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Spontaneous Lymphocyte Proliferation Is Elevated in Asymptomatic HTLV-I–Positive Jamaicans

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INTRODUCTION

The normal immune response involves a delicately balanced interplay of regulatory and effector cells. Included in this repertoire is the controlled expansion or proliferation of subsets of T and B lymphocytes as part of the immune response to foreign antigens, such as viruses. Recently, Jacobson and colleagues reported elevated spontaneous proliferation of lymphocytes in patients with HTLV-I–associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) who were infected with human T-cell leukemia virus (HTLV-I) (1). However, this study did not address the issue of whether this phenomenon was associated with disease (HAM/TSP) or exposure (HTLV-I). The current study was undertaken to characterize spontaneous lymphocyte proliferation in HTLV-I–infected normal persons free of neurologic disease and to ascertain whether other factors (e.g., demographic, socioeconomic, etc.) influence this phenomenon.

METHODS

Subject Selection

Whole blood samples were obtained from four groups of subjects. All the Jamaican subjects were enrolled in epidemiologic studies of HTLV-I sponsored by the National Cancer Institute in collaboration with the University of the West Indies in Kingston, Jamaica, and the Jamaican Ministry of Health. These studies

had been approved by the protocol review committees of both institutions, and all subjects gave informed written consent. The American control subjects were blood donors at the National Institutes of Health Blood Bank whose blood samples were designated for research purposes. The four experimental groups were

Group 1. Healthy Jamaican HTLV-I–seronegative controls. The 15 subjects in this group were randomly chosen from a nationwide serologic survey of HTLV-I antibody in Jamaica in 1985–1986. Healthy individuals were enrolled when they applied for licenses required for employment in food-handling occupations. In collaboration with the Jamaican Ministry of Health, blood samples obtained for syphilis screening were separated into aliquots for the HTLV-I study. A questionnaire concerning demographic and life-style factors and medical history and a screening physical examination were administered by trained personnel. Median age of subjects was 40; four were male; all were black.

Group 2. Healthy Jamaican HTLV-I seropositives. Fifteen healthy subjects from the same survey who tested positive for HTLV-I antibodies were randomly included in this group. Median age was 41; four were male; all were black.

Group 3. Patients with adult T-cell leukemia/lymphoma (ATL). Twelve patients with ATL were enrolled in a case-control study of all hematologic malignancies diagnosed at the University Hospital of the West Indies from 1984 through 1986 (2). All 12 had a clinicopathologic diagnosis of diffuse lymphoma, T-lymphocyte phenotype of the malignant cells, and antibodies to HTLV-I. Skin infiltration and hypercalcemia were common clinical findings in this group. Median age was 40; three were male; all were black.

Group 4. Healthy American controls. Thirteen healthy blood donors from the National Institutes of Health Blood Bank served as controls. All were seronegative for HTLV-I and HIV-1. Median age was 40; three were male; all were white.

Virologic Studies

Sera were tested for the presence of antibodies to HTLV-I with a research enzyme-linked immunosorbent assay (ELISA) that used purified, disrupted whole virus particles as antigen (DuPont Corp., Wilmington, DE). All positive samples were confirmed with a Western blot assay that used HUT102 cells as the source of antigen (Biotech Laboratories, Rockville, MD). The minimum criteria for a positive Western blot consisted of the presence of bands specific to the HTLV-I *gag* gene proteins p19 and p24 (3). Additionally, an envelope ELISA (Cambridge Bioscience, Cambridge, MA) was performed for confirmation, thus satisfying current Food and Drug Administration criteria that antibodies to two gene groups are detected in positive samples.

Immunologic Studies

Lymphoproliferative Response

Peripheral blood lymphocytes (PBL) were isolated by standard methods and viably frozen in vapor-phase liquid nitrogen until use. Cryopreserved PBL were thawed and plated in triplicate at a concentration of 3×10^5 cells per well in 96-well plates (Nunclon, Roskilde, Denmark). Media consisted of RPMI (GIBCO, Grand Island, NY) supplemented with 2% human AB serum. After 4, 5, and 6 d, wells were pulsed with 1 μCi of ^{3}H-thymidine for 4 h and harvested on a Scatron harvesting system (Skatron Inc., Sterling, VA) and counted per minute (CPM) in a liquid scintillation counter.

Statistical Methods

Spontaneous lymphoproliferation counts were log-transformed for analysis. Student's *t* test was used for comparing means. To evaluate risk factors for high lymphoproliferation among the 30 healthy Jamaicans, odds ratios (OR) and the appropriate 95% confidence intervals (CI) were calculated for a variety of demographic and life-style factors, including medical history. Logistic regression was carried out to determine the separate effects of HTLV-I serostatus and income on spontaneous lymphoproliferation. For these purposes, variables were dichotomized at their median.

