IgM Antibodies Specific for Epstein-Barr Virus in Infectious Mononucleosis without Heterophil Antibodies

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Summary

IgM antibodies specific for Epstein-Barr (E.B.) virus were demonstrable in all but one out of 46 patients diagnosed as having infectious mononucleosis wihout heterophil antibodies; cytomegalovirus aetiology was excluded. In all but two cases the highest titre was found in the first sample. In 21 patients a significant decrease was seen within a few weeks. IgG antibodies to E.B. virus, mostly remaining at a constant level, were demonstrable in all cases. IgM antibodies to E.B. virus were found in only five out of 300 controls.

The results suggest that in a disease similar to infectious mononucleosis without heterophil antibodies testing of transient E.B. virus-specific IgM antibodies makes a rapid aetiological diagnosis possible and that in clinically well-defined cases viruses other than E.B. virus and cytomegalovirus are unlikely to be causal agents.

Introduction

Abundant data indicate that the Epstein-Barr (E.B.) virus is the cause of infectious mononucleosis. Tests for heterophil antibodies are positive in about 80-90% of patients with clinical and haematological symptoms of this disease. In keeping with previous clinical and epidemiological observations (Evans, 1960) tests for specific antibodies to E.B. virus suggest that most cases without heterophil antibodies are also caused by E.B. virus (Niederman *et al.*, 1968; Evans *et al.*, 1968; Klemola *et al.*, 1970). Cytomegalovirus can also give rise, especially in adults, to an illness clinically indistinguishable from infectious mononucleosis but without a raised heterophil antibody titre (Klemola and Kääriäinen, 1965; Klemola, 1973). In some patients with a disease similar to infectious mononucleosis without heterophil antibodies, however, the aetiology has remained obscure (Evans *et al.*, 1968; Banatvala and Grylls, 1969; Klemola *et al.*, 1970).

In clinical practice tests for fluorescent IgG antibodies to E.B. virus are of little value for specific diagnosis of infectious mononucleosis since increases in titre seldom occur during the clinical illness and by the age of 20 most people have antibodies to E.B. virus. Unlike IgG antibodies IgM antibodies disappear soon after the disease and therefore make specific diagnosis possible (Hampar *et al.*, 1971; Banatvala *et al.*, 1972; Henle, 1973). Recently tests for IgM antibodies to E.B. virus suitable for routine use have been developed (Schmitz and Sherer, 1972; Nikoskelainen, 1973).

Using an E.B. virus-specific IgM test we studied the involvement of this virus in the aetiology of diseases with clinical and haematological features of infectious mononucleosis that do not give a positive heterophil antibody test result by the Davidsohn modification of the Paul-Bunnell reaction. Since there is no general agreement about when the heterophil antibody test result is to be considered positive these antibodies were assayed by other techniques too.

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Patients and Methods

PATIENTS WITH INFECTIOUS MONONUCLEOSIS

A prospective study on infectious mononucleosis was begun at the Aurora Hospital in 1965. During the subsequent nine-year period 517 patients (269 men and 248 women) were diagnosed as having infectious mononucleosis (table I). These patients had an acute febrile disease without an apparent cause and associated with relative and absolute lymphocytosis and with abundant atypical lymphocytes persisting more than a few days. Most—90%—of the patients had the pharyngeal type of the disease, 5% had the glandular, and 5% had the typhoidal. None had been given blood transfusions before the onset of the disease. Serum samples taken on admission, during the acute phase, and after the recovery were stored at −20°C.

