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Intraepithelial γ/δ T Cells in Duodenal Mucosa Are Related to the Immune State and Survival Time in AIDS

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The proportion of T-cell receptor γ/δ^+ cells and the CD4/CD8 ratio relative to all $CD3^+$ intraepithelial lymphocytes (IEL) were determined by immunofluorescence in duodenal mucosa of late-stage (mostly CDC IVC1/D) subjects ($n = 21$) infected with human immunodeficiency virus type 1 (HIV-1). The γ/δ fraction (median, 14.2%; range, 1.7 to 59.8%) was increased ($P < 0.03$) compared with that in HIV^- controls ($n = 11$; median 2.8%; range, 0.3 to 38%). Also, the number of γ/δ^+ IEL per mucosal unit was increased ($P < 0.05$) in the HIV^+ subjects (median, 11.1/U) compared with the controls (3.2/U). Approximately 100% of the γ/δ^+ IEL were $CD8^-$, and most expressed the Vδ1/Jδ1-encoded epitope (median, 90.9%). The total number of $CD3^+$ IEL tended to be lower than in the controls (67.4 versus 72.9/U). Both the epithelium and the lamina propria contained mainly $CD8^+$ T cells, the median ratios of $CD4^+$ T cells being 1 and 7.6%, respectively. This result accorded with the reduced CD4 cell number in blood (median, 18×10^6/liter). The HIV^+ subjects had increased serum levels of neopterin and β_2-microglobulin (both $P < 0.0001$), probably reflecting immunostimulation. Serum neopterin and β_2-microglobulin were inversely related to duodenal γ/δ IEL, particularly in the premortal group ($r = -0.97$ and $r = -0.58$, respectively). The increased γ/δ IEL might reflect enhanced intestinal protection in late-phase HIV infection. Short survival expectancy (<7 months) was associated not only with high levels of neopterin and β_2-microglobulin but also with a reduced number of duodenal γ/δ^+ cells ($P < 0.03$).

Human intraepithelial lymphocytes (IEL) are mainly T-cell receptor α/β^+ ($TCR\alpha/\beta^+$) $CD8^+$ in the small intestine, and only a small fraction (<8%) usually express TCRγ/δ (8, 9). An increased proportion of γ/δ IEL is present in patients with celiac disease (25, 44) and dermatitis herpetiformis (50). $TCR\gamma/\delta^+$ cells are also increased in the lesions of cutaneous leishmaniasis (37), tuberculous lymphadenitis (20), leprosy (37), discoid lupus erythematosus (24), rheumatoid arthritis (32), multiple sclerosis (29), and polymyositis (27) as well as in the blood of patients with infectious mononucleosis (17), malaria (42), or various immunodeficiency states (2, 12, 15, 48). Whether this increase is of immunopathogenetic importance or only a consequence of the disease remains unclear.

Previous studies of γ/δ T cells in both primary and secondary immunodeficiency have been performed primarily on blood from children (12, 15, 48), but we recently reported an increased proportion of $TCR\gamma/\delta^+$ duodenal IEL in patients with hypogammaglobulinemia associated with mild to moderate intestinal villus atrophy (40) and in immunoglobulin A (IgA)-deficient (IgAD) subjects without infections (38).

The chief aim of this study was to examine the proportion of $TCR\gamma/\delta^+$ IEL in the intestinal mucosa of subjects with human immunodeficiency virus type 1 (HIV-1) infection. We also determined the CD4/CD8 ratio of IEL and lamina propria T lymphocytes and related the distribution of these subsets to the number of circulating $CD4^+$ and $CD8^+$ cells as well as to clinical manifestations and serum levels of neopterin and β_2-microglobulin (β_2-M). These immunologic variables are increased in sera of patients with autoimmune disease (51), malignancy (23), common variable immunodeficiency (CVID) (1), and HIV infection (5). It was of interest to see whether such activation markers of cellular immunity are increased in serum from patients with advanced AIDS and if there exists any time-related association or difference between these markers, duodenal $TCR\gamma/\delta^+$ IEL, clinical variables, and mucosal CD4/CD8 ratios.

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MATERIALS AND METHODS

Patients. Duodenal biopsies and peripheral blood samples were obtained from 21 HIV^+ subjects (4 women and 17 men; median age, 39 years [range, 21 to 58 years]); 15 were homosexual men, 3 were intravenous drug abusers, and 3 had experienced heterosexual virus transmission. All patients had serum antibodies to HIV-1 as determined by enzyme-linked immunosorbent assay (Organon Teknika, Boxtel, The Netherlands) and confirmed by Western blotting (immunoblotting) (DuPont, Wilmington, Del.). Control samples were obtained from 11 HIV^- subjects (seven females and four males; median age, 21 years [range, 4.5 to 76 years]). Dyspepsia, growth retardation, irritable bowel syndrome, and hypothyroid disease were the main reasons for endoscopy in these subjects.

