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Intestinal Mucosal Inflammation Associated with Human Immunodeficiency Virus Infection

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The role of the human immunodeficiency virus type-1 (HIV) in producing intestinal disease was studied prospectively in 74 HIV-infected individuals with (43) or without (31) the acquired immunodeficiency syndrome (AIDS). Thirty-one subjects had enteric infections; all but one had AIDS. Alteration in bowel habits was the most common symptom and occurred independently of enteric infections. Abnormal histopathology was present in 69% of cases, and the finding was associated with altered bowel habits. An HIV-associated protein, p24, was detected in 71% of biopsies by ELISA assay. Tissue p24 contents varied with disease stage and were highest in HIV-infected individuals without AIDS (Walter Reed classes 3 and 4). Tissue p24 detection was associated with both altered bowel habits and histologic mucosal abnormalities. Tissue contents of the cytokines, tumor necrosis factor-α and interleukin-1β, were higher in HIV-infected individuals than in controls and their elevations were independent of enteric infection. We conclude that HIV reactivation in the intestinal mucosa may be associated with an inflammatory bowel syndrome in the absence of other enteric pathogens.

KEY WORDS: human immunodeficiency virus; AIDS; inflammation; colitis; cytokines; prostaglandins; leukotrienes; ELISA; radioimmunoassay; diarrhea.

Diarrhea is a common symptom in patients infected with the human immunodeficiency virus type-1 (HIV) (1). A wide variety of microorganisms and other complications have been shown to cause gastrointestinal dysfunction and diarrhea in AIDS patients (2). However, a substantial proportion of patients have no etiologic explanation for their symptoms after diagnostic evaluation (3–8), reaching 50% in some series. In these patients, nonspecific inflammation may be seen on mucosal biopsy, and there is no sustained clinical response to routine antibiotic and antiparasitic regimens (9, 10).

Several authors have suggested that intestinal inflammation is a direct consequence of HIV infection (11, 12). Previous studies have established that the gastrointestinal tract contains cellular reservoirs for HIV. Evidence of HIV has been found using the polymerase chain reaction technique, RNA and DNA in situ hybridization, immunohistologic studies using monoclonal antibodies to HIV-associated protein antigens, and antigen capture ELISA (12–15).

The relationship between HIV infection and intestinal disease has been obscure. Retroviral infections are widespread in the body (16), and the detection of infected cells in the gastrointestinal tract

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TABLE 1. WALTER REED CLASSIFICATION SYSTEM*

Class 2: CD4 cells >400,	persistent generalized lymphadenopathy
Class 3: CD4 cells <400,	normal skin reactivity
Class 4: CD4 cells <400,	partial anergy
Class 5: CD4 cells <400,	oral candidiasis
Class 6: CD4 cells <400,	complications diagnostic of AIDS

*Adapted from Ref. 18.

may represent no more than background infection. On the other hand, certain animal retroviruses have been shown to be tropic for the gastrointestinal tract and to be associated with an enteropathy (17).

The aims of this study were to determine the presence of an HIV-associated antigen, p24, in rectal mucosa and its association with clinical symptoms, enteric pathogens, histologic changes, and biochemical evidence of mucosal inflammation.

MATERIALS AND METHODS

Study Subjects. This was a prospective, sequential study of 74 evaluations in 71 HIV-infected patients who were referred for unexplained gastrointestinal or proctologic symptoms or who volunteered for study (three patients). Studies were performed twice in three patients, after a change in the clinical disease stage (see below). Seven patients with known cryptosporidiosis who were referred for a treatment trial and had baseline evaluations also were included. There were 67 men and four women. Sixty-three men were homosexual or bisexual males, five patients were intravenous drug abusers, two females were presumed infected during heterosexual sexual intercourse, and one male was infected during blood transfusion. The presence of HIV infection was determined by ELISA testing with confirmation by western blot analysis and the diagnosis of AIDS was based upon criteria established by the Centers for Disease Control (18). Many of the subjects in this study were used in a parallel study of intestinal histopathology (19). The results from 16 HIV-seronegative heterosexuals without intestinal symptoms who had been evaluated as paid volunteers were used as the control group.

The HIV-infected individuals were subclassified using a modified Walter Reed classification scheme (Table 1) (20). Ten subjects were in class 2, 11 were in classes 3 or 4, 10 were in class 5, and 43 patients had AIDS and were in class 6. Walter Reed classes 3 and 4 differ only in the presence or absence of skin reactivity to recall antigens. Since this parameter was not measured, the two subgroups were combined.

