

Abnormalities in subset distribution, activation, and differentiation of T cells isolated from large intestine biopsies in HIV infection

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SUMMARY

Intestinal T cells have a unique state of activation and differentiation which might specifically affect or be affected by HIV infection. Lymphocyte subsets in the peripheral blood are well characterized, but our knowledge about intestinal lymphocytes in HIV infection is incomplete. We therefore analysed lymphocytes isolated from large intestine biopsies of AIDS patients and controls by three-colour cytofluorometry. In the large intestine of HIV-infected patients CD4 T cells were reduced and CD8 T cells were increased compared with controls. Most of the CD8 T cells in the colorectal mucosa of AIDS patients were of the cytotoxic phenotype. Activated and resting CD4 T cells were similarly reduced, the expression of CD25 and HLA-DR of CD8 T cells was unaltered and increased, respectively. In intestinal CD4 T cells the expression of CD29 was decreased, but the expression of CD45RO and HML-1 was normal. CD8 T cells had a decreased expression of all these differentiation markers. Our findings demonstrate substantial alterations in subset distribution, activation, and differentiation of large intestine T cells, which may contribute to the secondary infections and malignancies commonly observed in the gut of AIDS patients.

Keywords T cell subsets T cell differentiation large intestine AIDS mucosal immune system

INTRODUCTION

Intestinal T cells constitutively express activation markers like CD25 or HLA-DR [1] and are assumed to be tissue-specific memory cells differing in phenotype and function from circulating memory T cells. Intestinal T cells are virtually all CD45RO⁺, but do not express CD29 in high density [2], and they do not proliferate when stimulated by recall antigen [3] or antibodies to CD3 [4]. HIV infection, replication, and cytopathogenicity are substantially influenced by the state of activation [5,6] and differentiation of T cells, since memory T cells have been found to be preferentially infected by HIV [7,8], and HIV replication is triggered by T cell activation [5]. The rectal mucosa is probably an important portal of entry for HIV, and high levels of HIV core protein p24 have been detected in intestinal biopsies [9]. Abnormalities of T cells in the large intestine of HIV-infected patients have been investigated so far only in a few immunohistological studies demonstrating an inverse CD4/CD8 ratio in the lamina propria [10-12]. To characterize alterations of T cell subsets, activation, and differentiation we analysed T lymphocytes isolated from endoscopical biopsies by three-colour cytofluorometry.

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PATIENTS AND METHODS

Patients

Eighteen HIV-infected patients undergoing diagnostic colonoscopy in two major referral-based clinics in Berlin because of gastrointestinal symptoms were studied. All patients had AIDS according to the definition of the Centres for Disease Control [13]. Examination of large intestine biopsies and at least three stool samples revealed infectious agents in five of 11 patients with diarrhoea and in none of the seven patients without diarrhoea at the time of study. In two patients cytomegalovirus (CMV) was detected in large intestine biopsies, one patient had coronavirus-like particles and cryptosporidia, one patient had *Salmonella sp.*, and one patient had atypical mycobacteria in stool samples. Sixteen patients were homosexual or bisexual men, one woman and one man were intravenous drug abusers; 15 patients received zidovudine therapy at the time of study. The AIDS patients were 27-57 years (median 42 years) old. Biopsies were taken from macroscopically normal areas.

Biopsies were also obtained from 12 patients not at risk for HIV infection undergoing routine diagnostic lower endoscopy (six male, six female) because carcinoma or colitis ulcerosa was suspected but could not be confirmed. The controls were 35-85 years (median 62 years) old. Control patients had no visible

abnormalities in colonoscopy and histologic examination of biopsies.

The study was approved by the local ethics committee.

