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Short Communication

Differential effects of glycoprotein B epitope-specific antibodies on human cytomegalovirus-induced cell–cell fusion

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Attachment of, and cell–cell fusion induced by, human cytomegalovirus were studied in the presence of neutralizing monospecific antibodies against antigenic domains 1 (AD-1) or 2 (AD-2) of glycoprotein B (gB, gpUL55). Efficient inhibition of the virion-mediated fusion event was consistently observed for the human AD-2-specific antibody as determined by a reporter gene activation assay based on permissive astrocytoma cells. In contrast, antibodies directed against the major neutralizing gB epitope AD-1 reduced fusion only by 20–60 %. Virus attachment via heparan sulfate was unaffected by the antibodies under the conditions used. Virus receptor binding as examined by heparin treatment of adsorbed virus was significantly reduced only if the virus had been coated with the AD-2-specific antibody. Neutralization of virus infectivity by the AD-2-specific antibody thus seems most likely to result from interference with a receptor-binding event during initial virus–host cell interaction.

Glycoprotein B (gB) is an abundant component of the envelope of human cytomegalovirus (HCMV) and a major target of neutralizing antibodies that develop after natural infection (Britt *et al.*, 1990; Gonczol *et al.*, 1991; Marshall *et al.*, 1992). Three antibody-binding sites have been mapped on the gB molecule, which is a type I transmembrane protein: the extracellular immunodominant antigenic domain 1 (AD-1) is located between amino acids 552 and 635; AD-2 (aa 50–86) at the N terminus is composed of two sites, aa 50–54 (site I) and 67–86 (site II); and AD-3 is located between aa 798 and 805 (Silvestri *et al.*, 1991; Meyer & Radsak, 2000) at the extreme cytoplasmic C terminus. Of the three domains, only AD-1 and site II of AD-2 have been shown to induce virus-neutralizing antibodies (Kniess *et al.*, 1991; Meyer *et al.*, 1992; Wagner *et al.*, 1992).

HCMV infection requires viral envelope glycoproteins and the respective cellular receptors to engage in a series of interactions, ultimately resulting in fusion of the viral envelope with the plasma membrane. The mechanisms by which gB-specific antibodies interfere with the complex processes involved leading to loss of viral infectivity have not been precisely defined so far, although a previous report suggested that antibodies may neutralize infection before or after adsorption to the cell surface (Ohizumi *et al.*, 1992). In order to analyse further antibody-mediated HCMV neutralization – an activity with potential therapeutic and protective function *in vivo* – the effects of human monoclonal antibodies (mAbs) against AD-1 and AD-2 of HCMV gB on virus attachment as well as on virus-induced cell–cell fusion were investigated. Virus induced cell–cell fusion was measured by a new reporter gene activation assay (Gicklhorn *et al.*, 1999) based on two stably transfected astrocytoma cell lines, U373-CAT and U373-VP16, in which induction of the chloramphenicol acetyltransferase (CAT) gene is triggered on experimentally induced cell–cell fusion by the transactivator GAL4-VP16. For the fusion assay, U373-CAT and U373-VP16 cells were seeded at a ratio of 1 : 1 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % foetal calf serum (FCS) into 15 mm dishes to obtain a subconfluent monolayer the next day (2×10^5 cells). To induce cell–cell fusion, cell-free HCMV strain AD 169, grown and titrated on human foreskin fibroblasts (HF) as previously described (Eggers *et al.*, 1992), was routinely used at an m.o.i. of 0·5. Higher m.o.i.s were previously found to result in reduced CAT activity, most likely as a result of pronounced cytopathogenicity (Gicklhorn *et al.*, 1999).

The neutralizing gB AD-1-specific mAbs ITC48, ITC52, ITC63B, or the AD-2-specific mAb ITC88 (recognizing site II of AD-2) and the non-neutralizing AD-1-specific mAb ITC39, as control antibody, were used (Ohlin *et al.*, 1993) after standard ammonium sulfate precipitation and dialysis against PBS. Human IgG was quantified with a human IgG–Fc ELISA Quantification kit (NatuTec) according to the manufacturer's instructions. For routine fusion-inhibition experiments, the virus inoculum was incubated with the

