ULTRASTRUCTURE OF THE JEJUNAL MUCOSA IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Received 6 January 1989
Accepted 22 January 1990

SUMMARY

The ultrastructural changes in the jejunal mucosa of 11 male patients, three with clinical AIDS, five with AIDS-related complex—progressive generalized lymphadenopathy (ARC—PGL), and three who were only HIV antibody positive, were studied. In the enterocytes, major abnormalities were proliferation of smooth endoplasmic reticulum mitochondrial changes, vacuolization of cells, and fat hold up. In the lamina propria, degeneration of enteric nerve axons and smooth muscle were seen. Microvasculature showed both endothelial cell degeneration and hyperplasia. The presence of tubuloreticular inclusions in endothelial cells paralleled the stage of the disease. Since none of the 11 patients had any opportunistic infection, these changes are likely to be the effect of HIV infection.

KEY WORDS—HIV infection, acquired immune deficiency syndrome, jejunal biopsy, ultrastructure.

INTRODUCTION

In patients with the acquired immunodeficiency syndrome (AIDS), chronic diarrhea and malabsorption can lead to marked weight loss as indicated by the local name ‘slim disease’ in Africa. Although nucleotide probing has demonstrated incorporation of the human immunodeficiency virus (HIV) genome into crypt enterocytes, it is still not clear whether HIV infection by itself causes a primary lesion in the intestinal mucosa. The histopathologic changes reported in the small and large bowel epithelium in patients with AIDS have been attributed to opportunistic infections, inflammation, and neoplasia, and do not fully explain the pathogenesis of the diarrhea. In some patients with AIDS, chronic diarrhea and malabsorption are present without any identifiable enteric infectious agent. We therefore studied the ultrastructural morphology of jejunal biopsies from 11 adult homosexual males infected with HIV, none of whom had evidence of other viral, bacterial, or parasitic infection of the gut. We present ultrastructural evidence to show that HIV infection is associated with striking abnormalities, both in the enterocytes and in several of the lamina propria constituents.

MATERIALS AND METHODS

Jejunal biopsies were obtained with Crosby capsules under fluoroscopic control, from the proximal jejunum from 11 HIV antibody-positive male homosexual subjects, with diarrhea of more than 2 weeks’ duration, attending HIV outpatient clinics in London. Three of these patients had AIDS based on CDC criteria, four patients had AIDS related complex (ARC), one patient had persistent generalized lymphadenopathy (PGL), and three of the subjects were asymptomatic except for mild intermittent diarrhea. Opportunistic gastro-intestinal infections were excluded at the time of biopsy by repeated examination of wet smears of stool, appropriate
stool cultures, and examining paraffin-embedded tissue with the modified Ziehl-Neelsen stain for acid fast organisms, PAS for fungi, and Giemsa and Gram stains for protozoa and bacteria.

The biopsies were rapidly retrieved and a portion was immediately fixed in 2.5 per cent glutaraldehyde in cacodylate buffer and processed for transmission electron microscopy, and another portion was fixed in 10 per cent formal saline for light microscopic examination. The biopsies were post-fixed in osmium tetroxide, enblock stained in uranyl acetate, and embedded in epoxy resin. Sections were cut on a LKB ultratome IV with a diamond knife and stained with lead citrate. The biopsies were coded and the electron microscopist was unaware of the HIV antibody status or clinical classification of the patients. Control jejunal biopsies were obtained from ten male subjects, not belonging to accepted high-risk categories for acquiring HIV infection, in whom enteric infection had been excluded by methods identical to those used in the HIV-positive patients. The light microscopic appearance of these jejunal biopsies was normal. The HIV status of the controls was not tested because of ethical consideration.

The study was approved by the Ethical Committees of the St George’s and St Mary’s Hospitals, London and written consent was obtained from the patients.

RESULTS

The biopsies from the HIV-infected patients showed a variety of changes in the epithelium and lamina propria, in striking contrast to the biopsies from controls, which were within normal limits when examined by the electron microscope.

Epithelium

Proliferation of the smooth endoplasmic reticulum (SER) was a striking feature in all but one (asymptomatic) subject (Fig. 1a). In enterocytes on the upper third of the villi, the SER occupied up to half the area of the cell. Electron-dense inclusions with occasional tubular structures were present as clusters of varying sizes within dilated tubules of endoplasmic reticulum at the periphery of the area of SER proliferation (Fig. 1b). These inclusions...
were present in all the patients with AIDS and in one patient with ARC, but were not found in any of the three asymptomatic individuals. SER proliferation and the presence of inclusions did not correlate with the presence of fat particles in enterocytes (Table 1).

