Cell-mediated immunity to human CMV infection: a brief overview

Nadia Terrazzini and Florian Kern*

Address: Pathogen Host Interaction Group (PHI), Immunology, Division of Medicine, Brighton and Sussex Medical School, Biology Road, Brighton, BN1 9PS, UK

* Corresponding author: Florian Kern (f.kern@bsms.ac.uk)

The electronic version of this article is the complete one and can be found at: http://f1000.com/prime/reports/b/6/28

Abstract

The cellular immune response to human cytomegalovirus (HCMV) has different components originating from both the adaptive and innate immune systems. There is a significant global interest in understanding how the immune system keeps HCMV under control, in particular with a view to situations where HCMV infection causes severe damage. Such settings include HIV infection, transplantation, and maybe most importantly perinatal medicine, HCMV being a major cause of sometimes catastrophic birth defects. The development of an active HCMV vaccine has proven very difficult but some recent successes raise hope that this might be available in the future. However, adoptive transfer of HCMV-specific T cells has been successfully used to prevent CMV disease after bone marrow transplantation for many years. In fact, the CD8 T cell response has been thought to be the most important effector response, with numerous reports focusing on specific T cell subsets recognizing select peptides in select human leukocyte antigen (HLA) contexts. However, it is becoming increasingly clear now that other cells, first and foremost CD4 T cells, but also gamma/delta (γ/δ) T cells and natural killer cells, are critically involved in the cellular immune response to HCMV. This commentary aims to provide a brief overview of the field.

Introduction

This short commentary will attempt to provide an up-to-date overview of the contribution of various immune cell types to the defense against HCMV, a very common human DNA virus, which has probably co-evolved with its host since the very beginning of human life [1]. However, although HCMV is almost a "part" of humankind, not everybody is infected. On a global scale, HCMV's most damaging impact is related to birth defects, including gross abnormalities but also sensorineural hearing loss leading to a learning disability that is sometimes discovered quite late [2-4]. In addition, HCMV infection is dangerous if immunity is compromised (for example, in the post-transplant setting or HIV infection, situations in which it may cause severe end-organ disease) [5,6].

Over the last decade or so, HCMV has been linked to premature aging of the immune system ("immunosenescence") as well as premature clinical manifestations of vascular pathology, particularly in the context of HIV infection [7-11]. HCMV is noted for sometimes inducing very large T cell responses but at the same time for possessing multiple immune evasion mechanisms [12]. The best known of these is probably latency, a "sleep-like" state with limited protein expression [13].

For a long time, "classic" cytotoxic alpha/beta (α/β) CD8 T cells, recognizing lytic-phase HCMV antigens, were thought to be the mainstay of cellular anti-HCMV immunity [14,15]; however, over the last decade or so, HCMV-specific CD4 T cells have been given increasing attention as they not only contribute cytotoxic activity in addition to "classic" helper function but also are a lot more numerous than previously thought. Of note, CD4 T cells recognizing HCMV proteins expressed during latency were very recently described. A subpopulation of these are thought to help HCMV remain sheltered from the immune response [16].

A more recent discovery is a subset of HCMV-induced regulatory T (iTreg) cells that probably develop from conventional antigen-activated CD4 T cells [17,18]. However, this area is controversial as no widely accepted general definition of inducible iTregs exists in the literature, especially not in humans. Among T cells, γ/δ T cells have been characterized that initially appeared to be specific for HCMV but now are understood to recognize stress antigens expressed (for example, by HCMV-infected cells) [19,20]. While natural killer (NK) cells are widely recognized as a first-line innate defense against HCMV, recent reports provide scope, at least in theory, for an HCMV-specific major histocompatibility complex/killer cell immunoglobulin-like receptor (MHC/KIR)-mediated interaction contributing to silencing this response [21].

To briefly mention technology, much of the work done in this area over the last 20 years or so has hugely benefitted from the advancements in antigen-specific immunology made in the 1990s. The approaches that have made most of the difference exploring T cell responses in the HCMV T cell field were MHC multimer-based approaches [22] and functional approaches like intracellular cytokine staining following *in vitro* stimulation with HCMV antigens (in particular, protein-spanning peptide pools) or infected cells [23-27]. The MASIR (measuring antigen-specific immune responses) series of five conferences between 2005 and 2013 was focused on the measurement of antigen-specific immune responses and produced a number of reviews providing a good overview of current technology in this field [28-30].

Classic effector and helper T cell responses to human cytomegalovirus infection

Throughout the 1990s, research exploring human HCMV T cell target antigens focused essentially on two major protein targets: the pp65 lower matrix phosphoprotein (UL83, referred to as "pp65" in this review) and the 72 kDa immediate early protein, IE-1 (UL123, referred to as "IE-1" in this review) [31-34]. The focus of research during this period was clearly on the "cytotoxic" CD8 T cell response. It is now clear that a broader range of antigens and cellular functions has to be taken into account. In the past, responses to epitopes presented by HLA-alleles common in Western Europe and parts of the United States (e.g. HLA-A*0201) were prioritized in research because they are easy to study.

