Role of Neutralizing Antibodies in CMV Infection: Implications for New Therapeutic Approaches

Virginia Sandonís , Estéfani García-Ríos, Michael J McConnell, Pilar Pérez-Romero.

Trends Microbiol. 2020 Nov;28(11):900-912

which has been published in final form at

https://doi.org/10.1016/j.tim.2020.04.003

Role of Neutralizing Antibodies in CMV Infection: Implications for New Therapeutic Approaches

Virginia Sandonís[1]*, Estéfani García-Ríos[2]*, Michael J. McConnell[2†], Pilar Pérez Romero[2†]

[1] Unit of Infectious Diseases. Hospital Universitario "12 de Octubre". Instituto de Investigación Hospital "12 de Octubre" (i+12), Madrid, Spain.

[2]National Center for Microbiology, Instituto de Salud Carlos III. Majadahonda, Madrid, Spain.

*V.S. and E.G.R. contributed equally to this work and should be considered co-first authors.

[†]M.J.M. and P.P.R. contributed equally to this work and should be considered co-last authors.

Correspondence: mpperez@isciii.es (P. Pérez-Romero)

Keywords: Cytomegalovirus; CMV-specific immune response; Neutralizing antibodies; Vaccine design; Antibody-based therapeutics

Abstract

Cytomegalovirus infection elicits a potent immune response that includes the stimulation of antibodies with neutralizing activity. Recent studies have focused on elucidating the role of neutralizing antibodies in protecting against CMV infection and disease, and characterizing viral antigens against which neutralizing antibodies are directed. Here, we provide a synthesis of recent data regarding the role of neutralizing antibodies in protection against CMV infection/disease. We consider the role of humoral immunity in the context of the global CMV-specific immune response, and the implications that recent findings have for vaccine and antibody-based therapy design.

General aspects of CMV infection

Although cytomegalovirus (CMV) establishes benign infections in immunocompetent hosts, in individuals with an immature or dysfunctional immune system, CMV is a major cause of morbidity and mortality [1–3]. Congenital CMV infection results in intrauterine growth restriction and miscarriage of the infected fetus, as well as neurological sequela in newborns [4]. Immunosuppressed individuals such as transplant recipients [5], cancer patients receiving cytoreductive therapy that significantly suppresses cellular immunity [6], HIV infected patients [7] and critically ill patients without immunosuppression [8,9] are also at increased risk of CMV replication and disease.

The cell mediated immune response, primarily T-cells and NK cells, have been considered the most important components of the immune system contributing to protection against CMV infection [10–18]. The role of antibodies in protection against CMV infection and, in particular neutralizing antibodies, is still debated. A number of recent studies have provided evidences indicating that the humoral response may play a critical role in protecting against CMV disease, particularly antibodies that are able to neutralize viral infection. This evidence includes studies demonstrating that a proportion of individuals with a CMV-specific T-cell response are not fully protected against new episodes of CMV infection and disease [10,19], and recent studies indicating that neutralizing antibody titers correlate with protection from infection in transplant recipients [11]. In contrast, other studies showed that T-cell response, but not neutralizing antibodies, is associated with protection from CMV infection in transplant recipients [20,21].

In addition to these results in humans, a recent publication by Martins et al., (2019) demonstrated that humoral immunity was sufficient for providing protection against viral

reactivation in a post-transplant animal model [22]. The authors found that mouse CMV was not detected in latently infected mice transplanted with T-cell replete grafts, despite the complete absence of T and NK cells. In addition, the adoptive transfer of immune serum protected mice from viral reactivation [22]. While humoral immunity alone may be protective against murine CMV, it may act differently against human CMV. Thus, the precise role of neutralizing antibodies in humans has still not been definitively characterized.

Together, these studies are providing new insights into the mechanisms underlying how protective immunity is achieved during CMV infection, and have sparked a high level of interest in characterizing antibodies able to neutralize CMV infection and their role in protective immunity. Here we provide a synthesis of recent data regarding the role of neutralizing antibodies in protection against CMV infection/disease in the context of the global CMV-specific immune response, and the implications that recent findings have for vaccine and antibody-based therapy design.

The CMV envelope and cell-entry

CMV can infect a variety of cell types including fibroblasts, endothelial cells, epithelial cells and cells of the myeloid lineage, among others [23]. Due to differences between cell types, the final composition of the mature virion depends on the nature of the infected cell [24,25]. CMV is a highly complex virus with multiple proteins embedded in the viral envelope, with at least four distinct types of covalently linked glycoprotein complexes required for CMV infectivity [26,27] (Figure 1).

The gCI complex includes gB, which is the major envelope glycoprotein of CMV and it is involved in membrane fusion [28]. Glycoprotein B is expressed as a precursor molecule that is glycosylated and then cleaved to form a disulfide-linked complex of

gp116 linked to gp55 transmembrane component [29]. The gCII complex, which consists of gM and gN [30], interacts with heparan sulfate proteoglycans in the cell membrane, indicating that may contribute to initial binding to the cell surface [31]. The gCIII complex includes gH, gL, and gO as a covalently linked trimer [26]. While gH and gL are involved in activating the fusogenic activity of gB and are necessary for the formation of infectious viruses, gO may act as a coreceptor cooperating with the fusion-competent gH [28,32,33]. However, disruption of the gene encoding gO yield viable virus, suggesting that gO is not essential for the infectious cycle [34,35]. The pentameric complex is composed of the gH/gL heterodimer bound to three small glycoproteins encoded by UL128, UL130, and UL131 [26,36]. In addition, up to 20 putative non-complexed envelope proteins have not been fully characterized, and may also be involved in the interaction between the virus and host cells during infection.

Additional complexity of the CMV envelope is attributable to amino acid variability of the glycoproteins gB and gH, which are known to induce neutralizing antibodies. In SOT recipients, variations in gB and gH sequences are used to establish CMV genotypes: four gB variants: gB1–gB4 and two gH genotypes: gH1 and gH2 [37]. The presence of mixed genotypes is associated with greater replication of the virus, slower viral clearance, progression to CMV disease, and a higher frequency of recurrence [37]. During infection, CMV uses different entry mechanisms [27] (Figure 2). The initial attachment of CMV to the cell may be mediated by the interaction between gM/gN and glycosaminoglycans on the cell membrane [38,39]. After initial recognition in fibroblasts and Langerhans cells, entry occurs through the interaction between gH/gL/gO and platelet-derived growth factor receptor-alpha (PDGFRα) [40–43] and integrins [44]. This is followed by a pH-independent fusion of the virion envelope with the cellular membrane probably mediated by gB and gH/gL/gO (Figure 2A) [32]. A recent study suggested that

CMV has evolved to utilize THY-1, a cargo protein of clathrin-independent endocytotic vesicles, to facilitate efficient entry into the cell by a macropinocytosis-like process [45,46]. THY-1 is expressed in numerous cell types, including fibroblast and epithelial cells and interacts with both gH and gB [45,46].

Entry into epithelial, endothelial and myeloid cells (after initial recognition) occurs mainly through the interaction between the pentameric complex and cell receptors, triggering pentameric complex-mediated endocytosis (Figure 2B). Among the cell receptors involved, the recently discovered neuropilin 2 (Nrp2) should be highlighted due to its role as the functional cell entry receptor for the pentameric complex [47]. The olfactory receptor family member *OR14I1* [48], and *CD147* are required for pentamer-dependent entry into epithelial cells however, in the case of *CD147* does not involve direct interaction with the pentamer complex [49]. The fusion of the virion envelope with cellular membranes is pH-dependent and occurs at the endosomal membrane, and gB and gH/gL/gO are thought to be involved [32].

The recently identified cell membrane receptor *CD46* may be involved in a *CD46*-dependent entry pathway during virus infection of epithelial cells during congenital infection, although may not be involved in fibroblast infection, highlighting the complexity of CMV entry [50].