respective mAb (6·2 μg IgG ml^{-1} each, diluted in DMEM) for 60 min at 37 °C. This concentration was selected as it is within the range of concentrations of AD-1-specific antibodies seen *in vivo* (Ohlin *et al.*, 1997). As negative and positive controls, a human anti-pp65 mAb (MO58) (Ohlin *et al.*, 1991) and an HCMV-positive human serum, respectively, were used. To obtain a baseline for the fusion process, virus was preincubated without antibody. Co-cultivated U373-CAT/VP16 cells were incubated with 200 μl of virus/antibody mixture for 60 min at room temperature to allow virus adsorption. The inoculum was removed and culture medium (DMEM supplemented with 2 % heat-inactivated FCS) was added. At 48 h post-infection, cell monolayers were solubilized in 50 μl 1 × lysis buffer (Promega) and analysed for CAT activity by thin-layer chromatography according to a standard protocol (Gorman *et al.*, 1982). Since total cell-free virus preparations were used, the possibility that dense bodies and non-infectious enveloped particles contributed to the observed fusogenic activity (Schmolke *et al.*, 1995) in addition to virions cannot be ruled out.

Efficient fusion inhibition by the standard IgG concentration (6·2 μg IgG ml^{-1}) was obtained for the human control serum as well as for the neutralizing AD-2-specific mAb ITC88, whereas with AD-1-specific antibodies (ITC52, ITC48, ITC63B) only 20–60 % fusion inhibition was found (Fig. 1). Since all of these antibodies exhibited neutralizing activities (Ohlin *et al.*, 1993), this result underlines the relevance of AD-2 for the fusion process. HCMV-mediated cell–cell fusion was unaffected by the control mAb MO58 as well as by non-neutralizing mAb ITC39.

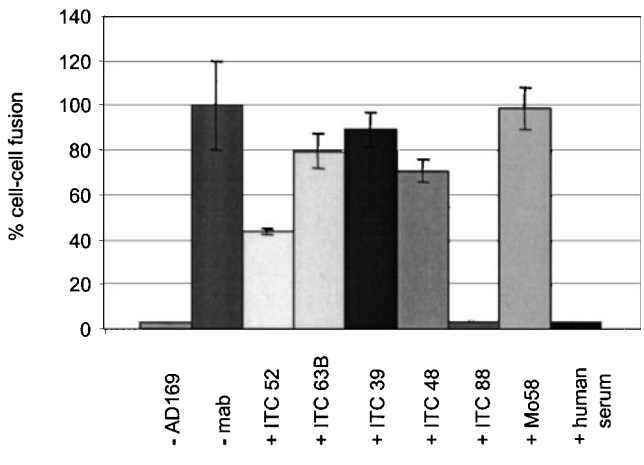

Fig. 1. Inhibition of cell–cell fusion activity of HCMV AD 169 by gB-specific mAbs. Cell–cell fusion activity of HCMV AD 169 was measured by a reporter gene activation assay after preincubation with gB-specific mAbs at final concentrations of 6·2 μg IgG ml^{-1} (+ITC52, ITC63B, ITC39, ITC48, ITC88). As controls, virus without preincubation (−mAb), after preincubation with the pp65-specific control antibody (+MO58), or with HCMV-positive human serum (+human serum) was used. As an internal assay control, uninfected co-cultivated U373-CAT/VP16 cells were used (−AD 169). AD 169-mediated cell–cell fusion without mAb preincubation (−mAb) was defined as 100 % fusion. All samples were tested in triplicate. The error bars indicate standard deviation.

The observed inhibitory effects suggested that the gB-specific mAbs interfered with the HCMV–cell interaction either at the level of binding to cellular surface receptors and/or with the consecutive fusion event. Initial attachment of HCMV to permissive host cells is dependent on the presence of cell-surface heparan sulfate proteoglycans (HSPGs) and can be competed by heparin (Compton *et al.*, 1993). HCMV gB and primarily glycoprotein complex II of the HCMV envelope (Kari & Gehrz, 1992, 1993) have been shown to mediate interaction with cell-surface HSPGs. With regard to virus binding, in particular via the gB protein, an additional heparin-independent receptor binding has been postulated (Boyle & Compton, 1998).

