Elevated Mucosal Addressin Cell Adhesion Molecule–1 Expression in Acquired Immunodeficiency Syndrome Is Maintained during Antiretroviral Therapy by Intestinal Pathogens and Coincides with Increased Duodenal CD4 T Cell Densities

Yin M. Miao, Peter J. Hayes, Frances M. Gotch, Mike C. Barrett, Nick D. Francis, and Brian G. Gazzard

Reduced intestinal CD4 T cell numbers and gastrointestinal disease are common features of acquired immunodeficiency syndrome (AIDS). Duodenal lymphocyte densities and mucosal addressin cell adhesion molecule (MAdCAM)–1 expression were analyzed in patients with AIDS after highly active antiretroviral therapy (HAART). Compared with human immunodeficiency virus (HIV)–seronegative individuals, HAART-naive patients with AIDS displayed reduced duodenal CD4 T cell densities. After HAART, AIDS patients with opportunistic intestinal pathogens displayed greater increases in duodenal lamina propria (LP) CD4 T cell densities than patients without such infections. Duodenal MAdCAM-1 expression was elevated in all HAART-naive patients with AIDS but remained elevated only in the intestinal pathogen group after HAART. The data suggest that, in HIV-1 infection, lymphocyte migration to the intestine may be promoted by increased MAdCAM-1 expression. After HAART, opportunistic intestinal pathogens maintain elevated MAdCAM-1 expression, which results in prominent increases in LP CD4 T cell densities in the absence of HIV-mediated CD4 T cell destruction.

Before the advent of highly active antiretroviral therapy (HAART), intestinal protozoal infections, such as infection with Cryptosporidium parvum or Enterocytozoon bieneusi, were major causes of mortality and morbidity in AIDS [1]. In AIDS, intestinal CD4 T cell numbers are disproportionately reduced, compared with circulating numbers [2, 3]. After the initiation of HAART, protozoal infections can be eradicated, presumably by immune mechanisms [4, 5]. Studies with murine models of C. parvum infection have demonstrated roles for both CD4 T cells and interferon (IFN)–γ in pathogen elimination [6].

The immune system of the gastrointestinal tract is quantitatively the largest in the body, containing over half of the total lymphocyte pool [7], and is a site for human immunodeficiency virus (HIV)–1 replication [8–12]. In addition to HIV-mediated CD4 T cell destruction, lymphocyte migration to the intestine may influence intestinal CD4 T cell numbers. Blood lymphocyte migration is governed by adhesion molecule and chemokine/receptor interaction between the lymphocyte and tissue endothelium [13]. The mucosal addressin cell adhesion molecule (MAdCAM)–1 is expressed predominantly on intestinal endothelia [14], with the integrin α4β7 ligand expressed on subpopulations of blood T cells, providing a mechanism for intestinal-specific lymphocyte migration [15–17]. Intestinal MAdCAM-1 expression is constitutive but is increased in inflammatory bowel disease [14]. Intestinal inflammation is common in AIDS, even in the absence of an intestinal pathogen [18]. Therefore, intestinal inflammation in AIDS may lead to increased MAdCAM-1 expression and thus may influence lymphocyte migration and lymphocyte numbers within the gastrointestinal tract.

The present study examined duodenal T cell densities and MAdCAM-1 expression in HAART-naive patients with AIDS and after the initiation of HAART. The effect of intestinal pathogens on these parameters was assessed, along with findings related to blood T cell counts, plasma virus loads, duodenal inflammation, and pathogen eradication.

Subjects and Methods

Subjects and study design. Twelve HIV-seropositive (HIV+) patients with CDC classification stage IV disease were recruited prospectively. Inclusion criteria consisted of a blood CD4 lymphocyte count < 200 cells/μL, no history of antiretroviral therapy, and a wish to initiate HAART. Patients were not selected from a larger group but were recruited at presentation at outpatient clinics at the Chelsea and Westminster Hospital, London, during 1998–1999.
The clinician in charge of the patient determined the choice of combination antiretroviral therapy. Patient characteristics are displayed in Table 1. Recruited patients included those who presented without (group 1) or with (group 2) opportunistic intestinal pathogens.