Isolation of intestinal lymphocytes

Lymphocytes were isolated from four to seven biopsies of the sigma or rectum by a modified method used for the isolation of mucosal lymphocytes from resected specimens as previously described [1]. Biopsies were washed five times in 30 ml PBS to remove adherent blood and mucus and cut into small fragments. After another washing in PBS the fragments were incubated overnight at 4°C in 10 ml RPMI 1640 medium containing 10% fetal calf serum (FCS; Gibco BRL, Berlin, Germany), 100 U/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml gentamycin, 2.5 µg/ml amphotericin (Seromed Biochrom KG, Berlin, Germany), 25 mM HEPES buffer, 0.05 M 2-mercaptoethanol, 0.01% collagenase CLS III (Worthington Diagnostic Systems, Inc., Freehold, NJ), 0.01% deoxyribonuclease I (Boehringer, Mannheim, Germany), and 0.01% soybean trypsin inhibitor (Sigma, Deisenhofen, Germany). This was followed by a 3-h incubation of the mucosal fragments in the same medium at 37°C on an orbital shaker. For further disintegration the suspension was passed first three times through a spinal needle and then through a 60-µm nylon mesh to obtain single cells. After washing in RPMI 1640 containing 10% FCS, cells were resuspended in 30% isotonic Percoll (Pharmacia, Uppsala, Sweden), underlayered with 70% isotonic Percoll and centrifuged at 1200 g for 25 min. Lymphocytes were harvested from the interface, washed in RPMI 1640 containing 10% FCS, immediately fixed for 30 min with 1% paraformaldehyde, and resuspended in PBS containing 2% FCS and 0.01% sodiumazide (staining buffer). The number of mononuclear cells obtained by this method was 0.9–1.5 × 10⁶, and viability as determined by trypan blue dye exclusion was always greater than 70%. Viability was not different between cells isolated from controls or HIV-infected patients, and most dead cells were assessed microscopically as epithelial cells which were not removed by further purification to avoid cell loss.

In prior experiments we found no differences in the expression of the antigens investigated between peripheral blood lymphocytes isolated by Ficoll density gradient centrifugation or the isolation procedure described above, or between lymphocytes isolated from biopsies without or with the overnight incubation step, which, however, increased cell yield (data not shown).

Triple-staining of isolated lymphocytes

Three-colour staining of intestinal lymphocytes was performed using FITC- or PE-labelled MoAbs to CD4, CD8, CD20, CD25, CD45RO, HLA-DR (all from Dakopatts, Hamburg, Germany), CD11b, CD16, CD57 (all from Becton Dickinson, Heidelberg, Germany), CD11a, CD29, CD45RA (all from Coulter, Krefeld, Germany), HML-1 (Dianova, Hamburg, Germany), TCRδ1 (T Cell Sciences, Cambridge, MA), and Peridinin chlorophyll protein reagent (PerCP)-labelled anti-CD3 (Becton Dickinson) in saturating concentrations as determined in previous experiments. Isotype-matched antibodies not reactive with human leucocytes were used as controls. Briefly, various combinations of three differently conjugated antibodies were pipetted into a 96-well round-bottomed plate, 50 µl staining buffer containing 3–10 × 10⁴ cells were added and incubated for 30 min at 4°C in the dark. Cells were washed two

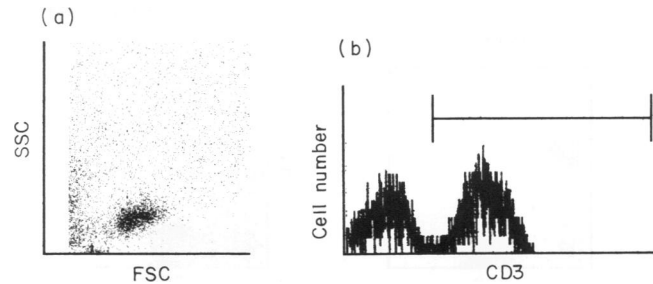


Fig. 1. Cytofluorometric analysis of intestinal T cells was done after gating (a) for viable lymphocytes in the forward (FSC) and side scatter (SSC) light diagram and (b) by a second gate including only CD3⁺ lymphocytes.

times and resuspended in 2–300 µl staining buffer for analysis. Preceding experiments revealed no significant differences in the percentage of positive cells between fixed and unfixed cells or between simultaneous and successive addition of antibodies (data not shown).

Flow cytometric analysis

At least 5000 cells were analysed by a FACScan flow cytometer (Becton Dickinson) using LYSYS II software (Becton Dickinson). Electronic compensation was used to eliminate spectral overlap between the three fluorochromes. An electronic gate was set to include only viable lymphocytes by their characteristic localization in the forward/side scatter diagram (Fig. 1a). Another gate was set to include only T cells as determined by the detection of bound PerCP-conjugated anti-CD3 (Fig. 1b); expression of CD20 (B cells) and of CD16 or CD57 (natural killer (NK) cells) was determined in the CD3⁻ lymphocyte population. Gates for analysis were always set to exclude more than 98% of cells stained with conjugated isotype-matched control antibodies.

Results are given as percentages of positive cells per CD3⁺ T cell (or per T cell subset), since the absolute number of positive cells per µl cannot be calculated in duodenal biopsies. To limit the effect of small measurement errors the percentage of positive cells per CD4⁺ T cell was only calculated in patients with at least 3% CD4⁺ T cells.

