American Journal of Pathology, Vol. 139, No. 4, October 1991

Detection, Localization, and Quantitation of HIV-associated Antigens in Intestinal Biopsies from Patients with HIV

Donald P. Kotler,* Safak Reka,* Anthony Borcich,* and William J. Cronin,†

Gastrointestinal Division, Department of Medicine, St. Luke's-Roosevelt Hospital Center, Columbia University, College of Physicians and Surgeons, New York, and the Department of Pathology,† Lenox Hill Hospital, New York, New York*

This study determined the prevalence, cellular localization, and content of human immunodeficiency virus (HIV)-associated antigens in intestinal mucosa from HIV-infected subjects. Studies were performed in 168 subjects with gastrointestinal, nutritional, or proctologic complaints, and HIV-seronegative controls. The polymerase chain-reaction technique, which detects viral DNA, was used in 20 subjects and was positive in 70%. In situ hybridization studies, using RNA probes, were employed in 48 cases and were positive in 31%. Immunohistologic studies using monoclonal antibodies to HIV p24 antigen were employed in 73 cases and were positive in 67%. Quantitative ELISA assays for tissue p24 content were performed in 168 cases and was positive in 68%. Evidence of HIV was found throughout the intestine and in different disease stages. The quantitative ELISA studies correlated significantly with in situ hybridization, implying a possible association between the presence of viral RNA and protein expression. The authors conclude that HIV is present in intestinal mucosa from most, if not all HIV-infected subjects. The relationship to intestinal disease currently is unclear. (Am J Pathol 1991, 139:823–830)

Gastrointestinal dysfunction is prevalent in patients infected with the human immunodeficiency virus (HIV).[1,2] A majority of patients experience diarrhea or other intestinal symptoms at some point in the disease course. Many enteric diseases occur in patients with the acquired immunodeficiency syndrome (AIDS).[3] The important pathogens include those that cause infections irrespective of immune competence, such as *Cryptosporidium parvum*, as well as pathogens that cause disease only in immune-deficient people, such as *Mycobacterium avium intracellulare*. However, the etiology of diarrhea is unexplained in 15 to 50% of patients in various series.[1–6] Patients with early AIDS-related complex and diarrhea are especially likely to have only a nonspecific inflammatory bowel disease on evaluation (DPK, personal observations).

In situ hybridization and immunohistologic studies have localized HIV to cells in the gastrointestinal (GI) tract. It has been suggested that HIV might play a pathogenetic role in GI disease.[7–9] HIV infection of the intestinal mucosa may be important in disease transmission as well as the pathogenesis of intestinal dysfunction. However, the precise cellular reservoirs for HIV in the intestine are not fully defined. In addition, the prevalence of HIV in the GI tract as well as the intensity of infection, clinical, and pathologic associations, and mechanisms of possible injury all are unknown.

This study determined the prevalence and location of HIV in the intestines of infected patients and applied a quantitative technique for measuring an HIV-associated protein antigen in mucosal tissue.

Methods

Experimental Subjects

The studies performed were approved by the Institutional Review Board at the St. Luke's–Roosevelt Institute of Health Sciences, and all patients gave specific informed consent. Studies were performed as part of the evaluation of gastrointestinal or proctologic symptoms in 168 HIV-infected persons. One hundred twenty six patients had CDC-defined AIDS (Walter Reed Class 6), whereas 42 patients had either persistent generalized lymphadenopathy (Walter Reed Class 2), laboratory evidence of

Supported in part by a grant from the National Institutes of Health (DPK-AI 21414).

Accepted for publication June 10, 1991.

Address reprint requests to Dr. Donald P. Kotler, GI Division/S&R 12, St. Luke's-Roosevelt Hospital Center, 114th St. and Amsterdam Ave., New York, NY 10025.

immune dysfunction such as lymphopenia, decreased numbers of CD4+ cells in peripheral blood with or without anergy (Walter Reed Class 3–4), or AIDS-related complex (Walter Reed Class 5).[10] There were seven females, six intravenous drug abusers, 2 of whom were female, and one patient who was presumed infected via blood transfusion. The other patients were homosexual or bisexual males. Biopsies from 20 normal heterosexual controls and 15 patients with idiopathic inflammatory bowel disease (IBD) were used as HIV-seronegative controls.

Tissue Specimens

Jejunal biopsy specimens were obtained either by Crosby capsule or by direct endoscopic biopsy of the jejunum using a pediatric colonoscope (Olympus PCF 10, Olympus Inc, Lake Success, NY). The latter method allows multiple biopsies to be obtained easily and safely. Colonic biopsies were obtained by mucosal biopsy during either proctoscopy, flexible sigmoidoscopy, or colonoscopy, with or without ileoscopy. Biopsy specimens were placed in 10% neutral buffered formalin or immediately frozen on dry ice and stored at −85C. The formalin-fixed tissue was processed within 24 hours, and the tissue was embedded in paraffin.