Specific informed consent was obtained before any study parameters were determined. The studies and consent form were approved by the Institutional Review Board at the St. Luke's-Roosevelt Institute for Health Sciences.

Clinical Protocol. All subjects completed a symptom questionnaire, which included detailed questions about bowel habits, sexual history, and medical history, including enteric diseases. In this study, altered bowel habits was defined as having three or more stools per day of altered consistency for at least three days per week for at least one month. Stool examination for routine enteric pathogens and ova and parasites had been performed or were done at least three times if negative in patients complaining of altered bowel habits. Twenty milliliters of blood was drawn immediately prior to sigmoidoscopy and placed in plain glass tubes and in tubes containing EDTA (5 mM) and aprotinin (0.7 units/ml, Sigma Corp, Worthington, New Jersey). The blood was centrifuged at 4° C within 5 min and the plasma stored at −80° C.

Sigmoidoscopy with or without colonoscopy was performed. Biopsies from the distal 15 cm of colon were pooled and processed as a separate tissue block. In addition, up to eight additional biopsies were taken from the rectum and frozen on dry ice within 30 sec, then kept at −80° C. Other biopsies were oriented on gelfoam and placed in culture media for incubation. Other gastrointestinal and clinical evaluations were based upon the presenting symptoms and signs.

Histopathology. Histopathologic assessment was made by a blinded observer (FC). Quantitative estimates of lymphoid cellularity were made in 10 high-power fields. The cells counted included lymphocytes, plasma cells, eosinophils, macrophages, neutrophils, and intraepithelial lymphocytes. In preliminary studies, the results correlated well with a point counting technique (19).

For the purposes of this study, a biopsy was classified as abnormal and demonstrating inflammation if it met one of several criteria. These included mucosal infiltration of one or more types of lymphoid cells, degranulating eosinophils, or detectable enteric pathogens, which are known to cause mucosal inflammation. The organisms detected, cryptosporidium, cytomegalovirus, *Mycobacterium avium intracellulare,* all were associated with other evidence of mucosal inflammation in this study. Abnormal infiltration of lymphocytes, macrophages, neutrophils, and plasma cells was defined as more than two standard deviations from the mean of the results in the control group. Abnormal infiltration of eosinophils was defined as more than twice the median in the control group, as the data were skewed. Other histopathologic findings such as focal epithelial cell necrosis (apoptosis), prominent lymphoid aggregates, histiocytic aggregates, patchy lymphoid atrophy, nuclear debris, and edema were noted but were not used as evidence of mucosal inflammation, as other studies in HIV infection did not establish a relationship between their occurrence and quantitative histopathology (19).

Hematoxylin and eosin stains were used for the detection of cytomegalovirus inclusions and cryptosporidia, while acid-fast staining was used for the detection of mycobacteria. In seven cases, the diagnosis of CMV colitis was confirmed using a commercially available *in situ* DNA hybridization kit (Enzo Biochemicals, New York, New York). All cases of small intestinal microsporidiosis were confirmed by electron microscopy (courtesy of Dr. J. Orenstein).

Assays. Mucosal HIV p24 antigen content was determined in rectal biopsies frozen at the time of sigmoidoscopy, using a quantitative antigen capture ELISA kit

(Coulter Immunologicals, Hialeah, Florida), as described in detail previously (12). The assay is linear between 10 and 125 pg/ml, and control tissues are uniformly negative. Standard curves made in media, serum, or tissue homogenate are virtually identical. Addition of known amounts of p24 to a tissue containing p24 yielded greater than 75% recovery. The coefficient of variation for repeated rectal biopsies is about 30% (12). Protein content was determined using the Folin reagent, and the data are expressed as picograms of p24 per milligram of protein.

Tumor necrosis factor-α (TNF-α) content of rectal mucosa was determined in 55 patients and seven controls by a quantitative ELISA technique as recommended by the manufacturer (T Cell Sciences, Cambridge, Massachusetts) on homogenates made from different biopsies than used for the p24 determination. Aprotinin (6.7 units/ml homogenate) (Sigma) and EDTA (5 mM) were added to the tissue before homogenization. All steps were carried out at 4° C. Tissues were homogenized in phosphate-buffered saline, pH 7.4, centrifuged at 13,000 rpm for 1 min, then passed through a 0.2-μm filter. The recovery of exogenous TNF added to control specimens prior to homogenization was about 60%, while the recovery of exogenous TNF added after homogenization was about 80%. Standard curves were linear between 20 and 1000 pg/ml. The data are expressed as picograms per milligram of protein.

