Quantitative histological study of enteropathy associated with HIV infection

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Abstract

A quantitative histological study was performed on small intestinal biopsies from eight ambulatory patients with HIV infection (AIDS/AIDS-related complex, ARC) and compared with those from 16 normal subjects. Enteropathy was assessed by measurement of villus area, crypt length and mitotic count, as well as duodenal counts of intraepithelial lymphocytes, mucosal mast cells and goblet cells. Enteropathy in subjects with AIDS/ARC was shown by reduced mean villus area of 0·363 (SD 0·081) compared with 0·500 (SD 0·064) mm² in control subjects (p<0·0001), while intestinal crypts were of similar length with 239 (SD 36) compared with 225 (SD 28) μm, but mitotic count was increased to 3·8 (SD 1·2) compared with 2·4 (SD 0·8) (p=0·01) in the same control subjects. These results indicate villous atrophy with impaired crypt hyperplasia. Duodenal cell counts showed similar numbers of mucosal mast cells, intraepithelial lymphocytes and goblet cells in AIDS/ARC patients and fifteen control subjects.

Gastrointestinal symptoms are a common feature and diarrhoea a common management problem in patients infected with the human immunodeficiency virus (HIV).[1,2] In a proportion of patients, an infective cause due to common or exotic organisms is found,[1-4] while there is also evidence for an enteropathy associated with HIV infection.[1,2,5] Enteropathy associated with graftversus-host reaction (GvHR) in animals and coeliac disease in man are believed to be mediated by activated mucosal T lymphocytes which lead to villous atrophy and crypt hyperplasia.[6,7] Because of immunodeficiency, this mechanism would be impaired in the enteropathy of HIV infection. Thus, it is important to define quantitatively the exact pattern of enteropathy of HIV infection and, in particular, whether there is crypt hyperplasia. Similarly, increases in intraepithelial lymphocytes,[8] mucosal mast cells,[9] and goblet cells[10,11] are associated with T cell mediation, but one study has found that mucosal mast cells were deficient in AIDS.[12] The aim of this study is to apply quantitative histological techniques to assess enteropathy and the number of duodenal mucosal mast cells, intraepithelial lymphocyte and goblet cells in patients with HIV infection and resulting AIDS or AIDS-related complex (ARC).

Methods

SUBJECTS

AIDS or ARC was diagnosed in eight patients by antibody to HIV as determined by an enzyme linked immunosorbent assay which was confirmed by Western blotting, by depletion of CD4 peripheral blood lymphocytes, and by clinical features (Table I). Seven of eight subjects received zidovudine (AZT) at the time of the

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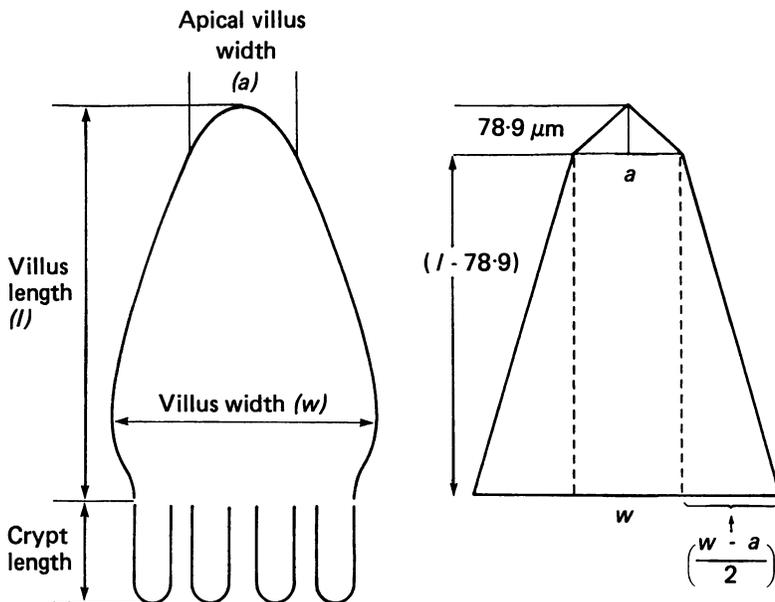Road, Woodville, Adelaide SA
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TABLE I *Clinical features of the eight patients with AIDS or ARC*

