Dynamics of T cell memory in human cytomegalovirus infection

Edward C. P. Waller · Elizabeth Day ·
J. G. Patrick Sissons · Mark R. Wills

Received: 22 January 2008 / Published online: 27 February 2008
© Springer-Verlag 2008

Abstract Primary human cytomegalovirus (HCMV) infection of an immunocompetent individual leads to the generation of a robust CD4+ and CD8+ T cell response which subsequently controls viral replication. HCMV is never cleared from the host and enters into latency with periodic reactivation and viral replication, which is controlled by reactivation of the memory T cells. In this article, we discuss the magnitude, phenotype and clonality of the T cell response following primary HCMV infection, the selection of responding T cells into the long-term memory pool and maintenance of this memory T cell population in the face of a latent/persistent infection. The article also considers the effect that this long-term surveillance of HCMV has on the T cell memory phenotype, their differentiation, function and the associated concepts of T cell memory inflation and immunosenescence.

Keywords Cytomegalovirus · T cell memory · T cell phenotype · Memory inflation · Immunosenescence

Introduction

The general course of a primary T cell response to a virus infection can be summarized thus: the naïve virus-specific T cells encounter viral antigens presented by activated dendritic cells (DC) that have migrated from peripheral sites where these professional antigen presenting cells have phagocytosed virus infected cells or cell fragments into

local draining lymph nodes in order to present antigen to naïve T cells. The virus-specific T cells subsequently undergo an extensive clonal expansion (which may exceed a 1,000-fold). Following this extensive T cell proliferation and activation of effector functions (such as cytokine production and expression of cytotoxic granules), the effector CD8+ T cells leave the lymphoid tissue and migrate to sites of viral infection via chemotactic gradients (detected by surface expression of e.g. CXCR3 and/or CCR5 receptors) in combination with local changes in adhesion molecule expression on blood vessel endothelium. Following this, primary T cell activation cells are selected to become memory cells, the expanded virus-specific T cells undergo a contraction phase, in which, more than 90% of the cells are lost and the remaining cells constitute a memory T cell pool. A number of factors have been suggested that may determine selection of T cells from the initial expanded population into the long-term memory pool, including expression of receptors for the IL-2 cytokine family (in particular, the Interleukin-7 receptor IL7-R), TNF family receptors, perforin and IFN γ as well as the upregulation of anti-apoptotic factors, (reviewed in [1, 2]).

Our understanding of the T cell responses following primary HCMV infection is far less detailed than our knowledge of the memory T cell response that we can observe in long-term virus carriers. In fact, all of the tools that we have to investigate the primary response to infection were derived from initial studies of the memory T cells in seropositive long-term virus carriers. This being the case, this article will start with a description of the specificities, phenotype and function of memory T cells before discussing what we currently know about the development and selection of these cells following primary infection. The article also examines the relationship between memory T cell phenotype and function and the

E. C. P. Waller · E. Day · J. G. P. Sissons · M. R. Wills (✉)
Department of Medicine, Level 5,
Addenbrookes Hospital, University of Cambridge,
Hills Rd, Cambridge CB2 2QQ, UK
e-mail: mrw1004@cam.ac.uk

concepts of virus-driven memory inflation and the potential for immunosenescence.

The memory CD8+ and CD4+ T cell response to HCMV in long-term virus carriage

CD8+ T cells specific for HCMV were first described around 25 years ago, cultured by stimulation with HCMV infected fibroblasts and shown to be cytotoxic [3]. Both the immediate early (IE) protein (UL123) [4] and the pp65 tegument protein (UL83) [5] were identified as important targets for the CD8+ T cell response. Extensive peptide epitope mapping of both the pp65 and IE proteins has been undertaken by many groups and as such, a detailed map of the minimal peptides and their HLA restrictions has been published, in addition, many of the epitopes have been constructed as MHC Class I tetramers or multimers some of which are commercially available (reviewed in [6]). Most individuals exhibit significant pp65 responses and these were shown to be significantly larger than other detectable responses to pp150, gB and gH, pp50 and pp28 [7], leading to suggestions that pp65 was the immunodominant antigen for the HCMV-specific CD8+ T cells.

