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Jejunal enteropathy associated with human immunodeficiency virus infection: quantitative histology

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SUMMARY Jejunal biopsy specimens from 20 human immunodeficiency virus (HIV) positive male homosexual patients were analysed and compared with those of a control group to determine whether the abnormalities were caused by the virus or by opportunistic infection. The degree of villous atrophy was estimated with a Weibel eyepiece graticule, and this correlated strongly with the degree of crypt hyperplasia, which was assessed by deriving the mean number of enterocytes in the crypts. The density of villous intraepithelial lymphocytes fell largely within the normal range, either when expressed in relation to the number of villous enterocytes or in relation to the length of muscularis mucosae. Villous enterocytes showed mild non-specific abnormalities. Pathogens were sought in biopsy sections and in faeces.

Crypt hyperplastic villous atrophy occurred at all clinical stages of HIV disease and in the absence of detectable enteropathogens. An analogy was drawn between HIV enteropathy and the small bowel changes seen in experimental graft-versus-host disease. It is suggested that the pathogenesis of villous atrophy is similar in the two states, the damage to the jejunal mucosa in HIV enteropathy being inflicted by an immune reaction mounted in the lamina propria against cells infected with HIV.

Malabsorption, diarrhoea, and weight loss are common features of human immunodeficiency virus (HIV) disease, and may occur in the absence of identifiable opportunistic infections or neoplasia.[1] Previous studies have described villous atrophy with increased intraepithelial lymphocyte density and mild damage to surface enterocytes in jejunal biopsy specimens from such patients with HIV enteropathy.[2] The present study assesses quantitative variables of jejunal mucosal morphology at different clinical stages of HIV infection in male homosexuals.

HIV can infect CD4 positive lymphocytes, cells of the macrophage lineage (also CD4 positive), and possibly gut epithelial or enterochromaffin cells.[3] As all are present in the jejunum, HIV infection of such cells may have a pathogenic role in jejunal mucosal damage of HIV enteropathy.

Material and methods

Twenty HIV positive homosexual male patients attending outpatient clinics at St George's and St Mary's Hospitals, London were investigated. All subjects were HIV antibody positive (Wellcome Diagnostics).

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Patients were divided into three HIV clinical groups according to the Centers for Disease Control criteria[4]: five patients were asymptomatic, nine had persistent generalised lymphadenopathy or AIDS-related complex (ARC), and six had acquired immune deficiency syndrome (AIDS) (table).

Jejunal biopsy specimens were taken from 10 age and sex matched controls and subjected to the same examinations. Jejunal biopsy specimens were taken from these subjects during the investigation of diarrhoea and were judged to be normal at light microscopic examination by two experienced histopathologists. HIV antibody testing was not carried out on control subjects for ethical reasons but no control subject was in a high risk group.

Stools collected at the time of jejunal biopsy were cultured and examined microscopically for enteropathogens. Stool specimens were cultured for Salmonellae, Shigellae, *Campylobacter* sp, *Aeromonas* sp, *Plesiomonas* sp, *Clostridium difficile*, and a special culture on Lowenstein-Jensen medium was carried out. Stool samples were examined microscopically for neutrophils, ova, and parasites. Ziehl-Neelsen and modified Ziehl-Neelsen stain were used to identify Mycobacteria, Cryptosporidia, and Isospora.

Table *Clinical data of subjects studied*

Case No	*Age (years)*	*Clinical HIV state*	*Subjective presence of diarrhoea*
1	36	Asymptomatic	Mild
2	26	Asymptomatic	Moderate
3	52	Asymptomatic	Mild
4	20	Asymptomatic	Mild
5	28	Asymptomatic	Moderate
6	30	Persistent generalised lymphadenopathy	Moderate
7	38	Persistent generalised lymphadenopathy	Nil
8	39	Persistent generalised lymphadenopathy	Mild
9	38	ARC	Severe
10	34	ARC	Moderate
11	31	ARC	Severe
12	27	ARC	Moderate
13	32	ARC	Nil
14	44	ARC	Moderate
15	43	AIDS	Severe
16	40	AIDS	Nil
17	33	AIDS	Mild
18	56	AIDS	Severe
19	36	AIDS	Mild
20	52	AIDS	Nil

Mild < 3 stools/day, moderate 4–6 stools/day, severe > 6 stools/day.

