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CONTRIBUTION TO RAPID DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS

V. FRAŇKOVÁ, *M. STAŇKOVÁ, *K. KOUBA, V. HÁSKOVÁ, E. PAVLÍK

Institute for Medical Microbiology and Immunology, Faculty of Medicine, Charles University, 128 00 Prague,
and *III Clinic of Infectious Diseases, Faculty of Medicine, Charles University, Prague, Czechoslovakia

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Summary. — By indirect immunofluorescence (IF) technique humoral antibodies to Epstein-Barr virus capsid antigen (EB--VCA) and to cytomegalovirus (CMV) were detected in 47% and 9% of persons with infectious mononucleosis (IM), respectively. In 23% of the patients examined, IgM antibodies to both viruses were detected, while in 8% of them high titres of IgG only were found in the absence of IgM class antibodies to EB-VCA or to CMV. The finding of IgM antibody to EB-VCA was in good correlation with the persisting symptoms of the disease. Discrepancy between the presence of specific IgM and the absence of heterophilic antibodies was observed in some children and in all persons with persistent or recurrent signs of IM. In the latter, specific IgM was found only during exacerbation of the disease, but during remissions IgG antibodies persisted in high levels. Antibodies to Epstein-Barr virus nuclear antigen (EBNA) were detected in all chronically ill persons and antibodies to the R-component of Epstein-Barr virus early antigen (EA) were present in the majority of them.

Key words: infectious mononucleosis; Epstein-Barr virus; cytomegalovirus; rapid virus diagnosis; chronic EBV infection

Introduction

The symptoms of IM are most often caused by Epstein-Barr virus (EBV) or CMV infections. Both viruses exert an immunosuppressive effect (Magni *et al.*, 1974; Carney and Hirsh, 1981). Small surgery or contemporary bacterial or viral infection in the course of unrecognized IM can lead to unexpected and serious consequences (Fanta *et al.*, 1984). Hence, the rapid laboratory diagnosis of IM is of substantial importance. The subject of this paper are the routine diagnostic results on the detection of IgM and IgG class antibodies to EBV and CMV in sera of patients with IM.

Materials and Methods

IgM and IgG class antibodies to EB-VCA and to CMV were detected in single serum samples of 448 persons with IM. In 54 individuals we investigated the development of the antibody response to EBV in correlation with the heterophilic antibodies and recession of clinical signs. Two to seven serum samples were obtained during 2—9 months since onset of the illness. Six persons with signs of chronic infection were under observation for more than one year. Sera of these patients and control sera of 10 persons with acute primary EBV infection and of 10 blood donors, selected at random, were examined for antibodies to EBV-EA and to EBNA (Henle *et al.*, 1971; 1974*a*).

Altogether 667 serum samples were investigated by indirect IF. Before examination, each serum was inactivated and rheumatoid factor removed by absorption to polyacrylamide gel-bound human IgG (Fraňková *et al.*, 1985).

For detection of antibodies to EB-VCA we used acetone fixed P3HR1 cells induced to increase expression of EB-VCA by n-butyrate (Luka *et al.*, 1979). Substrates for detection of antibodies to CMV were prepared according to Hanshaw *et al.* (1968). For detection of different Ig classes the commercial conjugates SwAHu/IgM and SwAHu/IgG (Sevac, Praha) were used. Staining by the sera and conjugates was performed in wet chamber at 37 °C for 30 min. For detection of IgM the incubation time was prolonged to 60 min. The preparations were counterstained with Evans blue, mounted into Tris-buffered glycerine pH 8 (1 part of 0.1 mol/l Tris-HCl buffer mixed with 3 parts of glycerine) and viewed in Fluoval 2 (Zeiss, Jena) microscope.

Antibodies to EB-VCA were determined according to specific IF of the whole cell surface of about 10% at random distributed cells within the fixed sediment). The assessment of antibodies to CMV was based on the presence of specific IF of the cytoplasmic or nuclear membranes, of the typical intranuclear and perinuclear inclusions, or on IF of whole infected cell surfaces as described by Horodniceanu and Michelson (1980). Nonspecific IF caused by the Fc-receptors was readily distinguished and disregarded. Heterophilic antibodies were detected in parallel by agglutination of horse erythrocytes and by the classical Paul-Bunnell reaction (PBR) (Davidsohn and Lee, 1964).