CD4 T cells have not usually been implicated in HCMV-specific cytotoxicity to the same extent as CD8 T cells, despite the fact that lysis of HCMV-infected cells by CD4 T cells was described as early as 1986 [35]. CD4 T cell cytotoxicity was discovered even earlier [36]. Using modern technology, Appay *et al.* confirmed in 2002 that CD28⁻ CD4 T cells (a hallmark of HCMV infection) had the ability to lyse target cells [37], and in the last 10 years or so, more and more researchers "rediscovered" CD4 T cell responses to HCMV. Experimental adoptive T cell transfer in bone marrow transplant patients in the early 1990s had already suggested that CD4 T cells are very important but their main task was believed to be the upkeep of CD8 T cell immunity [38]. In 1996, it was shown that stimulation of peripheral blood mononuclear cells with HCMV viral lysate was able to activate very large numbers of CD4 T cells based on intracellular cytokine staining (ICS); however, the full extent of CD4 T cell involvement in the immune response to HCMV was not clear until a landmark publication in 2005 [27] changed our perspective entirely, in regard to both CD4 and CD8 T cells. By screening the HCMV proteome using 213 protein-spanning peptide pools [26], it was revealed that human T cells recognized at least 151 proteins in a donor population of 33 individuals of mixed MHC backgrounds. CD4 T cells recognized 125 proteins, CD8 T cells recognized 107 proteins, and 81 proteins were recognized by both. Of note, until recently, the size of the HCMV proteome was somewhat underestimated. A recent publication estimated that the HCMV genome provides more than 700 protein open reading frames, potentially coding for many previously untested proteins as well as smaller proteins overlapping with previously known ones [39]. Being a very large DNA virus, HCMV adapts to the host through immune evasion rather than rapid mutation of target proteins [40]. Nevertheless, the HCMV-specific T cell response targets mostly proteins that are highly conserved between different HCMV strains [27]. There is a tendency for CD4 T cells to predominantly recognize structural (virion) proteins and for CD8 T cells to predominantly recognize non-structural proteins; however, there is huge overlap between the compartments. In addition to dendritic cell cross-presentation, there are ways in which a HCMV antigen is "packaged" to overcome the divide of exogenous and endogenous antigen-presenting pathways. For example, dense bodies are particles full of HCMV proteins that are secreted by infected cells. They are internalized by antigen-presenting cells [41,42] essentially moving endogenous antigens into the exogenous pathway. Non-infectious enveloped particles secreted by infected endothelial cells appear to have a similar function [43].

Preferences for proteins of certain kinetic classes to some extent relate back to their structural and non-structural nature (for example, a preference for [non-structural] immediate early proteins to be recognized by CD8 T cells); however, CD4 T cells can also recognize these and no strict divide seems to exist [27].

T cell responses to HCMV latency-associated proteins were somewhat "neglected" in the past, mostly because the identification of latency antigens was delayed compared with lytic-phase antigens [44]. Not many latency-associated antigens are known to date, but Mason *et al.* recently reported that some of these are recognized by CD4 T cells [16]. Their work focused on two proteins in particular—UL138 and LUNA (latency-associated unidentified nuclear antigen)—both of which are expressed during lytic infection and latency. CD4 T cells recognizing UL138 and LUNA were detected in the first instance by interferon-gamma (IFN-γ) enzyme-linked immunosorbent spot (ELISPOT) assays. In lieu of using complete proteins, the authors used protein-spanning overlapping peptide pools for stimulation [26]. More detailed analysis, however, revealed that, unlike CD4 T cells specific to a lytic-phase antigen like glycoprotein B, *ex-vivo* expanded CD4 T cells specific for LUNA or UL138 also produced IFN-γ when stimulated by latently infected autologous monocytes. Interestingly, some of these cells also produced cellular IL-10 (cIL-10) and transforming growth factor-beta (TGF-β), suggesting that they would subdue T cell responses rather than accelerate them. These findings were confirmed for two additional latency-associated proteins, US28 and vIL-10, which in summary suggests that latency-associated antigens prime subpopulations of CD4 T cells with a functional pattern "protective" of HCMV.

Despite huge efforts, the overall workings of the T cell response to HCMV are still understood poorly at best. If there is anything, such as a "formula", that will allow us to recognize whether the HCMV-specific T cell response in a given individual is in control of HCMV or not, it certainly has not been discovered yet. In humans, one usually has to work with phenomenological/correlative results. If such results are good enough to develop reliable clinical diagnostic tools, they are indeed very useful. This should, however, not be confused with an understanding of basic underlying mechanisms. For example, the monitoring of HCMV-specific CD8 T cells after bone marrow transplantation with a set of class-I MHC multimers provided a reasonable surrogate for "protective" immunity; however, it did not demonstrate that the T cells being monitored actually had any effect [45]. In a different study, the adoptive transfer of MHC-multimer-selected pp65-specific T cells after bone marrow transplantation provided protection from HCMV disease, suggesting that pp65-specific T cells can control HCMV infection [46]. Conversely, however, patients may develop overt HCMV disease in the face of even large pp65-specific T cell responses, suggesting that these cells do not necessarily always control infection [47].

Immune evasion mechanisms versus success of the cellular immune response to human cytomegalovirus

CMV disposes of a large array of strategies to avoid an efficient immune response and these are thought to be the reason for its successful survival in both human and animal populations. Latency appears to be a particularly important part of the ability of HCMV to hide from the immune system; however, the number of evasion mechanisms is such that this fascinating topic has warranted more reviews than can possibly be referenced here [12,48-54]. The fact that HCMV-infected people usually do not have HCMV-related health problems seems to confirm that the immune response generally keeps the upper hand, while the success of adoptive T cell transfer therapy in preventing acute HCMV-related complications after bone marrow transplantation underscores the efficiency of T cells in controlling CMV [55-57]. However, large investigations into different populations of older people have shown that being HCMV-positive at an older age may be a considerable disadvantage [7,58-61]. At this time, though, it is unclear to what extent this is related to the virus itself or to HCMV-specific immunity. It may be that very large HCMV-specific T cell responses, which are found in many (particularly older) individuals, are neither required nor beneficial but actually cause damage [62-64]. Some researchers believe that excessive T cell responses may be the cause of HCMV-associated premature immune senescence [11], and there is some evidence to suggest that how the immune system responds to HCMV determines the degree of such immune senescence rather than infection per se [65]. Large T cell expansions are often oligoclonal [66,67] and their phenotype is CD57+ (a marker associated with HCMV carrier status) [68] and CD28- [69]. The presence of a terminally differentiated CD4+CD28- ("CD28 null") subpopulation is actually a hallmark of HCMV infection [70], and as a result, the appearance of such cells in vascular autoimmune disease (for example, Wegener's granulomatosis) has drawn attention to a possible role of HCMV in these situations [71,72].

