EBV Antibodies in Family Contacts of Patients with Infectious Mononucleosis (34598)

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(Introduced by J. L. Melnick)

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The studies of Henle *et al.* (1), showing that antibodies to Epstein-Barr virus (EBV) develop during and persist after the course of infectious mononucleosis (IM), have opened up an excellent means of studying epidemiologic features of EBV infection. A previous report (2) showed that approximately one-third of the contacts of patients with IM developed symptoms such as pharyngitis, abnormal lymph glands, palpable spleen or liver. At that time, however, there was no means of specifically verifying an EBV infection.

We have studied seven families in which a child or young adult (the index case) was admitted to Danderyd's Hospital, Stockholm, and diagnosed as IM. Several members of the families developed EBV antibody titer rises indicative of EBV infection.

The study included measurements of bone marrow colony stimulating activity (3) with sera from all the families, since an increased growth-stimulating activity has been noted in several diseases, including IM.

Patients and methods. Clinic. Twenty-eight persons, constituting seven Swedish families with 2–5 members in each, were followed for 2–7 months after the index case had fallen ill. The first serum from the index cases was taken as soon as the patient entered the hospital. Venous blood was collected at the intervals shown in Table I. Smears were made for differential counts and the serum was then stored at −20° until required for further tests. All index cases displayed a typical clinical picture of IM, with irregular fever, lymphadenopathy, hepatosplenomegaly, increased liver transaminases, hematological findings of lymphocytosis with atypical cells, and heterophile antibodies persisting after guinea pig kidney absorption of the serum (4) (Fig. 1).

Serology. Heterophile antibodies. Sheep red cell agglutinins were assayed by the presumptive test according to Paul and Bunnell (5). Differential absorption according to Davidsohn (4) was performed to distinguish the heterophile antibody typical for IM from

TABLE I. Blood Sampling Data for Family Members.

Family:	sex,	age	Serum taken after clinical symptoms of index case (no. days)			
1Kj	F	2	5	34	120	
	F	23	5	34	120	
	M	4[a]	5	14	34	120
	M	24	5	34	120	
2B	F	44	18	107		
	M	50	18	107		
	F	16	17	31	107	
	F	5	18	107		
	F	12	18	107		
3Ka	F	12	7	130		
	F	43	7	130		
	M	20	5	24	130	
	M	59	7	120		
	M	23	7	130	207	
4J	M	44	4	121		
	F	44	4	121		
	F	21	4	121		
	M	15	4	14	121	
	M	14	4	121	207	
5P	M	38	5	38	71	
	F	34	5	38	71	
	F	5	5	38	71	
	M	2	5	38		
6F	F	17	61	93		
	F	33	61	92		
	M	8	61	72	92	
7G	F	46	17	60		
	F	17	17	26	60	

[a] Index cases underlined.

