American Journal of Pathology, Vol. 151, No. 1, July 1997

Active Replication of HIV-1 at the Lymphoepithelial Surface of the Tonsil

Sarah S. Frankel,* Klara Tenner-Racz,† Paul Racz,† Bruce M. Wenig,* Christian H. Hansen,‡ Dennis Heffner,* Ann M. Nelson,* Melissa Pope,§ and Ralph M. Steinman§

From the Armed Forces Institute of Pathology and the Division of Retrovirology, Department of HIV Vaccine Development, Walter Reed Army Institute of Research, Rockville, Maryland; the Bernard-Nocht-Institute for Tropical Medicine,† Hamburg, Germany; the Department of Pathology,‡ Georgetown University Medical Center, Washington, DC; and the Laboratory of Cellular Physiology and Immunology,§ Rockefeller University, New York, New York*

Cells that are infected with HIV-1 were visualized at the mucosal surface of the nasopharyngeal and palatine tonsils in 14 specimens from patients with CD4$^+$ T-cell counts of 200 to 900/μl and 2- to 10-year histories of HIV-1 infection. Most of the cells with intracellular HIV-1 protein were small but multinucleated. The majority of these syncytia could be double labeled for HIV-1 RNA and a dendritic cell marker S100. In the palatine tonsil, the infected cells were not found in the stratified squamous epithelium that is adjacent to the pharynx. Instead, the S100$^+$ infected syncytia were localized to the surface of tonsil invaginations or crypts. This mucosa, termed lymphoepithelium, contains antigen-transporting M cells that lie above regions where S100$^+$ dendritic cells are juxtaposed with CD4$^+$ lymphocytes. Likewise, infected cells were found in lymphoepithelium and not respiratory epithelium of nasopharyngeal tonsils or adenoids. We propose that lymphoepithelia, the histological term that describes the specialized regions where antigens access mucosa-associated lymphoid tissue, are sites where HIV-1 replication can be enhanced in syncytia derived from dendritic cells. (Am J Pathol 1997, 151:89–96)

A recent study[1] of nasopharyngeal tonsils (adenoids) uncovered some new features of HIV-1 replication *in situ*. In all 13 cases, which had been referred to the Armed Forces Institute of Pathology for consultation, it was possible to identify cells that stained strongly for intracellular HIV-1 gag protein. These cells were localized primarily at the mucosal surface rather than the underlying lymphoid tissue. Moreover, the infected profiles often were multinucleated, or syncytia, and carried markers found on dendritic cells (DCs) but not other leukocytes. The results implicated syncytia, DCs, and mucosal surfaces during active HIV-1 replication *in situ*. We have extended these findings to a new set of 14 specimens that included both adenoid and palatine tonsils. In the current set, more clinical data were available, and the specimens included four unselected samples that were uncovered in a search of hospital records for tonsillectomies in the setting of HIV-1 infection. We will show that cells with intracellular HIV-1 gag protein are readily identified but that these are restricted to one part of the tonsil surface termed lymphoepithelium.[2–7] Lymphoepithelial tissue is found at sites where antigens and infectious agents cross mucosal surfaces. We find that this tissue contains both DCs and T cells, which are known to promote HIV-1 replication *in vitro*.

Materials and Methods

Histology

Five-micron sections were prepared from formalin-fixed, paraffin-embedded tissue blocks. Each case was stained with hematoxylin and eosin (H&E) to rule out the presence of tonsillitis and with a number of different stains to rule out other infectious microor-

Supported by National Institutes of Health grants AI40045 and AI40874.

Accepted for publication March 24, 1997.

Address reprint requests to Dr. Ralph M. Steinman, Laboratory of Cellular Physiology and Immunology, Rockefeller University, New York, NY 10021.

ganisms. The stains included Gram (Brown-Hopps), mucicarmine, periodic acid-Schiff, Warthin-Starry, Gomori methenamine silver, and Ziehl-Neelson (acid-fast bacilli).

Immunocytochemistry

Five-micron sections were prepared as above but applied to positively charged slides coated with 3-amino-propylethoxysilane. The sections were dewaxed in xylene, treated with 3% hydrogen peroxide, and rinsed in PBS. Immunostaining was performed for 60 minutes at room temperature, and the stain was developed by the peroxidase/anti-peroxidase method. All antibodies were obtained from commercial sources and recognized CD20 (monoclonal antibody (MAb) L-26; Dako, Carpinteria, CA; 1:200), CD4 (MAb OPD4; Dako; 1:100), CD45RO (MAb UCHL-1; Dako; 1:200), CD3 (rabbit polyclonal; Dako; 1:500), cytokeratin cocktail (AE1/AE3 and CK1 mAbs; Dako and Boehringer Mannheim, Indianapolis, IN; 1:400), S100 (rabbit polyclonal; Dako; 1:100–1:800), HIV-1 p24 (MAb; Dako; 1:100), Epstein-Barr virus latent membrane protein (MAb; Dako; 1:50), herpes simplex virus (rabbit polyclonal; Dako; 1:200), and cytomegalovrius (MAb; Chemicon, Temecula CA; 1:800). Of the above antibodies, staining with cytokeratin and p24 required predigestion for 3 minutes with 0.05% protease VIII (Sigma Chemical Co., St. Louis, MO) in 0.1 mol/L phosphate buffer, pH 7.6, at 37°C). Sections were counterstained with Gill's hematoxylin. Nonreactive mouse and rabbit Ig were used as negative controls.