The immune response to CMV infection

Although the immune response to CMV infection has been extensively studied, the precise role of each immune effector function in controlling CMV infection has not been fully elucidated [13,51]. Both innate and adaptive immunity contribute to the response against CMV infection. The innate immune system is activated upon the recognition of pathogen-associated molecular patterns and involves the activation of Toll-

like receptors (TLRs). A heterodimer of TLR1 and TRL2 was found to bind glycoproteins B and H produced by CMV, stimulating dendritic cells as antigen-presenting cells (APCs), resulting in the secretion of inflammatory cytokines and recruiting natural killer cells [52]. In addition to TLRs, *DAI*/*ZBP1* and *AIM2* sensors are activated during CMV infection through detection of cytoplasmic dsDNA. Upon activation, *DAI/ZBP1* can interact with *RIPK3*, inducing receptor interacting protein kinase (PKR)-dependent necroptosis [53]. How PKR activation mediates cell death has not been completely elucidated, but activation of NF-kB and/or FADD-dependent caspase-8 activation may be involved [54].

Adaptive immunity is induced upon the recognition of CMV proteins during primary infection, triggering the activation and expansion of functional CMV-specific T-cells. Antigen-presenting cells process and display CMV antigens in the major histocompatibility complex (MHC) class I, resulting in activation of CD8+ T-cells that induce the suppression of intracellular virus replication through the secretion of interferon (IFN)-γ or tumor necrosis factor (TNF)-α, or lysis of virus-infected cells through the secretion of granzymes and perforins [55,56]. Presentation of CMV antigens by APCs via the MHC class II pathway activates CD4+ T-cells with cytotoxic activity, inducing the suppression of intracellular virus replication through the secretion of INF-γ and interleukin 2 (IL-2), leading to the proliferation of CD8+ T-cells and macrophages [57]. In addition, activated CD4+ T-cells induce B-cell activation, resulting in the production of CMV-specific antibodies against multiple viral proteins. A subset of the antibodies that are produced during infection recognize glycoproteins located at the CMV envelope and have potent virus neutralizing activity [58,59]. Neutralizing antibodies act by blocking the interaction between CMV envelope glycoproteins and their cellular receptors, thus preventing both CMV entry and cell to cell spread (Figure 3, Key Figure). Another

important function of antibodies that recognize proteins expressed on the surface of the virus or the target antigen when expressed on the surface of the infected cell is to recruit complement to promote lysis of the pathogen [60]. Alternatively, the infected cell can undergo antibody dependent cell cytotoxicity, promote phagocytosis of the pathogen, and modulate the downstream response of both the adaptive and innate immune responses [61,62]. Additional antibody-dependent cellular mechanisms (non-neutralizing) such as antibody-dependent cellular cytotoxicity or antibody-dependent cellular phagocytosis or others might also be involved in protection against CMV infection (Figure 3).

Based on what was described in the preceding section, antibodies targeting the gH/gL/UL128-131 pentameric complex should be able to block CMV entry into epithelial, endothelial and myeloid cells, and also fibroblasts and Langerhans cells [26, 40], by blocking penetration and cell-to-cell spread [41, 42]. Additionally, it was recently found that gH polymorphisms had no effect on neutralization of epithelial cell-tropic CMV infection, suggesting that gH polymorphisms are located in regions not recognized by neutralizing antibodies, and supporting the idea that antibodies against Ul128-131 are the most important for virus neutralization [63]. Antibodies targeting gB and the trimeric complex gH/gL/gO block entry into fibroblasts, Langerhans and epithelial cells (although with lower potency than anti-pentamer antibodies) [25] and some antibodies to gB inhibit the attachment of virions to cells, whereas others block the fusion of infected cells [24, 32, 33, 39], suggesting that gB might participate in multiple functions during the first steps of infection.

Role of neutralizing antibodies in protection against CMV

Natural infection

Neutralizing antibodies against epitopes in the CMV envelope glycoproteins are produced during natural infection, and depending on the glycoprotein recognized, may block infection of fibroblasts vs. non-fibroblast cell types [64]. Following natural infection, antibodies able to neutralize CMV infection of endothelial/epithelial cells are detected in serum samples earlier and at higher levels compared to antibodies neutralizing fibroblast infection. CMV hyperimmunoglobulin preparations have, on average, 48-fold higher neutralizing activity against epithelial cell entry than against fibroblast entry [65]. In addition, levels of antibodies able to neutralize CMV infection of endothelial/epithelial cells were significantly increased in a study performed in pregnant women after primary infection, exhibiting a >128-fold higher neutralizing titer for blocking epithelial cell infection compared to antibodies blocking infection of fibroblasts [64]. Similar results were observed in solid organ transplant recipients with primary CMV infection after transplantation, where levels of antibodies neutralizing epithelial cell infection depended on the number of CMV replication episodes, with significantly higher titers (median of 2560; IQR, 160-2560) compared to levels of antibodies neutralizing fibroblast infection (median of 40; IQR, 5-160). Additionally, levels of antibodies neutralizing fibroblast infection did not correlate with the number of CMV replication episodes [11].

Different authors have demonstrated a protective role for neutralizing antibodies against CMV infection. Neutralizing antibodies against the pentameric complex gH/gL/pUL128-131, and in particular to the UL128-131 proteins, have been associated with lower rates of CMV transmission from mother to fetus [59,66,67]. However, a recent study has shown that antibodies against the gH/gL/gO trimer and gH/gL/UL128-131 pentamer do not correlate with transmission of CMV from mothers to newborns during non-primary infection [68]. In addition, the increase in anti-UL128L antibodies within 30 days after CMV infection in pregnant women was associated with a decreased risk of

viral transmission to the fetus [66], suggesting that anti-UL128L antibodies play a critical role in protection against congenital infection. Although, individual monoclonal antibodies against UL130 or UL131A showed no inhibition of CMV infection in human trophoblast progenitor cells [69], a recent publication has shown that anti-pentamer antibodies block infection in cytotrophoblast [70].

In solid organ transplant recipients a protective role for epithelial cell neutralizing antibodies has also been suggested, since antibody titers ≥480 correlated with decreased CMV infection, fewer days of treatment, and complete protection from CMV disease, while antibodies neutralizing fibroblasts had no correlation with protection [11].

Passive immunization

The protective role of neutralizing antibodies against CMV infection has been suggested by some non-controlled studies through passive immunization using intravenous CMV hyperimmunoglobulin preparations. CMV hyperimmunoglobulin preparations are obtained from pooled adult human plasma selected for high concentrations of anti-CMV specific antibody titers. A nonrandomized study demonstrated that administration of hyperimmunoglobulin therapy to pregnant women reduced maternofetal-CMV-transmission and was associated with increased CMV-specific IgG concentrations and avidity, and lower risk of congenital CMV disease [71,72]. Other nonrandomized studies demonstrated a significant decrease in the number of infected newborns from mothers treated with hyperimmunoglobulin, or improved outcomes in CMV-infected infants [73–76]. In contrast, the only recent randomized controlled phase 2 clinical trial (NCT00881517; EudraCT2008-006560-11) did not show a decrease in the rate of CMV transmission among women treated with hyperimmune globulin compared to women receiving placebo [77]. Differences between studies such

as timing of vertical transmission, amniotic fluid viral loads or timing when amniocentesis was performed during pregnancy may explain why administration of hyperimmunoglobulin has not been associated with prevention of congenital CMV disease. Thus, further studies are necessary to assess the role of hyperimmunoglobulin administration in pregnant women with non-primary infection.