Very large expansions of HCMV-specific T cells are sometimes referred to as "memory inflation", a concept originally derived from inbred mouse models. Many authors have indeed used inbred mice infected with murine CMV (MCMV) to model the situation that occurs in HCMV-infected humans. "Memory inflation" thus describes the increase of T cell responses to certain epitopes over time and their long-term maintenance at high frequencies [73-75]. In mice, unlike in humans (where the time of infection is usually not known), this phenomenon can be studied longitudinally. There are a number of obvious differences between inbred mice and

humans as there are between MCMV and HCMV, but essential response mechanisms are still thought to be very similar. Compared with human models, inbred mouse models have the advantage that they produce predictable T cell response patterns that are useful for studying T cell immunity, albeit in a reductionist way [73,76]. Other animal models are also being used to study T cell immunity after CMV infection, including rats [77,78] and non-human primates [79]. Using rhesus CMV (RhCMV) as a vector in a novel and highly effective simian immunodeficiency virus (SIV) vaccine has exploited the fact that CMVs attract large and persistent T cell responses [80,81].

Cytomegalovirus-induced regulatory T cells

Recent years have seen an explosion of information on the critical role of regulatory T cells (Tregs) in the control of T cell effector responses, peripheral tolerance, and immune homeostasis. Tregs can develop in the thymus (natural Tregs, or nTregs) or can be induced in the periphery by the antigen activation of naïve T cells (inducible Tregs, or iTregs) in response to a variety of conditions, as recently reviewed by Bilate *et al.* [82]. In particular, iTregs can be generated in response to sub-immunogenic doses of antigen [83] and chronic inflammation, as has been observed in several infections [84-86]. It is believed that the expansion of iTregs in infection has the beneficial role to limit the magnitude and duration of inflammatory responses and consequential tissue damage and at the same time drive the establishment of chronic infection. Tregs appear to control the activation of effector T cell responses by competition for growth factors, secretion of soluble factors, and cell-cell interaction. The expression of the high-affinity IL-2 receptor CD25 allows Tregs to decrease the local concentration of IL-2, an essential growth factor for effector cells. Among other mechanisms, Tregs also express relatively high levels of cytotoxic T-lymphocyte antigen 4 (CTLA-4), an extrinsic regulator of T cell responses [87,88], and through CTLA-4 stimulate dendritic cells (DCs) to produce the enzyme indoleamine 2,3-dioxygenase, which degrades the essential amino acid tryptophan into pro-apoptotic kynurenine [89]. The ectonucleotidase, CD39, equally expressed on Tregs, catabolises inflammatory ATP to anti-inflammatory adenosine [90,91].

We currently lack reliable phenotypic markers for the discrimination of iTregs and nTregs. Moreover, the identification of iTregs is complicated by the existence of alternative subpopulations that include but are not limited to IL-10-producing Tr1 and TGF-β-producing Th3 cells [92,93]. Among the expression markers associated with Tregs, the forkhead transcription factor FoxP3 (forkhead box P3) was once considered a "benchmark" identifier as it was believed to be essential for Treg development [94]. However, FoxP3 is also (transiently) upregulated on activated CD4 T cells and not necessarily stably expressed in populations of suppressive iTregs [95-97]. Alternative markers, such as the transcription factor Helios (Izf2) [98] and the surface protein neuropilin-1 [99-101], have, likewise, remained unsatisfactory for the discrimination of nTregs and iTregs.

We have recently identified a population of antigen-activated iTreg cells by combining canonical Treg markers, CD25 and CD39, with the activation marker CD134 (OX40) [17,102]. Interestingly, iTregs defined this way were increased in HCMV-infected older people and associated with increased resting blood pressure, suggesting a role of HCMV in driving vascular changes in older life. These findings add to a number of published articles associating HCMV infection with vascular complications [9,10]; however, no direct link between the T cell response to HCMV and blood pressure had previously been made.

Other cellular responses to human cytomegalovirus infection: γ/δ T cells and natural killer cells

γ/δ T cells are not normally frequent in peripheral blood but are very frequent in gut epithelium [103]. Among γ/δ T cells, the Vδ2⁻ population is of particular interest in HCMV infection because its long-term expansion is almost like a signature of HCMV infection [20]. Recently, major inroads into the cognate ligands of this population in the context of HCMV infection were made [19]. While these cells are not strictly speaking HCMV-specific, they can effectively lyse HCMV-infected fibroblasts and endothelial cells as a result of a cellular stress response that leads to the upregulation of endothelial protein C receptor (EPCR) plus co-stimulatory molecules like CD54 (ICAM-1). Earlier reports suggested that a Vγ9⁻ γ/δ T cell population including Vγ8 Vδ1 cells with a "public" Vδ1 complementarity determining region 3 T cell receptor (TCR) sequence plays a role *in utero* in congenital HCMV infection; however, the antigen recognized by these cells has not been identified yet [104].

NK cells belong to the innate immune response and are equipped with receptors exhibiting much less polymorphism than the TCR. They express a range of inhibitory and activating surface receptors, including lectin-like receptors and Ig-like receptors (KIRs). The inhibitory lectin-like receptor CD94/NKG2A/B interacts with the (non-classic) class Ib MHC molecule HLA-E, stable expression of which depends on the presentation

of (classic) class Ia MHC molecule leader sequences. However, HLA-E can also be upregulated/stabilized by an HCMV-encoded peptide that is part of the glycoprotein UL40 sequence, a protein involved in immune evasion [105,106]. It is interesting that HCMV infection leaves a stable imprint on the KIR repertoire in infected individuals involving the activating KIRs, KIR2DS4, KIR2DS2, and KIR2DS1. This suggests that certain NK cells are indeed selected by HCMV infection [107]. These mechanisms are nowhere nearly as specific as TCR/MHC/peptide interactions; however, there is clearly an element of specificity and "memory" usually not associated with innate immunity. NK cell memory is a fairly recent discovery in a rapidly evolving field [108]. Recently, NK cell specificity was reported from the HIV field as it was shown that an HIV-1 p24-derived epitope can be presented by HLA-Cw*0102 and modulate the NK cell response via the ligand KIR2DL2 [21]. Similar mechanisms may be active in HCMV infection, but this field is only beginning to be explored.

