Exploiting viral natural history for vaccine development

Peter A. Barry

Abstract  The partial successes of the Phase 2 gB-based vaccine trials for HCMV highlight the very real likelihood that vaccine-mediated induction of antibodies that neutralize the fusion pathway of fibroblast infection is not sufficient as a singular strategy to confer protective efficacy against primary HCMV infection. Alternative strategies that serve as adjuncts to gB-based vaccines are likely required to target different aspects of the complex lifecycle of HCMV infection. There has been considerable recent interest in targeting the gH/gL/UL128/UL130/UL131 pentamer complex (gH/gL-PC) to neutralize the endocytic pathway of HCMV infection of epithelial and endothelial cells. Since both cell types are critical during primary mucosal infection, intrahost spread, and shedding of HCMV in an infected host, the gH/gL-PC represents a high-value target for vaccination to interrupt the HCMV lifecycle. The natural history of HCMV is exceedingly complex and incompletely resolved, and the protective efficacy generated by gH/gL-PC remains to be validated in clinical trials. Yet, there are salient aspects of its lifecycle that offer clues about how other novel vaccine strategies can be targeted to especially susceptible parts of the viral proteome to significantly disrupt HCMV’s ability to infect susceptible hosts. In particular, the protracted evolution of Herpesvirales has endowed HCMV with two remarkable properties of its natural history: (1) lifelong persistence within immune hosts that develop extraordinarily large antiviral immune responses and (2) the ability to reinfect those with prior immunity. The latter phenotype strongly implies that, if HCMV can overcome prior immunity to initiate a new infection, it is likely irrelevant whether prior immunity derives from prior infection or prior vaccination. Both phenotypes are unified by the extensive devotion of the HCMV coding repertoire (~50%) to viral proteins that modulate host cell signaling, trafficking, activation, antigen presentation, and resistance to apoptosis. Collectively, these viral proteins are the likely reason for the high barrier to success for the 4-decade effort to design an HCMV vaccine, and they represent the viral proteins that make HCMV be the virus that it is. James Hanshaw wrote in 1971 that, based on a 15-year retrospective of congenital HCMV cases, “… any thoughtful program designed at prevention or treatment deserves consideration”. Drawing upon natural history data from the nonhuman primate model of HCMV persistence and pathogenesis, a “thoughtful program” is put forth that HCMV immune-modulating proteins should be considered as vaccine candidates.

Keywords  Cytomegalovirus vaccine · Viral interleukin-10 · Immune modulation · Viral natural history

Introduction

Some of the earliest observations of CMV infections in nonhuman primates were remarkably prescient about contemporary CMV research occurring eight decades later (Table 1). Three years after it was demonstrated (1926) that the salivary gland agent of guinea pig was a virus that stimulated pathognomonic intracellular inclusions in infected cells, similar inclusion-bearing cells were observed in rhesus macaques acutely infected with poliovirus [1].

This article is part of the Special Issue on Cytomegalovirus.

Peter A. Barry
pabarry@ucdavis.edu

1 Center for Comparative Medicine, University of California, Davis, One Shields Avenue, Davis, CA 95616-5270, USA

Received: 3 March 2015 / Accepted: 9 March 2015 / Published online: 21 March 2015 © The Author(s) 2015. This article is published with open access at Springerlink.com
Inclusion-bearing cells were observed in the majority of study animals. The authors concluded that the intracellular lesions, phenotypically consistent with those described in guinea pigs, were unrelated to poliovirus infection. The authors further noted that cytomegalic cells were often associated with mixed lymphocytic infiltrates but sometimes were unaccompanied by any signs of a local inflammatory response. The occasional absence of inflammatory cells localized to a cytomegalic cell that we now recognize as one expressing the full complement of viral proteins is consistent with viral modulation of host immune responses in the microenvironment of that infected cell. These observations were extended 2 years later in 1931 by Covell [2] who concluded that the lesions, found in epithelial cells of multiple tissues, were not associated with clinical signs of infection and were “caused by some virus of low virulence”. Finally, Cowdry and Scott [3] noted that treatment of macaques with irradiated ergosterol, a sterol found in the cell membranes of fungi and yeasts, stimulated increased numbers of cytomegalic cells compared with untreated animals. The authors concluded that treatment with ergosterol “may have activated or intensified a process already latent in the kidneys” and that the “association between persistence of inclusions and presence of active virus may be stressed”.

