Association of human cytomegalovirus (HCMV) neutralizing antibodies with antibodies to the HCMV glycoprotein complexes

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Abstract
Background
Human cytomegalovirus (HCMV) causes asymptomatic infections, but also causes congenital infections when women were infected with HCMV during pregnancy, and life-threatening diseases in immunocompromised patients. To better understand the mechanism of the neutralization activity against HCMV, the association of HCMV NT antibody titers was assessed with the antibody titers against each glycoprotein complex (gc) of HCMV.

Methods
Sera collected from 78 healthy adult volunteers were used. HCMV Merlin strain and HCMV clinical isolate strain 1612 were used in the NT assay with the plaque reduction assay, in which both the MRC-5 fibroblasts cells and the RPE-1 epithelial cells were used. Glycoprotein complex of gB, gH/gL complexes (gH/gL/gO and gH/gL/UL128-131A [PC]) and gM/gN were selected as target glycoproteins. 293FT cells expressed with gB, gM/gN, gH/gL/gO, or PC, were prepared and used for the measurement of the antibody titers against each gc in an indirect immunofluorescence assay (IIFA). The correlation between the IIFA titers to each gc and the HCMV-NT titers was evaluated.

Results
There were no significant correlations between gB-specific IIFA titers and the HCMV-NT titers in epithelial cells or between gM/gN complex-specific IIFA titers and the HCMV-NT titers. On the other hand, there was a statistically significant positive correlation between the IIFA titers to gH/gL complexes and HCMV-NT titers.

Conclusions
The data suggest that the gH/gL complexes might be the major target to induce NT activity against HCMV.

Introduction
Human cytomegalovirus (HCMV) is a member of the betaherpesvirinae sub-family of Herpesviridae, and is a cause of congenital transplacental infection of the fetus. Children with congenital HCMV infection have significant morbidity and mortality with symptoms that include permanent neurological defects, such as sensorineural deafness, developmental delay, dysopia, and epilepsy. HCMV also causes severe and sometimes lethal diseases in immunocompromised patients [1, 2].
HCMV glycoprotein (gP) complexes (gcs) expressed in the virion envelope function in the process of cell attachment and entry, and they are predominant targets of the virus-neutralization (NT) antibodies of HCMV. Among 23 gPs that are encoded in the HCMV genome, 9 gPs form 4 gcs: gB, gM/gN, gH/gL/gO and pentamer complex; gH/gL/pUL128/pUL130/pUL131A (PC), and all of these gcs are reported to have the potential to induce HCMV-NT antibodies [3–6].

Before the early 2000s, gB had been considered the main antigen in the development of the HCMV vaccine [7]. At that time, HCMV-NT activities were measured using fibroblast cells and the highly passaged laboratory HCMV strain, which lacked the expression of PC [8–11]. PC has recently been shown to be indispensable for the entry of HCMV to epithelial cells (ECs), endothelial cells, and monocytes [12, 13], while the gH/gL/gO is required for the infection of both fibroblast cells and ECs [14]. Furthermore, it was reported that PC-specific antibodies might be a major component of HCMV-NT antibodies [6, 15, 16].

For the development of an HCMV vaccine, the strong induction of NT activity against HCMV is desired. HCMV has many intricate evasion strategies against humoral immunity; these impede the development of an HCMV vaccine [17]. On the other hand, it is known that potent HCMV-NT activity is required to protect a fetus from transplacental HCMV infection [18, 19]. Therefore, understanding the mechanism of HCMV NT antibody induction is necessary. In this study, the correlation of anti-HCMV-NT antibody titers of healthy adult sera with the antibody titers against each of the following 4 gc antigens, gB, gM/gN, gH/gL/gO and PC was assessed to identify the “authentic NT epitope” in Japanese adults.

Materials And Methods

Cells

Fibroblast MRC-5 cells (American Type Culture Collection [ATCC® CCL-171]) were used. The MRC-5 cells were grown in Eagle’s minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Carlsbad, CA), L-glutamine and sodium bicarbonate and 1% penicillin-streptomycin (MEM-10FBS). The retinal pigment epithelial cell line ARPE-19 (ATCC® CRL-2302™) and hTERT (human telomerase reverse transcriptase)-immortalized RPE-1 (ATCC® CRL-
both of which were epithelial cell lines, were cultured in Dulbecco’s modified Eagle medium (DMEM)/F-12 (1:1), including L-glutamine and 2.438 g/L sodium bicarbonate (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco or Hyclone, GE Healthcare UK Ltd, UK) (DMEM-10FBS). The culture medium for RPE-1 was additionally supplemented with hygromycin B at a concentration of 0.01 mg/mL. 293FT cells (Thermo Fisher Scientific, Waltham, MA) were grown in DMEM (Wako, JAPAN) supplemented with 5% fetal bovine serum (Gibco) (DMEM-5FBS). The 293FT cells were cultured in collagen-coated plates (TOYOBO, Osaka, JAPAN).

