REVIEW

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The immunological burden of human cytomegalovirus infection

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Abstract

Cytomegalovirus (CMV) is a persistent DNA virus that has evolved with humans to establish a finely balanced host-virus relationship. This balance is maintained by host immune surveillance since deficiencies in these processes can result in life-threatening disease, as observed in immunologically immature neonates and pharmacologically immunosuppressed transplant recipients. Both T cells and natural killer cells are intimately involved in maintaining asymptomatic infection by specific and non-specific recognition of infected cells. Under pressure from such host immune responses, CMV appears to have evolved elaborate strategies to subvert these responses in order to persist in the host. CMV target antigens are well characterized, with many CD8 T cell and CD4 T cell epitopes reported. This information is now being exploited to treat immunocompromised patients in order to boost virus-specific immunity. This review also discusses our current understanding of how virus carriage may skew lymphocyte populations in immunocompetent subjects and the association of CMV-seropositivity with immunosenescence.

Key words: cytomegalovirus, immune responses, therapy, immunosenescence.

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Cytomegalovirus (CMV), also known as human herpesvirus-5, belongs to the β-herpesvirus sub-family of the *Herpesviridae* family of viruses [61]. These are genetically stable agents that have co-evolved with their hosts for millions of years, establishing a subtle host-virus arrangement of mutual coexistence. Herpesviruses are enveloped viruses with an icosahedral capsid that encloses a double-stranded DNA genome. CMV is the largest of the eight human herpesviruses, with a genome of 230 kb. Transmission of CMV may occur through bodily fluids such as saliva, breast milk, placental transfer, and blood transfusions and also via solid organ transplantation or stem-cell transplantation. Seroprevalence varies between 50 to 90% depending on the socioeconomic status of the population. Primary CMV infection usually occurs by early childhood without any signs of disease. This is followed by a lifelong persistent phase of asymptomatic latent infection in cells of the myeloid lineage. Latency in monocytes involves CMV genome persistence without viral gene expression, but monocyte differentiation into macrophages can induce viral reactivation and productive replication, generating new virions transmissible to a secondary host [76]. Although our understanding of the precise mechanisms involved in viral latency is incomplete, it is clear that host immune responses are critical in keeping viral replication in check, as life-threatening CMV disease may occur in the immunocompromised or immunologically immature host. Symptoms include malaise, fever, hepatitis, interstitial pneumonia, gastrointestinal disease, and/or retinitis. Immunity against CMV in humans appears to multifaceted, with a central role for both CD8 and CD4 T cells and also importance for natural killer (NK) cells, γδ T cells, and antibodies. This review focuses on T cell and NK cell responses against CMV and examines progress in immunotherapy for the treatment of patients at risk of CMV disease. Finally, the recent association of CMV carriage with immunosenescence is discussed.

CD8 T CELL RESPONSES

Evidence for a central role for CD8 T cells in protection against disease came from work with murine CMV (mCMV), the animal model for CMV infection, and the inverse correlation between CMV disease and CD8 T cell reconstitution post stem cell transplant

(SCT) in humans [67, 69]. As CMV has a high protein-coding capacity, estimated at 165 open reading frames (ORFs) [16], it offers a huge number of potential antigenic targets, which may be proteolytically processed into short peptides and presented by MHC molecules for T cell recognition. Like other herpesviruses, CMV replication involves the sequential expression of immediate early (IE), early (E), and late (L) proteins. Early work with mCMV showed that cytotoxic CD8 T lymphocytes (CTLs) targeted the IE1 protein and that this response alone could confer protection [35]. More recent work has shown that IE1-specific CTLs can act as an immunological checkpoint by restricting viral gene expression to the IE phase [75].