With the advent of efficient methods to construct pools of multiple overlapping peptides which span whole proteins and advances in detecting and enumerating response by intracellular cytokine production (e.g. IFN γ) and flowcytometry [8, 9], our understanding of the breadth of T cell responses to the proteins encoded by HCMV has been further advanced. A significant recent study has looked at IFN γ responses to 213 predicted HCMV encoded open reading frames (ORFs) using around 13,687 peptides of 15 amino acids in length, which overlapped by ten amino acids in a panel of 33 seropositive donors with disparate MHC Class I types [10]. One hundred and fifty-one ORF's were shown to elicit a CD4+ or CD8+ T cell response in at least one donor. Three ORF's were recognised by more than half of the cohort, UL48, UL83 (pp65), and UL123 (IE). Individuals made CD8+ T cell responses to a median of eight ORFS. It has been suggested that the size of IE and pp65 responses may be a result of apoptosis of virus infected cells, which generates antigen for cross-presentation with proteins from the entry virion (pp65) and IE proteins being most abundant very early postinfection [11]. Cross-presentation of pp65 derived from internalised apoptosing fibroblasts has been demonstrated in vitro [12, 13].

The size of the T cell response to HCMV is significant with an average of 10% of CD8+ memory T cells being directed against the virus [10]. In addition, the immune response to a given HCMV peptide in a latently infected individual is characteristically highly focussed, being composed of very few CD8+ T cell clones, which have

expanded massively [14, 15]. In elderly individuals, oligoclonal expansions are more pronounced, with up to 23% of CD8+ memory cells directed against a single HCMV epitope [16]. It has been suggested that clonal expansions may be a result of repeated exposure to antigen during reactivation, perhaps explaining the frequency of HCMV-specific cells in the elderly.

In healthy long-term carriers of HCMV, pp65-specific CD8+ T cells are present in both the CD45RA+ and CD45RO+ populations [15, 17–19]. The same T cell clone can be found expressing either isoform of the CD45 molecule suggesting a common origin [17]. CD45RA+ memory cells have been termed “revertant” memory cells to denote their return to the CD45RA+ status of a naïve CD8+ T cell and are also referred to as T_{EMRA} (discussed in detail later in this article).

The protective nature of the large CD8+ T cell response to HCMV infection is inferred by a number of studies. In human bone marrow transplantation, patients who have received adoptive transfer of ex vivo expanded HCMV-specific T cells were protected from both primary infection and reactivating virus [20–22]. As few as 1×10^6 HCMV-specific T cells per litre was shown to be sufficient to protect from disease [23]. CD4+ T cells may however be critical in the reconstitution of immunity in humans [24]. In the murine model, MCMV, IE-specific CD8+ T cells protected mice from a lethal challenge dose of virus without CD4+ T cell help, both in acute infection [25] and following bone marrow transplantation with simultaneous MCMV infection [26]. In an MCMV model of virus reactivation in B cell deficient mice, a hierarchy of importance of cellular response exists (CD8+ T cells, NK cells, CD4 T cells). Depletion of CD8+ T cells alone increases frequency of recurrent infection but deletion of all three populations leads to uncontrolled reactivation and dissemination [27].

HCMV-infected cells express lower levels of surface MHC Class I molecules [28] due to the action of a number of HCMV immune evasion proteins. US2 [29] and US11 [30, 31] dislocate MHC class I heavy chains from the endoplasmic reticulum to the cytosol, where they are degraded by the proteasome. US3 causes MHC Class I peptide complexes to be retained in the ER [32] and US6 prevents the translocation of peptides into the ER by interaction with the cytosolic face of TAP [33, 34]. However, even in the presence of these mechanisms to interfere with antigen presentation, it is clear from the analysis of long-term virus carriers that a robust CD8+ T cell responses was mounted to virus infection. The in vivo significance of these genes is unknown, in the murine model deletion of multiple genes that downregulate MHC Class I had little effect on viral persistence [35]. It seems likely that they may exist to provide a window of opportunity for replication to occur undetected by CD8+ T cells following reactivation from latency. When

fibroblasts infected with HCMV lacking US2-11 were used to stimulate CD8+ T cells, IFN γ responses in up to tenfold more responding cells were detected [36]. This suggested that evasion may operate at the level of recognition rather than priming as has been suggested for MCMV [37].

Viral evasion of antigen processing may have implications for studies of CD8+ T cell specificity and function in vitro. There is a discrepancy between the frequency of IE-specific CD8+ T cells detected using specific peptides and HCMV infected fibroblast stimulators whilst frequencies of pp65-specific cells are comparable [38]. This may reflect the need for IE proteins to be synthesised before presentation at a time when viral proteins that disrupt antigen processing and presentation are operating. However, a significant amount of pp65 is present in the tegument of the incoming virion and may be presented without interference. Alternatively, disruption of presentation may occur at different efficiencies for different proteins. Deletion of US2-11 enhanced recognition of IE peptides from very low backgrounds in a way not seen for pp65, suggesting a selective effect of these genes on IE [36]. In support of a differential effect of US2-11 on IE and pp65 was a recent study, in which, pp65 was shown to be presented in infected cells when IE presentation was inhibited. This mechanism depended on proteosomal activity and could account for the discrepancy in responses detected to IE in infected cells [39].