In Situ *Hybridization (ISH) and Double Labeling*

Mixtures of DCs and T cells were obtained from human skin as described,[8,9] pulsed with HIV-1 IIIB for 90 minutes, washed, and recultured 1 to 4 days before preparing cytospins of the cells. Alternatively, fixed, paraffin-embedded sections were processed. The slides were immunolabeled brown for S100 using a polyclonal antibody (above) applied after antigen retrieval by enzyme treatment (2 mg of proteinase K, 200 μl of 1 mol/L Tris/HCl, 20 μl of 1 mol/L $CaCl_2$, and 20 ml of distilled water) for 10 minutes. After immunolabeling, the slides were hybridized to ^{35}S-labeled antisense cRNA probes specific for HIV-1 (Lofstrand Labs, Gaithersburg, MD). The probe was synthesized from five DNA templates that collectively represent 90% of the HIV-1 genome. The sections were incubated for 2 hours at 45°C in prehybridization mixture consisting of 50% formamide, 10% Denhardt's solution (0.3 mol/L NaCl, 10 mmol/L Tris/HCl, pH 7.4, 0.5 mmol/L EDTA, 5 mmol/L dithiothreitol, and 50 mg of yeast tRNA/ml). Thereafter, the slides were layered with hybridization cocktail (prehybridization mixture, 20% dextran sulfate in formamide, 2×10^6 dpm of ^{35}S-labeled probe/ml), boiled for 1 minute and then chilled, covered with a coverslip, sealed with rubber cement, placed in a moist chamber, and hybridized at 45°C overnight. The slides were washed sequentially in 50% formamide/2X SSC, in 0.01% Triton/2X SSC, and in 0.01% Triton/SSC and finally digested with RNAse (Boehringer Mannheim, Mannheim, Germany) at 37°C for 40 minutes. The slides were then washed in 2X SSC, dehydrated in 0.3 mol/L ammonium sulfate in 70 to 96% ethanol, dipped in Kodak NTB-2 emulsion, exposed for 6 days at 4°C, developed in Kodak D-19 developer, counterstained with hematoxylin, and mounted. As a positive control, paraffin sections of infected H9 cells were hybridized with the same HIV-1-specific probe. As a negative control, one section of each tonsil and of infected H9 cells were hybridized with a sense-strand probe. The sections were examined with an Axiophot Zeiss microscope equipped with epiluminescent illumination. Cells with at least 20 silver grains were scored as HIV-1 RNA positive. This grain count corresponded to a sixfold increase over the background level.

Counts of Infected Cells

Quantification and localization of infected cells were performed as follows. The sections were separated by region into lumen, surface epithelium, crypt epithelium, T zone, and follicle. For each compartment, the number of 40× fields was counted. The number of infected syncytia and single cells was then divided by the number of fields, and both numbers were included in the results (see Table 2).

Results

Patients

The 14 new specimens in this study included 7 nasopharyngeal tonsils and 7 palatine tonsils, hereafter termed adenoids and tonsils, respectively. Several were referred to the Armed Forces Institute of Pathology for consultation, but four came from a search of the records of the Georgetown University hospital for tonsillectomies that were performed on HIV-1-infected individuals between 1992 and 1995.

Table 1. *HIV-1-Infected Adenoids and Tonsils: Clinical Data*

Patient	Age (years)	Sex	Diagnosis of HIV	Surgery	Transmission	CD4 count	OIs
1	26	F		1996	Heterosexual	529	
2	37	M		1996	Homosexual	191	
3	36	M	1991	1995	Homosexual	326	
4	25	M		1996	Homosexual	200	
5	38	M	1986	1996	IVDU	931	
6	57	M		1996	IVDU		
7	48	M	1992	1995		230	
8	36	M	1986	1994	Homosexual	340	
9	34	M	1989	1995	Homosexual, IVDU	285	Candida esophagitis PCP, and lymphoma
10	37	M		1989			
11	11	M	1984	1994	Homosexual	422	
12	?	F		1993	Heterosexual		
13	38	M	1995	1995		340	
14	38	M		1996			

F, female; M, male; IVDU, IV drug user.