TABLE I—*Result of Heterophil Agglutination Test according to Age in 517 Patients with Infectious Mononucleosis*

Age (Years)	Result of Heterophil Agglutination Test					
	Positive		Negative		Total	
	No.	%	No.	%	No.	%
≤14	78	15·1	33	6·4	111	21·5
≥15	364	70·4	42	8·1	406	78·5
Total	442	85·5	75	14·5	517	100·0

TABLE II—*Clinical Type of Disease according to Age in 46 Patients with Infectious Mononucleosis without Heterophil Antibodies*

Age (Years)	Clinical Type of Disease			
	Pharyngeal	Glandular	Typhoidal	Total
≤14	11			11
≥15	29	3	3	35
Total	40	3	3	46

Tests for antibodies to cytomegalovirus were performed in all patients who were negative for heterophil antibodies and in those with glandular or typhoidal infectious mononucleosis. The latter were also tested for antibodies to toxoplasma. Since 1965 36 patients were diagnosed as having cytomegalovirus mononucleosis and another two patients, both with the typhoidal type of the disease, had a double infection with cytomegalovirus and toxoplasma. These 38 patients were not included in the series.

In 75 (14·5%) of the 517 patients tests for heterophil antibodies were repeatedly negative for three to six weeks (titre < 1/32 after guinea-pig kidney absorption according to the procedure of Davidsohn). The present series included 46 (26

males and 20 females) of these 75 patients. We had at least two serum specimens from 39 patients and one specimen taken during the febrile period from seven patients (table II). Of these patients 19 were included in a previous study (Klemola *et al.*, 1970). Suitable samples were not available from the rest of the patients negative for heterophil antibodies.

CONTROLS

The control series comprised 300 patients of whom 50 were blood donors and 250 were patients, mostly adults, with acute infectious diseases of miscellaneous aetiology—for example 77 patients with viral hepatitis, 20 with cytomegalovirus mononucleosis, patients with rubella, varicella, measles, mumps, mycoplasma infection, upper respiratory tract illness, pneumonia, salmonella infection, and malaria. All subjects were tested for IgG and IgM antibodies to E.B. virus and for heterophil antibodies in those instances where E.B. virus-specific IgM antibodies were demonstrable. A number of serum samples positive for heterophil antibodies from patients with infectious mononucleosis were randomly included in the control series when the sera were tested for antibodies to E.B. virus. They were all positive for both IgG and IgM antibodies to E.B. virus.

TESTS FOR IgM ANTIBODIES TO E.B. VIRUS

The indirect immunofluorescence test was a modification of the Henle technique (Henle and Henle, 1966) designed to determine IgG antibodies to viral capsid antigens of E.B. virus (Hopsu-Havu and Nikoskelainen, 1972). The Burkitt cell line P3HR-1 was used as antigen. For details of cultivation technique see Nikoskelainen *et al.* (1974).

TESTS FOR IgM ANTIBODIES TO E.B. VIRUS

The same cell line as in the IgG antibody tests was used as antigen in tests for IgM antibodies. Serum dilutions (1/10 to 1/80) to be tested were incubated with Burkitt cell slides for three hours at room temperature (instead of 45 minutes as in the IgG antibody test). The long incubation time seems to be essential for the detection of E.B. virus-specific IgM antibodies (Nikoskelainen, 1973; Nikoskelainen and Hänninen, 1973). After washing the slides were overlaid with anti-human IgM conjugate (Burroughs Wellcome, London) and incubated for 45 minutes. The results were read in the same way as for IgG antibody titration.

SPECIFICITY OF THE IgM TEST

Rheumatoid factors can give false-positive IgM fluorescence when present in serum simultaneously with specific IgG antibodies (Fraser *et al.*, 1971). Therefore, every serum with positive result in the E.B. virus-specific IgM fluorescence test was examined with latex slide agglutination test (R.A.-test, Hyland, Los Angeles). The sera containing rheumatoid factor were absorbed with heat-aggregated human γ-globulin at 4°C overnight (Shirodaria *et al.*, 1973). The latex test result is rendered negative by this procedure.

TESTS FOR HETEROPHIL ANTIBODIES

Initially the sera from all 517 patients were tested for heterophil antibody by sheep red blood cell agglutination after guinea-pig kidney absorption (Davidsohn and Lee, 1964). The tests were done in tubes and beef erythrocyte absorption was not performed at that time. Titres of 1:32 or higher were considered positive.