Subsequent experiments were thus designed to examine whether the observed inhibitory effect of gB-specific mAbs occurred at the level of virus attachment or at a later stage. To allow accurate comparisons, identical numbers of cells, inoculum volume and proportion of virus and antibody were used. Radioactively labelled [^{35}S]AD 169 was prepared and titrated in HF cultures after purification by sucrose cushion ultracentrifugation as previously described (Eggers *et al.*, 1992; Compton *et al.*, 1993). HCMV inoculums and mAbs were preincubated for 60 min at 37 °C prior to inoculation of duplicates of co-cultivated U373-CAT/VP16 cells and incubation for 90 min at 4 °C. As negative controls, heparin-sodium (Serva) at 100 IU ml^{-1} in DMEM was added for 60 min at 4 °C before addition of virus or during virus adsorption. The virus inoculum was removed and the cells were washed three times with PBS followed by lysis in lysis buffer (1 % Triton X-100, 1 % SDS in PBS). Cell extracts were prepared for scintillation counting (LS1801; Beckmann) as previously described (Radsak *et al.*, 1985).

Attachment of HCMV in the presence of heparin was strongly inhibited, whereas for mAbs ITC48, ITC52 and ITC39 and control mAb MO58, an increased attachment was observed compared with the positive control (virus without mAb) (Table 1). This enhancement of infectivity most likely reflects a phenomenon described mainly for neutralizing mAbs called antibody enhancement of infectivity (Dimmock, 1993). Attachment in the presence of mAb ITC88 and ITC63B was slightly reduced by 4–8 %. This minor effect, in particular for mAb ITC88, did not correlate with the strong inhibition observed in the fusion assay. Loss of infectivity of HCMV in the presence of AD-1-specific mAbs and AD-2-specific mAb ITC88 at concentrations of 6·2 μg IgG ml^{-1} appeared therefore to result primarily from inhibition of virus–cell fusion mechanisms and not from interference with HCMV attachment to HSPGs. This finding agrees with a previous report suggesting that another AD-2-specific antibody blocks virus penetration but not virus attachment to the cell surface (Ohizumi *et al.*, 1992),

Table 1. Attachment of HCMV AD 169 in the presence of gB-specific mAbs

^{35}S-labelled AD 169 (3×10^6 c.p.m. per well) was treated with mAbs (6·2 μg IgG ml^{-1}; m.o.i. = 0·5) for 60 min at 37 °C as described for the fusion assay prior to incubation of the mixture with co-cultivated U373-CAT/VP16 cells for 90 min at 4 °C. The virus inoculum was removed and cells were washed, then lysed in 50 μl lysis buffer and 10 μl of cell extract was prepared for scintillation counting. As negative attachment controls, cells were incubated with ^{35}S-labelled AD 169 in the presence of 100 (expt 1) or 10 (expt 2) IU heparin ml^{-1}. Attachment in the absence of mAb (−mAb) was defined as 100 %. The values shown represent the mean of duplicate determinations. Deviations of values are indicated by ± (%).

	Virus attachment (%)	
mAb	Expt 1	Expt 2
+Heparin	5±0·1	11±0·03
−mAb	100±1·6	100*
+ITC52	113±4·2	125±23·4
+ITC88	96±5·2	
+ITC48	107±5·6	
+ITC63B	92±3·9	
+ITC39	105±0·4	
+MO58	121±0·1	114±4·5

*Single determination.

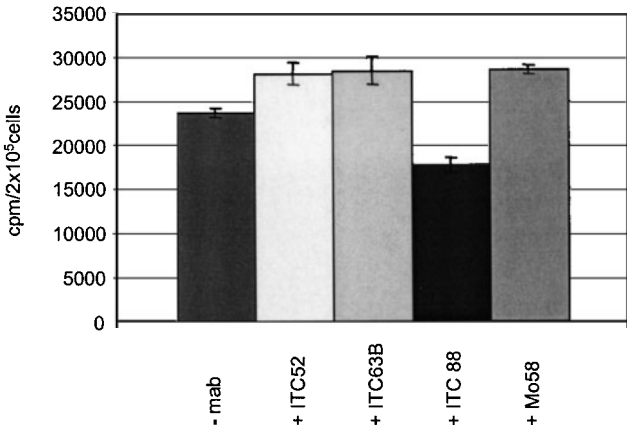

Fig. 2. Heparin-resistant cellular receptor binding of HCMV AD 169 in the presence of gB-specific mAbs. The HCMV attachment assay was as described in the legend to Table 1, except that after 90 min at 4 °C unattached virus was removed by extensive washing with PBS before cells were washed with 200 μl of heparin solution (100 μg ml^{-1}) for 1 h at 4 °C. Dissociated radioactive virus was removed, cells were washed with PBS and virus remaining adherent to the cell monolayers was solubilized by detergent, quantified by scintillation counting (c.p.m./2×10^5 cells) and defined as heparin resistant. The error bars indicate standard deviation.

and also extends such findings to AD-1-specific human antibodies. Inhibition of attachment at higher IgG concentrations, however, cannot be excluded but was not considered relevant in this context.