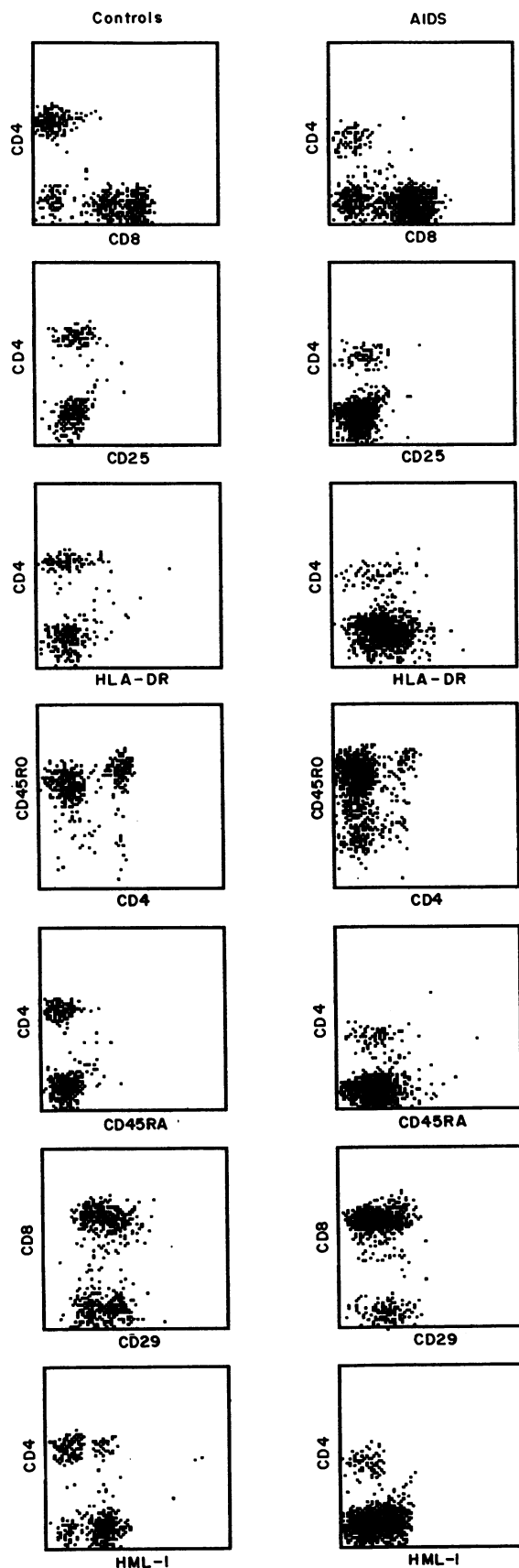
Statistical analysis

Measurement results were not normally distributed, and therefore described as median and 95% confidence interval. The non-parametric two-tailed Mann-Whitney *U*-test was used to evaluate comparative statistical significance. *P* < 0.05 was considered significant.

RESULTS

T cells in the large intestine of HIV-infected patients and controls

Intestinal lymphocytes were isolated from large intestine biopsies of 18 AIDS patients and 12 controls, and analysed by cytofluorometry. A representative set of two-parameter immunofluorescence profiles of intestinal T cells is shown in Fig. 2, and quantitative findings are given in Table 1. The cells in the lymphocyte gate were 45% (32–52%) and 41% (19–54%) CD3⁺ T cells in HIV-infected patients and controls, respectively. The proportion of γδ T cells was not different in HIV-infected patients and controls (14% (8–31%) versus 15% (7–26%)).



Expression of CD4 and CD8 on large intestine T lymphocytes

In the large intestine of HIV-infected patients the percentage of CD4⁺ T cells was reduced ($P < 0.001$) and the proportion of CD8⁺ T cells was increased compared with controls ($P < 0.001$). The percentage of CD8⁺ $\gamma\delta$ T cells was not different between controls and HIV-infected patients (8% (4–17%) versus 5% (3–8%)); but the proportion of CD8⁺ $\alpha\beta$ T cells was increased in the large intestine of HIV-infected patients to 67% (57–76%) compared with 35% (22–44%) in controls ($P < 0.001$). The percentage of CD4⁺CD8⁺ T cells was also increased in HIV infection ($P < 0.02$).