In contrast to the initial study of adenoids,[1] substantial clinical information was available on this cohort (Table 1). The histories indicated that the patients were asymptomatic, other than the presenting symptom that the tonsils were sufficiently enlarged to cause obstructive symptoms or raise the potential of malignancy. The submitting diagnosis was to rule out lymphoma. None of the patients had histories or histopathological indications of tonsillitis. A battery of special stains for infectious agents (see Materials and Methods) were negative. Two of the patients were females. Different risk factors were evident, ie, intravenous (IV) drug abuse, heterosexual transmission, and receptive anal intercourse. Several patients had been diagnosed with HIV-1 infection for several years (2 to 10 years) before tonsillectomy. Most had substantial numbers (200 to 900/μl) of $CD4^+$ T cells. Therefore, most of the adenoids and tonsils in this study had been removed from chronically infected individuals who were clinically well. Two tonsils from HIV-1 seronegatives that were also negative by ISH and antibody labeling for HIV-1 RNA and p24 were used as controls. These had follicular hyperplasia but no acute inflammation.

HIV-1-Infected Syncytia at the Surface of Tonsil Crypts

Cells containing intracellular p24 antigen were evident in all cases, both adenoids (not shown) and tonsils (Figure 1). The p24-positive cells could be found in groups (Figure 1A), and they always included multinucleated cells or syncytia (Figure 1, B–D). Some of the syncytia were large enough to be considered as giant cells in routine H&E-stained material. However, many had just two to four nuclei and required staining with anti-p24 to be easily detected (Figure 1D). A diffuse, generally less intense stain for HIV-1 p24 antigen was evident over the follicular dendritic cell network in the germinal centers that lie beneath the adenoid and tonsil mucosae (Figure 1, A and C). The depots of HIV-1 in germinal centers likely reflects extracellular virions that are trapped as immune complexes on follicular dendritic cells.[10]

When ISH was used to localize viral RNA, heavily labeled large cells were also noted in the mucosal surface. This labeling occurred only with antisense and not sense probes (Figure 1E). Scattered, heavily labeled cells also were noted in the germinal centers, but these were smaller in size than those in the crypt mucosa (Figure 1E).

Semiquantitative studies were carried out on the entire section from several of the patients (Table 2). Most $p24^+$ profiles were syncytia, containing two or more nuclei that were at least the size of lymphocyte nuclei, and the vast majority had three or more nuclei. Those cells that stained for p24 but had single nuclei were a distinct minority and were more readily detected by ISH (below).

A consistent finding was that the $p24^+$ syncytia were exclusively found in invaginations or crypts of the tonsil, rather than the surface adjacent to the pharynx (Figure 1, A and C; Table 2). The crypts are lined by a type of tissue called lymphoepithelium to be described below, whereas the tonsil surface is lined by a stratified squamous epithelium (labeled S in Figure 1A) that in many respects resembles other surfaces like vagina, ectocervix, skin, and pharynx.