HIV subject Age (y)/sex		CD4† (/μl)	CD4/CD8††	Body weight (kg)
1* 28M	Oral candidiasis Hairy leukoplakia	43	0·05	68·0
2 31M	Oral candidiasis *Herpes simplex* of buttocks (treated) Kaposi's sarcoma of oesophagus and stomach	99	0·30	64·5
3 35M	Oral candidiasis Weight loss Diarrhoea (repeated *Isospora belli* infection)	108	0·21	52·4
4 28M	Oral and oesophageal candidiasis Cerebral toxoplasmosis with transverse myelopathy (treated) Diarrhoea while on antibiotics Hepatitis B surface antibody positive	41	0·05	70·4
5 38M	Oral candidiasis Weight loss *Pneumocystitis carini* infection (treated) Kaposi's sarcoma of skin Hepatitis B surface antibody positive	21	0·02	54·0
6* 38M	Oral and oesophageal candidiasis Lethargy	35	0·36	65·0
7 34M	Weight loss Diarrhoea due to *Cryptosporidium* (treated) Cholestatic syndrome (normal ERCP)	126	0·66	59·8
8* 32M	Oral and oesophageal candidiasis Weight loss *Herpex simplex* of buttocks (treated) Diarrhoea	15	0·26	57·0

*AIDS-Related Complex (ARC); Normal range: †(405–2205); ††(0·72–3·6).

Figure 1: Calculation of villus area from measures of villus length, and apical and basal widths.

of these patients including enumeration of CD4 lymphocytes and CD4/CD8 ratio values in peripheral blood by flow cytometry. These values were recorded at investigations done within six weeks of intestinal biopsy. Another 16 'normal' subjects undergoing endoscopy whose biopsy and other investigations were later confirmed to be normal were used as control subjects. Three further subjects undergoing routine investigation for coeliac disease had additional biopsies taken for this study and these were used as examples of hyperplastic villous atrophy. Coeliac disease was confirmed in three subjects by clinical improvement, by decreased intestinal permeability on serial testing, and by improved intestinal morphology of a second biopsy while on a gluten free diet. Informed consent was obtained for endoscopy from each patient and the protocol for this study was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

study. For the eight subjects, the routes of HIV infection were heterosexual (one) or homosexual (seven). Two additional subjects were HIV antibody positive but had no clinical features of AIDS/ARC. Laboratory investigations were done regularly as part of medical management

SMALL INTESTINAL BIOPSY
Four small intestinal biopsies were taken at endoscopy from the distal second part of the duodenum. These biopsies were orientated on cardboard before two were placed in Clark's fixative (75% ethanol: 25% acetic acid, v/v) and briefly shaken to remove mucus for later microdissection, while the other two biopsies were fixed in Carnoy's fixative (60% ethanol: 30% chloroform: 10% acetic acid, v/v) for duodenal cell counts.

MEASUREMENT OF VILLUS AREA, CRYPT LENGTH AND MITOTIC COUNT
Intestinal biopsies were stained by the modified Feulgen technique and microdissected using a cataract knife as previously described.[13] These fragments were mounted in 45% acetic acid as a wet film on a slide and coverslipped. Using a calibrated microscopic graticule, the length (1), apical width (a), and maximal basal width (w) of eight to 15 villi and the length of 10 to 15 crypts were measured and the mean of each calculated (Fig 1). The apical width was measured 78·9 μm (10/100 graticule units) from the apex that is, approximately at one-tenth of the length villus. The tissue was squashed and mitotic figures counted in ten to fifteen crypts.