The HIV^+ patients were classified according to the Centers for Disease Control and Prevention (CDC) criteria as follows: 1 subject in group CDC III, 1 in group CDC IVA, 2 in group CDC IVC2, 12 in group CDC IVC1, and 5 in group CDC IVD; the latter 17 were classified as having AIDS, and the former 4 were classified as having AIDS-related complex (ARC). Because most of the patients were classified as having advanced AIDS, they were divided into three equal subgroups according to length of survival after biopsy: group I, ARC/AIDS (alive after 10 to 63 months); group II, late-stage AIDS (survival of 8 to 14 months); and group III, premortal AIDS (survival of <7 months). The median survival time after the first positive HIV test was 69 months (range, 22 to 119 months; $n = 14$).

Informed consent was obtained from all patients, and the study was approved by Regional Ethics Committee II.

Clinical features. The HIV^+ patients suffered from a large number of ongoing opportunistic and/or nonopportunistic infections, mostly in the respiratory and/or gastrointestinal tract: systemic cytomegalovirus (CMV) infection ($n = 4$), herpes simplex virus type 2 infection ($n = 3$), campylobacter enteritis ($n = 1$),

giardiasis ($n = 1$), schistosomiasis ($n = 1$), *Mycobacterium avium-M. intracellulare* infection ($n = 3$), and oral or esophageal candidiasis ($n = 8$). *Pneumocystis carinii* pneumonia was treated with trimetoprim-sulfa in one patient, and three others had recently received such treatment. Only four had verified Kaposi's sarcoma, and one had non-Hodgkin's lymphoma. Fifteen of the HIV^+ patients had chronic diarrhea. In addition to those mentioned above, four patients had a noninfectious watery diarrhea compatible with irritable bowel syndrome (three independent of antibiotic treatment), and one of these had partial intestinal villus atrophy which might indicate HIV-associated enteropathy (4). No other enteropathogens were observed by bacteriologic cultivation or microscopic stool examination for parasites.

Autoimmune disease was noted in one HIV^+ male with juvenile diabetes mellitus. Two HIV^+ patients had previously been treated for hepatitis B infection, one had been treated for hepatitis A virus infection, and five had received treatment for venereal diseases such as gonorrhea, chlamydia infection, and anogenital condylomata.

Seven of the HIV^+ patients were receiving zidovudine at the time of sampling, and eight others had discontinued such treatment because of side effects. None of them received immunoglobulin replacement therapy.

Quantitation of neopterin, β_2-M, and lymphocyte subsets in peripheral blood. Neopterin was measured by a commercial radioimmunoassay (IMMUtest Neopterin; Henning Berlin GMBH, Berlin, Germany), and β_2-M was measured by a microparticle enzyme immunoassay (Abbot IMX; Abbot Laboratories, Abbot Park, Ill.). Lymphocyte subsets ($CD4^+$ and $CD8^+$ T cells) were enumerated by an immunomagnetic method standardized in our hospital (10).

Biopsy specimens. Mucosal samples were obtained endoscopically (Olympus GIF 1T20) from the distal duodenum with a large biopsy forceps (Olympus FB-13K). Two or more specimens were placed in 0.5% formalin-saline solution of Alcian green for 5 to 10 min and then examined by stereomicroscopy. These specimens were further subjected to conventional tissue processing for histologic evaluation by light microscopy. Two additional specimens were fixed for 1 to 4 h at 4°C in 0.5% periodate-lysine-paraformaldehyde, washed for 1 to 4 h at 4°C in 0.01 M phosphate buffer (pH 7.5) containing 0.15 M NaCl and 5% sucrose, orientated on a thin slice of carrot, embedded in OCT (Tissue-Tek, Miles Laboratories, Elkhart, Ind.), snap-frozen in isopenthane cooled in liquid nitrogen, and stored at −70°C until cryosectioning was performed for immunohistochemistry. Other tissue specimens were fixed in 2% glutaraldehyde for electron microscopic evaluation.

Morphologic evaluation. A section from each series was stained with hematoxylin and eosin for histologic evaluation. In addition to histology, stereomicroscopy and scanning electron microscopy were performed to classify villus changes as previously described (40). Such combined evaluation showed normal villi in 18 and partial or subtotal villus atrophy in 3 HIV^+ patients. Two of the latter represented borderline partial atrophy (leaf-shaped villus changes), and overt subtotal villus atrophy was seen in only one (probably caused by CMV duodenitis). These three patients and two others had histologic signs of chronic inflammation. The control subjects had histologically normal duodenal mucosa.

