Epidemiologic Study of a Leukocyte-Transforming Agent in a General Population

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Studies by others have demonstrated a leukocyte-transforming agent(s) (LTA) in the oropharyngeal secretions of a significant number of individuals with Epstein-Barr virus-associated infectious mononucleosis, cancer, and, to a lesser extent, an outpatient population. This present study determines by systematic sampling the prevalence of LTA in 27 families of a semirural community. Throat swab inoculums from three of 54 adults and none of 44 children induced transformation of umbilical cord lymphocytes. Complement-dependent Epstein-Barr virus nuclear antigen was detected in two of the three transformed cell cultures. The three LTA-positive individuals were characterized by the absence of serologic evidence of a recent Epstein-Barr virus infection and the lack of elevated antibodies against the viral capsid antigen of Epstein-Barr virus.

A leukocyte-transforming agent(s) (LTA) has been demonstrated in the oropharyngeal secretions of a significant number of individuals with Epstein-Barr virus (EBV)-associated infectious mononucleosis (3, 8, 9, 16, 19), cancer (particularly those with leukemia or lymphoma; 6), and to a lesser extent in an outpatient population (6).

LTA is identified by its ability to transform human umbilical cord leukocytes into lymphoblastoid cells with unlimited growth potential in culture (3). The precise relationship of LTA to EBV is not well defined. Reservations about equating LTA with EBV have been well summarized by Chang and colleagues (6).

A survey determining the prevalence of oropharyngeal LTA in a general community has not been previously performed. A large-scale epidemiologic study of EBV infections conducted in southern Louisiana presented an opportunity for its investigation.

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MATERIALS AND METHODS

Study population. The study population has been previously described in detail (21). In summary, the area sampled was a semirural, predominantly sugar cane farming community in southern Louisiana. The first household was picked randomly and then every fourth household was systematically asked to participate in the study. During the visit an interview, blood sample, and throat culture were obtained from each individual. The throat culture procedure to detect oropharyngeal LTA was initiated in the latter part of the epidemiologic study.

Transformation analysis. The tonsillar and posterior pharyngeal areas were vigorously swabbed with a sterile cotton applicator that was then placed in 2 ml of RPMI 1640 medium with 20% fetal calf serum. The specimens were stored at -70°C and tested approximately 9 months after collection. For testing, the specimen was thawed and filtered ($0.45\ \mu\text{m}$). Three-tenths milliliter was inoculated into replicate umbilical-cord leucocyte cultures each containing 3×10^6 leukocytes in 1 ml of RPMI 1640 medium and observed for signs of transformation as described by others (2, 4, 5). In each assay, negative and positive controls (the latter kindly provided by R. Shihman Chang) were included. Cultures not transformed by week 12 were discarded.

Serology. Antibodies against the viral capsid antigen (anti-VCA) were determined by the indirect immunofluorescent technique described by Henle et al. (11, 12). In brief, smears of EB3 cells were incubated successively with the serially diluted test serum and goat anti-human immunoglobulin G. Antibodies against the early antigen complex (anti-EA) were determined by a similar technique utilizing Raji cells superinfected with EBV (13, 15).

The transformed cell cultures were evaluated for EBV nuclear antigen by the indirect immunofluorescent technique described by Reedman and Klein (20) and modified by Henle et al. (10). The transformed cells were concentrated, suspended in trisodium citrate (0.95%) for 5 s, and quickly placed onto slides. The smears were dried within 5 min in front of a cool fan, fixed for 150 s in an equal mixture of acetone and methanol at -20°C , and then air dried. They were stored at -20°C until used. For staining, the smears were overlaid with the serially diluted heat-inactivated (56 $^{\circ}\text{C}$ for 30 min) serum with known antibodies against EBV nuclear antigen (EBNA) at 37°C for 45 min. After washing with phosphate-buffered saline containing Ca^{2+} and Mg^{2+} , human complement, i.e., serum from an anti-

VCA negative donor diluted 1:10, was placed on the cell smears and incubated. After washing with the buffer, the cell smears were overlaid with fluorescein isothiocyanate-conjugated goat antibodies to human β_2C/β_2A globulins (Hyland Laboratories, Costa Mesa, Calif.) diluted 1:20. After incubation, the smears were washed in the buffer again, rinsed in distilled water, mounted onto slides with elvanol-glycerol medium, and examined under ultraviolet illumination.

RESULTS

Throat cultures were obtained from 54 adults and 44 children in 29 households (Table 1). There were three individuals whose throat swab washings transformed umbilical cord leukocytes. The ages of the LTA-positive individuals were 22, 24, and 34 years; all lived in different households.

The reciprocal anti-VCA titers of the LTA-positive individuals were 40, 80, and 80, respectively. The reciprocal geometric mean titer of the LTA-positive persons was similar to the LTA-negative group (Table 2). None of the LTA-positive individuals had anti-EA responses compared to five of 95 LTA-negative persons.