Method for Polymerase Chain Reaction

HIV DNA was detected in tissue specimens frozen at the time of biopsy in 20 patients using the technique as described by Saiki.[11] Studies were performed in the Laboratory of Immunoregulation at the National Institutes of Health. Briefly, tissue DNA was prepared by heating a crude homogenate in water to 95°C. Tissue DNA was amplified during 35 cycles of incubation in a DNA thermal cycler (Perkin Elmer Cetus) using the thermostable Taq DNA polymerase, with denaturation at 94°C, annealing at 55°C and extension at 72°C. HIV-specific oligonucleotide primers SK38/39, corresponding to gag 1151–1578 and 1638–1165, and SK68/89, corresponding to env 7801–7820 and 7922–7942 (Scientific Genetics, San Diego, CA), were used. An HLA DQa primer (QH 26/27) was used as a control. Final hybridization was performed using ^{32}P labelled HIV probes (SK 19, corresponding to gag 1595–1635, and SK 70, corresponding to env 7841–7875. Autoradiograms were performed after transfer to a 10% polyacrylamide gel.

Method for In Situ *RNA Hybridization*

In situ hybridization studies were performed on biopsy specimens from 48 HIV-infected patients using techniques that have been described in detail previously.[7] The results in 25 patients from the previous study[7] were included. Briefly, formalin-fixed, paraffin-embedded tissue sections were digested with proteinase K, incubated with the reducing agent, dithiothreitol, followed by the sulfhydryl modifying agent, iodoacetamide. In previous studies, these steps were important to reduce nonspecific binding of ^{35}S by eosinophils. The sections were acetylated, prehybridized, and incubated with a radiolabelled probe (^{35}S or ^{3}H) in a hybridization cocktail plus dextran sulfate in formamide, sealed, and hybridized at 45°C overnight. After hybridization, the slides were washed and single-stranded RNA was digested with RNAase. The slides were dipped in emulsion, exposed, developed, stained, and examined by dark-field and bright-field microscopy. Control tissues included an infected cell line and tissue from a brain known by electron microscopy to contain HIV. Hybridizations were performed with sense, antisense, and nonsense probes.

The specific probes used were isolated from H9 cells infected with HTLV III and obtained commercially (Lofstrand Labs Limited, Gaithersburg, MD); a gag-specific probe that also includes the 5′ LTR, a probe that includes most of the gag region as well as the pol region, a probe that includes all of the pol region, a probe that includes the sor region, and an env specific probe. Incubations were performed using a mixture of the probes.

Method for Immunoperoxidase Staining

Indirect immunoperoxidase stains were performed on formalin-fixed, paraffin-embedded tissue sections of biopsies from 73 AIDS patients and 10 controls using mouse monoclonal IgG antibodies to the HIV-associated proteins p17, p24, and gp120 (Dupont Laboratories, Wilmington, DE). After a 16- to 18-hour incubation at 4°C with primary antibody, the sections were further developed using an antimouse biotin-avidin-peroxidase technique (Vector Labs Inc, Burlingame, CA), stained using 3-amino-9-ethyl carbazole (AEC) (Ortho Pharmaceuticals, Raritan, NJ) as the chromagen, then counterstained using Mayer's hematoxylin. The controls consisted of formalin-fixed, paraffin-embedded sections from HIV-infected and noninfected HUT 78 cells (provided by Dr. David Volsky).

Several studies were performed to validate the immunohistologic method. No reactivity was seen in biopsies from five heterosexual controls or five patients with inflammatory bowel disease. Studies were performed on 25 biopsy specimens from HIV-infected patients using a monoclonal antibody to the p24 antigen of HTLV 1 (Dupont Laboratories), a human retrovirus related to HIV, and no staining was found. The specificity of the staining re-

action also was tested on eight biopsy specimens by blocking studies, in which slides were preincubated with serum from HIV-infected individuals with high titer anti-p24. These sera effectively prevented staining of cells with the mouse anti-p24 antibody, whereas preincubation with HIV-negative sera did not block subsequent staining.

Method for Quantitative ELISA

The content of HIV p24 in serum and tissue was determined using a commercially available antigen capture ELISA assay (Coulter Immunology, Hialeah, FL). The system utilizes a monoclonal anti-p24 antibody coated well and a polycolonal second-step antibody. The assay system was sensitive and specific, with a linear response between 10 to 125 pg/ml of antigen in serum or media. This assay system was adapted for use with tissue homogenates. Frozen tissue (approximately 3 mg) was thawed and homogenized in 1 ml of normal saline. An aliquot of 200 μl was assayed. There was essentially quantitative recovery of p24 when added to homogenates of HIV-seronegative individuals, and standard curves made in homogenate and serum were similar (Figure 1). Total protein concentration of tissue homogenates was assayed by the Lowry method.[12] Tissue p24 antigen content was expressed as picograms/mg tissue protein.

