Clin Exp Immunol 1993; 92:448–454

Loss of mucosal CD4 lymphocytes is an early feature of HIV infection

S. G. LIM, A. CONDEZ*, C. A. LEE†, M. A. JOHNSON‡, C. ELIA§ & L. W. POULTER* *Departments of Medicine and *Clinical Immunology, †Haemophilia Centre, ‡AIDS Unit, Royal Free Hospital and School of Medicine, London, UK, and §Department of Internal Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

(Accepted for publication 8 March 1993)

SUMMARY

T cell subsets in the gut mucosa are distinct populations and their imbalance in HIV has specific implications in infection. Alterations in T cell subsets in duodenal biopsies were investigated in 17 asymptomatic HIV patients, 24 AIDS patients and 10 controls with non-ulcer dyspepsia. Immunohistochemistry and immunofluorescence using MoAbs to CD3, CD4, CD8, CD68, CD45RA, CD45RO and gp120 were performed on frozen sections. In the lamina propria, there was a significant depletion of $CD4^+$ cells at all stages of HIV, but the density of CD8 lamina propria cells was increased. Intraepithelial lymphocytes were decreased in AIDS patients. There was a significant correlation between cellular density and mucosal $CD3^+$ lymphocytes, and between mucosal $CD3^+$ and $CD8^+$ lymphocytes. Although mucosal $CD4,CD45RO^+$ 'memory' cells were decreased, $CD8,CD45RO^+$ 'memory' cells were increased. Mucosal $CD4^+$ lymphocyte depletion occurred early in HIV, and thus their role in mucosal protection against opportunistic infection should be revised. Mucosal $CD8^+$ lymphocytes initially increased, but decreased when CD4 blood counts were depleted, perhaps contributing to loss of host protection against infection. Intraepithelial lymphocyte depletion may also contribute to opportunistic infection.

Keywords AIDS gastrointestinal immunology immunohistochemistry lymphocyte subsets

INTRODUCTION

The mucosal immune cells of the gut or gut associated lymphoid tissue (GALT) are a cellular population distinguished from immune cells in blood by specific phenotypic markers [1–3] and by a restricted migration pattern [4,5]. Although the proportion of $CD4^+$ and $CD8^+$ lymphocytes in the blood and the gut lamina propria in normals are similar, $CD4^+$ lymphocytes of the gut lamina propria are predominantly $Leu\text{-}8^-$ ('helper inducer') and $CD45RO^+$ ('memory'), and $CD8^+$ lymphocytes are similarly $Leu\text{-}8^-$ ('suppressor effector'), $CD45RO^+$ ('memory'), but are $CD16^-$ (absence of natural killer (NK) activity) [1]. While Leu-8 is thought to be a homing receptor for peripheral lymph nodes, it also appears to identify a subset of T cells that may have suppressor activity or induce CD8 suppressor cells [1]. Unstimulated lamina propria T lymphocytes in the gut have a higher expression of CD25 (IL-2 receptor) and MHC class II antigens compared with blood [6]. Lamina propria T lymphocytes also exhibit preferential migration pathways to mucosal tissues [4,7] by expressing selective adhesion molecules CD44 (Hermes 3) and very late activation antigen-4 (VLA-4). These phenotypic specificities suggest that GALT has specialized functions in immune surveillance and responses at the mucosal interface.

Immunohistochemical studies analysing immune cell subpopulations of the gut in HIV have shown that mucosal $CD4^+$ cells are decreased in AIDS patients, often with an increase in mucosal $CD8^+$ lymphocytes, resulting in an inversion of the normal CD4:CD8 ratio [8–11], but only two studies have investigated mucosal immune cells in early HIV disease. Zeitz and colleagues [11] found the number of mucosal $CD4^+$ cells to be no different from controls, while Schrappe-Bächer and colleagues [12] found mucosal $CD4^+$ cells in HIV^+ patients to be decreased compared with HIV^- controls, although patients with advanced AIDS (Walter-Reed stage 6) had a more profound decrease. Small bowel intraepithelial lymphocytes were found to be either increased [13,14], normal [15,16], or decreased [10].

Immune mechanisms [17] have been postulated to be involved in HIV-associated enteropathy [13,15,16,18,19], although the mechanisms are still unclear. There appears to be little relationship to the density of HIV-infected cells [18,20], nor has HIV conclusively been shown to infect enterocytes [20,21], and the depletion of T cell activation markers that are normally associated with immune-mediated mucosal damage [17,22] make this mechanism unlikely. CD4 lymphocyte counts have been used as a predictor for the development of opportunistic infection [23,24], but the mucosal events at the interface where

Correspondence: Dr L. W. Poulter, Department of Clinical Immunology, Lower Ground Floor, Royal Free Hospital School of Medicine, Pond Street, Hampstead, London NW3 2QG, UK.