Table 3 demonstrates the clinical manifestations that had occurred in the study group during the 2- to 3-month period prior to sampling. Two of the LTA-positive adults had experienced a mild cold. From the LTA-negative group of adults, six had had a cold, one a uri-

TABLE 3. *Correlation of recent acute illness with LTA*

Group	Number				
	Well	Cold	Sore throat	Chick-enpox	Other
LTA positive (adults)	1	2	0	0	0
LTA negative (adults)	43	6	0	0	2
LTA negative (children)	28	12	2	2	0

nary tract infection, and one acute severe arthritis. The children, although experiencing a high rate of mild upper respiratory infections, had no LTA activity detected. A statistical test is not available to appropriately compare the rates of clinical manifestations in these groups due to the small sample size of the LTA-positive persons.

Two of the three transformed cell cultures contained EBNA. The one transformed cell culture that did not give a positive reaction eventually degenerated after 12 subcultures. None of the nontransformed cell cultures contained EBNA.

DISCUSSION

It has been adequately established that primary EBV infections, i.e., heterophile-positive infectious mononucleosis, is associated with a variable but usually high frequency of oropharyngeal LTA (3, 8, 9, 16, 19). However, since this agent may be detected in patients with other disease states (6), and supposedly even in healthy individuals (6, 8, 9, 16), multiple factors or stresses may lead to its expression. The data provided by the present study permits us to speculate on various possibilities.

Anti-EA, antibodies which denote a current or recent EBV infection, were not present in the three LTA-positive adults. One explanation may be that LTA activity in this group manifested a reactivation of a previous EBV infection and was unassociated with an antibody booster response as can be seen with herpesvirus hominis (17). It is known that, in EBV-associated infectious mononucleosis, anti-EA often develop later than anti-VCA and are detected for a few months after the illness (14). The lack of anti-EA in this study's LTA-positive individuals may, therefore, indicate too early or too late testing in regard to the time of the acute infection. A corollary to this would be that LTA response occurs earlier or persists longer than anti-EA activity.

The lack of anti-EA in the LTA-positive persons also raises the possibility that non-EBV

TABLE 1. *Prevalence of oropharyngeal LTA in various age groups*

Age group (yr)	No.	Positive for LTA
0-6/12	4	0
7/12-1	1	0
2-5	12	0
6-10	14	0
11-15	13	0
16-30	25	2
31-50	16	1
≥50	13	0

TABLE 2. *Comparison of EBV antibodies in LTA-positive and LTA-negative individuals*

Subjects	No.	Anti-VCA geometric mean titer	No. with anti-EA
Children (<16 years)			
LTA positive	0		
LTA negative	44	20.4	2
Adults			
LTA positive	3	63.0	0
LTA negative	51	50.1	3

infections or other stresses may influence the presence of LTA, perhaps by impairing the cellular immune status of the individual. It is known that a variety of illnesses, severe infections, immunosuppressants, and other factors are associated with generalized delayed cutaneous unresponsiveness or anergy (18), and cell-mediated immunity appears to be of significant importance in protecting against viral replication (1, 7). In our study, two of the three LTA-positive individuals suffered from an upper respiratory infection shortly before the sampling. In the prevalence study of Chang et al. (6), the lowest rate of LTA was in outpatients receiving immunizations or undergoing a routine physical examination; the group whose diagnosis was trauma or more stressful diseases had higher prevalence rates. In their leukemia-lymphoma group, those who were critically ill (and receiving intensive chemotherapy) had LTA rates equivalent to that seen in infectious mononucleosis.

Five LTA-negative adults in our study had serological evidence of a current or recent EBV infection. Perhaps mildly symptomatic or asymptomatic EBV infections elicit no or minimal and short-lived LTA responses that were missed by the single surveillance attempt?

The presence of LTA may also be age dependent, the reasons for which are now known. None of the 44 children sampled in the present study (only nine of which had no antibodies against EBV) had LTA activity detected. These results contrast somewhat with the findings of Chang and associates (6).

It must be emphasized that the question whether a previous exposure to EBV is a prerequisite for detection of oropharyngeal LTA was not explored. Since EBV appears to induce EBNA (J. Wright, L. A. Falk, and F. Deinhardt, Abstr. Annu. Meet. Am. Soc. Microbiol., 1974, V 133, p. 222), the detection of EBNA in two of the transformed cell cultures in this study indicates that the throat swab specimen contained the virus. Why one of the transformed cell cultures did not contain EBNA is not clear but may be related to the fact that the test for the antigen was made after the cell culture had begun to deteriorate.

This preliminary prevalence study has found that a small but significant adult segment of a general population harbored oropharyngeal LTA. The performance of a large prospective study with serial sampling for LTA and EBV antibody reactivity would appear to be necessary to clarify the findings presently reported.

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