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Host Responses to Epstein-Barr Virus and Cytomegalovirus Infection in Leprosy

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A study was undertaken in patients with leprosy to assess the contribution of cell-mediated immunity to the host response to Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infection. Sixteen of 72 patients (22%) with lepromatous leprosy, with impaired cellular immunity, had anti-EBV titers of 1,640 or higher. Only 4 of 49 patients (8%) with tuberculoid leprosy, with intact cell-mediated immunity, attained the level of 1:640. The anti-EBV antibody titers were significantly higher in patients with lepromatous leprosy ($P \simeq 0.025$). No significant differences were found in the level of anti-CMV antibody titers in patients with the two types of leprosy. The presence of high anti-EBV antibody titers in lepromatous leprosy suggests that cell-mediated immunity is a significant factor in host response to EBV infection. Host immune responses should be taken into consideration when assignment of an etiological role to EBV is based upon seroepidemiological data.

Epstein-Barr virus (EBV; herpes-like virus, HLV), first detected in lymphoid cell cultures derived from Burkitt's lymphoma (6), has been suggested, mainly on seroepidemiological data, as the causative agent of diseases such as Burkitt's lymphoma (13), infectious mononucleosis (14, 21), and carcinoma of the posterior nasal space (22). Nevertheless, the ubiquity of the virus in the general population (8, 17) and the frequency of high antibody titers in patients with other diseases such as lupus erythematosus (7) and sarcoidosis (16) render the pathogenic nature of this agent uncertain (10).

Recently, cell-mediated immunity has been suggested to play a role in host reactions to EBV infection (10, 16, 23). A postulated role for cell-mediated immunity is the restriction of viral parasitism. Impairment of cellular immunity has been thought to result in increases in humoral antibody production (2, 28).

To further investigate the role of cellular immunity in host reactions to EBV infection, as well as to infection with other latent viral agents ubiquitous into the general population, we have studied the level of antibody titers against EBV and cytomegalovirus (CMV) in patients with leprosy. Leprosy, caused by *Mycobacterium leprae*, has a wide spectrum of clinicopathological presentations with two polar types: lepromatous type associated with impaired delayed hypersensitivity and the tuberculoid form usually associated with intact cutaneous reactivity (2, 4). (Presented in part at the 12th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, N.J., 26-29 Sept. 1972.)

MATERIALS AND METHODS

The sera of 122 patients with leprosy diagnosed in the hospital for infectious diseases "Aghia Barbara" Athens, Greece, were studied. The patients were classified according to the Ridley-Jopling scale (25, 26) modified by Ridley and Waters (27). Only patients with LL (extreme lepromatous) and TT (extreme tuberculoid) were included in the study because lepromin was not consistently available for immunological evaluation of the patients at the time of the serum sample collection. The LL and TT are the only immunologically stable points on the spectrum of the disease (31). The lepromatous group consisted of 72 patients, 32 females and 40 males, ranging in age from 12 to 78 years and the tuberculoid group contained 50 patients, 27 females and 23 males, ranging in age from 17 to 68 years. All the patients were under treatment with sulfones. The dose of various drugs was on the average similar in the lepromatous and tuberculoid groups. All patients with tuberculoid leprosy and most of the patients with lepromatous leprosy were confined in a residential area in Athens, Greece. Patients with severe lepromatous leprosy resided in the hospital located in the same residential area.

Approximately 10 ml of whole blood was collected from each patient, allowed to clot, and was centrifuged at 250 × *g* for 1 h. The serum was re-

moved and maintained at −20 C until use. The sera were tested for (i) anti-viral capsid antigen of the EBV by indirect immunofluorescent technique, as previously described, with EBV antigen present in the Jijoye cell line derived from a patient with African Burkitt's lymphoma (15) and (ii) CMV complement-fixing (CF) antibody by the microtiter CF technique (29). CMV CF antigen (AD 169 strain) was obtained from Microbiological Associates, Bethesda, Md. Samples of the same specimens were tested for the immunoglobulin G (IgG) concentration by the quantitative radial immunodiffusion method of Mancini (18) with plates from Meloy, Inc. Normal adult values obtained in our laboratory for IgG are 600 to 1300 mg/100 ml.

The tuberculoid group served as an internal control for the distribution of antibody titers in this particular population. Sera from normal individuals were not tested along with the patients studied.