Administration of CMV hyperimmunoglobulin has also been shown to be effective in preventing CMV disease in solid-organ transplant (SOT) patients [52, 53]. Results from a meta-analysis of 11 prospective randomized trials including 698 SOT, suggest that administration of hyperimmunoglobulin therapy significantly reduced the risk of CMV disease, CMV-related death, and all-cause mortality after SOT [78]. Analyses of large-scale transplant registry databases have confirmed that liver and heart transplant patients receiving CMV hyperimmunoglobulin also show improved survival rates [79]. In a recent study in pediatric hematopoietic stem cell transplantat recipients (HSCT), CMV infection at 1 year was 13.4% for hyperimmunoglobulin-treated *vs*. 44.4% with no hyperimmunoglobulin (p=0.001) [80]. Although administration of hyperimmunoglobulin has shown promising results, with lower rate of CMV infection and disease and improved survival, its use for CMV prevention in SOT recipients is controversial and not generally used in most transplant programs due to the lack of appropriately powered recent clinical trials and the availability of more convenient options, such as antiviral prophylaxis.

Reduced CMV transmission has been linked to CMV-specific neutralizing antibodies against the pentameric complex present in immunoglobulin preparations [81]. In fact, CMV immune sera contain two major cell type specific neutralizing activities able to neutralize infection of fibroblasts and epithelial/endothelial cells [40, 58-60]. While the majority of the antibodies present in CMV-hyperimmunoglobulin elicited against

CMV glycoproteins are directed toward gB, the majority of the antibodies neutralizing epithelial cell entry are directed against the gH/gL/UL128-131 complex [82,83].

Neutralizing antibodies and the development of vaccines and antibody-based therapies

Due to the significant morbidity and mortality caused by CMV infection, especially in transplant recipients and newborns, the Institute of Medicine identified CMV vaccine development as a top priority [84]. Numerous vaccine candidates based on attenuated viruses, recombinant proteins and DNA, among others, have been characterized in experimental models and clinical trials over the previous 40 years, however no vaccine has yet been approved for clinical use [85]. There is abundant evidence supporting the importance of cell mediated immunity, both CMV-specific CD4+ and CD8+ T-cells in protecting against CMV disease. This includes adoptive transfer studies in which administration of CMV-specific CD8+ T-cells limited CMV disease [86], and a strong association between CMV-specific CD8+ T-cells and protection against CMV reactivation in transplant patients [10,11,87]. While CD8+ T cells may be the ultimate effectors of the T-cell response, CD4+ T-cells are also critical for the long-term control of CMV [14–18]. Multiple vaccines aiming to stimulate cellular immunity are being developed, primarily for the prevention of CMV reactivation in transplant patients, and have been reviewed elsewhere [85,88].

As described in the preceding sections, both long-standing and recent evidence indicates that the humoral response, and particularly neutralizing antibodies, might provide protection against CMV. Vaccines that elicit a strong neutralizing antibody response against multiple proteins may therefore be effective in preventing CMV infection and disease. A key aspect of these vaccines is the inclusion of antigens capable

297 of stimulating antibodies that are able to neutralize infection, ideally in multiple target
298 cell types (i.e. fibroblasts, endothelial and epithelial cells). As detailed above, multiple
299 CMV proteins on the virion surface participate in virus entry including gB, the gH/gL/gO
300 complex, the gM/gN complex, the gH/gL/UL128-131 pentameric complex and
301 potentially other uncharacterized putative envelope glycoproteins, making them potential
302 candidates for vaccines eliciting neutralizing antibodies [89]. It is worth noting that
303 neutralizing antibody titers against individual antigens may not always correlate with
304 vaccine protection, as suggested by a study in which neutralizing antibody titers against
305 gB did not correlate with vaccine-induced protection [90].

306 The role of gB glycoprotein in receptor-mediated membrane fusion in fibroblasts
307 [29] and in pH-dependent virus entry in endothelial cells and epithelial cells [89] suggests
308 that anti-gB antibodies may be able to block infection in multiple cell types. Antibodies
309 against gB were shown to block in vitro infection of fibroblasts and epithelial/endothelial
310 cells [91], and prevent CMV infection in a guinea pig model of congenital infection [74].
311 Multiple vaccine candidates that employed gB as an antigen, either alone or in
312 combination with additional CMV antigens, have been evaluated in experimental models
313 and early stage clinical trials [90,92–96]. This has included purified recombinant gB,
314 virus vectors and DNA vaccines encoding gB, and virus like particles expressing gB,
315 among others [97]. Notably, a recombinant vaccine employing a monomeric form of gB
316 formulated with the MF59 adjuvant demonstrated 50% efficacy in preventing CMV
317 infection in seronegative postpartum women [98], 43% efficacy in seronegative
318 adolescent girls [92] and was associated with lower CMV viral loads and less antiviral
319 treatment in solid organ transplant recipients [94]. A recent study comparing
320 immunization with monomeric or trimeric gB in mice demonstrated that the trimeric form
321 induced higher neutralizing antibody titers, blocking both fibroblast and epithelial cell

infection [65]. Together these studies indicate that immunization with gB can contribute to the stimulation of neutralizing antibodies and provide at least partial protection against CMV infection. However, recent studies reported that gB non-neutralizing antibodies in both transplant patients and women may be important for antibody-mediated protection [90,93,99–101], suggesting a new protective mechanism for the gB/MF59 vaccine [102].

The envelope glycoprotein gH is present in both gH/gL/gO complex and the gH/gL/UL128-131 pentameric complex, and thus participates in cell entry of fibroblasts, myeloid, epithelial and endothelial cells [68]. While antibodies targeting distinct epitopes on gH demonstrated moderate neutralizing activity of fibroblasts, epithelial and endothelial cells [78, 79], other antibodies against epitopes of the pentameric complex that span different proteins in the complex [58,103], demonstrate potent neutralization of infection in epithelial cells, endothelial cells and macrophages, but not fibroblasts [104]. Multiple vaccines employing gH/gL and the pentameric complex are currently being developed in preclinical studies, including viral vectors and nucleic acid vaccines [97]. Results from clinical trials regarding their ability to induce neutralizing titers against different cell types will be of interest. Antibodies against gM/gN were shown to neutralize infection of fibroblasts, epithelial and endothelial cells in vitro [105], thus making them potential candidates for vaccines.

In light of these studies, a vaccine against CMV may need to include multiple antigens to induce antibodies capable of inhibiting infection of multiple cell types and achieve high levels of efficacy. Using monomeric gB may not be sufficient for achieving broad efficacy, but in combination with the gH/gL/UL128-131 pentameric complex may potently neutralize fibroblasts and non-fibroblast cells. Future studies are needed in order to clarify the potential of this combination and the role of other viral envelope antigens in inducing neutralizing antibodies. In addition, although it remains to be determined,

vaccines that employ antigens involved in different steps in viral entry (e.g. attachment, receptor binding, fusion) and different infection modalities (i.e. cell-free virus entry and cell to cell spread) may also increase efficacy compared to vaccines targeting only single entry or infection processes. Recently, a whole-virus vaccine (V160) was tested in a double-blind, randomized, placebo-controlled phase 1 clinical trial (NCT01986010), inducing both neutralizing antibody and T-cell responses. The vaccine is designed to have most of the antigens that are usually presented during natural CMV infection [106]. Currently the safety, tolerability, efficacy and immunogenicity of a 2-dose and a 3- dose regimen of V160 is being tested on an ongoing double-blind, randomized, placebo-controlled phase 2b clinical trial (EudraCT 2017-004233-86). Results of the clinical trial will help to determine whether the presence of multiple antigens, including most of the viral envelope antigens, is able to elicit a neutralizing immune response.