There was a reduction in the amount of rough endoplasmic reticulum with dropping off of ribosomes. The mitochondria were decreased in number, particularly in the enterocytes on the upper third of the villi. They were altered in shape with branching, irregular, or elongated forms as well as C- or cup-shaped mitochondria (Fig. 2). The mitochondrial cristae were longitudinally placed and the matrix appeared dense. In patients with ARC, PGL, and AIDS, these changes were present in 30–60 per cent of the mitochondria of cells in the upper third of the villi (Table 1).

Large vacuoles which were occasionally empty or contained membranous or proteinaceous debris were present in enterocytes in the upper and middle-third of the villi in half the subjects. A thin lining membrane could be visualized in some vacuoles (Fig. 3).

Throughout the villus and crypt epithelium there were scattered, single, or small groups of enterocytes which appeared to be degenerated, with well-preserved adjacent cells. The degenerated cells were darker staining with clumping of nuclear chromatin dilated rough endoplasmic reticulum and damaged mitochondria. There was an increase in lysosomal structures in most of the enterocytes on the villi.

Paneth cells of four patients showed alteration in the morphology of granules, with some of them showing crystalline inclusions (Fig. 4). Intra-epithelial lymphocytes with signs of activation were also increased.

Fat droplets were present in the apical cytoplasm, within the smooth endoplasmic reticulum, Golgi region, in dilated intercellular spaces, and beneath the basement membrane in the majority of patients (Table 1). In four subjects, the intercellular spaces were widely dilated and contained lipid material with a linear configuration.

**Lamina propria**

Plasma cells, macrophages, eosinophils, and mucosal mast cells contributed to the increased cellularity of the lamina propria in all the patients. The endoplasmic reticulum of plasma cells was dilated and many appeared to be undergoing cytolysis. Lysosomal particles were prominent in the macrophages, but viral particles could not be identified. Degranulation of eosinophils and mucosal mast cells was also observed. Scattered pyknotic and apparently degenerated cells were seen throughout the lamina propria, but the precise identification of the cell type was difficult.

Blood vessels were prominent and appeared to be increased in number. The endothelial cell cytoplasm contained tubuloreticular inclusions (TRIs), which were more prominent in patients with AIDS. The endothelial cells of some of the blood vessels showed
a varying degree of degenerative changes with marked swelling of the cells occluding the lumen and loss of cellular organelles, with dilatation of RER and swelling and loss of cristae of mitochondria (Fig. 5). Scattered endothelial cells were necrotic. Other vessels showed a hyperplastic response with an increase in cellular organelles, Weibel-Palade bodies, and multivesicular bodies. The nuclei of these cells were enlarged, more irregular, and contained prominent nucleoli. The basement membrane appeared thickened or reduplicated. Often small vessels with occluded lumens were found near crypts with focal degeneration of enteroctyes.

In all the patients, changes in the autonomic nerve fibrils of the intrinsic nerve plexus of the lamina propria of the intestinal mucosa were quite striking and have been reported in detail elsewhere. Briefly, there was proliferation of the nerve fibres and extensive degeneration of axons, which were swollen and electron-lucent with loss of cellular organelles. Schwann cells showed an increase in lipofuscin pigment (Table II).

Myofibrillar degeneration of smooth muscle cells in the lamina propria was a striking feature. The periphery of the cells showed large protrusions of sarcoplasmic membrane in between dense plaques. Degenerated axonal bundles were often found adjacent to such damaged smooth muscle fibres (Fig. 6).

DISCUSSION

The abnormalities detected in the epithelium and lamina propria of the jejunal mucosa of these patients, without evidence of opportunistic infection, suggest that HIV infection itself can damage this tissue. HIV genome incorporation has been demonstrated by nucleotide probing in jejunal and rectal mucosa. The failure to detect HIV particles in any of these biopsies may be because of a low rate of production of morphologically recognizable
particles or difficulty in distinguishing the particles from damaged cellular organelles. *In vitro* HIV can infect epithelial cells derived from colorectal carcinoma in continuous culture.\(^{12}\)

