Acta virol. **32** : 1—5, 1988

HUMAN ANTIBODY RESPONSE TO HUMAN CYTOMEGALOVIRUS-SPECIFIC DNA-BINDING PROTEINS

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Received April 24, 1987

Summary. — Human antibody responses to human cytomegalovirus (HCMV) specific DNA-binding proteins were studied in serum samples by the Western blot technique. The molecular weights of six DNA-binding proteins found in HCMV-infected cells, ranged from 52kD to 18kD. The sera obtained from patients with acute HCMV infections reacted well with the six HCMV specific DNA-binding proteins. The strongest reactivity was observed with the 52kD and 35kD proteins. The sera from healthy HCMV seropositive donors reacted only with the 52kD DNA-binding protein as visualized in Western blots, but 2 out of 8 sera failed to react with any HCMV specific DNA-binding proteins.

Key words: cytomegalovirus, DNA-binding proteins, antibody

Introduction

Human cytomegalovirus (HCMV) is an increasingly important aetiological agent in clinical virology. Broad spectrum of HCMV-associated diseases emphasizes the necessity for diagnostic techniques capable of early and reliable detection of HCMV infection. The host antibody responses to HCMV have been assessed by neutralization, complement fixation, indirect haemagglutination, and immunofluorescence tests, as well as by radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) (Horodniceanu and Michelson, 1980). Antibody responses to HCMV-specific polypeptides have been determined with immunoprecipitation (Pereira *et al.*, 1982; Pereira *et al.*, 1983) or enzyme immunoassay (Cremer *et al.*, 1985). It was shown that the late HCMV-infected cell polypeptides, in particular, the viral glycoproteins are highly immunogenic in the human host. Among the polypeptides detected in HCMV-infected cells, the DNA-binding proteins are of special importance.

Six DNA-binding proteins were found in HCMV-infected cells ranging in m.w. from 52kD to 18kD (Gergely *et al.*, 1985). In this paper we apply the Western blot technique to determine the antibody responses to HCMV specific DNA-binding polypeptides in acute and convalescent human sera.

Materials and Methods

Virus and cells. Human embryonic fibroblast cells were infected with the strain Ad169 of HCMV for the immunoblot assay.

Human sera. Acute and convalescent sera used in this study were submitted by the Departments of Medicine and Paediatrics, Medical University of Debrecen, for serological diagnosis of HCMV.

Preparation of HCMV specific DNA-binding proteins was described elsewhere (Gergely *et al.*, 1985). The proteins were eluted with 1 mol/l NaCl from native DNA cellulose column and used for immunoblotting analysis.

Immunoblotting procedure. The DNA-binding proteins were separated by polyacrylamide gel electrophoresis. Proteins from the acrylamide gel were transferred to nitrocellulose membranes according to the procedure described Towbin *et al.*, (1979). Immunostaining procedure was described previously (Gergely *et al.*, 1985). Briefly, after 2 hr transfer the nitrocellulose was removed; strips (8 mm in width) were cut, soaked in 10 mmol/l phosphate buffer containing 150 mmol/l NaCl and incubated overnight at +4 °C. The washed strips were incubated in the same buffer containing 0.05 % Tween 20 (PBS-T) and 5 % fraction V bovine serum albumin for 30 min at 37 °C to block nonspecific binding sites.

Serum specimens were diluted 1 : 10 in PBS-T and reacted with the strips for 60 min at 37 °C in small plastic bags rotated end—over—end. After incubation, strips were washed twice in PBS-T. The reacted strips were incubated with 1 : 500 dilution of a protein A-alkaline phosphatase conjugate (Sigma) in PBS-T supplemented with 2 % fraction V bovine serum albumin for 60 min at room temperature, and washed twice for 15 min in PBS-T. Finally, alpha-naphtyl phosphate substrate was added to each strip at room temperature (10 mg alpha-naphtyl phosphate (Sigma), 25 mg Fast Red (Sigma), 50 µl 1 mol/l $MgCl_2$, 50 ml 100 mmol/l Tris-HCl (pH 8.6). The stained strips were washed in water and dried on Whatman no. 2 filter paper.

Testing for IgG and IgM antibodies to HCMV late antigens. The indirect immunofluorescence method was performed as described previously (Gergely *et al.*, 1981).

Results

Sixteen sera were examined. Donors were classified as having acute HCMV infectionor apparently healthy HCMV seropositive persons. An HCMV infection was considered to be acute if IgM antibody test was positive. Acute sera were also characterized by the ralative high anti-HCMV IgG titres, together with the clinical signs of virus infection (usually mononucleosis). The immunoblotting technique allowed us to identify the size of virus-specific DNA--binding proteins recognized by antibodies in patients serum (Table 1).

Six DNA-binding antigens were detected, their molecular weights ranged from 52kD to 18kD. The broadest bands were represented by the 52kD and 35kD proteins. Sera from acute HCMV infection revealed strong reactivity to six DNA-binding proteins on Western blots. In one typical case (serum no. 1) the 52kD, 50kD, 45kD, 35kD, 25kD and 18kD DNA-binding proteins could be detected in immunoblot (Fig. 1, lane A). In the previous experiment the specificity of this method was tested, the sera failed to react with DNA--binding proteins separated from uninfected control cells (Gergely *et al.*, 1985).