In human CMV infection, the major viral phosphoprotein pp65 was the first identified CTL target antigen [56] and is thought to account for 70 to 90% of the total antiviral response [99]. IE1 was not considered as immunogenic as in mice and this was thought to be due to pp65-mediated inhibition of IE1 antigen presentation [29]. During the same period, reports emerged of viral proteins that interfere with antigen presentation by targeting MHC class I expression in infected cells (see Table 1). As pp65 is a major constituent of the incoming virus, it is processed and presented prior to viral gene expression [70] and is therefore not subject to such interference. The actual effectiveness of said immune subversion has been challenged with the development of single cell-based flow cytometry assays and recombinant CMV viruses lacking the immuno-subversive US2-US11 region. In many healthy virus carriers, IE1-specific and pp50 (E phase protein)-specific CD8 T cells are at frequencies comparable with pp65 [20, 39, 42, 43]. Furthermore, responses have been identified against antigens expressed throughout the viral replicative cycle [20, 41, 54] that were previously undetectable in restimulation experiments using the wild-type laboratory strains AD169 and Towne; it is thus clear that CMV immunosubversion does not prevent the maturation of a broad CD8 T cell response. This may be due to limited efficiency of viral interference with antigen presentation in different cell types *in vivo*. Secondly, some responses may be induced by cross-priming, in which professional antigen-presenting cells present antigens exogenously acquired from infected cells [83].

The biological value of responses generated and sustained by cross-priming is thus questionable, as antigen presentation by CMV-infected stromal cells may well be influenced by US2-US11 functions. There is also debate regarding the significance of measuring pp65 responses as a clinically useful marker. Some studies have shown pp65-specific CTL responses to be of importance [48, 71], whereas others suggest that IE1-specific CTLs are important and pp65-specific CTLs are not [6]. It is possible that pp65-specific and IE1-specific T cell responses are of benefit during distinct phases of viral activity. pp65 responses may be important in recognizing newly infected cells, as virion-derived pp65 is immediately available for processing before IE1 can be expressed. During viral reactivation from latency, IE1 responses may be preferentially expanded, as IE1 antigen would be available for processing and presentation much sooner than pp65. Indeed, IE1 responses do increase continually over time following primary CMV infection, whereas pp65 responses peak early on and then progressively decline [40].

Screening studies using peptide mixes that represent every ORF have shown that up to 150 ORFs were immunogenic in the study population, albeit at vastly different precursor frequencies [82]. However, pp65 and IE1 appear to be the most immunodominant CMV antigens, with most of our information on T cell epitopes being derived from these proteins (see Table 2). Individual epitope-specific CTLs are very high in fre-

Table 1. CMV gene products that subvert T cell and NK cell responses

ORF	Function	References
	T cell evasion	
US2	Causes MHC class I translocation to cytosol for proteasmoal degradation	33
	Destroys HLA-DRα and HLA-DMα	86
US3	Causes MHC class I retention in ER	34
	Impairs HLA-DR assembly	32
US6	Inhibits TAP-mediated translocation of peptides into ER	49
US10	Delays MHC class I trafficking out of ER	22
US11	Causes MHC class I translocation to cytosol	96
	NK cell evasion	
UL83 (pp65)	Inhibition of NKcell activating receptor NKp30	2
UL16	Sequesters MICB, ULBP1, ULBP2 (NKG2D ligands)	17
UL18	MHC class I homologue	12
	Putative inhibitory effect by engagement of LIR proteins	66
UL40	Enhances surface expression of HLA-E (ligand for CD94/NKG2A inhibitory NK receptors)	84, 92
UL112	Virally encoded microRNA that down-regulates MICB expression	80
UL141	Blocks surface expression of CD155, a ligand for activatory NK receptors	85
UL142	Inhibition of NK cell-mediated lysis	98