Viruses

HCMV strain Merlin (ME, ATCC® VR-1590™) was used as the source for constructing plasmids and for NT antibody analyses in MRC-5 cells. For the measurement of NT titers in ECs, HCMV clinical strain 1612, which was isolated in our laboratory from the urine of a 2-month-old baby with symptomatic HCMV disease was used. The HCMV ME and 1612 strains were propagated in MRC-5 cells and in ARPE-19 or RPE-1 cells, respectively. HCMV 1612 was used to measure the NT antibody titers in ECs, because the strain possessed the capacity to infect ECs.

Serum sampling (subject selection) and ethical considerations

Seventy-eight volunteers were recruited. The age of the volunteers ranged from 20 to 60 years (Table 1) All serum samples were first tested for HCMV IgG antibody positivity with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DENKA SEIKEN, Tokyo, Japan), and the HCMV-IgG ELISA titers were determined according to the manufacturer’s instructions. The HCMV-IgG-positive sera were then further tested for HCMV-antibody titers by an indirect immunofluorescence assay (IIFA) and an HCMV-NT assay, as described below. The serum samples were heat-inactivated at 56 °C for 30 min before testing antibody titers with any assays.

Table 1

| Age  | HCMV-IgG positivity [No. of positive/No. of tested (%)] | Male | Female | Total |
|------|---------------------------------------------------------|------|--------|-------|
| 20–29| 1/3 (33.3%)                                             | 2/4 (50%) | 3/7 (42.9%)   |
| 30–39| 10/15 (66.7%)                                           | 5/14 (33.3%) | 15/29 (51.7%) |
| 40–49| 8/11 (72.7%)                                            | 11/15 (73.3%) | 19/26 (73%)   |
| 50–59| 3/6 (50%)                                               | 7/9 (77.8%) | 10/15 (66.7%) |
| 60–69| 1/1 (100%)                                              | 0/0 | 1/1 (100%)   |
| Total| 23/42 (54.7%)                                           | 25/36 (69.4%) | 48/78 (61.5%) |

HCMV neutralization assay

HCMV-NT titers of each serum were assessed with a conventional plaque reduction assay. In brief,
serum samples were serially diluted with maintenance medium, MEM-2FBS or DMEM-2FBS. Sixty µL of the diluted serum sample and an equal volume of virus solution containing 60 plaque forming units (PFUs) of HCMV were mixed and incubated in a U-bottomed 96-well plate (Greiner Bio-One JAPAN, Tokyo, Japan) at 37 °C for 1 hour. One hundred microliters of the mixture was then added to monolayers of ECs (RPE-1) or fibroblast (MRC-5) cells (15,000 cells per well of the 96-well plate). HCMV ME was used for the HCMV-NT assay in MRC-5 cells, while HCMV 1612 was used for the HCMV-NT assays in RPE-1 cells and MRC-5 cells. Fixation, staining with trypan blue, formalin and methanol treatment, and washing of the HCMV ME-MRC-5 plates were carried out at 4 days post inoculation, while the processing and measuring of NT antibody titers against HCMV 1612 in RPE-1 cells and MRC-5 cells were fixed at 2 days post inoculation. The 50% virus-NT titers (NT₅₀) were defined as the reciprocal of the highest dilution level, at which the plaque number became less than half of the control. Each test was run in triplicate.

HCMV glycoprotein genome amplification with polymerase chain reaction for plasmid construction

Each open reading frame (ORF) of the gP of HCMV ME was amplified by polymerase chain reaction (PCR) using primer sets that were designed with reference to the ME sequence (GenBank Accession no; AY446894.2.). All primers and oligonucleotides were purchased from Eurofins Genomics (Tokyo, Japan). The 30-µL reaction was composed of 15 µL Q5 High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, MA), 0.5 µM of each primer, and template DNA. PCR amplicon bands were isolated from 10% agarose electrophoresis gel and were purified using the FastGene Gel/PCR Extraction Kit (NIPPON Genetics, Tokyo, JAPAN). Purified DNA was quantitated using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific).