Table 1. Clinical features of patients in the study

Patient no.	Age (years)	CDC	AIDS-defining illness	CD4 count ($\times 10^9/l$)	Endoscopic indications
4	32	II		0·548	Dysphagia
6	54	AIDS	PCP	0·008	Abdominal pain
8	43	AIDS	Oes. candida	0·064	Abdominal pain
9	41	AIDS	Oes. candida	0·028	Abdominal pain
10	22	II		0·294	Retrosternal pain
11	23	II		0·630	Dysphagia/abdominal pain
15	40	II		0·253	Abdominal pain
17	40	AIDS	PCP	0·001	Vomiting
19	31	II		0·005	Pharyngeal ulcers
20	19	II		0·360	Diarrhoea
21	37	II		0·350	Diarrhoea
26	35	AIDS	Oes. candida	0·004	Diarrhoea/abdominal pain
27	48	AIDS	PCP	0·011	KS assessment
29	38	II		0·338	Diarrhoea
30	40	AIDS	CMV	0·084	Abdominal pain
31	42	AIDS	PCP	0·130	KS assessment
32	50	AIDS	KS	0·013	Diarrhoea
34	31	AIDS	Oes. candida	0·004	Vomiting
35	45	AIDS	HIV neuropathy	0·020	Dysphagia
36	44	II		0·042	Dysphagia
37	24	II		0·240	Abdominal pain/diarrhoea
38	32	II		0·180	Dyspepsia
40	53	AIDS	KS	0·020	Abdominal pain
41	31	AIDS	Toxo myocarditis	0·500	Haematemesis
42	39	AIDS	PCP	0·020	Retrosternal pain
43	25	II		0·290	Abdominal pain
44	27	AIDS	Oes. candida	0·018	Dysphagia
45	23	II		0·480	Dyspepsia/vomiting
46	36	AIDS	PCP	0·006	Abdominal pain
47	33	AIDS	Oes. candida	0·090	Diarrhoea
48	22	AIDS	Oes. candida	0·030	Diarrhoea
49	30	II		0·000	Diarrhoea
51	28	AIDS	Oes. candida	0·040	Dysphagia
53	28	II		0·535	Abdominal pain
54	33	AIDS	Oes. candida	0·100	Dysphagia
55	42	II		0·210	Dysphagia
56	37	AIDS	PCP	0·020	Dysphagia
57	22	II		0·070	Diarrhoea
58	29	AIDS	Oes. candida	0·000	Dysphagia
59	38	AIDS	KS	0·530	Dysphagia
62	48	AIDS	Oes. candida	0·000	Diarrhoea/abdominal pain

PCP, *Pneumocystis carinii* pneumonia; KS, Kaposi's sarcoma; CMV, cytomegalovirus; oes. candida, oesophageal Candida; toxo, Toxoplasmosis.

immune cells encounter infectious agents have not been sufficiently explored.

The aim of the current study was to determine the alterations in mucosal immune cell subsets at different clinical stages of HIV infection, their tissue distribution, whether the alterations in immune subsets of GALT paralleled those in the blood in HIV infection, and their relationships to the development of opportunistic infection.

PATIENTS AND METHODS

Patients

Forty-one HIV$^+$ patients underwent upper gastrointestinal endoscopy for a variety of clinical indications (see Table 1). All patients had biopsies taken from the third part of the duodenum. The HIV$^+$ patients comprised 37 males and four females, and the risk groups were predominantly homosexual, with four haemophiliacs, three heterosexuals and five i.v. drug users. They formed two groups: group 1, those with asymptomatic (CDC II) HIV disease ($n=17$) and group 2, AIDS (CDC IV B/C/D) ($n=24$) as defined by the Centre for Disease Control [25]. The patients with asymptomatic HIV disease had a median age of 29 years (range 19–44 years) and median CD4 count of $0{\cdot}272 \times 10^9/l$ (range: $0{\cdot}0$–$0{\cdot}63 \times 10^9/l$), while those with AIDS had a median age of 39 years (range 27–54 years) and a median CD4 count of $0{\cdot}02 \times 10^9/l$ (range $0{\cdot}0$–$0{\cdot}53 \times 10^9/l$) (see Table 1). Twenty-two patients were taking antiretroviral therapy at the time of endoscopy (five asymptomatic and 17 AIDS). Patients who were

Table 2. Monoclonal antibodies used in the study

CD no.	Monoclonal antibody	Source	Specificity in normal tissues
CD3	UCHT1	Dr P. Beverly	All T lymphocytes
CD4	Leu-3a and Leu-3b	Becton Dickinson	T helper lymphocytes, macrophages
CD8	RFT8	In house	Suppressor/cytotoxic T lymphocytes
CD68	EBM11	Dako Ltd.	All macrophages
CD45RO	UCHL1	Dr P. Beverly	'Memory' T lymphocytes
CD45RA	SN130	In house	'Naive' T lymphocytes
nil	Anti-gp120	Du Pont	Epitope on HIV envelope

on chemotherapy or steroids were excluded from the study. Ten otherwise normal patients, eight males and two females, who were diagnosed as having non-ulcer dyspepsia after endoscopy and who were not taking medication, were used as controls. All controls had a normal upper gastrointestinal endoscopy and normal gut histology on biopsy.