Peptides used experimentally for T cell detection would not be subject to interference in their processing. However, interestingly a study using infected fibroblasts to stimulate CD8+ recall responses demonstrated that most detectable responses were directed to IE or E proteins [40] and CD8+ responses to IE proteins have been suggested to be two- to four-fold higher than for other classes of gene if epitope selection were random [10] suggesting responses in vivo are successfully mounted.

Evidence of the importance of CD4+ T cells for control of HCMV infection is provided in studies of renal transplant patients undergoing a primary infection (natural primary infections have not been studied). IFN γ -producing CD4+ cells precede the emergence of the CD8+ T cell response only in those with asymptomatic infection but their emergence is delayed where disease develops [41]. In patients undergoing bone marrow transplantation, whilst CD8+ T cell infusion was able to protect from disease, their maintenance was dependent on the presence of HCMV-specific CD4+ cells [22, 24]. This suggests that support provided by CD4+ helper T cells is essential for effective CD8+ T cell responses. In support of this, using the MCMV model, CD4+ T cell depletion only had a minimal effect on the control of acute infection, whilst depletion of both T cell subsets lead to significant disease [26]. Likewise, it should be noted that in a model of acute MCMV infection,

the protective effect of adoptive CD8+ T cell transfer was not dependent on the presence of CD4+ T cells [42].

Analysis of the CD4+ T cell responses to HCMV has lagged behind that of the CD8+ T cell response, in part, because MHC Class II tetramers have proven much more difficult to produce, however, antigen-specific cells have been identified by intracellular cytokine production. pp65 and IE-specific T cells as well as other specificities have been demonstrated [43–45]. Individual epitopes have been identified in pp65, IE, gB and gH [43, 46, 47] and others [6]. CD4+ T cells often respond to the same ORFs as CD8+ T cells, individuals respond to a median of 12 ORFs. Five ORFs [UL55, UL83 (pp65), UL86, UL99, and UL122/123 (IE)] were recognized by more than half the long-term carriers tested. Typically, 10% of the CD4+ T cell memory pool are specific for HCMV [10].

As has already been described for the CD8+ T cell response in long-term virus carriers, oligoclonal expansions are marked and the same clones are present in both CD45RO+ and CD45RA+ pools with some cells also lacking CD28 expression [48]. Most HCMV-specific CD4+ T cells secrete IFN γ on stimulation but may also secrete TNF α and IL-2 [49, 50]. In addition, direct cytotoxicity has been observed [48] and a subpopulation of CD4+ cells that increase in frequency following primary infection express granzyme B suggesting they may possess this capacity [51].

HCMV evades the CD4+ T cell response by disrupting the up-regulation of MHC Class II molecules that is induced by IFN γ signalling. This is achieved by modulation of the expression of Janus kinase 1 (JAK1) and repression of Class II transactivator (CIITA) mRNA. In addition, US2 can direct HLA-DR α and HLA-DM α to the cytosol where they are degraded [52].

The phenotype of HCMV-specific CD8+ T cells in long-term memory

In many healthy long-term carriers, both the CD8+ and CD4+ memory pools specific for HCMV contain large numbers of CD45RA+ re-expressing “revertant” memory cells (T effector memory CD45RA cells, T_{EMRA}) many of which lack the chemokine receptor CCR7 and the costimulatory receptors CD27 and CD28. It is of note that the number of CD45RA+ CD27– cells in the CD8+ pool directly correlates with HCMV infection but not with EBV or VZV [53]. At present no difference in the phenotype of CD8+ T cells responding to different epitopes has been consistently documented [15].

In healthy long-term carriers, the vast majority of CD45RA+, revertant memory cells also lack CD28 and CCR7 but are heterogeneous for expression of CD62L and

the costimulatory molecule CD27 [18]. Other studies have also demonstrated overlapping populations of CD45RA+ cells lacking combinations of CD28, CD27 and CCR7, and often expressing CD57 [19, 54–58]. In addition, CD8+ CD45RA+ cells often express CD56 [59], CD244 [60] and KLRG1 [61, 62]. The expression of the inhibitory receptor CD85j is increased on CD8+ T cells in parallel with aging or HCMV positivity and is often expressed in combination with CD57 and where CD28 expression is lacking [63, 64].