Interleukin-1β (IL-1β) content of rectal biopsies was determined by quantitative ELISA using the technique recommended by the manufacturer (Cistron, Pinebrook, New Jersey) in 59 patients and 10 controls. Interleukin-1 production also was assayed in 26 HIV-infected patients and seven controls in tissue samples incubated in supplemented RPMI media for 24 hr at 37° C and 5% CO_2–95% O_2. Tissues were homogenized in the incubation media at 4° C and pH 7.4, centrifuged at 13,000 rpm for 1 min, then passed through a 0.2-μm filter. The recoveries of exogenous IL-1 when added to samples from control tissues without detectable IL-1 were 68–90%. Standard curves were linear between 30 and 1000 pg/ml. The data are expressed as picograms per milligram of protein.

Prostaglandin E_2 (PGE_2) content was determined in rectal mucosal samples that had been rapidly frozen and stored at −80° C in 40 patients and five controls by ^3H radioimmunoassay as recommended by the manufacturer (Advanced Magnetics, Cambridge, Massachusetts). Tissue was homogenized in 1 ml 50 mM Tris buffer with 0.02 M EDTA, pH 7.4, acidified to pH 3.0; then PGE_2 was extracted with ethyl ether (21). Recovery was determined in every sample using [^3H]6-keto-prostaglandin $F_{1\alpha}$ (Amersham, Arlington Heights, Illinois) (22). The recoveries ranged from 57 to 75%. The cross-reactivity of antiserum to 6-keto-$PGF_{1\alpha}$ and other arachidonic acid metabolites was less than 1.0% at 50% B/B_0. The sensitivity of the assay was 8.2 pg/0.1 ml. Data are expressed as nanograms per milligram of protein.

Leukotriene B_4 (LTB_4) content was determined by ^3H radioimmunoassay in mucosal biopsies from 27 HIV-infected patients and six controls that had been rapidly frozen and kept at −80° C. Samples were homogenized in a buffer containing Tris HCl (50 mM) at pH 7.4. After the addition of absolute ethanol, the tubes were centrifuged at 3000 rpm for 15 min. The ethanol was evaporated and the precipitate dissolved in a Krebs–phosphate buffer, pH 3.5, containing 10% ethanol; then LTB_4 was extracted using Sep-Pak C-18 cartridge (Waters Inc., Milford, Massachusetts) (22, 23). The cartridge was washed with ethanol and petroleum ether; then the LTB_4 was collected using methyl formate. After evaporation under pure N_2, the precipitate was dissolved in RIA buffer and assayed using a [^3H]LTB_4 RIA kit (New England Nuclear, Boston, Massachusetts) (23, 24). The sensitivity of the assay was 12.5 pg/0.1 ml. Cross-reactivity of the antiserum to LTC_4, to metabolites of LTB_4, or to other arachidonic acid metabolites was less than 1% at B/B_0 = 50%. The amount of tracer used for the determination of recovery caused a negligible increase in background. Data are expressed as nanograms per milligram of protein.

Statistical Analysis. Comparison of the results of tissue p24, cytokine, PGE_2, and LTB_4 contents with disease stage was performed by ANOVA and by the Mann-Whitney test. Other specific associations between study parameters were determined by chi-square analysis.

RESULTS

Clinical Symptoms. The majority of patients (55 of 74, 75%) reported intermittent or persistent alterations of bowel habits with increased frequency and altered consistency of stools. Thirty-two of 43 AIDS patients, including 25 patients with weight loss, and 23 of 31 patients in Walter Reed classes 2–5 reported altered bowel habits. Other symptoms included abdominal pain in 24 patients, anorectal pain in 16, rectal bleeding in 4, visible mucus in the stools in 19, and mucoid or purulent drainage from the rectum in 17. Seventeen patients had a history of anorectal herpes simplex virus infection, although severe disease was present in only two patients at the time of evaluation. Thirteen of the 74 patients were taking AZT at the time of evaluation.