Antibodies to EBV-EA were determined by means of indirect IF (Henle *et al.*, 1971). Acetone- or methanol-fixed Raji cells induced by TPA (4-beta-phorbol 12-myristrate 13-acetate) to higher expression of EA were stained using the commercial conjugate SwAHu/Ig-FITC (Sevac, Praha). The R-component of EBV-EA was recognized by its selective destruction after methanol fixation. The complement-fixation reaction (CFR) with soluble antigen prepared from non-producer lines of human lymphoblastoid cells (SAL, Sevac, Praha) was performed for detection of anti-EBNA antibodies (Vonka *et al.*, 1972).

Results

At examination of single serum samples of IM patients, IgM antibodies to EB-VCA were found in 210 persons. In further titrations the IgM antibody levels ranged from 1 : 6 to 1 : 384. Titres 24 and 48 were the most common. In the same sera IgG antibodies to EB-VCA were regularly found in titres from 48 to 1536 or higher. In 39 patients specific IgM and IgG antibodies to CMV were detected. The titres of IgM did nor usually exceed 12, but in sporadic cases were as high as 96. The titres of IgG antibodies to CMV were 192 or higher in all sera containing relevant specific IgM. Sera of 103 persons contained IgM and high levels of IgG antibodies to both EB-VCA and CMV. In 58 patients antibodies to both EB-VCA and CMV were either absent, or their sera contained only IgG antibodies in titres not exceeding 192. High levels of IgG to EB-VCA and/or to CMV were found in sera of 34 and 4 patients, respectively, in the absence of relevant IgM antibodies. These results and our diagnostic conclusions are summarized in Table 1.

Table 1. Rapid diagnosis of IM in 448 patients by examination of single serum samples

Antibodies	Sample	Per cent	Conclusion
IgM and IgG to EB–VCA	210	46.8	Active EBV infection
IgM and IgG to CMV	39	8.7	Active CMV infection
IgM and IgG both to EB–VCA and CMV	103	22.9	Endogenous activation? Double infection?
IgG to EB–VCA (Titre $\geq$ 384)	34	8.4	Recent acute infection? Chronic infection?
IgG to EB-VCA, to CMV or to both (Titre $\leq$ 192)	58	12.9	Infection not confirmed

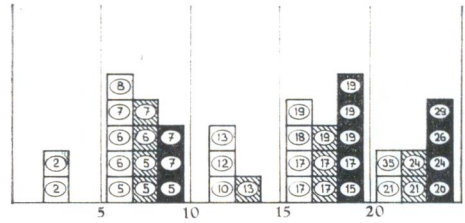
IgM antibodies to EB-VCA and heterophilic antibodies were compared in 54 IM patients. A good agreement in respect to the aetiology of the illness was found in 40 persons, namely in 5 the assays were negative (in 4 of these persons an active CMV infection was confirmed) and in 35 patients both, specific IgM to EB-VCA and heterophilic antibodies were detected. Nevertheless, in 13 patients of this group-specific IgM could be detected 3 weeks to 1 month longer than the heterophilic antibodies, thus it correlated better with the persisting clinical signs of the disease. In three persons liver dysfunction was observed after disappearance of specific IgM antibodies. Heterophilic antibodies were never found in the absence of IgM antibodies to EB-VCA. On the other hand, in 14 individuals there was a discrepancy between the finding of IgM to EB-VCA and a negative PBR. The disease and age of these patients are given in Table 2.

Table 2. Characterization of the disease and age of patients with IgM antibodies to EB-VCA in the absence of heterophilic antibody

Characterization of the disease	No. of patients	Age of individual patients (years)	Average age (years)
Acute IM (normal course)	8	13, 6, 8, 14 13, 12, 5, 2	9
Recurrence of IM 5 months after primary infection during Salmonellosis	1	5	5
Exacerbation of chronic recurrent EBV infection*	5	26, 29, 19, 19, 32	25

* Confirmed by subsequent long-term investigation

Fig. 1.
Persistence of IgM to EB-VCA in different age groups of 38 patients with IM
White squares: Individual with specific IgM detectable for less than1 month after the onset of IM
Striped squares: Subjects with specific IgM detectable for 1—3 months since the beginning of IM.
Black squares: Subjects revealing specific IgM antibody levels for longer than 3 months since the beginning of IM.
The age of individual patients given in years.



The 38 patients with confirmed EBV infection in whom precise information on the onset of IM symptoms was available, were investigated for IgM antibody persistence. The results are summarized in Table 3. The relation to the patients' age is shown in Fig. 1.