Two- or three-color immunohistochemical staining. Cryosections were cut at 4 μm and incubated for 1 h at ambient temperature with pairs of unlabeled murine monoclonal antibodies (MAbs) of the following human specificities: δ chain of TCRγδ (TCRδ1, IgG1, 1/20; T Cell Sciences Inc., Cambridge, Mass.) and CD3 (clone RIV9, IgG3, 1/10; Sanbio, Am Uden, The Netherlands); δ chain of TCRγδ and the CD8 α chain (BMA-081, IgG2a, 1/40; Behringwerke, Marburg, Germany); CD4 (anti-Leu-3a and -3b, IgG1, 1/40; Becton Dickinson, Mountain View, Calif.) and CD3 (RIV9, IgG3, 1/10; Sanbio); and CD8 (anti-Leu-2b, IgG2a, 1/20 [Becton Dickinson] mixed with BMA-081, IgG2a, 1/80 [Behringwerke]) and CD3 (anti-Leu-4, IgG1, 1/80; Becton Dickinson). Secondary antibody reagents were various combinations of biotinylated and fluorescein isothiocyanate-conjugated goat IgG (0.01 to 0.05 g/liter) with specificity for mouse IgG1, IgG2a, or IgG3 (Southern Biotechnology, Birmingham, Ala.), additionally mixed with rabbit antiserum to human cytokeratin (1/100) for delineation of the duodenal epithelium (28). These reagents were applied for 1.5 h, and then 7-amino-4-methylcoumarin-3-acetic acid-conjugated goat anti-rabbit IgG (1/20; Vector Laboratories, Burlingame, Calif.) in combination with streptavidin-Texas red (0.0025 g/liter; Bethesda Research Laboratories, Gaithersburg, Md.) was applied for 30 min. Human IgG at 0.8 g/liter (Kabi Vitrum, Stockholm, Sweden) was added to the goat reagents to block species cross-reactivity (25).

Because MAb TCRδ1 reacts with all $TCR\gamma/\delta^+$ cells, sequential staining was used to examine the subset expressing the Vδ1/Jδ1-encoded epitope for MAb δTCS1 (IgG1; T Cell Sciences) as detailed elsewhere (25).

Fluorescence microscopy and cell counting. Immunofluorescence was examined at a magnification of ×300 in a Leitz Orthoplan microscope equipped with a Ploem-type vertical illuminator with interference filters for selective observation of red, green, or blue emission. The results were recorded on Ectachrome professional 800/1600 ASA daylight film.

All sections were examined blind by the same investigator. The percentage distribution of $TCR\gamma/\delta^+$ IEL was determined by evaluating every $CD3^+$ IEL for TCRγ/δ expression. $TCR\gamma/\delta^+$ IEL were likewise examined for CD8 and Vδ1/Jδ1 expression.

The total number of $CD3^+$ and $TCR\gamma/\delta^+$ IEL was related to a 316-μm grid width, including all parts of the epithelium from the luminal face to the muscularis mucosae. The number of $CD3^+$ and $TCR\gamma/\delta^+$ IEL in four such defined parallel areas was determined and expressed per millimeter of muscularis mucosae (length unit). In addition, some cases ($n = 12$) were evaluated by paired staining for CD3 and CD4 or CD8 with additional cytokeratin staining to outline the epithelial compartment.

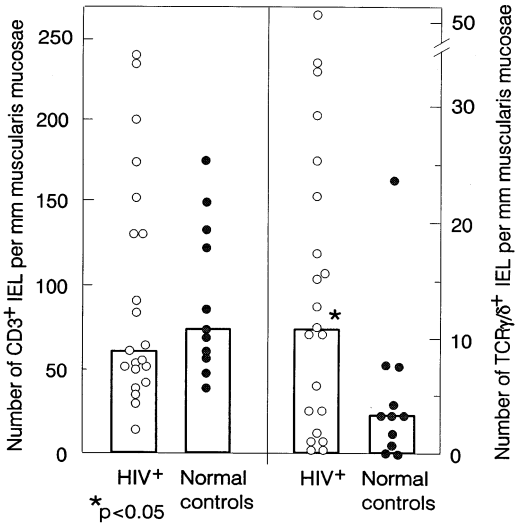

FIG. 1. Scatter diagram of the number of duodenal $CD3^+$ IEL (left panel) and $TCR\gamma/\delta^+$ IEL (right panel) per millimeter of muscularis mucosae in HIV^+ patients compared with normal controls. Columns indicate medians.