RESULTS

Distribution of anti-EBV antibody titers among patients with leprosy. Figure 1 illustrates the distribution of anti-EBV antibody titers among the patients with lepromatous and tuberculoid leprosy. All patients but three (two with lepromatous and one with tuberculoid leprosy) had antibody titers to EBV (98%). Titers $\geq$ 1:640 were considered to be significantly high for these studies. Sixteen of 70 patients with lepromatous leprosy or 22% had anti-EBV antibody titers of 1:640 or higher. Only 4 of 49 patients with tuberculoid leprosy (8%) attained the concentration of 1:640. By using a Kolmogorov-Smirnov analysis of the distributions, the concentrations of anti-EBV antibody were found to be significantly higher in patients with lepromatous leprosy (one-tail test: $P \simeq .025$) than those with tuberculoid leprosy. A 2 × 2 contingency chi-square test on titers $\geq$ 1:640 versus titers <1:640 also showed a significant difference [$\chi^2(1) = 7.06$, $P < 0.01$].

Distribution of anti-CMV antibody titers in patients with leprosy. Figure 2 presents the distribution of anti-CMV antibody titers among the patients with lepromatous and tuberculoid leprosy. Eighty-nine out of 99 patients tested had antibody to CMV as determined by complement fixation (90%). The levels of titer of $\geq$ 1:64 was considered significantly high. Four of 60 patients with lepromatous leprosy or 6.6% had titers of 1:64 and 5 of 39 patients with tubercu-