The sera with titres of 1/16 or less from 46 patients were tested by three techniques: sheep red blood cell agglutination after guinea-pig kidney absorption; horse red blood cell agglutination after guinea-pig kidney absorption; and horse red blood cell agglutination after absorption with papain-treated sheep erythrocytes (Davidsohn and Lee, 1964; Lee *et al.*, 1968). In each instance simultaneous absorption with beef erythrocytes was performed. The absorptions were done in tubes for three hours at room temperature and the agglutination experiments were performed on Microtiter plates. After four hours of incubation at room temperature the plates were shaken vigorously and read. Preliminary experiments had shown that this micromodification of the Paul-Bunnell-Davidsohn technique was about as sensitive as the conventional tube test.

Results

All 46 patients with infectious mononucleosis without heterophil antibodies had IgG antibodies to E.B. virus. In most cases the relatively high titre of the first sample remained at a constant level in subsequent specimens. Only two patients had a fourfold rise in IgG antibody levels, and they also had a clear IgM antibody response.

All but one of the patients had E.B. virus-specific IgM antibodies. A clear decrease in the IgM titres was seen in 15 of the 31 patients from whom only two successive samples were available; One patient had a seroconversion in IgM antibodies (fig. 1). Six of the eight patients from whom three or more serum samples were available had a decrease in IgM titre (fig. 2) and whereas one had seroconversion. From seven patients there was enough serum in only one sample, but in each instance we found IgM antibodies to E.B. virus. Three of the 46 patients had a positive latex test result; aggregated human γ-globulin rendered it negative but did not affect the IgM fluorescence to E.B. virus. Most sera were titrated only up to 1/80 when tested for IgM antibodies to E.B. virus since this titre was considered clearly positive. Some samples were further diluted and the maximum titre seen was 1/1,280.

One patient did not have IgM antibodies to E.B. virus in two samples taken six and 18 days after the onset of the illness. She was 15 years old and had a typical clinical picture of infectious mononucleosis. The maximam leucocyte count was 21,000/mm³ and 72% were lymphocytes with abundant atypical forms.

In 10 of the 300 control sera IgM fluorescent to E.B. virus was seen. This disappeared from five sera after absorption

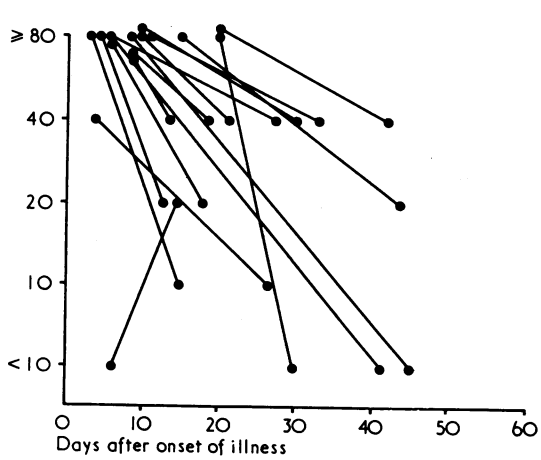

FIG. 1—IgM antibody titres to E.B. virus in two successive serum samples from 16 patients. The 15 cases without titre changes are not included.