Conclusions and outlook

In summary, we still lack a real understanding of how the cellular immune response to CMV is actually organized and functions as a whole to provide protection. Many inroads have been made into understanding individual immune response elements (commonly studied aspects involving target specificity, response size, avidity, functionality, phenotype, and clonality); however, the next step needs to be the integration of these elements into a bigger picture. In order to make a real difference, we need to understand the correlates of protection. This would critically improve the management of patients and assist an effective CMV vaccine design.

Abbreviations

CMV, cytomegalovirus; CTLA 4, cytotoxic T-lymphocyte antigen 4; Foxp3, forkhead box P3; HCMV, human cytomegalovirus; HLA, human leukocyte antigen; IFNγ, interferon-gamma; iTreg, induced regulatory T cell; KIR, killer cell immunoglobulin-like receptor; LUNA, latency-associated unidentified nuclear antigen; MCMV, murine cytomegalovirus; MHC, major histocompatibility complex; NK, natural killer; nTreg, natural regulatory T cell; TCR, T cell receptor; TGF-β, transforming growth factor-beta; Treg, regulatory T cell.

Disclosures

Florian Kern holds a patent related to the use of overlapping peptide pools for T cell stimulation from which he receives a financial return (EP 1 257 290 B1). The technology is referred to in references [23-27].

References

1. McGeoch DJ, Cook S, Dolan A, Jamieson FE, Telford EA: **Molecular phylogeny and evolutionary timescale for the family of mammalian herpesviruses.** *J Mol Biol* 1995, **247**:443-58.

2. Alford CA, Stagno S, Pass RF, Britt WJ: **Congenital and perinatal cytomegalovirus infections.** *Rev Infect Dis* 1990, **12**(Suppl 7): S745-53.

3. Stagno S, Reynolds DW, Amos CS, Dahle AJ, McCollister FP, Mohindra I, Ermocilla R, Alford CA: **Auditory and visual defects resulting from symptomatic and subclinical congenital cytomegaloviral and toxoplasma infections.** *Pediatrics* 1977, **59**:669-78.

4. Saigal S, Lunyk O, Larke RP, Chernesky MA: **The outcome in children with congenital cytomegalovirus infection. A longitudinal follow-up study.** *Am J Dis Child* 1982, **136**:896-901.

5. Rook AH: **Interactions of cytomegalovirus with the human immune system.** *Rev Infect Dis* 1988, **10**(Suppl 3):S460-7.

6. Griffiths PD: **Current management of cytomegalovirus disease.** *J Med Virol* 1993, **1**:106-11.

7. Solana R, Tarazona R, Aiello AE, Akbar AN, Appay V, Beswick M, Bosch JA, Campos C, Cantisán S, Cicin-Sain L, Derhovanessian E, Ferrando-Martínez S, Frasca D, Fülöp T, Govind S, Grubeck-Loebenstein B, Hill A, Hurme M, Kern F, Larbi A, López-Botet M, Maier AB, McElhaney JE, Moss P, Naumova E, Nikolich-Zugich J, Pera A, Rector JL, Riddell N, Sanchez-Correa B, et al.: **CMV and Immunosenescence: from basics to clinics.** *Immun Ageing* 2012, **9**(23).

8. Wang GC, Kao WHL, Murakami P, Xue Q, Chiou RB, Detrick B, McDyer JF, Semba RD, Casolaro V, Walston JD, Fried LP: **Cytomegalovirus infection and the risk of mortality and frailty in older women: a prospective observational cohort study.** *Am J Epidemiol* 2010, **171**:1144-52.

9. Aiello AE, Simanek AM: **Cytomegalovirus and immunological aging: the real driver of HIV and heart disease?** *J Infect Dis* 2012, **205**:1772-4.

   F1000Prime RECOMMENDED

10. Simanek AM, Dowd JB, Pawelec G, Melzer D, Dutta A, Aiello AE: **Seropositivity to cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the United States.** *PLoS ONE* 2011, **6**(e16103).

11. Pawelec G, Akbar A, Beverley P, Caruso C, Derhovanessian E, Fülöp T, Griffiths P, Grubeck-Loebenstein B, Hamprecht K, Jahn G, Kern F, Koch SD, Larbi A, Maier AB, Macallan D, Moss P, Samson S, Strindhall J, Trannoy E, Wills M: **Immunosenescence and Cytomegalovirus: where do we stand after a decade?** *Immun Ageing* 2010, **7**(13).

12. Reddehase MJ: **Antigens and immunoevasins: opponents in cytomegalovirus immune surveillance.** *Nat Rev Immunol* 2002, **2**:831-44.

13. Goodrum F, Caviness K, Zagallo P: **Human cytomegalovirus persistence.** *Cell Microbiol* 2012, **14**:644-55.

14. Quinnan GV, Kirmani N, Rook AH, Manischewitz JF, Jackson L, Moreschi G, Santos GW, Saral R, Burns WH: **Cytotoxic t cells in cytomegalovirus infection: HLA-restricted T-lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone-marrow-transplant recipients.** *N Engl J Med* 1982, **307**:7-13.

15. Quinnan GV, Burns WH, Kirmani N, Rook AH, Manischewitz J, Jackson L, Santos GW, Saral R: **HLA-restricted cytotoxic T lymphocytes are an early immune response and important defense mechanism in cytomegalovirus infections.** *Rev Infect Dis* 1984, **6**:156-63.

16. Mason GM, Jackson S, Okecha G, Poole E, Sissons JGP, Sinclair J, Wills MR: **Human cytomegalovirus latency-associated proteins elicit immune-suppressive IL-10 producing CD4$^+$ T cells.** *PLoS Pathog* 2013, **9**(e1003635).

    F1000Prime RECOMMENDED

17. Terrazzini N, Bajwa M, Vita S, Cheek E, Thomas D, Seddiki N, Smith H, Kern F: **A Novel Cytomegalovirus-Induced Regulatory-Type T-Cell Subset Increases in Size During Older Life and Links Virus-Specific Immunity to Vascular Pathology.** *J Infect Dis* 2013.

18. Tovar-Salazar A, Patterson-Bartlett J, Jesser R, Weinberg A: **Regulatory function of cytomegalovirus-specific CD4+CD27-CD28- T cells.** *Virology* 2010, **398**:158-67.

