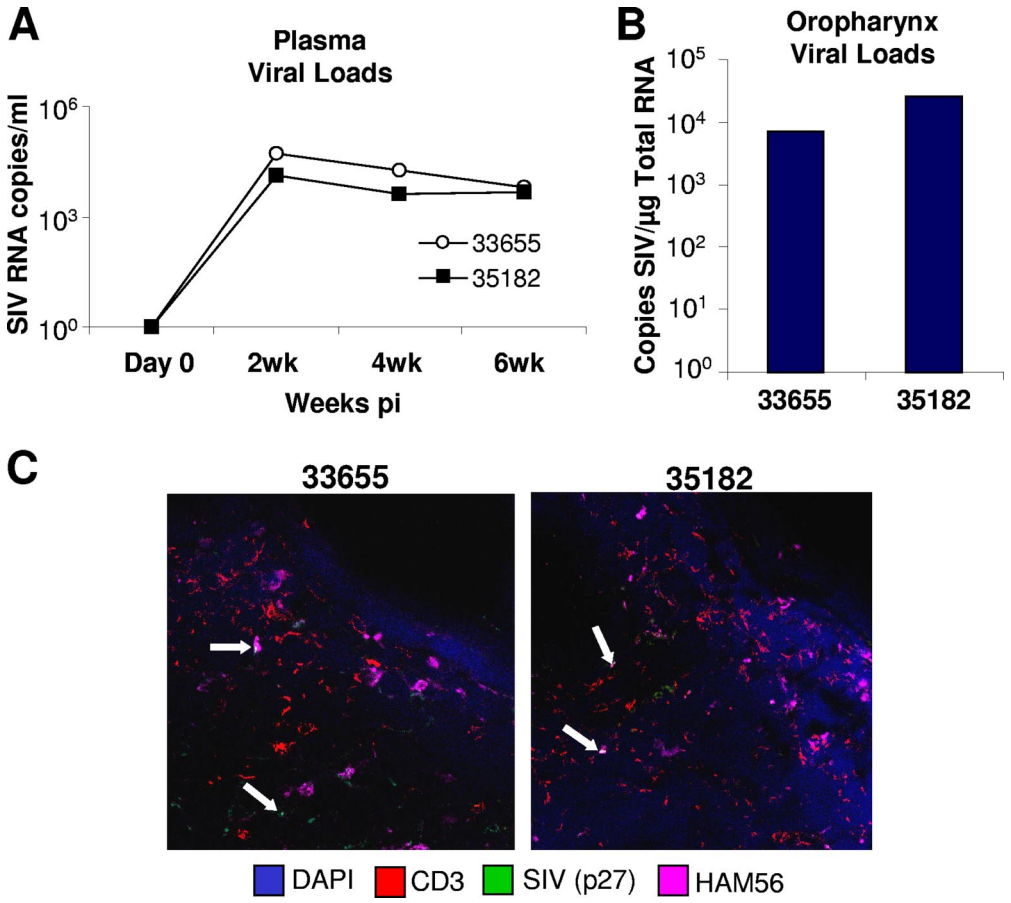

FIG. 1. SIV_{mac251} viral loads in plasma were determined at 2, 4, and 6 weeks p.i. (A) and those in the oropharynx were determined at 6 weeks p.i. (B) by RT-PCR. (C) Immunohistochemistry-based detection of SIV p27 in macrophages and T cells within oropharyngeal tissues at 6 weeks p.i.

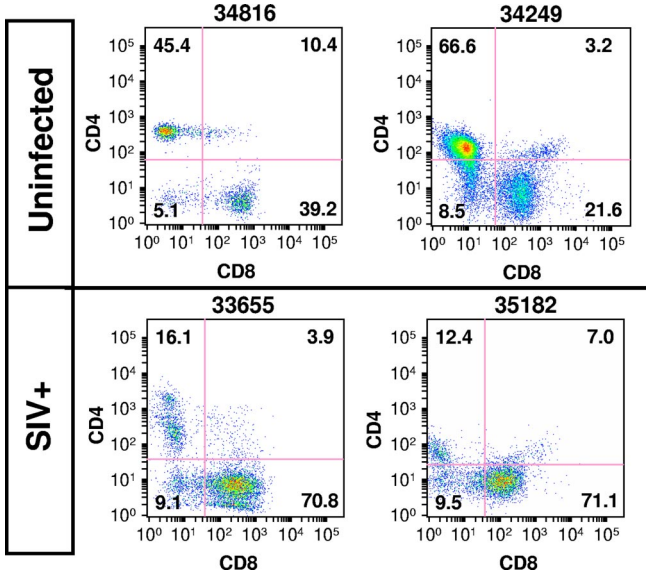

FIG. 2. Depletion of $CD4^+$ T cells and expansion of $CD8^+$ T cells in the oral mucosa of rhesus macaques 6 weeks following intravenous infection with SIV. SIV+, SIV infected.