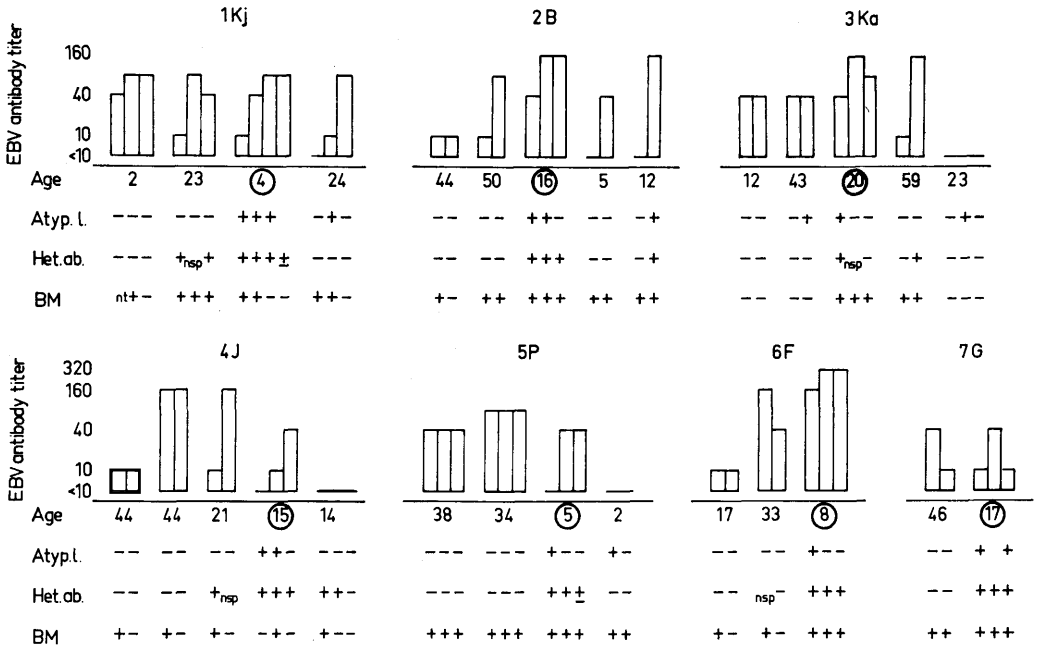

FIG. 1. EBV antibody titers for seven index cases of mononucleosis and their family members. Index cases are indicated by a ring around the age figure. The presence of atypical lymphocytes (Atyp. l.), heterophile antibodies according to the Monospot test (Het. ab.) and bone marrow stimulating activity (BM) is denoted beneath the curves for each serum sampling. The percentage of atypical lymphocytes for index cases varied between 11 and 50% in the acute phase, whereas only a few such cells (0–3%) were seen for other family members. The Paul-Bunnell heterophile titer after differential absorption varied between 20 and 320 in the acute phase; the Monospot reaction remained positive for longer than the Paul-Bunnell reaction. The highest Paul-Bunnell titer in family contacts was 20. Nsp designates nonspecific heterophile antibodies, not absorbed by beef erythrocytes. Nt = not tested due to lack of serum.

other heterophile antibodies. Higher sensitivity was obtained with the Monospot test (Johnson and Johnson), using horse erythrocytes as indicator cells (6, 7). In several family members, sheep agglutinins were not detected by the Paul-Bunnell test with the serum dilution used (1:20), but clear cut agglutinations were seen with undiluted sera and the horse red cells used in the Monospot test or with fresh horse erythrocytes. In such cases the test was regarded as positive only if the agglutinins were absorbed by beef erythrocytes but not by guinea pig kidney extract (4), although nonspecific reactions have been shown to occur even after cases of IM (7).

Antibodies to EBV. Serum dilutions were applied to smears of cultured Burkitt lymphoma cells (line Silfere, kindly supplied by Dr. A. Svedmyr) and an EBV positive cell line obtained from peripheral lymphocytes of a healthy donor (kindly supplied by Professor L. Philipson) according to the method of Henle *et al.* (1). When the slides had been incubated with serum dilutions for 30 min, the sera were washed off and the preparations exposed to a fluorescein-conjugated sheep-antihuman IgG. A fluorescein-conjugated IgG preparation obtained from pretested sera of patients with a recent history of mononucleosis was used for direct staining to control the frequency of EBV positive cells. Usually 1–3% of the cells stained specifically. The end point titer was set at the serum dilution at which brilliant fluorescence was still detectable. A titer of < 10 was regarded as negative and a fourfold titer increase as indicative of an EBV infection.

Additional viral serology. All sera were assayed for complement-fixing (CF) antibodies to cytomegalovirus (CMV), adenovirus, and influenza types A and B, since these infections were common in Sweden at the time when the sera were collected. The type of influenza A infection was assayed by hemagglutination-inhibition tests. The tests were either negative or revealed no titer differences except in the cases mentioned in the text.

Assay for bone marrow colony stimulation. Bone marrow plugs were collected from DBA/2 mice and single cell suspensions were prepared (3). Double strength modified Eagle's medium, containing 20% fetal calf serum and 20% trypticase soy broth, was mixed with an equal volume of 0.6% agar in water. The mixture was held at 37° and sufficient bone marrow cells were added to give a final concentration of 10^5 nucleated cells per ml in the agar-culture medium. Two ml of the bone marrow suspension in agar medium and 0.1 ml of the serum were mixed in 35-mm plastic petri dishes. Culture plates were then incubated for 10 days in humidified incubators at 37° with a continuous flow of 5% CO_2 in air. After incubation, plates were examined with a dissecting microscope and colony counts performed at × 30 magnification (3). To be counted, colonies had to contain 10 or more cells. Sera giving 10 or more colonies per plate with 0.1 ml of serum were considered positive. Known positive and negative sera were included as controls in each test.