Villus area was calculated using a geometric approximation of a small triangle mounted on a larger trapezoid, for which the major base was the maximal basal width (Fig 1). The surface area was computed by adding the area of this triangle and trapezoid. This method was simplified from a previous algorithm which also used a trapezoid approximation for calculating villus area, as described by Lorenz-Meyer, Köhn and Riecken.[14] Their alogrithm, which was validated planimetrically, calculates the surface area of both surfaces of the villus, whereas the present one calculates the surface area of one face. We have used data from another study by these authors in the rat,[15] and shown a correlation (r²) of 0·95 by the two methods. Surface area was calculated using a computer program written for the Apple Macintosh computer.
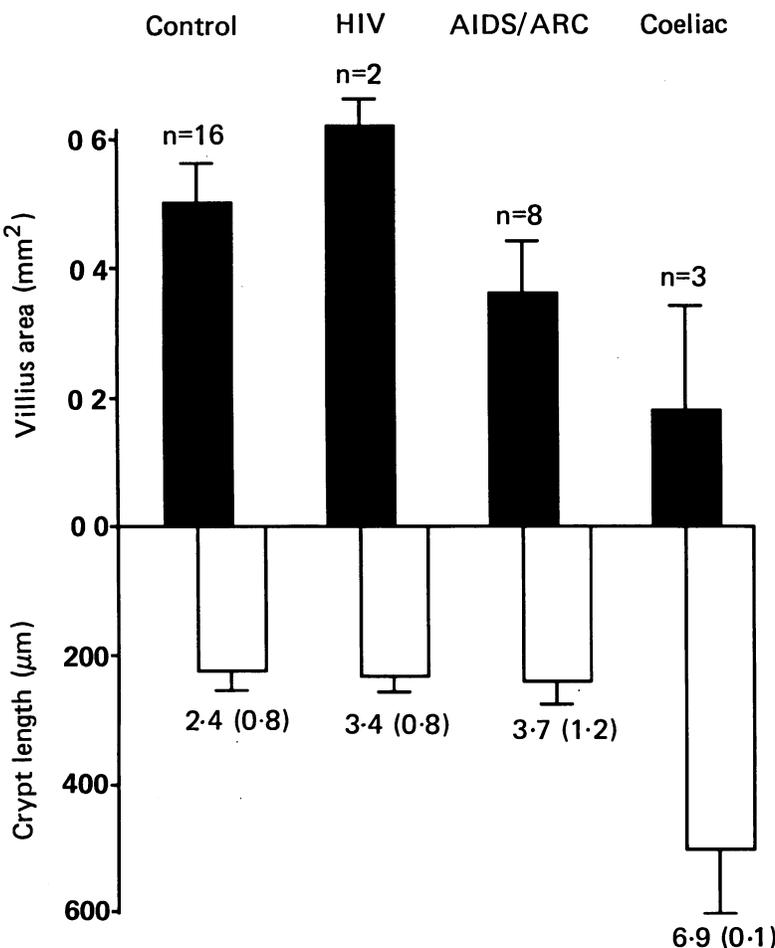

Figure 2: Villus area, crypt length and mitotic count of small intestinal biopsies from patients with AIDS/ARC, HIV infection, coeliac disease before treatment, and normal subjects.

TABLE II   *Duodenal counts of mucosal mast cells, intraepithelial lymphocytes and goblet cells in subjects with asymptomatic HIV infection, AIDS/ARC, coeliac disease before treatment, and normal subjects*

	Mucosal mast cells (/mm)	Intraepithelial lymphocytes (/mm)	Goblet cells (/mm)
Control (n=15)*	36 (15)	47 (21)	70 (17)
HIV (n=2)	41 (18)	37 (18)	71 (15)
AIDS/ARC (n=8)	41 (18)	37 (18)	71 (15)
Coeliac disease (n=3)	44 (12)	55†	51†

*One biopsy from the control subjects was unsuitable for cell counts.
†One biopsy from coeliac subjects was unsuitable for intraepithelial lymphocyte and goblet cell count.

DUODENAL CELL COUNTS

Tissue fixed in Carnoy's fixative was embedded in paraffin according to conventional techniques and sections (4 μm) cut. Intraepithelial lymphocyte and goblet cells were counted on sections stained with haematoxylin and eosin, and mucosal mast cells were counted in sections stained with Alcian blue (pH 0·3)/safranin. Cells were counted above the muscularis mucosae using a linear graticule (296 μm) and 25x objective lens.[16] Four to 10 counts was obtained for each subject. Cell counts were expressed as cells/mm of muscularis mucosae.

STATISTICAL ANALYSIS

Peritz' F test was used to compare the group means of the morphological measures and duodenal cell counts.[17] All results are expressed as the mean (SD).

Results

INTESTINAL MORPHOLOGY

In patients with HIV infection, villus area was reduced by 27% (p<0·0001), crypt length was similar (p=0·28) but mitotic count of crypts was increased to 3·7 (p=0·01), when compared to biopsies from control subjects (Fig 2). The increase in mitotic count was relatively impaired as we would have expected a value of approximately 5·4 for this degree of villous atrophy. This expectation is based on a curvilinear relationship of villous atrophy to mitotic count in coeliac subjects (unpublished observation). Thus, there was villous atrophy with impaired crypt hyperplasia. The only patient with AIDS, who was not receiving zidovudine, had a villus area of 0·306 mm², crypt length of 217 μm and mitotic count of 2·8 – that is, similar to the mean values for the AIDS/ARC group. This would indicate that zidovudine was not the cause of the enteropathy. Untreated coeliac patients had a 64% reduction in villus area (p<0001), crypt length increased by 224% (p<0·0001) and mitotic count was increased to 6·9 (p<0·0001), indicating villous atrophy with compensatory crypt hyperplasia.