Table 2. Commonly recognized CMV-encoded T cell epitopes

Antigen	Epitope sequence	Co-ordinates	HLA restriction	Immunogenicity	References
IE1	YILEETSVM	315–323	HLA-A2	+++	69
	YVLEETSVM	316–324	HLA-A2	+++	42
	VLEETSVML	316–324	HLA-A2	**++++**	42
	VLAELVKQI	81–89	HLA-A2	++	20
	ATTFLQTMLR	32–41	HLA-A68	**++++**	40
	EVISVMKRR	334–342	HLA-A68	+++	40
	CRVLCCYVL	309–317	HLA-B7	**++++**	39
	ELRRKMMYM	199–207	HLA-B8	**++++**	20
	ELKRKMIYM	199–207	HLA-B8	**++++**	73
	QIKVRVDMV	88–96	HLA-B8	**++++**	20
	CVETMCNEY	279–287	HLA-A1/B18	++	69
	RRKMMYMCY	201–209	HLA-B27	+++	20
	KRKMIYMCY	201–209	HLA-B27	+++	73
	RRIEEICMK	341–349	HLA-B27	++	73
	DELRRKMMY	198–206	HLA-A1/B18	+++	40
	KEVNSQLSL	42–50	HLA-B40	+++	40
	EEAIVAYTL	381–389	HLA-B44	+++	40
	FPKTTNGCSQA	221–231	HLA-B55	+++	40
	VRVDMVRHRIKEHMLKKYTQ	91–110	HLA-DR3	+	13
	NYIVPEDKREMWMACIKELH	156–175	HLA-DR8	+	26
IE2	IIYTRNHEVK	242–250	HLA-A2	++	72
	FEQPTETPP	381–389	HLA-B41	+++	72
pp50	VTEHDTLLY	245–253	HLA-A1	**++++**	20
	RGDPFDKNY	274–282	HLA-A1	++	20
	TVRSHCVSK	52–60	HLA-A3	++	20
pp65	YSEHPTFTSQY	363–373	HLA-A1	+++	52
	NLVPMVATV	495–503	HLA-A2	**++++**	15
	MLNIPSINV	120–128	HLA-A2	++	78
	RIFAELEGV	522–530	HLA-A2	++	20
	QYDVPAALF	341–349	HLA-A24	++	47
	FTSQYRIQGKL	369–379	HLA-A24	++	52
	VYALPLKML	113–121	HLA-A24	++	55
	QYVKVYLESF	222–231	HLA-A24	+++	40
	FVFPTKDVALR	186–196	HLA-A68	+++	52
	YTPDSTPCHR	61–70	HLA-A68	++	40
	DTPVLPHETR	31–40	HLA-A68	++	40
	TPRVTGGGAM	417–426	HLA-B7	**++++**	99
	RPHERNGFTVL	265–275	HLA-B7	+++	52, 99
	IPSINVHHY	123–131	HLA-B35	+++	27
	FPTKDVAL	188–195	HLA-B35	+++	20
	CPSQEPMSIYVY	103–114	HLA-B35	++	8
	QPSLILVSQY	52–61	HLA-B35	++	40
	SEHPTFTSQY	364–373	HLA-B44	++	20, 40
	EFFDANDIY	512–521	HLA-B44	+	94
	PQYSEHPTFTSQYRIQ	361–376	HLA-DR11	+++	38
	FTSQYRIQGKLEYRHT	369–383	HLA-DR11	+++	38
	LLQTGIHVRVSQPSL	41–55	HLA-DQ6	++	6, 38
	NPQPFMRPHERNGFT	259–273	HLA-DR13	+	44
	EPDVYYTSAFVFPTK	177–191	HLA-DR7	++	50
	IIKPGKISHIMLDVA	281–295	HLA-DR4/DR53	+++	38
	AGILARNLVPMVATV	489–503	HLA-DR3/DR11	+++	38, 46
	KYQEFFWDANDIYRI	509–523	HLA-DR52	+++	38, 46
pp150	TTVYPPSSTAK	945–955	HLA-A3	+	52
	QTVTSTPVQGR	792–802	HLA-A68	++	52
	NVRRSWEEL	212–220	HLA-B7	++	20
	KARDHLAVL	101–109	HLA-B7	++	20
gB	RIWCLVVCV	4–12	HLA-A2	++	20
	DYSNTHSTRYV	217–227	HLA-DR7	**++++**	19
	CMLTITTARSKYPYH	250–264	HLA-DR4	+	51
	VFETSGGLVVFWQGI	420–434	HLA-DR7	+	51
gH	HELLVLVKKAQL	276–287	HLA-DR11	++	19

Epitopes confirmed by CTL data or multiple studies are included. Additional immunogenic peptides are summarized in reference [24]. **++++** responses detected in >10% of the T cell subset, +++ 1–10%, ++ 0.1–1%, + <0.1%

