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Selective depletion of rectal lamina propria rather than lymphoid aggregate CD4 lymphocytes in HIV infection

F. CLAYTON, G. SNOW, S. REKA* & D. P. KOTLER* *Salt Lake VA Medical Centre and University of Utah School of Medicine, Salt Lake City, UT, and *St Luke's-Roosevelt Hospital and Columbia University School of Medicine, New York, NY, USA*

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SUMMARY

The goal of this study was to examine the changes in lymphocyte populations in rectal mucosa during HIV infection and to study their relationship to mucosal immunity and to systemic depletion of CD4 lymphocytes. Rectal biopsies from 58 HIV-infected subjects and eight controls were studied. Frozen rectal tissue sections were stained with antibodies to CD4, CD3, CD8, and markers for macrophages. HIV-infected subjects were divided into early stage (no opportunistic infections) and AIDS groups. There was profound depletion of rectal lamina propria CD4 lymphocytes (16% and 6% of normal content in early and AIDS groups, respectively). However, lymphoid aggregate CD4 lymphocytes were far less severely depleted (69% and 40% of normal content, respectively). The extent of lymphoid aggregate CD4 lymphocyte depletion generally parallelled the CD4 lymphocyte depletion in the blood. CD8 lymphocyte content in both the lamina propria and lymphoid aggregates usually were increased, particularly in early-stage patients. Macrophage contents were usually normal in the HIV-infected groups. We conclude that rectal lamina propria and lymphoid aggregates are distinct compartments differing markedly in their CD4 lymphocyte content during HIV infection. In light of this and an increased number of apoptotic cells which were noted in rectal lamina propria in HIV-infected subjects, we hypothesize that intestinal lamina propria could be a site of rapid CD4 lymphocyte destruction during HIV infection.

Keywords HIV AIDS rectum intestine immunology T lymphocytes

INTRODUCTION

Altered bowel habits and mucosal opportunistic infections are very common and often life-threatening in HIV-infected subjects [1]. Our prior studies of rectal mucosa have shown three or four distinct histological patterns of inflammatory cell populations which correlate strongly with the stage of infection [2]. Changes in mucosal lymphoid subpopulations during HIV infection are complex and presumably reflect the pathogenesis of mucosal immunodeficiency.

The importance of blood CD4 lymphocyte content in determining the stage and prognosis of HIV infection is well recognized [3], but changes in intestinal mucosal lymphocyte content have been less thoroughly studied. There are conflicting reports on the extent of CD4 lymphocyte depletion in small intestinal or colorectal mucosa; some have found severe depletion of intestinal lamina propria CD4 lymphocytes even in early HIV infection [4–8], while others have not [9,10]. The mucosal immune system encompasses several distinct compartments, including individual and grouped (Peyer's patches) lymphoid follicles, the lamina propria, and the epithelium. The goal of this study was to determine the changes in lymphocyte subpopulations in the various compartments of rectal mucosa and to correlate these with the stage of HIV infection.

Correspondence: Dr F. Clayton, Pathology Department, Salt Lake VA Hospital, 500 Foothill Drive, Salt Lake City, Utah 84148, USA.

MATERIALS AND METHODS

Rectal biopsies from 58 HIV^+ subjects and eight HIV^- healthy volunteer controls were studied. The HIV-infected group consisted of 56 male homosexuals and two females with presumably sexually acquired HIV infection. These were essentially sequential patients presenting with enteric or proctologic complaints. All had a blood CD4 lymphocyte content determination within 6 months of biopsy. Rectal biopsies were performed and promptly frozen in embedding medium, and stored at -70°C.

Sections (5 μm) were cut, fixed in acetone for 5 min, and allowed to air dry. Immunoperoxidase staining was done with antibodies to CD3 (UCHT1; Dako Corp., Santa Barbara CA), CD4 (MT310, Dako; and Leu-3a, Becton Dickinson, San Jose CA), CD8 (DK25; Dako), and macrophages (HAM56; Dako). The MT310 antibody to CD4 was used in all cases; a preliminary study that the proportion of cells stained by Leu-3a (which stains a different CD4

epitope) was similar. Secondary staining was done with biotin–$F(ab')_2$ fragment antibodies to mouse IgG or IgM, and streptavidin–horseradish peroxidase (all from Jackson Immunoresearch, West Grove, PA) according to the manufacturer's instructions and stained with diaminobenzidine. Haematoxylin and eosin-stained slides were also prepared from all blocks.