Routine analyses of plasma virus loads (branched DNA assay; Chiron version 3.0) and blood lymphocyte subsets were performed in the Department of Immunology, Chelsea and Westminster Hospital. All HIV+ patients with diarrhea at the Chelsea and Westminster Hospital undergo a standardized investigation protocol, with 3 stool samples and duodenal and rectal biopsy specimens analyzed for pathogens [5, 19]. This protocol was applied to patients recruited to this study, although rectal biopsies were not performed on group 1 patients. Stool samples were subjected to bacteriological culture, polymerase chain reaction, and routine light microscopy. Distal duodenal biopsy specimens were analyzed by histopathology, immunohistochemistry (for cytomegalovirus), and electron microscopy (EM). The histopathologist (N.D.F.) was blinded to patient clinical status. Eight distal duodenal biopsy specimens were obtained from each patient and formalin-fixed for histopathology (2 specimens), glutaraldehyde-fixed for EM (2 specimens), or embedded in optimal cutting temperature compound (R. A. Lamb) and snap-frozen in liquid nitrogen (3 specimens). One biopsy specimen was placed in 10 mL of Hanks’ balanced salt solution/0.001 M EDTA for processing to single-cell suspension. Formalin-fixed duodenal specimens from 9 age-matched (median, 53 years; P = .135, compared with patients with AIDS) and sex-matched (1 woman and 8 men) control subjects were obtained from the archives of the Histopathology Department, Charing Cross Hospital, London. These control subjects were investigated for unexplained anemia but were subsequently found to have normal duodenal histopathology. Archival and patient samples were processed in an identical manner in one histopathology laboratory. Two duodenal biopsy specimens per patient were obtained for frozen sectioning and single-cell suspensions from 6 other similar control patients (median age, 55 years; P = .22, compared with patients with AIDS). For ethical reasons, these patients were not tested for HIV status but were presumed to be HIV seronegative (HIV-).

**Immunohistochemistry.** Distal T cell densities were assessed in 4-µm formalin-fixed tissue sections, according to standard immunohistochemical techniques [20, 21]. Monoclonal mouse CD8 (Dako), CD4 (Novo Castria), and isotype control antibodies and biotinylated polyclonal secondary antibody (Serotec) were used according to the manufacturer’s instructions. Endogenous peroxidase was inactivated (30 min, 2% H2O2/methanol), and slides were washed with tap water and placed in boiling 0.01 M citrate buffer in a pressure cooker. Pressure was reached and maintained for 90 s. Slides were incubated with 10% horse serum/TRIS-buffered saline (TBS) prior to application of primary antibody. Antibodies and peroxidase-avidin-biotin complex (ABC; Vector Laboratories) were applied for 1 h each with TBS washing. Sections were treated with diaminobenzidine/H2O2 and were hematoxylin stained.

MAdCAM-1 expression was assessed with antibody 10G3 (provided by Michael Briskin and Walter Newman, Millennium Pharmaceuticals) [14]. Cryostat sections of 7 µm were air-dried and formalin-fixed (10 min), endogenous peroxidase was inactivated with 4 M sodium azide/0.5% H2O2 in PBS (30 min), and rabbit serum/TBS was applied prior to 10G3 or isotype antibodies (4°C overnight). Biotinylated rabbit secondary antibody (Dako) and ABC were applied sequentially for 1 h each. Sections were treated with diaminobenzidine/H2O2 and hematoxylin stained. For assessment of lamina propria (LP) areas and stained cell densities, slides were viewed via a microscope and drawing tube attachment positioned over a digitizer tablet. Low-angle tablet illumination allowed both section obser-

### Table 1. Patient characteristics, blood CD4 cell count, and plasma virus load with highly active antiretroviral therapy (HAART).

