Characterization of Intestinal Disease Associated with Human Immunodeficiency Virus Infection and Response to Antiretroviral Therapy

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Combination antiretroviral therapies suppress human immunodeficiency virus (HIV) in peripheral blood, but the effect in gastrointestinal mucosa is uncertain. The occurrence of pathogen-negative diarrhea led to speculation that local HIV infection is etiologic. Mucosal cellular reservoirs for HIV were documented by use of several techniques. Correlations were found among gastrointestinal symptoms, histopathologic findings, cytokine expression, lymphoid apoptosis, and HIV RNA and protein expression in rectal mucosa. Disproportionate depletion of mucosal CD4+ lymphocytes also was found. The short-term effects of antiretroviral therapies were examined to test the hypothesis that these changes are directly related to mucosal HIV infection. Therapy was associated with decreased symptoms, with comparable drops in peripheral blood and mucosal HIV RNA contents, and by increases in blood and mucosal CD4+ lymphocyte contents. In addition, the number of apoptotic cells also declined during therapy. These results suggest that HIV plays a direct role in producing intestinal dysfunction.

There have been rapid advances toward understanding the basic biology of human immunodeficiency virus (HIV) and its response to antiretroviral therapies, especially in the development and clinical application of combination therapies. Marked suppression of viral replication in peripheral blood can be achieved, and studies to determine the potential for viral eradication are ongoing. With effective viral suppression came the realization that CD4 lymphocyte production must be ongoing throughout the disease course, since virus-infected cells often die within a few days. The prompt rise in circulating CD4+ lymphocytes early in the treatment of many patients is related to a decrease in the death of infected cells, as opposed to an increase in cell production [1]. These important studies highlighted the need to examine viral production during disease progression in lymphoid tissue compartments other than blood. The gastrointestinal tract is the largest lymphoid organ in the body, in physical terms, and contains up to half the total pool of lymphocytes. The aim of this presentation is to review studies from my laboratory concerning the significance of HIV infection in intestinal mucosa.

Pathogen-Negative Diarrhea

Pathogen-negative diarrhea in HIV-infected persons has been reported frequently and has led to the speculation that local HIV infection is etiologic. However, in a study done between 1984 and 1987 by my laboratory, the diagnostic yield varied from 75% in patients with an established diagnosis of AIDS to 9% in HIV-infected, non-AIDS patients. Gastrointestinal symptoms represented one of the “constitutional symptoms” presaging the development of AIDS in the preantiretroviral era. In New York City in the early 1980s, such symptoms usually were ascribed to giardiasis, amebiasis, or the “gay bowel syndrome.”

Cellular Reservoirs for HIV in the Intestine

Many laboratories, using a variety of immunochemical and molecular techniques, have reported HIV infection of mucosal lymphocytes and macrophages [2–4]. Most studies localized HIV to cells in lamina propria and mucosal lymphoid follicles, while some suggested that epithelial cells may become infected [5]. Virions have also been shown to be trapped in immune complexes and adherent to follicular dendritic cells in lymphoid follicles, as in peripheral lymph nodes [6].

Studies of Mucosal Inflammation

My colleagues and I prospectively examined the relationships between mucosal HIV content and intestinal disease in patients referred for gastrointestinal evaluation [7]. Altered bowel habits in these patients correlated significantly with histopathologic changes on rectal biopsy. Symptoms also correlated with the
presence of HIV p24 antigen in mucosa but not with identifiable enteric pathogens. Histopathologic studies demonstrated lymphoid infiltration in the intermediate stage of disease (CD4 cell counts of between 200 and 500 cells/mm³). Rectal p24 antigen contents varied significantly with clinical disease stage and were highest in the intermediate stage. The number of activated T cells (CD45RO⁺) also varied during progression, and the results correlated with p24 expression. However, our patient group was evaluated because of clinical symptoms; thus, extrapolation of the results to all HIV-infected persons is precluded.

Further evidence of a relationship between HIV infection and mucosal inflammation was obtained by use of an in vitro organ culture technique that was adapted for rectal biopsies. Increases in tissue HIV p24 content of up to 10-fold greater than those in unincubated tissue were seen over 48 h, and progressive incorporation of [³⁵S]methionine into p24 was demonstrated (unpublished data). Incubation with inflammatory mediators affected tissue HIV p24 content [8, 9]. Hydrocortisone significantly inhibited p24 production, while prostaglandin E₂ and leukotriene B₄ both significantly stimulated p24 production. Divergent effects were seen in incubations with 5-aminosalicylic acid (5-ASA), an inhibitor of both lipoxygenase and cyclooxygenase, and indomethacin, a cyclooxygenase inhibitor. 5-ASA significantly inhibited and indomethacin stimulated p24 production, suggesting that the specific mediator of p24 production could be a lipoxygenase product. 5-ASA also inhibited a stimulatory effect of tumor necrosis factor (TNF) on p24. These results suggest that elements of an inflammatory response can modulate HIV protein production in rectal mucosa in vitro.

The interrelationships between HIV and cytokine expression in rectal mucosa were studied by use of RNA in situ hybridization [10]. The cytokines studied included those mediating delayed hypersensitivity (interleukin [IL]-2 and interferon-γ and -α), humoral responses (IL-4, IL-5, and IL-10), and proinflammatory cytokines (TNF-α, IL-1β, and IL-6). Both HIV RNA and cytokine mRNA expression varied during disease progression. Studies from HIV-seronegative volunteers and early cases implied predominant delayed hypersensitivity in the rectum. The pattern of cytokine mRNA expressed during the middle stage of disease indicated generalized immune activation, with expression of all classes of cytokines. The late stage of the disease, AIDS, was characterized by the presence of proinflammatory cytokines. The results of the these studies were consistent with a progressive decline in delayed hypersensitivity over time.