Thus, within 10 years of the recognition that a filterable agent of animals induced cytomegalic cells that were identical in description to those identified in infants who died shortly after birth [4, 5], the essence of CMV natural history was set forth. By the end of 1935, CMV was characterized as a ubiquitous virus with a broad tissue tropism that stimulates protective immune response in infected hosts, resulting in low pathogenic potential, yet persists in that same immune host, in part through establishment of and reactivation from latency, and in part through immune modulation.

This duality of CMV’s effect on the host immune system, stimulating immune responses that protect against clinical disease yet fail to clear persistent and active virus, represents something of a Gordian knot that requires thinking outside of the box to solve the underlying conundrum. Much as Winston Churchill recognized in 1939 that there was a key to predicting Russia’s actions (“a riddle wrapped in an mystery inside an enigma”), there, too, is a key to the seeming paradoxes of CMV natural history. For Churchill, the key was Russian national interests.

For CMV, the key is likely found within CMV and Herpesvirales evolution from a progenitor herpesvirus, which has been estimated to have arisen 200–400 M years ago [6, 7]. As obligate intracellular pathogens, physical, innate, and adaptive barriers to infection represent exceedingly high thresholds for a virus to enter a cell and subsume host machinery to generate progeny virions that can spread both within the infected host and between hosts. Given that herpesviruses infect countless vertebrate species, and at least one invertebrate species, the 200–400 M years of intervening time since the progenitor herpesvirus represents 200–400 M years of Darwinian selection, and that for every variant that arose, “rejecting that which is bad, preserving and adding up all that is good; silently and insensibly working [at] the improvement of each organic being” [8]. Put another way, an order of viruses that has evolved on a geologic timescale (since Devonian to Jurassic periods) has to be exceedingly good at what it does to still be around to infect us, let alone be fascinating topics of research.

While all herpesviruses establish and maintain lifelong persistence within infected hosts, the virus–host relationship between HCMV and infected humans appears to be unique among the herpesviruses, and definitely unlike any other characterized pathogen–host interaction. The pioneering study of Sylwester et al. [9], in which the T cell response to the HCMV proteome was quantified, revealed that there is an extraordinarily large devotion of the T cell repertoire (~10 % of both memory CD4+ and CD8+ T cells) to a single pathogen. This massive T cell response is undoubtedly essential for ensuring that HCMV is a virus with low pathogenic potential in immune competent hosts. This interpretation is substantiated by the fact that impairment of immune functionality, such as that following iatrogenic immunosuppression or HIV–AIDS, is associated with dramatically increased risks of HCMV morbidity and mortality. The T cell response to HCMV may have been the result of an evolutionary arms race with the coding sophistication within the HCMV genome. Between 56 and 72 % of the Towne and AD169, open reading frames (ORF) can be individually deleted, respectively, without impairing replication in fibroblasts [10, 11]. The CMV family has evolved redundant and/or overlapping functions for many viral proteins, thus enabling the deletion of one ORF without compromising replicative fitness within fibroblasts. However, another interpretation of ORF being dispensable for replication in cultured cells is that they are indispensable for viral infection within the host. While functions for

| Phenotype                        | Year | Reference |
|----------------------------------|------|-----------|
| Inclusion bodies                 | 1929 | [1]       |
| Ubiquitous                       |      |           |
| Immune modulation                |      |           |
| Low virulence                    | 1931 | [2]       |
| Broad tissue tropism             |      |           |
| Reactivation from latency        | 1935 | [3]       |
| Persistence                      |      |           |