JEJUNAL BIOPSY

Jejunal biopsy specimens were taken by Crosby capsule just distal to the ligament of Treitz and under fluoroscopic control. The specimens were orientated on paper, fixed overnight in 4% neutral buffered formaldehyde, processed routinely to paraffin wax, and sections 5 μm thick were cut from the blocks. Ten sections taken at regular intervals throughout the whole biopsy specimen were stained with haematoxylin and eosin for subjective light microscopic examination and for quantitation of mucosal surface area to volume ratio and mucosal crypt length. One section from each biopsy specimen was stained with periodic acid Schiff (PAS), Giemsa, and modified Ziehl-Neelsen for detection of mucosal enteropathogens. Transmission electron microscopy of villous enterocytes was performed for detection of mucosal enteropathogens. One section from each biopsy specimen was stained with a leucocyte common antigen (Dako) by the indirect immunoperoxidase technique for quantitation of intraepithelial lymphocytes. Negative controls were treated in the same way except for omission of the primary antibody.

MUCOSAL QUANTITATION

Observations were carried out by a histopathologist unaware of the clinical HIV state of patients.

Mucosal architecture index

The ratio of surface area of villi to volume of lamina propria in each jejunal biopsy specimen was estimated by a Weibel eyepiece graticule.[5] The graticule was viewed superimposed on the jejunal mucosa at a constant magnification (× 100) with the light microscope at several fields along the length of the biopsy specimen and at 10 levels through the block. The surface area to volume ratio was calculated for each biopsy specimen and expressed as the mucosal architecture index (S:V).

Crypt length

Crypt length was assessed by counting the number of enterocytes along one side of the crypt from its base to its junction with a villus. This count was repeated in at least 30 crypts from each biopsy specimen and a mean crypt length calculated. Only crypts whose full extent was visible in the plane of section were included in quantitation and care was taken not to measure the same crypt more than once in serial sections through the biopsy specimen.

Intraepithelial lymphocytes

The number of surface intraepithelial lymphocytes (IEL) in each biopsy specimen was estimated by two independent tests. The ratio of lymphocytes to enterocytes in surface epithelium was obtained by counting the number of immunologically stained lymphocytes per 500 enterocytes in three random areas of the biopsy specimen and expressed as number of lymphocytes per 100 enterocytes. The ratio of lymphocytes to length of muscularis mucosae was obtained by counting the number of stained lymphocytes in villous epithelium of a well orientated biopsy specimen overlying a length of muscularis mucosae measured using a Reichert-Jung Kontron IBAS 1 Image Analyser. This ratio was expressed as number of lymphocytes per millimetre of muscularis mucosae. IEL were distinguished from the occasional polymorph leucocyte in surface epithelium by their different nuclear morphology.

Results

HISTOLOGY

Jejunal biopsy specimens from HIV positive male homosexuals showed variable degrees of villous blunting and broadening, or were normal (figs 1 and 2). Villous enterocytes showed only mild focal nuclear irregularity. Vacuolation of apical cytoplasm of enterocytes was seen in some areas of a few biopsy specimens. This phenomenon is poorly understood,[6] but may be related to fixation artefact. Villous and crypt enterocytes showed no other cytological abnormalities. Mucosal crypts in some specimens seemed to be elongated, but this was difficult to assess accurately due to convolutions in the crypts. Crypt mitoses did not seem to be more or less common in test biopsy specimens. Apoptotic cells were observed in very few

crypts. Cellularity of lamina propria in some specimens fell within normal limits. In others there was a mild or moderate increase in the density of lymphocytes and plasma cells. Granulomatous inflammation was not a feature of any of the biopsy specimens. Foci of neutrophil polymorphs were seen in the epithelium of very few crypts or villi. No evidence of neoplasia was present in any biopsy specimen.