Enteric Pathogens. Enteric pathogens were found in 31 patients, including 30 AIDS patients and one Walter Reed class 5 patient. All patients with enteric pathogens complained of altered bowel habits, while 24 patients with altered bowel habits had no enteric pathogens identified, including two patients with AIDS and 22 patients without AIDS. The enteric pathogen was found in the colon in 24 cases and was found only in the small intestine in seven cases. The enteric pathogens included cytomegalovirus in 17 cases, cryptosporidia in 14 cases, microsporidia in the small intestine in 5 cases, the latter confirmed by electron microscopy, and *Mycobacterium avium intracellulare* in 4 cases. Some patients had more than one pathogen. One Walter Reed class 5 patient had a *Campylobacter* species isolated on stool culture. The symptoms were un-

TABLE 2. MUCOSAL INFLAMMATORY CELL POPULATIONS IN HIV+ SUBJECTS AND CONTROLS*

	HIV+	*Controls*
Lymphocytes	149 ± 14 (36)	220 ± 10
Plasma cells	81 ± 8 (41)	80 ± 10
Eosinophils	22 ± 4 (49)	10 ± 1
Macrophages	22 ± 2 (42)	20 ± 4
Neutrophils	10 ± 3 (22)	4 ± 0.4
Degranulating eosinophils	28 (38)	0

*Data as mean ± SE of cells per 10 high-power fields. The data for degranulating eosinophils represent the number showing this pathologic finding. The numbers in parentheses represent the percentage of biopsies from HIV+ subjects that were >2 SD from the mean of the controls (lymphocytes, macrophages, plasma cells, neutrophils), >2× the median of the controls (eosinophils), and degranulating eosinophils.

changed after effective treatment of the infection with erythromycin.

Thus, many HIV-infected patients have altered bowel habits in the absence of an enteric pathogen. The majority of patients with altered bowel habits and no identifiable pathogen in this series were HIV-infected patients without AIDS.

Gross and Histopathologic Examinations. The epithelium generally was intact, on endoscopic examination of the colon and rectum, except in cytomegalovirus infections, where erosions and shallow ulcerations of varying sizes were seen. Many cases demonstrated patchy or diffuse mild erythema with edema or spasm in the sigmoid colon. In several cases, scattered tiny vesicles or minute erosions of 1–2 mm with a thin erythematous rim but no viral inclusions on histologic examination were noted. Intramucosal nodules approximately 1–2 mm in size were seen in the rectal mucosa of several patients. These were shown on biopsy to represent enlarged mucosal lymphoid follicles. Abnormal findings on anorectal examination included hemorrhoids with erosions, condylomata, leukoplakia of the anal canal, perianal herpes simplex infection, anal fissures, and anal fistulae.

On histopathologic assessment, 69% of the rectal biopsies were classified as abnormal, including 71% of AIDS patients and 66% of patients without AIDS (Table 2). Mucosal histologic changes were equally prevalent in patients with or without colonic pathogens. The presence of histopathologic abnormalities was associated with altered bowel habits ($\chi^2 = 16.6$, $P < 0.001$).

Thus, HIV-infected patients may have histologic evidence of mucosal injury and inflammation associated with alterations in bowel habits, in the presence or absence of enteric pathogens.

