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Human cytomegalovirus co-opts FcRn-mediated IgG transport across trophoblast barriers

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Abstract

Congenital human cytomegalovirus (HCMV) infection can occur despite maternal antibodies, but how infectious virions cross the placental trophoblast barrier remains unclear. Here we show that HCMV co-opts physiological IgG transport through the neonatal Fc receptor (FcRn) to traverse polarized trophoblasts. In primary human trophoblast organoids and cell-based transcytosis models, IgG enhances the transfer of infectious virions. This process requires host FcRn and the viral Fcγ-gamma receptors (vFcγRs) gp34 and gp68. Genetic deletion of FcRn or vFcγRs abolishes transcytosis and antibodies targeting gp34 and gp68 can block transcytosis. Sera from women with primary HCMV infection transfer similar quantities of viral genomes irrespective of fetal transmission outcome. However, non-transmitter sera support transcytosis of fewer infectious virions, indicating that neutralization is retained during transcytosis but does not prevent virion passage. Mechanistically, gp34 promotes virion attachment to FcRn, whereas gp68 modulates FcRn-IgG interaction, supporting a model of coordinated entry and release. Rhesus cytomegalovirus (RhCMV) shows analogous vFcγR-dependent transcytosis, suggesting conservation among cytomegaloviruses. These findings identify vFcγRs as molecular determinants of placental HCMV passage and potential targets for preventing congenital infection.

Introduction

Congenital infections remain a major cause of neonatal morbidity and mortality worldwide [1,2]. Despite the diverse biology of pathogens that cross the placenta, they share the ability to breach the placental barrier and establish infection in the developing fetus. The precise mechanisms underlying transplacental passage are not fully understood [3]. The syncytiotrophoblast, a multinucleated cell layer which forms the primary interface between maternal and fetal compartments, acts as both a barrier to pathogens and a selective transport system for maternal IgG via the neonatal Fc receptor (FcRn). This FcRn-mediated pathway is essential for passive immunity of the newborn but also represents a potential route for pathogen exploitation.

Among congenital infections, congenital CMV (cCMV) is the most common and clinically significant as it is the leading infectious cause of sensorineural hearing loss and neurodevelopmental impairment in newborns [4]. The burden of disease is particularly severe when maternal infection occurs in the first trimester, a window of heightened fetal vulnerability [5–7]. Importantly, cCMV can occur despite the presence of maternal neutralising antibodies[8–11], highlighting a critical gap in our understanding of protective versus pathogenic immune mechanisms. Accordingly, trials with hyperimmune globulins (HIG) showed variable efficacy [12–18], underscoring the need for deeper mechanistic insight to guide successful intervention. Experimental evidence supports this paradox as HCMV can spread directly from cell to cell in the presence of neutralising antibodies [19]. In the non-human primate (NHP) model of infection, horizontal transmission of rhesus CMV (RhCMV) is observed despite robust antibody responses [20] and vertical transmission is observed in the absence of an epithelial entry receptor [21]. An earlier study

suggested that antibody-dependent transcytosis may contribute to placental HCMV transmission [22,23], but the underlying molecular mechanism remained unresolved for two decades.

CMVs encode a set of viral IgG binding glycoproteins (viral Fc-gamma receptors, vFcγRs) that bind to the Fc-part of IgG and contribute to persistence and immune evasion *in vitro* and in the non-human primate model of rhesus CMV (RhCMV) infection [24–28]. In HCMV and RhCMV, vFcγRs are incorporated into the virion envelope [27,29,30], which has raised the hypothesis that these glycoproteins may modulate IgG trafficking at the maternal-fetal interface.

In this study, we demonstrate that HCMV exploits maternal IgG to mediate transcytosis of infectious virions across the syncytiotrophoblast layer, providing a mechanistic explanation for how FcRn-mediated IgG transport can facilitate the passage of infectious virions even in the presence of antibodies.

Results

HCMV virions traverse the fetal trophoblast via IgG-dependent transcytosis.

To study HCMV virion traversal of the fetal syncytiotrophoblast, we used a two-chamber permeable support culture of primary human trophoblast cells. We used primary trophoblast organoids (TO) isolated from term placentas [33]. TO were isolated and continuously expanded as described previously [33]. This model recapitulates key features and the cellular composition of the trophoblast barrier. Dissociated trophoblast organoids were cultured until a 2D trophoblast barrier was formed and the syncytiotrophoblast (STB) was developed (Fig. S1A). Barrier tightness was monitored prior to and after each experiment (Fig. S1B). To allow for virion migration, no matrix coating was used in 2D cultures. HCMV virions of the TB40/E strain [31] were added into the top chamber in the absence or presence of human IgG (Fig. 1A). TB40/E is able to enter the syncytiotrophoblast but infection is abortive in trophoblast cells [37] (Fig. S1C). Transcytosed infectious virions were quantified by transferring basolateral samples to MRC-5 fibroblasts and staining for nuclear HCMV IE-1; viral genome transfer was quantified by qPCR of apical and basolateral samples. HCMV virions showed significantly enhanced transcytosis in the presence of human IgG1 compared to the absence of IgG (Fig. 1B). Quantification of viral genomes mirrored the quantification of infectious virions (Fig. 1C). The two tested TO from two individual women showed differences in transcytosis efficiency, which could be linked to different levels of FcRn expression (Fig. S1D) or differences in syncytia formation dynamics. Our study was designed to establish proof of principle rather than compare individual placentas; donor-specific differences were therefore not investigated further. Consistent with a previous report of first-trimester trophoblast expression [38], reanalysis of bulk RNA-seq data showed strong first-trimester placental FCGRT expression relative to fetal brain (Fig. S2A). Imaging cytometry further detected FcRn in the syncytiotrophoblast of two rhesus macaque placentae at nine weeks gestation and in a term human placenta [39]. Given the low frequency of infectious-virion transcytosis observed in our experiments, the limited maternal-to-fetal IgG transfer reported during early pregnancy [40] could be sufficient to support viral transmission. Further, in

immunocompetent pregnant women, substantial DNAemia is mainly detectable in blood during the first several weeks to months after primary infection. Most circulating viral DNA is associated with blood cells rather than the cell-free compartment [42]. To further assess the quantitative plausibility of this mechanism in humans, we extrapolated from our experimental measurements to approximate transcytosis rates across the placenta surface area (Fig. S2C). Although measured virion transcytosis was low relative to the input, even limited cell-free viraemia could, over time, permit sufficient transfer of infectious virions to seed fetal infection.

Virion transcytosis depends on FcRn and HCMV-encoded glycoproteins gp34 and gp68.