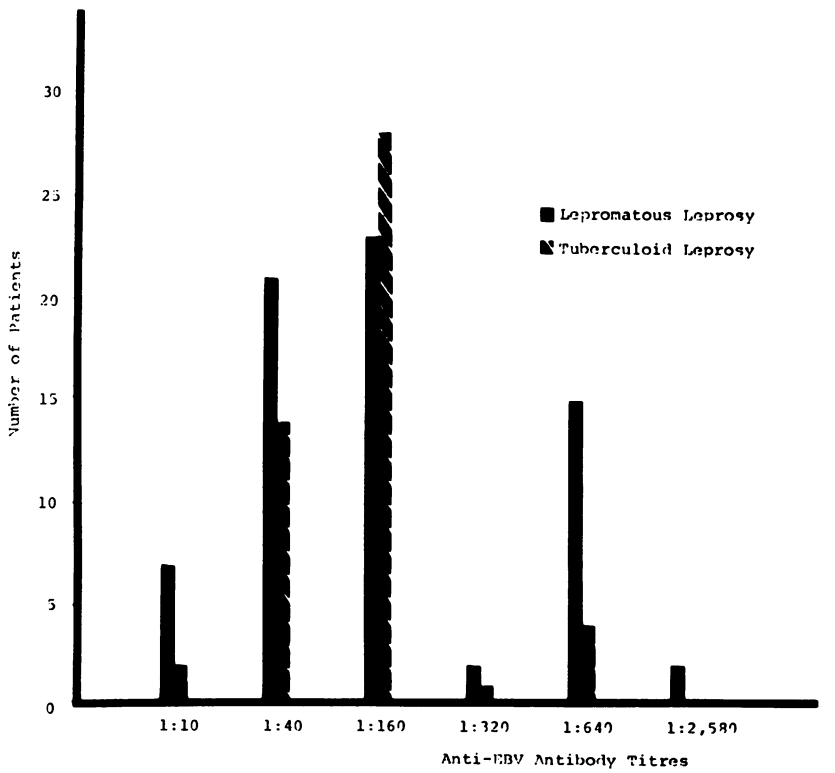

FIG. 1. *Distribution of anti-EBV antibody titers in patients with leprosy.*

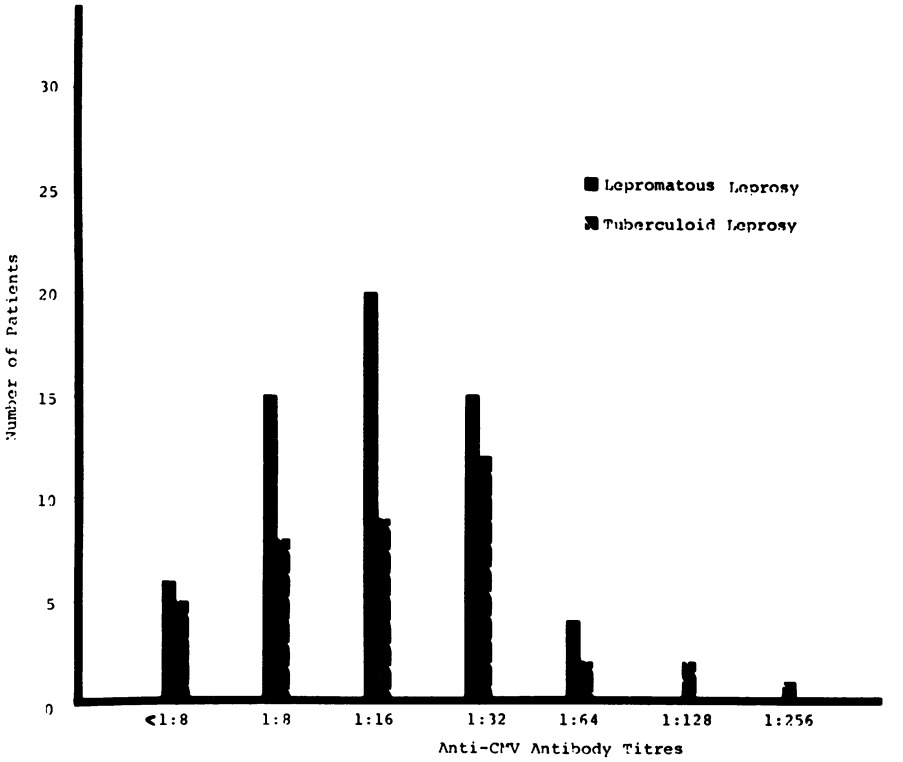

FIG. 2. *Distribution of anti-CMV antibody titers in patients with leprosy.*

loid leprosy or 12.5% had anti-CMV antibody titers $\geq$1:64. No significant difference was found in the level of anti-CMV antibody titers in patients with the two types of leprosy, as determined by either a Kolmogorov-Smirnov analysis (one-tail test: $0.10 \leq P \leq 0.5$) or a contingency chi-square test on titers $\geq$1:64 or <1:64.

Correlation of the IgG level with high anti-EBV titers in the patients with leprosy. Fifty-two of 71 patients (73%) with lepromatous leprosy had elevated IgG levels. Twenty-five of 50 patients (50%) with tuberculoid type of the disease had high IgG levels. A 2 × 2 contingency chi-square test showed significantly higher levels of IgG in patients with lepromatous leprosy [$\chi^2(1) = 6.2, 0.01 < P < 0.02$].

Antibody to EBV is restricted to the IgG class of immunoglobulins by the method used for these studies (15). Table 1 compares the IgG concentration with the corresponding anti-EBV antibody titer in a representative group of patients with high anti-EBV antibody titers. There was no correlation between the IgG concentration and EBV antibody titer. Some sera with normal IgG concentration had antibody titers as high as 1:1,280, whereas others with high IgG levels had antibody titers as low as 1:40.

TABLE 1. *Absence of correlation between anti-EBV antibody titer and IgG concentration in a representative group of patients with lepromatous leprosy*

No. of patient	Anti-EBV Titer	IgG concn
15	1:640	1,400
33	1:10	2,000
37	1:10	2,000
49	1:40	2,000
59	1:40	2,000
62	1:640	800
68	1:640	1,300
70	1:1,280	1,300

DISCUSSION

Recent studies of the in vitro response of peripheral lymphocytes from patients with lepromatous leprosy to *M. leprae* have shown specific lack of response to *M. leprae*. These data suggest that the lepromatous patients lack circulating lymphocytes responding to *M. leprae* (11), manifesting immunological tolerance specifically to this bacterial agent. The in vitro reactivity of

lymphocytes from lepromatous patients to nonspecific mitogens such as phytohemagglutinin has been shown to be suppressed as well (5). This depression has been found to be caused by a serum factor (3, 20). In vivo studies indicate that there is a partial nonspecific depression of all cell-mediated immune responses in lepromatous leprosy (2, 3, 12, 30), possibly from the inability to dissociate cells and serum factors in vivo. The two polar types of leprosy, therefore, offer a useful system for the study of the effects of cell-mediated immune responses of the host to various infections. The patients included in the present study were selected as belonging to the polar types of the disease. Treatment with medications such as sulfones could possibly alter the immunological status of the patients studied. However, the kind of medication given, the dose, and route of administration were similar in the patients with either type of leprosy.