Plasmid construction

First, the ORF of each gP of HCMV ME was amplified from cDNA, which was derived from RNA purified from the HCMV-ME-infecting MRC-5 cells by two step conventional reverse transcription. The synthetic DNA oligonucleotide of ME-UL128 wt (G > A in UL128 was fixed) was purchased from Integrated DNA Technologies (Coralville, IA). Each of the ORF genes was cloned into the cloning site of the modified pHEK293 ULTRA Expression vector II (Takara Bio Inc., Shiga, Japan), which was used for the
recombinant protein expression in mammalian cells as a form of fusion protein with a designated tag at the carboxy-terminal (Fig. 1A). The insertion of the gene of interest was carried out using Fusion™ HD (Takara Bio Inc) according to the manufacturer's instructions.

**Sanger DNA sequencing**

The nucleotide sequence was determined using an ABI Prism 3130 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequences were aligned to the reference using DNA Dynamo (Blue-Tractor Software, North Wales, UK). The nucleotide sequence of the constructed plasmids was confirmed to be the original sequence by Sanger DNA sequencing.

The sequence of the UL128L (UL128-131A locus) in strain 1612 was determined by Sanger sequencing, in which the PCR product was amplified using primers UL128L-F (GCGTATTTCGGACAAACACACA) and UL128L-R (CGCATGTTCAGACTGAGAAAGA) [20]. It was confirmed that there were no mutations in the UL128L gene of the HCMV 1612.

**Antigen preparations for the indirect immunofluorescence assay**

293FT cells were transfected with pHEK293-gB for the expression of recombinant gB. The same cells were also co-transfected with pHEK-gM and pHEK-gN, with pHEK-gH, pHEK-gL, and pHEK-gO, and with pHEK-gH, pHEK-gL, pUL128, pUL130, and pUL131A for the expression of gM/gN, gH/gL/gO, and PC, respectively, using pHEK293 Enhancer Vector (Takara Bio Inc.) and HuGENE HD (Promega).

The expression of gc was confirmed by IIFA by detecting the respective tags. The antibodies, which were used for the detection of histidine affinity tag (HAT)-fusion protein, c-myc-fusion protein, and FLAG-tag fusion protein, were rabbit anti-HAT-tag polyclonal antibody (GenScript, Piscataway, NJ), mouse MYC-monoclonal antibody (Aviva Systems Biology, San Diego, CA), and anti-FLAG M2 monoclonal antibody (Sigma-Aldrich Japan, Tokyo, Japan), respectively. The secondary antibodies were Alexa Fluor DyLight 488-conjugated goat anti-mouse IgG H + L antibody or DyLight 594-conjugated goat anti-rabbit IgG H + L antibody (Invitrogen).

The 293FT cells transfected with each designated plasmid or the combination of the plasmids were washed with phosphate buffered saline (PBS) (-), spotted on glass slides (Matsunami Glass IND., Ltd., Osaka, Japan), and fixed with a methanol and acetone mixture mixed at a ratio of 1:1.

**Detection of antibodies to each gc in indirect immunofluorescence assay**
To measure each gc-specific IIFA titer in sera, the serum samples were two-fold serially diluted with PBS and added onto the glass slides. After incubation at 37 °C for 1 hour, the cells were washed with PBS 3 times, and were then reacted with fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG H + L (Invitrogen, Carlsbad, CA). The antibody titer was defined as the reciprocal of the highest dilution level, at which the specific fluorescent signal was detected.

Statistical analysis
Statistical analyses were performed using the Stata15 software program (STATA Corporation, College Station, TX). Non-parametric analyses of the correlations were performed using Spearman’s test. P values of < 0.05 were considered to indicate statistical significance.