Next, we transitioned to a more adaptable model by using the BeWo b30 cell line [43,44], a clone from the BeWo trophoblast cell line selected for optimised monolayer formation and diaplacental transport studies, in conjunction with HCMV BAC2 (AD169varL [25]) to allow for genetic deletions (Fig. 2A). We used variants lacking expression of the previously reported IgG-binding glycoprotein gp95 (*RL12*) [25] to focus on the IgG-binding glycoproteins gp34 and gp68 of the virion envelope [30]. gp95 was not found to impact infectious virion transcytosis (Fig. S3A). Using this model, we reproduced the significant increase in transcytosis when IgG is added to virions (Fig. 2B). Further, using only $F(ab)_2$ fragments of human IgG or a formulation of HCMV hyperimmunoglobulin (HIG) that contains neutralising antibodies (Cytotect®), both abrogated transcytosis of infectious virions to background levels (Fig. 2B). As this identified IgG and Fcγ in particular to govern HCMV virion transcytosis, we then tested a genetic deletion variant that lacks expression of individual (Fig. S3A) or all vFcγRs (Fig. 2C). Again, this abrogated virion transcytosis and identified gp34 (*RL11*) and gp68 (*UL119-118*) as essential determinants. In turn, when we tested vFcγR-expressing virions on BeWo b30 cells with a genetic deletion in the FCGRT gene (FCGRT-KO), we observed abrogation of transcytosis as well (Fig. 2D, Fig. S3B). This identified FcRn as the indispensable host receptor mediating HCMV virion transcytosis. Quantification of HCMV genome transcytosis aligned with the above observations regarding IgG-mediated enhancement (Fig. 2E) and vFcγR-dependency (Fig. 2F). Strikingly, however, and in contrast to the data presented in Fig. 2B, we observed efficient transcytosis of HCMV genomes in the presence of HIG. We conclude that neutralisation of virions does not impair the process of transcytosis but that neutralisation is retained during transcytosis. Deletion of vFcγRs again abrogated genome transcytosis, indicating that vFcγRs are required whether virions are coated with virus-specific or non-specific IgG. Finally, we repeated the experiment in Fig. 2B using gp34- or gp68-specific monoclonal antibodies selected for their ability to inhibit vFcγR binding to IgG. Pre-incubation of HCMV virions with individual mAbs reduced ($p > 0.05$) transcytosis compared to a mouse IgG2a control (Fig. 2G). Equimolar mAb combination or a gp34-specific Fab fragment alone (recombinant wt.11) reduced transcytosis significantly ($p < 0.05$). A recombinant nanobody-like Fab fragment of wt.11 formed only from the heavy chain variable region (VH-Fab) also significantly reduced HCMV virion transcytosis ($p < 0.05$). A gp68-specific $F(ab')_2$ fragment from mAb digestion also significantly reduced transcytosis ($p < 0.05$, Fig. S3C).

Neutralisation is retained during but does not limit HCMV virion transcytosis.

We demonstrated that HCMV virions can traverse fetal trophoblast cells and reach the basolateral compartment by employing the glycoproteins gp34 and gp68. These glycoproteins facilitate transcytosis through the FcRn-IgG pathway. Further, we observed that virion neutralisation is retained during transcytosis. Consequently, we hypothesised that the neutralising potential of serum IgG determines if infectious virions can be transcytosed or not. To test this hypothesis, we assessed AD169varL transcytosis across BeWo b30 cells in the presence of sera collected at amniocentesis from women with primary gestational HCMV infection (Fig. 3A). Previously identified study participants [15,16] were divided into two groups; Transmitter (T) or non-transmitter (NT) based on congenital infection (cCMV) of the fetus, determined after amniocentesis. We found that T-sera enhance infectious HCMV virion transcytosis to a significantly greater extent than NT-sera ($p = 0.0109$), while transcytosis of genome copies remains comparable between the two groups ($p > 0{\cdot}05$) (Fig. 3B). Despite comparable genome transfer, transmitter sera showed significantly lower neutralising capacity than non-transmitter sera ($p = 0.046752$) (Fig. 3C). However, neutralisation did not correlate with infectious-virus transcytosis within either group (Fig. 3D). We conclude that neutralising activity can persist throughout transcytosis without preventing virion transport.

HCMV encoded glycoproteins gp34 and gp68 mediate attachment to and release from the neonatal Fc receptor.

As our previous work identified the distinct non-overlapping IgG-regions recognised by gp34 (hinge) or gp68 (CH2-CH3 interdomain) [25], we hypothesised that both molecules are also required for efficient transcytosis and that their respective roles are determined by their interaction with FcRn. FcRn binds the CH2-CH3 interdomain on IgG and, like gp68, binds at a 2:1 ratio[45,46]. We therefore expected gp68 to block FcRn binding to IgG, while gp34 would increase attachment via simultaneous binding to IgG. To test this, we employed a previously established model for the assessment of FcγR binding to immune complexes using flow cytometry [25,27]. In this approach, 293T cells stably expressing the model antigen CD20 are incubated with CD20-specific human IgG (I) or non-specific human IgG (II) or no IgG (III) and probed for FcRn binding (Fig. 4A). This setup simulates the interaction of FcRn with the virion envelope (I) in the presence of HCMV specific IgG or (II) in the absence of HCMV specific IgG or (III) in the absence of IgG. Further, as FcRn binds IgG at acidic $pH < 6$, we tested whether initial incubation at physiological pH 7.2 or continuous acidic pH 5.8 will impact gp34 or gp68 function. We found that gp34 enhanced binding to FcRn in all settings and independent of pH, while gp68 reduced FcRn binding in the presence of antigen-specific IgG. This effect was weaker when initial binding was performed at low pH as well, in line with the reported pH sensitivity of gp68 [45] (Fig. 4A). Strikingly, gp34 also showed direct and IgG-independent binding to FcRn, which was recapitulated in an ELISA approach (Fig. 4B). Here, we also observed that a gp34 point mutant, $gp34_{1-179\ W65F}$, with a strongly reduced IgG binding capacity (gp34mtrp [25]), still retains FcRn binding, implying separate binding regions for IgG and FcRn on gp34. Having previously identified Rh05 as a functional gp34 homologue in RhCMV [27], we tested its binding to human FcRn, which is highly conserved among mammals. Rh05 bound FcRn directly at levels comparable to gp34 (Fig. S4A). RhCMV 68-1R with repaired Rh152–Rh151 [27,32] also underwent IgG-

dependent transcytosis across human BeWo b30 cells, which was abolished in the absence of vFcγRs (Fig. S4B).