The complete data files utilized for the microarray analysis are available through the Gene Expression Omnibus database (GEO14351).

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).

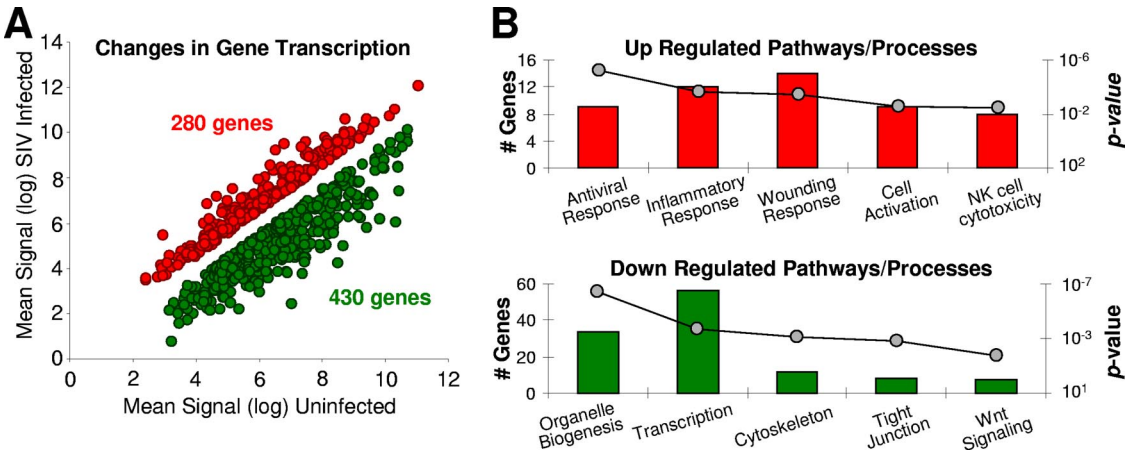

FIG. 3. Microarray analysis of changes in gene transcription in the rhesus oropharynx after 6 weeks of SIV infection. (A) Up- and downregulated genes were identified by comparison of SIV-infected animals ($n = 2$) to healthy uninfected control animals ($n = 3$). (B) Pathways statistically overrepresented in the up- and downregulated gene list.

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 1A1, 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. Logically,

	Gene Title	Mean Fold Increase	*p*-value
Antiviral Response	Trim5 alpha	1.8	0.0268
	CCL5	5.6	0.0032
	SAMHD1	2.0	0.0446
	IFN alpha receptor 2	2.2	0.0215
	lactotransferrin	3.9	0.0525
	leukocystatin	4.7	0.0259
	viperin, cig5	4.5	0.0423
	interferon gamma	2.7	0.0449
	CCL4L	4.3	0.0495
Cell Activation	CD8 alpha (p32)	6.3	0.0364
	CD8 beta (p37)	2.8	0.0083
	CD38 (p45)	3.8	0.0048
	FCER1G	2.6	0.0457
	CRTAM	4.7	0.0256
	von Willebrand factor	1.9	0.0338
	T-box 21	1.5	0.0243
	HLA-DRB3	1.6	0.0345
	STAT2	1.7	0.0143
NK Cell Activity	CD16	3.3	0.0020
	FAS antigen	2.3	0.0181
	FCER1G	2.6	0.0457
	granzyme A	6.4	0.0092
	granzyme K	14.4	0.0107
	HLA-B	2.4	0.0401
	HLA-C	2.1	0.0308
	HLA-G	2.3	0.0228
	IFN alpha receptor 2	2.2	0.0215
	integrin, beta 1	2.3	0.0368
	interferon-gamma	2.7	0.0449
	KIR3DH	2.1	0.0439
	NKG2-C	2.2	0.0272
	NKG7	6.3	0.0096
	perforin 1	2.5	0.0125