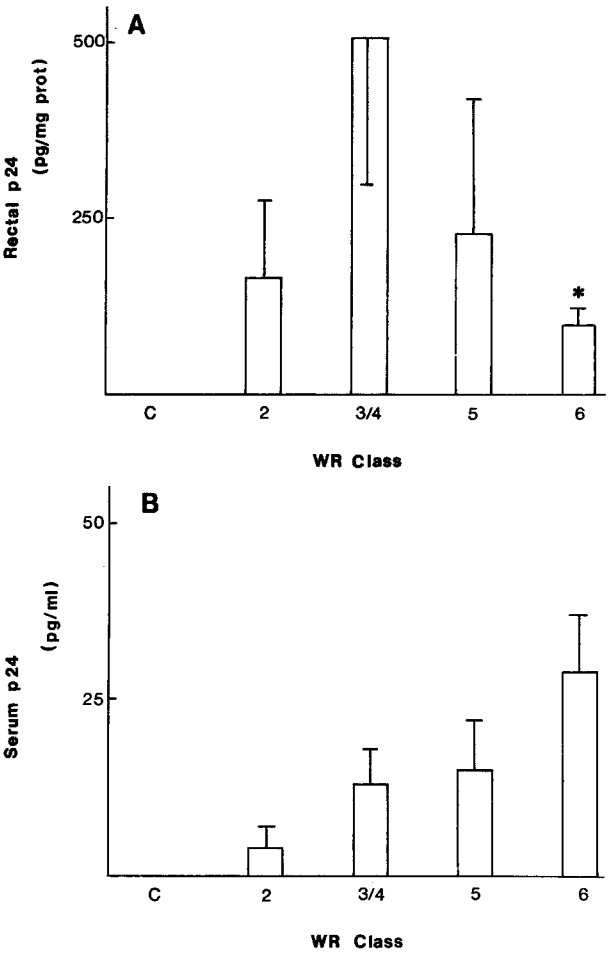

Fig 1. (A) Rectal mucosal HIV p24 content, determined by quantitative ELISA, as a function of disease stage, using the Walter Reed classification system. Rectal p24 content varied with disease stage ($P < 0.02$, WR class 3/4 vs WR class 6). (B) Same as A: mean serum p24 content was higher in more advanced disease stages.

Quantitative ELISA for HIV p24. HIV p24 antigen was detected in 71% of rectal biopsies from HIV-infected subjects, and no controls. HIV p24 content varied with disease stage (Figure 1). Tissue contents were low in Walter Reed (WR) class 2 patients, then rose to peak values in patients with WR class 3 or 4. HIV p24 contents also were high in WR class 5 but were significantly lower in WR class 6, representing AIDS patients, than in WR class 3 or 4 ($P < 0.02$). No differences in p24 content were found in patients taking or not taking AZT.

Tissue p24 contents were higher in subjects with altered bowel habits than in those without this symptom in the whole group (208 vs 109 pg/mg

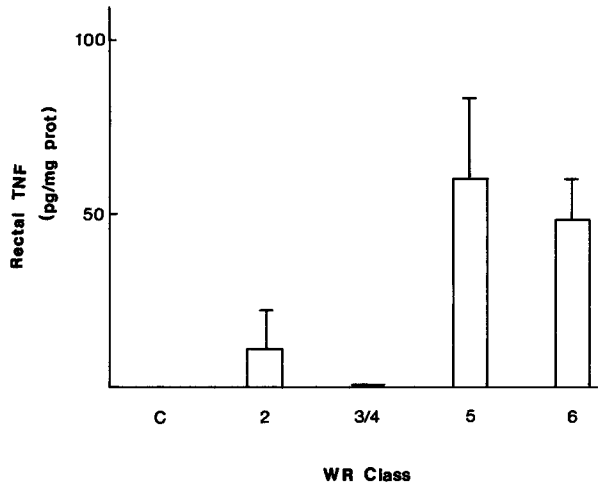

Fig 2. Same as Figure 1A. Rectal mucosal TNF contents were higher in HIV-infected subjects than in controls ($P = 0.05$).

protein, 55 vs 19 subjects) and in the subgroup without enteric pathogens (352 vs 109 pg/mg protein, 24 vs 19 subjects). The differences were not statistically significant. However, HIV p24 detection was associated with histologic alterations as defined in the methods (Mann-Whitney; $Z = 2.81$, $P < 0.005$). The relationship was stronger when patients with identifiable colonic pathogens were excluded from analysis (Mann-Whitney; $Z = 3.12$, $P < 0.001$). Serum samples contained detectable p24 in 47% of HIV-infected subjects (Figure 1). Serum p24 contents rose with disease progression. There was no association between the detection of p24 in serum and in rectal mucosa.

Thus, HIV-associated antigen production, as detected by antigen capture ELISA, is associated with histopathologic changes in the presence or absence of other enteric pathogens.

Tissue Cytokine Contents. Tumor necrosis factor was detected in 40% of the mucosal samples from HIV-infected patients (Figure 2) and contents were highest in WR class 5. In comparison, TNF was found in two of nine plasma samples from HIV-infected patients tested. TNF was not detected in rectal mucosa or plasma from normal volunteers.

Interleukin-1β was detected in tissue specimens from 61% of HIV-seropositive patients, and tissue contents were higher than in controls ($P < 0.01$ by ANOVA) (Figure 3A). The variation of tissue IL-1 content with disease stage was similar to rectal p24, with the highest values detected in WR class 3 or 4, and lower values in WR class 6. Interleukin-1 was not detected in plasma samples from 12 HIV-infected subjects.