<table>
<thead>
<tr>
<th>Group, patient, and original AIDS-defining illness</th>
<th>Sex/age, years</th>
<th>HAART regimen</th>
<th>Blood CD4 cell count, cells/µL</th>
<th>Log_{10} plasma virus load, copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td>Pre-HAART</td>
<td>At 1 month</td>
</tr>
<tr>
<td>1. Esophageal candidiasis</td>
<td>M/45</td>
<td>d4T, 3TC, Rtv, Sqv</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>2. Esophageal candidiasis</td>
<td>F/35</td>
<td>ddl, 3TC, DMP266</td>
<td>108</td>
<td>183</td>
</tr>
<tr>
<td>3. <em>Pneumocystis</em> pneumonia</td>
<td>M/54</td>
<td>d4T, ddl, Nvf</td>
<td>3</td>
<td>107</td>
</tr>
<tr>
<td>4. <em>Salmonella</em> septicemia (resolved 12 months prior to study)</td>
<td>M/40</td>
<td>d4T, 3TC, Rtv, Idv</td>
<td>4</td>
<td>64</td>
</tr>
<tr>
<td>5. <em>Pneumocystis</em> pneumonia</td>
<td>M/35</td>
<td>d4T, 3TC, Rtv, Sqv</td>
<td>110</td>
<td>114</td>
</tr>
<tr>
<td>6. Kaposi’s sarcoma</td>
<td>M/40</td>
<td>3TC, Zdv, DMP266</td>
<td>7</td>
<td>59</td>
</tr>
<tr>
<td>Group 1 median</td>
<td></td>
<td></td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td>Pre-HAART</td>
<td>At 1 month</td>
</tr>
<tr>
<td>7. Cryptosporidium parvum</td>
<td>M/40</td>
<td>d4T, 3TC, Idv</td>
<td>38</td>
<td>142</td>
</tr>
<tr>
<td>8. Enterocytotoxin bieneusi</td>
<td>M/33</td>
<td>d4T, 3TC, Rtv, Idv</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>9. <em>E. bieneusi</em></td>
<td>M/45</td>
<td>d4T, 3TC, Idv</td>
<td>67</td>
<td>132</td>
</tr>
<tr>
<td>10. Giardia and cytomegalovirus</td>
<td>M/30</td>
<td>d4T, 3TC, Idv</td>
<td>19</td>
<td>55</td>
</tr>
<tr>
<td>11. Campylobacter</td>
<td>M/32</td>
<td>d4T, ddl, Nvf</td>
<td>122</td>
<td>781</td>
</tr>
<tr>
<td>12. <em>C. parvum</em> and <em>E. bieneusi</em></td>
<td>M/39</td>
<td>3TC, Zdv, Idv</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>Group 2 median</td>
<td>36</td>
<td>29</td>
<td>94</td>
<td>172</td>
</tr>
</tbody>
</table>

*NOTE:* “At 1 month” and “At 6 months” indicate time since initiation of HAART. Patients have (group 2) or do not have (group 1) an opportunistic intestinal pathogen. P values (Mann-Whitney U test) represent significance levels between the 2 groups. 3TC, lamivudine; d4T, stavudine; ddl, didanosine; DMP266, efavirenz; Idv, indinavir; Nvf, nelfinavir; Rtv, ritonavir; Sqv, saquinavir; Zdv, zidovudine.
vation and tablet tracing. Tablet data were analyzed by use of the National Institutes of Health IMAGE program (available at http://rsb.info.nih.gov/nih-image). The area measured was calibrated with a calibrated microscope slide. T cells were counted and were converted to cells per square millimeter. The numbers of intraepithelial lymphocytes (IEL) were counted and were expressed per 100 epithelial cells. MadCAM-1 immunohistochemistry was expressed as stained vessels per square millimeter. Two observers performed comparable counts, and a mean was taken.

**Flow cytometric analysis of duodenal cell suspensions.** The epithelium was removed from biopsy specimens by shaking for 1 h at 37°C in Hanks’ balanced salt solution/0.001 M EDTA, the supernatant was retained, and the tissue was passed through a 100-μm cell strainer with PBS by use of a syringe barrel. The resulting epithelial and LP cells were resuspended in 200 μL of 0.1% wt/vol bovine serum albumin (BSA)/PBS; 50-μL cell suspensions were incubated (30 min on ice) with 5 μL of the antibodies CD3 fluorescein isothiocyanate, CD4 phycoerythrin (PE), and CD8 PE-Cy5 (all Dako), washed with 0.1% BSA, and fixed in 2% paraformaldehyde. Cells were analyzed with a FACS Calibur (Becton Dickinson). Duodenal lymphocytes were gated by forward/side scatter and CD3 expression. CD4 and CD8 T cell percentages of the CD3 T cell population were determined.

**Statistics.** Medians and interquartile ranges (IQRs) are presented. Data were analyzed with SPSS (version 10) software. The existence of a significant difference between unpaired data from the 3 groups (groups 1 and 2 and HIV+ controls) was assessed with a non-parametric Kruskal-Wallis test. When the Kruskal-Wallis test demonstrated the existence of a significant difference, Mann-Whitney U tests were used to assess differences between unpaired data from 2 separate patient groups. Paired data from within 1 group (pre- and post-HAART) were analyzed with a nonparametric Wilcoxon signed-rank test. Significant differences were assumed if $P < .05$.