MICROBIOLOGY

No mucosal enteropathogens were detected in any biopsy specimen on staining with haematoxylin and eosin or special stains. Care was taken to exclude infection with Cryptosporidia, Isospora, and Mycobacteria. Transmission electron microscopy of villous enterocytes also failed to detect mucosal enteropathogens, and in particular, Microsporidia were absent. No pathogens were identified on stool examination or culture.

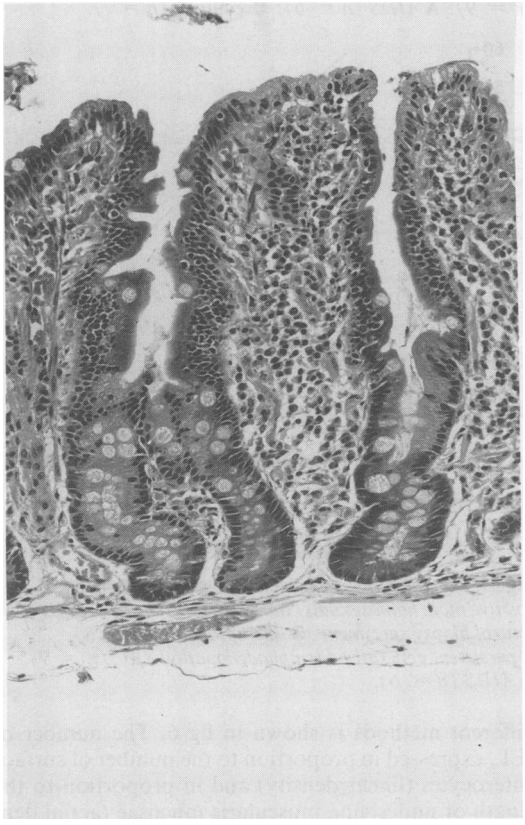

Fig 1 *Jejunal mucosa from patient with ARC showing mild atrophy of villi and hyperplasia of crypts. Surface enterocytes show mild nuclear irregularity. (Haematoxylin and eosin.)*

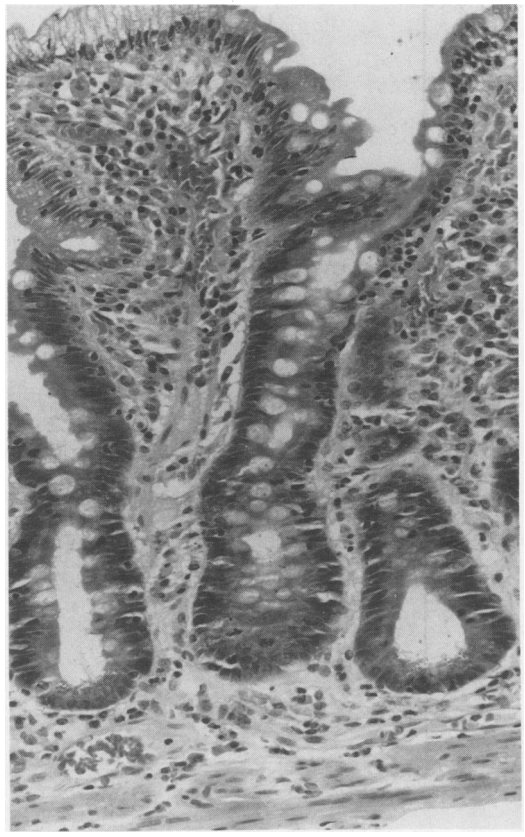

Fig 2 *Jejunal mucosa from patient with AIDS showing severe atrophy of villi and hyperplasia of crypts. (Haematoxylin and eosin.)*

MUCOSAL QUANTITATION

Mucosal architecture index

Results of quantitation of S:V are shown in fig 3. Sixteen of the 20 biopsy specimens from HIV positive subjects had a mucosal architecture index below one standard deviation from the mean of control biopsy specimens and were judged to show villous atrophy. There was no correlation between the degree of villous atrophy and clinical stage of HIV disease. Four biopsy specimens (two asymptomatic, one AIDS, and one persistent generalised lymphadenopathy/ARC) did not show villous atrophy.