19. Willcox CR, Pitard V, Netzer S, Couzi L, Salim M, Silberzahn T, Moreau J, Hayday AC, Willcox BE, Déchanet-Merville J: **Cytomegalovirus and tumor stress surveillance by binding of a human γδ T cell antigen receptor to endothelial protein C receptor.** *Nat Immunol* 2012, **13**:872-9.

F1000Prime RECOMMENDED

20. Pitard V, Roumanes D, Lafarge X, Couzi L, Garrigue I, Lafon M, Merville P, Moreau J, Déchanet-Merville J: **Long-term expansion of effector/memory Vdelta2-gammadelta T cells is a specific blood signature of CMV infection.** *Blood* 2008, **112**:1317-24.

F1000Prime RECOMMENDED

21. Fadda L, Körner C, Kumar S, van Teijlingen NH, Piechocka-Trocha A, Carrington M, Altfeld M: **HLA-Cw*0102-restricted HIV-1 p24 epitope variants can modulate the binding of the inhibitory KIR2DL2 receptor and primary NK cell function.** *PLoS Pathog* 2012, **8**(e1002805).

F1000Prime RECOMMENDED

22. Altman JD, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM: **Phenotypic analysis of antigen-specific T lymphocytes.** *Science* 1996, **274**:94-6.

23. Waldrop SL, Pitcher CJ, Peterson DM, Maino VC, Picker LJ: **Determination of antigen-specific memory/effector CD4+ T cell frequencies by flow cytometry: evidence for a novel, antigen-specific homeostatic mechanism in HIV-associated immunodeficiency.** *J Clin Invest* 1997, **99**:1739-50.

24. Kern F, Surel IP, Brock C, Freistedt B, Radtke H, Scheffold A, Blasczyk R, Reinke P, Schneider-Mergener J, Radbruch A, Walden P, Volk HD: **T-cell epitope mapping by flow cytometry.** *Nat Med* 1998, **4**:975-8.

25. Maecker HT, Dunn HS, Suni MA, Khatamzas E, Pitcher CJ, Bunde T, Persaud N, Trigona W, Fu TM, Sinclair E, Bredt BM, McCune JM, Maino VC, Kern F, Picker LJ: **Use of overlapping peptide mixtures as antigens for cytokine flow cytometry.** *J Immunol Methods* 2001, **255**:27-40.

26. Kern F, Faulhaber N, Frömmel C, Khatamzas E, Prösch S, Schönemann C, Kretzschmar I, Volkmer-Engert R, Volk HD, Reinke P: **Analysis of CD8 T cell reactivity to cytomegalovirus using protein-spanning pools of overlapping pentadecapeptides.** *Eur J Immunol* 2000, **30**:1676-82.

27. Sylwester AW, Mitchell BL, Edgar JB, Taormina C, Pelte C, Ruchti F, Sleath PR, Grabstein KH, Hosken NA, Kern F, Nelson JA, Picker LJ: **Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects.** *J Exp Med* 2005, **202**:673-85.

F1000Prime RECOMMENDED

28. Kern F, LiPira G, Gratama JW, Manca F, Roederer M: **Measuring Ag-specific immune responses: understanding immunopathogenesis and improving diagnostics in infectious disease, autoimmunity and cancer.** *Trends Immunol* 2005, **26**:477-84.

29. Li Pira G, Kern F, Gratama J, Roederer M, Manca F: **Measurement of antigen specific immune responses: 2006 update.** *Cytometry B Clin Cytom* 2007, **72**:77-85.

30. Gratama JW, Kern F, Manca F, Roederer M: **Measuring antigen-specific immune responses, 2008 update.** *Cytometry A* 2008, **73**:971-4.

31. Alp NJ, Allport TD, van Zanten J, Rodgers B, Sissons JG, Borysiewicz LK: **Fine specificity of cellular immune responses in humans to human cytomegalovirus immediate-early I protein.** *J Virol* 1991, **65**:4812-20.

32. Borysiewicz LK, Hickling JK, Graham S, Sinclair J, Cranage MP, Smith GL, Sissons JG: **Human cytomegalovirus-specific cytotoxic T cells. Relative frequency of stage-specific CTL recognizing the 72-kD immediate early protein and glycoprotein B expressed by recombinant vaccinia viruses.** *J Exp Med* 1988, **168**:919-31.

33. Wills MR, Carmichael AJ, Mynard K, Jin X, Weekes MP, Plachter B, Sissons JG: **The human cytotoxic T-lymphocyte (CTL) response to cytomegalovirus is dominated by structural protein pp65: frequency, specificity, and T-cell receptor usage of pp65-specific CTL.** *J Virol* 1996, **70**:7569-79.

34. Kern F, Surel IP, Faulhaber N, Frömmel C, Schneider-Mergener J, Schönemann C, Reinke P, Volk HD: **Target structures of the CD8(+)-T-cell response to human cytomegalovirus: the 72-kilodalton major immediate-early protein revisited.** *J Virol* 1999, **73**:8179-84.

35. Lindsley MD, Torpey DJ, Rinaldo CR: **HLA-DR-restricted cytotoxicity of cytomegalovirus-infected monocytes mediated by Leu-3-positive T cells.** *J Immunol* 1986, **136**:3045-51.

36. Krensky AM, Reiss CS, Mier JW, Strominger JL, Burakoff SJ: **Long-term human cytolytic T-cell lines allospecific for HLA-DR6 antigen are OKT4+.** *Proc Natl Acad Sci USA* 1982, **79**:2365-9.

37. Appay V, Zaunders JJ, Papagno L, Sutton J, Jaramillo A, Waters A, Easterbrook P, Grey P, Smith D, McMichael AJ, Cooper DA, Rowland-Jones SL, Kelleher AD: **Characterization of CD4(+) CTLs ex vivo.** *J Immunol* 2002, **168**:5954-8.

F1000Prime RECOMMENDED

38. Riddell SR, Greenberg PD: **Principles for adoptive T cell therapy of human viral diseases.** *Annu Rev Immunol* 1995, **13**:545-86.