**Results**

**Subjects.** Six patients with a prior AIDS-defining illness did not have concurrent detectable opportunistic intestinal pathogens at initiation of HAART (group 1, table 1). Five of these 6 patients had no symptoms of diarrhea prior to HAART. Patient 5 did present with mild symptoms of diarrhea, which rapidly resolved after HAART was initiated; however, no intestinal pathogen was detected, despite the rigorous investigation protocol. At initiation of HAART, the 6 other HIV+ patients had diarrhea secondary to an opportunistic intestinal pathogen diagnosed either on stool samples or on endoscopic biopsy specimens (group 2, table 1).

**Blood T cell counts and plasma virus loads after HAART.** All patients symptomatically improved after HAART. Pre-HAART blood CD4 counts of the 2 groups of patients with AIDS were similar ($P = .58$; table 1). After HAART, all patients displayed increased blood CD4 T cell counts, with no significant differences between the 2 groups ($P > .75$). Both patient groups displayed similar blood CD8 T cell counts at all time points ($P > .34$; data not shown). Grouping all 12 patients with AIDS together, median blood CD8 T cell counts increased from 471 cells/μL pre-HAART to 913 cells/μL at 6 months post-HAART. Pre-HAART plasma virus loads were similar in the 2 groups ($P = .75$) and were reduced after HAART in all patients (table 1). However, at 1 month post-HAART, group 2 patients with intestinal pathogens displayed moderately but significantly higher virus loads, compared with group 1 patients ($P = .027$). At 6 months, differences were not significant ($P = .18$), but plasma viremia was detectable in 3 patients from group 2 but in only 1 patient from group 1.

**Opportunistic intestinal pathogens promote restoration in LP CD4 T cells after HAART.** Among 9 HIV- individuals, the median (IQR) LP CD4 T cell density was 1170 (1060–1670) cells/mm² (figure 1A). Kruskal-Wallis analysis revealed significant differences in LP CD4 T cell densities among the 3 groups (HIV+ and AIDS patient groups 1 and 2) at all time points ($P < .002$). All patients with AIDS demonstrated severe pre-HAART depletions of LP CD4 T cell densities ($P < .001$, compared with HIV- individuals). Pre-HAART LP CD4 T cell densities were similar in the 2 AIDS patient groups (group 1, 13 [12–105] cells/mm²; group 2, 33 [13–120] cells/mm², $P = .62$). At 6 months, HAART group 2 patients displayed a median of 410 (290–510) LP CD4 T cells/mm², a significant increase compared with pre-HAART values ($P = .017$). These densities were significantly higher ($P = .017$) than those in group 1 patients, who displayed a median density of 48 (30–80) CD4 T cells/mm², an insignificant increase over pre-HAART values ($P = .19$).

Pre- and post-HAART LP CD8 T cell densities are elevated in all patients with AIDS. Among 9 HIV- individuals, the median (IQR) LP CD8 T cell density was 1390 (1230–1460) cells/mm² (figure 1B). Pre-HAART LP CD8 T cell densities of both AIDS patient groups were higher than those in HIV- individuals; however, Kruskal-Wallis analysis demonstrated that these differences were not significant ($P = .12$). Significant differences were demonstrated among the 3 groups at the 1- and 6-month post-HAART time points ($P < .014$), with both groups of patients with AIDS displaying elevated LP CD8 T cell densities, compared with HIV- individuals ($P < .018$). There were no significant differences in CD8 T cell densities between the 2 AIDS patient groups at any time point ($P > .5$).

**The duodenal epithelium is devoid of CD4 IEL in AIDS, with little restoration after HAART.** Among 9 HIV- individuals, median (IQR) IEL densities were 3.0 (2.7–3.2) and 10.7 (7.4–20.5) per 100 epithelial cells for CD4 and CD8 IEL, respectively. Prior to HAART, CD4 IEL were undetectable (median [IQR], 0 [0–0]) in all but 1 of the patients with AIDS (patient 3), even when entire tissue sections were examined. By 6 months of HAART, there was an insignificant ($P > .11$) increase in CD4 IEL densities in the patients with AIDS (median [IQR], 0 [0–0.4]). No discernible difference in CD4 IEL densities was apparent between the 2 AIDS patient groups, with CD4 IEL detectable in only 2 patients in each group. CD8 IEL densities were not significantly different from those of HIV- individuals.
at any time point. However, group 2 patients did display higher CD8 IEL densities (pre-HAART, 18.2 [13.8–31.5], and 6 months, 30.7 [13.4–34.4]) than group 1 and HIV− individuals. The lack of statistical significance appears to be due to the wide ranges in CD8 IEL densities in both patients with AIDS and HIV− individuals.