Crypt length

Results of crypt length measurements are shown in fig 4. Eighteen of the 20 biopsy specimens had a mean crypt length which was at or above the mean crypt length of those of controls. Of these 18, 10 had a mean crypt length which fell above one standard deviation from the mean of the control biopsy specimens. Thus

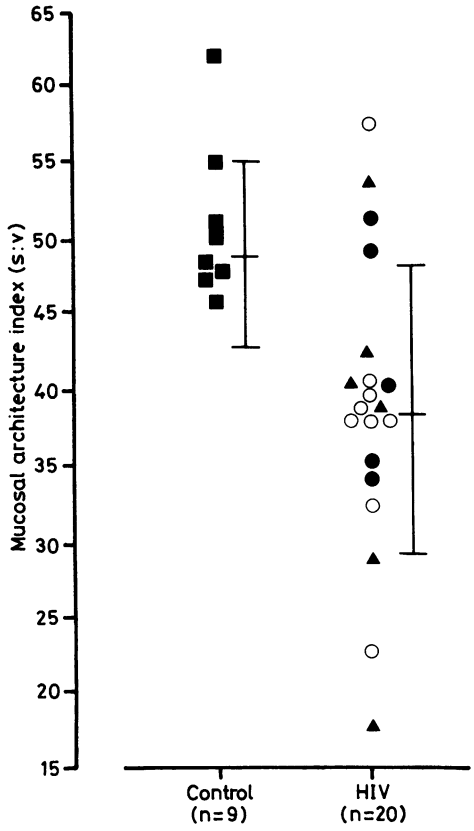

Fig 3 *Jejunal mucosal architecture in HIV antibody positive male homosexuals. Bars represent mean ± 1 SD. ● Asymptomatic (n = 5); ○ persistent generalised lymphadenopathy/ARC (n = 9); ▲ AIDS (n = 6); ■ controls (n = 9).*

the specimens exhibited a tendency to crypt hyperplasia. There was no correlation between the degree of crypt hyperplasia and the clinical stage of HIV disease. Two biopsy specimens from patients with AIDS had a mean crypt length which fell below the mean crypt length of the normal range.

Correlation between mucosal architecture index and crypt length

The correlation between S:V and crypt length is shown in fig 5. There was a highly significant inverse correlation ($p < 0.0005$) between S:V and mean crypt length in biopsy specimens from HIV antibody positive subjects—that is, atrophy of villi (decreasing mucosal architecture index) correlated strongly with hyperplasia of crypts (increasing mean crypt length).

Intraepithelial lymphocytes

Graphic representation of IEL quantified by two

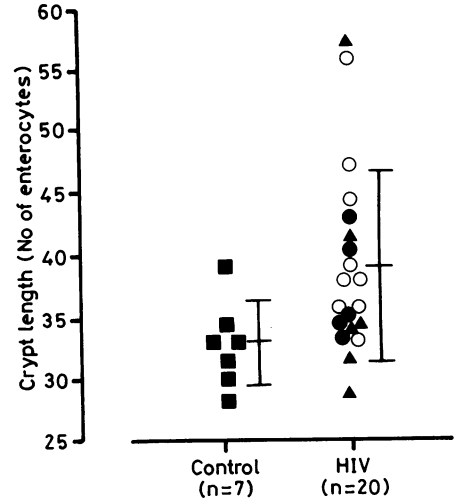

Fig 4 *Jejunal crypt length in HIV antibody positive male homosexuals. Bars represent mean ± 1 SD. ● Asymptomatic (n = 5); ○ persistent generalised lymphadenopathy/ARC (n = 9); ▲ AIDS (n = 6); ■ controls (n = 7).*