Mucosal CD4⁺ Lymphocyte Content

Several previous studies have suggested that lamina propria CD4⁺ lymphocytes are depleted disproportionately early during the disease course. The effect of disease stage upon mucosal lymphoid composition was examined in collaboration with Frederic Clayton and colleagues [11]. In the early patient group, the percentages and numbers of lamina propria CD4⁺ cells were markedly decreased compared with changes in the peripheral blood. In contrast, the decline in CD4⁺ lymphocytes in lymphoid follicles more closely reflected the changes in peripheral blood.

Studies of Apoptosis

Previous studies from our laboratory identified apoptosis as a common finding in mucosal biopsies from HIV-infected individuals. Apoptosis initially was identified in rectal crypt epithelial cells and was later detected in lamina propria and lymphoid follicles [7], where its detection in hematoxylin-eosin–stained slides correlated with peak expression of p24 and maximal lymphoid infiltration.

Apoptosis is the morphologic representation of programmed cell death and represents the final few minutes of a process that may take several hours to occur. Most instances of programmed cell death include activation of an endonuclease and cleavage of cellular DNA into sequences of ~200 bp or its multiples. Its presence can be detected by gel chromatography, which shows a stair pattern of DNA, with weights in multiples of ~200 bp. Cells primed to undergo apoptosis also can be identified in situ by hybridizing a reporter nucleotide to the ends of cleaved, single-stranded fragments of DNA. In preliminary studies, we showed that apoptosis was found diffusely in lamina propria in relatively similar amounts throughout the length of the intestine. We also showed that the numbers of apoptotic cells in lamina propria were elevated in the intermediate stage of disease and in late-stage patients with colonic opportunistic infections. Apoptotic cells in the lamina propria were relatively uncommon in most HIV-seronegative volunteer controls and in patients with early disease. The numbers of apoptotic cells in lymphoid aggregates also were highest in the intermediate group, although apoptosis was common in lymphoid aggregates in all groups.

Effect of Therapy

Taken together, these data suggest that there are significant associations among clinical symptoms, histopathologic alterations, mucosal inflammation, and increased rates of cell death in lamina propria. In addition, the contents of HIV RNA and proteins in blood and mucosa vary inversely at different disease stages. On the basis of these data, my colleagues and I hypothesized that clinical symptoms and intestinal injury are directly related to the presence of HIV in the mucosa and that the intestinal lamina propria could be a site of accelerated infection and destruction of CD4⁺ lymphocytes. It is not known whether the situation in the intestinal mucosa is the same or different from that in other tissue compartments. The proximity to foreign antigens is the most characteristic distinguishing feature of mucous membranes. Since bacterial lipopolysaccharides
stimulate the release of cytokines, such as TNF, which promotes HIV replication and inflammation [12], penetration of foreign antigens could affect the microenvironment within lamina propria. The process may become chronic and promote persistent recruitment of CD4+ lymphocytes from the systemic circulation and promote their activation and death.

To test this hypothesis, my colleagues and I took advantage of the sudden shift in clinical practice toward the use of combination antiretroviral therapy to rapidly suppress HIV virus burden. By examining the short-term effect of a sudden drop in HIV replication and tissue content upon clinical histopathologic and immunologic parameters, we hoped to obtain important information about disease pathogenesis [13]. The study also permitted a more basic question to be answered (i.e., whether the mucosal compartment responds to therapy in the same way as peripheral blood). In addition, the potential relationships among HIV, CD4+ lymphocytes, and apoptosis were evaluated. Rectal tissue was sampled before and after 7 days of therapy in a prospective, open-label study of subjects initiating or changing antiviral therapy because of a clinical indication, including decreasing CD4+ lymphocyte count, high plasma virus burden, constitutional symptoms, or a combination of these indications. Measurements included lymphocyte subset analysis, HIV RNA in plasma and tissue by reverse transcription–polymerase chain reaction, HIV p24 antigen content by ELISA, apoptosis by in situ end-labeling, and immunohistochemistry.

Therapy was associated with detectable improvement in symptoms, as determined by use of a standardized questionnaire. Peripheral blood studies showed a >1-log drop in virus burden and a rise in CD4+ lymphocyte counts, findings that are roughly comparable to results in other combination antiretroviral studies. Similar decreases in virus burden in rectal mucosa and rises in lamina propria CD4+ lymphocyte counts were found. In addition, the number of apoptotic cells also declined during the week of therapy. These results, if sustained in a larger trial, would lend support to the contention that HIV plays a direct role in promoting the intestinal alterations seen in the disease as well as the hypothesis that the intestine is a site of lymphocyte destruction.

Conclusion

Further studies are required to define the precise mechanism for HIV-associated intestinal injury and its relationship to HIV replication. Evidence from this study and others [14] suggests that bystander cells rather than HIV-infected cells are undergoing apoptosis. Protection of virus-infected cells against destruction has been reported for other viruses, such as adenovirus [15]. If this is the case for HIV, specific therapies directed at the apoptotic process may have potential benefit in some patients. Other evidence in support of cytokine activation as an important component of disease pathogenesis could lead to increased interest in the therapeutic potential of anticytokine therapies in the treatment of HIV infection. If eradication of HIV infection is a true possibility, then examination of tissue compartments, such as the intestinal mucosa, will be necessary to fully understand the kinetics of HIV replication and CD4+ lymphocyte production and turnover.

References