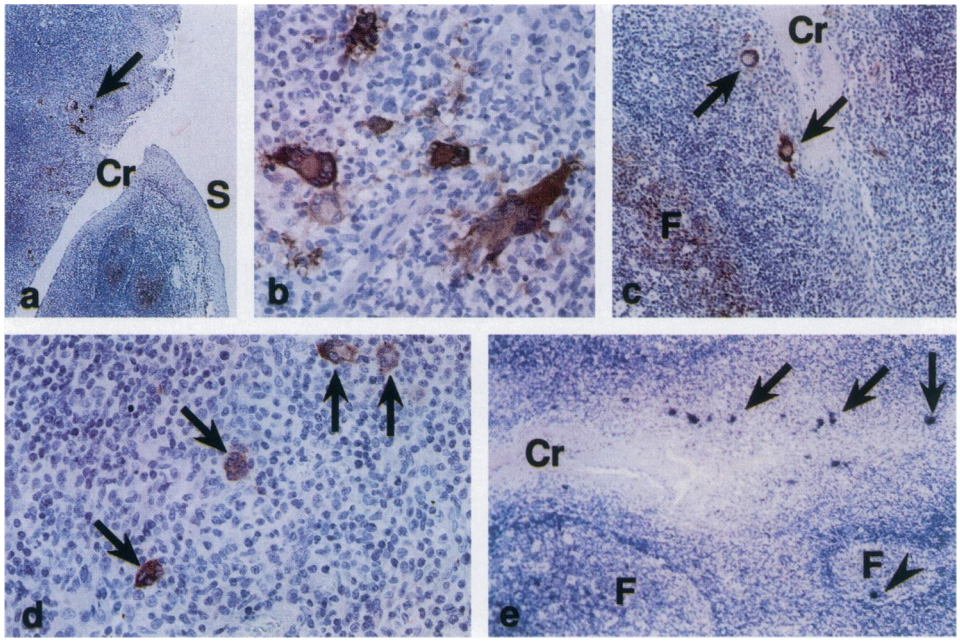

Figure 1. *HIV-1-infected cells and syncytia in tonsillar crypts.* **A:** *Low-power view (×10) stained for HIV-1 p24 antigen. A cluster of large p24⁺ cells* (**arrow**) *is found within the mucosa lining the crypt (Cr). Diffuse p24 staining is evident in the underlying follicle. p24⁺ cells are not found in the surface stratified squamous epithelium (S).* **B:** *Higher-power view of the arrowed area in* **A** *showing the infected cells to be irregular in shape and multinucleated.* **C:** *Another area (×40) of a tonsil showing two infected giant cells* (**arrows**) *in the mucosa and diffuse stain in the germinal center of an enlarged follicle (F). The infected cells lie in the lymphoepithelium of the crypt (Cr). Diffuse p24 staining is evident in the underlying follicle (F).* **D:** *Lymphoepithelium (×40) with small multinucleated cells or syncytia that can be identified with p24 staining* (**arrows**). **E:** *Low-power view of ISH with antisense probes showing large, HIV-1-infected cells* (**arrows**) *in the mucosa of the crypts (Cr) and smaller profiles in the follicles (F,* **arrowhead**).

Expression of S100 Antigen and HIV-1 RNA in Infected Syncytia

The S100 protein is expressed at high levels in many members of the DC system, including the Langerhans cells of the epidermis and the DCs, or interdigitating cells, of the T-cell areas in lymphoid organs.[11] For leukocytes, anti-S100 primarily stains DCs, but S100 is also expressed at high levels on $CD4^-$ cells such as neurones and glia. When our set of tonsils and adenoids were stained for S100, numerous positive syncytia were apparent in the crypt mucosa and many had dendritic shapes (Figure 2A, arrows). Macrophages, including giant cells derived from macrophages as in leprosy and tuberculosis, do not stain for S100. S100 is expressed by other cells, especially Schwann cells surrounding peripheral nerves and melanocytes, but these cells do not express CD4 and are unlikely to be infected by HIV-1. Peripheral nerves were seen in our tonsils but stained very much more intensely for S100 than syncytia.

As multinucleated cells develop with other infections, eg, measles and influenza, double-labeling studies for S100 and HIV-1 RNA were carried out. A control experiment was first performed to verify that S100 expression was maintained by HIV-1-infected DCs in tissue culture. Mixtures of skin DCs and T cells were isolated and infected with HIV-1 as described.[8,9] One to four days later, cytospins were made and stained sequentially with anti-S100 (brown) and radiolabeled antisense probe for HIV-1

Table 2. *HIV-1-Infected Cells in Oral Lymphoid Mucosa Are Primarily Syncytia that Localize to Lymphoepithelium*

Patient	Organ	Number of Syncytia/Number of Fields (single cells)				
		Surface	Lymphoepithelium	Lumen	T zone	Follicle
-8174	Adenoid	0/52	32/71 (2)	3	0/60	0/10
-8173	Adenoid	0/30	64/22	2	1/80 (1)	0/41
-5817	Adenoid	1/16 (2)	53/48	0	0/43 (10)	0/12 (1)
-4459	Tonsil	0/105 (2)	21/254 (3)	3 (1)	1/155 (2)	0/170
-4681	Tonsil	0/65	375/182 (2)	4	2/239 (7)	0/240
-	Tonsil	0/109 (5)	373/265 (47)	0	0/242 (36)	0/363 (9)
-5296	Tonsil biopsy	0/24	12/19 (6)	0	0/5 (1)	0/4 (1)