Control reference data. The proportion of $TCR\gamma/\delta^+$ intraepithelial T cells as well as the numbers of $CD3^+$ and $TCR\gamma/\delta^+$ IEL per mucosal length unit were determined as described above in duodenal mucosa of the HIV^- controls (40). Reference data were available in our laboratory for the normal expression of the Vδ1/Jδ1-encoded epitope on γ/δ IEL (25). The normal duodenal lamina propria and intraepithelial CD4/CD8 proportions were based on published data (43).

As normal reference data for circulating lymphocyte subsets, we used results from healthy (HIV^-) blood donors ($n = 60$) (11) recorded as described previously (10). Reference data from healthy blood donors were also available in our laboratory for serum concentrations of neopterin ($n = 15$) and β_2-M ($n = 18$). Control levels of neopterin were comparable to results previously published from a large group of healthy individuals (51).

Statistical analyses. Differences among the various categories of HIV^+ subjects and controls were determined by the nonparametric (two-tailed) Wilcoxon's test for unpaired samples (Mann-Whitney) as well as by the Kruskal-Wallis test (chi-square approximation) for multiple samples. Correlations between mucosal $CD3^+$, $CD4^+$, $CD8^+$, or $TCR\gamma/\delta^+$ IEL and circulating T-cell subsets, neopterin, or β_2-M in the HIV^+ subjects and the HIV^- controls were calculated by the Spearman rank correlation test (SPSS for Windows), as was the relationship between proportions and numbers of $TCR\gamma/\delta^+$ IEL. The reproducibility of γ/δ IEL enumerations by paired immunofluorescence staining was excellent, as documented in our previous studies (25, 38, 40).

RESULTS

Distribution of mucosal $CD4^+$ and $CD8^+$ T-lymphocyte subsets. The total number of $CD3^+$ IEL per mucosal unit (Fig. 1) tended to be slightly lower in the HIV^+ patients than in the controls (67.4 versus 72.9/U). As expected, the HIV^+ patients had a marked reduction in $CD4^+$ IEL (median, 1%; range, 0 to 20%; $n = 19$) compared with data (~20%) from normal subjects (43). The proportion of intraepithelial $CD8^+$ T cells (median, 78.9%; range, 41.5 to 93.5%; $n = 19$) was similar to that for healthy subjects (median, 84%; range, 72 to 96%). We arbitrarily divided the HIV^+ subjects into two subgroups: one ($n = 9$) with low proportions of γ/δ IEL (median, 2.1%), in which the sum of $CD8^+$ and $CD4^+$ intraepithelial subsets on average approached (85.6%) the number of $CD3^+$ IEL; and another ($n = 10$) with high proportions of γ/δ IEL (median, 22.3%), in which the $CD8^+$ and $CD4^+$ subsets on average added up to only 75.1% of the $CD3^+$ IEL. This discrepancy corresponded well to the expected $CD3^+$ $CD4^-$ $CD8^-$ per-

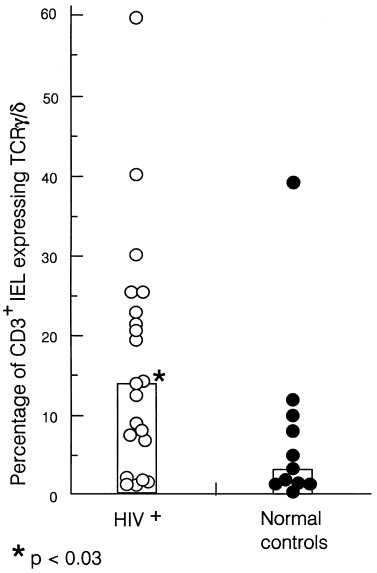

FIG. 2. Scatter diagram of the percentage of duodenal TCRγ/δ$^+$ IEL in HIV$^+$ patients compared with normal controls. Columns indicate medians.

centage represented by the major fraction of γ/δ IEL (see below).

If one assumes that 80% of the CD3$^+$ IEL express CD8 in subjects with a normal gut mucosa (normal controls) (43), we might have expected a median of 58.3 CD8$^+$ IEL per U in the 11 controls. Estimates in the 19 HIV$^+$ patients (see above) showed a similar number of CD8$^+$ IEL (median, 57.6 cells per U) and almost a total depletion of CD4$^+$ IEL (0.8 cells per U, compared with an expected 14.6 cells per U in controls). Likewise, the lamina propria CD3$^+$ cells were mainly CD8$^+$ (median, 79.6%; range, 55.6 to 95.2%; $n = 19$) and showed a greatly reduced CD4 fraction (median, 7.6%; range 1 to 15.7%) compared with that expected in healthy duodenal controls (69% CD4$^+$ and 31% CD8$^+$) (43).