Table 1 Early recognition of key features of CMV natural history in nonhuman primates
A central feature of microbial pathogenesis, including CMV, is that pathogens have evolved a myriad of virulence strategies involving attenuation, exploitation, and/or cooption of normally protective immune functions to enable the completion of the pathogen’s life cycle within the host and dissemination to the next susceptible host. While there are pathogen-specific mechanisms for altering pathogen–host interactions, there are also common immune-modulating pathways shared among evolutionarily diverse pathogens. In particular, a large number of viruses, protozoa, pathogenic and commensal bacteria, helminths, and fungi all utilize manipulation of the interleukin-10 (IL-10) signaling pathway as part of microbial natural history [14]. Given that IL-10 is a master immune regulator with pleiotropic effects on multiple cell types [15], it is no surprise that pathogens target this singular pathway to subvert protective effector functions. One emerging theme is that pathogens and commensal bacteria have coopted IL-10 signaling to enable states of immune tolerance, immune suppression, and immune evasion, strategies that are particularly salient for those pathogens that establish lifelong persistence [16, 17].

The vast majority of pathogens that exploit the IL-10 signaling pathways have evolved strategies that upregulate IL-10 expression by host cells, although the precise mechanism by how this occurs remains incompletely resolved. However, certain members of Herpesviridae and Poxviridae transduced cellular IL-10 (cIL-10) genes at some point in their evolution and now express the viral IL-10 (vIL-10) orthologs in the context of viral infection [18]. [NOTE: vIL-10 is used as a generic term for the cIL-10 ortholog encoded within viral genomes.] Identification of a multiply sliced vIL-10 transcript in RhCMV (rchmvlIL-10) was a serendipitous discovery. Based on the presence of the rchmvlIL-10 gene within the RhCMV genome, reanalysis of the AD169 coding content revealed that the original annotation for potential ORF within the AD169 sequence [19] missed the presence of the HCMV-encoded cmvIL-10 (UL111a) due to the sizes of the three exons of cmvIL-10 falling below the 100 amino acid threshold for identifying ORF [20]. Both HCMV- and RhCMV-encoded vIL-10 orthologs are about as divergent from the cIL-10 proteins of their host (27 and 25 % identity, respectively), as they are from each other (31 % identity). Sequencing of the vIL-10 gene from multiple strains indicates that the vIL-10 protein is very stable for intraspecies comparisons of HCMV or RhCMV strains [21]. cmvIL-10 and rchmvlIL-10 do not represent a hyper-variable region within the HCMV and RhCMV genomes. The extensive drift from the cIL-10 proteins of their respective host species strongly alludes to the functional dominance of this viral protein in HCMV and RhCMV natural histories.

Manipulation of the IL-10 signaling pathway

A central feature of microbial pathogenesis, including CMV, is that pathogens have evolved a myriad of virulence strategies involving attenuation, exploitation, and/or cooption of normally protective immune functions to enable the completion of the pathogen’s life cycle within the host and dissemination to the next susceptible host. While there are pathogen-specific mechanisms for altering pathogen–host interactions, there are also common immune-modulating pathways shared among evolutionarily diverse pathogens. In particular, a large number of viruses, protozoa, pathogenic and commensal bacteria, helminths,
the host cIL-10 must exceed the negative selection resulting from an increased immunogenic profile. To date, there is no evidence that cmvIL-10 drift has resulted in acquisition of new functionalities beyond those of cIL-10 [18]. It should be emphasized that this refers to the full-length cmvIL-10 protein. Notably, an incompletely spliced variant of vIL-10 found during latent infection (LaCmv-IL-10) does have novel functionalities that are independent of binding to the high-affinity IL-10R1 receptor [13, 18, 24]. The strongest clue to what might account for the extensive genetic drift of cmvIL-10 and rhcmvIL-10 relates to the relative binding affinities of cmvIL-10 and cIL-10 for the cellular IL-10R1 receptor. cmvIL-10 has higher binding affinity for IL-10R1 than cIL-10, and one possibility is that sequence differences between HCMV and RhCMV vIL-10 proteins were compensatory changes to differences between human and macaque IL-10R1 proteins [25]. One hypothesis that remains to be tested is that the ability of vIL-10 to outcompete cIL-10 for IL-10R1 confers greater fitness to HCMV and RhCMV than the negative consequences of drifting to an immunogenic form of the protein [21, 25].