In six persons investigated, reappearance of IM signs (lymphadenitis tonsilitis, low-grade fever) was observed. As seen from the results of repeated examinations given in Tables 4 and 5, IgM antibodies to EB-VCA were found for a prolonged time or the IgM antibodies were alternatively positive or negative in accordance with the exacerbation or recession of symptoms. In patients showing chronic symptoms high IgG antibody levels exceeding the titre of 192 were found for a prolonged period.

Investigations of antibodies to CMV in persons with chronic illness revealed the following results: in one patient (No. 1, Table 5), an invariably low level of anamnestic IgG (1 : 12—1 : 24) was found. In three patients (Nos 2, 3 and 6, Table 5) the IgG titres to CMV exceeded 192 in all examined sera. In the next two chronic patients no antibodies could be detected in the earlier period of their disease. Later exacerbation of the signs of IM with reappearance of IgM antibodes to EB-VCA was connected with seroconversion to CMV, accompanied by appearance of IgM and with high titre of IgG antibody to the latter virus.

Examinations for antibodies to EBV-associated nonstructural antigens EA and EBNA, which were conducted in six persons with prolonged or

Table 3. The persistence of IgM antibodies to EB-VCA after the onset of IM (38 persons examined)

Period	No. of patients	Per cent	Average age (years)	No. of parallel CMV IgM findings
1 month	16	42	12:4	0
2—3 months	13	34	14:3	4
More than 3 months	9	24	17:3	5

Table 4. The dynamics of antibody response to chronic EBV infection in the patient H. R. (Clinical signs: Recurrent tonsillitis, lymphadenitis, arthritis, fever, drug allergy)

Examination date	Reciprocal titre of EB–VCA antibody IgM/IgG	Reciprocal titre of CMV IgG*
May 17, 1982	48/> 1536	384
June 22, 1982	6/384	192
September 6, 1982	384/1536	not done
October 25, 1982	192/768	192
February 1, 1983	12/384	192
March 15, 1983	192/768	192
October 25, 1983	6/1536	96
November 14, 1983	48/768	96
December 6, 1983**	48/768	192
January 24, 1984	6/> 1536	192
March 20, 1984	6/768	384
October 16, 1984	6/384	192

* CMV IgM was never found in this patient
** Antibodies to EA (anti-R) and to EBNA were found in a titre of 32 and 64, respectively.

recurrent signs of IM, as well as in a control group of patients with usual course of IM and in a group of healthy blood donors are summarized in Table 6.

Discussion

In routine diagnostic work indirect IF or enzyme-linked immunosorbent assay (ELISA) are the most convenient techniques for the detection of different class antiviral antibodies. Because the preparation of standard purified antigens determinates the specificity and sensitivity of ELISA tests (Schmitz *et al.*, 1978), most routine diagnostic laboratories are dependent on expensive commercial sets. On the other hand the preparation of antigen-containing substrates for the detection of antiviral antibodies by means of indirect IF is accessible even to modestly equipped laboratories. The method is highly sensitive (Gerna *et al.*, 1976) and in our experience it seemed more convenient for prompt examination of single serum samples than the ELISA technique. As follows from our results, summarized in Table 1, the investigation of specific IgM and IgG antibodies to EB-VCA or to CMV with use of indirect IF in single serum samples of IM patients confirmed or excluded active EBV or CMV infections in nearly 70% of the persons examined. In the rest of them the interpretation of single examination results was not final.

For better understanding of the significance of these results, we compared the IgM anti-EB-VCA results with the positivity of PBR and correlated them with the duration of clinical signs in a group of repeatedly examined patients. In most of them, specific IgM and heterophilic antibodies were detectable over the period of persisting signs of IM, but in 1/3 of these patients, the positivity of PBR receded earlier than the specific IgM. This

Table 5. The results of long-term investigation of antibody response to EBV in persons with prolonged or recurrent signs of IM

Patient No., birth, interval of investigation (month/year)	IgM to VCA present (month/year)	IgM to VCA absent (month/year)	IgG to VCA in a titre $\geqq$ 384 (month/year)	Ig^{+} titre to EA (Anti-D/Anti-R)	CFR^{+} titre
1, (1945), 4/82–2/85	4/83, 1/84	5–7/84, 2/85	4/83–5/84	< 6/12***	1 : 16
2, (1957), 3/83–3/84	3, 6, 10/83	2, 3/84	3–10/83	6/< 6	1 : 16
3. (1966). 5/83–11/84	5–11/83	12/83 10, 11/84	5–12/83	< 6/6	1 : 64
4, (1964), 12/81–11/83^{++}	12/81, 1/82 11/83^{++}	4, 6, 9/82	12/81–1/83	< 6/6	1 : 16
5, (1932) 3/82–2/85	3–12/82 1–11/83^{++} 2, 3/84, 2/85	2, 8, 9, 12/83 5, 8, 11/84	3/82–2/85	< 6/12	1 : 128
6, (1956) 5/82–10/84	5, 9, 10/82 2, 3, 11, 12/83	6/82, 10/83 1, 3, 10/84	5/82–10/84	< 6/48	1 : 64