Next generation sequencing
Amino-acid sequence homology of HCMV ME and HCMV 1612 was confirmed following base sequence determination using a next generation sequencer (NGS). Genomic DNA of HCMV 1612 was extracted from ARPE-19 cells infected with HCMV 1612 using a QIAmp DNA Mini Kit (QIAGEN, Hilden, Germany) after repeated freeze-thaw cycle treatment 2 times. The sequencing libraries were then prepared by an Ion Xpress Plus Fragment Library Kit (Thermo Fisher Scientific) in accordance with the manufacturer’s instructions. The library concentration was quantified using the Ion Library TaqMan Quantification Kit (Thermo Fisher Scientific). An emulsion PCR was performed on the library, which was adjusted to a 50-pM concentration, pooled in equimolar amounts, and mixed with capture beads on the Ion Chef System (Thermo Fisher Scientific) supplemented with the Ion Torrent Personal Genome Machine (PGM) template 200 kit (Thermo Fisher Scientific). The template libraries were sequenced with the Ion Torrent PGM using the Ion 314 Chip Kit v2 (Thermo Fisher Scientific) and the Ion Torrent PGM Sequencing 200 Kit v2 (Thermo Fisher Scientific), according to the manufacturer's instructions. The resulting FASTQ format files were imported into CLC Genomics Workbench 9.0.1 (QIAGEN) for a homology analysis. Each gP sequence of 1612 strains were registered in GenBank with the accession numbers LC425070-LC425078.

Results
Confirmation of the gc expression for the detection of antibodies in IIFA antigen
The expression of all 4 types of gc was confirmed by IIFA through the detection of each of the fused
HCMV IgG prevalence among volunteers
The HCMV ELISA-IgG prevalence rate determined in the ELISA is shown in Table 1. The overall positive rate was 61.5%. The antibody prevalence in the > 40 age group was significantly higher than in the 20–40 age group (p = 0.02). There was no significant difference in the prevalence of IgG between men and women.

Correlation between the HCMV ELISA-IgG titers and the HCMV-NT antibody titers
There was a relatively moderate statistically significant correlation between the HCMV ELISA-IgG titers and the HCMV-NT titers, regardless of the combinations between cells and virus types (Fig. 2, R = 0.44 to 0.58, all p < 0.01).

Relevance of using HCMV strain 1612
The amino acid sequence homology of gB, gH, gL, gO, gM, gN, pUL130, and pUL131A between HCMV1612 and HCMV ME was 99.4%, 99.2%, 98.9%, 99.6%, 100%, 99.3%, 98.6%, and 100%, respectively. The amino acid sequence homology of pUL128 between strains 1612 and ME wild-type (Merlin BAC in which UL128 is repaired [21]) was 99.4%. In addition to the amino acid homology, we also evaluated the phenotypic homology of strain ME with strain 1612 (i.e., the NT antibody titers to each strain determined in MRC-5 cells were compared). There was a positive correlation between the NT$_{50}$ determined in fibroblasts against HCMV ME and those against HCMV 1612 (Fig. 3), indicating the validity of using the clinical isolate HCMV 1612.

Correlation between NT$_{50}$ and gc specific antibody titers determined with IIFA
The correlation between the HCMV-NT$_{50}$ and each gc-specific IIFA antibody titers is shown in Fig. 4. gB-specific IIFA antibody titers showed a statistically significant positive correlation with the HCMV-NT$_{50}$ titers to both HCMV ME and 1612 determined in fibroblast cells (Spearman R = 0.36, p = 0.011), but not with the HCMV-NT$_{50}$ to 1612 strain determined in ECs (R = 0.19, p = 0.17). Anti-gM/gN IIFA antibody titers did not contribute to eliciting either the HCMV-NT$_{50}$ in fibroblasts or ECs ($r_s$=-0.09 and p = 0.533 in fibroblast cells; $r_s$=-0.25 and p = 0.092 in ECs) (Fig. 4B). In contrast, gH/gL complex-specific IIFA titers had a statistically significant correlation with HCMV-NT$_{50}$ titers (Fig. 4C and 4D), as
a correlation analysis, gave Spearman’s R values of 0.58, 0.69, 0.56, and 0.78 for
gHLO/NT(Fibroblasts), gHLO/NT(ECs), PC/NT(Fibroblasts), and PC/NT(ECs), respectively; all p values
were < 0.05. The highest correlation was demonstrated between the HCMV-NT_{50}s determined in ECs
and PC-specific IIFA titers (R = 0.78, p < 0.0001, Fig. 4D, right panel). Both gH/gL/gO-specific IIFA
antibody titers and PC-specific IIFA titers showed a higher correlation with the HCMV-NT_{50}s
determined in ECs in comparison to those determined in fibroblasts. There was also a statistically
significant positive correlation between the gH/gL/gO-specific IIFA titers and the PC-specific IIFA titers
(R = 0.5967, p < 0.001).