The identification of potent neutralizing antibodies and their corresponding epitopes raises the possibility of developing novel antibody-based therapeutics for the treatment and prevention of CMV disease. As suggested by the results from passive immunization studies, antibody-based therapeutics could potentially have an important clinical impact given concerns related to of the toxicity of drug-based therapies and the potential for the emergence of resistance associated with currently-used antiviral compounds (ganciclovir and valganciclovir). However, variability between CMV-hyperimmunoglobulin production lots is a current limitation for its use as a standard treatment, which could be overcome with therapies based on monoclonal antibodies. The development of therapeutic monoclonal antibodies against multiple CMV envelope antigens has been previously reported, including antibodies against gB, gH, and the UL128-131 trimer of the pentameric complex [58,107]. RG7667 (Genentech), which consists of a combination of two monoclonal antibodies (MCMV5322A and

MCMV3068A) that bind neutralizing epitopes on the CMV complexes gH/gL and gH/gL/UL128-131, was tested in a randomized, double-blind, placebo-controlled phase 1 clinical trial (NCT01496755) [108],and it has recently been advanced to a phase 2 clinical trial (NCT01753167) in renal transplant patients [109]. In this study, CMV disease was less common in patients that received RG7667 compared to the placebo group (3.4% versus 15.8%; $p = 0.03$), and time to viremia was delayed [109]. Compared to polyclonal immunoglobulin preparations, monoclonal antibodies offer several advantages including higher target specificity, the potential for greater potency with the ability to administer higher doses of antibody, and lower toxicity. As with prophylactic vaccine development, antibodies against multiple epitopes/antigens may be necessary in order to achieve broad efficacy.

Concluding Remarks and Future Directions

Recent studies have greatly increased our understanding of the role played by neutralizing antibodies in providing protection against CMV infection and disease. Insights have also been gained regarding the viral envelope proteins that contribute to eliciting these antibodies, although further work is clearly needed in order to fully understand how individual viral components contribute to the neutralizing antibody response (Box 1). A few studies have begun to define how neutralization of infection of different cell types correlates with protective immunity. Applying this information to vaccine design and development may yield promising vaccine candidates. In addition, further preclinical and clinical studies are needed to identify promising viral antigens and the neutralizing antibody response they elicit in order to define the optimal combination of antigens that are needed for vaccine and antibody-based therapy development.

Acknowledgements

This study was supported by the Spanish Ministry of Science, Innovation and University, Instituto de Salud Carlos III Grant/Award Numbers: PI17CIII-00014 (MPY110/18); DTS18CIII/00006 (MPY127/19). This work was supported by Plan Nacional de I + D+i 2013-2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministry of Science, Innovation and University, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0009), co-financed by European Development Regional Fund 'A way to achieve Europe'. E.G-R is supported by the Sara Borrell Program (CD18CIII/00007), Instituto de Salud Carlos III, Ministerio de Ciencia, Innovación y Universidades.

Conflicts of interest

MJM and PPR are founders and shareholders of Vaxdyn, S.L. a biotechnology company developing vaccines.

References

1 Murphy, E. and Shenk, T.E. (2008) *Human cytomegalovirus*, 325

2 Boeckh, M. and Geballe, A.P. (2011) Science in medicine Cytomegalovirus : pathogen , paradigm , and puzzle. *J. Clin. Invest.* 121, 1673–1680

3 Seitz, R. (2010) Human Cytomegalovirus (HCMV)-Revised. *Transfus. Med. Hemotherapy* 37, 365–375

4 Britt, W.J. (2017) Congenital Human Cytomegalovirus Immunity. *J. Virol.* 91, 1–7

5 Roddie, C. and Peggs, K.S. (2017) Immunotherapy for transplantation-associated viral infections. *J. Clin. Invest.* 127, 2513–2522

6 Kuo, C.P. *et al.* (2008) Detection of cytomegalovirus reactivation in cancer patients receiving chemotherapy. *Clin. Microbiol. Infect.* 14, 221–227

7 Deayton, J.R. *et al.* (2004) Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. *Lancet* 363, 2116–2121

8 Li, X. *et al.* (2018) Cytomegalovirus infection and outcome in immunocompetent patients in the intensive care unit: A systematic review and meta-analysis. *BMC Infect. Dis.* 18, 1–10

9 Lachance, P. *et al.* (2016) Impact of cytomegalovirus reactivation on clinical outcomes in immunocompetent critically ill patients: protocol for a systematic review and meta-analysis. *Syst. Rev.* 5, 127

10 Mena-Romo, J.D. *et al.* (2017) CMV-specific T-cell immunity in solid organ transplant recipients at low risk of CMV infection. Chronology and applicability

in preemptive therapy. *J. Infect.* 75, 336–345

11 Blanco-Lobo, P. *et al.* (2016) Use of antibodies neutralizing epithelial cell infection to diagnose patients at risk for CMV Disease after transplantation. *J. Infect.* 72, 597–607

12 López-Oliva, M.O. *et al.* (2014) Pretransplant CD8 T-cell response to IE-1 discriminates seropositive kidney recipients at risk of developing CMV infection posttransplant. *Transplantation* 97, 839–845

13 Picarda, G. and Benedict, C.A. (2018) Cytomegalovirus: Shape-Shifting the Immune System. *J. Immunol.* 200, 3881–3889

14 Walter, E.A. *et al.* (1995) Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of t-cell clones from the donor. *N. Engl. J. Med.* 333, 1038–1044

15 Gamadia, L.E. *et al.* (2001) Differentiation of cytomegalovirus-specific CD8 + T cells in healthy and immunosuppressed virus carriers. *Blood* 98, 754–761

16 Sester, M. *et al.* (2020) Correlate with Cytomegalovirus control and predict-induced disease after renal transplantation. *Transplantation* 71, 1287–1294

17 Pourgheysari, B. *et al.* (2009) Early reconstitution of effector memory CD4+ CMV-specific T cells protects against CMV reactivation following allogeneic SCT. *Bone Marrow Transplant.* 43, 853–861

18 Gabanti, E. *et al.* (2014) Human Cytomegalovirus (HCMV)-specific CD4+and CD8+ T cells are both required for prevention of HCMV disease in seropositive solid-organ transplant recipients. *PLoS One* 9,

19 Díaz, J. *et al.* (2014) Incidence and risk factors for cytomegalovirus disease in a

Colombian cohort of kidney transplant recipients. *Transplant. Proc.* 46, 160–166

20 Lilleri, D. *et al.* (2018) Human cytomegalovirus (HCMV)-specific T cell but not neutralizing or IgG binding antibody responses to glycoprotein complexes gB, gHgLgO, and pUL128L correlate with protection against high HCMV viral load reactivation in solid-organ transplant recipients. *J. Med. Virol.* 90, 1620–1628

21 Giménez, E. *et al.* (2015) Role of cytomegalovirus (CMV)-specific polyfunctional CD8+ T-cells and antibodies neutralizing virus epithelial infection in the control of CMV infection in an allogeneic stem-cell transplantation setting. *J. Gen. Virol.* 96, 2822–2831

22 Martins, J.P. *et al.* (2019) Strain-specific antibody therapy prevents cytomegalovirus reactivation after transplantation. *Science (80-.).* 363, 288–293

23 Li, G. and Kamil, J.P. (2016) Viral Regulation of Cell Tropism in Human Cytomegalovirus. *J. Virol.* 90, 626–629

24 Tandon, R. and Mocarski, E.S. (2012) Viral and host control of cytomegalovirus maturation. *Trends Microbiol.* 20, 392–401

25 Das, S. *et al.* (2014) Identification of Human Cytomegalovirus Genes Important for Biogenesis of the Cytoplasmic Virion Assembly Complex. *J. Virol.* 88, 9086–9099

26 Ciferri, C. *et al.* (2015) Structural and biochemical studies of HCMV gH/gL/gO and pentamer reveal mutually exclusive cell entry complexes. *Proc. Natl. Acad. Sci. U. S. A.* 112, 1767–1772

27 Nguyen, C.C. and Kamil, J.P. (2018) Pathogen at the gates: Human cytomegalovirus entry and cell tropism. *Viruses* 10,