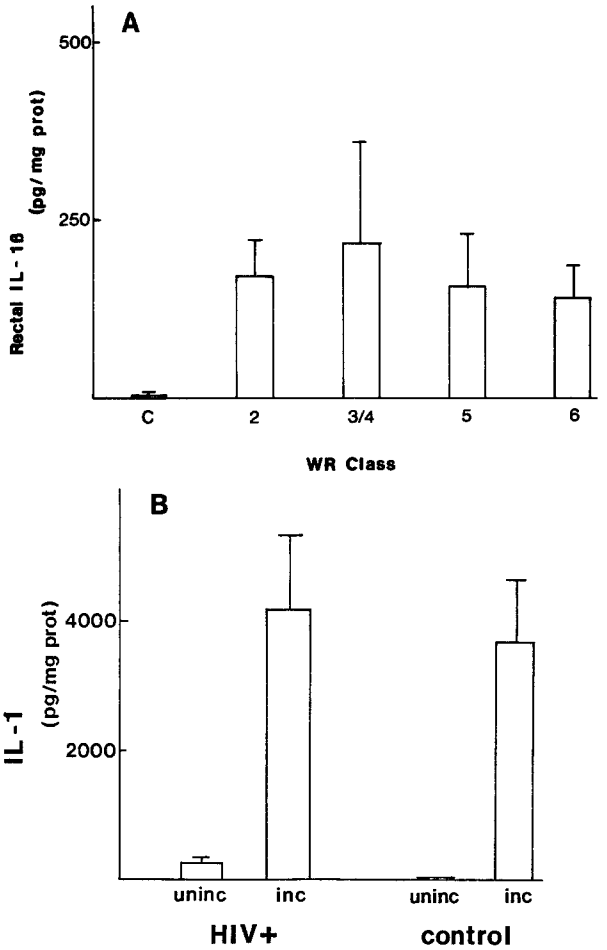

Fig 3. (A) Same as Figure 1A. Rectal mucosal IL-1β contents were higher in HIV-infected subjects than in controls ($P < 0.01$). (B) Rectal mucosal IL-1β contents were compared in HIV-infected subjects and controls. Tissue IL-1β contents in unincubated tissues were higher in HIV-infected subjects than in controls ($P < 0.02$). IL-1β contents rose markedly during 24 hr of incubation to similar levels in HIV-infected subjects and controls.

In order to assess the relative magnitude of the increase in tissue IL-1, its contents in tissue and media also were determined, after short-term incubation, in subgroups of 26 HIV-infected patients and seven controls. Interleukin-1 content of unincubated tissue was higher in HIV-infected subjects than in controls ($P < 0.02$ by ANOVA, Figure 3B). Twenty-four-hour incubation resulted in a marked increase in the IL-1 content in tissue and media in both subgroups to a similar level. The difference in results from unincubated tissues between HIV-positive and controls were small compared to the increases in both groups seen after 24 hr of incubation. These data suggest that baseline production of

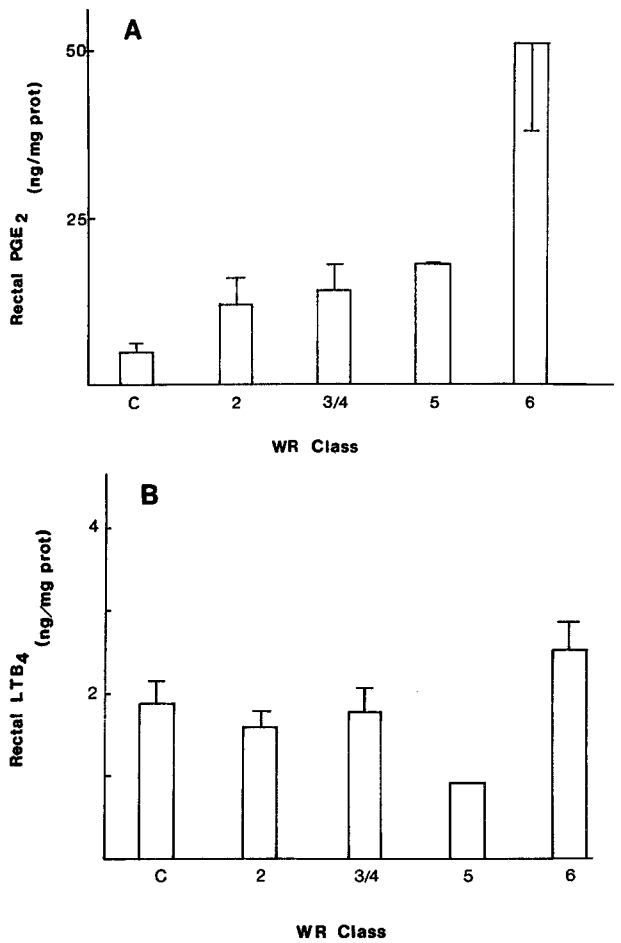

Fig 4. (A) Same as Figure 1A. Rectal mucosal PGE_2 contents were higher in HIV-infected subjects than in controls ($P < 0.002$). (B) Same as Figure 1A. Rectal mucosal LTB_4 contents were higher in WR class 6 patients than in other groups (NS).