Preparation and analysis of biopsy material

All HIV patients had duodenal biopsies routinely sent for histology, bacteriological and mycobacterial culture, and for cytomegalovirus (CMV) and herpes simplex virus culture. Biopsies sent to histopathology were fixed in 10% buffered formalin, embedded in paraffin blocks and 6 μm sections were cut and stained with haematoxylin-eosin and special stains (Giemsa, Ziehl/Neilsen, Gram, periodic acid-Schiff). In addition, immunohistochemistry was performed for CMV. Biopsies were sent to virology in viral transport medium. The biopsies were homogenized and cultured on human embryonic lung fibroblasts. Detection of early antigenic fluorescent foci (DEAFF) was performed after 12–16 h, to identify infection with CMV. In addition, cultures were maintained for 21 days, or until a positive result, whichever occurred first. Impression smears of biopsies sent to microbiology were assessed microscopically by Auramine (cryptosporidium) and Giemsa (giardia) stains for ova, cysts and parasites. They were then placed on Sabouraud's medium for yeasts and Robertson's cooked meat medium for bacterial pathogens; these cultures were read after 24–48 h. Biopsies sent for mycobacterial culture were placed on two Lowenstein–Jensen slopes, one supplemented with pyruvate and one with glycerol. Biopsy specimens were, in addition, cultured in Kirchner's broth. Patients with intestinal pathogens on biopsy or stool microscopy were excluded. In addition, biopsies from each patient were placed on saline-soaked gauze, and transferred to cork and embedded in Optimal Cutting Temperature Compound (Miles Scientific Labs, Kankakee, IL) after orientation, and snap frozen in isopentane cooled in a liquid nitrogen bath. Cryostat sections (6 μm) were cut, air dried overnight, and then fixed in acetone:chloroform (1:1) for 15 min. Slides were coded and later examined by a blinded observer.

Immunoperoxidase method

Sections were incubated with MoAbs against CD3 (Table 2) in a dilution of 1:5, using a previously described indirect immunoperoxidase method [26], and a second layer antibody (P161, rabbit anti-mouse, Dako Corp., Santa Barbara, CA). A positive control section (human palatine tonsil) and a patient's negative control section was used with each staining procedure.

Alkaline phosphatase method [26]

Sections were incubated with the following MoAbs: anti-CD4, anti-CD8, anti-CD68 (Table 2), all at a dilution of 1:5, followed by second and third layer antibodies (Z259 and D651; Dako) conjugated to calf alkaline phosphatase. Appropriate positive and negative controls as in the peroxidase method were used.

Double alkaline phosphatase method

This method [26] was employed to differentiate cells expressing CD4 alone (lymphocytes) and CD4 together with CD68 (macrophages) (Table 2). The first monoclonal (CD4) is fully developed in blue using the previously described alkaline phosphatase method, then the slides washed in running water before applying the second MoAb (CD68) and then fully developed in red.

Cell counting

Cell counting was performed using an image analyser (Seescan Ltd, Cambridge, UK) which allowed fields seen under the microscope ($\times 40$ magnification) to be separated by means of outlining areas of lamina propria, and either excluding epithelium or outlining epithelium alone. Positive cells were identified and counted within each field, recording the number of cells and the field area. Three to five representative fields were counted on each specimen.

Double immunofluorescence method

MoAbs used in combination [27] at a dilution of 1:5 include CD8(IgM)/CD45RO(IgG) and CD45RO(IgG1)/CD45RA (IgG2) (Table 2) followed by a second layer mixture of anti-IgG or anti-IgG1 FITC and anti-IgM or anti-IgG2 tetraethyl rhodamine isothiocyanate (TRITC) purchased from Southern Biotechnology Inc, Birmingham, AL. Immunofluorescent counting was performed on a fluorescence microscope (Zeiss, Oberkochen, Germany) at $\times 40$ magnification with selective filters for FITC and TRITC. Cells were either positive for FITC, TRITC, or both. As before, three to five representative fields were counted. Single immunofluorescence staining was performed with gp120 (Du Pont, Boston, MA) with FITC-conjugated anti-IgG. The number of positive cells/high power field were counted, a positive control being cytospins of an HIV-infected lymphocyte cell line.

