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Serum antibody response to the gH/gL/pUL128–131 five-protein complex of human cytomegalovirus (HCMV) in primary and reactivated HCMV infections

Emilia Genini[a], Elena Percivalle[a], Antonella Sarasini[a], M. Grazia Revello[b], Fausto Baldanti[a], Giuseppe Gerna[c,*]

[a] SC Virologia e Microbiologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[b] SC Ostetricia e Ginecologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[c] Laboratori Sperimentali di Ricerca, Area Trapiantologica, Fondazione IRCCS Policlinico San Matteo, v. Taramelli 5, 27100 Pavia, Italy

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ABSTRACT

Background: Recently, a new human cytomegalovirus (HCMV) glycoprotein complex has been identified and potentially proposed as a vaccine.
Objective: The aim of this study was to determine whether the HCMV gH/gL/pUL128–pUL130–pUL131 (gH/gL/pUL128–131) 5-protein (pentameric) complex (which has been recently found to be indispensable for the infection of endothelial and epithelial cells) is able to elicit a consistent antibody response in both primary and reactivated HCMV infections.
Study design: The antibody response was determined by both indirect immunofluorescence (IFA) and ELISA, using fixed (IFA) or lysed (ELISA) epithelial (ARPE-19) cells infected with one or more adenoviral vectors, each carrying one HCMV gene and, in parallel, with a control adenovirus vector.
Results: The specificity of results was determined by the reactivity of human neutralizing mAbs recognizing two, three, or four proteins of the complex. In 14 cases of primary infection, an IgG antibody seroconversion to the UL128–131 gene products was consistently detected within 2–4 weeks after onset of infection, while antibodies persisted for at least 12 months. The IgG antibody response to UL128–131 gene products was generally superior to the response to gH and appeared to follow the neutralizing antibody response (as determined in epithelial cells). In reactivated infections, the antibody response showed a trend reminiscent of a booster response. IgG antibodies were detected in HCMV-seropositive healthy adult controls, but not in HCMV-seronegative individuals.
Conclusions: The IgG antibody response to the pentameric complex could be a major target for the evaluation of the antibody response to a pentamer-based vaccine.

1. Background

In recent years, it has been shown that human cytomegalovirus (HCMV) entry into endothelial and epithelial cells requires the presence of UL128–UL130–UL131 (UL128–131) locus gene products in the virus envelope.[1–3] In addition, UL128–131 gene products have been shown to assemble with glycoprotein H (gH) and L (gL) to form a 5-protein complex (gH/gL/UL128–131), which is required for HCMV infection of epithelial cells.[4] Recent findings suggest that gH/gL and not gH/gL/gO[5] mediates entry into fibroblasts, and both gH/gL and gH/gL/pUL128–131 are required for virus entry into epithelial/endothelial cells.[6,7] The HCMV neutralizing antibody response, as measured in human embryonic lung fibroblasts (HELF), has been considered in the past to be primarily directed to gB and gH and to appear late (2–4 months) after the onset of primary infection. In contrast to the past,[8–11] recently, it was shown that the neutralizing antibody response appears early during the convalescent-phase of a primary infection when determined on endothelial/epithelial cells.[12–14]

2. Objectives

These results prompted us to attempt to dissect the antibody response to the different components of the 5-protein complex, in particular, to pUL128–131 and gH, during natural infection. Initially, the antibody response was determined by immunofluorescence (IFA) using, as a cell substrate, epithelial cells (ARPE-19) infected with one or more adenoviral vectors carrying HCMV genes, each expressing one of the five proteins of the complex,[4] or with a control adenoviral vector carrying the transactivator alone. In parallel, an ELISA assay was developed using infected ARPE-19 cell lysate expressing one or more proteins of the complex as well as

* Corresponding author. Tel.: +39 0382 503314; fax: +39 0382 502648.
E-mail addresses: g.gerna@smatteo.pv.it, giuseppe.gerna@libero.it (G. Gerna).

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a cell control lysate carrying the control adenovirus vector. Preliminary results indicate that in primary HCMV infections an IgG response to pUL128–131 is consistently detected and persists for at least 12 months, while a booster-type response is observed in reactivated infections.

3. Study design

3.1. Patients

The study was approved by the local Ethics Committee, and written informed consent was obtained from the subjects included in the study. Serum samples for the kinetics study of the antibody response to HCMV infection were taken at different intervals concomitantly with medical visits.