HIV p24 was absent in biopsy specimens from 10 heterosexual controls and 12 patients with symptomatic inflammatory bowel disease. The variability of the results was tested by measuring p24 content in six biopsy specimens each of rectal mucosa from six patients. The relative localization of HIV-infected cells in different areas of the gut was evaluated in six patients by examining biopsy specimens obtained from the ileum and various areas of the colon, and by comparing the prevalence and tissue content of p24 in biopsy specimens obtained from different areas of the small intestine and colon using all tissues.

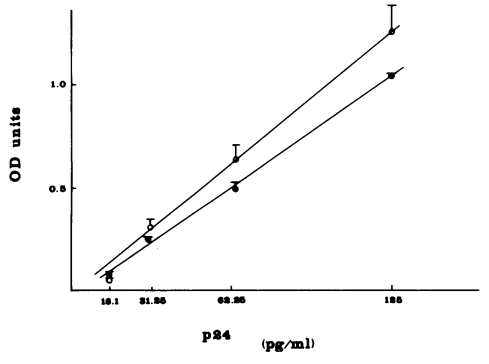

Figure 1. *Standard curves made using known quantities of HIV p24 antigen dissolved in serum from HIV seronegative subjects and in tissue homogenates from HIV-seronegative subjects. The curves did not differ, statistically. Clear circles = serum, solid circles = tissue homogenates.*

Data Analysis

Tissue p24 content as determined by quantitative ELISA was compared in different regions of the intestine using analysis of variance. The correlations between quantitative tissue p24 content and the positivity of PCR, *in situ* hybridization, and immunoperoxidase studies was performed by the Student's *t*-test. All other correlations between the nonquantitative parameters were performed by chi-square and by McNemar's test[13] and reliability was evaluated by calculating the kappa value.

Results

The results of the various analyses are summarized in Table 1.

Polymerase Chain Reaction

The polymerase chain-reaction technique was employed on rectal biopsy specimens from 20 HIV-infected patients and results were positive in 14 patients indicating a 70% positivity rate in HIV-infected patients.

RNA In Situ *Hybridization*

In situ RNA hybridization studies were performed on 48 biopsy specimens and were positive in 15 (31%). All hybridizing cells were located in the lamina propria and resembled lymphocytes or macrophages (Figure 2).

Immunohistochemical Stains

Immunohistologic studies were performed using monoclonal antibodies to an HIV-associated protein in 73

Table 1. *Prevalence of Positive Tests*

PCR	70%	(20)
In situ hybridization	31%	(48)
Immunoperoxidase (any cell)	67%	(73)
Immunoperoxidase (lamina propria mononuclear cell)	52%	
Immunoperoxidase (intraepithelial mononuclear cell)	34%	
Immunoperoxidase (epithelial cell)	37%	
Quantitative ELISA	68%	(168)

PCR = polymerase chain reaction. The numbers in parentheses represent the number of patients studied.

