

Serological follow-up of children with infectious mononucleosis caused by Epstein-Barr virus

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Ninety children, most under 6 years of age, 73 of whom were suffering from a disease suspect of infectious mononucleosis, were examined serologically for EBV infection. Acute EBV infection was demonstrated in 40 cases. These children were followed up until the end of the second year by examining serum samples taken every month to every third month for anti-VCA-IgM, anti-VCA-IgG, anti-VCA-IgA, anti-EBNA, and for the specific antibodies to the components D and R of the early antigen. Heterophil antibodies were examined by agglutination of sheep and horse erythrocytes. The temporal course of the antibody response was similar to those reported in adults while anti-EA-D and heterophil antibodies were demonstrated less frequently.

Primary Epstein-Barr virus (EBV) infection in adolescents and adults gives rise to the clinical picture of infectious mononucleosis (IM) [6, 9], but in children it is often symptomless, or atypical in course [1, 14]. This atypical course may explain why only few EBV infections of children have been analysed clinically [5, 7, 12, 13, 16, 18, 19] and why no serological follow-up studies of acutely infected children have been published.

MATERIAL AND METHODS

Ninety children were examined for EBV infection serologically. Of these, 41 showed the typical clinical picture of IM, 32 appeared to be suspect of IM, and in 17

cases the EBV test was performed for some other cause. A case was regarded as typical IM if at least four of the five symptoms of IM, *viz.* fever, pharyngeal changes, lymphadenopathy, hepatomegaly and splenomegaly, were observed, one or more of them in a very pronounced form (fever lasting for a week or longer; confluent tonsillitis; generalized lymphadenopathy; hepato- or splenomegaly exceeding 2 cm). The case was suspect if the symptoms were mild, or fewer than four symptoms were present.

Serological tests. Anti-EB-VCA IgG, IgM and IgA antibodies were examined by the indirect immunofluorescence (IF) method. The marmoset cell line B95-8 [11] was used as antigen. Anti-EBNA was determined by the anti-complement IF (ACIF) test, using an antigen prepared from Raji cells [3]. The early EBV antigens were induced with IUdR in Raji cells as described by Simonova et al. [17]. The IgG preparations against components D and R

of the early antigen (EA-D and EA-R) were assessed by indirect IF. In the anti-EA-D assay, methanol-treated preparations were used, whereas a positive reaction with acetone-treated cells parallel with a negative methanol-treated preparation was indicative of the presence of anti-EA-R [17]. Heterophil antibodies were demonstrated by the Paul—Bunnell—Davidsohn (P—B—D) test and the MITEST slide reaction, the latter being a horse-erythrocyte agglutination test.

A serological test was regarded as positive if its titre was 1 : 10 or higher. If, however, anti-EBNA had been absent in the acute phase, a subsequent positive reaction even in the 1 : 2 serum dilution was accepted as positive. The P—B—D reaction was regarded as positive at a titre of 1 : 64 or more.

The 90 cases under study were divided into four serological groups. Those positive for anti-VCA-IgM were regarded as acute EBV infections (40 cases), those positive for anti-EBNA as old EBV infections (20 cases). If neither of these antibodies was present while any other EBV antibody could be demonstrated, the EBV infection was designated as not old (14 cases). In the EBV-negative group (16 cases) EBV-specific antibodies could not be demonstrated.

The children suffering from acute EBV infection were followed up for 24 months except those who did not present at the

given time. In the first six-month period, a serum sample was examined in every month to every second month. Several samples were not examined for anti-VCA-IgA and/or anti-EA, due to technical difficulties. From the 7th month on, only those children were followed up by examining a serum sample every second or third month, whose last reaction pattern did not agree with the pattern characteristic of an old EBV infection.

RESULTS

In Table 1, the results of the first virus-serological tests are compared to the actual clinical symptoms. Distribution by age is shown in Table II, and the frequencies of virus-specific and heterophil antibodies in acute EBV are shown in Table III. Serological follow-up of the same cases is shown in Table IV.

The following serological changes were registered during the 24-month observation period.