3.2. Diagnosis and timing of primary HCMV infection

Diagnosis of primary HCMV infection was based on one or more of the following criteria: HCMV-specific IgG seroconversion, HCMV-specific IgM antibody detection and low IgG avidity, and the presence of HCMV or HCMV products in blood.[15,16] Timing of primary HCMV infection was based on the previously reported criteria.[17] In addition, sera from the acute- and the convalescent-phase of reactivated infections were collected from 12 solid organ transplant (SOT) recipients undergoing heart ($n = 6$) or kidney ($n = 6$) transplantation (one HCMV-seronegative and 11 ELISA HCMV IgG-seropositive before transplantation).[18]

3.3. Preparation of adenoviral vectors, each carrying a single gene of the gH/gL/pUL128–131 complex

Adenoviral vectors carrying HCMV genes as well as their transactivator alone[4] were propagated in HEK 293T cells (ATCC) grown in DMEM Dulbecco's medium (GIBCO) and collected 96 h post-infection in the presence of 100% cytopathic effect. The infectivity titration of adenoviral vector harvests resulted in a titer of $(1–3) \times 10^7$ p.f.u./ml for each vector, while real-time PCR detected 10^8 to 10^9 HCMV DNA copies/ml.[19]

3.4. Immunofluorescence assay

Suspensions of ARPE-19 cells (ATCC) propagated in F-12K medium (GIBCO) were infected with adenoviral vector preparations (MOI 3-5) and the relevant transactivator (same MOI) in single or multiple combinations. Control cell preparations were infected with transactivator only (control adenovirus vector). After 72 h, cells were fixed with paraformaldehyde and permeabilized with NP40 according to a published procedure.[20] Gene expression of the 5-protein complex was verified with human mAbs reactive with one, two or three proteins of pUL128–131 complex or with gH.[21] As negative controls, anti-gB human mAbs were used. Serum dilutions of 1:20 in PBS were generally used for testing serum reactivity. After 30 min incubation, cells were washed and incubated for 60 min with a fluorescein-conjugated goat IgG fraction to human IgG (Fc-chain-specific, Cappel Laboratories, MP Biomedical, LLC, Solon, OH).

3.5. ELISA

Crude antigens for ELISA IgG determination were prepared by infecting ARPE-19 cells with all five adenovirus vectors (MOI 3–5) expressing the complete 5-glycoprotein complex, or different combinations of adenoviral vectors carrying single genes, or the transactivator alone (control adenovirus). Infected cells were harvested 10 days p.i. and sonicated. About 1.0 ml of crude antigen was obtained by sonicating cells from a 150 cm^2 flask. In parallel, a control adenovirus antigen was prepared. Coating was done using 1:100 dilution of antigen and control antigen preparations. Test sera (1:50 dilution) were then incubated for 60 min and reacted with the peroxidase-conjugated-goat IgG fraction to human IgG (Fc-chain-specific, Cappel Laboratories) for 60 additional min. Positive and negative control sera were included in each test run. Net OD was obtained by subtracting OD of the control adenovirus cell lysate from OD of the HCMV protein-expressing cell lysate. ELISA reactivity was considered positive when the net OD was greater than or equal to 0.10, as determined based on the reactivity of HCMV-seronegative control sera.

3.6. Neutralization assay

Serial dilutions of heat-inactivated human sera were incubated in duplicate for 60 min at 37 °C with an equal virus (VR1814) volume containing 100 p.f.u.[12] Virus-antibody mixtures were then added in duplicate to monolayers of ARPE-19 or HELF cells and centrifuged at $700 \times g$ for 30 min, as reported.[12] After 48 h incubation, cells were fixed and stained for HCMV p72 using a pool of murine monoclonal antibodies.[22] The serum dilution inhibiting virus infectivity by 50% or more with respect to virus controls (no serum) was reported as the neutralizing-antibody titer.

3.7. Statistical analysis

Sera from pregnant women with primary HCMV infection were grouped into different time intervals after onset of infection. ELISA reactivities and neutralizing antibody titers relevant to sera collected at two consecutive time points, as well as sera collected at the early (within 20 days) vs late stage (after 60 days) of primary and reactivated infections, were compared by the *t*-test for paired data. Neutralizing antibody titers were log-transformed prior to analysis.

4. Results

4.1. IFA and ELISA reactivity of human monoclonal antibodies with proteins of the pentamer complex expressed in ARPE-19 cells infected with adenoviral vectors

Recently, we were able to isolate a number of human monoclonal antibodies (mAbs) that potently neutralize HCMV infection of epithelial, endothelial and myeloid cells by targeting different epitopes of the gH/gL/pUL128–131 complex.[21] Preliminary results showed that the reactivity of human mAbs with epithelial (ARPE-19) cells infected with adenoviral vectors was in full agreement with that previously shown in 293 HEK T cells transfected with different constructs.[21]

Human mAbs previously shown to recognize the dimer pUL130/UL131, the trimer (pUL128/UL130/UL131), or the pentamer (gH/gL/pUL128/UL130/UL131) using different constructs,[21] were found to react similarly by IFA and ELISA using adenoviral vectors. On the basis of these results, it was verified that the various protein components of the complex were correctly and sufficiently expressed in ARPE-19 cells to allow testing of human sera.

4.2. Antibody response to the complex in primary HCMV infection

The IgG antibody response was investigated in sequential serum samples from 14 cases of primary HCMV infection occurring in pregnant women. All 14 women seroconverted for IgG to the complex at a median time of 55.5 (17–68) days after onset of infection. All 14 cases of seroconversion were detected by both IFA and ELISA