In a comprehensive study of CD8+ T cell responses specific for HCMV, EBV, HIV and HCV [56], it was suggested that virus-specific CD8+ cells underwent a linear differentiation programme in which, CD45RA+ CD28+ CD27+ CCR7+ naïve cells became CD45RO+ following activation and sequentially lost expression of CCR7, CD28 and CD27. It was suggested that the phenotypes that predominate for each virus represented an accumulation at different points along the pathway, with HCMV-specific cells being predominantly of the late differentiation phenotype when CD45RA is reacquired. Loss of CD28 and CD27 also occurs when HCMV seropositive patients receive HCMV positive stem cell transplants with the reconstituted HCMV-specific CD8+ T cell population being relatively enriched for CD28– and CD27– cells compared to the donor [65]. HCMV-positive transplant patients who received an HCMV-positive organ also showed a decrease in CD27+ cells and increase in CD45RA+ cells as a percentage of their CD8+ T cell pool a year after transplantation, perhaps as a result of just HCMV antigen exposure, although it must be noted that these patients were immunosuppressed [66].

In addition to surface phenotype analysis, differences in cytokine profiles and the cytotoxicity of CD8+ memory subsets have been described. CD45RO+ cells produce a range of cytokines (IL-2, IL-4, IFN γ and TNF- α) and are not directly cytotoxic but on re-stimulation produce cytotoxic progeny. CD45RA+ CD27– cells (which largely overlap with CD45RA+ CD28– and CD45RA+ CCR7– populations) have features of effector cells but are resting, lacking the activation markers CD38, Ki67, CD69 and HLA-DR found on CD45RO+ effector cells during an acute response. They express high levels of FAS-L mRNA and the cytotoxic molecules perforin and granzyme B, and are cytotoxic without re-stimulation. CD45RA+ cells were shown to be reliant on exogenous growth factors like IL-2 and IL-15 and to produce only IFN γ and TNF- α , not IL-2 or IL-4 [67]. A study of rhesus CMV using the CD107a assay for degranulation showed that CD8+ T cells that degranulated but did not produce cytokines were mainly CD45RA+ CD28– [68].

Murine CD8+ responses to MCMV are a mixture of T_{CM} and T_{EM}, which are separated by CD62L high and low expression, respectively. The T_{EM} phenotype also expressed low levels of CD27 and CD28 but those com-

pletely lacking CD28 do not develop. The regulation of CD28 expression on murine and human T cells is distinct; in mice, CD28 expression is maintained for life but its expression on human (and non-human primate) T cells is less stable [69]. CD8+ CD28– T cells are therefore not only found in HCMV infection. Although all T cells in the human newborn express CD28 [70, 71] CD28– T cells (especially CD8+) accumulate in both the aged [70–73] and in various chronic viral infections [56, 74–76].

The generation of T cell responses following primary infection

Primary HCMV infections in otherwise healthy individuals are difficult to identify and this is reflected in the small number of published studies on the T cell immune response early postinfection [18, 77]. Given these difficulties, a number of studies have examined the response in cohorts that are highly likely to contract primary HCMV infection, either following kidney transplantation from a seropositive donor into a seronegative recipient [41, 78] or following congenital infection [79] or in early childhood in areas with a high HCMV seroprevalence [80].

Taking all the studies together there is a consensus that HCMV CD8+ T cells generated early postinfection express perforin and granzymes and can be shown to have direct *ex vivo* cytotoxicity. The cells express CD45RO but have lost expression of CCR7 and CD62L. At this early time point, there is no IL7R α expression and this molecule is not expressed until much later on memory T cells in agreement with another study [81]. These findings are in contrast to a number of mouse models, which suggest that its expression is required for selection [82, 83]. All the studies describe the loss of surface expression of both CD27 and CD28 molecules, however, the kinetics of this appear to be slower in the kidney transplantation patients as compared to the other cohorts. The appearance of CD45RA+ CD27–/CD28– positive HCMV-specific T cells following primary infection as T cells are selected into memory is also a notable observation in all the studies [18, 41, 77, 79, 80].

We were able to follow the evolution of the memory response to two different pp65 epitopes in a small number of otherwise normal healthy people who acquired natural HCMV infection, Fig. 1 summarizes the key findings. In these patients, the HCMV DNAemia in plasma peaked 2–3 weeks postinfection and reduced to background levels within 4–5 weeks following the onset of symptoms. Peak HCMV-specific T cell levels of 10–20% were measured during primary infection to a single pp65 peptide and as such, it was concluded that the total HCMV-specific T cell frequency in peripheral blood during primary infection is likely to be very large indeed [77].