IL-1 in rectal mucosa of HIV-infected patients is mildly increased.

There were no significant associations between the detection of the cytokines with stage of disease, colonic pathogens, mucosal inflammation, rectal p24 content, or with each other.

Tissue PGE_2 and LTB_4 Content. Prostaglandin E_2 was detected in rectal mucosal samples in all study subjects, including HIV-seronegative controls. Tissue PGE_2 contents were higher in HIV-infected patients than in controls ($P < 0.002$ by ANOVA, Figure 4) and contents were highest in WR class 6. Tissue LTB_4 contents were highest in WR class 6 patients, but the results were not significantly different from results in WR classes 2–5 and controls. There were no associations between PGE_2 or LTB_4 contents and colonic pathogens, mucosal inflammation, p24 content, or cytokine contents.

Thus, tissue contents of selected mediators of immune and inflammatory responses are higher in rectal mucosa of HIV-infected patients than controls, in the presence or absence of identifiable enteric pathogens.

DISCUSSION

The results of these studies suggest that HIV-associated antigen expression is associated with an inflammatory disease of intestinal mucosa in patients with or without AIDS. Interrelationships among mucosal HIV p24 antigen, altered bowel habits, and mucosal inflammation were noted and were independent of the presence of identifiable enteric pathogens. Tissue contents of the cytokines, TNF-α and IL-1β, and the inflammatory lipid mediators, PGE_2 and LTB_4, generally were higher in tissues from HIV-infected and AIDS patients than in controls, providing biochemical evidence of inflammation. While no asymptomatic HIV-seropositive or AIDS control group was available for study, 19 HIV-infected subjects had been referred for evaluation of proctologic complaints or volunteered for the study and did not complain of altered bowel habits. This group was used as an HIV-positive control group for those with altered bowel habits. In a companion study examining quantitative rectal histopathology, results from HIV-seronegative homosexual controls and heterosexual controls did not differ (19).

It is difficult to rule out the possibility of unidentified pathogens. Thirty of 33 AIDS patients with altered bowel habits harbored an enteric pathogen, while only one of 23 non-AIDS patients with altered bowel habits had an identifiable pathogen. It is unlikely that chronic infection with an unidentifiable pathogen would occur most commonly in WR class 3 or 4 patients. In other studies, specialized techniques for the detection of unusual enteric pathogens did not find evidence for such infections in HIV-infected individuals (25, 26). Some studies detected unusual organisms such as *Campylobacter*-like organisms (26) and spirochetes (27), but their presence was not limited to symptomatic patients.

The range of enteric pathogens found in the AIDS patients in this study differs from most published studies, in which bacteria such as *Salmonella, Shigella,* and *Campylobacter,* and parasites such as *Giardia lamblia* or *Entameba histolytica* are found in a substantial proportion of patients (3–8). The

explanation may lie in the pattern of referral of patients for evaluation. In this study, performed between September 1988 and March 1990, the referring physicians were experienced in the management of HIV-infected patients, and patients with enteric pathogens identifiable on stool examination were treated successfully and not referred. Many patients gave histories of amebiasis, giardiasis, or anorectal herpes simplex virus infection that was treated at some time in the past. Of the four pathogens found in AIDS patients, three (CMV, MAI, and microsporidia) require histologic confirmation for diagnosis.

The presence of HIV in rectal mucosal was determined by quantitative antigen capture ELISA. Previous studies demonstrated similar contents of p24 at different sites in the small intestine and colon, implying that HIV infection is diffuse in the gastrointestinal tract (12). Several other studies also have demonstrated HIV genome or protein in intestinal mucosa (13–15). Studies in cell lines using primary culture or established tumor cell lines demonstrate that epithelial cells can be productively infected with HIV under experimental conditions (28–30).