Only one patient showed no reduced intraepithelial CD4 ratio. This 58-year-old homosexual man had been HIV$^+$ for the last 6 years and was currently being treated with Dapson. He had earlier suffered from periodic diarrhea, probably caused by systemic CMV infection, and had signs of duodenitis and subtotal villus atrophy. Although his circulating CD4 cells were low (22×10^6/liter), he showed a normal CD4$^+$ IEL fraction (20%), rather few CD8$^+$ IEL (41.5%), and relatively many γ/δ IEL (40%), but the total CD3 IEL number was considerably reduced (29.3 cells per U).

No striking difference between the survival time-related subgroups was noted with regard to intraepithelial and lamina propria counts for CD3$^+$, CD4$^+$, or CD8$^+$ lymphocytes.

Distribution of TCRγ/δ$^+$ IEL. The γ/δ IEL proportions (Fig. 2) in the 21 HIV$^+$ patients (median, 14.2%; range, 1.7 to 59.8%) were significantly increased ($P < 0.03$) compared with the control values (median, 2.8%; range, 0.3 to 38%). Also, the number of γ/δ IEL per U (Fig. 1) was increased ($P < 0.05$) in the HIV$^+$ patients (median, 11.1 versus 3.2/U). The proportions and total numbers of γ/δ IEL were well correlated ($r = 0.76$, $P < 0.001$), substantiating the validity of the two counting methods.

Patients with chronic diarrhea ($n = 15$) had an increased median proportion of γ/δ IEL (15.9%) compared with normal controls (2.8%), particularly the three with ongoing *M. avium-M. intracellulare* infection (median, 22.6%), the one with campylobacter enteritis (20.9%), the three with herpes simplex virus type 2 infection (median, 19.4%), and the four with systemic CMV infection (median, 17.5%). We also found high γ/δ IEL proportions in four patients with histologically verified duodenitis (median, 31%), in three with villus changes (median, 21.9%), and also in the four that had Kaposi's sarcoma (median, 20.2%). However, as a group, the patients with diarrhea showed values for γ/δ IEL similar to those for patients who had normal bowel function (median, 15.9%, 10.7/U versus 11.5%, 14.3/U), but the former group had a much lower number of CD3$^+$ IEL (median, 62.7 versus 140.8 cells per U). The patient with possible HIV-associated enteropathy also had a high γ/δ IEL proportion (21.9%) and a rather large increase in CD3$^+$ IEL (248 cells per U), probably reflecting his duodenitis. No relationship was found between CD3$^+$ or TCRγ/δ$^+$ IEL and the number of CD4$^+$ and CD8$^+$ IEL or the CD4/CD8 ratio in peripheral blood.

Regarding the relationship to survival time, we found increased proportions and total numbers of γ/δ IEL both in group I (alive; median, 21.9% and 15.1 cells per U) and group II (<14 months' survival; median, 20.9% and 23 cells per U), in contrast to a large reduction ($P < 0.03$) in group III (<7 months' survival; median, 7.5% and 4 cells per U). The proportions and numbers of γ/δ IEL in groups I and II were significantly increased ($P < 0.04$ and $P < 0.02/P < 0.03$, respectively) compared with control levels, but only group II had a significantly higher γ/δ IEL proportion ($P < 0.03$) than group III (Fig. 3). Longitudinal γ/δ cell counts in all available duodenal biopsies taken at different times in four patients (Fig. 4) revealed the same tendency, with mostly high but varying γ/δ IEL ratios in groups I and II and low ratios in group III.

Seven patients receiving treatment with zidovudine had only slightly increased γ/δ IEL proportions (median, 8.2%), almost similar to those found in five patients on antibiotics (mostly for respiratory tract infections) at the time of biopsy (median, 7.2%).

Two-color staining for CD8 revealed that almost all TCRγ/δ$^+$ IEL were CD8$^-$ in the HIV$^+$ patients (median, 100%; range, 88.5 to 100%) as reported previously for patients with celiac disease (median, 90%) (25) and primary immunodeficiencies (median, 94 to 99%) (38, 40) as well as normal controls (median, 75%) (40).