In villous enterocytes, proliferation of the SER was a striking feature. Proliferation of the SER has been reported in enterocytes and liver cells secondary to drug-induced enzyme production.\(^{13,14}\) These patients were not on any drugs, in particular Azidothymidine or barbiturates, during the month prior to the biopsy, nor were they consuming alcohol in excess. SER proliferation can also occur during active fat absorption,\(^{15}\) but these patients had been fasting for at least 7 h prior to biopsy. The presence of fat particles in the epithelium and lamina propria indicates derangement of fat transport but the prominent SER proliferation, not correlated with intracellular lipid, is not found in other malabsorptive conditions such as tropical sprue.\(^{16}\) The nature of the particles seen within the SER is not clear and some of these could be lipoprotein particles.\(^{17}\) However, the prevalence of particles with a tubular configuration within the SER mainly in patients with AIDS suggests that these could be associated specifically with HIV infections. A variety of tubular complexes have been described in viral infections and neoplasia.\(^{18,19}\)

Morphologic abnormalities in mitochondria with abnormal orientation of cristae have been reported in association with alteration of mitochondrial enzymes following exposure to toxins, neoplasia, hypoxia, and delayed fixation.\(^{20}\) Delayed fixation is an unlikely cause of the mitochondrial alterations, since the control biopsies with normal mitochondria were processed in exactly the same fashion. The mitochondrial abnormalities in these patients are likely to be from the alterations responsible for SER proliferation, although hypoxia
secondary to microvascular alterations may also play a part.

Scattered damage and necrosis of enterocytes was maximal in patients with AIDS, confirming reports by others. This enterocyte damage is due to cell-mediated cytolysis of virus-infected cells, with an additional contribution by ischaemia due to vascular occlusion. The Paneth cell alteration could be secondary to malabsorption of nutrients, especially zinc, as has been observed in other gastro-intestinal disorders.

Striking abnormalities were seen in the lamina propria. The involvement of the nerves to a similar extent in all the groups of patients (Table II) suggests that this tissue may be one of the primary targets of the virus. The enteric nervous system is important in controlling blood flow, motility, and epithelial transport. The neural damage in the intrinsic plexuses can partly explain the diarrhoea in patients with AIDS.

The smooth muscle myofibrillar degeneration and prominent sarcoplasmic protrusions are similar to the changes described in isolated smooth muscle fibres in vitro, as a result of electrical or agonist stimulation. Such changes have not been reported previously in intact smooth muscles. These sarcoplasmic protrusions are similar to the sarcolemmal pads occurring during myofibrillar degeneration of striated muscle secondary to denervation, deprivation of vascular supply, metabolic insufficiency, cachexia, mechanical compression, drug action, or muscle disorders. In the present group of patients, the smooth muscle abnormalities are most likely secondary to denervation caused by damage to the intrinsic autonomic plexus. Motility abnormalities consequent on this smooth muscle damage may also contribute to diarrhoea.

The endothelial abnormalities and the reduplication of the vascular basement membrane in the lamina propria confirm that the endothelium is
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TRI = Tubuloreticular inclusion.

Fig. 6—A cross-section (C) and longitudinal section (L) of smooth muscle cells with sarcoplasmic membrane projections (P) and adjacent non-myelinated nerve (N) with axonal degeneration. × 15,000
another tissue which is affected early by HIV. Although endothelial degeneration and necrosis were present, no virus particles were found in endothelial cells or in adjacent macrophages, as described in necrotizing vasculitits of peripheral nerves in AIDS. 13 Prevalence of TRI paralleled the disease stage (Table II) as observed by other workers also. 18 The proliferation and hyperplasia of the endothelium are similar to pre-Kaposi sarcoma changes seen in skin capillaries. 29 This is of significance, since gut mucosa is the most common site of visceral Kaposi's sarcoma in AIDS. 31

Two possible factors explain the pathogenesis of fat malabsorption in these patients. Metabolic alterations in the enterocytes, as evidenced by the SER and mitochondrial changes, may interfere with triglyceride synthesis in enterocytes. The linear arrays in lateral intercellular spaces of enterocytes and lamina propria may be the product of this alteration. Additionally, the failure of pumping action due to smooth muscle and enteric nerve axonal damage may be an important factor, as evidenced by the empty and dilated lymphatics, with large amounts of fat held up in the lamina propria spaces. 32-36

ACKNOWLEDGEMENTS

The Wellcome Research Unit is supported by the Wellcome Trust, London in association with the Christopher Medical College, Yellore. E.G.E. is a Wellcome Trust Senior Lecturer. This work was supported in part by the Medical Research Council, U.K.

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