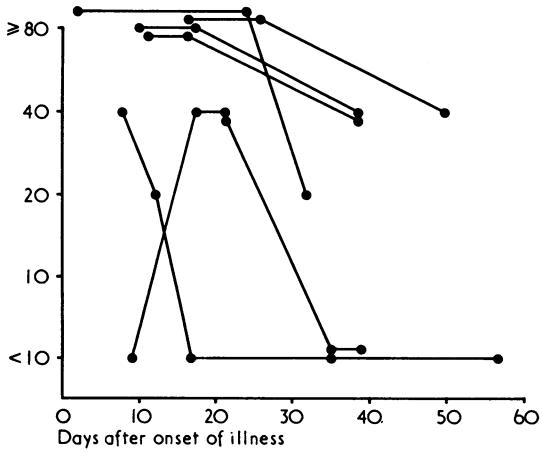

FIG. 2—IgM antibody titres to E.B. virus in sera of seven patients with three or more serum samples. One case without titre change is not included.

with aggregated γ-globulin. Only one of these sera gave a positive result in the latex test. Of the remaining five patients with IgM fluorescence to E.B. virus after absorption three had cytomegalovirus mononucleosis (with titres of IgM antibodies to E.B. virus 1/80, 1/20 and 1/20), one had hepatitis A (with a titre of 1/20), and one was a healthy blood donor (with the titre of 1/20). Only one of these patients had a positive latex test result.

HAEMAGGLUTINATION EXPERIMENTS

Altogether 94 serum samples from the 46 patients of the present series were carefully retested for sheep and horse red blood cell agglutination after differential absorptions. With sheep erythrocytes the sera from 33 patients had a titre of 1/16 or less after absorption with guinea-pig kidney, in eight cases at least one serum sample had a titre of 1/32, and in five cases the titre was 1/64. In these instances beef erythrocytes were able to remove the sheep agglutinins in at least three tubes.

When horse erythrocytes were used instead of sheep red blood cells only 14 cases had a titre of 1/16 or less after guinea-pig kidney absorption and in 26 patients the titre was 1/64 or more. Again, beef erythrocytes reduced the titre by at least three twofold dilution steps. If guinea-pig kidney was replaced by papain-treated sheep erythrocytes as absorbent the results were similar except that two additional patients had titres of 1/64 and one patient a titre of 1/32.

The sera from two patients had relatively high titred horse agglutinins (1/128 and 1/256) even after absorption with guinea-pig kidney or papain-treated sheep erythrocytes, but differential absorption with beef erythrocytes reduced the titres in only two tubes. One of these patients was the only one in whom no IgM antibodies to E.B. virus were found.

The haemagglutination experiments with horse red blood cells were also done on 20 serum samples from patients with cytomegalovirus mononucleosis and on 77 sera from viral hepatitis patients. Only one of the former group and none of the latter had a titre exceeding 1/16 after guinea-pig kidney absorption, and two hepatitis sera gave a titre of 1/32 after absorption with papain-treated sheep erythrocytes.

Discussion

Generally two laboratory criteria should be fulfilled before a diagnosis of infectious mononucleosis is made: there should be relative and absolute lymphocytosis in the peripheral blood with abundant atypical forms of long duration and heterophil antibodies to sheep or horse erythrocytes should be present in abnormally high concentrations. Thus, a disease similar to infectious mononucleosis without a positive heterophil antibody test result is a special diagnostic problem and, as Evans (1972) states, a challenge to the virologists and clinicians. The clinical presentation and the haematological findings are all the more important because in many infectious diseases there is a relative increase in the number of peripheral lymphocytes which to a certain degree may be transiently atypical. All the 46 patients of the present series were carefully examined both clinically and haematologically, and cytomegalovirus as well as toxoplasma were excluded as possible aetiological agents before the diagnosis was made.

All patients with infectious mononucleosis with heterophil antibodies have transient IgM antibodies to E.B. virus which as a rule disappear within two to three months (Nikoskelainen, 1973; Nikoskelainen and Hänninen, 1973). When we used the same technique in our study of 46 patients who in the initial serological tests did not have specific sheep erythrocyte agglutinins in titres over 1/16 all but one had IgM antibodies to E.B. virus. The transient character of these antibodies was demonstrable in 21 patients. In the control series a specific IgM fluorescence to E.B. virus was demonstrable in only five cases, a finding that naturally may have been due to a recent or current infection with E.B. virus.