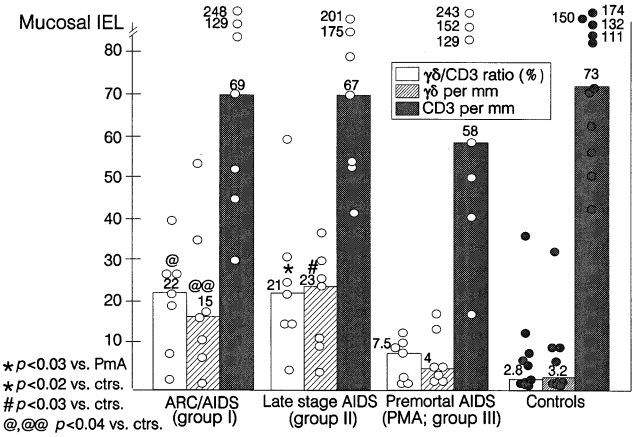

FIG. 3. Scatter diagram of the mucosal γ/δ/CD3 IEL ratio, γ/δ cells per millimeter, and CD3 cells per millimeter in three survival time-related subgroups compared with normal controls (ctrs.). Columns indicate medians (value indicated above each column). PMA, premortal AIDS.

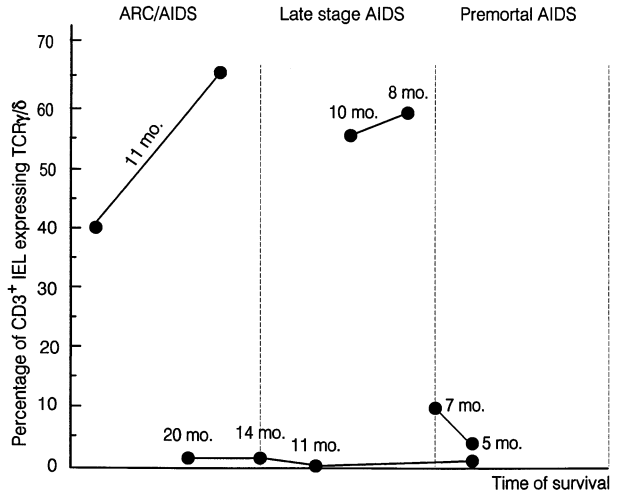

FIG. 4. Distribution of the percentage of duodenal TCRγ/δ^+ IEL in four HIV^+ patients determined from longitudinal studies in all available biopsy samples from the same patients. Vertical broken lines indicate subgroup categories.

A large fraction of γ/δ IEL (median, 90.9%; range, 33.3 to 100%) expressed the Vδ1/Jδ1-encoded epitope revealed by MAb δTCS1, somewhat higher than that reported previously for patients with celiac disease (67%) and primary immunodeficiencies (63 to 84%) (38, 40) as well as controls (75%) (25, 40). No difference was found among the three patient subgroups.

T-cell subsets in peripheral blood in relation to TCRγ/δ^+ IEL. When the results for circulating T-lymphocyte subsets in HIV^+ patients were compared with values for healthy blood donors (11), a tendency toward reduced numbers of $CD8^+$ cells (median, 370×10^6/liter, range, [85 to 2,120] $\times 10^6$/liter versus median, 405×10^6/liter, range, [180 to 1,230] $\times 10^6$/liter) was observed, and the $CD4^+$ lymphocytes were, as expected, significantly ($P < 0.0001$) reduced (median, 18×10^6/liter, range, [2 to 510] $\times 10^6$/liter versus median, 815×10^6/liter, range, [500 to 1,580] $\times 10^6$/liter). No subgroup contributed more than the others to this low figure.

No relationship was found between the relative or absolute numbers of γ/δ IEL and the numbers of circulating $CD4^+$ or $CD8^+$ T cells or the CD4/CD8 ratio in peripheral blood, nor was there any relationship between the number of $CD3^+$ IEL and circulating T cells.

Neopterin and β_2-M in serum. HIV^+ patients (median, 38.1 nmol/liter; $n = 17$) had significantly increased ($P < 0.0001$) serum concentrations of neopterin (Fig. 5) compared with controls (median, 9.0 nmol/liter). Among clinical or histological subgroups, only the four patients with verified CMV infection (median, 52 nmol/liter) and those with various degrees of villous atrophy (median, 14.1 nmol/liter) had particularly elevated levels. Serum neopterin showed an inverse relationship ($r = -0.32$) to duodenal γ/δ IEL, particularly in group III ($r = -0.97$, $P < 0.002$; $n = 7$). A tendency to increasing terminal neopterin concentrations was observed in all three subgroups (Fig. 5). No relationship was found between serum neopterin and circulating $CD4^+$ and $CD8^+$ cells, duodenal $CD3^+$ IEL, or any of the other immunological variables.