RESULTS

In vitro spontaneous lymphocyte proliferation (in the absence of exogenous antigens) at days four and five was highly correlated with proliferation at day six ($r = 0.75$, $p = 0.0001$; and $r = 0.90$, $p = 0.0001$, respectively). The findings at day 6 are shown in Fig. 1. Mean proliferation rate (±standard error) was 62 852 (±12 569) CPM in HTLV-I–seropositive Jamaicans compared with 18 483 (±4371) CPM in HTLV-I–seronegative Jamaican controls, 12 629 (±2860) CPM in HTLV-I–seropositive ATL, and 2345 (±436) CPM in HTLV-I–seronegative American controls. Comparisons of mean responses between the various groups showed three significant differences: 1. HTLV-I–seropositive versus HTLV-I–seronegative Jamaicans ($p = 0.003$), 2. HTLV-I–seropositive Jamaicans versus HTLV-I–seropositive ATL ($p = 0.0004$), and 3. HTLV-I–seronegative Jamaicans versus American controls ($p = 0.0002$). There was no significant difference between rates of HTLV-I–seronegative Jamaican controls and HTLV-I–seropositive ATL ($p = 0.7$). There was no correlation between proliferation rate and white blood cell count or lymphocyte count for any of the four groups.

Additionally, among the 30 healthy seropositive and seronegative Jamaicans,

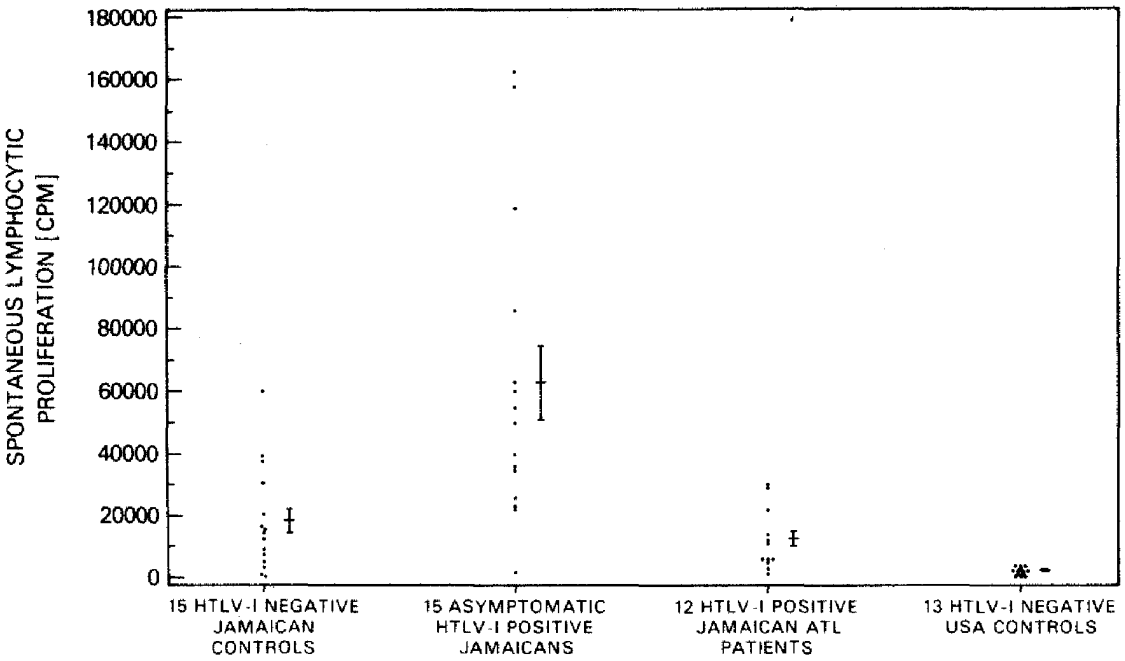

FIG. 1. Spontaneous lymphocytic proliferation rates (counts per minute [CPM] of ^{3}H-thymidine intake of lymphocytes at day 6) in the four study groups; 15 HTLV-I–seronegative Jamaicans, 15 asymptomatic HTLV-I–seropositive Jamaicans, 12 HTLV-I–seropositive Jamaican patients with ATL, and 13 HTLV-I–seronegative American controls. Vertical bars indicate mean values ± SEM. HAM/TSP patients (7) had a mean lymphocytic proliferation rate of 57 927 (±9916) which thus was similar to a rate of 62 852 (±12 569) of asymptomatic HTLV-I carriers in this study. Similar values for the controls in the two studies assure comparability of the results.

the relationship between lymphocyte proliferation and a number of demographic features (age, sex, and socioeconomic status) was explored. Of these, only low income was associated with high lymphoproliferation. However, logistic regression analysis revealed that HTLV-I seropositivity was a stronger risk factor for high lymphoproliferation (OR = 17.2, 95% CI 1.8, 164.7) than was low income (OR = 8.5, 95% CI 0.9, 81.6).

DISCUSSION

The clinical outcomes of HTLV-I infection include a type of T-lymphoproliferative malignancy (ATL) and a chronic degenerative neurologic disease (HAM/TSP). Clinical immunodeficiency associated with HTLV-I has been anecdotally reported and myriad in vitro immunologic perturbations observed (4).