Emerging evidence for the vIL-10 proteins of both HCMV and RhCMV highlights that vIL-10 broadly targets innate immune cells. Based on in vitro assays following exposure of macrophages (Mφ), peripheral blood mononuclear cells (PBMC), monocyte-derived dendritic cells (MDC), and plasmacytoid dendritic cells (PDC) to vIL-10 [18, 27–32], expression of vIL-10 in vivo should have pleiotropic immunosuppressive effects on multiple cell types (Table 2). It is especially notable that the functionality of vIL-10 is directed at key innate cell types, particularly monocytes/Mφ, MDC, and PDC, that are links between the innate and adaptive immune responses. vIL-10-mediated modulation of the functionality of these cells should have the effect of directly influencing the quality of the adaptive antiviral immune responses.

### Table 2 Functional activity of vIL-10 on lymphoid cells in vitro

| Cell type(s) | Phenotype | Ref. |
|--------------|-----------|-----|
| Monocytes    | ↑ Differentiation into inflammatory macrophages ↓ HLA-DR ↑ CD64, CCR5 | [26] |
| PBMC         | ↓ Mitogen-stimulated proliferation, MHC-I and –II ↓ IFN-γ | [27, 28] |
| Monocytes    | ↓ IL-6, IL-1α, GM-CSF, TNF-α; MHC class I and II ↑ HLA-G | [29, 30] |
| MDC          | ↓ TNF-α, IL-6 IL-12, Bcl-2, Bcl-XL ↑ cIL-10 | [29, 30] |
| PDC          | ↓ INF-α, IFN-β | [31] |

Based on the functionalities of vIL-10 on the different cell types in Table 2, it is proposed that vIL-10, likely in conjunction with other viral proteins, exerts a viral adjuvant-like effect on long-term antiviral immunity. While it remains to be determined whether other HCMV proteins exert comparable effects on innate cells, the hypothesis leads to a model whereby lifelong persistence is initiated at the earliest virus–host interactions following primary mucosal infection [16, 26]. Support for this comes from inoculation of naïve rhesus macaques with the 68-1 strain of RhCMV, a fibroblast-adapted strain, and an engineered variant of 68-1 in which the rhcmvIL-10 gene had been deleted (RhCMV ΔvIL-10) [26]. Prominent distinctions were observed in both the acute and long-term immune responses, including the extent and cell composition of the cellular infiltrate at the site of subcutaneous (SC) inoculation, and the early and long-term anti-RhCMV adaptive immune responses. Based on this result, a converse approach, i.e., vaccination against rhcmvIL-10, was evaluated to determine to what extent rhcmvIL-10 influences the virus–infected host relationship.

### Vaccination against rhcmvIL-10

As noted, rhcmvIL-10 is strongly immunogenic when expressed in the context of RhCMV infection [22], establishing a precedent to immunize macaques with a recombinant form of rhcmvIL-10. However, vaccination of naive animals with fully immunosuppressive vIL-10 would likely stimulate immune responses very distinct, and likely undesirable, from those induced by vaccination with a non-functional form of rhcmvIL-10. To maximize immunogenicity of rhcmvIL-10, minimal alterations were engineered into rhcmvIL-10 (2 amino acids) to create a biologically inactive form of the protein, by abrogating binding to IL10R1 [22, 33]. The specific amino acids that were changed were based on the by crystal structures of cIL-10/IL-10R1 and cmvIL-10/IL-10R1 complexes [25]. Immunization of RhCMV-uninfected macaques by a combined DNA prime/protein boost immunization strategy stimulated both rhcmvIL-10-binding and rhcmvIL-10-neutralizing antibodies that did not exhibit any cross-reactivity with rhesus macaque cIL-10 [34]. Vaccinated animals were subsequently challenged with the epitheliotropic UCD59 strain of RhCMV and evaluated for molecular and immunological parameters of infection. The results demonstrated that vaccination against a single RhCMV immune-modulating protein, rhcmvIL-10, significantly altered viral parameters of challenge infection, including reductions locally at the site of SC inoculation, and in the frequency and magnitude of RhCMV shedding in saliva and urine. This proof-of-principal study demonstrated for the first time for CMV...
that vaccine-induced neutralization of the functionality of a single immune-modulating protein (rhcmvIL-10) confers protective efficacy in the absence of vaccine-induced antibodies that neutralize viral attachment to susceptible cells. Extrapolating from this result in macaques to human, vaccination against cmvIL-10 should have comparable efficacy in protecting against primary HCMV challenge and the establishment of a persistent infection. In addition, vaccination could also protect against the maintenance of persistence, based on recent studies on cmvIL-10 expression during latency.