The specificity of the fluorescence test is of great importance. Antiglobulins in the serum to be tested may react with IgG antibodies to E.B. virus and, since the antiglobulins themselves are mostly of the IgM class, they give a false-positive result when the fluorescent anti-IgM is added. We found this false-positive IgM fluorescence, which could be removed by absorption with aggregated human γ-globulin, in five of our control sera.

In another five of the control sera of the present series the IgM fluorescence could not be absorbed with γ-globulin—that is, it seemed to be specific. Interestingly enough, three of them were samples from patients with cytomegalovirus mononucleosis. One of the three had a high IgM antibody titre (over 1/80) to E.B. virus. The sera of this patient had a maximal agglutination titre of 1/64 with horse erythrocytes, and the agglutinins were completely removable with beef erythrocytes but not with guinea-pig kidney—that is, the sera contained a typical heterophil antibody. Since there was virological and serological evidence of cytomegalovirus infection this patient apparently had two viral infections simultaneously. IgM antibodies to E.B. virus have been suggested to cross-react with other herpes viruses, especially with cytomegalovirus (Schmitz and Scherer, 1972). The weak IgM fluorescence seen in the sera of two patients with cytomegalovirus mononucleosis may have been due to this phenomenon.

The sensitivity of the fluorescence technique is dependent on the nature of the antigen and on the methods and equipment used in the test. We used interference filters in the fluorescent microscope. They are known to enhance the sensitivity twofold to fourfold as compared with conventional glass filters (Nikoskelainen, 1973). In addition, the P3HR-1 line may contain more specific antigenic determinants than some other cell lines. These points may explain why five of the sera that gave strong IgM and IgG fluorescence in the present study had very low or absent IgG titres in a previous investigation (Klemola *et al.*, 1970). At that time glass filters and the E.B. 3 line were used.

Some patients with infectious monocleosis either have no heterophil antibodies to sheep red blood cells or the titres are low. Using sheep red cells in the agglutination test and an absorption with guinea-pig kidney we found that 30% of the children and 10% of the adults had a titre of 1/16 or less, which was considered negative. When the negative sera were carefully retested some of the borderline cases had a

slightly higher titre, probably reflecting the usual laboratory errors. Of these negative sera, however, more than half had considerable titres (1/64) with horse red blood cells. In one case the sheep agglutinin titre was less than 1/4 and the horse agglutinin titre was 1/512. This is in accordance with the findings of Lee *et al.* (1968). We think that every case that clinically resembles infectious mononucleosis should be tested with horse erythrocytes and differential absorptions if the classical Paul-Bunnell-Davidsohn test is negative.

Our results indicate that testing for E.B. virus-specific IgM antibodies is of great value for the aetiological diagnosis in diseases resembling infectious mononucleosis but without a positive heterophil antibody test result. One of the great advantages of this IgM test is that diagnosis as a rule can be made from a single sample. Viruses other than E.B. virus and cytomegalo-virus seem unlikely to be causal agents in diseases similar to infectious mononucleosis without heterophil antibodies, provided that the diagnosis is based on well defined clinical and haematological criteria.