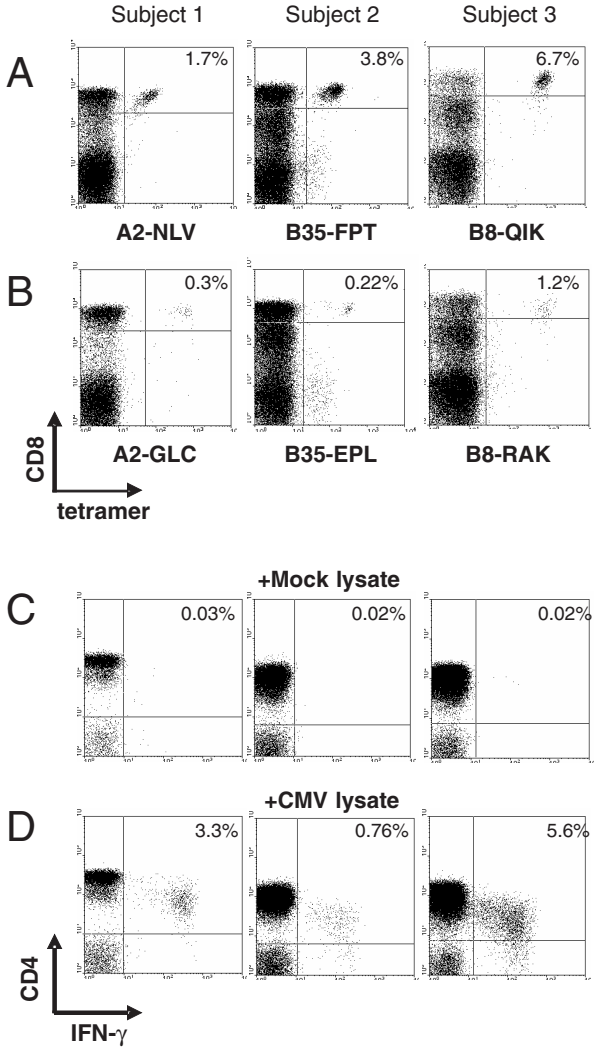

Fig. 1. The immunogenicity of CMV in healthy virus carriers. Both CD8 and CD4 T cell subsets invest heavily in CMV-specific responses, as shown by these examples of flow cytometric analysis of three healthy asymptomatic CMV-seropositive individuals. (**A** and **B**) Virus-specific CD8 T cells made visible by staining with fluorescent class I MHC-peptide tetramers. The selected individuals show higher frequencies of CMV-specific CD8 T cells (**A**) than EBV-specific CD8 T cells (**B**) for epitopes restricted by the same HLA allele. (**C** and **D**) Detection of virus-specific CD4 T cell responses using cytoplasmic IFN-γ staining after brief *in vitro* stimulation with either mock lysate (**C**) or CMV lysate (**D**). CMV lysates specifically induce a cytokine response in all cases.

quency, often well exceeding 1% of the CD8 subset, even in healthy hosts [42, 20]. This contrasts with responses against non-persistent viruses such as influenza A, which are typically 1–2 orders of magnitude lower. Interestingly, CMV-specific CD8 T cells also appear to be higher in frequency than CD8 T cells specific for Epstein-Barr virus (EBV), another chronic herpesvirus infection, suggesting that persistence alone does not explain the unique immunogenicity of CMV (see Fig. 1). Studies of the clonal composition of CMV-specific CD8 T cell responses show highly restricted T cell receptor (TCR) usage that often involves a single TCR β chain [42, 65, 87, 94]. Furthermore, these responses are predominantly composed of highly differentiated effector memory cells ($CD57^+$, $CD27^-$, $CD28^-$) that are high-avidity and perform multiple functions. This is argued to bestow a competitive advantage over lower-avidity T cells with the same peptide-MHC specificity, allowing selection of high-avidity responses into the memory pool [37].