DUODENAL CELL COUNTS

Satisfactory duodenal counts were done on biopsies of 15 of the 16 control, one of two HIV-infected subjects who were asymptomatic, and eight subjects with AIDS/ARC. Counts of mucosal mast cells, intraepithelial lymphocyte and goblet cells were similar in the HIV-infected subjects compared to those in control subjects (Table II). In coeliac subjects, mucosal mast cells were done in three subjects, and intraepithelial lymphocyte and goblet cell counts in two subjects. Coeliac subjects did not differ in counts of mucosal mast cells, intraepithelial lymphocyte or goblet cells, although the number of subjects was small.

Discussion

We have shown that enteropathy associated with HIV infection (resulting in AIDS or ARC) has villous atrophy but impaired crypt hyperplasia, and that duodenal counts of mucosal mast cells, intraepithelial lymphocyte and goblet cells were similar to normal subjects. This is an unusual pattern of enteropathy and suggests that villous atrophy in these patients was not mediated by mucosal T lymphocytes which are deficient in AIDS,[18] even though these cells mediate villous atrophy experimentally in GvHR, which is the model of a T cell mediated response.

Villous atrophy could be mediated by direct viral infection of villous epithelial cells, or of supportive cells in the underlying lamina propria such as lymphocytes, macrophages or fibroblasts. Small virus-like particles have been seen by electron microscopy in intestinal or colonic epithelial cells and in histiocytes of the lamina propria in two of six AIDS subjects at autopsy,[19] although another study failed to find virus particles in rectal biopsy material.[20] HIV has been detected by *in situ* hybridisation in the lamina propria of the duodenum, and in enterochromaffin cells and possibly other cells of rectal crypts.[21] Two asymptomatic HIV-infected subjects, however, did not have villous atrophy which might indicate that HIV infection does not directly the villus, although this view would depend on the number and variety of cells infected at different clinical stages of the disease. Another suggested mechanism for villous atrophy is immune complex reaction which, together with T cells may mediate villous atrophy in coeliac disease.[22] AIDS is associated with hypergammaglobulinaemia,[5] which potentially could react with gut luminal antigens to form such immune complexes. A further mechanism for villous atrophy is secondary to reduced mitotic activity of intestinal crypts that may or may not be associated with active destruction of villous epithelial or supportive cells in the lamina propria. It is unlikely that zidovudine treatment caused villous atrophy as the one AIDS subject, who was not receiving the drug, had villous atrophy.

Evidence suggests that the mitotic rate of intestinal crypts is regulated by the activity of mucosal T lymphocytes. This has been principally shown experimentally in rodents with possible involvement of T cell activation with crypt hyperplasia at weaning,[16] in mucosal GvHR,[8 9 23] and during immune expulsion of the *Nippostrongylus* – nematode from the small intestine.[6] The principle of T cell dependence of crypt hyperplasia has been confirmed in human gut using the technique of explant foetal culture; MacDonald and Spencer[7] have shown that crypt

hyperplasia in human fetal intestinal explant culture is induced by activation of T cells with pokeweed mitogen or with anti-CD3 antibody, and conversely that cyclosporin A treatment inhibited crypt hyperplasia. Thus a variety of studies in rodents and man have demonstrated the T cell dependence of intestinal crypt hyperplasia. The present findings of impaired intestinal crypt hyperplasia in the enteropathy of AIDS/ARC would be expected from the immunodeficiency of mucosal T cells, and further supports the general notion that mucosal T cells control the mitotic rate of intestinal crypts.

This impaired crypt hyperplasia should be regarded as relative because crypt length was similar to those of normal subjects rather than being hypoplastic. This could be because of the influence of residual T cells, or because of non-immune factors also influencing the proliferation of intestinal crypts. The similarity in intestinal crypt length of HIV-infected and control subjects would indicate equivalent crypt cell population as crypt length and crypt cell count are directly correlated. The discrepancy between this and some (but impaired) increase to mitotic activity suggests that there was crypt cell destruction due to HIV infection, as otherwise crypt length would be increased in HIV subjects. This notion has some support by the finding of HIV infection in intestinal and rectal crypts in an *in situ* – hybridisation study.[21] This would mean there is decreased crypt cell efflux onto villi of HIV-infected subjects from reduced mitotic rate as well as from destruction of crypt cells within the intestinal crypt.

