Damage to jejunal intrinsic autonomic nerves in HIV infection

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Jejunal biopsies from 11 HIV-infected male homosexuals, without secondary enteropathogen infection, were examined at ultrastructural and light microscope level. Subjects were clinically categorized into four groups: asymptomatic (3), AIDS-related complex (4), persistent generalized lymphadenopathy (1), and AIDS (3). All 11 biopsies, including the three from asymptomatic HIV-infected individuals, showed extensive damage to autonomic nerve fibres in the lamina propria. These findings show that HIV infection damages the enteric autonomic nerves. Since asymptomatic HIV-infected individuals had similar damage, this appears to be an early event in the course of HIV infection.

Keywords: Ultrastructure, autonomic nerves in jejunal mucosal, HIV infection.

Introduction

There is currently no direct information concerning autonomic nervous system function or structure in HIV disease in any organ system. However, indirect evidence, obtained from recording cardiovascular autonomic responses [1,2] suggests malfunction of the autonomic nervous system early in HIV infection.

Chronic diarrhoea is often a dominant clinical feature of HIV infection [3] with [4] or without [5] secondary opportunistic infection. While partial villus atrophy and fat malabsorption have been documented [6], nothing is known about intestinal autonomic nervous function in HIV infection, although such dysfunction can be associated with chronic diarrhoea [7]. Since the morphology of intestinal autonomic nerve fibres can be readily visualized in jejunal biopsies [8], 11 male homosexual patients at different clinical stages of HIV infection were studied in an effort to identify morphological alterations in the autonomic nervous tissue.

Subjects and methods

Eleven HIV-antibody-positive male homosexual subjects attending HIV outpatient clinics in London volunteered to take part in the study. Three patients were asymptomatic, four patients had AIDS-related complex, one patient had persistent generalized lymphadenopathy, and three patients had AIDS, based on the Centers for Disease Control criteria [9]. Subjects were asked whether at any time in the previous 2 weeks they considered that they had no, mild, moderate or severe diarrhoea based on recalled stool frequency (Table 1).

Each subject underwent Crosby capsule biopsy of the proximal jejunum under fluoroscopic control. A portion of the biopsy was immediately fixed in 2.5% glutaraldehyde in cacodylate buffer and processed for transmission electron microscopy. Another portion of the biopsy was fixed in formal saline for paraffin wax embedding and light microscopy. Paraffin sections were stained with haematoxylin and eosin and graded as normal or showing partial villous atrophy by a consultant pathologist who was unaware of the clinical diagnosis. Enteric infection was searched for in specially stained (Ziehl–Neilsen, periodic acid-Schiff, Giemsa and Gram) jejunal biopsy sections in stool culture and microscopy. Transmission electron microscopic examination of coded jejunal biopsies were carried out at Vellore and the examiner (MM) was unaware of the HIV antibody status or clinical classification of the patients. Jejunal biopsies from 10 male subjects, who were investigated for diarrhoea but had

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Table 1. HIV infection classification and clinical details of 11 HIV-antibody-positive male homosexuals studied.

<table>
<thead>
<tr>
<th>HIV clinical classification</th>
<th>Age (years)</th>
<th>Subjective presence of diarrhoea</th>
<th>Villous morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Asymptomatic</td>
<td>26</td>
<td>Moderate</td>
<td>Normal</td>
</tr>
<tr>
<td>2. Asymptomatic</td>
<td>52</td>
<td>Moderate</td>
<td>PVA</td>
</tr>
<tr>
<td>3. Asymptomatic</td>
<td>28</td>
<td>Mild</td>
<td>Normal</td>
</tr>
<tr>
<td>4. ARC</td>
<td>34</td>
<td>Moderate</td>
<td>Normal</td>
</tr>
<tr>
<td>5. ARC</td>
<td>27</td>
<td>Moderate</td>
<td>PVA</td>
</tr>
<tr>
<td>6. ARC</td>
<td>44</td>
<td>Moderate</td>
<td>PVA</td>
</tr>
<tr>
<td>7. ARC</td>
<td>32</td>
<td>Nil</td>
<td>Normal</td>
</tr>
<tr>
<td>8. PGL</td>
<td>39</td>
<td>Moderate</td>
<td>PVA</td>
</tr>
<tr>
<td>9. AIDS</td>
<td>40</td>
<td>Moderate</td>
<td>PVA</td>
</tr>
<tr>
<td>10. AIDS</td>
<td>52</td>
<td>Nil</td>
<td>Normal</td>
</tr>
<tr>
<td>11. AIDS</td>
<td>44</td>
<td>Severe</td>
<td>PVA</td>
</tr>
</tbody>
</table>

ARC, AIDS-related complex; PVA, partial villous atrophy. The clinical scoring of subjective diarrhoea was based on stool frequency: mild, <three stools/day; moderate, four-to-six stools/day; severe, >six stools/day.

normal light microscopic jejunal histological appearance, served as controls. HIV serological testing was not carried out on control subjects for ethical reasons but none of the control subjects was in an accepted high-risk category.

The study was approved by the ethical committees of St George's and St Mary's Hospitals and written consent was obtained from patients.