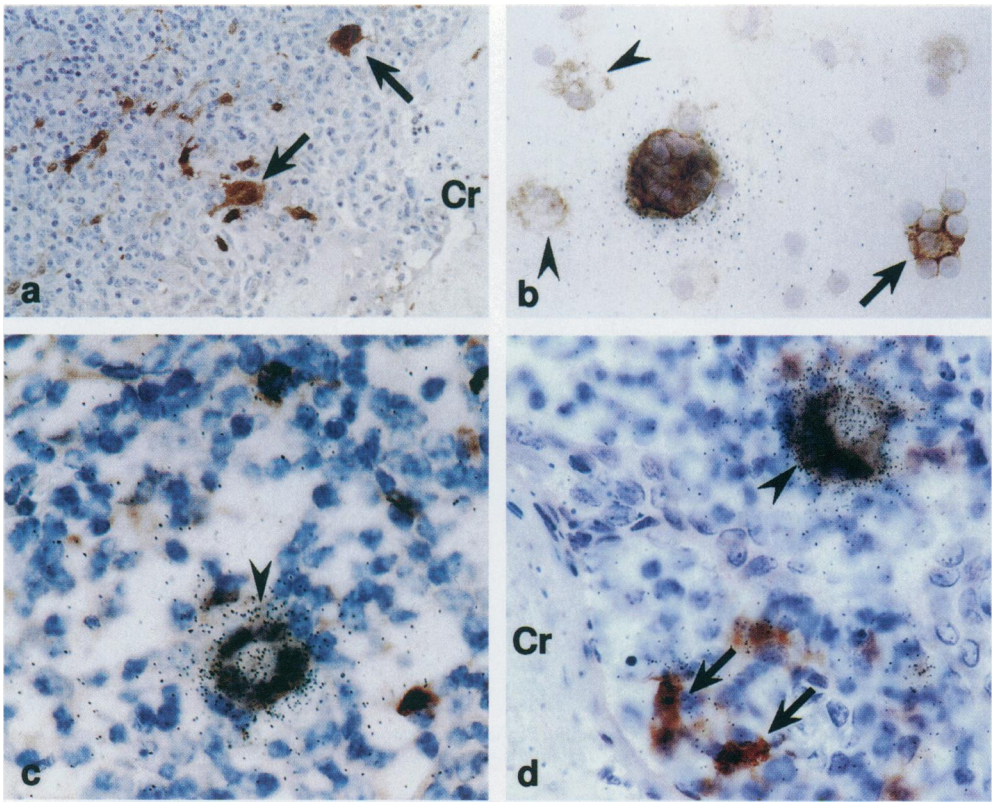

Figure 2. *Immunolabeling for S100 antigen and ISH for HIV-1 RNA.* **A:** *$S100^+$ cells, including irregularly shaped syncytia* (**arrows**) *in the mucosa of tonsil crypts (Cr). Magnification, ×40.* **B:** *Combined ISH (antisense probe) and S100 stain of HIV-1-infected mixtures of cultured skin DCs and T cells. The large infected multinucleated cell or syncytium in the center is covered with black silver grains and stains strongly for S100 (brown). Other DCs (brown) and T cells (blue) are ISH negative including a DC-T cell conjugate* (**arrow**). **C** *and* **D:** *Combined S100 staining (brown) and ISH (black silver grains) in the tonsil crypt mucosa from two patients. The giant cells are $S100^+$ and labeled with silver grains* (**arrowheads**), *whereas single $S100^+$ cells lack HIV RNA* (**arrow**).

mRNA (black silver grains from the autoradiography). As illustrated in Figure 2B, the profiles with HIV-1 mRNA (covered with silver grains) always had two or more nuclei, and all of the syncytia in the culture were covered with silver grains. No labeling was seen with sense probes (not shown). All syncytia stained strongly for S100. As expected, many uninfected DCs (no silver grains, arrowhead in Figure 2B) also were $S100^+$, including uninfected DCs that were attached to T cells (Figure 2B, arrow).

We proceeded to double label sections of HIV-1-infected tonsil in the two cases for which we had abundant tissue. The sections were labeled first with anti-S100 and a peroxidase anti-Ig (brown color) and then with ^{35}S-radiolabeled, HIV-1-specific cRNA probes (black silver grains). No labeling was evident with sense probes. The heaviest labeling for viral RNA (antisense probes) was seen in giant cells (Figure 2, C and D). The majority of cells with viral RNA also labeled brown for S100, as quantified in Table 3.

Table 3. *Combined ISH for HIV-RNA and Immunolabeling for S-100 Protein*

Type of cell	Number of cells counted	
	Tonsil 1	Tonsil 2
Giant cells (≥4 nuclei)		
RNA^+ $S100^+$	67	27
RNA^+ $S100^-$	23	14
RNA^- $S100^+$	15	10
RNA^- $S100^-$	3	4
Bi- or trinucleated		
RNA^+ $S100^+$	34	18
RNA^+ $S100^-$	33	12
RNA^- $S100^+$	4	5
RNA^- $S100^-$	ND	ND
One nucleus		
RNA^+ $S100^+$	50	32
RNA^+ $s100^-$	127	42

Combined labeling was performed as in Figure 2, C and D, on tonsil sections from patients 8 and 9. No labeling was seen in the sense controls. Most of the RNA^+ $S100^-$ cells had large numbers of silver grains, which would obscure the detection of the S100 double label. ND, not determined.