**Immunohistochemical T cell densities confirmed with flow cytometric analysis.** Analysis of CD4 expression on blood leukocytes demonstrated that monocytes express CD4 at a mean fluorescent intensity approximately one-tenth of that of CD4 T cells (data not shown). To assess the possibility that immunohistochemical determinations of duodenal CD4 T cell densities may be influenced by low-level expression of CD4 on tissue macrophages, the percentages of intestinal CD3+ T cells expressing CD4 and CD8 were assessed by flow cytometry (figure 2). CD4 and CD8 T cell percentages demonstrated an almost identical pattern to immunohistochemical densities, with reduced pre-HAART LP CD4 T cell percentages in all patients with AIDS of 0%–2% of CD3+ T cells, compared with 34% in HIV− individuals. However, it should be noted that this analysis used a separate HIV− control group rather than that used for immunohistochemistry. After 6 months of HAART, there was a 12-fold increase in LP CD4 T cell percentages, to 11% of CD3 T cells, in the intestinal pathogen group 2. However, group 1 patients displayed only a 2-fold increase, to 3.8% of CD3 T cells. LP CD8 T cell percentages in patients with AIDS ranged from 83% to 91% of CD3+ T cells and were similar in both groups of patients with AIDS at all times points. The median LP CD8 T cell percentage in HIV− individuals was 51%. Flow cytometric analysis of epithelial cells was in agreement with the immunohistochemical analysis (data not shown); therefore, flow cytometric analysis confirmed the validity of the immunohistochemical analysis of duodenal T cell densities. However, flow cytometry alone cannot establish that the high intestinal CD8 T cell percentages were due to increases in such cells, because elevated percentages may merely reflect the depletion in intestinal CD4 T cells. Immunohistochemical detection allowed a true assessment of intestinal T cell populations, expressed as cells per square millimeter.

**Duodenal LP MadCAM-1 expression is elevated in all HAART-naive patients with AIDS and is maintained after HAART by opportunistic intestinal pathogens.** MadCAM-1 expression was detected by immunohistochemistry in both patients with AIDS and HIV− individuals with a clear LP location (figure 3A–3E). Kruskal-Wallis analysis revealed significant differences in MadCAM-1+ vessel densities among the 3 groups at all time points (P < .007). Pre-HAART MadCAM-1+ vessel densities were significantly elevated in both group 1 (P = .004) and group 2 (P = .004), compared with HIV− individuals (figure 4). The 2 groups of patients with AIDS displayed similar MadCAM-1 vessel densities pre-HAART (P = .69). At 6 months, MadCAM-1+ vessel densities remained elevated in group 2 patients (P = .004, compared with HIV− individuals). At 6 months, group 1 MadCAM-1 expression was significantly lower than those in group 2 patients at this time point (P = .01) and in group 1 patients pre-HAART (P = .026). Group 1 MadCAM-1 expression at 6 months was similar to that of HIV− individuals (P = .13).

**Duodenal histopathological abnormalities were still evident after 6 months of HAART in patients diagnosed as having an opportunistic intestinal pathogen, despite apparent pathogen eradication.** Prior to HAART, group 2 patients displayed duodenal histopathology that was reported by the clinical pathologist (N.D.F.) to consist of inflammation and villus blunting. Such duodenal histopathology has been reported elsewhere in HIV-1 infection [22]. At 1 month post-HAART, histopathological abnormalities and opportunistic intestinal pathogens were still detected in group 2 patients 7–10 and 12. However, after 6 months of HAART, despite intestinal pathogens being undetectable, apart from a few dead *E. bieneusi* spores observed in patient...
8, duodenal histopathological abnormalities were still apparent in patients 7, 8, 9, and 12. Prior to HAART, duodenal histopathology was normal in 5 of 6 group 1 patients. Patient 5, who presented with mild diarrhea but no opportunistic pathogen, displayed mild villus blunting that resolved with HAART.