CD8⁺ T cells were further analysed by their coexpression of CD11a, which is found on cytotoxic T cells [14], and of CD11b, which is expressed on suppressor T cells [15]. HIV-infected patients had an increased proportion ($P < 0.01$) of CD8⁺CD11a⁺ T cells (61% (50–74%)) compared with controls (46% (37–54%)), while the proportion of CD8⁺CD11b⁺ T cells was not different (3% (2–8%) versus 4% (1–11%)). The percentage of CD8 cells expressing CD11a (91% (88–96%) versus 4% (1–11%)) and CD11b (4% (3–17%) versus 7% (3–21%)) in AIDS patients compared with controls was not different.

Activation of large intestine T cells

The percentage of CD25⁺ as well as of CD25⁺CD4 T cells, and the proportion of HLA-DR⁺ as well as of HLA-DR⁺CD4 T cells was decreased in HIV infection (each $P < 0.01$), and the proportion of CD4 T cells expressing CD25 or HLA-DR was not different in the large intestine of HIV-infected patients compared with controls. The percentage of intestinal CD8⁺CD25⁺ T cells was normal in HIV infection, but the proportion of CD8⁺CD25⁺ T cells was increased in HIV-infected patients compared with controls ($P < 0.002$). In contrast, only the percentage of HLA-DR⁺ ($P < 0.002$) but not of HLA-DR⁺CD8 T cells was significantly increased in the large intestine of HIV-infected patients. The proportion of CD8 T cells expressing CD25 was unaltered, but the proportion of CD8 T cells expressing HLA-DR was increased in the large intestine of HIV-infected patients compared with controls ($P < 0.002$).

Differentiation of large intestine T cells

To characterize alterations in the state of differentiation of intestinal T cells in HIV infection we analysed the expression of CD45RO, CD45RA, CD29, and of the mucosa-specific T cell differentiation antigen HML-1. Virtually all intestinal T cells of controls were CD45RO⁺ and CD45RA⁺, and 68% and 41% expressed CD29 and HML-1, respectively. In HIV infection the percentage of T cells expressing CD45RO, CD29, and HML-1 was reduced (each $P < 0.01$), and CD45RA⁺ T cells were increased ($P < 0.05$).

Fig. 2. Representative two-colour immunofluorescence profile of intestinal CD3⁺ T cells isolated from large intestine biopsies of AIDS patients (right) and controls (left). Isolated cells were stained with peridinin chlorophyll protein reagent (PerCP)-conjugated anti-CD3, and with FITC- or PE-conjugated antibodies to CD4, CD8, CD25, HLA-DR, CD45RO, CD29, CD45RA, and HML-1 as indicated. Logarithmic fluorescence intensity for green (abscissa) and red (ordinate) fluorescence was determined on gated CD3⁺ T cells as shown in Fig. 1.

Table 1. Subsets, activation, and differentiation in T cells isolated from the large intestine of 18 AIDS patients and 12 controls