Cases were reviewed without knowledge of the HIV status or stage of infection. In each case the lamina propria and the lymphoid aggregates (if present) were examined separately. If lymphoid aggregates were not present in the initial specimen, an additional block was sectioned and stained in order to find them. Cells were counted as a proportion of all mononuclear cells in the lamina propria or the peripheral (T cell-rich) zone of the lymphoid aggregate, as appropriate. The number of lamina propria mononuclear cells per high power field (HPF) (0·38 mm diameter) was also counted. Counts were adjusted for slide thickness [11]. Most CD4 staining cells were small and had scanty cytoplasm. A few had more abundant cytoplasm and, in adjacent sections, stained with the HAM56 macrophage antibody. These were considered macrophages and not counted as CD4 lymphocytes. CD3 staining (pan T cells) was done as a control for the accuracy of CD4 and CD8 lymphocyte counting.

Cases were divided into controls and two patient groups for analysis. Subjects were considered to have AIDS if they had had either an AIDS-defining opportunistic infection or a blood CD4 lymphocyte content <200/mm^3; the other HIV$^+$ subjects were considered to have early-stage infection. Statistical analysis was performed using BMDP (BMDP Statistical Software, Los Angeles, CA) with ANOVA using Scheffe statistics, analysis of covariance, *t*-tests using the non-parametric Mann–Whitney statistic, and bivariate correlation using the product moment correlation.

RESULTS

Blood T lymphocyte subsets

Blood CD4 lymphocyte content was decreased in both HIV-infected groups and the AIDS group had lower CD4 lymphocyte counts than the early HIV group ($P < 0{\cdot}01$ for each comparison). The mean CD4 lymphocyte contents in the early HIV and AIDS groups were 37% and 6% of the mean normal content, respectively. The mean CD8 lymphocyte content was increased to 240% of mean normal value in the early HIV group ($P < 0{\cdot}01$), but was not significantly different from normal in the AIDS group (see Fig. 1).

Lamina propria T lymphocyte subsets and macrophages

There were striking differences in lamina propria CD4 lymphocyte content among the different groups (Figs 2a and 3a). In normal controls, the content of CD4 lymphocytes per HPF varied between 13 and 34, while all HIV-infected subjects had 14 or fewer CD4 lymphocytes. Only two cases (both early HIV stage) barely overlapped the normal range. The mean CD4 lymphocyte contents in the early HIV and AIDS groups were 16% and 6% of the HIV$^-$ controls ($P < 0{\cdot}01$ for all comparisons except early HIV *versus* AIDS, where $P < 0{\cdot}05$). The mean proportion of CD8 lymphocytes was markedly increased in HIV-infected groups relative to controls, particularly the early HIV group ($P < 0{\cdot}01$ for controls *versus* early HIV, $P < 0{\cdot}05$ for the other comparisons). CD3 lymphocyte content minus the sum of the CD4 and CD8 lymphocyte content had a mean of 0·6%, with a s.d. of 6%.

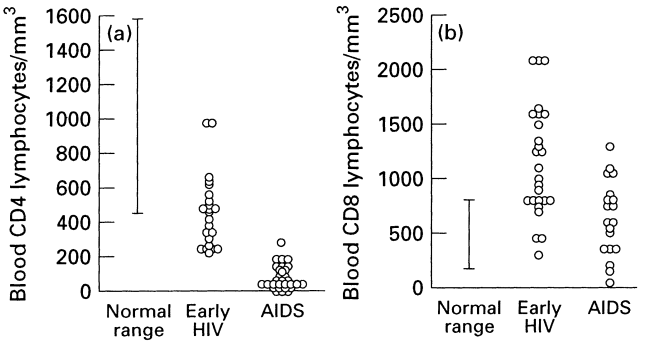

Fig. 1. Blood CD4 and CD8 lymphocyte content per mm^3 for the HIV-infected groups compared with the normal range. Each HIV-infected group is significantly different from the other ($P < 0{\cdot}01$, Student's *t*-test).

There were a mean of 15 or 16 macrophages per HPF in the HIV$^-$ control subjects and in both the HIV-infected groups. There was no correlation between the content of macrophages (CD56) and the presence or stage of HIV infection.

Lymphoid aggregates

As in the lamina propria, there was significant depletion of CD4 lymphocytes in both HIV-infected groups, particularly AIDS patients (see Figs 2b, 3b). However, the decrease in the proportion of CD4 lymphocytes was far less severe than in the lamina propria,