28 Malito, E. *et al.* (2018) From recognition to execution-the HCMV Pentamer from receptor binding to fusion triggering. *Curr. Opin. Virol.* 31, 43–51

29 Burke, H.G. and Heldwein, E.E. (2015) Crystal Structure of the Human Cytomegalovirus Glycoprotein B. *PLoS Pathog.* 11, 1–21

30 Kropff, B. *et al.* (2012) Glycoprotein N of Human Cytomegalovirus Protects the Virus from Neutralizing Antibodies. *PLoS Pathog.* 8,

31 Kari, B. and 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

32 Zhou, M. *et al.* (2015) Human Cytomegalovirus gH/gL/gO Promotes the Fusion Step of Entry into All Cell Types, whereas gH/gL/UL128-131 Broadens Virus Tropism through a Distinct Mechanism. *J. Virol.* 89, 8999–9009

33 Schultz, E.P. *et al.* (2016) Scanning Mutagenesis of Human Cytomegalovirus Glycoprotein gH/gL. *J. Virol.* 90, 2294–2305

34 Wille, P.T. *et al.* (2010) A Human Cytomegalovirus gO-Null Mutant Fails To Incorporate gH/gL into the Virion Envelope and Is Unable To Enter Fibroblasts and Epithelial and Endothelial Cells. *J. Virol.* 84, 2585–2596

35 Ryckman, B.J. *et al.* (2010) Human Cytomegalovirus TR Strain Glycoprotein O Acts as a Chaperone Promoting gH/gL Incorporation into Virions but Is Not Present in Virions. *J. Virol.* 84, 2597–2609

36 Ryckman, B.J. *et al.* (2008) Characterization of the Human Cytomegalovirus gH/gL/UL128-131 Complex That Mediates Entry into Epithelial and Endothelial Cells. *J. Virol.* 82, 60–70

37 Manuel, O. *et al.* (2009) Impact of Genetic Polymorphisms in Cytomegalovirus Glycoprotein B on Outcomes in Solid-Organ Transplant Recipients with Cytomegalovirus Disease. *Clin. Infect. Dis.* 49, 1160–1166

38 Vanarsdall, A.L. and Johnson, D.C. (2012) Human cytomegalovirus entry into cells Adam. *Curr. Opin. Virol.* 2, 1–7

39 Gerna, G. and Lilleri, D. (2019) Human cytomegalovirus (HCMV) infection/re-infection: Development of a protective HCMV vaccine. *New Microbiol.* 42, 1–20

40 Stegmann, C. *et al.* (2017) A derivative of platelet-derived growth factor receptor alpha binds to the trimer of human cytomegalovirus and inhibits entry into fibroblasts and endothelial cells. *PLoS Pathog.* 13, 1–27

41 Wu, Y. *et al.* (2017) Human cytomegalovirus glycoprotein complex gH/gL/gO uses PDGFR-α as a key for entry. *PLoS Pathog.* 13, 1–24

42 Soroceanu, L. *et al.* (2008) Platelet-derived growth factor-α receptor activation is required for human cytomegalovirus infection. *Nature* 455, 391–395

43 Kabanova, A. *et al.* (2017) Platelet-derived growth factor-α receptor is the cellular receptor for human cytomegalovirus gHgLgO trimer. *Nat. Microbiol.* 1, 16082

44 Feire, A.L. *et al.* (2004) Cellular integrins function as entry receptors for human cytomegalovirus via a highly conserved disintegrin-like domain. *Proc. Natl. Acad. Sci. U. S. A.* 101, 15470–15475

45 Li, Q. *et al.* (2016) Cell Surface THY-1 Contributes to Human Cytomegalovirus Entry via a Macropinocytosis-Like Process. *J. Virol.* 90, 9766–9781

46 Li, Q. *et al.* (2015) THY-1 Cell Surface Antigen (CD90) Has an Important Role

in the Initial Stage of Human Cytomegalovirus Infection. *PLoS Pathog.* 11, 1–26

47 Martinez-Martin, N. *et al.* (2018) An Unbiased Screen for Human Cytomegalovirus Identifies Neuropilin-2 as a Central Viral Receptor. *Cell* 174, 1158-1171.e19

48 Xiaofei, E. *et al.* (2019) OR14I1 is a receptor for the human cytomegalovirus pentameric complex and defines viral epithelial cell tropism. *Proc. Natl. Acad. Sci. U. S. A.* 116, 7043–7052

49 Vanarsdall, A.L. *et al.* (2018) CD147 Promotes Entry of Pentamer-Expressing Human Cytomegalovirus into Epithelial and Endothelial Cells. *MBio* 9, 1–19

50 Stein, K.R. *et al.* (2019) CD46 facilitates entry and dissemination of human cytomegalovirus. *Nat. Commun.* 10, 1–13

51 Brune, W. and Andoniou, C.E. (2017) Die another day: Inhibition of cell death pathways by cytomegalovirus. *Viruses* 9, 1–17

52 Boehme, K.W. *et al.* (2006) Human Cytomegalovirus Envelope Glycoproteins B and H Are Necessary for TLR2 Activation in Permissive Cells. *J. Immunol.* 177, 7094–7102

53 Kaiser, W.J. *et al.* (2013) Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. *J. Biol. Chem.* 288, 31268–31279

54 Dauber, B. and Wolff, T. (2009) Activation of the antiviral kinase PKR and viral countermeasures. *Viruses* 1, 523–544

55 Takeuchi, A. and Saito, T. (2017) CD4 CTL, a cytotoxic subset of CD4+ T cells, their differentiation and function. *Front. Immunol.* 8, 1–7

549 56 van Lier, R.A.W. *et al.* (2003) HUMAN CD8+ T-CELL DIFFERENTIATION
550 IN RESPONSE TO VIRUSES. *Nat. Rev. Immunol.* 3, 913–918

551 57 Chiu, Y.L. *et al.* (2016) Cytotoxic polyfunctionality maturation of
552 cytomegalovirus-pp65-specific CD4+ and CD8+ T-cell responses in older adults
553 positively correlates with response size. *Sci. Rep.* 6, 1–11

554 58 Gerna, G. *et al.* (2016) Monoclonal Antibodies to Different Components of the
555 Human Cytomegalovirus (HCMV) Pentamer gH/gL/pUL128L and Trimer
556 gH/gL/gO as well as Antibodies Elicited during Primary HCMV Infection
557 Prevent Epithelial Cell Syncytium Formation. *J. Virol.* 90, 6216–6223

558 59 Lilleri, D. *et al.* (2012) Antibodies against neutralization epitopes of human
559 cytomegalovirus gH/gL/pUL128-130-131 complex and virus spreading may
560 correlate with virus control in vivo. *J. Clin. Immunol.* 32, 1324–1331

561 60 Lu, L.L. *et al.* (2018) Beyond binding: Antibody effector functions in infectious
562 diseases. *Nat. Rev. Immunol.* 18, 46–61

563 61 Ohta, A. *et al.* (2009) Recombinant human monoclonal antibodies to human
564 cytomegalovirus glycoprotein B neutralize virus in a complement-dependent
565 manner. *Microbes Infect.* 11, 1029–1036

566 62 Li, F. *et al.* (2017) Complement enhances in vitro neutralizing potency of
567 antibodies to human cytomegalovirus glycoprotein B (Gb) and immune sera
568 induced by Gb/MF59 vaccination /631/250/590/2294 /692/53/2423 article. *npj*
569 *Vaccines* 2,

570 63 Kobayashi, R. *et al.* (2018) Analysis of relationships between polymorphisms in
571 the genes encoding the pentameric complex and neutralization of clinical

cytomegalovirus isolates. *Vaccine* 36, 5983–5989

64 Gerna, G. *et al.* (2008) Human cytomegalovirus serum neutralizing antibodies block virus infection of endothelial/epithelial cells, but not fibroblasts, early during primary infection. *J. Gen. Virol.* 89, 853–865