Anti-VCA-IgG. Each case remained seropositive throughout the whole period. The titre was $\geq 1:160$ in 32

TABLE I

Clinical picture of cases grouped according to the phase of EBV infection (90 children)

Clinical picture	EBV infection \square				Totals
	acute	not old	old	EBV-negative	
Typical IM	35	6	0	0	41
Suspect of IM	5	5	15	7	32
Not IM	0	3	5	9	17
Totals	40	14	20	16	90

- \square Acute: anti-VCA-IgM positive, anti-VCA-IgG positive, anti-EBNA negative
 Not old: anti-VCA-IgM negative, anti-VCA-IgG positive, anti-EBNA negative
 Old: anti-VCA-IgM negative, anti-VCA-IgG positive, anti-EBNA positive

TABLE II

EBV infectedness and age distribution of 73 children with clinically typical, or suspect of IM

Age	EBV infection \square				Altogether
	acute	not old	old	EBV-neg-ative	
10-23 months	0	4	5	3	12
2 years	6	4	3	0	13
3-5 years	17	3	3	4	27
6-9 years	10	0	4	0	14
12-14 years	7	0	0	0	7
10 months - 14 years	40	11	15	7	73

 \square Explanation see footnote to Table I

TABLE III

Demonstrability of EBV-specific and heterophil antibodies (HA) in acute EBV infection (40 cases)

Antibodies	No. of children found positive/examined in age groups (Years)				Totals
	2	3-5	6-9	12-14	
anti-VCA-IgG	6/6	17/17	10/10	7/7	40/40
anti-VCA-IgM	6/6	17/17	10/10	7/7	40/40
anti-VCA-IgA*	2/4	8/16	6/8	5/6	21/34
anti-EA-D*	0/4	6/16	3/8	3/6	12/34
anti-EA-R (exclusively)*	1/4	1/16	0/8	0/6	2/34
anti-EBNA	0/6	0/17	0/10	0/7	0/40
HA P-B-D	0/6	2/17	4/10	6/7	12/40
HA MITEST	1/6	9/17	7/10	7/7	24/40

* six cases have not been tested

cases in the first serum samples already. A four-fold rise in titre occurred in 8 cases, usually after the acute symptoms had disappeared. High serum titres tended to decline 6 months after onset of the illness, but in 50% of the cases the titre did not fall below 1:160 by the end of the first year, and values less than 1:40

were never observed at the end of the observation period.

Anti-VCA-IgM. In the first two-week period a titre of 1:40 or higher was measured in 32 cases. In further 8 cases, the titre was lower, 1:10 or 1:20. Sixteen to 45 days after the appearance of the first symptoms, the reaction was already negative. In 7

TABLE IV
Course of serological response after EBV infection of children

Antibodies	No. of children found positive/examined at indicated time (months) after onset of illness								
	1	2	3	4	5	6	7-9*	10-12*	13-18*
anti-VCA-IgG	40/40	34/34	28/28	30/30	26/26	24/24	18/18	14/14	7/7
anti-VCA-IgM	40/40	6/34	0/28	0/30	0/26	0/24	nt.	nt.	nt.
anti-VCA-IgA	21/34	1/23	0/17	0/16	0/22	0/24	nt.	nt.	nt.
anti-EA-D	12/34	10/23	3/17	2/16	1/22	0/24	0/18	0/14	0/7
anti-EA-R (exclusively)	2/34	7/23	10/17	10/16	15/22	18/24	10/18	6/14	3/7
anti-EBNA	0/40	4/34	5/28	12/30	15/26	14/24	13/18	12/14	6/7
HA P-B-D	12/40	1/34	0/28	0/30	0/26	0/24	nt.	nt.	nt.
HA MITEST	24/40	17/34	3/28	3/30	2/26	2/24	1/18	1/14	0/7

* selected patients, distorted numerical data
nt. = not tested

cases, the first serum sample taken after clinical recovery was still positive while in 5 cases the serum became negative in spite of the protracted course.

Anti-VCA-IgA. Both the frequency and the titres were lower than for anti-VCA-IgM. The two curves ran nearly parallel.

Anti-EA-D. In the acute stage, 35% of the cases showed titres of $\geq 1:15$. Anti-EA-D, however, was demonstrated in no case under 3 years of age. In the second month after onset of illness, four previously negative children developed anti-EA-D, i.e. 45% of the cases under study were positive in this period. Subsequently, the frequency curve dropped abruptly, but in one case anti-EA-D was still present even in the 5th month.