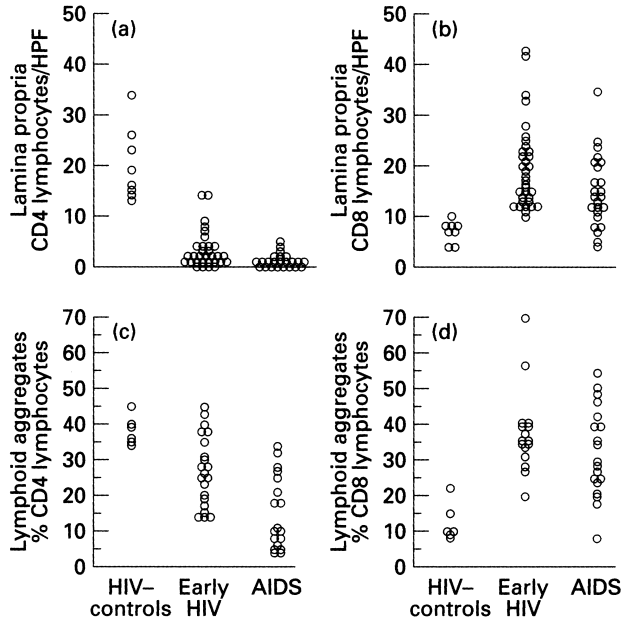

Fig. 2. (a,b) Lamina propria CD4 and CD8 lymphocyte content expressed as cells per high power field (HPF). Note the marked depletion of CD4 lymphocytes in the HIV-infected groups. CD8 lymphocyte content is increased in both HIV-infected groups. (c,d) Lymphoid aggregate CD4 and CD8 lymphocyte content expressed as percentage of cells in the peripheral zone of lymphoid aggregates. While the CD4 lymphocytes are often depleted relative to HIV$^-$controls, the depletion is far less common and less severe than in the lamina propria ($P < 0{\cdot}01$, compare with Fig. 2a). CD8 lymphocyte content is increased in many of the HIV-infected subjects, particularly the early HIV stage group.

