JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1979, p. 149-151
0095-1137/79/01-0149/03$02.00/0

Vol. 9, No. 1

Comparison of Occurrence of Antibodies to Human Cytomegalovirus as Demonstrated by Immunofluorescence and Indirect Hemagglutination Techniques

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Received for publication 25 September 1978

Indirect immunofluorescence and hemagglutination study of reactivity to human cytomegalovirus immediate early, early, and late antigens revealed that antibody responses to these antigens varies greatly and that hemagglutination is positive when sera contain only antibody to one or both early antigens.

A new human cytomegalovirus (HCMV)-specific antigen, immediate early antigen (IEA), has recently been described (3). As detected by indirect immunofluorescence (IIF), it appears within 20 min after infection and is homogeneously distributed throughout the nuclei of 100% of human diploid lung fibroblasts infected at a multiplicity of 1 plaque-forming unit (PFU)/cell. IEA is no longer detectable 45 h after infection. In light of the rapidity of its appearance and its transitory nature, it seemed of interest to determine (i) whether in humans antibodies to IEA occur in any particular pattern with relation to other CMV-specific antigens, (ii) whether IEA positivity corresponds to any particular stage of disease, and (iii) whether indirect hemagglutination (IHA) positivity correlated with the presence of any or all CMV-specific antigens as detected by IIF.

To determine whether IEA antibodies appeared in a particular pattern, sera from various populations were tested by IIF for the presence of antibodies to three different CMV-specific antigens: IEA, early antigen (EA) (6), and late antigen (LA). IIF was carried out on human diploid lung fibroblasts grown in Leibovitz L-15 medium supplemented with 10% calf serum. Cells (2×10^5/well) were plated onto 16-by-16-mm glass cover slips placed in wells of 25-well Sterilin culture plates and infected with 1 PFU of the Mira strain of HCMV per cell. Plates were sampled at 3 h after infection for IEA and 4 to 5 days postinfection for LA. EA cover slips were prepared by growing infected cells in the presence of cytosine arabinoside (20 μg/ml) for 3 days from the time of virus adsorption. Plates were washed three times in phosphate-buffered saline, fixed in cold methanol, and stored in methanol at −30°C until use. Each serum was also tested on uninfected cells, and each IIF test run included a CMV-negative serum and one positive for CMV-specific IEA, EA and LA. Cover slips were air-dried, incubated with test sera for 30 min at 37°C, washed three to four times in phosphate-buffered saline, incubated with a 1:100 dilution of fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G (IgG) (Behring or Melory) for 30 min at 37°C, washed with phosphate-buffered saline, and mounted in glycerol-phosphate-buffered saline (9:1). Unless otherwise indicated, sera were tested at a 1:20 dilution, this being the minimum dilution considered positive. Preliminary investigations showed that sera which were IIF negative at 1:20 were not positive at lower dilutions, even if IHA results were positive.

Among blood donors, 62.5% (65/104 tested) were positive for at least one CMV antigen (Table 1). Of these positive sera, as many as 44.6% had antibodies to IEA, either exclusively or in combination with antibodies to other antigens. Response to EA was even higher (49.1%), and, as would be expected, 64.7% had antibodies to LA. There was no combination of antigens to which blood donors reacted preferentially. It can only be said that in a "normal" (blood donor) population, individuals may be responding to any given combination of antigens at any one time (Table 1). Nor was there any particular pattern of response among renal transplant patients, congenitally infected children, pregnant women, or multiply transfused patients. It is noteworthy that in none of these populations were there sera positive against only IEA or EA or both together (Table 1). However, since the number of cases in each of the latter groups was quite small, it cannot be concluded that these groups lack reactivity to early antigens except in combination with antibodies to LA.

To see if a particular antibody response could

be associated with recent and/or ongoing infection, a group of 95 sera randomly selected from various populations were tested for the presence of CMV-specific IgM using LA cell preparations as antigen and an incubation time of 3 h at 37°C with test sera, followed by 30 min with fluorescein isothiocyanate-conjugated goat anti-human μ chains (Behring; diluted 1:50). Both IgM-positive and IgM-negative individuals had antibodies to all combinations of CMV antigens (Table 2). However, a significantly greater percentage (36%, $P < 0.005$) of IgM-positive sera reacted with all three CMV antigens than did IgM-negative sera (11.8%).