	Controls	AIDS
CD4	53 (43–59)*	8 (2–21)†
CD8	43 (37–52)	74 (64–78)†
CD4 ⁻ CD8 ⁻	7 (3–19)	12 (9–21)†
CD25	9 (3–29)	5 (4–11)
CD4 ⁺ CD25 ⁺	5 (2–7)	1 (1–2)†
CD4 ⁺ CD25 ⁻	43 (27–52)	6 (1–20)†
CD25 ⁺ /CD4 ⁺	10 (5–12)‡	9 (3–33)†
CD8 ⁺ CD25 ⁺	1 (0–7)	2 (1–5)
CD8 ⁺ CD25 ⁻	47 (43–60)	86 (73–93)†
CD25 ⁺ /CD8 ⁺	3 (0–13)§	3 (1–5)§
HLA-DR	19 (7–27)	40 (23–45)†
CD4 ⁺ HLA-DR ⁺	7 (4–12)	2 (1–8)†
CD4 ⁺ HLA-DR ⁻	43 (35–47)	3 (0–19)†
HLA-DR ⁺ /CD4 ⁺	15 (10–22)‡	27 (11–89)‡
CD8 ⁺ HLA-DR ⁺	11 (3–15)	32 (19–46)†
CD8 ⁺ HLA-DR ⁻	35 (33–52)	48 (34–76)
HLA-DR ⁺ /CD8 ⁺	24 (7–32)§	35 (23–52)†§
CD45RO	99 (91–99)	91 (89–95)†
CD4 ⁺ CD45RO ⁺	51 (29–58)	9 (3–22)†
CD4 ⁺ CD45RO ⁻	1 (0–1)	0 (0–1)
CD45RO ⁺ /CD4 ⁺	98 (96–100)‡	100 (88–100)‡
CD8 ⁺ CD45RO ⁺	49 (42–60)	82 (69–86)†
CD8 ⁺ CD45RO ⁻	1 (0–4)	8 (5–10)†
CD45RO ⁺ /CD8 ⁺	98 (94–100)§	90 (88–93)†§
CD45RA	3 (2–7)	4 (1–15)†
CD4 ⁺ CD45RA ⁺	2 (1–4)	1 (0–2)
CD4 ⁺ CD45RA ⁻	50 (38–54)	6 (1–19)†
CD45RA ⁺ /CD4 ⁺	4 (2–10)‡	8 (0–100)‡
CD8 ⁺ CD45RA ⁺	1 (0–3)	4 (1–7)†
CD8 ⁺ CD45RA ⁻	47 (44–55)	89 (70–95)†
CD45RA ⁺ /CD8 ⁺	2 (0–5)§	4 (2–9)§
CD29	68 (55–77)	35 (17–60)†
CD4 ⁺ CD29 ⁺	26 (14–50)	4 (1–14)†
CD4 ⁺ CD29 ⁻	21 (11–33)	8 (1–18)†
CD29 ⁺ /CD4 ⁺	57 (40–82)‡	33 (13–56)†‡
CD8 ⁺ CD29 ⁺	32 (27–40)	16 (9–28)†
CD8 ⁺ CD29 ⁻	12 (5–31)	47 (27–68)†
CD29 ⁺ /CD8 ⁺	74 (48–87)§	27 (15–55)†§
HML-1	41 (31–54)	22 (16–59)†
CD4 ⁺ HML-1 ⁺	9 (2–40)	1 (1–3)†
CD4 ⁺ HML-1 ⁻	45 (5–61)	7 (0–21)†
HML-1 ⁺ /CD4 ⁺	18 (4–89)‡	10 (3–100)‡
CD8 ⁺ HML-1 ⁺	36 (20–51)	51 (14–78)
CD8 ⁺ HML-1 ⁻	11 (2–18)	40 (21–68)†
HML-1 ⁺ /CD8 ⁺	74 (66–96)§	57 (20–79)†§

* Median (95% confidence interval) proportion of T cell subset per intestinal CD3⁺ T cell if not indicated otherwise (see ‡ and §).

† $P < 0.05$ compared with controls.

‡ Median (95% confidence interval) proportion of indicated T cell subset per intestinal CD4⁺ T cell. Patients with less than 3% CD4⁺ T cells were excluded.

§ Median (95% confidence interval) proportion of indicated T cell subset per intestinal CD8⁺ T cell.

The percentage of CD45RO⁺ and CD45RA⁻ CD4 T cells was reduced in HIV-infected patients compared with controls ($P < 0.002$), but since CD45RO⁻ or CD45RA⁺ CD4 T cells were nearly absent in HIV-infected patients, as in controls, intestinal CD4 T cells in HIV infection were still virtually all CD45RO⁺ and CD45RA⁻. In contrast, the percentage of CD45RO⁻ and CD45RA⁺ CD8 T cells was clearly increased in HIV infection ($P < 0.01$), although the CD45RO⁺ and CD45RA⁻ subset still predominated in intestinal CD8 T cells of HIV-infected patients, as in controls.

The percentage of CD29⁻ CD4 T cells ($P < 0.05$) and even more CD29⁺ CD4 T cells was reduced ($P < 0.002$) in large intestine of HIV-infected patients compared with controls, resulting in a decreased proportion of CD4 T cells expressing CD29 in HIV infection ($P < 0.05$). The expression of CD29 on CD4 and CD8 T cells was reduced ($P < 0.05$) in the large intestine of HIV-infected patients compared with controls. Only the percentage of CD29⁻ CD8 T cells was increased ($P < 0.01$), but the proportion of CD29⁺ CD8 T cells was reduced in HIV infection ($P < 0.05$).

The proportion of HML-1⁺ and HML-1⁻ CD4 T cells was reduced in HIV infection (each $P < 0.05$), but the expression of HML-1 on intestinal CD4 T cells was highly variable, and not significantly different between HIV-infected patients and controls. In contrast, the proportion of intestinal CD8 T cells expressing HML-1 was reduced in HIV infection ($P < 0.05$), since HML-1⁺ CD8 T cells were normal and HML-1⁻ CD8 T cells were increased in HIV-infected patients compared with controls ($P < 0.01$).

Natural killer cells and B cells

The percentage of NK cells, i.e. CD3⁻ cells expressing CD16 or CD57, in intestinal lymphocytes was significantly reduced in HIV-infected patients compared with controls (6% (1–8%) versus 20% (13–23%); $P < 0.01$). The proportion of CD20⁺ B cells in large intestine lymphocytes was similar in HIV-infected patients (10% (2–16%)) and controls (7% (3–13%)).