Results. Index cases. The clinical, hematological, and serological (Fig. 1) findings in the index cases confirm that these patients had an acute infectious mononucleosis (1, 2, 8, 9). The EBV antibody titers for index cases and family members are shown in Fig. 1. The presence of atypical lymphocytes, heterophile antibodies to horse cells after differential absorption, and EBV antibody is indicated for each sample.

Family members. Family 1Kj. A 2-year-old sister of the patient displayed upper respiratory disease with exanthema but no fever 9 days before her brother fell ill. She had a rising percentage of lymphocytes (42, 57, and 64%) but no atypical cells. The two young parents had fever and sore throats 2 weeks and 1 week before the index case. Three members of this family displayed increasing titers to EBV during the observation period and one was negative from the beginning. The father also had a significant increase of antibodies to influenza A virus (A2-Hong Kong 1968). All family members had low antibody titers to CMV, not indicative of recent infections.

Family 2B. The 44-year-old mother already had EBV antibodies and she was healthy before and during the observation period. The father had a sore throat a week before the index case fell ill. One 5-year-old sister had fever, exanthema, and a sore throat immediately after the index case. The 12-year-old sister was ill with fever and upper respiratory symptoms 6 weeks after the index case. Both sisters had increases in EBV antibody titers. In this family only the parents had low persistent CMV antibody titers indicative of a previous infection.

Family 3Ka. One sister and the mother had probably had an EBV infection previously. The father was the only person, apart from the index case, to have significant titer rises to EBV. He had had an anemia since childhood and became seriously ill, 6 weeks after the index case, with fever, myocarditis, and an Adams-Stoke's attack and was hospitalized for more than 1 month. Furthermore, he developed titers to influenza A (type A2-HongKong 1968) and CMV. The mother had relatively high (80), later decreasing, titers to CMV, possibly indicative of a recent CMV infection (Wahren, Espmark, and Walldén, to be published). In this family the infections with CMV and EBV appeared simultaneously. One brother was still negative to EBV 7 months after the index case had fallen ill. Only two members of the family were clinically ill and had EBV antibody titer rises. They were the only ones whose sera stimulated bone marrow cell growth (see below).

Family 4J. The parents had EBV antibody titers indicative of an earlier infection. Both, however, had recently had upper respiratory

symptoms or developed such during the observation period. The father had a significant titer rise to influenza A virus (HongKong). A 21-year-old sister, without clinical symptoms, developed a high EBV antibody titer and had heterophile antibodies in the first serum sample. The 14-year-old brother had two instances of a sore throat and a titer increase to influenza A (HongKong) but was still negative for EBV antibodies 7 months after the index case fell ill. Low titers of CMV antibodies were found in the sera of the parents only.

Family 5P. The father was ill immediately before the index case, from an unknown infection. The parents had apparently had the EBV infection earlier. The 2-year-old brother was negative for EBV antibodies for 2 months and then had two fever periods, but it was not possible to obtain further sera from him. All family members were negative for CMV antibodies.

Family 6F. The 17-year-old maid had already had the infection. In this family the index case had two periods of clinical disease, the first 2 months before and the second the day before admission to hospital. By that time the EBV antibody titers were high. The mother displayed high, later decreasing, titers possibly indicative of a recent EBV infection. She too, had had an infection with fever and sinusitis at the time of the index case's first symptoms. CMV antibodies were found in the sera of the two oldest family members.

Family 7G. This family consisted of two members only. The mother displayed decreasing EBV antibody titers but had no apparent clinical symptoms. Both were negative for CMV antibodies.

Discussion. Hospitalized index cases had heterophile antibodies typical of mononucleosis, 11–50% atypical lymphocytes, and increasing or high EBV antibody titers. The index cases (except in family no. 6) had not had any prolonged disease when the first serum sample was taken, which may explain the increasing titers. Such increases are not always observed (1), since many patients are ill for some time before being admitted to hospital. Of the other 21 family members, 7 had significant EBV antibody increases and 3 of these were initially negative. Two others had decreasing titers possibly indicating a recent EBV infection. In three of these nine, we found neither atypical lymphocytes nor heterophile antibodies. Since both atypical lymphocytes and Paul-Bunnell heterophile antibodies are present for only a relatively short period during the acute stage of mononucleosis, the diagnosis of subclinical or otherwise mild mononucleosis depends on the appearance of antibodies specific to EBV (1, 10).

