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The initial goals of this project are to collect autopsy tissues from HIV-positive asymptomatic drug addicts who die unexpectedly from drug overdoses. A wide sampling of autopsy tissue will be subjected to various techniques in identifying and localizing HIV-1. The viral burden, cellular sites of replication and progression of viral disease will be studied.

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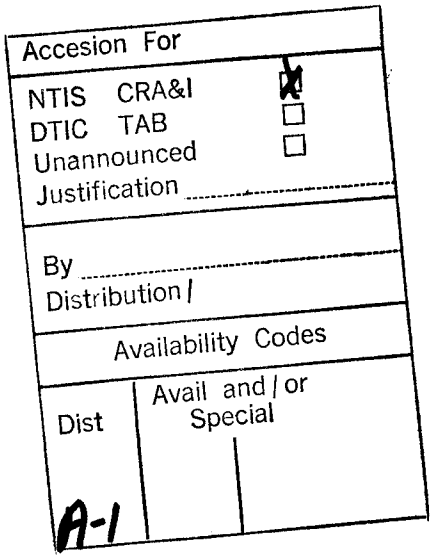

Allen Berube 15 Dec 94

Principal Investigator's Signature Date

INTRODUCTION

Nature of Problem. The long-term goal of this work is to establish a laboratory capable of detecting HIV-1 in tissue samples. Cellular localization as well as (semi) quantitation are parts of this goal. These techniques may be useful in staging disease and monitoring therapy. The tissues studied in this proposal will be exclusively from patients with early (i.e., non-symptomatic) HIV disease and will exclude AIDS patents.

Background of previous work. There have been several studies that have identified HIV-1 in tissues by several techniques. Immunohistochemistry and in situ hybridization have been shown to be successful in demonstrating HIV RNA in lymph nodes and central nervous system tissues. The polymerase chain reaction (PCR) has also demonstrated HIV-1 in autopsy as well as surgical tissues from patients with HIV disease. Most of these studies have been performed on tissues from patients with symptomatic HIV disease.

Purpose of present work. The initial goals of this grant project are to collect autopsy tissues from HIV-positive asymptomatic drug addicts who die unexpectedly from drug overdoses. A wide sampling of autopsy tissues will be subjected to various techniques in identifying and localizing HIV-1. The viral burden, cellular sites of replication and progression of viral disease will be studied.

Methods of approach. Three technologies will be employed: immunohistochemistry, in situ hybridization, and polymerase chain reaction. The first two techniques demonstrate viral antigens and nucleic acids, respectively, and provide information on cellular localization. PCR provides a sensitive technique for viral DNA detection and can be modified for semi-quantification of viral burden. In this study year, emphasis was placed on in situ hybridization for the localization of HIV-1 RNA in the gastrointestinal tract.

Case Selection. Sera from cases of unexpected death among intravenous drug abusers in the state of Maryland were screened for the presence of HIV-1 antibodies with a rapid ELISA kit (Genetic Systems Corporation, Redmond WA) and confirmed by Western blot testing performed at the Department of Health and Mental Hygiene, State of Maryland. The gastrointestinal tracts of 26 consecutive autopsies were studied. Only individuals without a history of HIV-1 disease or seropositivity were considered for study.

In situ hybridization. The protocol of Cecil Fox was followed with minor modifications for the detection of viral RNA (1). Positive control sections included lymph nodes with known viral RNA (7) and cell suspensions as previously described (1,3). Negative control probes were used as previously published (1,3) and consistent of sense probes; each slide was run in parallel using sense and anti-sense probes.

Immunohistochemistry. The avidin-biotin complex method was applied to deparaffinized sections to tissues from all cases. The following antibodies (dilutions in parentheses) were purchased from Dako Corporation (Indianapolis, IN): CD35 (1:20), CD21 (1:400), p24 (1:200). For CD35 and p24, tissue sections were predigested with protease K at 37C (0.1 mg/ml) for 20 minutes (Sigma Chemical Co., St. Louis, MO).

Histologic assessment of tissues. In each case, representative sections of gastric body, fundus, and antrum; distal esophagus; and proximal, mid and distal rectum were fixed in 10% buffered formalin for not more than 12 hours. The number of lymphoid aggregates within the mucosa, or at the mucosal-submucosal interface was counted in each section. Those lymphoid aggregates that contained a follicular dendritic cell network, as evidenced by positive germinal center staining for CD21 and CD35, were tabulated separately. The density of lymphoid aggregates (LA) and germinal centers (GC) within the mucosa and submucosa was assessed by

relating the number of each to the length of mucosa studied (cm).

RESULTS

Patient data. 17 of the autopsy cases were seronegative (group 1), and nine were seropositive (group 2). The mean age of individuals in group 1 was 37.2 +/- 2.9 years, and the mean age of individuals in group 2 was 35.0 +/- 7.1 years. 5/17 drug addicts in group 1 were female, compared to 3/9 addicts in group 2. Complete autopsies in all cases revealed no evidence of opportunistic infections, and the cause of death in each case was classified as undetermined (narcotic intoxication).