CD4 T CELL RESPONSES

Although CD8 T cells appear to be critical in the recognition of CMV-infected cells, a growing body of data are highlighting the important role for MHC class II-restricted CD4 T cell responses in controlling infection. Initial indicators for a CD4 role came from adoptive transfer trials of CMV-specific CD8 T cell clones in bone marrow transplant patients. While these clones persisted in all patients, CD8 T cell activity was lower in patients deficient in virus-specific CD4 T cells, suggesting that T helper cell activity was required for CTL persistence [91]. Due to the greater difficulty in the synthesis of MHC class II tetramers, present methods to measure virus-specific CD4 T cells mainly involve detecting cytokine responses against CMV antigens (lysates of CMV-infected fibroblasts) or libraries of synthetic peptides. Studies of healthy immunocompetent children that acquired CMV have shown that deficiencies in CD4 T cells appear to correlate with prolonged viral shedding [89], whereas CD8 T cell deficiencies do not [9]. In immunosuppressed renal allograft recipients, the early formation of CMV antigen-specific CD4 T cells producing interferon (IFN)-γ correlated strongly with improved recovery from infection, whereas functional CD8 T cells alone were insufficient to control viral replication [23].

CD4 T cells specific for CMV show striking similarities with their CD8 T cell counterparts: CMV-specific CD4 T cells can reach very high frequencies (often >1%) (see Fig. 1), display a highly differentiated membrane phenotype ($CD57^+CD27^-CD28^-$) [21, 64, 75], and secrete high levels of IFN-γ, tumor necrosis factor (TNF)-α, and MIP-1β, in the absence of interleukin (IL)-2 [8]. This profile contrasts with viral infections such as HIV and vaccinia, against which CD4 T cells secrete high levels of IL-2 and minimal IFN-γ and TNF-α, a functional profile more typical of helper T cells. In fact, CMV-specific CD4 responses are characterized by high levels of cytoplasmic granzyme B and perforin [90], which implies a direct role in reducing viral burdens by killing infected cells in addition to cytokine-mediated effects and T helper function. CD4 T cell responses also appear to be very restricted in TCR usage [5, 95], suggesting that certain CD4 clonotypes are selected into the memory pool, probably on the basis of higher avidity for MHC class II-peptide ligands. CMV has evolved the ability to interfere with the MHC class II antigen-

-presentation pathway (see Table 1), but clearly this does not appear to have restricted the host's ability to mount a substantial MHC class II-restricted CD4 T cell response. Indeed this suggests that CMV has been subject to considerable immunological pressure from virus--specific CD4 T cells during its co-evolution with the host.

CD4 T cell responses (based on IFN-γ staining) can be detected against up to 40 different ORFs representing antigens expressed across the viral replicative cycle [82]. pp65 and the glycoprotein B (gB) are probably the most immunodominant antigens in terms of the frequency of responding CD4 T cells, with IE1 being less immunogenic. Most of the HLA class II-restricted peptides described in the literature are derived from pp65 with some epitope information from gB and IE-1 also (see Table 2). Individual epitope-specific responses can represent a large component (>90%) of the total CMV--specific CD4 T cell response (detected using CMV antigens) in some donors (unpublished observations), but in many cases, epitope specificity is undefined. Interestingly, endogenous gB appears to be processed and presented by MHC class II molecules in infected cells, allowing direct recognition by cytotoxic CD4 T cells [31]. Thus, further epitope-screening and processing studies need to be undertaken with other antigens to improve our understanding of CD4 T cell immunogenicity.

NK CELLS

NK cells are lymphocytes of the innate immune system that are involved in the recognition of pathogen--infected cells and tumors. NK cells express a range of inhibitory and activatory receptors which mainly recognize MHC class I molecules or molecules related to MHC class I. The balance of signals from these inhibitory and activatory receptors allows NK cells to discriminate between healthy and unhealthy cells. Under normal conditions, such as the absence of infection, NK cell activation is prevented by maintaining the balance towards inhibitory receptor engagement. To avoid T cell recognition, CMV causes MHC class I down-regulation; however, this abrogates the engagement of MHC class I by inhibitory NK cell receptors such as killer-cell immunoglobulin-like receptors (KIRs), CD94/NKG2A and CD85j. This renders target cells more vulnerable to an NK cell response that is driven by activatory receptors. The importance of NK cells in viral infection has been elegantly demonstrated in the mCMV model using different strains of mice. Certain strains appear to be resistant to mCMV infection but this property is abrogated upon NK cell depletion. This resistance has been mapped to the murine Ly49H receptor (a NK cell-surface antigen) and the viral ligand identified to be m157 [1, 77].