To distinguish further between primary HSPG binding and specific heparin-independent receptor binding, dissociation of bound HCMV (in the presence of mAbs) by heparin was measured. Co-cultivated U373-CAT/VP16 cells in duplicate were incubated for 90 min at 4 °C with radioactive virus/antibody mixtures (m.o.i. = 0·5) as described above to allow attachment. Unattached virus was removed by extensive washing with PBS before cells were washed with 200 μl of heparin solution (100 μg ml^{-1}) for 1 h at 4 °C to dissociate (heparin-soluble) virus that was most likely only attached to HSPGs. Radioactive virus remaining adherent to the cell monolayers at 4 °C was solubilized by detergent treatment and defined as heparin resistant.

In the presence of the control mAb MO58 or the AD-1-specific mAbs ITC52 or ITC63B, heparin-resistant receptor binding of HCMV did occur and was enhanced by up to 20 %, whereas the AD-2-specific mAb ITC88 reduced heparin-resistant receptor binding by 30 % (Fig. 2). These results suggested that the neutralizing activity of AD-1- and AD-2-specific mAbs may involve different stages of the virus adsorption process to cells.

Taken together, at the biologically relevant concentrations used, the inhibitory effect on HCMV-mediated cell–cell fusion by the AD-2-specific mAb exceeded that by AD-1-specific mAbs. Furthermore, none of the antibodies affected virus attachment via HSPGs under the conditions used. This observation is in contrast to that of Silvestri & Sundqvist (2001) who determined HSPG binding of AD-2 using a synthetic AD-2 peptide. Inhibition of HCMV-specific receptor binding by the ITC88 antibody used here indicates its interference with the fusion process at an early stage consecutive to virus attachment via HSPGs. The reduced heparin-resistant receptor-binding in the presence of mAb ITC88 may either result from blocking the receptor binding domain of HCMV gB directly or may be an indirect consequence of antibody binding, which may induce a conformation of the gB molecule that is no longer competent to interact with the putative cellular receptor. Interestingly, in the case of herpes simplex virus 1 glycoprotein D, the neutralizing activity of group Ib mAbs was suggested to reflect an overlap between the epitopes recognized and the receptor-binding domain (Whitbeck *et al.*, 1999). Investigations are therefore under way to elucidate whether the AD-2 epitope of HCMV gB also overlaps the receptor-binding domain of gB.

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REFERENCES

Boyle, K. A. & Compton, T. (1998). Receptor-binding properties of a soluble form of human cytomegalovirus glycoprotein B. *J Virol* **72**, 1826–1833.

Britt, W. J., Vugler, L., Butfiloski, E. J. & Stephens, E. B. (1990). Cell surface expression of human cytomegalovirus (HCMV) gp55-116 (gB): use of HCMV-recombinant vaccinia virus-infected cells in analysis of the human neutralizing antibody response. *J Virol* **64**, 1079–1085.

Compton, T., Nowlin, D. M. & Cooper, N. R. (1993). Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparin sulfate. *Virology* **193**, 834–841.

Dimmock, N. J. (1993). Neutralization of animal viruses. *Curr Top Microbiol Immunol* **183**, 1–149.

Eggers, M., Bogner, E., Agricola, B., Kern, H. F. & Radsak, K. (1992). Inhibition of human cytomegalovirus maturation by brefeldin A. *J Gen Virol* **73**, 2679–2692.

Gicklhorn, D., Eickmann, M. & Radsak, K. (1999). Chloramphenicol acetyltransferase expression as a sensor for fusion activity. *Biotechniques* **27**, 902–904, 906, 908.

Gonczol, E., deTaisne, C., Hirka, G., Berencsi, K., Lin, W. C., Paoletti, E. & Plotkin, S. (1991). High expression of human cytomegalovirus (HCMV)-gB protein in cells infected with a vaccinia-gB recombinant: the importance of the gB protein in HCMV immunity. *Vaccine* **9**, 631–637.

Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982). Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol Cell Biol* **2**, 1044–1051.