Gastric histology. Mucosal and submucosal lymphoid nodules were present in every group 2 stomach, and 15/17 group 1 stomachs. Germinal centers indicative of follicular gastritis were present in 11/17 group 1 stomachs and every group 2 stomach. The mean density of lymphoid nodules was 1.01 +/- 0.29 for group 1, and 3.27 +/- 0.63 for group 2 ($p = 0.002$). The mean density of germinal centers was 0.45 +/- 0.20 for group 1 and 1.2 +/-0 0.6 for group 2 ($p = 0.05$).

Rectal histology. Mucosal and submucosal lymphoid nodules were present in every rectum of either group. Germinal centers were present in 11/17 group 1 rectums and every group 2 rectum. The mean density of lymphoid nodules was 1.1 +/- 0.3 for group 1, and 2.3 +/- 0.46 for group 2 ($p = 0.05$). The mean density of germinal centers was 0.58 +/- 0.26 for group 1 and 1.0 +/- 0.27 for group 2 ($p = 0.25$).

Esophageal histology. Mucosal and submucosal lymphoid nodules were present in all esophagi from group 2 and 15/17 esophagi from group 1. Germinal centers were present in 9/17 group 1 esophagi and every group 2 esophagus. The mean density of lymphoid nodules was 1.1 +/- 0.4 for group 1, and 2.2 +/- 0.6 for group 2 ($p = 0.16$). The mean density of germinal centers was 0.76 +/- 0.34 for group 1 and 1.25 +/- 0.55 for group 2 ($p = 0.44$).

In situ hybridization. No signal was noted in any seronegative case (17/17) in any of the three organs studied. In three of the nine seropositive cases, positive signal, consisting of marked increased density of silver grains, was noted over lymphoid aggregates and germinal centers. The distribution was similar to that previously reported in lymph nodes (1). In two of the three cases, signal was present in esophagus, stomach, and rectum; in one case, signal was present in stomach and rectum only.

DISCUSSION

This study complements our previous findings that, in early HIV-1 infection, there is diffuse hyperplasia of the lymphoid system consisting primarily of follicular hyperplasia and expansion of the B-cell areas (2). We have shown that the distribution of viral RNA and p24 antigen is predominantly within germinal centers in a distribution of the follicular dendritic cell network (1). HIV-1 RNA is present in virtually all lymph nodes in early stage disease and DNA viral burden is quite high, approximating 1/10 cellular DNA levels within lymph nodes showing characteristic follicular alterations (4). Not only lymph nodes, but thymus glands show typical histologic alterations with RNA localized to germinal centers (3).

Our recent studies show that there is also diffuse follicular hyperplasia in the esophagus, stomach, and rectums of drug addicts who are seropositive and who show no signs of AIDS. Compared to non-infected controls, the density of lymphoid aggregates and germinal centers within the mucosa of HIV-1 infected drug addicts was greater for all three organs; the difference was statistically significant for gastric lymphoid nodules and germinal centers, as well as rectal lymphoid nodules.

By in situ hybridization, HIV-1 RNA was found to be present within the lymphoid nodules and germinal centers of only 3 of 9 seropositive cases. This result is in contrast to our findings in lymph nodes in a similar population, which demonstrate HIV-1 viral RNA within follicle centers in over 90% of cases (4). The reason for this discrepancy is unclear. It is possible that the majority of lymphoid hyperplasia in the gut in asymptomatic patients infected with HIV-1 is not due to direct viral infection. Alternatively, there may be selective autolysis within the mucosa of the gut which exceeds that of lymph nodes; this autolysis may result in the degradation of RNA. In support of the latter hypothesis, qualitative histologic assessment of gut mucosa indicates that autolytic changes are significantly greater than those of lymph nodes. Because of the small numbers of individuals studied, it is further possible that a rate of 3/9 positivity wit in situ hybridization is not representative of the population as a whole. To attempt to verify one or more of these hypotheses, future studies will address expanding the total number of cases studied, as well as probing for control RNA (e.g. alpha actin) within the tissues to assess the degree of RNA degradation secondary to post-mortem autolysis.

Conclusions. Asymptomatic infection with HIV-1 in intravenous drug addicts is characterized by a generalized mild lymphadenopathy and hyperplasia of the B-cell areas of lymph nodes, mucosa-associated lymphoid tissue, and the thymus. Viral RNA burden is concentrated in lymphoid follicles in these tissues. Because of the diffuse nature of lymphoid proliferation as well as viral RNA accumulation within these tissues, the viral burden in early HIV-1 disease appears to be staggeringly high.

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TABLE

Histologic findings, esophagus:

	Seropositive	Seronegative
Lymphoid aggregates/cm	2.0±0.6	1.1±0.4
Germinal centers/cm	1.25±0.6	0.76±0.3

Histologic findings, stomach:

	Seropositive	Seronegative	p value
Lymphoid aggregates/cm	3.3±0.6	1.0±0.3	0.01
Germinal centers/cm	1.2±0.3	0.5±.2	0.01

Histologic findings, rectum

	Seropositive	Seronegative
Lymphoid aggregates/cm	2.3±0.46	1.1±.3
Germinal centers/cm	1.0±.27	0.58±0.3