Anti-EA-R. Two children under six years of age showed an antibody

response to component R but not to component D. Later, when the majority of the samples had become negative for anti-EA-D, more and more of the patients developed an anti-EA-R response. In months 5-7, positive results were obtained in 60-70% of the cases. From the 8th month on, this antibody, too, tended to disappear, and only a single serum sample was positive 2 years after the end of the acute phase.

Anti-EBNA. This antibody was absent in every acute-phase serum sample. In the second month, it appeared with titres not exceeding 1:5. Later, the titres increased up to 1:20 or even higher. The frequency of positive serum samples increased slowly, reaching 50% in the 5th or 6th month and 90% in the 10th month. In two cases even the 15-month sample was negative; in one, anti-EBNA appeared in the 16th month while in the other the

reaction remained negative throughout the observation period.

Heterophil antibodies (HA). The P—B—D reaction reached a titre of $\geq 1:64$ during the acute phase of IM. The MITEST reaction, on the other hand, often remained positive during convalescence, in one case until the 15th month.

DISCUSSION

It is known that in children most of the primary EBV infections manifest themselves with a mild disease or remain symptomless; typical IM occurs rarely [1, 18].

According to the present observations, typical IM may be caused by EBV also in childhood. The probability of an EBV aetiology is the higher the older the child. In accordance with observations reported from other countries [2, 4], we have failed to find acute infections showing the typical clinical picture of IM under 2 years of age. In children between 12 and 14 years, on the other hand, all of our suspect patients proved to be suffering from acute EBV infection.

The immune response to EBV seems to be age-dependent. Besides the presence of anti-VCA-IgM, anti-VCA-IgG and anti-VCA-IgA and the absence of anti-EBNA, the appearance of anti-EA-D is thought to be characteristic of an acute EBV infection [10]. We have, however, failed to find even a single anti-EA-D-positive patient among those of the 2 to 3 year age group, whereas the result was positive for 50% of the adolescent patients.

Fleischer et al [4] called attention to their experience suggesting that under two years of age instead of anti-EA-D, the presence of anti-EA-R was characteristic of acute EBV infections; older children have not been examined in this respect. According to our experience, not only the anti-EA-D response, but also the anti-VCA-IgA response seems to be to some extent age-dependent, although the number of our cases prevents us from drawing conclusions. The P—B—D test for heterophil antibodies was consistently negative under 3 years of age, and it was rarely positive between 3 and 6 years. Similar observations were published by Schmitz et al [16].

MITEST, a slide test based on horse erythrocyte agglutination, proved to be more sensitive than the P—B—D reaction, though the frequency of the positive MITEST reaction was diagnostically utilizable only above 6 years. In an earlier study [5], the incidence of heterophil antibody responses in children with IM was studied comparatively by using various reactions. Most of the patients were more than 6 years old. With the rapid slide test, positive results were obtained in 79%.

According to the present results, the course of the specific anti-EBV response in children resembles those reported in adults [10]. The anti-VCA-IgG reaction was positive in all of our patients even two years after recovery from acute IM. The presence of anti-VCA-IgM is characteristic of the acute phase of IM. A negative result of this

test does not, however, exclude an EBV infection contracted 2 to 3 weeks earlier and being still active. The presence of anti-EA-D is of short duration. In our experience, it is present most frequently, in the present study in 45% of the children, near the end of the acute phase. The anti-EA-R antibody appeared later, mostly in the 5th to 7th month after onset of the illness, in about $\frac{2}{3}$ of our patients.

The presence of anti-EBNA excludes a recent EBV infection. The fact that anti-EBNA had not appeared by the end of the second year after recovery suggests that if anti-EBNA is absent in the presence of anti-VCA-IgG, the time of infection may vary between broad limits.

A positive P-B-D reaction for heterophil antibodies is suggestive of an acute EBV infection. The MITEST reaction should be evaluated cautiously because it may occasionally remain positive for months. Similar experiences have been published for other methods based on horse erythrocyte agglutination [1].