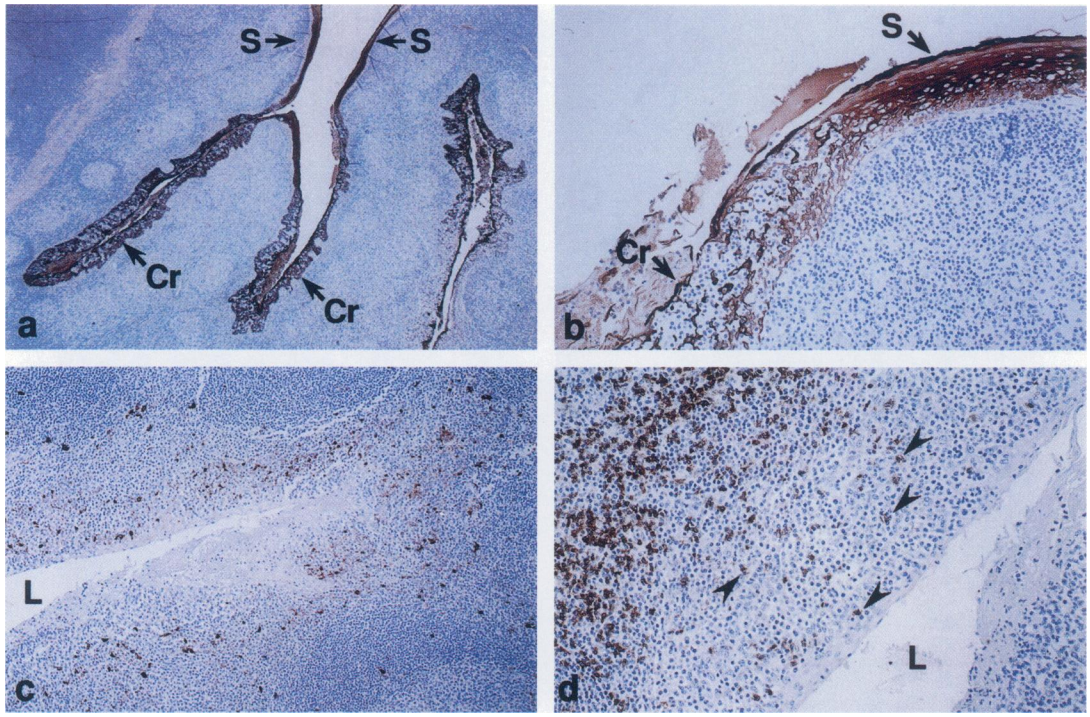

Figure 3. *Comparison of surface (S) and crypt (Cr) epithelia.* A *and* B: *Cytokeratin stains showing the compact* versus *ramifying keratin in surface (S)* versus *crypt (Cr) epithelium.* C: *Numerous S100 profiles surround the crypt lumen (L).* D: *CD4$^+$ cells* (arrowheads) *in the lymphoepithelium.*

Properties of Infected Lymphoepithelia

As it was clear that HIV-1-infected cells localized in the tonsil to crypts rather than the surface adjacent to the pharynx (Figure 1, A and C; Table 2), we compared the histology of these two epithelial surfaces. Previous histological studies have shown that the tonsil surface is covered by squamous epithelium that is similar in many respects to the lining of the mouth, vagina, and skin. The crypts in contrast are lined by lymphoepithelium, so termed because the epithelial cells form a meshwork surrounding many lymphocytes.[2–4,6,7] Crypt epithelium also contains M cells[2] that mediate transport of antigens, infectious agents, and particulates.[12,13]

Several antibodies nicely distinguished the histology of surface and crypt epithelium in our material. The stratified squamous, surface epithelium stained in a dense compact pattern with anti-keratin antibodies. The lymphoepithelium of the crypts was reticular in organization with a loose arrangement of keratin-stained epithelial cells often in a mesh-like fashion surrounding lymphocytes (Figure 3 A,B). Another distinction was the frequency of S100$^+$ DCs. These were much more numerous in the crypt (Figure 3C), as was also evident in the two control HIV-1-negative specimens. Lastly, in both controls and HIV-1-infected specimens, lymphocytes were infrequent in surface epithelium but were abundant within the interstices of crypt epithelium. Most were CD20$^+$ B cells as has been described,[3,6] but CD4$^+$ T cells were present as well in crypt epithelium (Figure 3D).

The surface of the adenoids also is a patchwork of two types of epithelium: respiratory ciliated epithelium and lymphoepithelium. The latter contained many lymphocytes and S100$^+$ cells and was the region where the HIV-1-infected profiles were found.

Discussion

These observations extend to the palatine tonsil many of the features of HIV-1 infection that were previously noted in HIV-1-infected, nasopharyngeal tonsil or adenoid.[1,14] Cells containing intracellular HIV-1 protein were readily identified in tonsil, predominantly at the mucosal surface. The infected cells were syncytia that stained for the S100 marker. Among CD4$^+$ cells, S100 is expressed at high levels primarily in DCs, but S100 is also abundant in CD4$^-$ neurones and glia. HIV-1-infected syncytia previously were noted in brain.[15,16] Our findings apply to enlarged, HIV-1$^+$ tonsils, and we have not examined the larger population whose tonsils are not enlarged. Several new findings became apparent in the current paper.

At the clinical level, histories were available for the current cohort of patients to show that most had

substantial $CD4^+$ T cells, were clinically asymptomatic, and had been infected for years before tonsillectomy (Table 1). This indicates that heavily infected DCs are characteristic of chronic HIV-1 infection. In our previous work, specimens were exclusively referred to the Armed Forces Institute of Pathology for consultation,[1] whereas 4 of 11 of the current specimens were unselected and identified in searches for tonsillectomies that had been performed on HIV-1-infected individuals in two large University hospitals.