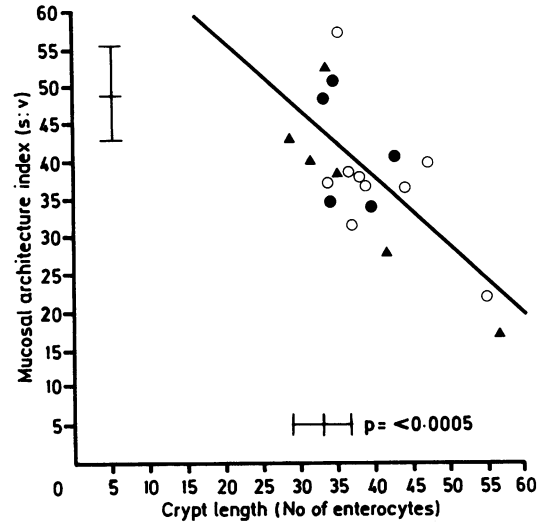

Fig 5 *Jejunal villus/crypt morphology in HIV antibody positive male homosexuals. Bars represent mean ± 1 SD of control biopsy specimens. ● Asymptomatic (n = 5); ○ persistent generalised lymphadenopathy/ARC (n = 9); ▲ AIDS (n = 6).*

different methods is shown in fig 6. The number of IEL, expressed in proportion to the number of surface enterocytes (linear density) and in proportion to the length of underlying muscularis mucosae (aerial density), fell within the normal range in most biopsy specimens from HIV antibody positive subjects.

There was no correlation between the aerial density

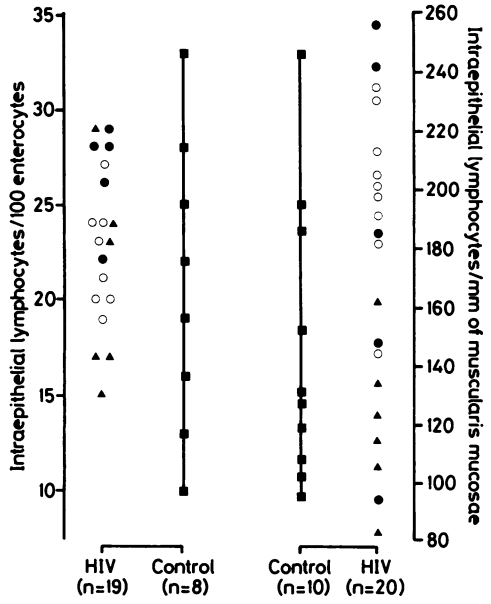

Fig 6 *Jejunal intraepithelial lymphocytes in HIV antibody positive male homosexuals, expressed in relation to number of villous enterocytes and to length of muscularis mucosae.* ●*Asymptomatic;* ○*persistent generalised lymphadenopathy/ ARC;* ▲*AIDS;* ■ *controls.*

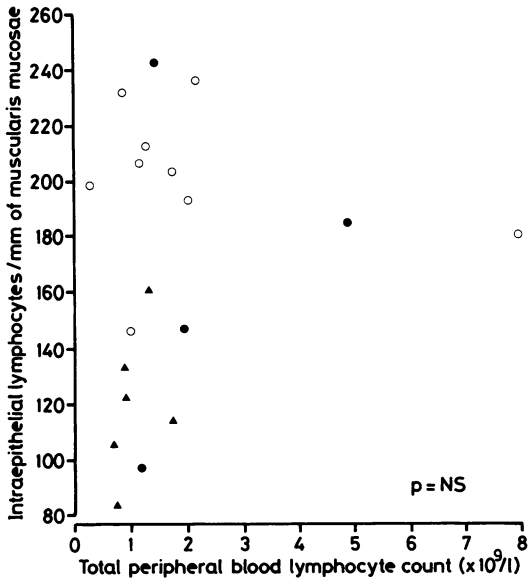

Fig 7 *Correlation between aerial density of jejunal intraepithelial lymphocytes and total peripheral blood lymphocyte count in HIV antibody positive male homosexuals.* ●*Asymptomatic (n = 4);* ○*persistent generalised lymphadenopathy/ARC (n = 9);* ▲*AIDS (n = 6).*

of IEL and the total peripheral blood lymphocyte count in HIV antibody positive subjects (fig 7).