	Gene Title	Mean Fold Increase	*p*-value
Inflammatory Response	CCL5	5.6	0.0032
	CCL4L	4.3	0.0495
	CD163 antigen	3.4	0.0497
	FCGR3A (CD16)	3.3	0.0020
	CXCR6	3.1	0.0004
	IL-8R beta	2.3	0.0453
	FCGR1A (CD64)	2.1	0.0318
	CXCR3	1.6	0.0380
	PLA2 activator	1.5	0.0189
Interferon Induction	GBP1	4.2	0.0327
	GBP2	3.3	0.0407
	IFI44	8.1	0.0010
	IFN gamma	2.7	0.0449
	IFIT2	4.4	0.0247
	IFIT3	4.2	0.0536
	IFIT4	3.7	0.0363
	IFITL1	3.0	0.0450
Response to Wounding	FVIII	1.6	0.0395
	FVIII associated	1.7	0.0285
	CCL5	5.0	0.0151
	SERPING1	2.4	0.0215
	NMI	2.3	0.0298
	CCL4L	4.3	0.0495
	CCL5	5.6	0.0032
	MNDA	4.6	0.0222
	granulysin	3.6	0.0432
	CD8 alpha	6.3	0.0364
	IL-8Rbeta	2.3	0.0453
	LILRB2	2.6	0.0479
	HLA-G	2.3	0.0228
	integrin, beta 1	2.3	0.0368
	Sialoadhesin	1.8	0.0077

FIG. 4. Mean fold increases in the transcription of genes associated with upregulated pathways in the oropharynx of SIV-infected animals.

	Gene Title	Mean Fold Decrease	*p*-value
Organelle Biogenesis	ARID1B	-2.1	0.0170
	churchill domain containing 1	-4.0	0.0126
	c-Maf-inducing protein	-2.1	0.0088
	DAAM1	-6.6	0.0167
	Decorin	-2.4	0.0031
	ectodermal-neural cortex	-2.4	0.0344
	Ephrin-A5	-4.0	0.0353
	Exostoses 1	-3.7	0.0089
	GalNAc-T1	-2.8	0.0474
	GNRH	-4.0	0.0052
	HNRPD	-3.2	0.0427
	IRA1	-1.9	0.0404
	Kruppel-like factor 6	-3.9	0.0337
	MLL3	-3.7	0.0253
	NSD3	-6.5	0.0511
	ret finger protein 2	-19.7	0.0097
	SET translocation	-2.4	0.0290
	TIP5	-2.2	0.0064
	Wiskott-Aldrich syndrome-like	-10.4	0.0527
Tight Junctions	ash1	-2.3	0.0168
	TJ protein 4	-2.2	0.0364
	protein kinase C eta	-2.9	0.0245
	K-RAS2A	-6.6	0.0049
	F11 receptor	-3.5	0.0159
	PTEN	-2.6	0.0062
	EPB41	-1.5	0.0094
	desmocollin 2	-10.0	0.0124
	plakophilin 4	-4.3	0.0261
Cytoskeleton	ABI1	-1.6	0.0445
	ArgBP2	-2.5	0.0299
	ACTR2	-2.1	0.0226
	ENC1	-2.4	0.0344
	EPB41	-1.5	0.0094
	myosin IE	-1.5	0.0266
	spectrin B1	-1.7	0.0207
	WASL	-10.4	0.0527

		Gene Title	Mean Fold Decrease	*p*-value
Wnt Signaling		casein kinase 1A1	-3.9	0.0164
		catenin beta 1	-2.2	0.0183
		SFRP1	-2.9	0.0253
		TCF4	-5.3	0.0395
		TBL1X	-3.5	0.0220
		GSK3B	-1.7	0.0275
		TBL1Y	-4.9	0.0175
		DAAM1	-6.6	0.0167
		IRA1	-1.9	0.0404
Transcription	Morphogenesis	YY1 transcription factor	-2.0	0.0369
		zinc finger protein 161	-2.8	0.0404
		itchy homolog E3	-2.2	0.0101
		Kruppel-like factor 6	-3.9	0.0337
		WHSC1L1	-6.5	0.0511
		mortality factor 4 like 1	-3.0	0.0106
		ets homologous factor	-2.1	0.0484
		PBX1	-3.4	0.0316
	DNA Packaging	ash1-like	-2.3	0.0168
		transducin (beta)-like 1XR1	-1.9	0.0404
		MLL3	-3.7	0.0253
		BAZ2A	-2.2	0.0064
		SAFB	-1.7	0.0515
		EMSY protein	-2.3	0.0534
		NR3C1	-8.7	0.0277
		CHD2	-1.6	0.0506
	Initiation	HBXAP	-2.3	0.0464
		THRAP3	-1.8	0.0141
		general transcription factor II	-2.1	0.0265
		TRAP240	-4.1	0.0347

FIG. 5. Mean fold decreases in the transcription of genes associated with downregulated pathways in the oropharynx of SIV-infected animals.

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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