Tissue p24 contents varied with disease stage, with the highest levels found in WR class 3 or 4. Tissue p24 content was significantly higher in this group than in AIDS patients (WR class 6) (Figure 1). A possible explanation for this finding is that WR class 3 or 4 patients have more intestinal lymphocytes able to be infected and to produce p24 than do WR class 6 patients. The companion study demonstrated significant associations between tissue p24 content and lymphocyte counts in the lamina propria, determined morphometrically, but no association between p24 content and the macrophage counts (19).

Tissue p24 contents were higher in patients with altered bowel habits than in those without this symptom. Rectal p24 content also was associated significantly with altered mucosal histology, especially when patients with colonic pathogens identified during evaluation were excluded. On the other hand, rectal p24 content had no association with serum p24 content. This result implies either that serum p24 is derived from other sites or that its measurement is affected by other factors, such as antibody binding or clearance.

The results of the cytokine assays provide biochemical evidence for the presence of intestinal inflammation. While not directly associated with rectal p24, mucosal TNF-α and IL-1β contents were higher in HIV-infected patients than in controls. The levels of IL-1 found in this study are low compared to studies done in patients with active ulcerative colitis and Crohn's disease (data not shown) as well as biopsies incubated for 24 hr (Figure 3B), suggesting that the inflammatory process associated with HIV infection usually is mild to moderate. The results suggest an association between biochemical and histological changes, although the limited examination of cytokines in this study precludes any interpretation of cause and effect.

There are several reasons why tissue contents of cytokines and inflammatory mediators might not correlate with p24 content. Other pathogens besides HIV, such as CMV (31), cryptosporidium, or MAI, affect cytokine production and decrease the specificity of the p24 measurement. In addition, the episodic nature of cytokine secretion and short half-lives of cytokines and inflammatory mediators decrease the sensitivity for detection. Furthermore, prostaglandin and leukotriene production might be nonspecifically increased as a result of tissue injury during biopsy, thus narrowing the differences between HIV-infected subjects and controls. Other techniques for examining cytokines and other inflammatory mediators will be needed to define their relationship to HIV.

HIV infection may lead to intestinal cellular injury by several mechanisms. Viral cytopathy may occur, although the presence of virions in intestinal biopsies is disputed (8, 32). HIV could interfere with normal cell function and lead to cell demise without virion assembly. This could occur especially if HIV acts as a defective virus in the gut, a situation shown for epithelial cell infection in an animal retrovirus model (33). Mucosal injury also could occur as a result of anti-HIV immunity and immune-mediated lysis of cells expressing viral antigens. Several potential cytotoxic mechanisms against HIV-infected target cells have been demonstrated *in vitro* (34–37). HIV infection of mononuclear cells may affect the regulation of cytokine gene expression, as has been shown experimentally (38, 39). Further studies will be needed to define the specific role of HIV and anti-HIV immunity in the pathogenesis of intestinal inflammation in HIV-infected patients.

There are several potential adverse effects of the mucosal inflammatory response. Since ion and water fluxes through the epithelial cell are modulated

by cytokines and inflammatory mediators (40), it is possible that the altered bowel habits are a direct consequence of altered local cytokine release.

Several studies, including one study performed in rectal mucosa, have shown that cytokines and inflammatory mediators may promote HIV production *in vitro* (41–47). Intestinal inflammation may lead to increased mucosal permeability and uptake of luminal contents, including bacterial lipopolysaccharides (endotoxins), which are powerful immunomodulatory agents and stimulate cytokine production (48). Altered intestinal permeability has been demonstrated in AIDS patients (49).

Mucosal inflammation also leads to recruitment of cells from peripheral blood by altering endothelial cell function and the expression of specific receptors on high endothelial venules (50, 51). Lymphocyte recruitment and activation, related to mucosal inflammation, increase the vulnerability to HIV (52) and may lead to spread of infection. Thus, a chronically inflamed intestinal mucosa may be one site where recruitment, primary infection, and death of lymphocytes takes place. If this is so, antiinflammatory therapy might play a role in the treatment of HIV-associated intestinal inflammation, as a decrease in inflammation should lower the tissue contents of promoters of HIV production. An open-label clinical study demonstrated clinical benefit and significant decreases in rectal p24 content after two months of therapy with 5-aminosalicylic acid (Asacol, Norwich Eaton) (53).

SUMMARY

The results of these studies demonstrate associations between HIV antigen expression in intestinal mucosa, alterations in bowel habits, and histologic and biochemical evidence of inflammation in HIV-infected individuals with or without AIDS, unrelated to other enteric pathogens. The possibility that HIV production in intestinal mucosa is a significant pathogenetic process in HIV infection requires further study.

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