Discussion

The pathogenesis of small bowel abnormalities occurring in HIV disease in the absence of opportunistic infection or neoplasia is not clearly understood. Several recent studies of the small bowel have described villous atrophy with little evidence of abnormality in surface enterocytes. Crypt hyperplasia has been described subjectively but not quantitated, and an increased linear density of lymphocytes in villous epithelium has been reported.[2 7-9] The finding of this study shows that the villous atrophy of HIV enteropathy is accompanied by a corresponding degree of crypt hyperplasia, and yet occurs in the absence of any detectable abnormality in enterocyte cytology or change in intraepithelial lymphocyte ratio. Furthermore, HIV enteropathy occurs at all stages of HIV infection, while the degree of mucosal damage seems to bear no relation to the clinical stage of disease.

Crypt hyperplastic villous atrophy is characteristic of enteropathies that may have an immunological basis (such as coeliac disease,[10] graft-versus-host disease[11]) and infectious aetiology (such as stasis syndromes,[12] post-infective malabsorption[13]). Luminal bacterial overgrowth in the small bowel secondary to impaired mucosal immunity in HIV disease has been postulated as the cause of HIV enteropathy by some.[1 8 14] This remains a possibility, although luminal bacterial pathogens have not been shown consistently in the bowel in HIV disease. We have recent evidence using breath hydrogen monitoring on 20 subjects with AIDS that small intestinal bacterial overgrowth is not a feature of HIV disease (our unpublished observations). We were unable to identify infectious agents in our test subjects in biopsy specimens or in faeces. Furthermore, evidence of surface enterocyte damage is a feature of infective enteropathies[12 13] yet strikingly few changes are found in the villous epithelium in HIV disease, even at ultrastructural level.[9]

The increased density of lymphocytes in the surface epithelium of the jejunum in coeliac disease[10] has long been regarded as central to the pathogenesis of this disease. The physiological role of intraepithelial lymphocytes in normal jejunum is poorly defined. The predominantly T suppressor cell (CD8) population of these lymphocytes may maintain a state of immune tolerance to dietary antigens, possibly regulating the induced T helper cell (CD4) immune response in the lamina propria beneath.[15 16] The part played by surface lymphocytes in the damage to the mucosa in coeliac disease is also obscure. Although these cells seem to be morphologically activated and show changed surface antigen expression,[17 18] there is no proof that they are

responsible for the abnormalities seen in the villous enterocytes.[18] Indeed, it is now thought that the lesion of coeliac disease results from both humoral and T cell-mediated hypersensitivity reactions, probably initiated in the lamina propria.[16 18]

A recent study of villous intraepithelial lymphocytes in HIV disease has shown "activated" ultrastructural appearance of these cells[7]; the changes resemble in many ways those seen in the surface lymphocytes in coeliac disease.[18] This and other studies[2 7 8] have recorded an increased density of lymphocytes in surface epithelium in HIV enteropathy, expressed as a ratio comparing the number of lymphocytes against a fixed number of enterocytes (linear density). This method of quantifying lymphocytes has been called into serious question, however, as the "constant" against which the lymphocyte population is compared is itself changed by the same pathological process that affects the number of surface lymphocytes.[15 17–19] A more valid comparison is made by counting the number of lymphocytes against a structure which is not damaged by the disease process, such as muscularis mucosae (aerial density).

Regardless of these theoretical considerations, our study has shown that the size of the intraepithelial lymphocyte population falls within normal limits by both methods of quantitation. The population of lymphocytes in the surface epithelium of the jejunum in HIV disease may fall merely in parallel with the depletion of circulating T lymphocytes which occurs in these patients; thus the observed density of surface lymphocytes may underestimate their functional importance. We were, however, unable to show a correlation between the aerial density of intraepithelial lymphocytes and the peripheral blood lymphocyte count in our patients. Analysis of the subsets of intraepithelial lymphocytes in HIV enteropathy may clarify this point.