**IL-10 signaling during latency**

It is well established that HCMV establishes a latent infection in CD34+ hematopoietic progenitor cells [35], and recent studies indicate that persistence is maintained, in part, through HCMV-induced generation of CD4+ T cells that secrete cIL-10 in response to HCMV antigens. Two hallmarks of latency in these cells are an extremely restricted pattern of viral gene expression and the absence of infectious virion production [36, 37]. The viral transcriptome defining latently infected CD34+ cells includes cmv-IL-10 [24] and LAcmv-IL-10 [38], the aberrantly spliced transcript that results in a truncated, but biologically functional, form of cmvIL-10 that does not engage IL-10R1 [39, 40]. Expression of LAcmv-IL-10 in latently infected CD34+ cells stimulates expression of cIL-10 in a mechanism that appears to involve suppressed expression of the microRNA hsa-miR-92a [38]. The authors note that expression of LAcmv-IL-10 in a paracrine and/or autocrine fashion “modulates the microenvironment of the infected cell, leading to evasion of the immune system”. They further speculate that LAcmv-IL-10 represents a potential therapeutic target since LAcmv-IL-10 is very distinct in sequence from cIL-10. The limited expression of the HCMV proteome in latently infected cells also elicits a very specific effect on the type of antigen-specific T cells. Expression of cmvIL-10, like that of LUNA, UL138, and US28, stimulates antigen-specific CD4+ T cells that secrete cIL-10 in response to antigen exposure, leading to the suggestion that “HCMV skews the T cell responses to latency-associated antigens to one that is overall suppressive in order to sustain latent carriage in vivo” [41]. Viral induction of cIL-10 signaling parallels MCMV-mediated induction of CD4+ T cells secreting cIL-10 [42], lending support to the hypothesis that disruption of the cIL-10 signaling pathway in persistent infections may be one way of altering the virus–host homeostasis in persistently infected hosts to a state favoring host immune-mediated clearance of virally infected cells [16]. The mechanisms that HCMV appears to utilize in skewing host immune responses during a persistent infection may also be operative during reinfection.

It is now accepted that HCMV can reinfect those with prior immunity, which assumes clinical importance in the context of congenital infections in populations with near universal HCMV seroprevalence [43–47]. Despite the extraordinarily large T cell response devoted to HCMV antigens and the broad neutralizing responses against multiple HCMV glycoproteins involved in attachment and entry [9, 48–52], HCMV can reinfect immunocompetent individuals with preexisting immunity to HCMV. At this point, there is no evidence indicating that reinfection involves selection for distinctly antigenically variant strains of HCMV, which leaves the hypothesis that modulation of host immunity during reinfection enables the reinfesting strain to disseminate the mucosal surface to distal sites, particularly the maternal/fetal interface. While studies investigating the role the vIL-10 may play during HCMV and RhCMV reinfection are ongoing, results from the rhesus model unequivocally demonstrate that modulation of host immunity is essential for RhCMV reinfection.

**Attenuation of host immunity during RhCMV reinfection**

Rhesus CMV (RhCMV) encodes sequence and functional orthologs of HCMV US2, 3, 6, and 11, and like their HCMV counterparts, the RhCMV proteins disrupt antigen presentation by major histocompatibility complex (MHC) class I [53]. The RhCMV orthologs of US2-11 are not required for the establishment of a persistent infection following SC inoculation of RhCMV-uninfected macaques with an engineered variant of 68-1, in which the RhCMV US2-11 orthologs were deleted, and the SIV gag gene was coexpressed [54]. Animals developed long-term RhCMV- and Gag-specific T cell responses, and input virus could be recovered from the urine of inoculated animals almost 2 years after primary inoculation. However, when animals with preexisting immunity to RhCMV were inoculated with the same US2-11-deleted variant (and co-expressing SIV gag), there was absolutely no evidence of reinfection. Animals did not develop Gag-specific T cell immune responses, and the input virus was never recovered from the urine. In contrast, inoculation of immune animals with the parental construct expressing US2-11 efficiently resulted in reinfection. This study emphasizes that in this model, antigenic variation is not the critical factor for reinfection. Rather, attenuation of host antiviral immune responses, in this case through disruption of MHC class I antigen presentation, is essential for reinfection.
Summary