Levels of β_2-M (Fig. 5) in HIV^+ patients (median, 2,979 μg/liter; $n = 18$) were significantly increased ($P < 0.0001$) compared with control levels (median, 1,200 μg/liter). Among clinical categories, this increase was especially marked in the four patients with CMV infection (median, 4,010 μg/liter), in the one with schistosomiasis (3,500 μg/liter), and in the three with *M. avium-M. intracellulare* infection (median, 3,475 μg/liter); β_2-M also tended to be elevated in six patients with candidiasis (median, 3,267 μg/liter) and in the four with duodenitis (median, 3,266 μg/liter). Serum β_2-M showed an inverse relationship ($r = -0.41$) to duodenal γ/δ IEL, particularly in group III ($r = -0.58$), but this association was not significant ($P = 0.22$). A tendency to increasing β_2-M concentrations toward the end of life was observed in all three clinical subgroups (Fig. 5). No relationship was observed between serum β_2-M and circulating $CD4^+$ and $CD8^+$ cells, duodenal $CD3^+$ IEL, or any of the other immunological variables.

DISCUSSION

The HIV^+ patients showed an increased occurrence of duodenal TCRγ/δ^+ IEL, especially those with histologically verified duodenitis, villus changes, or Kaposi's sarcoma, as well as those with ongoing *M. avium-M. intracellulare* infection, systemic CMV infection, or campylobacter enteritis. Therefore, if γ/δ cells contribute to a first line defense against infectious agents (30), this protective measure appears to be intact and perhaps enhanced in most HIV^+ patients. Comparisons among three survival time-related stages of HIV infection revealed a significantly reduced proportion of γ/δ IEL in patients with the shortest life expectancy (Fig. 3). To our knowledge, such a variation of intestinal γ/δ cells has not been observed previously, although a recent study (26) reported a similar reduction of γ/δ cells in peripheral blood from patients with advanced AIDS (CDC IVC/D) compared with levels from those in earlier stages (CDC II to III).

Longitudinal studies of individual patients with AIDS regarding their duodenal γ/δ cells during various clinical stages might be more convincing, but such data are difficult to obtain both for practical and ethical reasons. However, longitudinal γ/δ IEL counts performed in a few patients supported our observation of a decreasing tendency toward the end of life (Fig. 4). It is not possible to know whether this decrease (or rather normalization) of intraepithelial γ/δ cells is merely a consequence of a general breakdown of mucosal defense or contributes to the end stage of the disease.

TCRγ/δ^+ cell levels are increased in the lesions of a variety of infectious diseases (20, 24, 37, 50), suggesting either a protective or an immunopathological role. Cytotoxic effects of γ/δ cells against natural killer cell-sensitive and natural killer cell-resistant target cells have been demonstrated, at least in vitro

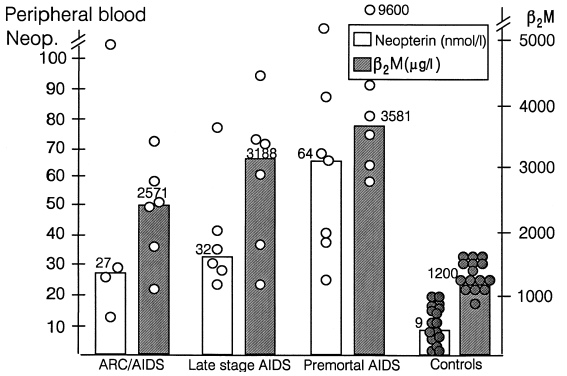

FIG. 5. Scatter diagram of serum concentrations of neopterin ($n = 17$) and β_2-M ($n = 18$) indicating an increasing terminal trend in all three AIDS subgroups compared with healthy controls. Medians are indicated by columns (value indicated above each column).

(30). A recent study (35) regarding the cytokine profile and ultrastructural features of γ/δ cells in inflamed gingiva suggested that their main effector function is that of cytotoxicity. Thus, this IEL subset may prevent the entrance of pathogens by attacking stressed epithelial cells and by controlling epithelial cell growth through secretion of regulatory cytokines and keratinocyte growth factor (6, 19). Although the antigenic repertoire and putative restriction element(s) of γ/δ cells are still obscure, they may recognize allospecific major histocompatibility complex class I (14), class II (7), and class I-like molecules (46), including different CD1 isoforms (41). Recent evidence, moreover, suggests that γ/δ cells may directly react with unprocessed microbial antigens (33).