Discussion

Our findings provide mechanistic insight into how HCMV exploits maternal IgG antibodies and Fc-receptor pathways at the placental interface to facilitate viral dissemination. In agreement with recent studies, which reported that antibody neutralisation does not correlate with protection from cCMV in humans [8,47,48], we show that neutralising capacity of human serum does not correlate with a reduction in infectious virion transcytosis. This observation may explain the limited efficacy of hyperimmune globulin (HIG) therapy in clinical studies: insufficient dosing may fail to achieve complete neutralisation but instead promote IgG decoration of infectious virions, thereby facilitating viral passage rather than blocking it [12–17]. This provides a plausible explanation for why maternal immunity reduces, but does not eliminate the risk of transplacental transmission and congenital infection [9–11]. Our findings suggest that FcRn-mediated IgG transport is inherently permissive to immune complexes regardless of their neutralisation status, thereby creating a vulnerability that can be exploited by pathogens. This distinction between neutralisation and transport provides a conceptual framework for understanding how antiviral antibodies can fail to prevent, and in some cases facilitate, pathogen dissemination across barrier tissues. The essential roles of gp34 and gp68 in transcytosis, together with their ability to simultaneously engage distinct sites on IgG [25] provide a plausible molecular basis for HCMV's apparent specialization in exploiting this pathway. Whether other vertically transmitted pathogens similarly interact with IgG to cross the human placenta remains to be determined.

We propose that FcRn-dependent HCMV transcytosis may not be a placenta-specific adaptation, but instead reflect opportunistic exploitation of an IgG-trafficking pathway shared by multiple FcRn-expressing epithelia. Congenital transmission could therefore represent an incidental consequence of a broader capacity to traverse FcRn-expressing barriers. The low transcytosis efficiency observed in our experiments is compatible with this view. The syncytiotrophoblast normally constitutes a strong antiviral barrier, and most internalised IgG-virion complexes are likely retained in non-productive infection as we and others observed [37] (Fig. S1C). However, HCMV-encoded vFcγRs may allow a low but sufficient number of virions to escape intracellular retention. Further, low but detectable IgG transfer in early pregnancy [42] is compatible with rare but effective transcytosis, whereas increasing IgG transfer during gestation may contribute to higher transmission rates despite lower disease severity. Low amounts of cell-free as well as leukocyte-associated HCMV may both supply this pathway. However, leukocyte DNAemia did not correlate with fetal transmission in a previous cohort study [49] and pentamer-independent congenital transmission in non-human primates [21] further indicates that pentamer-dependent leukocyte or endothelial passage is not likely.

RhCMV employs homologous vFcγRs to mediate immune evasion *in vivo* [27], and we demonstrate that one of its vFcγRs, Rh05, directly binds FcRn. Moreover, RhCMV is capable of transcytosis even across

human cells and in the presence of human IgG, underscoring the close evolutionary relationship between these systems. Importantly, loss of all vFcγRs eliminated transcytosis of both HCMV and RhCMV, independent of IgG, showing that the IgG-vFcγR-FcRn axis is required for transcytosis across the syncytiotrophoblast.

We found that gp34 and gp68 each contribute to efficient transcytosis: loss of either gene, or blocking either protein with monoclonal antibodies, reduced transcytosis. The overlapping and simultaneous binding activities of FcRn, gp68, and gp34 further support a cooperative mechanism of vFcγRs. Based on our data, we propose the following working model of transcytosis: (1) gp34 mediates IgG-independent attachment to FcRn at physiological pH on the maternal trophoblast surface; (2) IgG enhances FcRn-gp34 binding in the acidic environment of the early endosome; and (3) gp68 then engages and antagonises FcRn-IgG interactions, which likely promotes virion release at the fetal trophoblast side (Fig. 5). This requirement of gp68 is supported by the observation that transcytosis is abolished in the absence of gp68 alone. Further, as transcytosis is abolished in the absence of vFcγRs regardless of virus-specific or non-specific IgG being present, we conclude that vFcγRs are also required for virion release. This coordinated mechanism would enable efficient and directional transcytosis of infectious virions across the trophoblast barrier.

This study has limitations. Although our findings establish a mechanistic route for placental traversal of infectious virions, the quantitative contribution of FcRn-mediated transcytosis to congenital infection in humans remains to be determined. In addition, trophoblast organoid and transcytosis models cannot fully recapitulate the complexity of the maternal-fetal interface during pregnancy. Future studies will therefore be required to define how antibody specificity, Fc glycosylation, and maternal immunity influence transplacental viral dissemination. Although experimental *in vivo* validation would be desirable, existing animal models present substantial limitations for studying congenital CMV transmission. Murine CMV (MCMV) does not accurately model transplacental transmission following maternal infection, whereas congenital infection in immunocompetent nonhuman primates occurs at low frequency, like in humans [50]. Although immunocompromised rhesus macaque models support increased congenital transmission [39], severe immune perturbation induced by CD4 depletion may influence IgG-dependent barrier traversal mechanisms. The guinea pig CMV (GPCMV) model does support congenital infection, however, vFcγRs encoded by GPCMV are insufficiently defined. These limitations underscore the value of human trophoblast organoids and polarised transcytosis systems for mechanistic studies of placental viral dissemination.

Our data identify new molecular determinants of congenital HCMV infection and detail the mechanism of a previously proposed pathway that may be exploited by pathogens beyond HCMV. In the context of HCMV, we uncover new potential targets for intervention and prevention of congenital infection.

Methods

Ethics

Human placental tissues and sera were obtained with ethics approval from the University Medical Center Freiburg (24-1573-S1) and University of Tübingen (111/2025BO2, 053/2024BO2, and 237/2019BO). All participants provided written informed consent in accordance with the 2000 Declaration of Helsinki, and samples were anonymised before analysis.

Human cohort samples

Sera collected at amniocentesis from women with primary CMV infection who transmitted HCMV to the fetus (T; n = 13) or did not (NT; n = 12) were obtained from the CMV Consiliary Laboratory, University Hospital Tübingen. None had received CMV-specific hyperimmune globulin, and primary infection was defined as described previously [15,16]. Congenital CMV infection was diagnosed by amniotic-fluid culture and quantitative CMV PCR. Transmitter viral loads (genome copies/mL amniotic fluid) were retrieved from laboratory records.