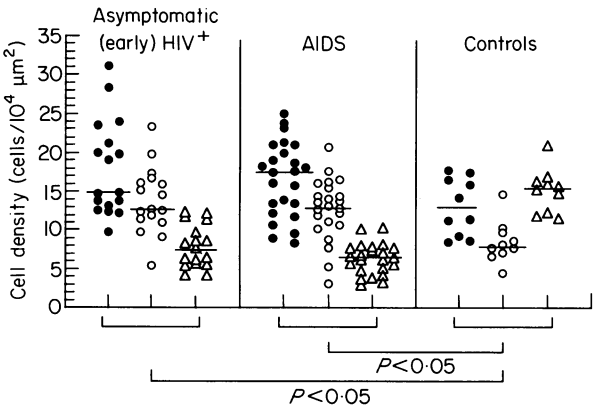

Fig. 1. Density of $CD3^+$, $CD8^+$ lamina propria lymphocytes and $CD4^+$ lamina propria cells (cells/10^4 μm^2) in patients with asymptomatic HIV infection and AIDS compared with controls. Horizontal bars denote medians. There were significant differences in patients with asymptomatic HIV infection and AIDS compared with controls, in all three cell types ($CD3^+$, $CD4^+$ and $CD8^+$) (Wilcoxon rank sum test). ●, CD3; ○, CD8; △, CD4.

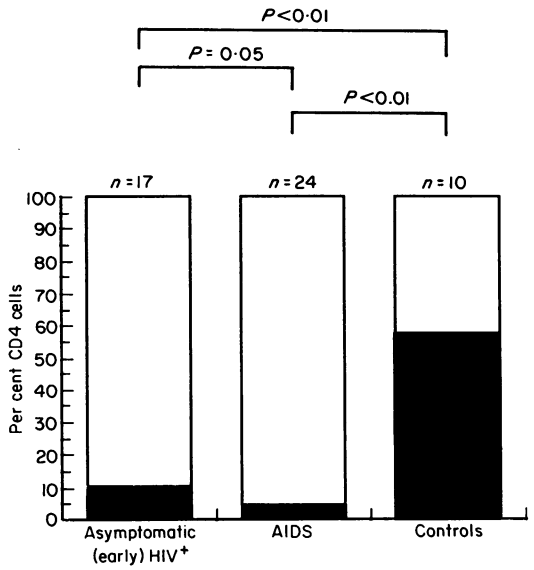

Fig. 2. Proportion of $CD4^+$ lymphocytes *versus* macrophages in patients with asymptomatic HIV infection and AIDS compared with controls. There were significant differences between all those with HIV and controls (χ^2-test, $P \leq 0{\cdot}05$), and between HIV^+ asymptomatics and AIDS patients ($P < 0{\cdot}05$). □, Per cent $CD4^+$, $CD68^+$ cells (macrophages); ■, per cent $CD4^+$, $CD68^-$ cells (lymphocytes).

Statistical analysis

All data are presented as cells per unit area except for doubly stained cells (CD45RO/CD45RA, CD8//CD45RO, CD4/CD68) which were represented as a percentage of total positive staining cells present, and $CD3^+$ intraepithelial lymphocytes represented as number of positive staining cells per 100 epithelial cells. All graphical data are presented as scattergrams, except for doubly stained cell populations which were represented as bar histograms. Data analysis was performed using the Wilcoxon rank sum test, comparison of proportions was performed using the χ^2-test, and correlations performed using Spearman correlation analysis. Comparisons between groups, and correlations were considered to be statistically significant if $P < 0{\cdot}05$.

RESULTS

Total cellularity

There was no difference in total cellularity of the lamina propria per unit area between those with asymptomatic disease, AIDS and normal controls (data not shown).

Lamina propria $CD3^+$ and $CD8^+$ lymphocytes

There was a significant increase in $CD3^+$ and $CD8^+$ lamina propria lymphocytes in all patients with HIV compared with controls (Fig. 1).

Lamina propria $CD4^+$ cells

There was a significant and profound decrease in lamina propria $CD4^+$ cells in patients with HIV compared with controls (Fig. 1). Double staining for CD4 and CD68 demonstrated two distinct populations of $CD4^+$ cells—those that were $CD4^+$, $CD68^+$ representing macrophages, and those that were $CD4^+$, $CD68^-$ representing lymphocytes. Double staining with CD4 and CD68 revealed a depletion of $CD4^+$ lymphocytes at all stages of HIV infection, even in the early asymptomatic stage with better preserved blood CD4 counts (χ^2-test, $P < 0{\cdot}01$), those with AIDS having significantly lower lamina propria CD4 than those in early HIV infection ($P = 0{\cdot}05$) (Fig. 2). Spearman analysis revealed no correlation between lamina propria $CD4^+$ lymphocyte density and peripheral blood CD4 count (data not shown), the lamina propria CD4 count being considerably depleted even in patients with peripheral blood CD4 counts of $0{\cdot}5 \times 10^9/l$.