Three persons remained negative for EBV antibodies; two of them were tested for the last time 7 months after the respective primary case had fallen ill. The appearance of heterophile antibodies or atypical lymphocytes in blood samples from these three persons (Fig. 1) may indicate that they had a subclinical IM and would possibly develop antibodies later. More probably, they may have had other complaints, since both heterophile antibodies and atypical lymphocytes have occasionally been described in other diseases (11).

Bone marrow colony stimulating activity (Fig. 1 and Table II) has been found in sera from patients with several types of leukemias (12), IM (3), and several other infectious diseases (13). Such activity may well be a normally occurring factor regulating the growth of white blood cells (12, 14), an increase reflecting a disturbance of this mechanism. All the index cases and family members displayed such stimulating activity in one or more serum samples with the exception of family no. 3, in which only the sera of the index case and the diseased father were active. It is not yet known whether an increased serum activity reflects an *in vivo* stimulation of bone marrow or other cells. This may, however, be the case, since the majority of persons in the families with mononucleosis had highly active sera, which is usually not seen in healthy persons (Table II).

Out of 12 parents, 4 showed increases in EBV antibody titer. This is a high proportion according to other investigators (15, 16)

TABLE II. Comparison of EBV Antibody Findings with the Presence of Atypical Cells, Heterophile Antibodies, and Bone Marrow Colony Stimulating Activity in Blood from Patients with Mononucleosis and from Their Relatives.

	Atypical cells	Heterophile antibodies	Bone marrow colony stimulation	Mean no. (range) of colonies
Index cases	7/7	7/7	7/7	106 (63–185)
Other patients with mononucleosis (14)	14/14	14/14	14/14	137 (58–250)
Family members with				
EBV titer increases	2/7	4/7	7/7	81 (34–105)
titer decreases	0/2	0/2	2/2	63 (40– 85)
no titer differences	1/9[a]	0/9	7/9	55 (0–113)
negative EBV antibody test	2/3	1/3	2/3	27 (0– 42)
Healthy controls	0/30[b]	0/25[c]	1/25[d]	3 (0– 30)

[a] This patient had a recent CMV infection but no indications of a recent EBV infection.

[b] Thirty persons who had had various diseases (not IM) chosen at random from recovered patients. Sera from the acute phase of infectious diseases often have bone marrow stimulating activity (13).

[c] Healthy adult blood donors and healthy school children.

[d] Same as [c]. A previous report (13) also showed that only 1 out of 85 sera from healthy school children gave a weak colony stimulation.

and the explanation may lie in the high social status of the present families. It has been shown that members of families with a high socioeconomic background come into contact with the EBV later in life than those from low social levels (10, 17, 18). Similarly, the proportion of patients with CMV antibodies was lower than expected (Wahren, Espmark, and Walldén to be published). The finding of one case with a titer rise to CMV as well as to EBV in family no. 3 is of interest as indicating that these two infections are much more common than used to be thought.

It is difficult to draw any conclusions about the incubation period of IM from a family study, since the members were in repeated contact. It is probable, moreover, that the index cases were not the real primary cases. A latent period of 33–49 days has been suggested by Hoagland (19) and we found two persons who developed fever and EBV antibodies 6 weeks after the index case had fallen ill.

Altogether, the spread of EBV infection within the families studied seems fairly high, although few members developed the characteristics of a clinical mononucleosis.

Summary. Seven Swedish families, altogether 28 persons, were kept under observation for 2–7 months for the spread of EBV infections. The seven index cases, aged 4–20 years, all presented a typical clinical picture of IM with atypical cells and heterophile antibodies. They all displayed increases in EBV antibody titers. Seven of the 21 contacts had significant EBV antibody titer rises, indicating an EBV infection. Five of these seven individuals also had atypical cells or heterophile antibodies. One case was found of a concomitant titer rise to EBV and CMV. Sera from all but 3 of the 28 persons stimulated the growth of bone marrow colonies *in vitro*.

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