Since it appears from the literature that CMV-specific IgM and CMV-specific EA antibodies appear in both primary and secondary infection and persist for relatively long periods (4, 5), testing for one or the other does not seem to be helpful in differentiating primary from secondary infections. The fact that in the present studies a significantly greater percentage of IgM-positive individuals reacted simultaneously to all three CMV-specific antigens suggests that testing for IEA antibodies together with antibodies to the other antigens and CMV-specific IgM antibodies might be helpful in differentiating between initial and reactivated infection. A prospective study to investigate this possibility is currently underway.

To determine whether IHA positivity correlated with the presence of any or all CMV antibodies as detected by IIF, all sera tested by IIF were also titrated by IHA. IHA was performed as previously described (1) using either serum or blood specimens collected on blotting cards used for PKU tests. Each serum was titrated at least from 1:10 to 1:5,120, and further if necessary.

IHA was positive for sera which had antibodies to only one or both early antigens as determined by IIF. IHA titers were significantly increased ($P < 0.0001$) when sera contained antibodies to both EA and LA (Table 1). Geometric mean IHA titers were higher among IgM-positive individuals (Table 2) undoubtedly because IgM, as well as IgG, participates in the IHA reaction. In general, sera with high IHA titers were most likely to have high IIF titers. IHA detected more positive individuals (74%) than did IIF (62.5%), although in seven cases IIF was positive while IHA was negative. This confirms the results of Fuccillo et al. (2).

In conclusion, according to our observations, over 40% of individuals in any given population may react to IEA at any one time, either alone or in combination with other antigens. The fact

TABLE 1. *Distribution of CMV-specific antibodies in the sera of various groups of CMV-positive individuals*

IIF detection of IgG antibodies against:	Population[a]					Populations as a whole	
	Blood donors	Renal transplant patients	Congenitally infected children	Pregnant women	Multiply transfused patients	%	Geometric mean IHA titers
LA	38.5	33.3	53.8	72.8	50.0	48.7	567
EA + LA	6.2	0	15.4	13.6	10.0	8.4	2,229[b]
IEA + EA + LA	13.8	50.0	23.1	13.6	40.0	18.5	2,347[b]
IEA + EA	18.4	0	0	0	0	10.1	639
IEA	6.2	0	0	0	0	5.9	637
IEA + LA	6.2	16.7	7.7	0	0	3.4	806
EA	10.7	0	0	0	0	5.0	579
No. of CMV-positive sera tested	65	6	13	22	10	116	
Total no. of sera tested	104	9	20	32	18	183	

[a] Percentage of CMV-positive sera reacting to indicated antigen.
[b] Difference significant; $P < 0.0001$.

TABLE 2. *Antibody response to CMV-specific antigens as detected by IIF*

Sera	% Positive against:							Geometric mean IHA titer	Total no. of sera tested
	LA	IEA	EA	EA + LA	IEA + LA	IEA + EA	IEA + EA + LA		
IgM positive	30.9[a]	2.4	2.4	11.9	4.8	9.5	38.1[b]	1,586	42
IgM negative	47.2[a]	3.8	11.3	5.7	5.7	15.0	11.3[b]	648	53

[a] Difference not significant; $P > 0.05$.
[b] Difference significant; $P < 0.005$.

that some sera had antibodies to one or both early antigens and no LA antibodies might possibly be explained on the basis that if viral replication in vivo were arrested (as can be done in vitro with various drugs [cytosine arabinoside, PPA]) at an early stage, LA-antibody production might not reach levels detectable by IIF. Actively infected (IgM-positive) individuals reacted more frequently to all three antigens than did those chronically infected (IgM-negative). Since IHA detected CMV antibodies when IIF was positive only against early antigens, hemagglutinating antibodies are probably some of the first to appear after CMV infection. Differential diagnosis of primary and reactivated infection may be possible by using in combination the sensitive IHA for detection of CMV-specific antibodies and the IIF to determine the types of CMV-specific antibodies present and the IgM status of an individual.

We thank A. Eyquem of the Centre de Transfusion Sanguine, Institut Pasteur, for supplying blood donor sera and Michel Barme, Virology Department of Institut Pasteur, for his advice on statistical analysis.

This work was partially supported by contracts AT 50-77-82 and CRL 76-5-149-8 from the Institut National de la Sant é et de la Recherche Médicale.

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