Discussion

Both duodenal LP and IEL CD4 T cells were depleted in patients with AIDS, as reported elsewhere [2, 3]. Four of 6 intestinal infections were due to the protozoon *C. parvum* or *E. bieneusi*. Murine models suggest a role for CD4 T cells in eradicating such organisms [6], but a role for these cells in human infection is uncertain. Our data support such a role, since, after HAART, group 2 intestinal pathogen patients displayed prominent increases in LP CD4 T cell densities that coincided with pathogen eradication. However, there were minimal increases in CD4+ IEL densities. *C. parvum* infection is restricted to the enterocyte [23], and, in mice, CD4+ IEL play a role in *Cryptosporidium* eradication via IFN-γ [6, 24, 25]. However, in the present study, eradication of *C. parvum*, and other pathogens coincided with increased LP, but not IEL, CD4 T cell densities. Intestinal IEL make up a distinct lymphoid compartment [26], which may explain the contrasting LP and IEL CD4 T cell densities. In addition, IFN-γ production has been demonstrated within the LP but not the epithelium after experimental human *C. parvum* infection [27]. These data suggest that LP CD4 T cells play an important role in human intestinal pathogen eradication, even when infection is restricted to the enterocyte. HIV replication has been detected in the LP rather than the epithelium [8], and a lack of association of HIV with the epithelium may be due to a lack of HIV-susceptible CD4+ IEL. In contrast to group 2, group 1 patients displayed minimal increases in LP CD4 T cell densities after HAART. This could not be explained by differing pre-HAART LP T cell densities or blood T cell counts at any time, which were similar between the 2 groups (figure 1 and table 1).

Pre-HAART LP MAdCAM-1+ vessel densities were elevated in both patient groups. Elevated MAdCAM-1 expression has been demonstrated in inflammatory bowel disease [14, 28] and would promote lymphocyte migration to the intestine [15–17].

Figure 2. Representative flow cytometry profiles of duodenal lamina propria cells. A. Forward scatter (FSC) against side scatter (SSC; patient 9). CD3+ lymphocytes were found to reside within region 1 (R1). B. CD3 expression (R2) in R1-gated cells. C–G, CD4 and CD8 profiles of R1- and R2-gated lymphocytes. Percentages of the CD3+ lymphocytes residing within the CD4 and CD8 populations are displayed for patient 9 (group 2) before (C) and 6 months after (D) beginning highly active antiretroviral therapy (HAART) and for patient 2 (group 1) before (E) and 6 months after (F) beginning HAART. Reduced CD4 but elevated CD8 T cell percentages were evident in all patients with AIDS, compared with human immunodeficiency virus–seronegative individuals (G). Some HAART-induced restoration in CD4 T cell percentages is evident in panels D and F.
Higher, although not significantly so, densities of LP CD8 T cells were observed in all patients with AIDS prior to HAART. The intestine is a site of HIV replication [8–12], which would result in CD4 T cell destruction. Presumably, intestinal CD4 T cell destruction would cease during HAART, allowing CD4 T cell densities to rise. With HAART, group 2, but not group 1, patients maintained elevated MAdCAM-1 expression, which coincided with prominent increases in LP CD4 T cell densities in group 2 patients. Intestinal pathogens were detectable at 1 month post-HAART but were largely undetectable at 6 months. Elevated MAdCAM-1 expression at 6 months might be due to an undetectable level of pathogen infection. The detection of dead *E. bieneusi* spores in patient 8 would support this.

The elevated pre-HAART MAdCAM-1 expression in group 1 implies that lymphocyte migration to the intestine would be promoted in AIDS, even in the absence of opportunistic intestinal pathogens. Although abnormal duodenal histopathology was observed in only one group 1 patient, intestinal histopatholog-

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**Figure 3.** Immunoperoxidase detection of mucosal addressin cell adhesion molecule expression (brown) in the duodenum of AIDS patient 12, infected with *Cryptosporidium parvum* and *Enterocytozoon bieneusi*, before highly active antiretroviral therapy (HAART) (A) and at 6 months (B). Expression in patient 1 (group 1), with esophageal candidiasis, is shown before HAART (C) and at 6 months (D). Endothelial expression is apparent within the lamina propria in both patients, with increased expression in panels A–C compared with panels D and E, which shows a specimen from a human immunodeficiency virus–seronegative individual. Blue hematoxylin counterstain; bar, 50 μm.
Duodenal lamina propria (LP) mucosal addressin cell adhesion molecule (MAdCAM-1) vessel densities/mm² before and 1 month and 6 months after highly active antiretroviral therapy (HAART) in patients with AIDS diagnosed as having (group 2; black squares, n = 6) or not having (group 1; white squares, n = 6) an opportunistic intestinal pathogen and in human immunodeficiency virus–seronegative (HIV−) individuals (gray squares, n = 6). The bar represents the median value of the group.

Figure 4.