Epstein-Barr Virus Infections in Hairy Cell Leukemia Patients in the Presence of Complement-dependent Neutralizing Antibody

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ABSTRACT

Immune system status was characterized in patients with hairy cell leukemia (HCL) with respect to explaining their chronic or recurrent infections with Epstein-Barr virus. Measures of cellular immune responsiveness for a group of 11 HCL patients were, in general, decreased when expressed as the proportion of tested patients with values less than 2 S.D. below mean values for a group of 17 healthy adults: T-cell enumeration, seven of 13; mitogen responsiveness of phytohemagglutinin, 10 of 11; concanavalin A, 10 of 11; pokeweed mitogen, 10 of 11; B-cell responsiveness to anti-immunoglobulin immunobead stimulation, two of six; responsiveness to streptolysin O antigen, four of seven; mixed-lymphocyte reaction, six of seven; natural killer cell activity, six of eight. Specific immunity to Epstein-Barr virus was measured by complement-independent, antibody-mediated virus neutralization (mean index for HCL patients being 56% of control value) and complement-dependent virus neutralization (98% of control value). We concluded that, in spite of depressed levels of immune responses measured with general, cellular assays, functional levels of complement-dependent virus-neutralizing antibody were present in these HCL patients.

INTRODUCTION

Many patients with HCL appear to be infected with EBV chronically or recurrently. Such infections are reflected in their elevated antibody titers to EA³ and VCA (30), precipitating antibodies to EBV antigens (3), the establishment of EBNA-positive, spontaneously transformed lymphoblastoid cell lines (25, 28), and rescue of EBV by cocultivation of their peripheral blood mononuclear cells with cord blood lymphocytes (28). Those patients' EBV-receptor-positive, B-lineage, leukemic cells could be infected with EBV in vitro, as indicated by low levels of EBNA induction in enriched leukemic cell populations upon infection with EBV in vitro (27). However, a latent EBV infection of leukemic cells in vivo could not be demonstrated by sensitive Southern hybridization assays of leukemic cells for the EBV genome BamHI reiterated W region (27). Other B-lineage leukemic cells can be "transformed" in vitro to EBNA*, EBV genome* lines expressing the idiotype or chromosomal abnormality of the leukemic cell (7, 13, 16). Similar studies with HCL (28) led to EBNA* lymphoblastoid cell lines which expressed abundant levels of p35 (a marker for a subset of HCL patients [32]) and tartrate-resistant acid phosphatase (a histochemical marker for HCL [37]). However, those lines did not express the B-lym transforming gene function of hairy leukemic cells and, thus, were considered to arise from B-lymphocytes of the HCL patients (26). It is of interest, therefore, to explain why these HCL patients have chronic or recurrent EBV infections, but do not demonstrate EBV* hairy leukemic cells or cell lines in vivo or in vitro. Such explanations could be related to their mechanisms of EBV-specific immunity in the face of apparent general immunodeficiency or to restrictions upon infection and "transformation" of their leukemic cells. Toward these goals, we have assessed the general status of the cellular immune system in these patients and, more specifically, their levels of complement-independent and complement-dependent EBV-neutralizing antibodies. We have found that, in spite of depressed general measures of cell-mediated immunity and complement-dependent EBV-neutralizing antibodies, these patients had functional levels of complement-dependent virus-neutralizing antibody and elevated anti-EA and anti-VCA titers.

MATERIALS AND METHODS

Leukemic Cells. Peripheral blood samples were obtained from HCL patients whose diagnoses were established on clinical and pathological grounds (as discussed previously in Refs. 32 and 33). These patients have been identified with one series of unique numbers in our reports (3, 25–29, 32–34).

Cellular Immune Functions. T-cells were enumerated by rosette formation with sheep erythrocytes (15). Proliferative responses were assayed to PHA (0.1 to 2.0 µg/culture; Burroughs Wellcome, Research Triangle Park, NC), Con A (0.1 to 3.0 µg/culture; Calbiochem-Behring Corp., San Diego, CA), PWM (0.1 to 10 µg/culture; Grand Island Biological Co., Laboratories, Grand Island, NY), anti-human F(ab')² of IgG-coated polystyrene beads [the gift of Dr. David Parker (8)], streptolysin O antigen (1:2 dilution; Difco, Detroit, MI), and to allogeneic human B-lymphoblastoid cell line 8392. These responses were measured by tritiated thymidine incorporation for a 16-hr period after 3 or 5 days of stimulation (22). Natural killing to the K562 human myeloid cell line was measured in a 4-hr radiochromium-release, cytotoxicity assay (1). Results were presented as means of triplicate proliferative or killing assays with subtraction of appropriate control values. Geometric means, S.D.s, and 95% confidence intervals were calculated as described previously (35).

Antibody Titers to EBV-related Antigens. Indirect immunofluorescence tests for anti-EA and anti-VCA were performed (9, 12, 30). Antibody-mediated virus neutralization measured abrogation of virus-induced EA expression (29). Briefly, a mixture of 0.2 ml of virus suspension and 0.2 ml of diluted, heat-inactivated serum was incubated overnight at 4°. Raji cells (0.1 ml; 10⁶ cells/ml) were then exposed for 90 min

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RESULTS

Proliferative Immune Responses. General measures of cell-mediated immunity in these patients were decreased (Table 1). Proliferative responses to lectin mitogens (PHA, Con A, PWM) were significantly decreased in most patients. The fractions of patients below the lower 95% confidence interval (2 S.D.s below the mean value for a group of 17 healthy adults) were: PHA, 10 of 11 (91%); Con A, 10 of 11 (91%); and PWM, 10 of 11 (91%), respectively. The B-cell proliferative response to anti-immunoglobulin-coated beads was depressed in 2 of 6 patients (33%) relative to the lower 95% confidence interval for similar determinations with healthy individuals. Proliferative responses to a recall antigen (streptolysin O) were decreased relative to controls [4 of 7 (57%) being less than the lower 95% confidence interval]. Mixed-lymphocyte culture responses to allogeneic antigens presented on the B-lymphoblastoid cell line 8392 were also depressed relative to those of healthy controls [5 of 7 (71%) being below the lower 95% confidence interval]. In part, these reductions probably represented lessened T-cell numbers in these patients [7 of 13 (54%) being less than the lower 95% confidence interval]. Natural killing in 2 patients (Patients 12 and 22) was within the normal range, but values obtained with the other 6 of 8 patients were depressed below the range of normal values.