Discussion
The HCMV IgG seroprevalence rate of subjects (approximately 60%) in the present study is
compatible with previously reported data in Japan [22, 23]. The HCMV-NT_{50} titers showed a weak to
moderate correlation with EIA IgG-antibody titers in either virus–cell combination (Fig. 2), as reported
previously [24, 26]. When pregnant women are infected with HCMV as the primary infection, the IgG
antibody, as determined by the EIA, become positive. The levels are parallel to the NT-antibody titers,
and the antibody level peaked at approximately 3 months after infection, and are maintained for
approximately 1 year [27]. Little is known about the strength of the correlation between NT-antibody
titers and EIA-HCMV IgG titers when several years or more have passed since the primary infection.
The present study demonstrated a moderate correlation between NT-antibody titers and EIA-HCMV
IgG titers.

This study confirmed the expression of each gP, with the exception of gO, by Western blotting (data
not shown). On the other hand, the single expression of gO was not observed by any methods (Fig. 2).
The gH/gL complex is stabilized as a complex form by binding with gO or ULs; thus, tags and peptide
antigens were considered to be exposed after conformational changing of the expressed protein [28, 29].

In the last 20 years, it has been recognized that gB might be the main target with which NT antibodies
react [7]. Originally, the development of gB-based vaccines has been driven by the observation that
the gB-specific antibodies were dominantly present in the IgG fraction of HCMV-seropositive
individuals and that they neutralize infectious HCMV [4, 30, 31]. It was also reported that gB-specific antibody titers were strongly correlated with NT$_{50}$ titers [32]. This evidence was obtained from experiments in which the serum NT activity was explored using fibroblasts (mainly, human embryo lung fibroblast cells) and highly-passaged laboratory HCMV strains, such as the HCMV Town strain. As HCMV-NT$_{50}$ titers differ greatly depending on the type of cells and HCMV strains used, we measured the HCMV-NT$_{50}$ titers using 3 types of HCMV-cells combination as described above. It was revealed that there was a positive correlation between gB-specific IIFA titers and the HCMV-NT$_{50}$ titers when the HCMV-NT$_{50}$ was determined in MRC-5 fibroblast cells but not in RPE-1 ECs (Fig. 4A), suggesting that the correlation is dependent on the virus strain and cells used for measuring HCMV-NT antibodies. In addition, it was revealed that antigenic target sites, with which NT antibodies react, were more heavily glycosylated than those that elicit non-NT antibodies, suggesting that HCMV-gB shields NT epitopes by taking advantage of glycosylation [33]. It has also been shown that the partial effect of the gB subunit vaccine is independent on NT antibody induction [34, 35], and that the vast majority (> 90%) of gB-specific antibodies secreted from B-cell clones do not have NT activity against HCMV [36], supporting the results obtained in the present study that anti-gB specific IIFA titers in sera showed little correlation with the NT antibody titers determined in the HCMV 1612/RPE-1 ECs combination.

Although the function of gM/gN is largely unknown, it has been reported that NT antibodies can be induced by the immunization of subjects with gM/gN [5, 37, 38]. However, no correlation was found between anti-gM/gN IIFA antibody titers and NT$_{50}$ titers (Fig. 4B). It was reported that immune evasion from NT antibodies became possible by the glycosylation of gN [39]. The result that the IIFA gM/gN-IgG titers were not significantly correlated with the HCMV-NT$_{50}$ titers in this study might be due to the glycosylation of gN. Because the gM/gN is the glycoprotein complex that is the most abundantly expressed on viral particles [40], glycosylation of gM/gN could reduce the NT effect by NT antibodies, as in the case of gB [33]. Further studies are required.

In this study, the strongest correlation was found between anti-PC IIFA antibody titers and NT$_{50}$ titers
measured in ECs and the IIFA-anti-gH/gL complex antibody titers (Fig. 4D). Furthermore, the anti-gH/gL/gO IIFA titers were also significantly correlated with the HCMV-NT<sub>50</sub> titers (Fig. 4C). These glycoprotein complexes seem to be the most important antigen in the induction of NT antibodies, as reported previously [28, 41–45]. It is evident that a common epitope exists in these antigen complexes, gH/gL/gO and PC. Thus, it seems that the gH/gL common antigen in IIFA has considerable influence on the positive and significant correlation of the IIFA-PC antibody titers or the correlations of gH/gL/gO with the HCMV-NT<sub>50</sub> values (Fig. 4C and 4D), and that the common epitope did not induce the difference in correlation with the HCMV-NT<sub>50</sub> titers, as reported previously [29].