The important finding at the pathological level was that HIV-1-infected cells were restricted to select regions of the tonsil. These regions are termed lymphoepithelium because large numbers of lymphocytes, primarily B but also $CD4^+$ T cells, are abundant within a ramifying epithelial network. Jolly first used the term lymphoepithelium to describe epithelia that are permeated by lymphocytes, in his case, the bursa of Fabricius in birds and the appendix of rabbits.[17] Schmincke pointed out that a similar situation occurs in intestinal and pharyngeal-associated lymphoid tissue. Specifically, "the epithelia form the framework and the structure for the lymphocytes that imbed themselves".[18] Electron microscopy nicely visualized this mixing of lymphocytes and epithelial cells and further showed that the lymphoepithelium of the adenoid[4] and tonsil crypts[2] includes many microfold, or M, cells. M cells are sites for the entry of antigens and small particles, including HIV-1,[4,12] across the epithelium (reviewed in Refs. 13,19, and 20). DCs have been described beneath the epithelial and M cells.[7,21] With S100 staining, we could visualize DCs to be particularly abundant within the crypt mucosa. Lymphocytes, including $CD4^+$ T cells, also were more numerous in the crypt mucosa. In sum, the lymphoepithelium represents sites in which antigens gain access to a sub- or intraepithelial region that is rich in DCs and T cells.

What might distinguish $S100^+$ DCs within lymphoepithelium, which can be infected with HIV-1 in all 27 specimens that we have now examined, from DCs within the T cell areas of peripheral lymph nodes, which have not been shown to be stained for HIV-1 protein or mRNA?[10] One possibility is that the DCs and T cells in the adenoid and tonsil lymphoepithelium lie directly beneath M cells and therefore would be chronically presenting specific antigens and superantigens that enter from the nose and throat. A second possibility is that, in the lymphoepithelium, the DCs and T cells are interacting, but not necessarily in an antigen-specific way. Studies of HIV-1 infection *in vitro* have shown that DCs and T cells together drive viral replication. This infection can occur in the setting of an immune response, ie, presentation of alloantigens, superantigens, and protein antigens.[22–24] Alternatively, when these two cell types are isolated from skin[8,9] or other body surfaces,[25] the DCs and T cells form conjugates that are permissive for HIV-1 replication in syncytia in the absence of added stimuli.

At the level of pathogenesis, this paper raises some issues. First, it should be determined whether HIV-1 replicates in other mucosal-associated lymphoid tissues. The rectum, intestine, and airway, for example, contain sites where an epithelium with M cells overlies a lymphocyte and DC-rich, subepithelial zone. Second, because lymphoepithelium contains M cells,[2] transmission of HIV-1 could take place during oral sex or at birth when the neonate swallows infected fluids from the mother. Baba et al reported that SIV is transmitted efficiently by the oral route,[26] perhaps because the oral route allows virus to access M cells, DCs, and T cells in the tonsils and other lymphoid tissues of Waldeyer's ring. Third, pharyngeal lymphoid tissues could contribute to chronic viral replication, as all of our patients were chronically infected. The virus initially may not have infected our patients via the oral route. Instead, the virus may be transported to oral mucosae from other sites in the body, for example, from DCs or other cells that are initially infected in the genital tract as in recent experiments with SIV.[27] HIV-1 could be transported to the oral mucosa either as free virus, as virus adsorbed to CD4 on T cells, monocytes, or DCs, or as virus-infected $CD4^+$ cells. Once virus reaches the lymphoepithelium, the interaction of DCs with T cells would be expected to drive HIV-1 replication as has been documented in tissue culture systems.[8,9]

Our data do not provide information on the extent to which HIV-1 replicates at mucosal surfaces relative to peripheral lymph nodes. The latter contain doubly spliced transcripts,[28] so HIV-1 transcription and most likely protein synthesis is occurring in peripheral nodes. However, the findings in this paper draw attention to several points that are different from lymph nodes. Mucosa-associated lymphoid tissues contain cells with abundant intracellular HIV-1 protein. The infected cells are likely to be derived from DCs and include syncytia, features that have yet to be documented in peripheral lymph nodes.

Acknowledgments

We are grateful to Pamela S. Bell for the library work required to locate some of the referenced papers and to Judy C. Adams for assembling the figures.