Apoptoses, or indivdual cell necroses, in crypt epithelium are the histological hallmark of HIV disease in rectal mucosa.[20] This phenomenon is also typically seen in the rectum in graft-versus-host disease.[21] Graft lymphocytes eliminate host epithelial cells in this condition and cytotoxicity depends to some extent on differences in surface antigen components between host and graft tissue. Such changes may be induced in rectal epithelium in HIV disease, conceivably by infection with HIV.[22]

We observed just a few apoptotic enterocytes in only the occasional crypt in jejunal biopsy specimens from both the infected and control subjects. Human fetal small intestinal explants containing activated mucosal T cells,[23] experimental small intestinal graft-versus-host disease, and rejecting allografts of small bowel mucosa in the mouse, however,[24 25] are characterised by crypt hyperplasia, villous atrophy, but little evidence of enterocyte damage. These features bear a striking resemblance to the abnormalities seen in the jejunal biopsy specimens of HIV positive subjects reported in this study. The manipulations of one of these experiments in the mouse[25] cleverly avoided any effect of surface antigen incompatibility between small bowel epithelium and cytotoxic lymphocytes. The authors concluded that the intestinal mucosa was damaged innocently by an immune reaction occurring within the lamina propria, most likely mediated by soluble factors (lymphokines) which could induce crypt cell proliferation and which were released in a hypersensitivity reaction involving activated T lymphocytes. It is tempting to speculate that the abnormalities of HIV enteropathy may be produced by similar means.

Phenotyping of the T cell population of the lamina propria of small bowel mucosa in HIV disease has shown a reversal of the normal T helper:T suppressor (CD4:CD8) cell ratio.[8 14] The total T cell population and the component of T helper cells are depleted, while the component of T suppressor cells is proportionately increased. There is also mounting evidence that HIV may infect non-lymphoid cells in gut mucosa, possibly following an interaction with low affinity CD4 receptors, as well as T helper lymphocytes.[3 26] In situ hybridisation studies[27] have shown HIV infection of cells in the base of duodenal crypts, thought most likely to be neuroendocrine cells. Colorectal carcinoma cell lines have been successfully infected with the virus and CD4 RNA shown in normal colonic mucosa.[28 29] Furthermore, tubuloreticular structures, although non-specific products of viral infection, are often observed in a variety of non-lymphoid cells in the gut mucosa in HIV disease.[9]

It is evident that a variety of cells that are normal constituents of the jejunal mucosa may be infected by HIV. We postulate, however, that the structural abnormalities we have observed in HIV enteropathy are mediated not by the virus itself, nor by unidentified opportunistic pathogens. We suggest, rather, that they are caused by an immune reaction mounted in the mucosa by the host against its own HIV infected cells in a similar way to graft-versus-host disease. If this should prove to be the case then paradoxically the small bowel is damaged by autoimmunity against a background of profound immune deficiency.

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References

1 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**:619–22.

2 Kotler DP, Gaetz HP, Lange M, Klein EB, Holt PR. Enteropathy associated with the acquired immunodeficiency syndrome. *Ann Intern Med* 1984;**101**:421–8.

3 Weiss RA. Retroviruses and human disease. *J Clin Pathol* 1987;**40**: 1064–9.

4 Centers for Disease Control: classification system for human T-lymphotropic virus type III/lymphadenopathy-associated virus infection. *Ann Intern Med* 1986;**105**:234–7.

5 Dunnill MS, Whitehead R. A method for the quantitation of small intestinal biopsy specimens. *J Clin Pathol* 1972;**25**:243–6.

6 Isaacs PET, Sladen GE, Filipe I. Mefenamic acid enteropathy. *J Clin Pathol* 1987;**40**:1221–7.

7 Weber JR, Dobbins WO. The intestinal and rectal epithelial lymphocyte in AIDS. An electron-microscopic study. *Am J Surg Pathol* 1986;**10**:627–39.