Cells

MRC-5, HFF, TRF, BeWo b30, and BeWo b30 FCGRT-knockout cells were cultured at 37°C and 5% CO_2 in DMEM (anprotec) supplemented with 10% FCS, 0.5% penicillin–streptomycin, and 0.1% ciprofloxacin. BeWo b30 cells were passaged every 2–3 days at 1:2–1:3 and MRC-5 cells every 5–7 days at 1:3. All lines were routinely screened for mycoplasma by PCR, and contaminated cultures were treated with Plasmocure (InvivoGen).

Viruses

HCMV strains TB40/E and BAC-cloned AD169varL (BAC2), and BAC-cloned 68-1R RhCMV, are described elsewhere [25,27,31,32]. Mutants lacking vFcγRs RL11/gp34, UL119-118/gp68, or both were generated by BAC recombineering. Viral stocks were propagated in MRC-5 fibroblasts, and genomic integrity was confirmed by Illumina sequencing.

Human trophoblast organoids

Trophoblast organoids (TOs) were established from villous tissue of independent term placentas as described previously [33]. In brief, chorionic villi were sequentially digested with 0.2% trypsin/0.02% EDTA and 1 mg/mL collagenase V, mechanically dissociated, pelleted, and embedded in 40 µL growth factor-reduced Matrigel domes (Corning) in 24-well plates. Domes were overlaid with trophoblast organoid medium comprising Advanced DMEM/F12, B27, N2, 10% FBS, GlutaMAX, Primocin, N-acetyl-L-cysteine, A83-01, CHIR99021, EGF, R-spondin-1, FGF2, HGF, nicotinamide, Y-27632, and prostaglandin E2. Medium was refreshed every 2–3 days. Organoids appeared after approximately 12 days and were passaged every 7–10 days using TrypLE and mechanical dissociation, with additional Y-27632 for the first four

days. Organoids were cryopreserved in 10% DMSO, 30% FCS, and 60% Advanced DMEM/F12 in liquid nitrogen.

Two-chamber transcytosis assays

Transcytosis was assessed in 24-well Transwell® inserts (3 µm pores; Corning). BeWo cells (6×10^4) or dissociated, 70 µm-filtered organoid cells (8×10^4) were seeded apically and cultured for six or nine days, respectively, with medium changes every 2–3 days. Barrier integrity was verified before and after experiments by TEER and 110 kDa FITC–dextran permeability (Fig. S1B). After 1 h serum starvation, virions (typically 6×10^5 PFU) were incubated for 1 h on ice with or without antibody (50 µg/mL): rituximab, ofatumumab, trastuzumab, Cytotect, ofatumumab or gp68-specific $F(ab')_2$ fragments, or rhesusised Rh-CD4-specific IgG; serum was used at 1:200. Mixtures were applied apically in pH-adjusted buffer, incubated for 30 min on ice and 10 min at room temperature, then transferred to 37°C. Apical and basolateral samples were collected at 0, 12, 24, and 48 h for normalization, infection assays, IgG ELISA, and viral genome quantification.

Quantification of transcytosed HCMV

Basolateral samples of 100µl were applied to MRC-5 fibroblasts (2.5×10^4 cells per well). After 72 h, cells were fixed with ice-cold methanol and stained for HCMV immediate-early antigen IE-1 using a mouse monoclonal antibody (kind gift from William Britt, Birmingham, Alabama, USA) followed by FITC-conjugated secondary antibody; rabbit anti-mouse FITC-conjugated secondary antibody (Invitrogen, 31561). Infected nuclei were counted by fluorescence microscopy on a Zeiss Observer Microscope. RhCMV titration was performed on TRF after 24h using RhCMV-IE2 specific mouse mAb (OHSU, Portland OR, USA) and the above detection method. Total human IgG in supernatants was measured using a human IgG ELISA kit (Invitrogen). Viral DNA was extracted from supernatants and quantified by qPCR (AltoStar system).

Neutralisation assays

Neutralisation was assessed as described previously [34]. BAC2-derived AD169varL^25 (MOI 0.1) was incubated with serially diluted sera or IgG for 1 h at 37°C before infection of MRC-5 cells. After 48 h, infection was quantified by IE-1 immunofluorescence, and neutralisation titres were calculated relative to virus-only controls.

Genetic deletion of the FCGRT gene in BeWo b30 cells

BeWo b30 cells (1×10^6) were nucleofected with 1 µg donor DNA and 1 µg of each gRNA (CRISPR KN2.0; OriGene, KN400364) using a 4D-Nucleofector (Lonza; SF solution, pulse CM-138). GFP expression was monitored microscopically. At 80% confluence, cells were selected with 5 µg/mL puromycin (Sigma-Aldrich, P8833). Resistant clones were expanded, reselected, and subcloned by limiting dilution in conditioned medium containing 20% FCS. Single-cell clones were expanded and analysed for FcRn

expression by immunoblotting. Cells (5×10^6 per clone) were freeze–thaw lysed, heated at 95°C for 10 min, separated by 12% SDS-PAGE, and transferred to nitrocellulose membranes (Cytiva, A30764822). Membranes were probed with HRP-conjugated anti-FcRn (sc-271745; 1:2,000) and anti-GAPDH (HRP-60004; 1:10,000) antibodies. Signal was detected using SignalFire Elite ECL (12757S) and a ChemoStar Touch imager.

FcRn-vFcγR binding assays

Flow cytometry binding assays were performed as described previously [25]. Briefly, 293T cells expressing CD20 and individual vFcγRs were incubated with rituximab or infliximab, followed by recombinant His_6-tagged human FcRn ectodomain complexed with β_2-microglobulin (CT009-H08H, Sino Biological). Binding was detected by His-tag staining and flow cytometry (LSR Fortessa). For ELISA, FcRn or human IgG1 (I5154-1MG, Sigma-Aldrich) was immobilised overnight on 96-well plates (NUNC MaxiSorp). After blocking, V5-tagged vFcγRs or a V5-tagged control protein (Pr00106, Absolute Antibody) were added overnight, and binding was detected with HRP-conjugated anti-V5 antibody (R961-25, Invitrogen).

Immunofluorescence microscopy

For characterization, trophoblast organoids (TOs) were dissociated and seeded at 2.5×10^4 cells per well in 96-well plates. After 10 days, cells were fixed with 4% PFA, permeabilized, and stained for cytotrophoblasts with rat anti-ITGA6 (MA5-16884, Invitrogen) and syncytiotrophoblasts with rabbit anti-CGB3 (ab131170, Abcam). Alexa Fluor 488 goat anti-rat (A11006) and Alexa Fluor 594 donkey anti-rabbit (A21207; Invitrogen) secondary antibodies were used, with DAPI nuclear counterstaining. Images were acquired on a Keyence fluorescence microscope using a 10× objective. In infected TOs or BeWo b30 cells, IE-1 was detected using a mouse monoclonal antibody (gift from Bill Britt) and FITC-conjugated rabbit anti-mouse secondary antibody (31561, Invitrogen).