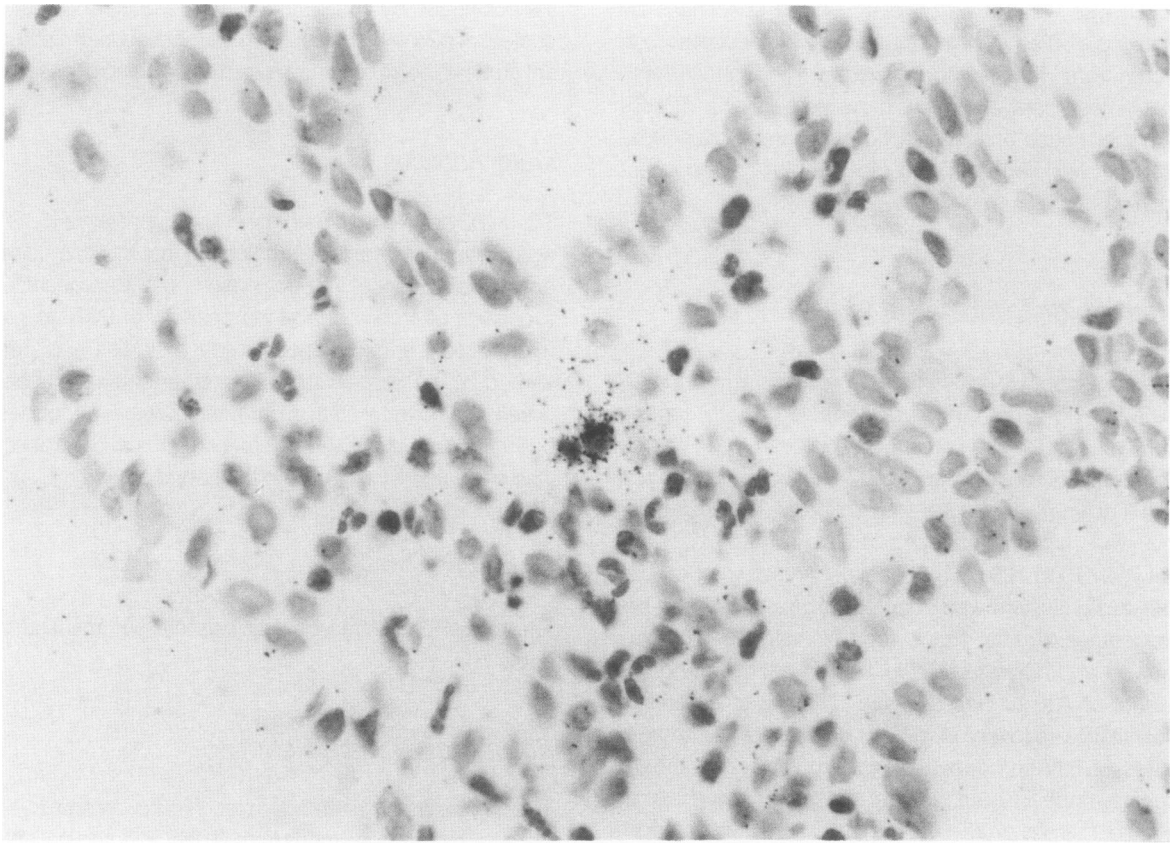

Figure 2. In situ *hybridization study showing silver grains over a mononuclear cell in the lamina propria, located within 30 microns from the base of a rectal crypt. The study indicates that the cell contains HIV RNA (H&E ×250).*

cases. In 25 cases, separate studies were done with antibodies to p17, p24, and gp120. Similar degrees of staining were found with p17 and p24, whereas no staining was seen with gp120. Antibody staining was focal, involving single cells or clusters of cells. Similar areas of focal staining were seen in serial sections.

Staining cells were found in the lamina propria or the epithelial layer in 67% of cases. The lamina propria cells resembled either lymphocytes and macrophages and were seen in 52% of biopsy specimens (Figure 3). Staining also was seen in the epithelium, in cells resembling intraepithelial mononuclear cells (34%) as well as epithelial cells (37%) (Figure 3). Cytoplasmic staining of varying intensity was seen in lamina propria mononuclear cells and cells in the epithelium. Some intraepithelial mononuclear cells had apparent crisp, curvilinear membrane staining similar to control positive tissue culture cells. Some cells also had nuclear staining. A wide variation in the number of staining cells per slide occurred, and many sections had only one or two stained cells.

Quantitative ELISA for HIV p24

Rectal biopsy specimens from all 168 patients were analyzed for p24 content by quantitative ELISA assay, and HIV p24 was detected in 68%. ELISA assays were performed on six separate rectal biopsy specimens from six patients to determine the intrinsic variability of HIV protein expression. No p24 was detected in any specimen in one patient. The coefficient of variation for the p24 assay in the other five patients was 30.0 ± 1.4% (mean ± SE, range, 25–36%).

Six patients had biopsy specimens taken from the ileum, right colon, left colon, and rectum during colonscopy, and their results were compared (Table 2). Statistically, HIV p24 content in the different areas did not differ, although p24 contents were higher in the ileum and rectum. Assays for p24 in samples from jejunum, ileum, right colon, and left colon also were performed on all specimens in the whole study group, and the results were analyzed. The prevalence and tissue content of p24 also were similar in different areas of the intestine (Table 2). Thus, HIV p24 antigen is found diffusely in the gastrointestinal tract.

Serum samples were analyzed for HIV p24 content in 148 patients, and the results were positive in 48%. The relative concentrations of p24 in serum and tissue were compared in 20 subjects after converting the results to p24 per wet weight of sample. HIV p24 content per wet weight of mucosa was much higher in tissue than in se-