8 Budhraja M, Levendogln H, Kocka F, Mangkornkanok M, Sherer R. Duodenal mucosal T cell subpopulation and bacterial cultures in acquired immune deficiency syndrome. *Am J Gastroenterol* 1987;**82**:427–31.

9 Dobbins WO, Weinstein WM. Electron microscopy of the intestine and rectum in acquired immunodeficiency syndrome. *Gastroenterol* 1985;**88**:738–49.

10 Lee FD, Toner PG. Diffuse villous abnormality: coeliac disease. In: *Biopsy pathology of the small intestine.* London: Chapman and Hall, 1980:97–104.

11 Ferguson A. Delayed-type hypersensitivity to alloantigens in small intestinal mucosa. In: March MN, ed. *Immunopathology of the small intestine.* Chichester: John Wiley and Sons, 1987:235–44.

12 Lee FD, Toner PG. Diffuse villous abnormality: The "blind loop" syndrome (stasis syndrome). In: *Biopsy pathology of the small intestine.* London: Chapman and Hall, 1980:108–11.

13 Schreiber DS, Trier JS, Blacklow NR. Recent advances in viral gastroenteritis. *Gastroenterol* 1977;**73**:174–83.

14 Rodgers VD, Fassett R, Kagnoff MF. Abnormalities in intestinal mucosal T cells in homosexual populations including those with the lymphadenopathy syndrome and acquired immunodeficiency syndrome. *Gastroenterol* 1986;**90**:552–8.

15 Dobbins WO. Human intestinal intraepithelial lymphocytes. *Gut* 1986;**27**:972–85.

16 Brandtzaeg P. The intestine as an immunological organ. In: *British Society of Gastroenterology/International Teaching Sessions.* Chichester: John Wiley and Sons, 1987:133–8.

17 Jenkins D, Goodall A, Scott BB. T-lymphocyte populations in normal and coeliac small intestinal mucosa defined by monoclonal antibodies. *Gut* 1986;**27**:1330–7.

18 Marsh MN. Functional and structural aspects of the epithelial lymphocyte, with implications for coeliac disease and tropical sprue. *Scand J Gastroenterol* 1985;**20**:55–75.

19 Corazza GR, Frazzoni M, Gasbarrini G. Jejunal intraepithelial lymphocytes in coeliac disease: are they increased or decreased? *Gut* 1984;**25**:158–62.

20 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.

21 Sloane JP, Dilly SA. Pathogenesis of graft versus host disease. *Histopathol* 1988;**12**:105–10.

22 Slavin RE. Lymphocyte-associated apoptosis in AIDS, in bone-marrow transplantation, and other conditions. *Am J Surg Pathol* 1987;**11**:235–8.

23 MacDonald TT, Spencer J. Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. *J Exp Med* 1988;**167**:1341–9.

24 Mowat AM, Ferguson A. Intraepithelial lymphocyte count and crypt hyperplasia measure the mucosal component of the graft-versus-host reaction in mouse small intestine. *Gastroenterol* 1982;**83**:417–23.

25 Elson CO, Reilly RW, Rosenberg IH. Small intestinal injury in the graft versus host reaction: an innocent bystander phenomenon. *Gastroenterol* 1977;**72**:886–9.

26 Klatzmann D, Gluckman JC. HIV infection: facts and hypotheses. *Immunology Today* 1986;**7**:291–6.

27 Nelson JA, Reynolds-Kohler C, Margaretten W, Wiley CA, Reese CE, Levy JA. Human immunodeficiency virus detected in bowel epithelium from patients with gastrointestinal symptoms. *Lancet* 1988;i:259–62.

28 Adachi A, Koenig S, Gendelman HE, *et al.* Productive, persistent infection of human colorectal cell lines with human immunodeficiency virus. *J Virol* 1987;**61**:209–13.

29 Adachi A, Gendelman HE, Koenig S, *et al.* Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J Virol* 1986;**59**:284–91.

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