In humans, NK cell abnormalities also appear to cause susceptibility to severe herpesvirus infections, including CMV [4]. A case report of abnormal expression of one inhibitory KIR on all NK cells was thought to be the cause for recurrent CMV infections [28]. It is argued that such blanket expression of inhibitory receptors may confer total NK cell inhibition, resulting in disease susceptibility. Activating KIR genotype can also be important, as significantly lower episodes of CMV reactivation occur in sibling-SCT where donors express more than one activatory KIR [11]. In healthy individuals, CMV appears to selectively modify the NK repertoire; increased levels of $NKG2C^+$ NK cells (and $NKG2C^+$ T cells) with reduced activatory receptor expression are observed in CMV-seropositive persons [30].

Since NK cells also play a critical role in controlling CMV infection, it is likely that this has forced the virus to evolve countermeasures, as it has done for T cell recognition. Indeed, research over the last decade has revealed a number of NK cell-subversive mechanisms that target activatory or inhibitory NK receptors (see Table 1). For example, UL16 sequesters stress-induced ligands for the activating receptor NKG2D, thus preventing NKG2D-mediated target cell lysis [17]. In contrast, UL40 up-regulates HLA-E, the ligand for the inhibitory NK receptor NKG2A/CD94. This modulation confers resistance against $NKG2A/CD94^+$ NK cells [92]. Interestingly two NK-subversion proteins, UL141 and UL142, were recently identified in low-passage strains of CMV, but are absent in commonly used high-passage strains (AD169 and Towne). It is possible that other genes present only in wild-type isolates may also have immunological significance, and further reports are anticipated in this field.

TREATMENT

Over the last few decades antiviral therapy with ganciclovir has helped reduce the incidence of CMV disease in transplant recipients. However, ganciclovir therapy is associated with significant drug-induced toxicity. Immunotherapeutic strategies to combat CMV disease offer an attractive alternative to antiviral drugs. Since cell-mediated immunity is impaired in pharmacologically immunosuppressed SCT patients, attempts to reconstitute immunity have featured the *in vitro* expansion of donor-derived virus-specific T cell clones/lines for infusion into patients. Initial work was done using CD8 T cell infusions [91], but lately the value of virus-specific CD4 T cell therapy, in combination with CD8 T cells, has been explored. These combined infusions successfully expand *in vivo* and coincide with reduced incidence of viral reactivation post-SCT [18,62], emphasizing the importance of CD4 T cell responses as described earlier. Most of these trials have proven safe, with impressive results; however, this strategy is limited to centers with the resources and expertise to undertake clinical-scale T cell culture. It has also become clear that T cells generated *in vitro* may not perform adequately *in vivo* [25]. To address such problems, an alternative approach was

recently pioneered by Cobbold et al. Donor CMV-specific T cells were selected to high purity (~98%) using MHC class I tetramers for direct infusion into SCT patients, all of whom showed a significant reduction in viral loads [10]. Although promising, this approach is currently only applicable to CD8 T cell infusions, as MHC class II tetramers are not widely available for adoptive transfer of purified virus-specific CD4 T cells.

Limited progress in adoptive immunotherapy has led to more concerted focus on the development of a CMV vaccine. Indeed, this has been assigned a major health priority by the US Institute for Medicine [81]. Since CMV has evolved to infect and persist in the host in the face of multiple arms of immune attack, the aim of CMV vaccine development has been to prevent symptomatic CMV-associated disease rather than to prevent infection. Results from vaccines based on live attenuated CMV isolates have been disappointing (reviewed in [45]) and such formulations also present long-term safety issues, most notably in pregnant women. Research has shifted to development of subunit vaccines that contain the most important antigens and induce responses that will be protective *in vivo*. This can be designed to target specific T cell epitopes by using recombinant viruses containing minimal CMV epitopes linked together, such as the poly-epitope-based recombinant adenovirus vaccine [72]. This constitutes over 30 different immunogenic peptides derived from eight CMV antigens, restricted by 16 HLA types covering >90% of the population. Alternatively, recombinant viruses expressing multiple full-length proteins such as IE1, pp65, pp150, and gB are being developed [93]. This would have broader population coverage and also include neutralizing antibody epitopes. Both systems efficiently restimulate peptide-specific T cells *in vitro*, and further evaluation of these strategies is expected.

