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ANTI-HUMAN CYTOMEGALOVIRUS NUCLEAR ANTIGEN ANTIBODIES OF DIFFERENT IMMUNOGLOBULIN CLASSES

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Summary. — IgG, IgM and IgA immunoglobulin classes of antibodies to human cytomegalovirus nuclear antigens (CMNA) were studied by the acid-fixed nuclear binding technique (AFNB) and combined anti-complement immunofluorescence (combined ACIF). In acute cases of infectious mononucleosis (IM) of human cytomegalovirus (HCMV) origin and in the so-called double virus infections (HCMV + Epstein-Barr virus), anti-CMNA IgM antibodies were detected. They were absent from both anti-HCMV positive sera of healthy donors and sera of patients suffering of IM caused by EBV used as controls. The presence of anti-CMNA IgM may thus serve as an additional evidence of acute HCMV infection. Non-complement-fixing IgA classes of the anti-CMNA antibodies were not found in some of the sera gathered during the acute phase of IM of EBV origin: in one fourth of the HCMV seropositive donors and in a number of late serum samples. But non-complement-fixing and complement-fixing anti-CMNA components of the IgG class were detected.

Key: words: human cytomegalovirus infections; nuclear antigens; antibodies to nuclear antigens; combined anti-complement immunofluorescence test

Introduction

Cytomegalovirus nuclear antigen (CMNA), the synthesis of which is independent from the DNA and RNA synthesis, can be detected in the nuclei of cells very early after in vitro infection with human cytomegalovirus (HCMV) (Michelson-Fiske *et al.*, 1977; Reynolds, 1978; Ohtsuka *et al.*, 1979). This antigen has a character of a DNA-binding protein (Gergely *et al.*, 1980). The biological role of the CMNA is unknown. Recent findings suggested that this protein modifies host cell chromatin-template activity and structure (Kamata *et al.*, 1978). Immediate early antigens [Epstein-Barr virus- (EBV)-determined nuclear antigen (EBNA)] are also demonstrable in the nuclei of EBV-infected cells (Reedman and Klein, 1973).

Antibodies of the various immunoglobulin classes against EBNA are formed and their presence can be detected by the anti-human globulin-anti-

complement immunofluorescence (combined ACIF) test (Czeglédy *et al.*, 1978).

In the present study we investigated the types of CMNA antibodies occurring in acute HCMV infections and in sera of healthy donors. We employed a recently developed method (Gergely *et al.*, 1980) which proved suitable for detection of complement-fixing and non-complement-fixing CMNA antibodies.

Materials and Methods

Serum samples. The following were used: (*1*) 20 samples from 5 patients free of heterophilic antibodies and possessing anti-CMV IgM; (*2*) 21 samples from 5 patients positive for heterophilic antibody and anti-EBV IgM; (*3*) 15 samples from 3 patients positive both for anti-CMV IgM and anti-EBV IgM. Each of the patients showed typical clinical and haematological symptoms of infectious mononucleosis (IM), and from each 2-3 blood samples were obtained during the acute phase and 1-3 samples during convalescence. We had received all the IM sera from the László Central Hospital for Infectious Diseases, Budapest. (*4*) 30 samples from HCMV seropositive healthy donors (Blood Supply Service, Debrecen). The sera were stored at −20° C until used. Fresh native human serum negative for HCMV antibodies served as source of complement.

Detection of complement-fixing CMNA antibodies by the acid-fixed nuclear binding (AFNB) technique. The method was described (Gergely *et al.*, 1980). The source of the antigen was purified CMNA bound to the acid-fixed nuclei. CMNA was purified by affinity chromatography as described (Gergely *et al.*, 1980). Smears for antibody titration were prepared as follows: Embryonic fibroblast cells were washed and suspended in hypotonic salt solution (0.062 mol/l KCl), kept for 15 min at room temperature and then disrupted in a Dounce homogenizer. The pelleted nuclei were fixed in cold methanol: acetic acid (3 : 1, v/v) for 1 hr. The nucleus suspension was then spread on chilled slides and air-dried. The smears were covered with 20 μl of purified CMNA extract and incubated in a humid chamber for 2 hr at 4° C. During this period, CMNA was bound to the acid-fixed nuclei. The unbound proteins were removed by washing the slides in BSS (0.8% NaCl; 0.014% $CaCl_2$; 0.04% KCl; 0.02% $MgSO_4$, $7H_2O$; 0.06% KH_2PO_4; 0.06% $Na_2HPO_4 . 2\ H_2O$).