Imaging Cytometry

Fresh-frozen, OCT-embedded human and rhesus macaque (RM) placental tissues were imaged as described previously [35]. Sections were fixed with acetone and ethanol, mounted on tissue chips (Bruker), and blocked with 5% goat serum in DPBS. Iterative background scanning, antibody staining, imaging, and photobleaching were performed using a Zellscanner microscope. Antibodies included pan-cytokeratin–AF488 (AE1-AE3, Novus), FCRN-PE (FCGRT/2832, Novus), CD45-BUV395 (human: 1D11; RM: D058-1283; BD Biosciences), and CD163-FITC (EDHu-1, Bio-Rad). Selected fields were exported as OME-TIFF files and analysed in QuPath v0.5.1 [36]

Monoclonal antibodies (mAbs)

Mouse IgG2a mAbs wt.11 (gp34-specific) and 68.04/68.05 (gp68-specific) were raised against vFcγR ectodomains and are described in EP21181202.9. Full-length mAbs were purified from hybridoma supernatants by protein G chromatography (ÄKTA, GE Healthcare). wt.11 Fab and nanobody-like fragments were expressed in 293T cells using VL-Fab/VH-Fab plasmids or VH-Fab alone, respectively,

and affinity-purified (plasmids provided by AG Kontermann). 68.04 $F(ab')_2$ fragments were generated from full-length mAb using the Pierce $F(ab')_2$ Preparation Kit (Thermo Fisher Scientific).

Statistics and reproducibility

Experiments were performed with independent experiments as indicated in the figure legends, typically including technical triplicates. Statistical analyses were performed using tests as indicated in the figure legends provided by Prism 10 Software.

Large Language Models

During the preparation of the original draft, the corresponding author used ChatGPT in order to help with German to English translation, grammar and wording. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the published article.

Data availability

Data supporting the findings of this study are provided in the Article and Supplementary Information. Additional experimental data and deidentified participant-level data may be obtained from the corresponding author upon reasonable request, subject to the original participant consent, applicable ethical and institutional approvals.

Declarations

Competing Interests

PK has received funding by Biotest GmbH & Co. KGaA and has received Cytotect® for research purposes. HH, KH and PK are joint holders of patent EP21181202.9 on gp34 and gp68 specific monoclonal antibodies. None of these activities have impacted this work beyond financial and material support. The remaining authors declare no competing interests.

Author contributions

Conceived and designed the experiments: PK, KH, RKR

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Figures

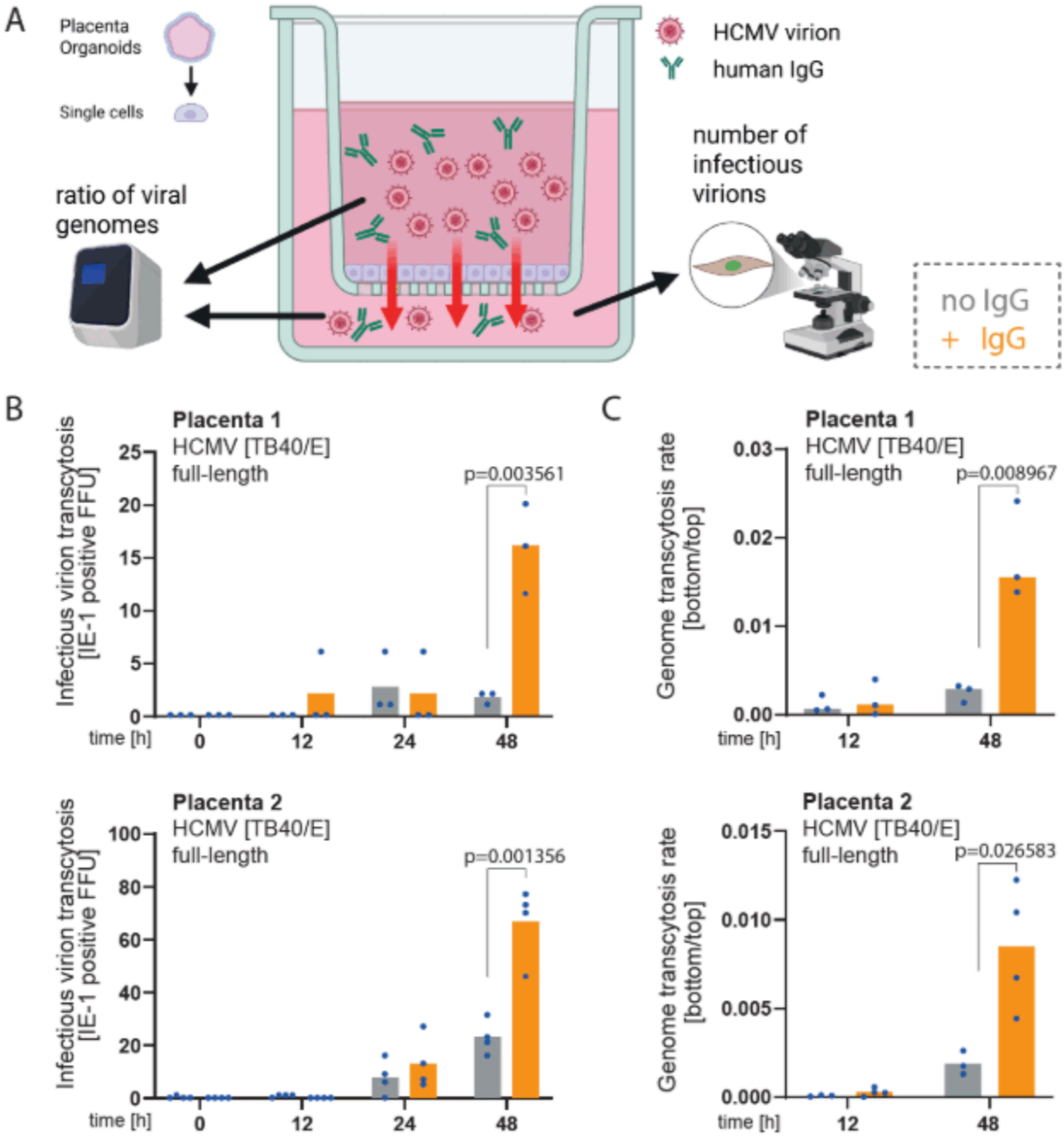


Figure 1

HCMV exploits IgG antibodies to pass the fetal syncytiotrophoblast via transcytosis. A) Experimental setup and legend. Trophoblast Organoids (TO) were homogenised into single cells, seeded into a two-chamber permeable support culture plate and cultured until formation of a tight monolayer. HCMV virions were added to the apical side and translocated infectious virions or HCMV genomes were quantified at the indicated time points. IgG is human IgG1. Created in BioRender. Kolb, P. (2025) https://BioRender.com/gs9ndbg. B) Infectious virion transcytosis across TO monolayers from two individuals. Counts in 100µl sample. Symbols indicate mean values from individual experiments measured in triplicates. Bars show mean across individual experiments. Unpaired t-test. C) HCMV genome transcytosis across TO monolayers from two individuals. Ratio between samples from bottom