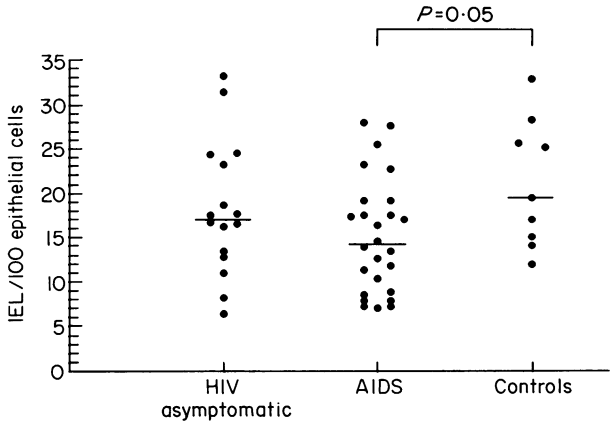

Fig. 3. Intraepithelial lymphocytes per 100 epithelial cells in patients with asymptomatic HIV infection and AIDS compared with controls. There was a significant difference between AIDS patients and controls ($P < 0{\cdot}05$, Wilcoxon rank sum test).

Intraepithelial lymphocytes

The number of intraepithelial lymphocytes (IEL)/100 epithelial cells was significantly decreased in AIDS patients compared with controls, but not in patients with asymptomatic HIV infection (Fig. 3).

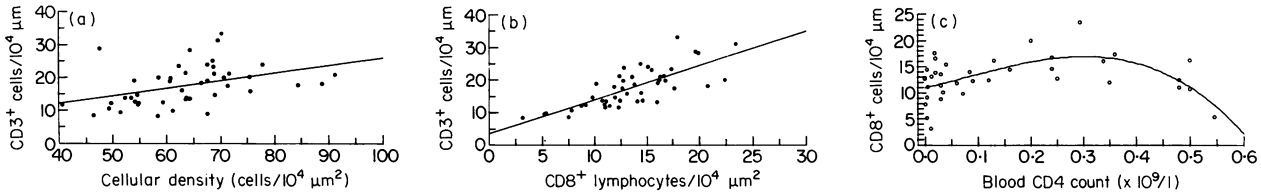

Fig. 4. (a) Spearman analysis showing significant correlation ($P<0.001$) between cellular density and lamina propria CD3+ lymphocytes in all HIV patients. (b) Spearman analysis showing significant correlation ($P<0.001$) between lamina propria CD3+ lymphocytes and lamina propria CD8+ lymphocytes. (c) Correlation analysis showing a second order polynomial relationship between lamina propria CD8+ lymphocytes and peripheral blood CD4 counts.

Correlations

Alterations in total lamina propria cell density in all HIV patients correlated with alterations in lamina propria CD3 density (Fig. 4a), i.e. the increase in density correlated with an increase in lamina propria CD3 density (Spearman correlation, $P<0.001$). This increase in lamina propria CD3 density directly correlated with increases in lamina propria CD8+ density (Fig. 4b, Spearman correlation, $P<0.001$). There was a second order polynomial relationship between decreasing peripheral blood CD4 counts and lamina propria CD8 density, rather than linear (Fig. 4c). A similar relationship exists between lamina propria CD8 density and blood CD8 counts (data not shown).

CD45 isoforms

There were no significant differences in the proportions of CD45RO+, CD45RA+ double staining T cells in patients with HIV or AIDS compared with controls (data not shown). Double staining with CD8 and CD45RO showed that HIV+ patients had a relative increase (χ^2-test, $P\leqslant 0.05$) in the CD8+, CD45RO− ('naive') population compared with the CD8+, CD45RO+ ('memory') population of CD8+ T cells (Fig. 5a). However, of the total CD45RO+ T cell population, compared with controls, there was a relative and dramatic increase (χ^2-test, $P<0.01$) in the CD8+, CD45RO+ population, due partly to the depletion of CD8−, CD45RO+ T cells (presumably CD4+, CD45RO+ lymphocytes) (Fig. 5b), and due partly to the increased density of mucosal CD8+ T cells (Fig. 1). Thus, there was an increase in total mucosal CD8+ density, to which 'naive' CD8+ T cells contributed significantly, but 'memory' CD8+ T cells increased markedly relative to CD8−'memory' T cells. The numbers of CD4 lymphocytes were too few to give meaningful CD45 isoform results (data not shown).