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References

Banatvala, J. E., and Grylls, S. G. (1969). *British Medical Journal*, **3**, 444.
Banatvala, J. E., Best, J. M., and Waller, D. K. (1972). *Lancet*, **1**, 1205.
Davidsohn, I., and Lee, C. L. (1964). *American Journal of Clinical Pathology*, **41**, 115.
Evans, A. S. (1960). *American Journal of Hygiene*, **71**, 342.
Evans, A. S., Niederman, J. C., and McCollum, R. W. (1968). *New England Journal of Medicine*, **279**, 1121.
Evans, A. S. (1972). *New England Journal of Medicine*, **286**, 836.
Fraser, K. B., Shirodaria, P. V., and Stanford, C. F. (1971). *British Medical Journal*, **3**, 707.
Hampar, B., *et al.* (1971). *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 1407.
Henle, G., and Henle, W. (1966). *Journal of Bacteriology*, **91**, 1248.
Henle, W., and Henle, G. (1973). *New England Journal of Medicine*, **288**, 263.
Hopsu-Havu, V. K., and Nikoskelainen, J. (1972). *Acta Dermatovenereologia*, **52**, 346.
Klemola, E., and Kääriäinen, L. (1965). *British Medical Journal*, **2**, 1099.
Klemola, E., *et al.* (1970). *Journal of Infectious Diseases*, **121**, 608.
Klemola, E. (1973). *Annals of Internal Medicine*, **79**, 267.
Lee, C. L., Zandrew, F., and Davidsohn, I. (1968). *Journal of Clinical Pathology*, **21**, 631.
Niederman, J. C., *et al.* (1968). *Journal of the American Medical Association*, **203**, 205.
Nikoskelainen, J., and Hänninen, P. (1973). *Scandinavian Journal of Clinical and Laboratory Investigation*, **31**, Suppl. No. 130, p. 32.
Nikoskelainen, J. (1973). *Serological and Epidemiological Studies on Epstein-Barr Virus Infections*, Academic Dissertation, Turku.
Nikoskelainen, J., Hannuksela, M., and Palva, T. (1974). *Scandinavian Journal of Infectious Diseases*. In press.
Schmitz, H., and Scherer, M. (1972). *Archiv für die gesamte Virusforschung*, **37**, 332.
Shirodaria, P. V., Fraser, K. B., and Stanford, F. (1973). *Annals of Rheumatic Diseases*, **32**, 53.

Anaesthesia during Raised Creatine Phosphokinase Activity

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Summary

No adverse effects were observed in a series of patients in whom he levels of creatine phosphokinase (C.P.K.) were known to be raised and who received anaesthetics. The need to exercise caution in the interpretation of screening test results for C.P.K. activity before anaesthesia is stressed.

Introduction

The association of malignant hyperpyrexia during anaesthesia and increased creatine phosphokinase (C.P.K.) activity has been reported (Ellis *et al.*, 1972; Isaacs and Barlow, 1970). Most of the patients described with this condition have received suxamethonium together with a volatile anaesthetic such as halothane. Great increases have also been noted in the level of serum aspartate aminotransferase and serum alanine aminotransferase during the course of hyperpyrexia in these cases. Studies to establish a familial basis for this disorder (Isaacs and Barlow, 1970) have left some doubt as to the aetiology of the condition. It has been shown (Gosling *et al.*, 1972; Meltzer *et al.*, 1969) that raised levels of C.P.K. may occur during the early stages of psychotic illnesses. There is some evidence to suggest that there might be some underlying myopathy in patients with raised C.P.K. (Meltzer and Moline, 1970; Denborough *et al.*, 1970). The role of hyperpyrexia and the resultant change in cell permeability makes interpretation of enzyme studies difficult. Screening for raised C.P.K. levels before operation has been discussed by Ellis *et al.* (1972). The number of cases in which an anaesthetic has been administered to patients known to have raised C.P.K. activity is very small. We report here a group of patients who received anaesthetics without untoward effect on several occasions when their C.P.K. levels were known to be grossly raised. They were suffering from affective disorders. Daily C.P.K. measurements were carried out as part of their psychiatric investigation. Their uneventful course under anaesthetics suggests that raised C.P.K. activity is not always associated with anaesthetic complications.

Patients and Methods

Ten female patients were anaesthetized at times when they had raised C.P.K. activity (no male patients were included as the C.P.K. data was available only on female patients). They were all receiving electric convulsion therapy for affective disorders. Altogether anaesthetics were given to patients with raised serum C.P.K. activity on 28 occasions. The C.P.K. levels were between 200 and 400 mU/ml.

The same drugs and techniques were used for all anaesthetics. The premedication was 0·6 mg of atropine sulphate given intramuscularly one hour before the anaesthetic.

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