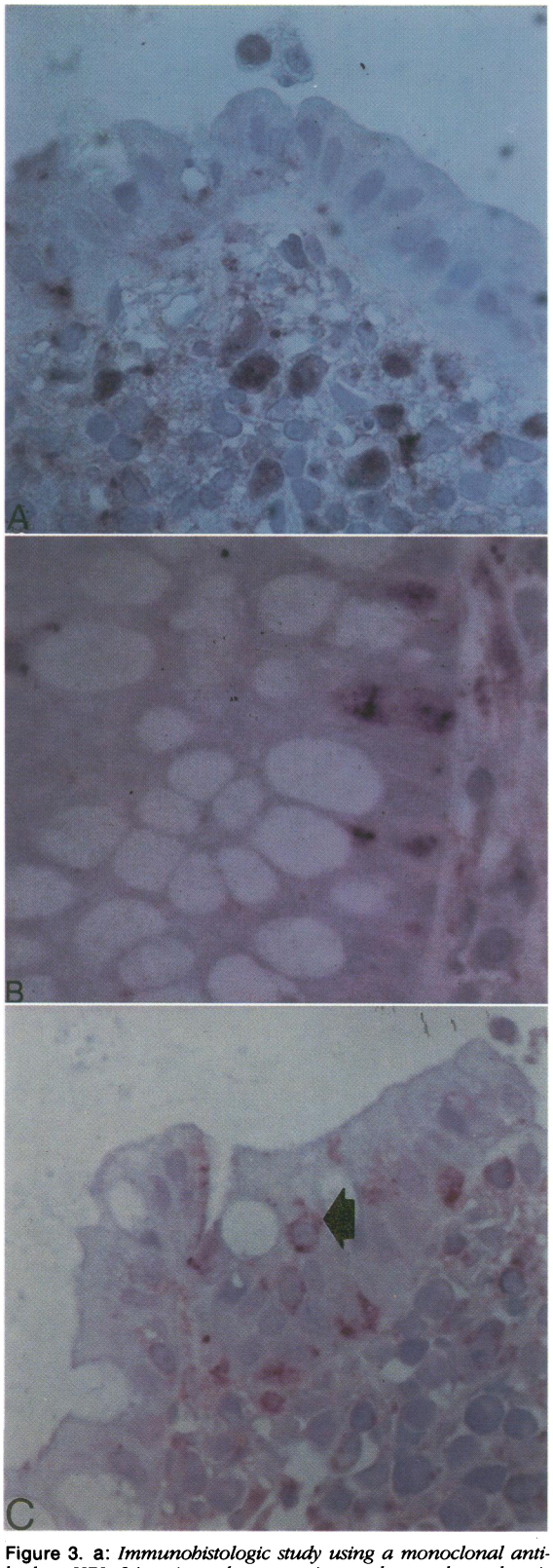

Figure 3. a: *Immunohistologic study using a monoclonal antibody to HIV p24 antigen, demonstrating nuclear and cytoplasmic staining of lamina propria mononuclear cells (×400).* b: *Immunohistologic study using a monoclonal antibody to HIV p24 antigen, demonstrating cytoplasmic staining in a crypt epithelial cell (×600).* c: *Immunohistologic study using a monoclonal antibody to HIV p24 antigen, demonstrating cytoplasmic staining in an intraepithelial mononuclear cell (arrow, ×400).*

rum, with results ranging between 200 to 1000 fold. The detection of p24 in serum and rectal mucosa did not correlate.

Correlations Between Tests for HIV in Intestinal Mucosa

The results of the quantitative ELISA studies were compared with the results of the *in situ* hybridization studies in the 48 patients having both studies (Table 3). The prevalence of positive *in situ* hybridization studies and positive ELISA assays correlated statistically ($P < 0.05$). In addition, the subgroup with positive *in situ* hybridization studies had significantly higher tissue p24 contents than patients with negative *in situ* hybridization studies (441 ± 114 vs. 148 ± 51 pg/mg prot, mean ± se, $P < 0.02$).

No statistical correlation was found between the results of PCR and *in situ* hybridization in the 20 patients having both studies performed. There also were no statistical correlations between the results of PCR and the immunohistologic studies. The prevalence of HIV as determined by ELISA and by *in situ* hybridization tissue content as determined by ELISA were compared with the prevalence of cellular staining by immunohistology in 48 and 73 cases, respectively, and did not correlate statistically with overall staining or with staining of any cell type. However, the presence of p24 by immunohistology was correlated in the three cell types detected (LPL vs. IEL, $P = 0.001$; LPL vs. EPI, $P = 0.004$; IEL vs. EPI, $P = 0.001$).

Discussion

The results of these studies show that HIV DNA, RNA, and protein antigens can be detected in mucosal tissues from the gastrointestinal tracts of HIV-infected patients, ranging in stages from persistent generalized lymphadenopathy to CDC-defined AIDS. HIV can be detected diffusely in the GI tract. Tissue content of p24 antigen in different areas did not differ statistically. When the results of all assays were combined, evidence of HIV was found in 166 of 168 rectal biopsy specimens.