39. Stern-Ginossar N, Weisburd B, Michalski A, Le VTK, Hein MY, Huang S, Ma M, Shen B, Qian S, Hengel H, Mann M, Ingolia NT, Weissman JS: **Decoding human cytomegalovirus.** *Science* 2012, **338**:1088-93.

F1000Prime RECOMMENDED

40. Holtappels R, Thomas D, Reddehase MJ: **The efficacy of antigen processing is critical for protection against cytomegalovirus disease in the presence of viral immune evasion proteins.** *J Virol* 2009, **83**:9611-5.

41. Sauer C, Klobuch S, Herr W, Thomas S, Plachter B: **Subviral dense bodies of human cytomegalovirus stimulate maturation and activation of monocyte-derived immature dendritic cells.** *J Virol* 2013, **87**:11287-91.

F1000Prime RECOMMENDED

42. Pepperl S, Münster J, Mach M, Harris JR, Plachter B: **Dense bodies of human cytomegalovirus induce both humoral and cellular immune responses in the absence of viral gene expression.** *J Virol* 2000, **74**:6132-46.

F1000Prime RECOMMENDED

43. Walker JD, Maier CL, Pober JS: **Cytomegalovirus-infected human endothelial cells can stimulate allogeneic CD4+ memory T cells by releasing antigenic exosomes.** *J Immunol* 2009, **182**:1548-59.

44. Kondo K, Xu J, Mocarski ES: **Human cytomegalovirus latent gene expression in granulocyte-macrophage progenitors in culture and in seropositive individuals.** *Proc Natl Acad Sci USA* 1996, **93**:11137-42.

45. Gratama JW, Boeckh M, Nakamura R, Cornelissen JJ, Brooimans RA, Zaia JA, Forman SJ, Gaal K, Bray KR, Gasior GH, Boyce CS, Sullivan LA, Southwick PC: **Immune monitoring with iTAg MHC Tetramers for prediction of recurrent or persistent cytomegalovirus infection or disease in allogeneic hematopoietic stem cell transplant recipients: a prospective multicenter study.** *Blood* 2010, **116**:1655-62.

46. Cobbold M, Khan N, Pourgheysari B, Tauro S, McDonald D, Osman H, Assenmacher M, Billingham L, Steward C, Crawley C, Olavarria E, Goldman J, Chakraverty R, Mahendra P, Craddock C, Moss PAH: **Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers.** *J Exp Med* 2005, **202:**379-86.

F1000Prime RECOMMENDED

47. Bunde T, Kirchner A, Hoffmeister B, Habedank D, Hetzer R, Cherepnev G, Proesch S, Reinke P, Volk H, Lehmkuhl H, Kern F: **Protection from cytomegalovirus after transplantation is correlated with immediate early 1-specific CD8 T cells.** *J Exp Med* 2005, **201:**1031-6.

F1000Prime RECOMMENDED

48. Reddehase MJ: **The immunogenicity of human and murine cytomegaloviruses.** *Curr Opin Immunol* 2000, **12:**390-6.

49. Mocarski ES: **Immunomodulation by cytomegaloviruses: manipulative strategies beyond evasion.** *Trends Microbiol* 2002, **10:**332-9.

50. Doom CM, Hill AB: **MHC class I immune evasion in MCMV infection.** *Med Microbiol Immunol* 2008, **197:**191-204.

51. Powers C, DeFilippis V, Malouli D, Früh K: **Cytomegalovirus immune evasion.** *Curr Top Microbiol Immunol* 2008, **325:**333-59.

52. Jackson SE, Mason GM, Wills MR: **Human cytomegalovirus immunity and immune evasion.** *Virus Res* 2011, **157:**151-60.

53. Noriega V, Redmann V, Gardner T, Tortorella D: **Diverse immune evasion strategies by human cytomegalovirus.** *Immunol Res* 2012, **54:**140-51.

54. Lu M, Guo Q, Pasternack RF, Wink DJ, Seeman NC, Kallenbach NR: **Drug binding by branched DNA: selective interaction of tetrapyridyl porphyrins with an immobile junction.** *Biochemistry* 1990, **29:**1614-24.

55. Riddell, Greenberg: **T cell therapy of human CMV and EBV infection in immunocompromised hosts.** *Rev Med Virol* 1997, **7:**181-92.

56. Riddell SR, Rabin M, Geballe AP, Britt WJ, Greenberg PD: **Class I MHC-restricted cytotoxic T lymphocyte recognition of cells infected with human cytomegalovirus does not require endogenous viral gene expression.** *J Immunol* 1991, **146:**2795-804.

57. Riddell SR, Reusser P, Greenberg PD: **Cytotoxic T cells specific for cytomegalovirus: a potential therapy for immunocompromised patients.** *Rev Infect Dis* 1991, **13**(Suppl 11):S966-73.

58. Derhovanessian E, Larbi A, Pawelec G: **Biomarkers of human immunosenescence: impact of Cytomegalovirus infection.** *Curr Opin Immunol* 2009, **21:**440-5.

59. Chen S, Craen AJM de, Raz Y, Derhovanessian E, Vossen ACTM, Westendorp RGJ, Pawelec G, Maier AB: **Cytomegalovirus seropositivity is associated with glucose regulation in the oldest old. Results from the Leiden 85-plus Study.** *Immun Ageing* 2012, **9**(18).

F1000Prime RECOMMENDED

60. Mortensen LH, Maier AB, Slagbom PE, Pawelec G, Derhovanessian E, Petersen I, Jahn G, Westendorp RGJ, Christensen K: **Early-life environment influencing susceptibility to cytomegalovirus infection: evidence from the Leiden Longevity Study and the Longitudinal Study of Aging Danish Twins.** *Epidemiol Infect* 2012, **140:**835-41.

F1000Prime RECOMMENDED

61. Derhovanessian E, Maier AB, Hähnel K, Zelba H, Craen AJM de, Roelofs H, Slagboom EP, Westendorp RGJ, Pawelec G: **Lower proportion of naïve peripheral CD8+ T cells and an unopposed pro-inflammatory response to human Cytomegalovirus**

proteins in vitro are associated with longer survival in very elderly people. *Age (Dordr)* 2013, **35:**1387-99.

