Incidence of Herpesvirus Antibody Among Leukemic and Nasopharyngeal Carcinoma Patients (36265)

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(Introduced by W. R. Dowdle)

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Previous investigators have reported an association between herpes-like particles and Burkitt's lymphoma and nasopharyngeal carcinoma (NPC) (1–3). Type 2 herpes simplex virus has been reportedly associated with carcinoma of the cervix, and a causal relationship has been suggested (4). These findings have prompted us to survey sera of patients with leukemia and NPC for antibody to types 1 and 2 HSV, varicella virus, cytomegalovirus, and Epstein Barr virus.

This report presents data which show complement fixation (CF) or fluorescent antibody (FA) titers to the above viruses in 60 sera from patients with leukemia, 22 sera from patients with NPC, and 82 sera from healthy individuals matched with cancer patients by age, sex, economic status, and geographic location.

Materials and Methods. Viruses. The viruses used in this study were: Strains MacIntyre VR No. 3 (type 1) and MS (type 2) herpes simplex viruses (HSV), strain CP5, 262 varicella virus (VV), and strain AD 169 cytomegalovirus (CMV). They were obtained from Dr. Robert E. Kissling, Center for Disease Control (CDC).

Virus propagation. Viruses were propagated in 32 oz prescription bottle cultures of cells maintained with a modified Eagle's essential medium (5) supplemented with 2% fetal calf serum. HSV strains were propagated in continuous African green monkey (Vero) cells. VV and CMV were propagated in human diploid fibroblasts of foreskin origin.

Complement fixation. CF antigens of type 1 and type 2 HSV were prepared from infected Vero cultures as previously described (6). CF antigens of VV and CMV were produced from monolayer cultures of human fibroblasts as described by Kissling *et al.* (7) for the production of VV CF antigen except that the buffer consisted of 0.85% NaCl, buffered at pH 9.0 with 0.05 *M* glycine–NaOH, instead of Veronal buffered saline at pH 7.2. Uninfected cell cultures, treated the same as virus-infected cells, were used as control antigens. CF tests were performed by the LBCF method (8). Reference antigens and antisera for these tests were obtained from the Biological Reagents Section, CDC.

Sera. The sera evaluated in this study were obtained from leukemic patients, patients with NPC, and healthy individuals chosen as controls. The leukemic patients ranged in age from 5 to 74 years and had been diagnosed as having acute myelogenous leukemia, acute or chronic lymphocytic leukemia, or acute or chronic granulocytic leukemia. All NPC patients were over 50 years old. Sera designated as controls were matched with sera from cancer patients by age, sex, economic status and geographical location.

Fluorescent antibody tests. The indirect fluorescent antibody (FA) test for Epstein Barr virus (EBV) antibody was performed by the method of Henle and Henle (9). Serum EBV FA titers were determined by testing against HR-1K cells. These cells were received from Dr. Werner Henle, Children's Hospital of Philadelphia. All testing was performed with the same reagents and the same FA microscope. Sera from cancer patients and their corresponding controls were tested for EBV antibody on the same day and were read by the same investigator.

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TABLE I. Herpesvirus Antibody Geometric Mean Titer[a] for Patients with Leukemia and NPC and in Normal Controls.

Disease group	No. of patients	Antigen						
		EBV	Varicella	HSV-1	HSV-2	CMV	Uninoculated Vero TC	Uninoculated human fibroblast
Leukemia ≤ 18 yrs	30	24	<8	<8	<8	<8	<8	<8
Control ≤ 18 yrs	30	21	<8	<8	<8	<8	<8	<8
Leukemia > 18 yrs	30	25	<8	9	<8	8	<8	<8
Control > 18 yrs	30	24	<8	11	<8	9	<8	<8
Nasopharyngeal carcinoma	22	416	20	49	<8	23	<8	<8
NPC control	22	32	<8	20	<8	20	<8	<8

[a] Geometric mean base titer 1:4.

Results. Sera from leukemic patients and their corresponding controls were divided into two groups on the basis of age. One group consisted of sera from persons 18 years old or younger and the other of individuals over 18 years old. Sera from NPC patients were not divided, because all were over the age of 50 years. Table I shows the geometric mean titer (GMT) of sera from leukemic and NPC patients and their corresponding controls to EBV, VV, HSV types 1 and 2, and CMV. Antibody GMT titers to these viruses in sera from leukemic patients were similar to those of their respective controls. GMT to HSV type 1 and CMV were higher with sera from both leukemic patients and controls over 18 years of age than with similar sera from younger persons. These results agree with the findings of others (10, 11).

The ratio of the GMT of sera from NPC patients to the GMT for the control group was 13 for EBV, 2.5 (at least) for VV, and 2.5 for HSV type 1. None of the sera tested reacted with control tissue culture antigens.

Table II shows the distribution of FA titers to EBV and CF titers to HSV-1 and VV in sera from patients with NPC indicate an increase over controls both in terms of numbers of sera containing antibody of 8 or greater (*i.e.*, EBV 22/22 vs 17/22 controls; VV 17/22 vs control 7/22; HSV-1 22/22 vs control 16/22) and GMT to those sera having serum titers of at least a 1:8 level (EBV 416 vs 55.7 control; VV 32 vs 10.6 control; HSV-1 64 vs 24.3 control).

Discussion. The data presented show no evidence that levels of humoral antibody to the herpesviruses were higher or lower in patients with leukemia than in their corresponding controls. However, this does not *a*

TABLE II. Distribution of EBV, VV, and HSV Antibody Titers in Patients with NPC and in Normal Adult Controls.

	Reciprocal of serum dilution										
	<8	8	16	32	64	128	256	512	1025	2048	Total having antibody titer of at least 8
NPC-EBV	0	0	0	0	1	2	4	10	5		22/22
Control-EBV	5	1	2	4	5	3	2				17/22
NPC-VV	5	3	4	4	3	2	1				17/22
Control-VV	15	4	3								7/22
NPC-HSV	2	0	2	4	8	5	1				20/22
Control-HSV	6	3	4	6	3						16/22

priori rule out an association between leukemia and herpesviruses, since a survey of serum antibody taken perhaps years after the initiation of leukemia might not reflect a subtle relationship.

The finding that sera from NPC patients had a higher GMT to EBV than their corresponding controls had is in agreement with the findings of others (3). But the finding that the GMT to HSV type 1 and VV were higher in NPC sera than in sera from healthy individuals had not been previously noted. Although this could be a response to a common herpesvirus antigen, other data do not support this contention. Some sera from ostensibly healthy humans have high EBV antibody titers but do not have high HSV or VV antibody titers, and sera with high HSV and VV titers do not necessarily have high EBV titers.

It is possible that NPC patients are more susceptible to all herpesvirus infections. However, it is also possible that during NPC illness, a latent HSV infection is reactivated and then produces a humoral antibody response to HSV and, in some cases, a concomitant heterotypic response to VV. The latter interpretation is supported by the finding that all sera from NPC patients had antibody to EBV; whereas only 20 of the 22 had HSV antibody and only 17 had VV antibody. No sera showed antibody to only VV. This lesser response to VV may reflect a lesser incidence of latent VV infection and therefore less chance for reactivation.

Summary. This report presents data which show complement fixation or fluorescent antibody titers to the herpes virus group in sera from patients with leukemia, patients with nasopharyngeal carcinoma, and healthy controls. Higher titers against EBV, herpes simplex type 1, and varicella were found in the sera from nasopharyngeal carcinoma patients.

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