and top chamber is indicated on the y-axis. Symbols indicate mean values from individual experiments measured in triplicates. Bars show mean across individual experiments. Unpaired t-test.

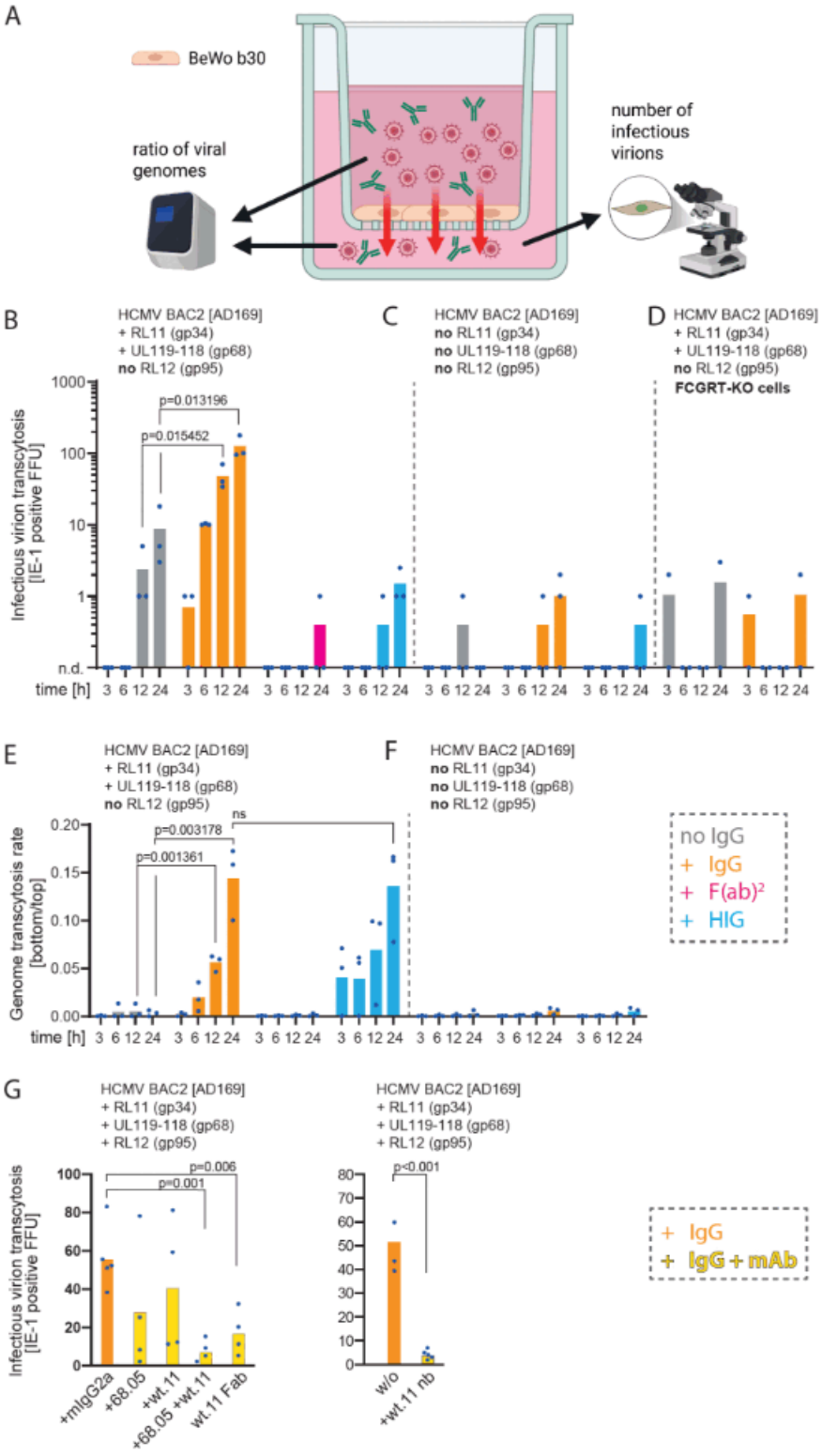


Figure 2

HCMV virion transcytosis requires FcRn, IgG and vFcγRs. A) Experimental setup and legend. Created in BioRender. Kolb, P. (2025) https://BioRender.com/gs9ndbg. BeWo b30 human syncytiotrophoblast cells

were seeded into a two-chamber permeable support culture plate and grown until formation of a tight monolayer. IgG is humanised IgG1 (trastuzumab) or a F(ab)$_2$ fragment thereof. mAbs are vFcγR-specific monoclonal antibodies raised in mice or fragments thereof, which were pre-selected for interference with IgG binding. HIG is Cytotect®. HCMV virions were added to the apical side and transcytosed infectious virions as counts in 100µl samples (B-D/G) or transcytosed HCMV genomes as ratio between samples from bottom and top chamber (C-F) were quantified at the indicated time points. B/E) Transcytosis of HCMV virions in the presence of vFcγRs gp34 and gp68, C/F) in the absence of vFcγRs, D) in the presence of vFcγRs but in the absence of FcRn, G) in the presence of trastuzumab +/- mAbs or Fab fragments or nanobody-like fragments (nb, VH-Fab only) as indicated. Unpaired t-test. Symbols show means of individual experiments measured in technical triplicates. Bars show mean across individual experiments.