65 Cui, X. *et al.* (2018) Novel trimeric human cytomegalovirus glycoprotein B elicits a high-titer neutralizing antibody response. *Vaccine* 36, 5580–5590

66 Lilleri, D. *et al.* (2013) Fetal Human Cytomegalovirus Transmission Correlates with Delayed Maternal Antibodies to gH/gL/pUL128-130-131 Complex during Primary Infection. *PLoS One* 8, 1–13

67 Chiuppesi, F. *et al.* (2015) Vaccine-Derived Neutralizing Antibodies to the Human Cytomegalovirus gH/gL Pentamer Potently Block Primary Cytotrophoblast Infection. *J. Virol.* 89, 11884–11898

68 Vanarsdall, A.L. *et al.* (2019) HCMV trimer- and pentamer-specific antibodies synergize for virus neutralization but do not correlate with congenital transmission. *Proc. Natl. Acad. Sci. U. S. A.* 116, 3728–3733

69 Zydek, M. *et al.* (2014) HCMV infection of human trophoblast progenitor cells of the placenta is neutralized by a human monoclonal antibody to glycoprotein B and not by antibodies to the pentamer complex. *Viruses* 6, 1346–1364

70 Tabata, T. *et al.* (2019) Neutralizing monoclonal antibodies reduce human cytomegalovirus infection and spread in developing placentas. *Vaccines* 7, 135

71 Nigro, G. *et al.* (2005) Passive immunization during pregnancy for congenital cytomegalovirus infection. *N. Engl. J. Med.* 353, 1350–1362

72 Planitzer, C.B. *et al.* (2011) Cytomegalovirus neutralization by hyperimmune and

standard intravenous immunoglobulin preparations. *Transplantation* 92, 267–70

73 Visentin, S. *et al.* (2012) Early primary cytomegalovirus infection in pregnancy: Maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. *Clin. Infect. Dis.* 55, 497–503

74 Buxmann, H. *et al.* (2012) Use of cytomegalovirus hyperimmunoglobulin for prevention of congenital cytomegalovirus disease: A retrospective analysis. *J. Perinat. Med.* 40, 439–446

75 Nigro, G. and Adler, S.P. (2013) Hyperimmunoglobulin for Prevention of Congenital Cytomegalovirus Disease. *Clin. Infect. Dis.* 57, S193–S195

76 Adler, S.P. (2012) Primary maternal cytomegalovirus infection during pregnancy: Do we have a treatment option? *Clin. Infect. Dis.* 55, 504–506

77 Revello, M.G. *et al.* (2014) A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. *N. Engl. J. Med.* 370, 1316–1326

78 Bonaros, N. *et al.* (2008) CMV-hyperimmune globulin for preventing cytomegalovirus infection and disease in solid organ transplant recipients: a meta-analysis. *Clin. Transplant.* 22, 89–97

79 Snydman, D.R. *et al.* (2011) The impact of CMV prevention on long-term recipient and graft survival in heart transplant recipients: analysis of the Scientific Registry of Transplant Recipients (SRTR) database. *Clin. Transplant.* 25, E455–E462

80 Goldstein, G. *et al.* (2017) The role of immunoglobulin prophylaxis for prevention of cytomegalovirus infection in pediatric hematopoietic stem cell transplantation recipients. *Pediatr. Blood Cancer* 64,

81 Schampera, M.S. *et al.* (2019) Role of pentamer complex-specific and IgG subclass 3 antibodies in HCMV hyperimmunoglobulin and standard intravenous IgG preparations. *Med. Microbiol. Immunol.* 208, 69–80

82 Kabanova, A. *et al.* (2014) Antibody-driven design of a human cytomegalovirus gHgLpUL128L subunit vaccine that selectively elicits potent neutralizing antibodies. *Proc. Natl. Acad. Sci. U. S. A.* 111, 17965–17970

83 Fouts, A.E. *et al.* (2012) Antibodies against the gH/gL/UL128/UL130/UL131 Complex Comprise the Majority of the Anti-Cytomegalovirus (Anti-CMV) Neutralizing Antibody Response in CMV Hyperimmune Globulin. *J. Virol.* 86, 7444–7447

84 Modlin, J.F. *et al.* (2004) Vaccine Development to Prevent Cytomegalovirus Disease: Report from the National Vaccine Advisory Committee. *Clin. Infect. Dis.* 39, 233–239

85 Plotkin, S.A. and Boppana, S.B. (2018) Vaccination against the human cytomegalovirus. *Vaccine* DOI: 10.1016/j.vaccine.2018.02.089

86 Cobbold, M. *et al.* (2005) Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. *J. Exp. Med.* 202, 379–386

87 Molina-Ortega, A. *et al.* (2019) Impact of pretransplant CMV-specific T-cell immune response in the control of CMV infection after solid organ transplantation: a prospective cohort study. *Clin. Microbiol. Infect.* 25, 753–758

88 Diamond, D.J. *et al.* (2018) A fifty-year odyssey: Prospects for a cytomegalovirus vaccine in transplant and congenital infection. *Expert Rev.*

Vaccines 17, 889–911

89 Gerna *et al.* (2019) Human Cytomegalovirus Cell Tropism and Host Cell Receptors. *Vaccines* 7, 70

90 Baraniak, I. *et al.* (2018) Protection from cytomegalovirus viremia following glycoprotein B vaccination is not dependent on neutralizing antibodies. *Proc. Natl. Acad. Sci. U. S. A.* 115, 6273–6278

91 Wussow, F. *et al.* (2017) Neutralization of human cytomegalovirus entry into fibroblasts and epithelial cells. *Vaccines* 5,

92 Bernstein, D.I. *et al.* (2016) Safety and efficacy of a cytomegalovirus glycoprotein B (gB) vaccine in adolescent girls: A randomized clinical trial. *Vaccine* 34, 313–319

93 Nelson, C.S. *et al.* (2018) HCMV glycoprotein B subunit vaccine efficacy mediated by nonneutralizing antibody effector functions. *Proc. Natl. Acad. Sci. U. S. A.* 115, 6267–6272

94 Griffiths, P.D. *et al.* (2011) Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: A phase 2 randomised placebo-controlled trial. *Lancet* 377, 1256–1263

95 Vincenti, F. *et al.* (2018) A randomized, phase 2 study of ASP0113, a DNA-based vaccine, for the prevention of CMV in CMV-seronegative kidney transplant recipients receiving a kidney from a CMV-seropositive donor. *Am. J. Transplant.* 18, 2945–2954

96 Kharfan-Dabaja, M.A. *et al.* (2012) A novel therapeutic cytomegalovirus DNA vaccine in allogeneic haemopoietic stem-cell transplantation: A randomised,

double-blind, placebo-controlled, phase 2 trial. *Lancet Infect. Dis.* 12, 290–299

97 Schleiss, M.R. *et al.* (2017) Progress toward development of a vaccine against congenital cytomegalovirus infection. *Clin. Vaccine Immunol.* 24, 1–20

98 Pass, R.F. *et al.* (2009) Vaccine prevention of maternal cytomegalovirus infection. *N. Engl. J. Med.* 360, 1191–1199

99 Baraniak, I. *et al.* (2018) Epitope-Specific Humoral Responses to Human Cytomegalovirus Glycoprotein-B Vaccine with MF59: Anti-AD2 Levels Correlate with Protection from Viremia. *J. Infect. Dis.* 217, 1907–1917

100 Nelson, C.S. *et al.* (2018) Intrahost Dynamics of Human Cytomegalovirus Variants Acquired by Seronegative Glycoprotein B Vaccinees. *J. Virol.* 93, 1–17

101 Baraniak, I. *et al.* (2019) Original Antigenic Sin Shapes the Immunological Repertoire Evoked by Human Cytomegalovirus Glycoprotein B/MF59 Vaccine in Seropositive Recipients. *J. Infect. Dis.* 220, 228–232