Kari, B. & Gehrz, R. (1992). A human cytomegalovirus glycoprotein complex designated gC-II is a major heparin-binding component of the envelope. *J Virol* **66**, 1761–1764.

Kari, B. & Gehrz, R. (1993). Structure, composition and heparin binding properties of a human cytomegalovirus glycoprotein complex designated gC-II. *J Gen Virol* **74**, 255–264.

Kniess, N., Mach, M., Fay, J. & Britt, W. J. (1991). Distribution of linear antigenic sites on glycoprotein gp55 of human cytomegalovirus. *J Virol* **65**, 138–146.

Marshall, G. S., Rabalais, G. P., Stout, G. G. & Waldeyer, S. L. (1992). Antibodies to recombinant-derived glycoprotein B after natural human cytomegalovirus infection correlate with neutralizing activity. *J Infect Dis* **165**, 381–384.

Meyer, G. A. & Radsak, K. D. (2000). Identification of a novel signal sequence that targets transmembrane proteins to the nuclear envelope inner membrane. *J Biol Chem* **275**, 3857–3866.

Meyer, H., Sundqvist, V. A., Pereira, L. & Mach, M. (1992). Glycoprotein gp116 of human cytomegalovirus contains epitopes for strain-common and strain-specific antibodies. *J Gen Virol* **73**, 2375–2383.

Ohizumi, Y., Suzuki, H., Matsumoto, Y., Masuho, Y. & Numazaki, Y. (1992). Neutralizing mechanisms of two human monoclonal antibodies against human cytomegalovirus glycoprotein 130/55. *J Gen Virol* **73**, 2705–2707.

Ohlin, M., Sundqvist, V. A., Gilljam, G., Ruden, U., Gombert, F. O., Wahren, B. & Borrebaeck, C. A. (1991). Characterization of human monoclonal antibodies directed against the pp65-kD matrix antigen of human cytomegalovirus. *Clin Exp Immunol* **84**, 508–514.

Ohlin, M., Sundqvist, V. A., Mach, M., Wahren, B. & Borrebaeck, C. A. (1993). Fine specificity of the human immune response to the major neutralization epitopes expressed on cytomegalovirus gp58/116 (gB), as determined with human monoclonal antibodies. *J Virol* **67**, 703–710.

Ohlin, M., Silvestri, M., Sundqvist, V. A. & Borrebaeck, C. A. (1997). Cytomegalovirus glycoprotein B-specific antibody analysis using electrochemiluminescence detection-based techniques. *Clin Diagn Lab Immunol* **4**, 107–111.

Radsak, K., Wiegandt, H., Unterdorfer, G., Wagner, C. & Kaiser, C. J. (1985). Sodium butyrate selectively inhibits host cell glycoprotein synthesis in human fibroblasts infected with cytomegalovirus. *Biosci Rep* **5**, 589–599.

Schmolke, S., Drescher, P., Jahn, G. & Plachter, B. (1995). Nuclear targeting of the tegument protein pp65 (UL83) of human cytomegalovirus: an unusual bipartite nuclear localization signal functions with other portions of the protein to mediate its efficient nuclear transport. *J Virol* **69**, 1071–1078.

Silvestri, M. E. & Sundqvist, V. A. (2001). An investigation into the heparin-binding properties of a synthetic peptide deduced from the antigenic domain 2 of human cytomegalovirus glycoprotein B. *Scand J Immunol* **53**, 282–289.

Silvestri, M., Sundqvist, V. A., Ruden, U. & Wahren, B. (1991). Characterization of a major antigenic region on gp55 of human cytomegalovirus. *J Gen Virol* **72**, 3017–3023.

Wagner, B., Kropff, B., Kalbacher, H., Britt, W., Sundqvist, V. A., Ostberg, L. & Mach, M. (1992). A continuous sequence of more than 70 amino acids is essential for antibody binding to the dominant antigenic site of glycoprotein gp58 of human cytomegalovirus. *J Virol* **66**, 5290–5297.

Whitbeck, J. C., Muggeridge, M. I., Rux, A. H., Hou, W., Krummenacher, C., Lou, H., van Geelen, A., Eisenberg, R. J. & Cohen, G. H. (1999). The major neutralizing antigenic site on herpes simplex virus glycoprotein D overlaps a receptor-binding domain. *J Virol* **73**, 9879–9890.