There was a statistically significant correlation between the prevalence of detecting HIV RNA by *in situ* hybridization and by quantitative ELISA assay. In addition, tissue p24 content was significantly higher in biopsy specimens with positive *in situ* hybridization results than in specimens with negative results. These results suggest that the presence of detectable HIV RNA is associated with translation and HIV protein synthesis *in situ*, which supports the contention that virus production occurs in the GI tract.[14] HIV p24 was detected more frequently by quantitative ELISA than by *in situ* hybridization. The reason may be that samples for ELISA assay

Table 2. *Tissue p24 Detection in Different Regions of the GI Tract*

	Rectum	Left colon	Right colon	Ileum	Jejunum	Serum
Number tested	6	6	6	6	—	6
Tissue p24	112 ± 26	42 ± 12	56 ± 17	318 ± 220		10 ± 5
Number tested	168	23	27	10	47	154
Prevalence	68%	68%	74%	70%	53%	48%
Tissue p24	144 ± 25	76 ± 35	62 ± 15	84 ± 35	75 ± 22	25 ± 3
Tissue p24*	213 ± 34	110 ± 47	87 ± 15	119 ± 45	141 ± 37	51 ± 6

* Positives only.
Data expressed as pg/mg prot for tissue and pg/ml for serum, in mean ± SE. The top panel shows the results in six patients in whom all areas were biopsied. The lower panel shows the results in all the tissues that were tested.

are derived from the whole tissue biopsy, whereas the *in situ* studies were performed on a small fraction of the biopsy specimen, thus increasing the possibility of sampling error using the latter technique. These results imply that tissue ELISA and *in situ* RNA hybridization assay are examining the same stage of HIV infection, and that tissue ELISA is a more sensitive technique for detecting HIV infection than is *in situ* hybridization.

A lack of correlation between *in situ* hybridization and PCR is expected since PCR detects HIV DNA, which represents latently infected cells, whereas *in situ* hybridization detects HIV RNA, which represents productively infected cells. The lack of correlation between the immunohistologic and ELISA studies suggests that immunohistology is not adequate for quantitative analysis, although it may be a valid technique for cellular localization of HIV-infected cells, especially if combined with cell surface marker analysis.

The histologic studies demonstrated HIV in several cell types. *In situ* hybridization localized HIV RNA to lamina propria cells, presumably lymphocytes and macrophages. The immunohistologic studies demonstrated staining in lamina propria mononuclear cells, but also detected p24 in occasional epithelial cells and intraepithelial mononuclear cells. There are several possible explanations for the difference in results. The detection of HIV RNA implies the existence of a productive infection with transcription and viral protein synthesis. Virus production may occur in the epithelial cell but that the number of genome copies are below the limits of detection by *in situ* hybridization. However, the presence of HIV antigens in the absence of RNA may represent phenomena other than viral reactivation. Epithelial cells can absorb intact molecules and present antigens in association with Class II histocompatibility antigens.[15–16] Specific binding of HIV gp120 to gut epithelial cells has been demonstrated *in vitro.*[17] A significant proportion of intraepithelial mononuclear cells bear surface antigens of monocytes and also could serve phagocytic and antigen-presenting roles.[18] Thus, cells in the epithelium may absorb antigens from the lumen or the interstitial compartment and not be involved directly in virus production. The possibility that epithelial cells absorb viral particles or cellfree viral antigens is consistent with recent electron microscopic studies that show internalization of viral particles during *in vitro* infection of an small intestinal epithelial cell line.[19]

Studies from other laboratories have documented the localization of HIV and other retroviruses to gastrointestinal tissues. Levy et al. isolated HIV from intestinal biopsy specimens.[15] Several investigators have documented HIV DNA in intestinal biopsy specimens by *in situ* hybridization.[8,20] In those studies, cells in the lamina propria and epithelium hybridized with the DNA probe. Some of the epithelial cells appeared to be neuroendocrine cells, whereas others appeared to be normal crypt epithelial cells. Other investigators also have demonstrated HIV

Table 3. *Correlations Between the Different Assays for HIV*

	PCR	*In situ*	IP (any)	ELISA
PCR	—	NS	NS	NS
In situ		—	NS	<0.02
IP (any)			—	NS
ELISA				—
		IP (LPL)	IP (IEL)	IP (EPI)
IP (LPL)		—	<0.001	<0.002
IP (IEL)			—	<0.001
IP (EPI)				—

PCR = polymerase chain reaction, *in situ* = RNA *in situ* hybridization, IP = immunoperoxidase, any = any staining cell by immunoperoxidase, LPL = lamina propria mononuclear cell, IEL = intraepithelial cell, EPI = epithelial cell, ELISA = quantitative ELISA.

protein antigens in mucosal tissue sections by immunohistologic techniques.[9,21]

Studies using experimental cell culture systems have shown the ability of colon lamina propria and epithelial cell primary cultures and established cell lines to be productively infected with HIV.[22–25] Other epithelial cells such as found in the kidney also may have detectable HIV antigens or genome.[26–27] Studies using a feline retroviral model of AIDS demonstrated a strain with specific tropism for the gut as well as cellular localization of retroviral genome in the lamina propria.[28] The possibility that variations in cell tropism of different strains of HIV modulates infection of different cell types in the intestine is in keeping with current concepts of antigenic variation of HIV.[29] In those studies a viral core protein was demonstrated in the epithelium by immunohistologic techniques. The finding of retroviral genome in the lamina propria and protein antigens in the epithelium is similar to the results of this study.