Although antibodies are important for the clearance of circulating microbial agents, cell-mediated immunological responses seem to be the principal method by which the immunocompetent host restricts intracellular parasitism (1). Therefore, impairment of cell-mediated immunity in vivo would be expected to decrease capacities for restriction of cells containing intracellular parasites, leading to increased production of the infectious agent and its antigens. The antigenic load on the antibody-forming system of the host would increase with the end result of increased production of humoral antibodies against these antigens. A number of clinical disorders with altered cell-mediated immunity and high concentration of humoral antibody have been reported. Patients with sarcoidosis have a marked deficiency in delayed-type hypersensitivity skin responses, accompanied by high concentrations of circulating immunoglobulins. These patients usually respond to immunization with an overproduction of antibody (28). Also, patients with the lepromatous form of leprosy have impaired delayed cutaneous reactivity to lepromin, yet the quantities of circulating antibodies to antigens of *M. leprae* are increased (2). Patients with tuberculoid leprosy, on the other hand, have intense delayed skin reactivity to lepromin, and antibodies to mycobacterial antigens are less frequently detected in these cases (4).

The increased incidence of high concentrations of IgG in the patients with lepromatous leprosy suggests the presence of a protective antibody escape mechanism which is mobilized in the face of impaired cell-mediated immunity. Our findings of significantly higher anti-EBV antibody titers in lepromatous leprosy with impaired delayed-type hypersensitivity suggest that cell-mediated immunity is a significant factor in host response to EBV. In a recent study (15) less than 10% of normal persons were found to have anti-EBV titers as high as 1:640. It may be suggested that a few of the high anti-EBV antibody titers in the lepromatous group resulted from acute infection rather than impaired immunity. Although we do not have serial determinations to detect seroconversions in these patients, none of the patients studied had clinical evidence of infectious mononucleosis.

Since EBV replicates only in lymphoblasts (9), lymphoreticular diseases like leprosy, sarcoidosis, Burkitt's lymphoma, carcinoma of the posterior nasopharynx should favor the replication of this virus. Without cell-mediated immune responses to restrict this replication, higher anti-viral antibody titers (antibody escape mechanism) might result. Burkitt's lymphoma, carcinoma of the posterior nasopharynx, and sarcoidosis are likely to be associated with impaired cell-mediated immunity, and high-titered anti-EBV antibody has similarly been demonstrated (13, 16, 22). Unpublished data from our laboratory suggest that significantly elevated titers against EBV tend to occur in newly diagnosed patients with sarcoidosis (without therapy) who have marked suppression of delayed-type hypersensitivity skin responses. EBV has been suggested mainly on seroepidemiological data, as the etiological agent of diseases like Burkitt's lymphoma and carcinoma of the posterior nasopharynx (13, 22). We would suggest that host immune responses be taken into consideration when assignment of an etiological role to EBV is based solely upon seroepidemiological data. The underlying mechanism leading to high anti-EBV antibody titers in a small number of patients with tuberculoid leprosy is unclear. Perhaps the classification of the patients as LL and TT was not absolutely correct.

Cytomegalovirus, a common latent viral infection in the general population (19), appears from our studies to be handled by other host mechanisms. Impairment of cell-mediated immunity did not result in high antibody titers to CMV in our lepromatous patients with the CMV CF antigen (AD 169 strain) used. Although it is possible that neutralization tests would be more sensitive, the distribution of the antibodies would not be significantly different. CMV is known to replicate within other tissues as well as in lymphoid tissue. A lymphoproliferative disease like leprosy may not favor excessive viral replication and marked increase of antigenic load of this virus. It would be useful to study the CMV titers in other patients with multitissue diseases with impaired cellular immunity to assess the role of

replicating tissue in CMV antigen production. Interestingly, high anti-CMV antibody titers have been reported by Prince et al. (24) in immunosuppressed patients with renal transplants who underwent seroconversion after blood transfusion.

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