102 Schleiss, M.R. (2018) Recombinant cytomegalovirus glycoprotein B vaccine: Rethinking the immunological basis of protection. *Proc. Natl. Acad. Sci. U. S. A.* 115, 6110–6112

103 Macagno, A. *et al.* (2010) Isolation of Human Monoclonal Antibodies That Potently Neutralize Human Cytomegalovirus Infection by Targeting Different Epitopes on the gH/gL/UL128-131A Complex. *J. Virol.* 84, 1005–1013

104 Freed, D.C. *et al.* (2013) Pentameric complex of viral glycoprotein H is the primary target for potent neutralization by a human cytomegalovirus vaccine. *Proc. Natl. Acad. Sci. U. S. A.* 110, 4997–5005

105 Shimamura, M. *et al.* (2006) Human Cytomegalovirus Infection Elicits a

Glycoprotein M (gM)/gN-Specific Virus-Neutralizing Antibody Response. *J. Virol.* 80, 4591–4600

106 Adler, S.P. *et al.* (2019) Phase 1 Clinical Trial of a Conditionally Replication-Defective Human Cytomegalovirus (CMV) Vaccine in CMV-Seronegative Subjects. *J. Infect. Dis.* 220, 411–419

107 Seedah, E.A. *et al.* (2015) Immunotherapeutic Approaches To Prevent Cytomegalovirus-Mediated Disease. *Antibodies Infect. Dis.* DOI: 10.1128/microbiolspec.aid-0009-2013

108 Ishida, J.H. *et al.* (2015) Phase 1 randomized, double-blind, placebo-controlled study of RG7667, an anticytomegalovirus combination monoclonal antibody therapy, in healthy adults. *Antimicrob. Agents Chemother.* 59, 4919–4929

109 Ishida, J.H. *et al.* (2017) Phase 2 Randomized, Double-Blind, Placebo-Controlled Trial of RG7667, a Combination Monoclonal Antibody, for Prevention of Cytomegalovirus Infection in High-Risk Kidney Transplant Recipients. *Antimicrob. Agents Chemother.* 61, e01794-16

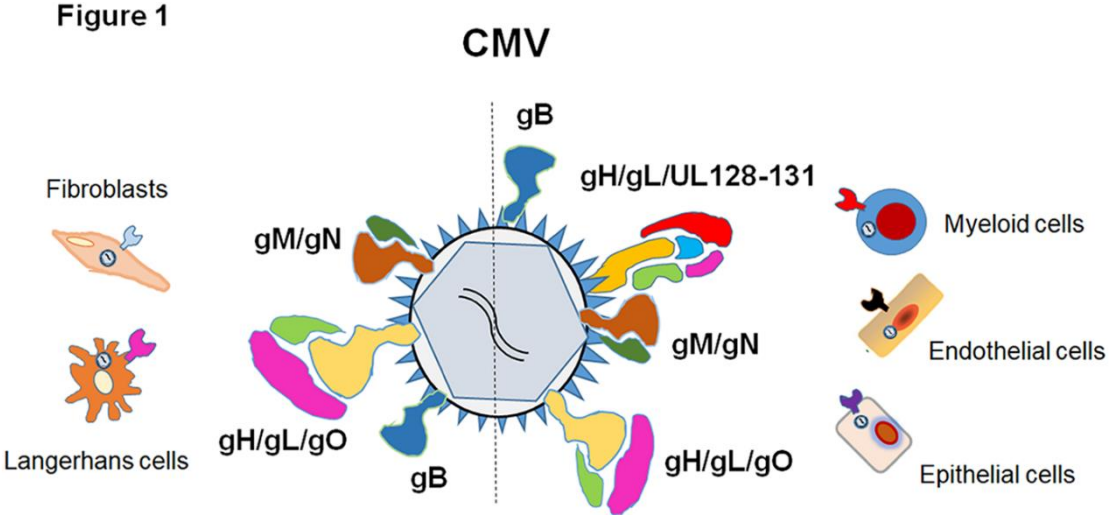
Figure Legends

Figure 1. CMV structure. Schematic representation of the CMV virion. The outer membrane of CMV has multiple embedded glycoprotein complexes. The gCI complex includes gB, the gCII complex consisting of gM and gN, the gCIII complex including gH, gL, and gO and the pentameric complex is composed of the gH/gL heterodimer bound to three small glycoproteins encoded by UL128, UL130, and UL131. The gCII (gM/gN) is involved in the initial attachment with the cell though interaction with glycosaminoglycans. For fibroblasts and Langerhans cells, viral entry is mediated by gB and gH/gL/gO, while entry into epithelial, endothelial and myeloid cells occurs through the interaction between the pentameric complex: gH/gL/UL128-131 and the cell receptor.

Figure 2. Mechanisms of CMV entry in the cells. A. CMV infection of macrophages endothelial and epithelial cells can occur using different pH-dependent pathways, including endocytosis and macropinocytosis-like pathway, followed by pH-dependent fusion of the viral membrane with the endosomal membrane in the cytoplasm. During endocytosis the pentameric complex gH/gL/pUL128-131 interacts with the cellular receptor triggering endocytosis. In addition CMV uses macropinocytosis-like pathway that in epithelial cells can be mediated through the interaction between either the cellular protein CD46 (highlighted in red) or THY-1 (highlighted in green) with viral glycoproteins gH and gB. B. CMV entry into fibroblasts occurs through two different mechanisms. The pH-independent pathway that occurs by direct fusion of the viral envelope with the cellular membrane. In addition during infection of fibroblasts CMV uses a macropinocytosis-like pathway, a pH-dependent fusion mechanism. This pathway involves the fusion of the viral membrane with the endosomal membrane and occurs though the interaction between the cellular protein THY-1 (highlighted in green) and gH or gB.

Figure 3. CMV-specific adaptive immune response. The recognition of CMV proteins during primary infection triggers the activation and expansion of functional CMV-specific T-cells. Antigen-presenting cells process and display CMV antigens in the major histocompatibility complex (MHC) class I, activating CD8+ T-cells that secrete interferon (IFN)-γ or tumor necrosis factor (TNF)-α that induce the suppression of intracellular virus replication, or that secrete granzymes and perforins that induce lysis of virus-infected cells. Presentation of CMV antigens via the MHC class II activates CD4+ T-cells with cytotoxic activity that secrete INF-γ inducing the suppression of intracellular virus replication or that secrete interleukin 2 (IL-2) that induce the proliferation of CD8+ T-cells and macrophages. Activated CD4+ T cells can also activate B-cells, inducing the production of CMV-specific antibodies against multiple viral proteins among which neutralizing antibodies act by blocking the interaction between CMV envelope glycoproteins and the cellular receptor preventing CMV entry into the target cell and cell to cell spread. Antibodies can also recruit complement, promoting antibody dependent cell cytotoxicity and phagocytosis of the pathogen.