Antibody titration was performed in three steps: (*1*) the smears were incubated with various dilutions of the serum at 37° C for 30 min in a humid chamber; (*2*) the preparations were washed and then incubated with human complement (1 : 5) at 37° C for 30 min in a humid chamber; (*3*) after washing, the smears were incubated with a 1 : 20 dilution of fluorescein isothiocyanate (FITC) labelled anti-human complement (Hyland) at 37° C for 30 min in a humid chamber. After washing, one drop of buffered glycerol was added, the smears were covered with cover slips and examined in the same microscope as described below.

Testing for anti-CMNA antibodies of different immunoglobulin classes. The preparation of smears was the same as above. Antibody titration was performed in four phases. (*1*) Smears were incubated with test sera at 37° C for 1 hr, except those serving for demonstration of complement-fixing IgM, which were incubated for 20 hr in a humid chamber; (*2*) smears were washed and incubated with 1 : 50 dilutions of either goat anti-human IgG serum (γ-chain specific) or anti-human IgA serum (α-chain specific) or anti-human IgM serum (u-chain specific) at 37° C for 1 hr in a humid chamber; (*3*) the preparations were washed again and covered with human complement (1 : 10) at 37° C for 1 hr in a humid chamber; (*4*) the preparations were washed, stained with a 1 : 20 dilution of FITC-labelled anti-human complement (Hyland) at 37° C for 20 min, then washed, dried, covered with buffered glycerol and examined in a Leitz Orthoplan UV microscope equipped with a Ploem Opak-illuminator.

The preparations were washed and the reagents diluted with BSS throughout.

Following the proposal of Schmitz *et al.* (1975), anti-CMNA antibodies were bound first and then anti-human IgG (or IgM or IgA) was added in the second phase.

The reasons for including this step are that the method thus (*1*) becomes suitable to detect both complement-fixing and non-complement-fixing antigens, and (*2*) makes possible the detection of antibodies present in a low concentration, since we are demonstrating a major quantity of human complement bound to globulin molecule-CMNA complex.

Table 1. Horizontal study with serum samples from patients with IM of HCMV origin and in double viral infections

Case*	Time of sampling	Anti-CMNA titres			
		AFNB	AFNB + combined ACIF		
			IgM	IgG	IgA
No. 1, 17 yr, M	0 day	< 3	< 3	8	< 3
	8 days	16	8	16	8
	10 months	4	< 3	8	< 3
No. 2, 15 yr, M	0 day	< 3	4	< 3	< 3
	20 days	4	16	3	8
	6 months	32	4	8	8
	10 months	16	3	8	4
No. 3, 25 yr, M	0 day	4	< 3	< 3	< 3
	20 days	32	8	8	4
	5 months	64	4	16	4
	9 months	32	< 3	8	4
No. 4, 14 yr, M	0 day	16	4	< 3	< 3
	19 days	32	4	4	4
	6 months	16	< 3	8	4
	10 months	16	< 3	4	4
No. 5, 24 yr, F	0 day	< 3	8	4	4
	11 days	32	4	16	8
	50 days	16	< 3	32	4
	7 months	8	< 3	16	4
	10 months	8	< 3	8	< 3
No. 6, 20 yr, M	0 day	8	< 3	< 3	3
HAB 512	10 days	16	8	4	4
	9 months	16	< 3	4	3
	14 months	8	<3	4	< 3
No. 7, 22 yr, M	0 day	16	4	4	3
HAb 64	6 days	32	4	16	8
	28 days	32	8	16	8
	5 months	32	<3	8	4
	8 months	16	< 3	4	< 3
No. 8, 20 yr, M	0 day	< 3	< 3	3	< 3
Hab 64	5 days	8	4	4	4
	10 days	16	8	8	4
	31 days	16	8	8	4
	5 months	8	< 3	3	< 3
	11 months	3	< 3	3	< 3

* No., age in years, sex (M — male, F — female,); HAB = titre of heterophil antibody.

Table 2. Horizontal study with serum samples from patients with IM of EBV origin

Case*	Time interval between first and actual sampling	Anti-CMNA titres		
		AFNB	AFNB + combined ACIF IgG	IgA
30 yr, M	0 day	4	4	3
	8 days	4	4	3
	10 months	3	4	3
	14 months	3	4	< 3
19 yr, F	0 day	3	3	
HAb 1024	8 days	3	3	
	26 days	3	3	negative
	6 months	3	3	
	9 months	3	3	
16 yr, M	0 day	8	3	4
HAb. 1024	6 days	8	3	4
	12 days	8	3	4
	1.5 months	8	3	4
	5 months	8	3	4
14 yr, M	0 day	4	3	3
Hab 4096	3 days	4	3	3
	1 month	4	4	4
	3.5 months	3	4	4
22 yr, F	0 day	3	3	3
HAb 512	4 days	3	3	3
	10 days	4	3	3
	4 months	4	4	3
	9 months	3	3	< 3

* See Table 1.
** Tests for IgM were invariably negative.