REFERENCES

- ANDIMAN, W. A.: The Epstein-Barr virus and EB virus infections in childhood. *J. Pediat.* **95**, 171 (1979).
- BIGGAR, R. J., HENLE, G., BÖCKER, J., LENNETTE, E. T., FLEISHER, G., HENLE, W.: Primary Epstein-Barr virus infections in African infants. II. Clinical and serological observations during seroconversion. *Int. J. Cancer* **22**, 244 (1978).
- EPSTEIN, M. A., ACHONG, B. G., BARR, Y. M., ZAJAC, B., HENLE, G., HENLE, W.: Morphological and virological investigations on cultured Burkitt tumor lymphoblasts (strain Raji). *J. nat. Cancer Inst.* **37**, 547 (1966).
- FLEISHER, G., HENLE, W., HENLE, G., LENNETTE, E. T., BIGGAR, R. J.: Primary infection with Epstein-Barr virus in infants in the United States: clinical and serologic observations. *J. infect. Dis.* **139**, 553 (1979).
- FLEISHER, G., LENNETTE, E. T., HENLE, G., HENLE, W.: Incidence of heterophil antibody responses in children with infectious mononucleosis. *J. Pediat.* **94**, 723 (1979).
- GERGELY L., CZEGLÉDI J., SZALKA A., VÁCZI L., BINDER L.: Epstein-Barr virus and cytomegalovirus antibodies in infectious mononucleosis. *Acta microbiol. Acad. Sci. hung.* **24**, 13 (1977).
- GINSBURG, C. M., HENLE, W., HENLE, G., HORWITZ, C. A.: Infectious mononucleosis in children. *J. Amer. med. Ass.* **237**, 781 (1977).
- HENLE, W., GUERRA, A., HENLE, G.: False negative and prozone reactions in tests for antibodies to Epstein-Barr virus-associated nuclear antigen. *Int. J. Cancer* **13**, 751 (1974).
- HENLE, G., HENLE, W.: The virus as the etiologic agent of infectious mononucleosis in: The Epstein-Barr virus. Ed. Epstein, M. A., Achong, G. B. Springer-Verlag, Berlin-Heidelberg-New York 1979 p. 297.
- HENLE, W., HENLE, G., HORWITZ, C. A.: Epstein-Barr virus specific diagnostic tests in infectious mononucleosis. *Hum. Path* **5**, 551 (1974).
- MILLER, G., SHOPE, TH., LISCO, H., STITT, D., LIPMAN, M.: Epstein-Barr virus transformation, cytopathic changes, and viral antigens in squirrel monkey and marmoset leukocytes. *Proc. nat. Acad. Sci. (Wash.)* **69**, 383 (1972).
- NIKOSKELAINEN, J., LEIKOLA, J., KLEMOLA, E.: IgM antibodies specific for Epstein-Barr virus in infectious mononucleosis without heterophil antibodies. *Brit. med. J.* **4**, 72 (1974).
- PELLER, P., GOETZ, I.: Die Diagnose von Epstein-Barr-Virus-Infektionen mit dem IgM - Immunfluoreszenztest. *Mscr. Kinderheilk.* **127**, 728 (1979).
- RAPP, C. E., HEWETSON, J. F.: Infectious mononucleosis and the Epstein-Barr virus. *Amer. J. Dis. Child.* **132**, 78 (1978).
- REEDMAN, B. M., KLEIN, G.: Cellular localization of an Epstein-Barr virus (EBV) associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int. J. Cancer* **11**, 499 (1973).
- SCHMITZ, H., VOLZ, D., KRAINICK-RIECHERT, C., SCHERER, M.: Acute Epstein-Barr virus infections in child-

- ren. Med. Microbiol. Immunol. **158**, 58 (1972).
17. SIMONOVÁ, I., ZÁVADOVÁ, H., VONKA, V.: Differential expression of D and R components of Epstein-Barr virus early antigen after superinfection and after induction with 5-Iododeoxyuridine. *Acta virol.* **21**, 184 (1977).
18. SUMAYA, C. V.: Primary Epstein-Barr virus infections in children. *Pediatrics* **59**, 16 (1977).
19. TAMIR, D., BENDERLY, A., LEVI, J., BEN-PORATH, E., VONSOVER, A.: Infectious mononucleosis and Epstein-Barr virus in childhood. *Pediatrics* **53**, 330 (1974).

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