The relative failure of crypt hyperplasia contrasts with the study by Kotler *et al.*[5] which reported crypt hyperplasia in biopsies of small intestine from AIDS patients. Their study was based on subjective assessment without quantitative measures because of acknowledged difficulties with imperfect orientation of biopsies. The use of a microdissection technique in our study has overcome this problem. It is also possible that their study may have had individuals who were not as immunodeficient as those in our present study and whose biopsy might have therefore shown some measure of crypt hyperplasia. Batman *et al*[24] also reported crypt hyperplastic villous atrophy in quantitative study of enteropathy of HIV infection. They measured the mucosal architecture index that is, the ratio of surface area to volume of lamina propria, and column count of the intestinal crypt. A lower architecture index confirmed villous atrophy in HIV subjects, particularly those with AIDS. The authors concluded that crypt length by column cell count in HIV subjects had a tendency to crypt hyperplasia, although their data overlapped with that of control subjects, as was the case for four of their AIDS individuals. No statistics were done to reach this conclusion for this particular measurement, and no comparison was made of crypt length in a disease group known to have crypt hyperplasia such as coeliac disease.

Irani *et al*[12] found that mucosal mast cells ('T mast cell') in the ileum of three patients with AIDS were reduced to 5% of the number in normal ileam, and similarly that mucosal mast cells were reduced (to less than 1%) in two patients with severe combined immunodeficiency syndrome. The majority of these specimens were taken at autopsy, and thus it is likely that these agonal patients were severely malnourished. This could be a confounding factor for the reduction of mucosal mast cell as malnutrition in the rat depletes mucosal mast cells, although, interestingly, it has less of an effect on functional mucosal mast cell activity.[25] While mucosal mast cell hyperplasia is T cell dependent,[26] nevertheless mucosal mast cells are not totally depleted in T cell immunodeficiency states.[27] Although the patients in this present study were immuodeficient with low CD4 cells (Table I), the number of mucosal mast cells was similar to normal subjects. Bishop *et al*[28] also found a similar number of mucosal mast cells in four jejunal biopsies of AIDS patients. At the present time, activity of human mucosal mast cells can only be measured *in vivo* by specific tryptase secretion which apparently is not sensitive at physiological levels.[29] Studies in rodents examining systemic release of a soluble mucosal mast cell granule protein, rat mucosal mast cell protease 11, have shown that a fall in mucosal mast cells may be associated with either decreased activity,[9] or with increased activity.[16][30] Thus, it is not prudent to rely on mucosal mast cell count to infer mucosal mast cell activity.

Kotler *et al*[5] reported that intraepithelial lymphocytes were increased in the enteropathy of AIDS when the count was expressed per 100 epithelial cells. Our present study showed that the IEL count was similar in control of AIDS subjects when expressed per mm of muscularis mucosae. One explanation may be that their AIDS patients were less immunodeficient and therefore showed a pattern of enteropathy similar to that seen in coeliac disease. The most likely explanation however, is the manner of expression of cell count. Both intraepithelial lymphocyte count/mm and /mm^2 of muscularis mucosae are not affected by spurious increase in count due to decrease in volume of tissue with the only assumption being that the muscularis mucosae remains unaltered.[31][32] Batman *et al*[24] also found that the intraepithelial lymphocyte count was similar to that in control subjects when expressed per mm of muscularis mucosae. This preservation of intraepithelial lymphocytes indicate they are not directly affected by HIV infection and contrasts with immunodeficiency of other lymphocytes in the lamina propria and systemically.[28] This may be because intraepithelial lymphocytes have the γδ T-cell receptor (at least in the mouse) rather than the αβ T-cell receptor of systemic lymphocytes,[33] and therefore are a fundamentally different (possibly more primitive) population of immune cells.

The number of goblet cells showed no decrease as might be expected from their possible dependence on T cells.[10][11] This presumably means that the number of goblet cells are influenced by other factors. Any inference of goblet cell function must, however, be cautious as the count may be underestimated because of mucus discharge and difficulty in counting goblet cells.

In conclusion, our present study has shown an unusual pattern of enteropathy in AIDS/ARC with villous atrophy with impaired crypt hyperplasia but normal duodenal counts of mucosal mast cells, intraepithelial lymphocytes and goblet cells. The failure to compensate by crypt hyperplasia in the intestine of these subjects would severely compromise repair of villous atrophy and function of the gut. In addition, HIV infection may possibly destroy intestinal crypt cells and reduce available crypt cells to populate adjacent villi.

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