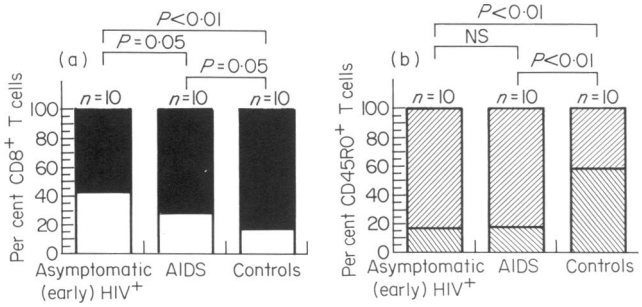

Fig. 5. (a) Proportion of CD8+ lymphocytes expressing or not expressing CD45RO in patients with asymptomatic HIV infection and AIDS compared with controls. Significant differences were found between controls and both asymptomatic HIV infection (χ^2-test, $P<0.01$) and AIDS ($P=0.05$); and between the two HIV+ groups ($P=0.05$). ■, Per cent CD8+, CD45RO+; □, per cent CD8+, CD45RO−. (b) Proportion of CD45RO+ lymphocytes expressing or not expressing CD8 in patients with asymptomatic HIV infection and AIDS compared with controls. Significant differences were found between controls and both HIV+ groups (χ^2-test, $P<0.01$). ▩, Per cent CD8+, CD45RO+; ▧, per cent CD8−, CD45RO+.

gp120 expression

Sixteen of 34 biopsies (47%) had 3–10 gp120+ positive cells per section in the lamina propria. Such numbers were also present in negative control sections (data not shown).

DISCUSSION

Alterations in subsets of CD4+ and CD8+ lymphocytes in HIV infection show that selective populations are affected in a time-dependent fashion. Amongst the CD4+ lymphocytes, 'memory' phenotypes (such as CD4, CD45RO) are selectively and profoundly lost even in the early stages of HIV infection [28–31]. This finding conforms with known defects in immune responses to recall antigens [29,32] in such individuals, and is compatible with data that indicate HIV replication occurs in activated cells [33]. In contrast, there is an expansion of the CD8+ lymphocytes, with an increase in the proportions of CD8+, CD45RO+ ('memory') and CD8+, CD38+ ('activated' or 'cytotoxic activity') subsets [34]. These phenotypic alterations may represent proliferation of CD8+ cells with both non-specific and HIV-specific cytotoxic activity [35,36].

Immunohistochemical studies on mucosal immune cells in the gut have shown that the CD4+ lymphocyte subset is depleted and the CD8+ lymphocyte subset is expanded in AIDS patients [8–10,37]. We have confirmed these findings, but have also found that the CD4+ lymphocyte subset is depleted in the earlier stages of HIV infection, a finding at variance with that of Zeitz and colleagues [11,17]. A particular problem in assessing mucosal CD4+ cells is the presence of CD4+ macrophages, which may distort the true density of mucosal CD4+ lymphocytes. However, these cells can be differentiated by double-staining with CD68, a pan-macrophage marker. Double staining confirmed that CD4+ lymphocytes were indeed severely depleted in all stages of HIV infection, while there was no alteration in density of CD68 staining mucosal macrophages in patients with HIV (Lim *et al.*, *Clinical and Experimental Immunology*, 1993; **92**:442–447). Supporting evidence for this was the linear relationship between CD3+ cells (pan-T cell marker) and CD8+ lymphocytes, confirming that the alterations in CD3+ cells was almost exclusively due to alterations in the CD8+ population, and not CD4. Furthermore, the possibility that CD4+ lymphocytes were 'masked' by gp120 was excluded

by the absence of significant gp120 staining in consecutive tissue sections. This early loss of CD4 mucosal lymphocytes is consistent with the depletion of peripheral blood $CD4^+$, $CD45RO^+$ 'memory' cells in early HIV disease [32,33], as this is the predominant (>90%) phenotype of $CD4^+$ mucosal lymphocytes [3]. Supporting evidence comes from the finding that the non-$CD8^+$ proportion of mucosal $CD45RO^+$ 'memory' cells (presumably $CD4^+$, $CD45RO^+$) is severely depleted early in HIV. Early depletion of $CD4^+$ mucosal lymphocytes may also be due to selective HIV replication in activated T cells ($CD25^+$) normally present in the gut [6]. This early loss of $CD4^+$ lymphocytes is not paralleled by early development of gut opportunistic infection. On the contrary, this occurs in the later stages of HIV disease, when more profound immunosuppression develops as blood CD4 counts are reduced [24]. This scenario suggests that other host defences (such as CD8 lymphocytes) play an equally important role in protection from gut infection.