Taken as a whole, accumulating data on the immune-modulating proteins of HCMV and RhCMV highlight their central role during all phases of the infectious cycle within an infected host, beginning with the immediate virus-mediated modulation of host immune responses at the mucosal surface of a naïve host, to maintenance of an immune suppressive environment in latently infected CD34+ cells, to suppression of effector/memory responses during reinfection of an immune host. HCMV has had eons to optimize an orchestrated symphony of viral functions that target almost every aspect of innate and adaptive immunity that we as hosts can throw against it. Because of this, HCMV immune modulation represents an opportunity to leverage some of these qualities to change human health. The dramatic and extremely promising results obtained using RhCMV-based vaccine vectors to protect against virulent SIV challenge are possible only because of RhCMV-encoded immune modulatory proteins [55–57]. These functions enable reinfecion of and long-term persistence in immune animals, facilitating long-term, non-classical CD8+ T cell responses that can protect against SIV challenge years after immunization. However, this armada of immune-modulating functions constitutes a significant challenge to overcome in designing vaccine strategies that prevent primary and non-primary infections. Taking Hanshaw’s declaration for “any thoughtful program designed at prevention” [58] to heart, the weight of evidence points to some of the viral immune-modulating proteins as especially susceptible targets of the viral proteome for vaccine strategies.

Acknowledgments This work was supported by grants from the National Institutes of Health (AI063356, AI097629, AI049342, DE021273), the NIH Office of Research Infrastructure Programs/OD P51OD011107 to the California National Primate Research Center, and the Margaret Deterding Infectious Disease Research Support Fund.

Conflict of interest The author has no conflicts of interest related to this manuscript.