Jacobson et al. have recently reported that patients with HAM/TSP have an array of immunologically abnormal cells, particularly increased numbers of activated T lymphocytes in fresh peripheral blood as measured by increased proportion of DR and Tac positive cells (1). Similarly, Yasuda et al. (5) and Usuku et al. (6) observed activated T cells in fresh isolates and cultured PBL. In addition

to these perturbations in T-cell subsets, Jacobson also reported elevated spontaneous proliferation of T lymphocytes. The current study was undertaken to evaluate in vitro lymphocyte proliferation in healthy HTLV-I–positive individuals from Jamaica.

Spontaneous lymphoproliferation in asymptomatic HTLV-I–positive Jamaicans (Fig. 1) was significantly higher than among HTLV-I–negative Jamaicans. This finding was not correlated with total white blood cell or lymphocyte counts, arguing that this association was not an artifact of an overabundance of dividing cells. Rather these data suggest that elevated spontaneous lymphoproliferation may be associated with HTLV-I exposure per se. Because the rate of proliferation was indistinguishable in HTLV-I positives and HAM/TSP patients (7), a possible mechanism that may also provide insight into the pathogenesis of HAM/TSP is provided by Sonoda and colleagues (6). In their in vitro studies, exogenous HTLV-I antigens were added to resting PBL and induced proliferation to HTLV-I antigen was measured. They reported that HAM/TSP patients had a high lymphocyte proliferative response to added virus, whereas those with ATL were low responders. Family members who shared certain HLA-haplotypes with HAM/TSP or ATL patients showed parallel responses, i.e., those with the HAM/TSP HLA-haplotype were high responders, whereas those with the ATL HLA-haplotype were low responders. Perhaps the presence of virus infection in our study subjects results in viral antigen expression in vitro which stimulates cells to spontaneous proliferation. Whatever the mechanism, such a perturbation could lead to altered cell growth as well as immunologic dysregulation, with obvious implications for HTLV-I disease pathogenesis.

The low spontaneous lymphocytic proliferation in HTLV-I–positive ATL likely reflects an overabundance of HTLV-I–positive tumor cells that have lost their capacity for proliferation. The frequent finding of opportunistic infections in ATL supports the concept that ATL patients have an altered immune response.

Recent reports have suggested that persons co-infected with HTLV-I and HIV-1 may progress to AIDS at an accelerated rate (8–10). HTLV-I–induced spontaneous proliferation of blood lymphocytes may provide a mechanism to explain this. Specifically, for HIV-1 to kill a T lymphocyte, current evidence suggests that that cell must undergo cell division, which promotes viral gene expression, an overproduction of viral particles, and cell death. HTLV-I may act as a T-4–cell mitogen that stimulates cell division, both accelerating cell death as well as creating activated T cells, which are suitable targets for HIV-1 infection.

Of the two HTLV-I–uninfected groups, HTLV-I–negative Jamaicans had significantly higher lymphoproliferation compared with American controls. The reason for this finding is unclear. Because all Jamaicans were black and all American controls were white, genetic factors may account for this difference (11). However, it is also possible that spontaneous proliferation is influenced by the level of acute and chronic exposure to a variety of different antigens and pathogens. Multiple infections are more common in the tropical country of Jamaica, which has a significantly lower per capita income and standard of living than

the United States. Because low income may be a surrogate marker for antigenic stimulation by various pathogens, it is interesting that in this study low income was an additional risk factor for high lymphoproliferation.

We conclude that 1. HTLV-I infection is the main source for increased spontaneous lymphoproliferation, a functional abnormality of T lymphocytes occurring in the absence of exogenous immune stimulators; and 2. environmental factors, such as other antigens (parasites, viruses, dietary components, and others) may modify this effect. Additional prospective data are needed to further clarify the significance of spontaneous lymphocytic proliferation in the natural history of HTLV-I infection and disease.

SUMMARY

In vitro elevated spontaneous lymphocytic proliferation (in the absence of exogenous antigens), first reported in patients with HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP), was measured in asymptomatic HTLV-I–seropositive and –negative Jamaicans. Mean spontaneous lymphocytic proliferation rate was 62 852 [±12 569 SEM] CPM in 15 seropositives compared with 18 483 [±4371] CPM in 15 seronegatives ($p = 0.003$). Among 12 HTLV-I–seropositive Jamaican patients with ATL, the counts were 12 629 (±2860) CPM, thus significantly lower than asymptomatic Jamaican HTLV-I seropositives ($p = 0.0004$), but similar to seronegative Jamaican controls ($p = 0.7$). Thirteen HTLV-I–seronegative American controls had a mean proliferation value of 2345 (±436) CPM, significantly lower compared with Jamaican seronegative controls ($p = 0.0002$). Elevated spontaneous lymphocytic proliferation in asymptomatic Jamaican seropositives appears to be due to HTLV-I infection per se, whereas the absence in seropositive ATL may reflect overproduction of functionally deficient tumor cells. The higher rate in seronegative Jamaicans compared with American controls may reflect racial or environmental exposure differences, because markers of lower social class in Jamaica were moderately associated with increased proliferation.

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