The present studies demonstrate that HIV antigens can be quantitated in tissues by ELISA assay. The proper interpretation of a p24 measurement in tissue or serum is not clear. Tissue p24 content undoubtedly is related to viral antigen production, but the relationship between p24 content and production rate can be modified by the rates of virus-induced cell lysis, cytotoxic reactions against HIV-infected cells,[30] antigen release from living cells, and clearance of p24 released from cells.

The presence of HIV in the intestine does not necessarily imply a pathogenetic role in intestinal dysfunction. It is likely that widespread, low-level reactivation is occurring throughout the body during the occult phase of HIV infection, with no immediate clinical consequences. On the other hand, unexplained chronic inflammation of intestinal mucosa without identifiable enteric pathogens[1,2] is a common finding. Several pieces of evidence suggest that HIV might be involved in this process. We have identified characteristic histopathologic features in HIV-infected patients with altered bowel habits and correlated these results with elevated tissue contents of HIV p24, interleukin-1 and tumor necrosis factor (TNF).[31] Elevated tissue cytokine contents also are a feature of the inflammatory bowel diseases, ulcerative colitis and Crohn's disease.[32] Specific injury to epithelial cells (apoptosis)[33] located at or near the crypt base has been noted in rectal biopsy specimens from AIDS patients.[1,34] Similar changes have been seen in acute graft-vs.-host disease after bone marrow transplantation and other immunologic reactions.[35] Many studies have demonstrated an active immune response to cells expressing HIV antigens in HIV-infected patients.[30] Cytokines or other mediators released as a result of the inflammatory response could promote HIV reactivation, as shown in *in vitro* studies using monocytic and lymphocytic cell lines.[36] Studies in progress correlating clinical symptoms and histopathologic findings with evidence of HIV infection will help clarify the possible pathogenetic role of HIV in gastrointestinal disease in HIV infection.

In other studies, we showed evidence that HIV p24 is produced during short-term (48 hr) incubation of rectal mucosa *in vitro*.[37] The ability to quantitate p24 antigen production in tissue explants *in vitro* may be an important tool to test the ability of antiviral and other agents to inhibit HIV production. Studies to correlate tissue p24 content with tissue contents of endogenous antiviral agents such as interferons, or putative reactivating agents such as cytokines also are possible.

In summary, HIV genome or protein antigens can be detected, localized to several cell types, and quantitated in the intestinal mucosa in most HIV-infected patients. The possibility that epithelial cells are naturally infected with HIV was not resolved in this study. The possible association between HIV expression and intestinal disease remains to be clarified.

Acknowledgment

The authors thank Dr. Cecil H. Fox of the Laboratory of Immunoregulation at the National Institutes of Health for performing the studies using the polymerase chain reaction technique.

References

1. Kotler DP, Gaetz HP, Klein EB, Lange M, Holt PR: Enteropathy associated with the acquired immunodeficiency. Ann Intern Med 1984, 101:421–8
2. Gillin JS, Shike M, Alcock N, et al: Malabsorption and mucosal abnormalities of the small intestine in the acquired immunodeficiency syndrome. Ann Intern Med 1985, 102:619–24
3. Kotler DP: Intestinal and hepatic manifestations of AIDS. Adv Intern Med 1988, 34:43–71
4. Laughon BE, Druckman DA, Vernon A, et al: Prevalence of enteric pathogens in homosexual men with and without acquired immunodeficiency syndrome. Gastroenterology 1988, 94:984–9
5. Dworkin B, Wormser GP, Rosenthal WS, et al: Gastrointestinal manifestations of the acquired immunodeficiency syndrome: A review of 22 cases. Am J Gastroenterol 1985, 80:774–9
6. Smith PD, Lane HC, Gill VJ, et al: Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS). Ann Intern Med 1988, 108:328–33
7. Fox CH, Kotler DP, Tierney AR, Wilson CS, Fauci AS: Detection of HIV-1 RNA in intestinal lamina propria of patients with AIDS and gastrointestinal disease. J Infect Dis 1989, 159:467–71
8. Nelson JA, Wiley CA, Reynolds-Kohler C, et al: Human im-