Correlation of T cell subsets with clinical characteristics

No significant correlation was found between the T cell subsets investigated and sex or age in HIV-infected patients or controls. No significant differences were seen in the proportion of CD4 T cells or the other subsets investigated between AIDS patients with and without diarrhoea (CD4 9% (1–40%) versus 6% (1–34%)), or with and without secondary intestinal infections (2% (1–14%) versus 9% (1–40%)).

DISCUSSION

The gastrointestinal tract is a common site of secondary infections and malignancies in patients with AIDS, indicating substantial impairment of the mucosal immune system in HIV infection. HIV might induce specific abnormalities of mucosal immunity, since mucosal lymphocytes differ phenotypically and functionally from circulating lymphocytes [1,2]. Furthermore, a considerably higher HIV content has been found recently in lymph nodes [16–18] and intestinal biopsies [9] compared with the peripheral blood, suggesting that HIV-induced defects

might also vary between lymphoid compartments. However, abnormalities of mucosal immunity in HIV infection are not well characterized.

We found a decrease in the percentage of CD4 T cells and an increase in the percentage of CD8 T cells in the large intestine of AIDS patients, in accordance with earlier immunohistological studies [11,12]. The higher proportion of intestinal CD4⁺ cells in both HIV-infected patients and controls reported in these studies could result from technical differences. First, the isolated lymphocytes in our study contained intraepithelial T cells which rarely express CD4 [19]. Second, the number of CD4 T cells might be overestimated by immunohistology, since intestinal macrophages which are preserved in HIV infection frequently express CD4 (manuscript submitted); in addition, intracellular trapping of CD4 which has been demonstrated in mononuclear cells infected with HIV *in vitro* [20] could also occur *in vivo* as indicated by the increase in CD4⁻CD8⁻ T cells detected by cytofluorometric analysis of surface molecules on isolated intestinal T cells. Intracellular expression of CD4 in such double-negative T cells would, however, lead to positive staining by immunohistology. Finally, although our control experiment did not reveal alterations in the T cell subsets investigated by our isolation procedure, we cannot exclude that some loss of CD4 T cells may occur during cell isolation.

Unfortunately we could not obtain biopsies from an appropriately matched control group. Differences in sex or age are unlikely to explain the differences between AIDS patients and controls in intestinal T cells which did not correlate with these parameters. Since HIV⁻ homosexual men have been investigated neither in earlier [11,12] nor in our studies it remains unclear to what extent homosexual behaviour *per se* leads to immunological abnormalities in the large intestine. However, we did not find any specific features in intestinal T cells of the two non-homosexual AIDS patients studied, suggesting that the abnormalities observed are due rather to HIV infection.

Activation of CD4 T cells is critical for HIV infection and replication [5,6]. However, as determined by the expression of the activation markers CD25 and HLA-DR, activated and resting CD4 T cells were similarly reduced in the large intestine of AIDS patients compared with controls, resulting in an unaltered proportion of activated cells in the CD4 T cell subset. This finding could nevertheless be consistent with a preferential loss of activated CD4 T cells in HIV infection, since the pool of activated CD4 T cells may be continuously replenished by the activation of resting CD4 T cells by HIV or HIV proteins.

It has been speculated that an increase in intestinal CD8⁺ suppressor T cells might contribute to mucosal immunodeficiency [10], but our findings demonstrate an increase only in the percentage of CD8⁺ cytotoxic T cells in the large intestine of AIDS patients, who like controls had low proportions of intestinal suppressor T cells. A considerable proportion of intestinal CD8 T cells is activated as determined by the expression of HLA-DR, but the specificity and function of these predominantly cytotoxic T cells remain to be identified. They may recognize HIV antigens [21–24] and play a role in the control of HIV infection, but they may also be harmful by damaging the intestinal epithelium as in HIV-associated alveolitis [25]. In fact, morphological changes resembling chronic inflammatory bowel disease can be induced by T cell activation in human fetal colon explants [26]. However, *in vitro* studies of circulating T cells have shown that the activity of cytotoxic T

cells is strongly dependent on the presence of CD4⁺ T cells [27], therefore the specific activity of the cytotoxic T cells in the mucosa may be impaired due to intestinal CD4 T cell depletion.