The increase in mucosal $CD8^+$ lymphocytes appears to parallel the expanded blood $CD8^+$ population as a result of HIV infection. However, MoAb-induced $CD4^+$ depletion in animals results in a surprising increase in mucosal $CD3^+$, $CD8^+$ and $CD3^-$, $CD8^+$ subsets [38]. This may be a compensatory mechanism for the loss of CD4, or a result of release from $CD4^+$ 'suppressor' cells [39]. Our finding of an initial increase in mucosal $CD8^+$ lymphocytes followed by a later decrease has also been reported by Schrappe-Bächer and colleagues [12]. This finding may have implications for mucosal defence when the increase in $CD8^+$ T cells is mainly due to increases in 'unprimed' $CD8^+$ T cells, as shown in our study, and if initial levels of protective $CD8^+$ lymphocytes are progressively lost.

Animal models have also clearly demonstrated that T helper (CD4) subsets and CD8 T cells play vital roles in host defences against a variety of pathogens [40], thus are of major relevance in HIV- associated opportunistic infections of the gut such as CMV, Cryptosporidium and mycobacterial infection. The importance of CD8 T cells in host protection has been increasingly recognized [40], and the loss of CD4 T cells in HIV implies an increased role for $CD8^+$ cells in host defences. The findings in this study support the hypothesis that mucosal $CD8^+$ lymphocytes may play a far more important part in host protection against gut infection in HIV than has previously been recognized. The decrease in intraepithelial lymphocytes (which are predominantly $CD8^+$) in AIDS patients in this study may support this hypothesis, although this is not a consistent finding in other studies [10,13,15,16]. Such differences may however lie in the methodology of calculating the number or density of intraepithelial lymphocytes, and in selection of the patient groups.

ACKNOWLEDGMENTS

The authors are pleased to acknowledge that this work was supported by Medical Research Council grant G9902951 to S.G.L. and L.W.P., and Medical Research Council grant G8910613 to L.W.P.

REFERENCES

1 Kanof ME, James SP, Strobert W. The phenotype and function of T cells in the lamina propria of the human intestine. Reg Immunol 1988; **1**:190–5.

2 Akbar A, Terry L, Timms A *et al.* Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. J Immunol 1988; **140**:217–23.

3 Janossy G, Bofill M, Rowe D *et al.* The tissue distribution of T lymphocytes expressing different CD45 polypeptides. Immunol 1989; **66**:517–25.

4 Chin Y-H, Sackstein R, Cai J-P. Lymphocyte-homing receptors and preferential migration pathways. Proc Soc Exp Biol Med 1991; **196**:375–80.

5 Duijvestijn A, Hamann A. Mechanisms and regulation of lymphocyte migration. Immunol Today 1989; **10**:23–28.

6 Zeitz M, Greene CG, Peffer NJ *et al.* Lymphocytes isolated from the intestinal lamina propria of normal non-human primates have increased expression of genes associated with T cell activation. Gastroentrol 1988; **94**:647–55.

7 Chin Y-H, Cai J-P, Hieslaar T. Lymphocyte migration into mucosal lymphoid tissues: mechanism and modulation. Immunol Res 1991; **10**:271–8.

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

9 Budhara MD, Levendoglu H, Krocka F *et al.* Duodenal mucosal T cell subpopulation and bacterial cultures in acquired immunodeficiency syndrome. Am J Gastroenterol 1987; **82**:427–31.

10 Jarry A, Cortez A, Rene E *et al.* Infected cells and immune cells in the gastrointestinal tract of AIDS patients. An immunohistochemical study of 127 cases. Histopathology 1990; **16**:133–40.

11 Zeitz M, Ullrich R, Reicken E-O. The role of gut associated lymphoid tissue in the pathogenesis of the acquired immunodeficiency syndrome (HIV-infection). In: T.T. M, Challacombe SJ, R.V. H, *et al*, eds. Advances in mucosal immunology. Lancaster: Kluwer Academic Publishers, 1990:655–9.

12 Schrappe-Bächer M, Salzberger B, Fätkenheur G *et al.* T-lymphocyte subsets in the duodenal lamina propria of patients infected with the human immunodeficiency virus type 1 and the influence of high-dose immunoglobulin therapy. J AIDS 1990; **3**:238–43.

13 Kotler DP, Scholes JV, Tierney AR. Intestinal plasma cell alterations in acquired immunodeficiency syndrome. Dig Dis Sci 1987; **32**:129–38.

14 Weber JR, Dobbins WO. The intestinal and rectal epithelial lymphocyte in AIDS. Am J Surg Pathol 1986; **10**:627–39.

15 Batman PA, Miller ARO, Forster SM *et al.* Jejunal enteropathy associated with human immunodeficiency virus infection: quantitative histology. J Clin Pathol 1989; **42**:275–81.

16 Cummins AG, Labrooy JT, Stanley DP *et al.* Quantitative histological study of enteropathy associated with HIV infection. Gut 1990; **31**:317–21.

17 Ullrich R, Zeitz M, Heise W *et al.* Mucosal atrophy is associated with loss of activated T cells in the duodenal mucosa of human immunodeficiency virus (HIV)-infected patients. Digestion 1990; **46** (Suppl. 2):302–7.