References

1. Frankel SS, Wenig BM, Burke AP, Mannan P, Thompson LDR, Abbondanzo SL, Nelson AM, Pope M, Steinman RM: Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid. Science 1996, 272:115–117
2. Owen RL, Nemanic P: Antigen processing structures of the mammalian intestinal tract: an SEM study of lymphoepithelial organs. Scanning Electron Micros 1978, 11:367–378
3. Ruco LP, Uccini S, Stoppacciaro A, Pilozzi E, Morrone S, Gallo A, De Vincentiis M, Santoni A, Baroni CD: The lymphoepithelia organization of the tonsil: an immunohistochemical study in chronic recurrent tonsillitis. J Pathol 1995, 176:391–398
4. Winther B, Innes DJ: The human adenoid: a morphologic study. Arch Otolaryngol Head Neck Surg 1994, 120:144–149
5. Fujiyoshi T, Watanabe T, Ichimiya I, Mogi G: Functional architecture of the nasopharyngeal tonsil. Am J Otolaryngol 1989, 10:124–131
6. Morente M, Piris MA, Orradre JL, Rivas C, Villuendas R: Human tonsil intraepithelial B cells: a marginal zone-related subpopulation. J Clin Pathol 1992, 45:668–672
7. Bani D, Gallo O, Fini-Storchi O: Intraepithelial lymphocyte subpopulations and dendritic accessory cells in normal and hypertrophic adenoids. Laryngoscope 1994, 104:869–873
8. Pope M, Betjes MGH, Romani N, Hirmand H, Cameron PU, Hoffman L, Gezelter S, Schuler G, Steinman RM: Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell 1994, 78:389–398
9. Pope M, Gezelter S, Gallo N, Hoffman L, Steinman RM: Low levels of HIV-1 in cutaneous dendritic cells initiate a productive infection upon binding to memory $CD4^+$ T cells. J Exp Med 1995, 182:2045–2056
10. Tenner-Racz K, Bofill M, Schulz-Meyer A, Dietrich M, Weber J, Pinching AJ, Veronese-Dimarzo F, Popovic M, Klatzmann D, Gluckman JC, Janossy G: HTLV-III/LAV viral antigens in lymph nodes of homosexual men with persistent generalized lymphadenopathy and AIDS. Am J Pathol 1986, 123:9–15
11. Takahashi K, Isobe T, Ohtsuki Y, Sonobe H, Takeda I, Akagi T: Immunohistochemical localization and distribution of S-100 proteins in the human lymphoreticular system. Am J Pathol 1984, 116:497–503
12. Amerongen HM, Weltzin R, Farnet CM, Michetti P, Haseltine WA, Neutra MR: Transepithelial transport of HIV-1 by intestinal M cells: a mechanism for transmission of AIDS. J AIDS 1991, 4:760–765
13. Neutra MR, Pringault E, Kraehenbuhl J-P: Antigen sampling across epithelial barriers and induction of mucosal immune responses. Annu Rev Immunol 1996, 14: 275–300
14. Rinfret A, Latendresse H, Lefebvre R, St Louis G, Jolicoeur P, Lamarre L: Human immunodeficiency virus-infected multinucleated histiocytes in oropharyngeal lymphoid tissues from two asymptomatic patients. Am J Pathol 1991, 138:421–426
15. Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour G, Yungbluth M, Janotta F, Aksamit A, Martin MA, Fauci AS: Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science 1986, 233:1089–1093
16. Budka H: Multinucleated giant cells in brain: a hallmark of the acquired immune deficiency syndrome (AIDS). Acta Neuropathol (Berlin) 1986, 69:353–358
17. Jolly J: La bourse de Fabricius et les organes lymphoepitheliaux: compt rend de l'assoc des anatomistes 1911, 164–176
18. Schmincke A: Classics in oncology: on lymphoepithelial tumors. Pathol Anat 1921, 68:161–170
19. Gebert A, Rothkotter H, Pabst R: M Cells in the Peyer's patches of the intestine. Int Rev Cytol 1996, 167:91–159
20. Neutra MR, Frey A, Kraehenbuhl J-P: Epithelial M cells: gateways for mucosal infection and immunization. Cell 1996, 86:345–348
21. Wilders MM, Sminia T, Plesch BEC, Drexhage HA, Welte-Vreden EF, Meuwissen SGM: Large mononuclear Ia-positive veiled cells in Peyer's patches. II. Localization in rat Peyer's patches. Immunology 1983, 48:461–467
22. Cameron PU, Freudenthal PS, Barker JM, Gezelter S, Inaba K, Steinman RM: Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to $CD4^+$ T cells. Science 1992, 257:383–387
23. Pinchuk LM, Polacino PS, Agy MB, Klaus SJ, Clark EA: The role of CD40 and CD80 accessory cell molecules in dendritic cell-dependent HIV-1 infection. Immun 1994, 1:317–325
24. Weissman D, Barker TD, Fauci AS: The efficiency of acute infection of $CD4^+$ T cells is markedly enhanced in the setting of antigen-specific immune activation. J Exp Med 1996, 183:687–692
25. Pope M, Elmore D, Ho D, Marx P: Dendritic cell-T cell mixtures, isolated from the skin and mucosae of macaques, support the replication of SIV. AIDS Res Hum Retroviruses 1997 (in press)
26. Baba TW, Trichel AM, An L, Liska V, Martin LN, Murphey-Corb M, Ruprecht RM: Infection and AIDS in adult macaques after nontraumatic oral exposure to cell-free SIV. Science 1996, 272:1486–1489
27. Spira AI, Marx PA, Patterson BK, Mahoney J, Koup RA, Wolinsky SM, Ho DD: Cellular targets of infection and route of viral dissemination following an intravaginal inoculation of SIV into rhesus macaques. J Exp Med 1996, 183:215–225
28. Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, Orenstein JM, Kotler DP, Fauci AS: HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 1993, 362:355–358