**Conclusion**

Both the anti-PC antibody titers and the anti-gH/gL/gO antibody titers determined in the IIFA were highly correlated with the HCMV-NT<sub>50</sub> titers determined in both HCMV ME-fibroblasts and HCMV 1612-epithelial cell combinations. By measuring the antibody titers to gH/gL/gO or PC in IIFA, it is possible to estimate the level of the HCMV-NT<sub>50</sub> titer. The data obtained in this study suggest that the induction of a strong immune response to gH/gL complexes is required for the development of HCMV vaccines, which might have an ability to reduce the risk of congenital HCMV infection.

**Abbreviations**

- **DMEM**: Dulbecco’s modified Eagle medium
- **EC**: epithelial cells
- **ELISA**: enzyme-linked immunosorbent assay
- **FITC**: fluorescein isothiocyanate
- **gc**: glycoprotein complex
- **gP**: glycoprotein
- **HCMV**: human cytomegalovirus
IIFA
indirect immunofluorescence assay
NGS
next generation sequencer
NT
neutralization
NT₅₀
fifty percent virus-NT titers
ORF
open reading frame
PC
pentamer complex
PCR
polymerase chain reaction
PFU
plaque forming unit
PGM
Personal Genome Machine

Declarations

Ethics approval and consent to participate

This study was carried out under the approval of the Medical Research Ethics Committee of the National Institute of Infectious Diseases (NIID) for the use of human subjects (Approval number: 797).

Informed consent was obtained from all volunteer blood donors.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
Competing interests

We have no competing interests.

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Authors’ Contributions

M Shibamura performed the majority of the experiments and wrote the manuscript. M Saijo, AO, and MM planned and guided the research and supported to write this manuscript. TY, TI, SY, PHAN, HF, SH, and SF supported performing the research under discussion on this research. All authors read and revised the manuscript and approved the final manuscript.

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Figures
Confirmation of the expression of each membrane glycoprotein complex in 293FT cells transfected with each expression vector complex with an indirect immunofluorescence assay (IIFA) using antibodies to the tags fused with each membrane glycoprotein complex.

Glycoproteins that were expressed on each IIFA plate were described on the first line. Tag antibodies used as the primary antibodies are rabbit anti-HAT-IgG, mouse anti-FLAG, mouse myc-IgG, and rabbit anti-gO peptide antibody. The expression of Tag was distinguished by a secondary antibody (anti-rabbit IgG Dylight594 or anti-mouse IgG Dylight488). The tags fused with each gc were HAT (-gB, -gH, -gM, -pUL128), FLAG (-gL, -gN, -pUL130) and c-myc (-gO, -pUL131A).
Figure 2

Correlation between the quantitative ELISA HCMV-IgG titers and the HCMV-NT titers (NT50). The correlations were analyzed using Spearman’s test. The strength of the correlation was expressed using the correlation coefficient “rs”. P values of <0.05 were considered to indicate statistical significance. The rs and p values were 0.58 (a; closed diamond), 0.51 (b; square) and 0.44 (c; closed circle) for HCMV 1612-cell PRE-1 (a), HCMV 1612-cell MRC-5 (b), and HCMV ME-cell MRC-5 (c) combinations, respectively.
The correlation between the NT antibody titers of 78 participants determined using HCMV ME in MRC-5 fibroblast cells and those determined using HCMV 1612 in MRC-5 fibroblast cells. A strong correlation was observed with an rs value of 0.85 (p<0.05).
Figure 4

The correlation of each anti-gc specific IIFA titer with HCMV-NT50 titers. The HCMV strain and cell types used in the neutralization test were HCMV ME/MRC-5 fibroblasts (left panels of A, B, C, and D), and HCMV 1612/RPE-1 epithelial cells (right panels of A, B, C, and D).

Each correlation coefficient $r_s$ and p-value is indicated in the figure. “Negative” in IIFA means a result of <8 (3 Log2). All samples were HCMV-NT50-positive (>8) and showed a positive CMV-IgG titer (EIA). The correlation coefficient was calculated regarding IIFA-negative as 0 (zero).