Memory T cells have been defined by their high expression of CD45RO and CD29 and the absence of CD45RA [28]. Intestinal T cells which are virtually all CD45RO⁺ and CD45RA⁻ differ from circulating memory T cells, as they do not express CD29 in high density but express other differentiation markers like HML-1, which is absent on peripheral blood T cells [2]. The reduced expression of CD45RO and CD29 on intestinal T cells could result from a preferential loss of differentiated CD4⁺ memory T cells as described in the peripheral blood [7]. The decreased proportion of intestinal CD4 T cells expressing CD29 in AIDS patients is in accordance with this hypothesis; however, the expression of CD45RO, CD45RA and HML-1 was not altered on intestinal CD4 T cells in HIV infection. Thus, in intestinal CD4 T cells, depletion seems to be the predominant effect of HIV infection, since the remaining CD4 T cells were phenotypically normal except for CD29 expression, the role of which on intestinal T cells is unclear.

Defective help of these reduced CD4 T cells for CD8 T cell differentiation is the likely cause for the reduced expression of differentiation markers on intestinal T cells of AIDS patients, which was predominantly due to the presence of CD8⁺CD45RO⁻CD45RA⁺ T cells and to the increase in CD8⁺CD29⁻ and CD8⁺HML-1⁻ T cells, while CD8⁺29⁺ T cells were even reduced. The functional consequences of this impaired differentiation of intestinal CD8 T cells have not been studied so far, but CD8⁺ cell anti-HIV activity in the peripheral blood has been found to decrease with disease progression [29]. Furthermore, it is obvious that these cells do not effectively control malignancies and infections in the gastrointestinal tract of AIDS patients. Insufficient helper function of intestinal CD4 T cells is also indicated by the reduced proportion of IgA plasma cells in the large intestine of AIDS patients reported by Kotler *et al.* [30], since terminal IgA B cell differentiation is CD4 T cell-dependent [31]. Defects in secretory immunity which may also contribute to intestinal disease in HIV infection could not be analysed in our study, since very few B cells were recovered by our isolation procedure. Finally, NK cells which can eliminate virally infected and tumour cells [32] were reduced in the intestinal mucosa of AIDS patients, which may further facilitate infections and malignancies in the large bowel.

We found similar proportions of intestinal T cell subsets in AIDS patients with and without diarrhoea. The median proportion of CD4 T cells was lower in AIDS patients with an intestinal infection than in uninfected AIDS patients, though this difference was not statistically significant. Due to the limited number of patients studied the clinical significance of the abnormalities detected in T cell subsets of the large intestine cannot be definitively assessed at present.

In conclusion, we found a severe reduction of the proportion of CD4 T cells in the large intestine of AIDS patients. Although the state of activation and differentiation of the remaining CD4 T cells appeared to be fairly normal, this reduced proportion of CD4 T cells probably leads to the impaired differentiation of the predominant cytotoxic CD8 T cells. In association with impaired secretory immunity and NK cell deficiency, these abnormalities demonstrate a break-down of the mucosal immune barrier, leading to the common secondary infections and malignancies of the large intestine in AIDS patients.