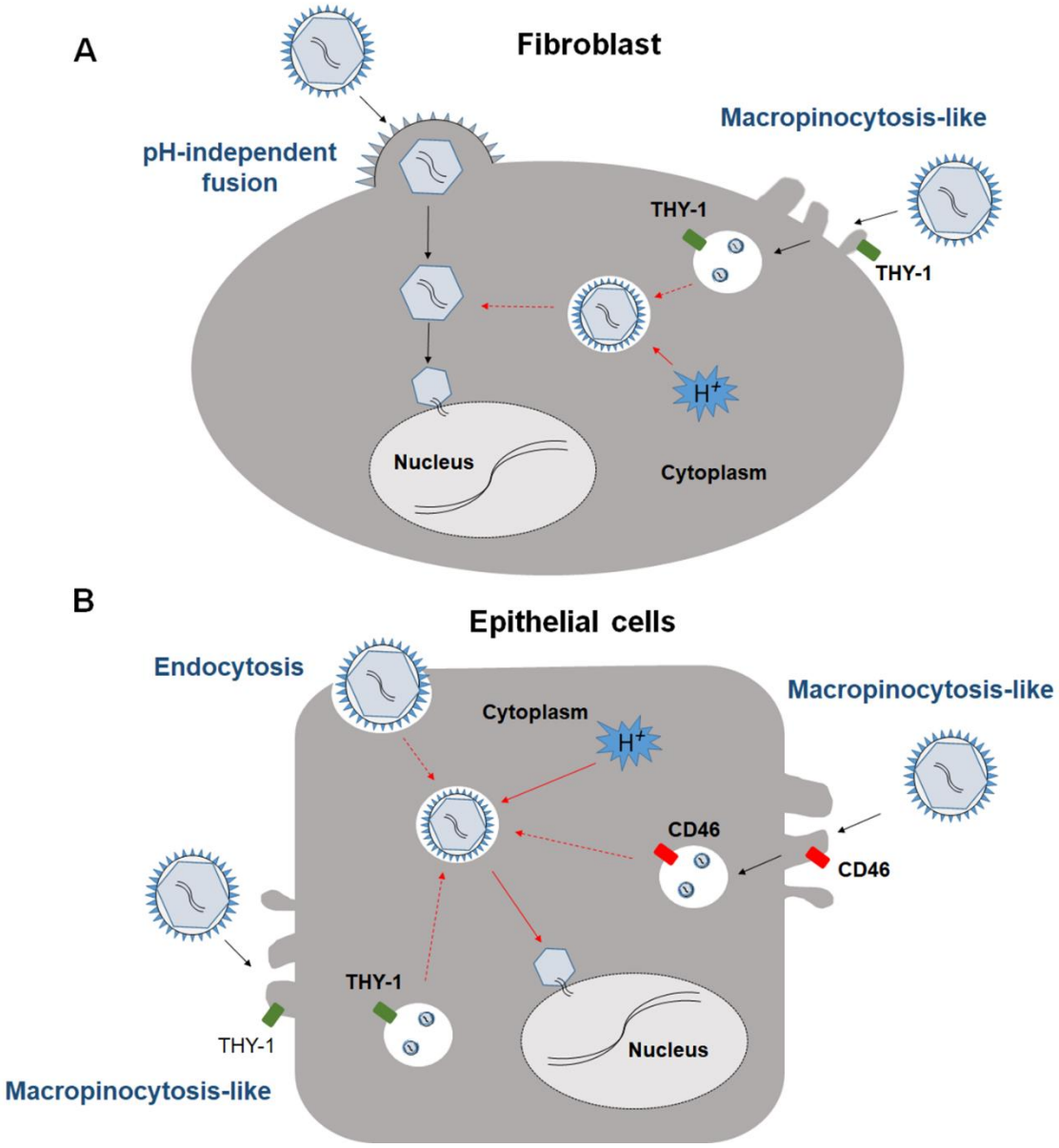
Figure 1



747

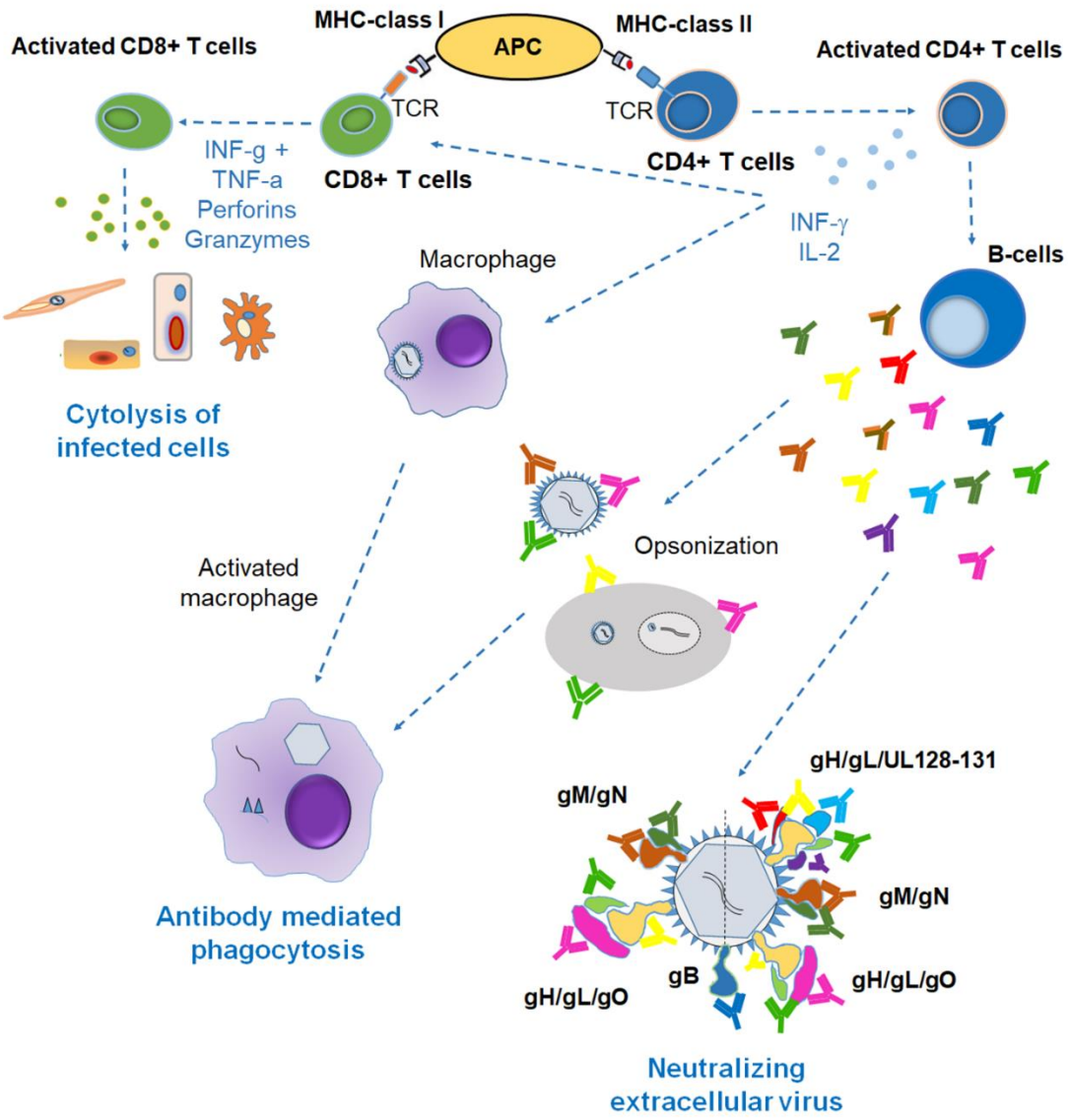
748

Figure 2


A
Fibroblast
pH-independent fusion
Macropinocytosis-like
THY-1
THY-1
H+
Nucleus
Cytoplasm
B
Epithelial cells
Endocytosis
Cytoplasm
H+
Macropinocytosis-like
CD46
CD46
THY-1
THY-1
Nucleus
Macropinocytosis-like

749

Figure 3


Activated CD8+ T cells
MHC-class I
APC
MHC-class II
Activated CD4+ T cells
TCR
TCR
INF-g +
TNF-a
Perforins
Granzymes
CD8+ T cells
CD4+ T cells
INF-γ
IL-2
B-cells
Macrophage
Cytolysis of
infected cells
Opsonization
Activated
macrophage
gH/gL/UL128-131
gM/gN
gM/gN
gB
gH/gL/gO
gH/gL/gO
Antibody mediated
phagocytosis
Neutralizing
extracellular virus

750