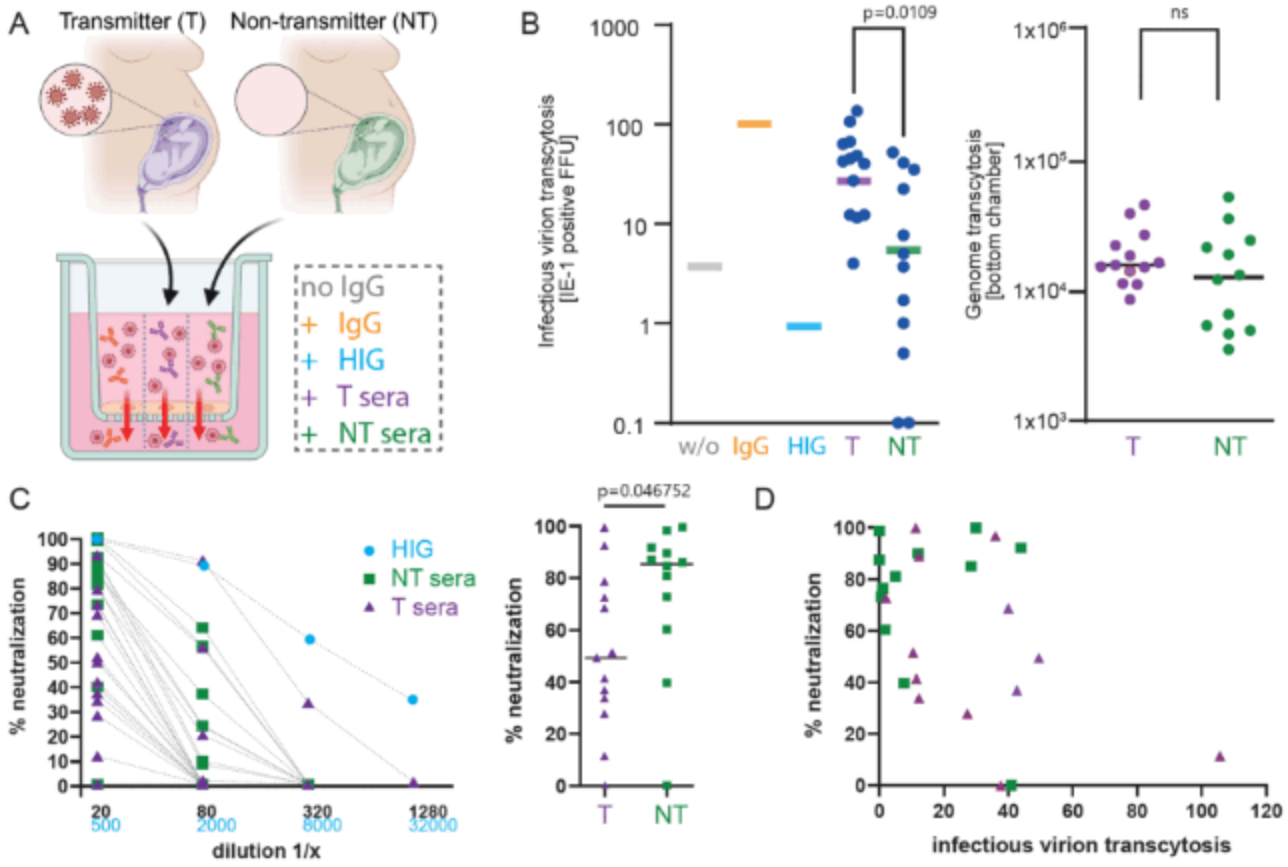


Figure 3

Virus neutralisation by maternal serum does not limit but is retained during transcytosis. A) Experimental setup. Created in BioRender. Kolb, P. (2025) https://BioRender.com/cn949ap. Sera from a previous study [15] with untreated women with primary infection early during pregnancy. Sera were taken at amniocentesis. 13 transmitter (T) vs 12 non-transmitter (NT). Per sample, a 1:200 dilution of serum was mixed with 3x10^6 PFU/ml of HCMV BAC2-AD169varL virions prior to apical chamber application and B) transcytosed infectious virions as counts in 100 µl samples (left) or transcytosed HCMV genomes from the bottom chamber (right) were quantified at the 24 h time point. Symbols show mean

values from individual serum samples measured in technical triplicates. Lines show median across individual serum samples. Controls (no IgG, +IgG, +HIG) are shown as median from 9 replicates. Mann-Whitney test. C) Neutralisation was assessed via titration on human MRC-5 fibroblasts and compared to HIG (50 mg/ml IgG, dilutions in blue). Symbols indicate individual serum neutralisation as mean of technical triplicates. Mann-Whitney test on 1/20 serum dilutions. Line shows median. D) Comparison of transcytosis of infectious virions to neutralisation potential of sera. Data was taken from B and C. Spearman correlations between neutralisation and transcytosis (NT sera, p=0.9739, r=-0.01399, 95%CI= -0.5958 to 0.5775; T sera, p=0.9063, r=-0.03846, 95%CI= -0.5893 to 0.5368).