18 Ullrich R, Zeitz M, Heise W *et al.* Small intestinal structure and function in patients infected with human immunodeficiency virus (HIV): evidence for HIV-induced enteropathy. Ann Intern Med 1989; **111**:15–21.

19 Editorial. HIV enteropathy. Lancet 1989; **2**:777–8.

20 Fox CH, Kotler D, Tierney A *et al.* Detection of HIV-1 RNA in the lamina propria of patients with AIDS and gastrointestinal disease. J Infect Dis 1989; **159**:467–71.

21 Fleming SC, Kapembwa MS, MacDonald TT *et al.* Direct *in vitro* infection of human intestine with HIV-1. AIDS 1992; **6**:1099–104.

22 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.

23 Masur H, Ognibene FP, Yarchoan R *et al.* CD4 counts as predictors of opportunist pneumonias in human immunodeficiency virus (HIV) infection. Ann Int Med 1989; **111**:223–31.

24 Crowe SM, Carlin JB, Stewart KI *et al.* Predictive value of CD4 lymphocyte numbers for the development of opportunist infections and malignancies in HIV-infected persons. J AIDS 1991; **4**:770–6.
25 MMWR. CDC revision of the CDC surveillance case definition for acquired immuno-deficiency syndrome. MMWR 1987; **36**:1–15.
26 Mason DY, Abdulaziz Z, Falini B *et al.* Double immunoenzymatic labelling. In: Polak JM, van Noorden S, eds. Immunocytochemistry: practical applications in pathology and biology. Bristol: J. Wright, 1983:113–7.
27 Janossy G, Bofill M, Poulter LW. Two colour immunofluorescence: analysis of the lymphoid system with monoclonal antibodies. In: Polak J, van Noorden S, eds. Immunocytochemistry: practical applications in pathology and biology. Bristol: J. Wright, 1983;438–55.
28 Gruters RA, Terpstra FG, De Goede REY *et al.* Immunological and virological markers in individuals progressing from seroconversion to AIDS. AIDS 1991; **5**:837–44.
29 Van Noesel CJM, Grutters RA, Terpstra FG *et al.* Functional and phenotypic evidence for a selective loss of memory T cells in asymptomatic human immunodeficiency virus-infected men. J Clin Invest 1990; **86**:293–9.
30 Giorgi JV, Nishhanian PG, Schmid I *et al.* Selective alterations in immunoregulatory lymphocyte subsets in early HIV infection. J Clin Immunol 1987; **7**:140–50.
31 Demartini RM, Turner RR, Formenti SC *et al.* Peripheral blood mononuclear cell abnormalities and their relationship to clinical course in homosexual men with HIV infection. Clin Immunol Immunopathol 1988; **46**:258–71.
32 Schnittman SM, Lane HC, Greenhouse J *et al.* Preferential infection of $CD4^+$ T-memory cells by human immunodeficiency virus type I: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc Natl Acad Sci USA 1990; **87**:6058–62.
33 Margolick JB, Volkman DJ, Folks TM *et al.* Amplification of HTLV III/LAV infection by antigen-induced proliferation of T cells and direct suppression by virus of lymphocyte blastogenic responses. J Immunol 1987; **138**:1719–23.
34 Vanham G, Kestens L, Penne G *et al.* Subset markers of CD8(+) cells and their relation to enhanced cytotoxic T-cell activity during human immunodeficiency virus infection. J Clin Immunol 1991; **11**:345–56.
35 Bogner JR, Matuschke A, Heinrich B *et al.* Expansion of activated T lymphocytes ($CD3^+HLA/DR^+$) detectable in early stages of HIV-1 infection. Klin Wochenschr 1990; **68**:393–6.
36 Mackewicz CE, Ortega HW, Levy JA. $CD8^+$ cell anti-HIV activity correlates with the clinical state of the infected individual. J Clin Invest 1991; **87**:1462–6.
37 Ellakany S, Whiteside TL, Schade RR *et al.* Analysis of intestinal lymphocyte subpopulations in patients with Acquired Immunodeficiency Syndrome (AIDS) and AIDS-Related Complex. Am J Clin Pathol 1988; **87**:647–55.
38 Mega J, Bruce MG, Beagley KW *et al.* Regulation of mucosal responses by $CD4^+$ T lymphocytes: effects of anti-L3T4 treatment on the gastrointestinal immune system. Int Immunol 1991; **3**:793–805.
39 Kanof ME, Strober W, Fiocchi C *et al.* The lamina propria is deficient in CD4, Leu-8^+ suppressor T cells. Gastroenterol 1987; **92**:1459.
40 Scott P, Kaufman SHE. The role of T-cell subsets and cytokines in the regulation of infection. Immunol Today 1991; **12**:346–51.