munodeficiency virus detected in bowel epithelium from patients with gastrointestinal symptoms. Lancet 1988, 2:259–62
9. Ullrich R, Zeitz M, Heise M, L'age M, Hoffken G, Rieken EO: Small intestinal structure and function in patients infected with human immunodeficiency virus (HIV): Evidence for HIV-induced enteropathy. Ann Intern Med 1989, 111:15–21
10. Redfield RR, Wright DC, Tramont EC: The Walter Reed staging classification for HTLVIII/LAV infection (special report). N Engl J Med 1986, 314:23–7
11. Saiki RK, Gelfand DH, Stoffel S, et al: Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988, 239:487–91
12. Lowry OH, Roseborough NJ, Farr AL, Randall RJ: Protein measurement with the folin reagent. J Biol Chem 1951, 193:265–75
13. Fleiss JL: Statistical Methods for Rates and Proportions. 2nd edition, New York: John Wiley, 1981
14. Barnett SW, Barboza AJ, Wilcox CM, Forsmark CE, Levy JA: Characterization of HIV-1 Strains Recovered From the Bowel. VI International Conference on AIDS, 1990, 3:112
15. Bland PW, Warren JG: Antigen presentation by epithelial cells of the small intestine. 1. Kinetics, antigen specificity and blocking by anti-Ia antisera. Immunology 1986, 58:1–7
16. Mayer L, Shlien R: Evidence for function of Ia molecules on gut epithelial cells in man. J Exp Med 1987, 166:1471–83
17. Xu YL, Ramirez A, Ernani F, Moyer MP: Human Immunodeficiency Virus (HIV) binding to gastrointestinal cells cultured *in vitro*. Gastroenterology 1990, 98:A481
18. Austin LL, Dobbins WO: Intraepithelial leucocytes of the intestinal mucosa in normal man and in Whipple's disease. A light and electron microscopic study. Dig Dis Sci 1982, 27:311–20
19. Bourinbaiar AS, Phillips DM: Transmission of human immunodeficiency virus from monocytes to epithelia. J Acq Immun Def Syn 1991, 4:56–63
20. Mathijs JM, Hing M, Grierson J, Dwyer DE, et al: HIV infection of rectal mucosa. Lancet 1988, 1:1111
21. Rene E, Jarry A, Brousse N, et al: Demonstration of HIV infection of the gut in AIDS patients: Relation with symptoms and other digestive infection. Gastroenterology 1988, 94:373A
22. Adachi A, Koenig S, Gendelman HE, et al: Productive, persistent infection of human colorectal cell lines with human immunodeficiency virus. J Virol 1987, 61:209
23. Moyer MP, Ramirez A Jr, McKee-Suarez MS, Gendelman HE: Human Immunodeficiency Virus (HIV) Infection of Gastrointestinal (GI) Cell Cultures. VI International Conference on AIDS, 1990, 2:140
24. Evans L, Corbell JE, Edwards P, Vasak E, Cooper D, Penny R: Infection of Colon Epithelial Cells by HIV-1. VI International Conference on AIDS, 1990, 2:141
25. Sierra-Madera J, Yeh-Lieberman B, Profitt MR, Fiocchi C: Enhanced replication of the human immunodeficiency virus (HIV) in intestinal mononuclear cells as compared to peripheral blood. Gastroenterology 1990, 98:A474
26. Cohen AH, Sun NCJ, Shapshak P, Imagawa DT: Demonstration of human immunodeficiency virus in renal epithelium in HIV-associated nephropathy. Mod Pathol 1989, 2:125–8
27. Cronin W, Shevchuk M, daSilva M, Bagchi S: The Prevalence of HIV Positivity in Kidneys of AIDS patients. VI International Conference on AIDS, 1990, 2:212
28. Hoover EA, Mullins JI, Quackenbush SL, et al: Experimental transmission and pathogenesis of immunodeficiency syndrome in cats. Blood 1987, 70,1880
29. Cordonnier A, Montagnier L, Emermen M: Single amino acid changes in HIV envelope affect viral tropism and receptor binding. Nature 1989, 340:571–4
30. Ealker BD, Plata F: Editorial review: Cytotoxic T lymphocytes against HIV. AIDS 1990, 4:177–84
31. Reka S, Borcich A, Kotler DP: Quantitation and modulation of HIV p24 antigen content in rectal mucosa. Gastroenterology 1989, 96:A412
32. Ligumsky M, Simon PL, Karmeli F, Rachmilewitz D: Role of interleukin 1 in inflammatory bowel disease—enhanced production during active disease. Gut 1990, 31:686–9
33. Weedon D, Searle J, Kerr JF: Apoptosis. Its nature and implications for dermatopathology. Am J Dermatopathol 1979, 1:133–44
34. Kotler DP, Weaver SC, Terzakis JA: Ultrastructural features of epithelial cell degeneration in rectal crypts of patients with AIDS. Am J Surg Pathol 1986, 10:531–8
35. Gallucci BB, Sale GE, McDonald GB, Epstein R, et al: The fine structure of human rectal epithelium in acute graft-versus-host disease. Am J Surg Pathol 1982, 6:293–305
36. Rosenberg ZF, Fauci AS: Minireview: Induction of expression of HIV in latently or chronically infected cells. AIDS Res Hum Retrovir 1989, 5:1–4
37. Reka S, Kotler D: Intestinal inflammation associated with HIV infection. VI International Conference on AIDS, 1990, 1:102