THE LONG-TERM COST OF CMV CARRIAGE IN IMMUNOCOMPETENT HOSTS

Aging is associated with a number of immunological changes such as reduced thymic output, weaker T cell proliferation and function, and sub-optimal responses to vaccination [14, 58, 79], which collectively lead to immunological deterioration termed immunosenescence. A common finding in many elderly subjects is the presence of clonal T cell expansions (TCEs), which can occupy a large proportion (up to 50%) of the T lymphocyte pool [63]. Such TCEs are also often highly differentiated effector memory cells ($CD28^{low}$), associated with increased mortality in 80 and 90 year olds and termed the immune-risk phenotype [59, 97]. CMV carriage also has profound effects on the T cell repertoire in healthy immunocompetent virus carriers. The CD8 subset is doubled in healthy elderly CMV-seronegative persons compared with elderly CMV-seronegative persons [53]. This increase could be directly attributed to antiviral immunity, as CMV-specific T cells (both IE1-specific and pp65-specific) increase with age, representing between 10–50% of total CD8 T cells in many elderly subjects [43, 44]. A similar increase in virus-specific CTLs, termed CD8 memory inflation, was shown in mCMV-infected mice [36]. CMV-specific TCEs can often be monoclonal [44], $CD28^{low}$, and the majority of TCEs appear to be non-functional [43, 60]. Similar, though lower-frequency, increases in CMV-specific CD4 T cells with a $CD28^{low}$ phenotype occur with aging (see Fig. 2) [64]; a population shown to have limited replicative capabilities [21]. Thus CMV drives the expansion of T cell subsets that are linked with immunosenescence, suggesting that this virus accelerates the process of age-associated deterioration of immune function.

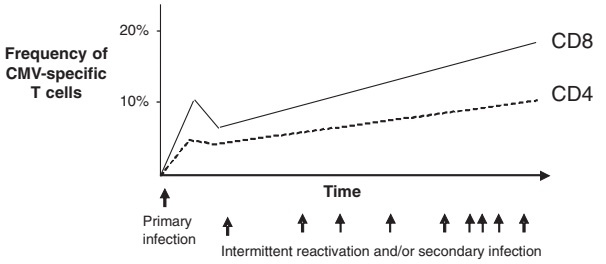

Fig. 2. CMV-specific T cell responses increase over time. Longitudinal studies of primary CMV infection and comparisons between CMV-infected persons of different ages suggest this model for virus-specific T cell responses. Viral antigens made available during occasional episodes of reactivation from latency and/or re-infection with secondary viral strains may stimulate virus-specific T cells, resulting in T cell expansions that constitute several percent of the total T cell subset. Both CD8 and CD4 T cell responses against CMV increase over time, although CD8 responses are generally stronger than CD4 responses and appear to undergo greater contraction following acute infection. It must be noted that increases in both CD8 responses and CD4 responses are not observed in all individuals and may occur independently of each other.

Such high levels of investment against a single virus may divert finite immunological resources away from other potential antigenic challenges by reducing the overall level of antigenic diversity, as shown with large TCEs in mice [57]. CMV carriage does appear to reduce the level of CTL immunity against another persistent herpesvirus, EBV; however, these individuals remained healthy without any symptoms of EBV-associated disease [43]. CMV status may also affect immunity against influenza, as high levels of $CD28^-$ CD8 T cells and CMV carriage coincide with weaker antibody responses post-influenza vaccination [88]. As influenza remains a major cause of morbidity and mortality in the elderly, these findings warrant further longitudinal studies to test if early intervention against CMV can alter the development of immunosenescence.

CMV carriage may also have potentially beneficial effects, as latent herpesvirus (mCMV and murine γ-herpesvirus 68) infections in mice cause prolonged IFN-γ production and systemic macrophage activation [3].

This modulation of the basal level of innate immunity generates an "antiviral state" that confers resistance to subsequent bacterial infections. As cross-protection is observed with two different herpesviruses, this may be an evolutionarily conserved feature of the host-virus balance, suggesting that further epidemiological studies are required to determine the significance of CMV carriage on general immune function in humans.

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