Research involving human participants and/or animals and informed consent The manuscript does not report new data from studies involving either human participants or research animals.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Stewart FW, Rhoads CP (1929) Lesions in nasal mucous membranes of monkeys with acute poliomyelitis. Proc Soc Exp Biol Med 26:664–665
2. Cowdry EV, Scott G (1935) Nuclear inclusions in kidneys of Macacus rhesus monkeys. Am J Pathol 11:659–668
3. Goodpasture EW, Talbot FW (1921) Concerning the nature of “protozoan-like” cells in certain lesions of infancy. Am J Dis Child 21:415–421
4. Ribbert D (1904) Uber protozoenartige zellen in der niere eines syphilitischen neugobernen und in der parotis von kindern. Zentralbl Allg Pathol 15:945–948
5. McGeoch DJ, Gatherer D (2005) Integrating reptilian herpesviruses into the family herpesviridae. J Virol 79:725–731
6. Darwin C (1859) On the order of species by means of natural selection. J. Murray, London
7. McGeoch DJ, Cook S, Dolan A, Jamieson FE, Telford EA (1995) Molecular phylogeny and evolutionary timescale for the family of mammalian herpesviruses. J Mol Biol 247:443–458
8. Dunn W, Chou C, Li H, Hai R, Patterson D, Stolic V, Zhe H, Liu F (2003) Functional profiling of a human cytomegalovirus genome. Proc Natl Acad Sci USA 100:14223–14228
9. Yu D, Silva MC, Shenk T (2003) Functional map of human cytomegalovirus AD169 defined by global mutational analysis. Proc Natl Acad Sci USA 100:12396–12401
10. Engel P, Angulo A (2012) Viral immunomodulatory proteins: usurping host genes as a survival strategy. Adv Exp Med Biol 738:256–276
11. McSharry BP, Avdic S, Slobedman B (2012) Human cytomegalovirus encoded homologs of cytokines, chemokines and their receptors: roles in immunomodulation. Viruses 4:2448–2470
12. Eberhardt MK, Barry PA (2014) Pathogen manipulation of cIL-10 signaling pathways: opportunities for vaccine development? Curr Top Microbiol Immunol 380:93–128
13. Saraiva M, O’Garra A (2010) The regulation of IL-10 production by immune cells. Nat Rev Immunol 10:170–181
14. Slobedman B, Barry PA (2010) Primate models of cytomegalovirus infection, prevention, and therapy. In: Norfolk RM (ed) Cytomegaloviruses: from molecular pathogenesis to therapy. Caister Academic Press/Horizon II, UK
15. Eberhardt MK, Chang WL, Logan JN, Yue Y, Walter MR, Barry PA (2012) Host immune responses to a viral immune modulating protein: immunogenicity of viral interleukin-10 in rhesus cytomegalovirus-infected rhesus macaques. PLoS One 7:e37931
23. de Lemos Rieper C, Galle P, Pedersen BK, Hansen MB (2011) Characterization of specific antibodies against cytomegalovirus (CMV)-encoded interleukin 10 produced by 28% of CMV-seropositive blood donors. J Gen Virol 92:1508–1518
24. Avdic S, Cao JZ, Cheung AK, Abendroth A, Slobedman B (2011) Viral interleukin-10 expressed by human cytomegalovirus during the latent phase of infection modulates latently infected myeloid cell differentiation. J Virol 85:7465–7471
25. Jones BC, Logsdon NJ, Josephson K, Cook J, Barry PA, Walter MR (2002) Structural crystal structure of human cytomegalovirus IL-10 bound to soluble human IL-10R1. Proc Natl Acad Sci USA 99:9404–9409
26. Chang WL, Barry PA (2010) Attenuation of innate immunity by cytomegalovirus IL-10 establishes a long-term deficit of adaptive antiviral immunity. Proc Natl Acad Sci USA 107:22647–22652
27. Nachtwy J, Spencer JV (2008) HCMV IL-10 suppresses cytokine expression in monocytes through inhibition of nuclear factor-kappaB. Viral Immunol 21:477–482
28. Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, Schall TJ (2002) Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. J Virol 76:1285–1292
29. Chang WL, Baumgarth N, Eberhardt MK, Lee CY, Baron CA, Gregg JP, Barry PA (2007) Expression of myeloid dendritic cells to exogenous or endogenous IL-10 during maturation determines their longevity. J Immunol 178:7794–7804
30. Chang WL, Baumgarth N, Yu D, Barry PA (2004) Human cytomegalovirus-encoded interleukin-10 homolog inhibits maturation of dendritic cells and alters their functionality. J Virol 78:8720–8731
31. Chang WL, Barry PA, Szubin R, Wang D, Baumgarth N (2009) Human cytomegalovirus suppresses type I interferon secretion by plasmacytoid dendritic cells through its interleukin 10 homolog, Virology. 390:330–337
32. Spencer JV, Cadaoas J, Castillo PR, Saini V, Slobedman B (2008) Stimulation of B lymphocytes by cmvIL-10 but not LAc-mvIL-10. Virology 374:164–169