F1000Prime RECOMMENDED

62. Steininger C: **Clinical relevance of cytomegalovirus infection in patients with disorders of the immune system.** *Clin Microbiol Infect* 2007, **13:**953-63.

63. Steininger C, Puchhammer-Stöckl E, Popow-Kraupp T: **Cytomegalovirus disease in the era of highly active antiretroviral therapy (HAART).** *J Clin Virol* 2006, **37:**1-9.

64. Griffiths PD: **CMV as a cofactor enhancing progression of AIDS.** *J Clin Virol* 2006, **35:**489-92.

65. Derhovanessian E, Maier AB, Beck R, Jahn G, Hähnel K, Slagboom PE, Craen AJM de, Westendorp RGJ, Pawelec G: **Hallmark features of immunosenescence are absent in familial longevity.** *J Immunol* 2010, **185:**4618-24.

F1000Prime RECOMMENDED

66. Weekes MP, Wills MR, Mynard K, Carmichael AJ, Sissons JG: **The memory cytotoxic T-lymphocyte (CTL) response to human cytomegalovirus infection contains individual peptide-specific CTL clones that have undergone extensive expansion in vivo.** *J Virol* 1999, **73:**2099-108.

67. Weekes MP, Carmichael AJ, Wills MR, Mynard K, Sissons JG: **Human CD28-CD8+ T cells contain greatly expanded functional virus-specific memory CTL clones.** *J Immunol* 1999, **162:**7569-77.

68. Gratama JW, Naipal AM, Oosterveer MA, Stijnen T, Kluin-Nelemans HC, Ginsel LA, den Ottolander GJ, Hekker AC, D'Amaro J, van der Giessen M: **Effects of herpes virus carrier status on peripheral T lymphocyte subsets.** *Blood* 1987, **70:**516-23.

69. Pawelec G, Akbar A, Caruso C, Effros R, Grubeck-Loebenstein B, Wikby A: **Is immunosenescence infectious?** *Trends Immunol* 2004, **25:**406-10.

70. van Leeuwen EMM, Remmerswaal EBM, Vossen MTM, Rowshani AT, Wertheim-van Dillen PME, van Lier RAW, Berge IJM ten: **Emergence of a CD4+CD28- granzyme B+, cytomegalovirus-specific T cell subset after recovery of primary cytomegalovirus infection.** *J Immunol* 2004, **173:**1834-41.

F1000Prime RECOMMENDED

71. Eriksson P, Sandell C, Backteman K, Ernerudh J: **Expansions of CD4+ CD28- and CD8+CD28- T cells in granulomatosis with polyangiitis and microscopic polyangiitis are associated with cytomegalovirus infection but not with disease activity.** *J Rheumatol* 2012, **39:**1840-3.

72. Morgan MD, Pachnio A, Begum J, Roberts D, Rasmussen N, Neil DAH, Bajema I, Savage COS, Moss PA, Harper L: **CD4+ CD28- T cell expansion in granulomatosis with polyangiitis (Wegener's) is driven by latent cytomegalovirus infection and is associated with an increased risk of infection and mortality.** *Arthritis Rheum* 2011, **63:**2127-37.

F1000Prime RECOMMENDED

73. Klenerman P, Hill A: **T cells and viral persistence: lessons from diverse infections.** *Nat Immunol* 2005, **6:**873-9.

F1000Prime RECOMMENDED

74. Munks MW, Cho KS, Pinto AK, Sierro S, Klenerman P, Hill AB: **Four distinct patterns of memory CD8 T cell responses to chronic murine cytomegalovirus infection.** *J Immunol* 2006, **177:**450-8.

75. Snyder CM, Cho KS, Bonnett EL, van Dommelen S, Shellam GR, Hill AB: **Memory inflation during chronic viral infection is maintained by continuous production of short-lived, functional T cells.** *Immunity* 2008, **29:**650-9.

F1000Prime RECOMMENDED

76. O'Hara GA, Welten SPM, Klenerman P, Arens R: **Memory T cell inflation: understanding cause and effect.** *Trends Immunol* 2012, **33**:84-90.

77. Meijer H, Dormans PH, Geelen JL, van Boven CP: **Rat cytomegalovirus: studies on the viral genome and the proteins of virions and nucleocapsids.** *J Gen Virol* 1984, **65** (Pt 4):681-95.

78. Stals FS, Bosman F, van Boven CP, Bruggeman CA: **An animal model for therapeutic intervention studies of CMV infection in the immunocompromised host.** *Arch Virol* 1990, **114**:91-107.

79. Price DA, Bitmansour AD, Edgar JB, Walker JM, Axthelm MK, Douek DC, Picker LJ: **Induction and evolution of cytomegalovirus-specific CD4+ T cell clonotypes in rhesus macaques.** *J Immunol* 2008, **180**:269-80.

80. 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, Piatak M, Lifson JD, Picker LJ: **Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine.** *Nature* 2011, **473**:523-7.

    F1000Prime RECOMMENDED

81. Hansen SG, Piatak M, Ventura AB, Hughes CM, Gilbride RM, Ford JC, Oswald K, Shoemaker R, Li Y, Lewis MS, Gilliam AN, Xu G, Whizin N, Burwitz BJ, Planer SL, Turner JM, Legasse AW, Axthelm MK, Nelson JA, Früh K, Sacha JB, Estes JD, Keele BF, Edlefsen PT, Lifson JD, Picker LJ: **Immune clearance of highly pathogenic SIV infection.** *Nature* 2013, **502**:100-4.

    F1000Prime RECOMMENDED

82. Bilate AM, Lafaille JJ: **Induced CD4+Foxp3+ regulatory T cells in immune tolerance.** *Annu Rev Immunol* 2012, **30**:733-58.

83. Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, Boehmer H von: **Inducing and expanding regulatory T cell populations by foreign antigen.** *Nat Immunol* 2005, **6**:1219-27.

    F1000Prime RECOMMENDED

84. Punkosdy GA, Blain M, Glass DD, Lozano MM, O'Mara L, Dudley JP, Ahmed R, Shevach EM: **Regulatory T-cell expansion during chronic viral infection is dependent on endogenous retroviral superantigens.** *Proc Natl Acad Sci USA* 2011, **108**:3677-82.