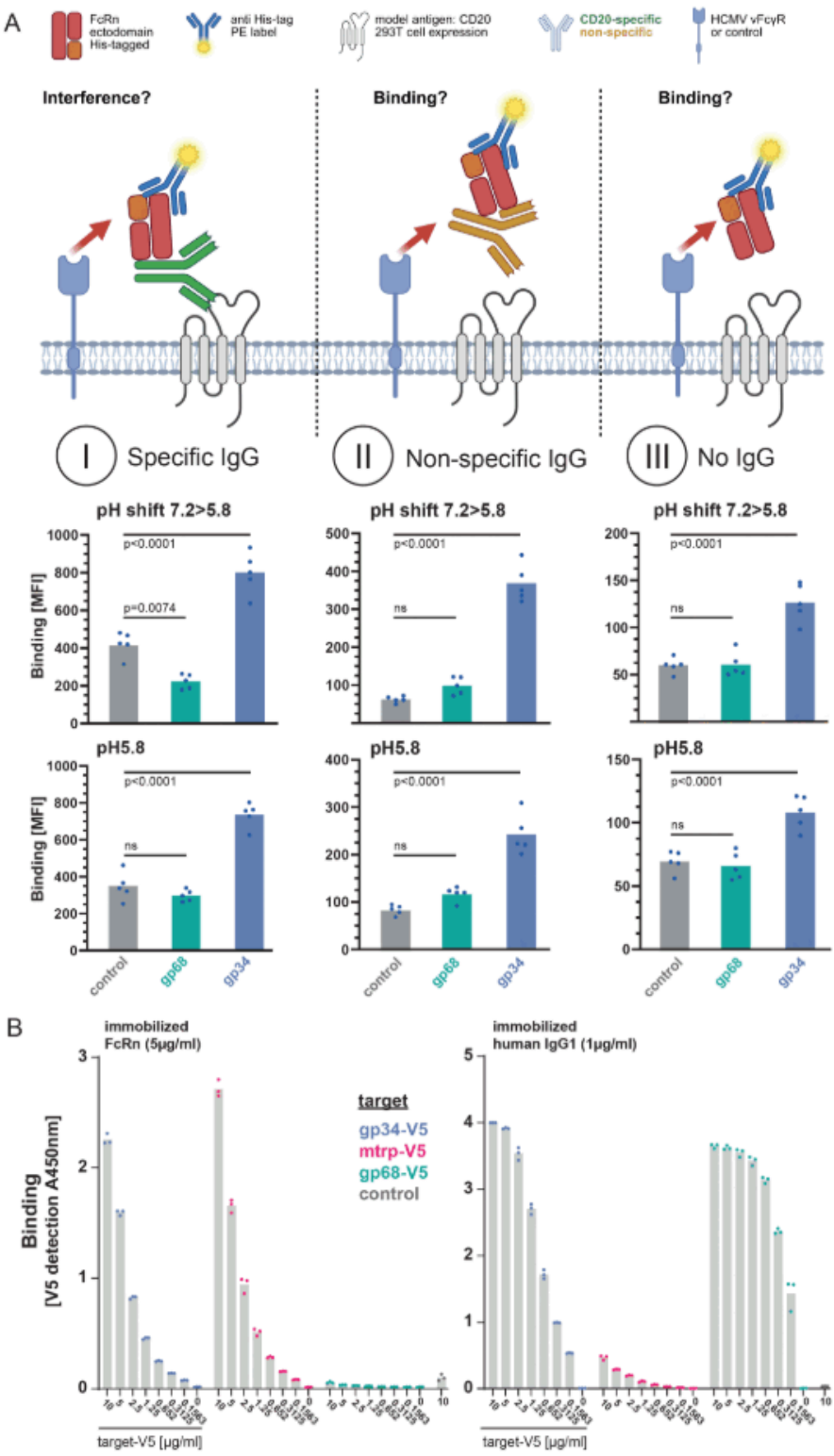


Figure 4

HCMV vFcγRs mediate attachment to and release from FcRn. A) Top: Experimental setup. Created in BioRender. Kolb, P. (2025) https://BioRender.com/zfav1nx. 293T cells stably expressing human CD20 were transfected to transiently co-express individual vFcγRs or a human CD99 control protein. Immune complexes were formed using either human IgG1 specific for CD20 (rituximab) or not (infliximab) or no IgG. Recombinant human FcRn (non-covalently complexed FCGRT and β2m) binding was detected via

6xHis-tag staining in flow cytometry. Bottom: Symbols show mean of individual experiments (mean fluorescence intensity, MFI) performed in technical triplicates. Bars represent mean across individual experiments. One-way ANOVA. B) Immobilised human FcRn or human IgG1 were probed with titrated amounts of V5-tagged vFcγR ectodomains, a tagged gp34 point mutant ($gp34_{1\text{-}179\ W65F}$) that is defective for IgG binding (mtrp) or a multitag protein control. Symbols show means of individual experiments performed in technical duplicates. Bars show means across individual experiments.

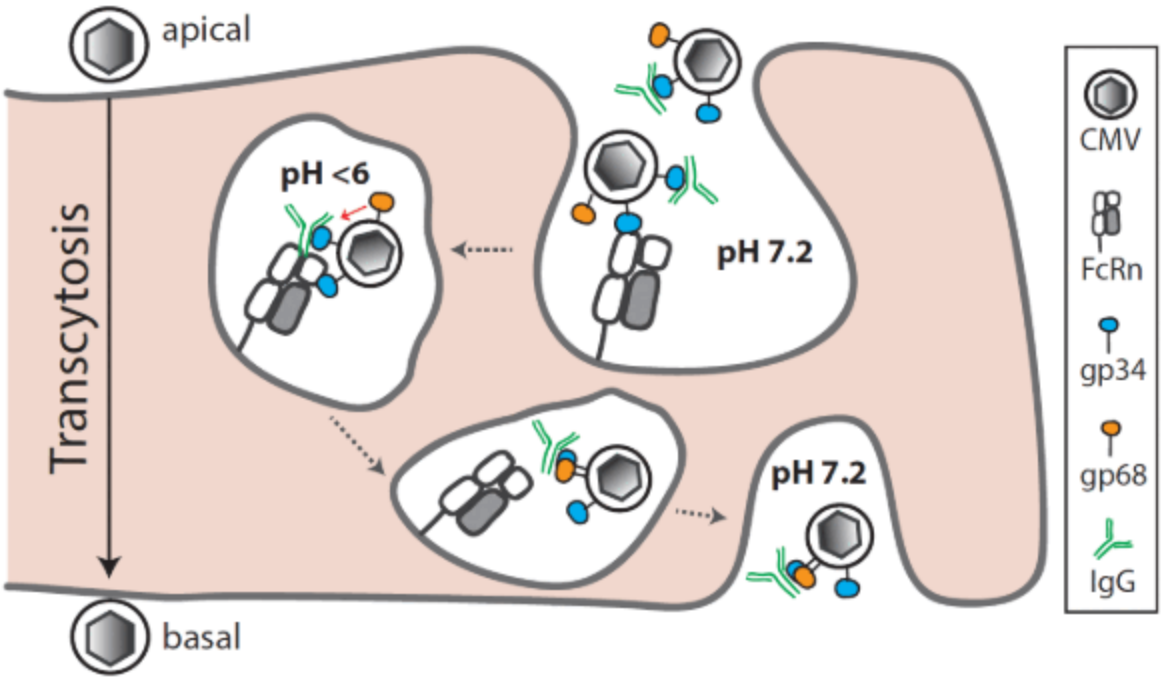


Figure 5

Model for vFcγR-mediated transcytosis of HCMV virions across a polarised cellular barrier. HCMV virions bind circulating IgG and the neonatal Fc receptor (FcRn) at physiological pH. Following endocytic uptake at the apical cell surface, FcRn engagement with IgG in early endosomes enhances retention of IgG-virion complexes. Virions subsequently traffic along the FcRn-mediated transcytosis pathway, which normally transports IgG, and are released at the basal cell surface. The viral Fcγ-binding glycoproteins gp34 and gp68 can bind IgG simultaneously. gp68 and FcRn interact with overlapping regions on the IgG Fc domain, which enables gp68 to antagonise FcRn binding while gp34 remains associated with IgG. Original illustration by Philipp Kolb.

Supplementary Files

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