33. Logsdon NJ, Eberhardt MK, Allen CE, Barry PA, Walter MR (2011) Design and analysis of rhesus cytomegalovirus IL-10 mutants as a model for novel vaccines against human cytomegalovirus. PLoS One 6:e28127
34. Eberhardt M, Deshpande A, Chang W-L, Barthold S, Walter M, Barry P (2013) Vaccination against a virally-encoded cytokine mutants as a model for novel vaccines against human cytomegalovirus. J Virol 87:13947–13955
35. Jenkins C, Abendroth A, Slobedman B (2004) A novel viral transcript with homology to human interleukin-10 is expressed during latent human cytomegalovirus infection. J Virol 78:1440–1447
36. Jenkins C, Garcia W, Godwin MJ, Spencer JV, Stern JL, Abendroth A, Slobedman B (2008) Immunomodulatory properties of a viral homolog of human interleukin-10 expressed by human cytomegalovirus during the latent phase of infection. J Virol 82:3736–3750
37. Mason GM, Jackson S, Okecha G, Poole E, Sissons JG, Sinclair JW, Wills MR (2013) Human cytomegalovirus latency-associated proteins elicit immune-suppressive IL-10 producing CD4+ T cells. PLoS Pathog 9:e1003635
38. Humphreys IR, de Trez C, Kinkade A, Benedict CA, Croft M, Ware CF (2007) Cytomegalovirus exploits IL-10-mediated immune regulation in the salivary glands. J Exp Med 204:1217–1225
39. Boppana SB, Rivera LB, Fowler KB, Mach M, Britt WJ (2001) Intracellular transmission of cytomegalovirus to infants of women with preconceptional immunity. N Engl J Med 344:1366–1371
40. Ross SA, Fowler KB, Askrith G, Stagno S, Britt WJ, Pass RF, Boppana SB (2006) Hearing loss in children with congenital cytomegalovirus infection born to mothers with preexisting immunity. J Pediatr 148:332–336
41. Sohn YM, Park KI, Lee C, Han DG, Lee WY (1992) Congenital cytomegalovirus infection in Korean population with very high prevalence of maternal immunity. J Korean Med Sci 7:47–51
42. Wang C, Zhang X, Bialek S, Cannon MJ (2011) Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. Clin Infect Dis 52:e11–e13
43. Yamamoto AY, Massi-Pinhata MM, Boppana SB, Novak Z, Wagatsuma VM, de Oliveira FP, Duarte G, Britt WJ (2010) Human cytomegalovirus reinfection is associated with intrauterine transmission in a highly cytomegalovirus-immune maternal population. Am J Obstet Gynecol 202(297):e291–e298
44. Britt WJ (1984) Neutralizing antibodies detect a disulfide-linked glycoprotein complex within the envelope of human cytomegalovirus. Virology 135:369–378
45. Britt WJ, Mach M (1996) Human cytomegalovirus glycoproteins. Intervirology 39:401–412
46. Cui X, Meza BP, Adler SP, Mccvoy MA (2008) Cytomegalovirus vaccines fail to induce epithelial entry neutralizing antibodies comparable to natural infection. Vaccine 26:5760–5766
47. Macagno A, Bernasconi NL, Vanzetta F, Dander E, Sarasini A, Revello MG, Gerna G, Sallusto F, Lanzavecchia A (2010) Isolation of human monoclonal antibodies that potentially neutralize human cytomegalovirus infection by targeting different epitopes on the gHgLUL128-131A complex. J Virol 84:1005–1013
48. Revello MG, Gerna G (2010) Human cytomegalovirus tropism for endothelial/epithelial cells: scientific background and clinical implications. Rev Med Virol 20:136–155
49. Pande NT, Powers C, Ahn K, Fruh K (2005) Rhesus cytomegalovirus contains functional homologues of US2, US3, US6, and US11. J Gen Virol 92:1508–1518
50. Hansen SG, Powers CJ, Richards R, Ventura AB, Ford JC, Siess D, Axthelm MK, Nelson JA, Jarvis MA, Picker LJ, Fruh K (2010) Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus. Science 328:102–106
51. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, Whizin N, Oswald K, Shoemaker R, Swanson T, Legasse AW, Chiuchiolo MJ, Parks CL, Axthelm MK, Nelson JA, Jarvis MA, Patak M Jr, Lilson JD, Picker LJ (2011) Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature 473:523–527
52. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Scholz I, Gilbride RM, Lewis MS, Gilliam AN, Ventura AB, MaloI D, Xu G, Richards R, Whizin N, Reed JS, Hammond KB, Fischer M, Turner JM, Legasse AW, Axthelm MK, Edlefsen PT, Nelson JA, Lilson JD, Fruh K, Picker LJ (2013) Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. Science 340:1237874
57. Hansen SG, Vieville C, Whizin N, Coyne-Johnson L, Siess DC, Drummond DD, Legasse AW, Axthelm MK, Oswald K, Trubey CM, Piatak M Jr, Lifson JD, Nelson JA, Jarvis MA, Picker LJ (2009) Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. Nat Med 15:293–299

58. Hanshaw JB (1971) Congenital cytomegalovirus infection: a fifteen year perspective. J Infect Dis 123:555–561