Results

HCMV antibodies in acute IM of HCMV origin

These patients had already been involved in a series of examination (Gergely *et al.*, 1977) which clearly revealed the aetiology of their disease. We demonstrated the presence of different immunoglobulin classes (IgM, IgG, IgA) of antibodies to CMNA (Table 1).

Components belonging to the IgM immunoglobulin class could only be detected in the acute phase. In 5 of the latest serum samples, the IgA antibodies were not detectable. Antibodies of the IgG type were present in sera taken in the phase of convalescence, but only in low titres.

The anti-CMNA IgG titre reached the maximum relatively late stage, subsequently decreased and since the 10th month probably reamined at a low level. The anti-CMNA IgM and IgA titres reached the highest level at a very early stage, thereafter started to decrease and finally were no more detectable.

Detection of CMNA antibodies in double viral infection (EBV + HCMV)

These cases may be regarded as acute HCMV infections (Gergely *et al.*, 1977).

The quantitative changes in anti-CMNA antibodies belonging to different subclases resembled antibody titre changes in the sera of patients with MI of HCMV origin (Table 1, cases 6-8).

Anti-CMNA antibody subclasses in MI of EBV origin and in HCMV seropositive individuals

We used both materials as controls. In the heterophil antibody-positive EBV-induced IM which besides clinical symptoms of mononucleosis, was manifested by the presence of anti-EBV IgM antibody and the absence of anti-HCMV IgM antibodies, the CMNA antibodies were present in low titres throughout (Table 2). Nor could we discover any rise of titres in sera of healthy donors.

A further common feature of the two materials examined was that they contained no anti-CMNA IgM antibodies. But all sera contained IgG type anti-CMNA antibody (the highest titre was 4).

We could not detect anti-CMNA IgA antibodies in one of 5 EBV-induced MI cases and in about one fourth of healthy seropositive donors. Of the latter 30 sera, all were positive for anti-CMNA (AFNB) and anti-CMNA IgG (AFNB + combined ACIF), none was positive for anti-CMNA IgM (AFNB + combined ACIF) and 22 were positive for anti-CMNA IgA (AFNB + combined ACIF).

Discussion

Our results indicate that anti-CMNA antibodies belonging to the IgM, IgG and IgA immunoglobulin classes are detectable in sera of HCMV seropositive persons.

Complement-fixing antibodies of the anti-CMNA-IgM class appeared only in acute HCMV infections. Their quantitative changes in time were the same as those of the so-called anti-EBNA-IgM antibody (Czeglédy *et al.*, 1978).

It seems that the presence of the non-complement-fixing anti-CMNA antibodies belonging to the IgG_4 subclass was not connected with the acute HCMV infection since they were detectable in small quantities months after the disease and also in sera of seropositive donors. This observation corroborates our finding that the anti-CMNA antibodies persist for lifelong (unpublished deta). Since the non-complement-fixing IgA antibodies appeared and were secreted irregularly or their quantities were below the level of detectability, their actual significance needs further examinations.

The very sensitive method used in the present study made it possible to differentiate between the early nuclear antigens demonstrated in earlier studies. An important feature of the nuclear antigen is that it is a protein bound to DNA (Gergely *et al.*, 1980). In view of this feature of the CMNA we used a novel immunofluorescence method to detect the antibodies to the

DNA-binding components of the early nuclear antigens. The essence of our method is that we use purified CMNA as antigen source. In this way we can eliminate the possible disturbing effect of other viral antigens — a danger to which the methods in which a virus-infected host cell is used as antigen source are exposed.

We modified an earlier method to make it suited for the detection of complement-fixing and non-complement-fixing antibodies also in extremely small quantities by combining it with the modified ACIF test of Schmitz *et al.* (1975).

The main mass of the anti-CMNA antibodies circulating in the blood serum of HCMV seropositive individuals for lifelong represents complement-fixing anti-CMNA IgG (IgG_1, IgG_2, IgG_3).

It is an important task in diagnostic practice to establish the acute character of a HCMV infection. From the serological viewpoint, this is based at present on the detection of HCMV-IgM antibody by immunofluorescence. By our method we succeeded in detecting the specific IgM component to CMNA which also reflects the acute phase.

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