$^{+}$ Determined in the last serum with positive IgM to VCA antibodies
$^{++}$ In this sample seroconversion to CMV along with CMV IgM antibodies were detected
*** Dilution reciprocals

Table 6. Antibodies to EA and EBNA in patients with acute and chronic signs of IM and in healthy subjects

Group	No. of persons	No. of persons with antibody to EA		No. of persons with antibody to EBNA
		Anti-D	Anti-R	
Acute primary IM	10	4	2	2
Prolonged or recurrent disease	6	1	4	6
Healthy blood donors	10	0	1	6

may indicate that disagreement between the presence of IgM antibodies to EB-VCA and heterophilic antibodies in some primary EBV infections described already by Henle *et al.* (1974*b*) and noted by many others, might be a consequence of earlier decrease of the heterophilic antibodies.

In our group under study a discrepancy between anti-EB-VCA IgM findings and the absence of heterophilic antibodies was found in 26% of persons. They were either children (in agreement with Schmitz *et al.*, 1972, and many others) or patients with prolonged or recurrent signs of IM. As evident from the literature, other authors did not find heterophilic antibodies in chronic EBV patients after the subsidence of primary acute stage of infection either (Horwitz *et al.*, 1975, Tobi *et al.*, 1983). Thus the specific IgM response is a sign of the activity of infection and does not allow differentiation between primary EBV infection or endogenous activation. On the other hand the presence of heterophilic antibodies is one of the markers of primary EBV infection (Nikoskeleinen *et al.*, 1974).

In accordance with the mild clinical course of IM in most children under age of 15 there was an early decline of specific IgM antibodies, often in the course of the first month after onset of disease. With increasing age of the patients, the illness and the detectability of specific IgM was prolonged, exceeding 3 months in 24% of the patients. In three persons symptoms of liver disorder lasted longer than detectability of IgM antibodies a high level of specific IgG being the only evidence of recent EBV infection in that stage.

It was remarkable that longer persistence of IgM antibodies to EB-VCA and to CMV were concomittantly found in accordance with higher age and prolonged duration of the disease. Both viruses, persisting regularly in the organism after primary infection, may be activated under conditions of immunosuppression (Nagington, 1971; Sumaya, 1977). A mutual activating effect among the Herpesvirus family was observed by Ross and McDavid (1972) and others. The reappearance of anti-EB-VCA IgM antibodies during primary CMV infection in two subjects under long-term observation likewise supports this assumption.

After disappearance of specific IgM a gradual decrease of specific IgG to anamnestic levels was observed in the most of patients (data not shown).

In persons with prolonged or recurrent illness IgG antibodies to EB-VCA were detectable in titres exceeding 192 for many months. Similar observations were made by Horwitz *et al.* (1975) and by Tobi *et al.* (1983) in their chronic patients. Albeit the titres of 320 or higher are considered the proof of recent EBV infection (Henle *et al.*, 1974), the above mentioned observations show that high IgG in a single serum sample does not substantiate the diagnosis of acute IM. This should be confirmed by a later decrease of the specific IgG antibody level.

The results of our preliminary examinations comparing the anti-EA and anti-EBNA antibodies in persons with prolonged illness, acute IM and healthy blood donors are consistent with the earlier experience (Henle *et al.*, 1974; Horwitz *et al.*, 1975) on the secondary importance of anti-EA tests in routine diagnostics. The antibodies to EA, if present, corroborate the diagnosis of recent active EBV infection, but negative results do not exclude it. As evident from our anti-R antibody findings, these may support the suspicion of chronic infection.

The antibodies to EBNA correspond to antibodies to soluble antigen from nonproducer lymphoblastoid lines (Reedman and Klein, 1973; Klein and Vonka, 1974). They can be easily determined by CFR if a proper antigen is used. The accessibility of the test to average laboratories makes it a useful complementary method for rapid diagnosis of IM. The absence of anti-EBNA in sera containing IgM to EB-VCA, or significantly high levels of specific IgG confirm acute or recent primary EBV infection.

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