**Results**

The age of the patients ranged between 26 and 52 years. All but two of the patients, whose jejunal biopsies were graded as normal on light microscopy, gave a history of diarrhoea (Table 1). Enteropathogens were not detected in the stool or jejunal biopsies of any of these patients. Six patients, with jejunal biopsies showing partial villous atrophy, had either moderate or severe diarrhoea.

Scattered nerve bundles were easily identifiable in the jejunal mucosal lamina propria at transmission electron microscopy. In the control subjects the nerve profiles consisted of axonal bundles surrounded by Schwann cells which enclose each axon by simple folding and overlapping of the cytoplasmic sheath (Fig. 1). The axonal cytoplasm contains mitochondria, microtubules and neurofilaments, tubular profiles of agranular endoplasmic reticulum and a variety of vesicles which help to identify neurotransmitters associated with the particular nerve [8]. The number of axons within each bundle and the diameter of the axon profiles were different in different bundles.

The nerve bundles in the biopsies from all the 11 patients infected by HIV showed extensive damage. At least 20 axonal bundles were examined in each biopsy. More than 70% of the fibres were swollen, ballooned and electron lucent (Fig. 2a, b, c) with loss of normal axonal organelles. Myelin figures and lysosomal particles were present in the axonal cytoplasm. The extent of damage was not related to the clinical status of HIV infection. The residual small granular vesicles in the damaged axons were characteristic of the neurosecretory granules of adrenergic nerves. The damaged axonal bundles were often located adjacent to smooth muscle cells, blood vessels and the base of crypts. The Schwann cells surrounding the axons showed degenerative changes with dilated endoplasmic reticulum and increased lipofuscin granules (Fig. 3). Virus particles could not be identified in axons or Schwann cells.

**Discussion**

The results presented here provide direct evidence for autonomic nerve damage in HIV infection and confirm earlier suggestions, based on physiological responses, of damage to the autonomic nervous system [1,2]. Degenerate axons were predominantly found around crypt bases,
Fig. 2. Transmission of electron micrograph of jejunal mucosal lamina propria nerve bundles from patients infected with HIV. (a) Biopsy from an asymptomatic HIV infected subject. The axons (A) are swollen and balloononed with loss of neurotubules and appear electron lucent (magnification 25,000). (b) Biopsy from a patient with AIDS-related complex. Appearance similar to (a) (magnification 25,000). (c) Biopsy from a patient with AIDS. Axons in addition to ballooning and loss of neurotubules shown myelin figures (arrows). A single axon with preserved neurosecretory granules (N) is also present (magnification 25,000).

Fig. 3. Biopsy from a patient with AIDS. Schwann cell (S) containing lipofuscin granules (arrow) and degenerated axonal bundles (A) (magnification 25,000).

an area of the jejunal mucosa where the HIV genome has been demonstrated [10]. Damaged autonomic nerves were detected in patients who were only HIV-antibody positive as well as in jejunal mucosa with normal villus architecture (Table 1), suggesting that autonomic nerve involvement occurs at a very early stage of HIV infection of the jejunum.

The failure to detect viral particles in the damaged nervous tissue may be because of genome incorporation [10], small numbers of viruses or difficulty in differentiating from other damaged organelles. HIV nucleic acids and proteins have been detected in capillary endothelium, macrophages, microglial cells and occasionally in neurones of the central nervous system in patients with AIDS [11–13]. The CD4 receptor is expressed on the membrane of neuroglial cells [14] but it is not known whether they are present in the Schwann cells of the autonomic system. Axonal degeneration has been demonstrated in slow virus infections such as Kuru [15] and Jakob–Creutzfeldt disease [16].

Axonal damage of the gut autonomic nervous system, morphologically similar to the changes found in these HIV infected patients, has been reported in a variety of conditions including long-term laxative abuse, diabetic autonomic neuropathy, chronic inflammatory bowel disease and amyloidosis [17]. In particular, morphologically similar changes have been reported in the jejunal mucosa in Crohn’s disease, where a viral aetiology has been suspected [18]. The pathogenesis of the damage to the lamina propria nerve bundles in these conditions is not understood. It appears that the intramural nervous system of the gut shows a uniform pattern of damage irrespective of the primary damaging agent, with swelling and ballooning of axons and loss of axon organelles. This degeneration of the bundle can lead to impaired function.

The autonomic nervous system of the gastrointestinal tract controls gut motility; there is evidence that absorption is also under autonomic control [19]. Sympathetic denervation of intestinal segments causes spontaneous secretion [20] and adrenergic agonists enhance absorption in humans in vivo [21]. It is possible, therefore, that malabsorption of fluid and electrolytes from the gastrointestinal tract, as part of the pathophysiological response to autonomic denervation reported in this study, may contribute to diarrhoea in HIV disease, in addition to the fat malabsorption which has been previously documented [6].

The current study with direct ultrastructural evidence of autonomic nerve involvement early in HIV infection, along with the indirect evidence provided by results of psychometric testing and magnetic resonance brain imaging of asymptomatic and AIDS-related complex sub-
jects [22] show that HIV infection involves the nervous system early in the course of the illness.

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References