    F1000Prime RECOMMENDED

85. Belkaid Y, Rouse BT: **Natural regulatory T cells in infectious disease.** *Nat Immunol* 2005, **6**:353-60.

86. Losikoff PT, Self AA, Gregory SH: **Dendritic cells, regulatory T cells and the pathogenesis of chronic hepatitis C.** *Virulence* 2012, **3**:610-20.

87. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, Baker J, Jeffery LE, Kaur S, Briggs Z, Hou TZ, Futter CE, Anderson G, Walker LSK, Sansom DM: **Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4.** *Science* 2011, **332**:600-3.

    F1000Prime RECOMMENDED

88. Walker LSK, Sansom DM: **The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses.** *Nat Rev Immunol* 2011, **11**:852-63.

    F1000Prime RECOMMENDED

89. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre M, Puccetti P: **Modulation of tryptophan catabolism by regulatory T cells.** *Nat Immunol* 2003, **4**:1206-12.

    F1000Prime RECOMMENDED

90. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, Chen J, Enjyoji K, Linden J, Oukka M, Kuchroo VK, Strom TB, Robson SC: **Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression.** *J Exp Med* 2007, **204**:1257-65.

    F1000Prime RECOMMENDED

91. Borsellino G, Kleinewietfeld M, Di Mitri D, Sternjak A, Diamantini A, Giometto R, Höpner S, Centonze D, Bernardi G, Dell'Acqua ML, Rossini PM, Battistini L, Rötzschke O, Falk K: **Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression.** *Blood* 2007, **110**:1225-32.

92. Peterson RA: **Regulatory T cells: diverse phenotypes integral to immune homeostasis and suppression.** *Toxicol Pathol* 2012, **40**:186-204.

93. Gregori S, Goudy KS, Roncarolo MG: **The cellular and molecular mechanisms of immuno-suppression by human type 1 regulatory T cells.** *Front Immunol* 2012, **3**(30).

94. Fontenot JD, Gavin MA, Rudensky AY: **Foxp3 programs the development and function of CD4+CD25+ regulatory T cells.** *Nat Immunol* 2003, **4**:330-6.

    F1000Prime RECOMMENDED

95. Roncarolo M, Gregori S: **Is FOXP3 a bona fide marker for human regulatory T cells?** *Eur J Immunol* 2008, **38**:925-7.

96. Allan SE, Song-Zhao GX, Abraham T, McMurchy AN, Levings MK: **Inducible reprogramming of human T cells into Treg cells by a conditionally active form of FOXP3.** *Eur J Immunol* 2008, **38**:3282-9.

97. Allan SE, Passerini L, Bacchetta R, Crellin N, Dai M, Orban PC, Ziegler SF, Roncarolo MG, Levings MK: **The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs.** *J Clin Invest* 2005, **115**:3276-84.

98. Akimova T, Beier UH, Wang L, Levine MH, Hancock WW: **Helios expression is a marker of T cell activation and proliferation.** *PLoS ONE* 2011, **6**(e24226).

    F1000Prime RECOMMENDED

99. Sarris M, Andersen KG, Randow F, Mayr L, Betz AG: **Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition.** *Immunity* 2008, **28**:402-13.

100. Weiss E, Schmidt A, Vobis D, Garbi N, Lahl K, Mayer CT, Sparwasser T, Ludwig A, Suri-Payer E, Oberle N, Krammer PH: **Foxp3-mediated suppression of CD95L expression confers resistance to activation-induced cell death in regulatory T cells.** *J Immunol* 2011, **187**:1684-91.

101. Yadav M, Louvet C, Davini D, Gardner JM, Martinez-Llordella M, Bailey-Bucktrout S, Anthony BA, Sverdrup FM, Head R, Kuster DJ, Ruminski P, Weiss D, Schack D von, Bluestone JA: **Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo.** *J Exp Med* 2012, **209**:1713-22S1-19.

102. Zaunders JJ, Munier ML, Seddiki N, Pett S, Ip S, Bailey M, Xu Y, Brown K, Dyer WB, Kim M, Rose R de, Kent SJ, Jiang L, Breit SN, Emery S, Cunningham AL, Cooper DA, Kelleher AD: **High levels of human antigen-specific CD4+ T cells in peripheral blood revealed by stimulated coexpression of CD25 and CD134 (OX40).** *J Immunol* 2009, **183**:2827-36.

    F1000Prime RECOMMENDED

103. Cheroutre H, Lambolez F, Mucida D: **The light and dark sides of intestinal intraepithelial lymphocytes.** *Nat Rev Immunol* 2011, **11**:445-56.

104. Vermijlen D, Brouwer M, Donner C, Liesnard C, Tackoen M, van Rysselberge M, Twité N, Goldman M, Marchant A, Willems F: **Human**

cytomegalovirus elicits fetal gammadelta T cell responses in utero. *J Exp Med* 2010, **207**:807-21.

F1000Prime RECOMMENDED

105. Tomasec P, Braud VM, Rickards C, Powell MB, McSharry BP, Gadola S, Cerundolo V, Borysiewicz LK, McMichael AJ, Wilkinson GW: **Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40.** *Science* 2000, **287**(1031).

F1000Prime RECOMMENDED

106. Ulbrecht M, Martinozzi S, Grzeschik M, Hengel H, Ellwart JW, Pla M, Weiss EH: **Cutting edge: the human cytomegalovirus UL40 gene product contains a ligand for HLA-E and prevents NK cell-mediated lysis.** *J Immunol* 2000, **164**:5019-22.

107. Béziat V, Liu LL, Malmberg J, Ivarsson MA, Sohlberg E, Björklund AT, Retière C, Sverremark-Ekström E, Traherne J, Ljungman P, Schaffer M, Price DA, Trowsdale J, Michaëlsson J, Ljunggren H, Malmberg K: **NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs.** *Blood* 2013, **121**:2678-88.

F1000Prime RECOMMENDED

108. Paust S, Andrian UH von: **Natural killer cell memory.** *Nat Immunol* 2011, **12**:500-8.