Sera 9—16 were obtained from healthy HCMV seropositive donors. Out of these 6 sera reacted only with the 52kD major HCMV specific DNA-binding protein and 2 of them failed to react with any DNA-binding protein. In two cases a weak reactivity with the 35kD DNA-binding protein was observed. The example of immunoblot from one patient (serum no. 9) revealed existing antibodies only to 52kD DNA-binding protein (Fig. 1,

Table 1. Reactivity of human sera in Western blots with HCMV-induced DNA-binding proteins

Serum	Anti-HCMV titre*		Antibody response to HCMV-induced DNA-binding protein					
	IgM	IgG	52kD	50kD	45kD	35kD	25kD	18kD
1	40	1280	+	+	+	+	+	+
2	80	1280	+	+	+	+	+	+
3	40	640	+	±	+	+	±	±
4	80	640	+	±	+	+	±	±
5	40	1280	+	+	+	+	+	+
6	40	1280	+	+	+	+	+	+
7	80	640	+	±	+	+	±	+
8	40	640	+	+	+	+	+	+
9	negative	640	+	−	−	−	−	−
10	negative	80	±	−	−	±	−	−
11	negative	160	+	−	−	±	−	−
12	negative	80	−	−	−	−	−	−
13	negative	160	+	−	−	−	−	−
14	negative	160	±	−	−	−	−	−
15	negative	640	+	−	−	−	−	−
16	negative	320	−	−	−	−	−	−

*Titres are expressed as reciprocals of the serum dilution.

lane B). In two patients (nos 2. and 5.) suffering from acute HCMV infection blood was drawn again 8 months after the onset of disease; only 52kD and anti-35kD antibodies were present in these late sera as judged by immunoblotting findings (data not shown). These data indicated a response similar to that seen in the sera of healthy HCMV seropositive donors. It was interesting that two HCMV seropositive sera failed to react with any HCMV-specific DNA-binding protein.

Discussion

We applied the immunoblotting technique in order to characterize antibodies to HCMV specific DNA-binding antigens. The use of immunoblotting and immunoprecipitation in assessing the specific host antibody response to herpesviruses was described by several investigators (Kahlon *et al.*, 1986; Pereira *et al.*, 1983; Dillner *et al.*, 1985; Lehtinen *et al.*, 1985). In our previous experiments six DNA-binding proteins of HCMV infected cells (52kD, 50kD, 45kD, 35kD, 25kD, and 18kD) were detected in Western blots (Gergely *et al.*, 1985). The major 52kD DNA-binding protein is a non-virion protein having a net basic charge present in large quantities in HCMV-infected cells. This protein has been designated as "delayed-early" protein, because for maximal expression it requires viral DNA synthesis (Gibson, 1984).

The 35kD protein is an important phosphoprotein (Gibson, 1983), while the other DNA-binding proteins are not yet characterized. Some of these

proteins probably fulfil a histon-like role during infection. In spite of the limited numbers of the patients evaluated, the overall pattern of response for HCMV specific DNA-binding proteins was defined. For acute HCMV infections the host response was directed against all six DNA-binding proteins. Later, antibodies to four DNA-binding proteins (50kD, 45kD, 25kD, 18kD) were not detected in Western blots. Sera from healthy HCMV seropositive donors reacted mainly with the 52kD protein, but 2 sera failed to react even with this protein. Antibodies against 35kD protein were demonstrated in 2 sera from 8 healthy HCMV seropositive donors.

Our data suggest that large quantities of virus or antigenic load are required to elicit good antibody response to HCMV specific DNA-binding proteins. The lower magnitude of antibody responses in healthy HCMV seropositive persons paralelling a diminished quantity of antigenic load might explain the observations that these sera reacted only with the 52kD and 35kD proteins, respectively.

Sera from symptomatic, HCMV-infected children precipitated a greater number of polypeptides, and in larger quantities, than asymptomatic, congenital and perinatally infected patients (Pereira *et al.*, 1983). Antibody to a 150kD nucleocapsid antigen persisted for a shorter time than did the antibody to the other antigens (Cremer *et al.*, 1985). Some of the DNA--binding proteins might belong to the early antigens (EA); some papers state that the percentage of reactivity ot EA is very high also in the sera from patients with current HCMV disease (Landini *et al.*, 1984). Our observations are comparable to the results obtained in acute herpes simplex virus (HSV) infections. Patients with an acute HSV-2 infection had clearcut antibody responses to the purified major HSV-2 specified DNA-binding protein (Lehtinen *et al.*, 1985). Similarities between the 52kD HCMV specific DNA-binding protein and the Epstein-Barr virus (EBV) nuclear antigen (EBNA) were suggested (Gibson, 1983), but according to the presented results, the antibody response is completely different. The delayed appearance of antibodies to EBNA (EBNA-1 and EBNA-2) during the course of acute EBV infection was demonstrated by immunoblotting technique (Dillner *et al.*, 1985).

Our experiments demonstrate the early appearance of antibodies to HCMV specific DNA-binding proteins and the usefulness of immunoblotting technique to detect antibodies to the different HCMV specific antigens.

Acknowledgements. This work was supported by a grant from the Hungarian Ministry of Health. We thank Mr. J. Márton for his excellent technical assistance.

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Explanation to the Figure (Plate I):

Fig. 1. Immunoblotting of DNA-binding proteins from HCMV-infected cells. Sera used for the different blots:
Lane A: Serum from a patient with acute HCMV infection, dil. 1 : 10.
Lane B: A healthy HCMV-positive donor serum, dil. 1 : 10.
Number at left indicate the apparent molecular weight in kilodaltons.