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REFERENCES

- 1 Zeitz M, Greene WC, Peffer NJ, James SP. Lymphocytes isolated from the intestinal lamina propria of normal nonhuman primates have increased expression of genes associated with T cell activation. *Gastroenterology* 1988; **94**:647-55.
- 2 Schieferdecker HL, Ullrich R, Hirsland H, Zeitz M. T cell differentiation antigens on lymphocytes in the human intestinal lamina propria. *J Immunol* 1992; **149**:2816-22.
- 3 Zeitz M, Quinn TC, Graeff AS, James SP. Mucosal T cells provide helper function but do not proliferate when stimulated by specific antigen in Lymphogranuloma venereum proctitis in nonhuman primates. *Gastroenterology* 1988; **94**:353-66.
- 4 Pirzer UC, Schürmann G, Post S, Betzler M, Meuer SC. Differential responsiveness to CD3-Ti vs CD2-dependent activation of human intestinal T lymphocytes. *Eur J Immunol* 1990; **20**:2339-42.
- 5 Nabel G, Baltimore D. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* 1987; **326**:711-3.
- 6 Rosenberg ZF, Fauci AS. Immunopathogenesis of HIV infection. *FASEB J* 1991; **5**:2382-90.
- 7 Van Noesel CJ, Gruters RA, Terpstra FG, Schellekens PT, van Lier RA, Miedema F. 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.
- 8 Schnittman SM, Lane HC, Greenhouse J, Justement JS, Baseler M, Fauci AS. Preferential infection of CD4⁺ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. *Proc Natl Acad Sci USA* 1990; **87**:6058-62.
- 9 Kotler DP, Reka S, Borcich A, Cronin WJ. Detection, localisation and quantitation of HIV-associated antigens in the intestinal biopsies from patients with HIV. *Am J Pathol* 1991; **139**:823-30.
- 10 Ellakany S, Whiteside TL, Schade RR, vanThiel DH. Analysis of intestinal lymphocyte subpopulations in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Am J Clin Pathol* 1987; **87**:356-64.
- 11 Jarry A, Cortez A, René E, Muzeau F, Brousse N. Infected cells and immune cells in the gastrointestinal tract of AIDS patients. An immunohistochemical study of 127 cases. *Histopathology* 1990; **16**:133-40.
- 12 Clayton F, Reka S, Cronin WJ, Torlakowic E, Sigal SH, Kotler DP. Rectal mucosal pathology varies with human immunodeficiency virus antigen content and disease state. *Gastroenterology* 1992; **103**:919-33.
- 13 CDC. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *MMWR* 1987; **36**(Suppl.1):3s.
- 14 Morimoto C, Rudd CE, Letvin NL, Schlossman SF. A novel epitope of the LFA-1 antigen which can distinguish killer effector and suppressor effector cells in human CD8 cells. *Nature* 1987; **330**:479.
- 15 Landay A, Gartland L, Clement LT. Characterization of a phenotypically distinct subpopulation of Leu-2⁺ cells which suppresses T cell proliferative responses. *J Immunol* 1983; **131**:2757.
- 16 Pantaleo G, Graziosi C, Butini L *et al.* Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proc Natl Acad Sci USA* 1991; **88**:9838-42.
- 17 Pantaleo G, Graziosi C, Demarest JF *et al.* HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 1993; **362**:355-8.
- 18 Embretson J, Zupanic M, Ribas JL *et al.* Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* 1993; **362**:359-62.
- 19 Ullrich R, Schieferdecker HL, Ziegler K, Riecken EO, Zeitz M. γ/δ T cells in the human intestine express surface markers of activation and are preferentially located in the epithelium. *Cell Immunol* 1990; **128**:619-27.
- 20 Schneider T, Hildebrandt P, Rokos K *et al.* Expression of nef, vpu, CA and CD4 during the infection of lymphoid and monocytic cell lines with HIV-1. *Arch Virol* 1992; **125**:161-76.
- 21 Walker BD, Flexner C, Paradis TJ *et al.* HIV-1 reverse transcriptase is a target for cytotoxic T lymphocytes in infected individuals. *Science* 1988; **240**:64-66.
- 22 Culmann B, Gomard E, Kieny M-P *et al.* An antigenic peptide of the HIV-1 nef protein recognized by cytotoxic T lymphocytes of seropositive individuals in association with different HLA-B molecules. *Eur J Immunol* 1989; **19**:2383-6.
- 23 Nixon DF, McMichael AJ. Cytotoxic T-cell recognition of HIV proteins and peptides. *AIDS* 1991; **5**:1049-59.
- 24 Kundu SK, Merigan TC. Equivalent recognition of HIV proteins, Env, Gag and Pol by CD4⁺ and CD8⁺ cytotoxic T-lymphocytes. *AIDS* 1992; **6**:643-9.
- 25 Autran B, Mayaud CM, Raphael M *et al.* Evidence for a cytotoxic T-lymphocyte alveolitis in human immunodeficiency virus-infected patients. *AIDS* 1988; **2**:179-83.
- 26 Evans CM, Phillips AD, Walker-Smith JA, MacDonald TT. Activation of lamina propria cells induces crypt epithelial proliferation and goblet cell depletion in cultured human fetal colon. *Gut* 1992; **33**:230-5.
- 27 Watret KC, Whitelaw JA, Froebel KS, Bird AG. Phenotypic characterization of CD8⁺ T cell populations in HIV disease and in anti-HIV immunity. *Clin Exp Immunol* 1993; **92**:93-99.
- 28 Sanders ME, Makgoba M, Shaw S. Human naive and memory T cells: reinterpretation of helper-inducer and suppressor-inducer subsets. *Immunol Today* 1988; **9**:195-9.
- 29 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.
- 30 Kotler DP, Scholes JV, Tierney AR. Intestinal plasma cell alterations in acquired immunodeficiency syndrome. *Digest Dis Sci* 1987; **32**:129-38.
- 31 Strober W, Harriman GR, Kunimoto DR. Early steps of IgA cell differentiation. *Immunol Res* 1991; **10**:386-8.
- 32 Herberman R. NK cells and other natural effector cells. New York: Academic Press, 1982.