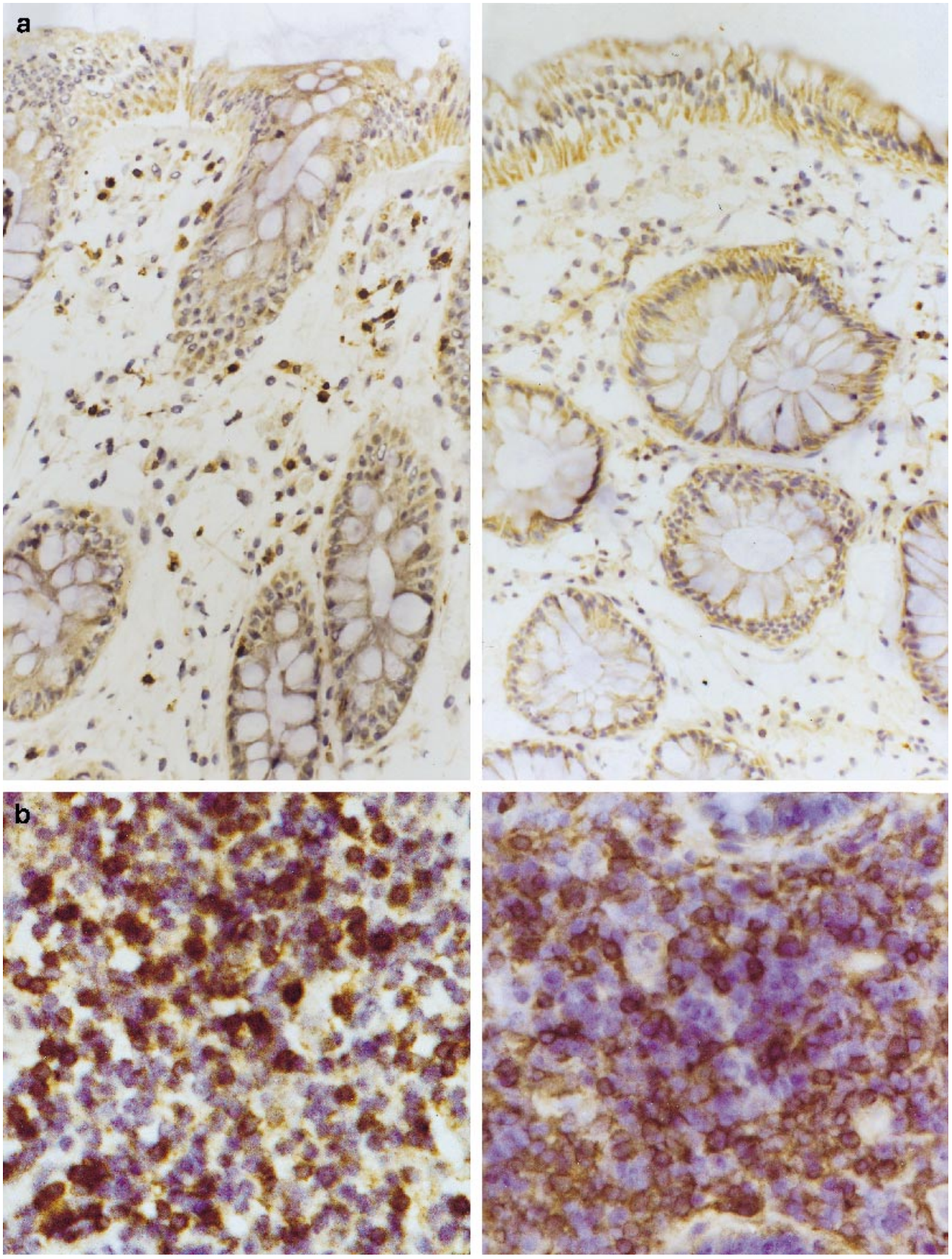

Fig. 3. (a) Lamina propria immunostained with antibodies to CD4, in an HIV$^-$ control (left) and an early HIV group subject (right). Notice the virtual absence of CD4-staining cells in the right panel. (b) Lymphoid aggregates immunostained with antibodies to CD4, showing a normal lymphoid aggregate (left) and an early HIV group subject (right). Notice the moderate but definite decrease in CD4-immunostaining cells in the right panel.

and many early stage subjects had CD4 cell contents in the normal range. The early HIV and AIDS groups had 69% and 40% the CD4 lymphocyte fraction of the HIV^- controls ($P < 0{\cdot}01$ for controls *versus* early HIV group, $P < 0{\cdot}05$ for the other comparisons). Analysis of covariance showed that the extent of CD4 lymphocyte depletion in the lymphoid aggregates was significantly different from in the lamina propria ($P < 0{\cdot}01$). Also, as in the lamina propria, there was an increase in CD8 lymphocyte content in the HIV-infected groups ($P < 0{\cdot}01$, each HIV-infected group *versus* controls) and no overall change in the proportion of macrophages in any of the groups.

DISCUSSION

The marked depletion of rectal lamina propria CD4 lymphocytes, even in early-stage HIV-infected subjects, is similar to prior small intestinal lamina propria results [4,5,7,8,12], and colonic lamina propria results [6,12]. These results differ from some earlier studies, which did not find severe CD4 lymphocyte depletion in early-stage subjects [9,10]. Possible reasons for the discrepancy include mistaking CD4-staining macrophages as lymphocytes or failing to distinguish lymphoid aggregate from lamina propria lymphocytes. Another potential explanation for apparent depletion of CD4 lymphocytes would be the masking of the CD4 antigen by HIV gp120. In the current study this is unlikely, for two reasons: (i) the CD4 lymphocyte content closely matched the CD3 lymphocyte content minus the CD8 lymphocyte content; and (ii) the proportion of staining cells was similar for both the Leu-3a and MT310 antibodies, which recognize different CD4 epitopes.

There is a striking disparity in the extent of CD4 lymphocyte depletion in the lamina propria as opposed to the blood or lymphoid aggregates. Lim *et al.* [4] and Schneider *et al.* [6] noted that CD4 lymphocyte depletion is predominantly a loss of $CD4^+CD45RO^+$ activated/memory Th cells. Particular loss of this subset, relative to $CD4^+DC45RA^+$ cells, is also seen in the blood. In intestinal mucosa, naive Th cells are found mainly in the lymphoid aggregates (the afferent limb of the mucosal immune system), while the $CD45RO^+$ activated/memory cells are mainly in the lamina propria (the efferent limb of the mucosal immune system) [13,14]. This could reflect both the propensity for $CD45RO^+$ HIV-infected CD4 lymphocytes to undergo cell death [15] and increased turnover of CD4 lymphocytes during HIV infection [16,17]. Lymphoid aggregate CD4 lymphocyte depletion is significantly less severe than in the lamina propria and more closely parallels the extent of CD4 lymphocyte depletion in the blood.

Increased apoptosis has been documented in rectal lamina propria in HIV-infected subjects, even well before the development of AIDS, indicating this is a site of abundant cell death [18]. Given this and the striking paucity of lamina propria CD4 lymphocytes even in early-stage subjects, it is plausible that the lamina propria is a site of accelerated loss of CD4 lymphocytes. Also, given the high content of HIV p24 antigen in rectal mucosa in many HIV-infected subjects who have not yet developed AIDS [2,19], it is plausible that this early depletion of lamina propria CD4 lymphocytes is directly mediated by HIV. Thus we hypothesize that rectal lamina propria is a site of accelerated depletion of CD4 lymphocytes, playing a causal role in the development of mucosal immunodeficiency.

In conclusion, rectal mucosa has distinct compartments—the lymphoid aggregates and lamina propria—which could be considered the afferent and efferent limbs of the mucosal immune system. The changes in CD4 lymphocyte populations are strikingly different in these different compartments, with substantial depletion of lamina propria CD4 lymphocytes even in early-stage subjects, but relative preservation of lymphoid aggregate CD4 lymphocytes even in AIDS patients. In HIV-infected subjects, rectal lamina propria are a site of early loss of CD4 lymphocytes, while CD4 lymphocytes are preserved and possibly produced in lymphoid aggregates.

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