Serological Responses to EBV Antigens. The immune responses to EA and VCA in these patients were elevated (30). The geometric mean titer of these patients' anti-EA response was 1:60 (compared to no titer, less than 1:10, for seropositive, healthy controls) (Table 1). An IgA anti-VCA response was noted in 2 patients (Patients 2 and 24), while seropositive healthy controls did not demonstrate significant IgA anti-VCA responses at all. The IgG anti-VCA response was elevated with a geometric mean titer of 1:960 (compared to seropositive, healthy controls, 1:80 (30)).

DISCUSSION

Patients with HCL appear to have chronic or recurrent EBV infections as reflected in rescue of infectious virus from their blood or spleens and in elevated anti-VCA and -EA titers (27, 30). Such elevated titers to EA, in particular the appearance of IgA antibodies to EA, reflect concurrent or recent immune response to EBV antigens (10). In studying the nature of this infection and the response to it, we have assessed the level of the patients' cellular immune responses generally and, in particular, their serological defense to EBV in the form of virus-neutralizing antibody. These patients had generally depressed levels of cellular immune response mechanisms, as demonstrated in decreased...
confidence limits (±2 S.D.) for T-cell percentage, proliferative responses, and natural killing.

values of proliferative response: to 3 lectin mitogens; to one recall antigen, streptolysin O; to allogeneic lymphocyte activating determinants; and to polyclonal B-cell stimulation. In addition, natural killing was low in most tested patients. These observations of depressed general measures of immune responsiveness in HCL patients parallel the findings of Hersh et al. (11), who demonstrated low lymphocyte-blastogenic responses to PHA, Con A, and PWM, and virtually absent antibody-dependent cellular cytotoxicity to human erythrocytes. Our studies extend their findings to analysis of natural killing activity, B-cell response to anti-immunoglobulin stimulation, and specific EBV responses. These quantitative measurements of immunodeficiency in HCL patients reflect previous finding of increased infection rates in patients with HCL (2, 19).

Since one can infer from the lowered proliferative responses to mitogenic stimulation and lowered natural killing levels that cellular immunity to EBV is probably depressed, permitting reactivation of the virus in these HCL patients, a primary objective of our study was the assessment of the serological defense against infectious virus in the form of virus-neutralizing antibody which we have described recently in analysis of anti-EBV defenses in patients with infectious mononucleosis (29). While complement-independent virus neutralization was depressed relative to that of EBV-seropositive healthy control subjects, complement-dependent neutralizing antibody was not similarly depressed. One can suggest that neutralization indices greater than 0.9 at 1:10 serum dilution would offer clinically significant protection against recurrent viremia in these patients. The effect of such antibodies against cell-to-cell spread of virus would be less certain. Thus, while decreased cellular responses to EBV might have permitted EBV reactivation and expression of EBV antigens which boosted
an immune response to these antigens, as reflected in anti-VCA and anti-EA titers, spread of the virus itself still appears to be subject to effective multiple restrictions.

Others have addressed the same general question which we pose: what determines the variable outcome of EBV infection in immunosuppressed patients, leading either to EBV-triggered, lymphoproliferative disease, or to chronic or recurrent EBV infection (4, 5, 14, 17, 18, 20, 21, 31, 36)? EBV can be associated with the development of lymphoid cancers in patients who are immunosuppressed for renal transplantation (4, 5), or who have the X-linked, lymphoproliferative syndrome (24). However, in other relatively immunodeficient patients with Hodgkin’s disease or non-Hodgkin’s lymphoma, only chronic or recurrent EBV infections occur with high VCA titers being inversely related to the overall level of general immune responses (14, 17, 18, 20, 21). Some hemophiliacs and renal transplant recipients also show evidence for primary and reactivated EBV infections associated with impaired cellular immunity (6, 31). In these various groups of patients, it is not clear how much the original immunodeficiency contributed to the potential for EBV reactivation, and how much immunodeficiency (secondary to EBV infection) has contributed to the maintenance of chronic EBV infection (6, 35).

Our study of HCL patients extends the type of observation of chronic or recurrent infection without the development of EBV-triggered cancer, to yet another group of patients. A general hypothesis put forth in the interpretation of such studies is that EBV, an ubiquitous, potentially oncogenic virus, is checked by polymorphic immune effector mechanisms, and only a breakdown in most or all of these mechanisms is associated with the outgrowth of malignant, transformed clones of cells (20, 21). A series of effector mechanisms does appear to be relevant to controlling EBV infection or the course of EBV+ cancers, as reviewed by others (10, 20, 21, 36). In this study, we have documented a general immunodeficiency of HCL patients who do not have EBV+, second cancers. We have also demonstrated another anti-EBV defense mechanism which is mediated by complement-dependent virus-neutralizing antibodies. These antibodies appear to be present at clinically effective levels and might significantly impede the hematogenous spread of virus.

REFERENCES

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