Carbonari et al. (12) found increased circulating γ/δ cells in patients with ataxia telangiectasia but normal levels in patients with CVID, Wiskott-Aldrich syndrome, and severe combined immunodeficiency. Other reports have described elevated circulating γ/δ cells only occasionally in patients with ataxia telangiectasia (48) but commonly in patients with severe combined immunodeficiency or the complete Di-George anomaly (15). All of these studies were performed exclusively on peripheral blood, in which most γ/δ T cells (60%) use Vδ2 TCR gene rearrangements (13). Conversely, γ/δ IEL in the adult intestine consist mainly of the Vδ1 phenotype (25, 36, 44). Therefore, it is difficult to compare our results for samples obtained from the gut with previously published results. Nevertheless, Autran et al. (2) found increased numbers of Vδ1 cells in the blood of some HIV^+ patients, particularly those with AIDS. De Paoli et al. (16) suggested that a similar Vδ1 increase may be due to a diminished retention within a thymus damaged by HIV infection or to stimulation by activated autologous B cells.

Our efforts to quantify on an absolute basis the number of γ/δ IEL in HIV infection provided an overall count higher than that in normal controls, especially in the two subgroups preceding the end stage. Provided that γ/δ cells really perform antimicrobial functions and that these cells are similarly increased at other epithelial surfaces, they might contribute to an important part of local resistance against pathogens in HIV^+ individuals. In a previous study (38), we found significantly increased duodenal γ/δ IEL in IgAD patients only when they had no infections, although the compensatory effect of mucosal IgM and IgG1 also might have contributed to their apparently well-functioning antimicrobial defense (39). The reasons why some HIV individuals remain healthy over a long period while others rapidly become infection prone have up till now been ascribed to variations in their CD4 cell numbers, but how HIV kills $CD4^+$ T cells is still a matter of controversy (21, 22).

About two-thirds of the γ/δ IEL, both in hypogammaglobulinemic (40) and IgAD (38) subjects as well as in controls (25), express a Vδ1/Jδ1-encoded epitope, in contrast to less than 30% in peripheral blood (8). More than 90% of the γ/δ IEL in HIV^+ patients expressed this epitope, perhaps reflecting an intraepithelial clonal expansion of particular T-cell subsets. It was recently shown that both jejunal (3) and colonic (49) $TCR\alpha/\beta^+$ IEL are oligoclonal under normal conditions.

The total number of $CD3^+$ IEL per mucosal length unit (Fig. 1) tended to be slightly lower in the HIV^+ patients than in the controls, but the fraction of $CD8^+$ IEL was similar to that in the controls. As expected in these patients (34), we found a marked reduction of $CD4^+$ cells, both in the epithelium and in the lamina propria. However, determination of $CD8^+$ IEL numbers per mucosal length unit revealed no compensatory increase of such cells, in agreement with previous reports (18, 31). Only one patient showed a normal intraepithelial CD4/CD3 ratio. Although this homosexual man (CDC IVC1) had a severely reduced number of circulating CD4 cells (22×10^6/liter), his ratio of lamina propria CD4 cells remained higher (19%) than in the other HIV^+ patients (7.6%). Nevertheless, he had the lowest number of $CD3^+$ IEL among all patients, suggesting a parallel reduction of both CD4 and CD8 cells intraepithelially. This patient had recently regained normal bowel function, probably as a result of immunomodulating Dapson treatment, perhaps combined with the fact that parts of his intestinal mucosa remained intact and contained potentially cytotoxic γ/δ cells as a protection against HIV. It has also previously been reported that mucosal CD4 cells may survive longer than their circulating counterparts in AIDS (47).

We also determined certain serum markers of immunostimulation such as β_2-M (increases with cellular turnover) and neopterin (monocyte/macrophage hyperactivity). Both markers probably reflect gamma interferon stimulation and were significantly increased in our HIV^+ patients (Fig. 5), as recently reported for patients with AIDS (5, 51) and other types of immunodeficiency such as CVID (1, 51) and IgAD (38, 45). Both β_2-M and neopterin (an intermediate in the biosynthesis of tetrahydrobiopterin) showed an inverse correlation with duodenal γ/δ IEL in end-stage AIDS but were not related to CD4 cell counts in peripheral blood, in contrast to our previous findings for primary immunodeficiency (1, 38). A strong tendency of increasing neopterin and β_2-M concentrations toward the end of life was observed.

In conclusion, we found increased numbers of γ/δ IEL in most HIV^+ patients, although with a wide range probably reflecting opportunistic infections. Conversely, the almost total depletion of mucosal $CD4^+$ cells was not compensated for by a numerical increase of duodenal $CD8^+$ cells. In HIV^+ patients with particularly short life expectancy, γ/δ IEL were decreased to virtually normal levels. Longitudinal studies in a few patients supported this observation, suggesting that γ/δ IEL might be involved in prolonging the life of patients with AIDS. Other consequences of the final breakdown of the immune system were increased serum levels of β_2-M and neopterin, probably reflecting overstimulation of cell-mediated components.

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