Fig. 1 T cell response following primary infection selection of T cells into memory and long-term maintenance. Following primary infection large expansion of polyclonal, activated and directly cytotoxic T cells are generated. Within a few weeks, the viral DNA load has been decreased and many clones of responding T cells have contracted in size and disappeared. An oligoclonal population of cells is selected into memory. Many cells re-express the CD45RA isoform and have perma-

nently lost CD28 and CD27 expression. These clones initially selected into memory are very stable for years, probably decades and for life; however, the size of the T cell population does increase with time (memory inflation) and in the very elderly can become very large. New T cell clonotypes can also arise with time, thus broadening the clonotypic repertoire

In long-term seropositive virus carriers, CD8+ T cell responses to both IE and pp65 antigens are often highly focused with T cells utilizing few or just a single TcR Vβ family and the sequence diversity within these families of T cell clones is often highly conserved [84]. This may be because the primary T cell response becomes focused with differential selection of certain clones into the memory T cell pool (in contrast to the random, non-selective retention of the broad repertoire of the primary response reported in a number of model murine virus infection systems [85–88]) or because long-term carriage of the virus with periodic reactivation could lead to a progressive focusing of T cell response with time.

In the small number of donors, where we have been able to follow the generation of the primary T cell response and the subsequent selection into long-term memory, the initial CD8+ T cell repertoire is very diverse but rapidly focuses as certain families of responding T cells become undetectable. The cells that are selected into the memory pool were a subset of the initial responding CD8+ T cell population. A somewhat surprising observation was that focusing onto particular TcR usage occurred during the resolution of

primary infection and much more rapid than expected, as such it is not long-term carriage and reactivation of the virus that drives clonal focusing. The dominant virus-specific clonotypes found in long-term memory often show conservation of amino acid motifs in their TcR hypervariable regions between unrelated donors (public clonotypes), whereas private clonotypes were generally subdominant. This phenomenon is in agreement with a previous report in HCMV carriers of public TcR usage by CD8+ T cells specific for the same peptide [89].

Do pp65-specific clones, which have public TcR usage, have a functional advantage, such as a greater avidity for the peptide MHC complex? Evidence from influenza-specific memory responses suggests that a dominant public TcR Vβ17 was up to 10,000-fold more avid than subdominant clones [90]. Determination of the functional avidity of clones early in the response and comparing them with clones selected into memory, suggests that the clones that are lost early had a 2–3 log lower functional avidity than the dominant clones. HCMV pp65-specific CD8+ T cell selection following primary HCMV infection may be based on affinity driving survival and favouring selection into the

T cell memory pool. Early infection generates a diverse virus-specific TcR repertoire, comprising clones with a range of affinities including cells with public TcR usage, however, analysis at this level of detail has only been performed in a single donor to date and a larger panel of contracted clones is required to substantiate this message and to definitively determine the mechanistic basis of clonal focussing during primary HCMV infection. The events following primary HCMV infection selection into memory and maintenance are summarized in Fig. 1.

What is the lineage relationship of CD45RA+ CD28– (revertant or T_{EMRA}) T cells to the effector cells generated during primary infection?

HCMV-specific CD8+ CD45RA+ CD28– T cells are detectable from about 4 weeks postprimary HCMV infection. Are they a separate lineage of memory cells distinct from the cells detected during primary infection or are they derived from the primary effector cells? We have previously shown that the same TcR sequence initially detected in CD45RO+ effector cells is also utilized by CD45RA+ memory cells suggesting a common precursor [17].

Several additional lines of evidence suggest that CD45RA+ cells derive from CD45RO+ precursors. The re-expression of CD45RA has been shown to occur following the activation of naïve CD8+ T cells to give CD45RO+ effector cells [91]. Analysis of the loss of unstable chromosomes in CD45RA+ and CD45RO+ cells using mathematical modelling suggests that the observations were consistent with CD45RO–CD45RA reversion [92]. In addition, experiments in a rat model have provided evidence that CD45RO and CD45RA isoforms are interchangeable [93, 94]. The gene expression profiles of CCR7– cells correlate with CD28 and CD27 loss but not with CD45RA re-expression such that, in fact, CD45RA/RO may be interchangeable [95].

The factors, which drive the development of CD8+ CD45RA+ CD28– T cells in HCMV infection are not well understood. However, CD8+ CD45RO+ CCR7+ memory T cells cultured with IL-7 or IL-15 in the absence of antigen (TcR stimulation), produce a significant number of CD45RA+ progeny [96]. Culture with IL-15 has also been shown to induce reversion of CD8+ CD45RO+ EBV-specific cells to CD45RA expression, but that this did not occur with IL-2 or IL-7 [97].

CD45RA+ expression has been shown to be a function of time since upon antigenic stimulation, CD8+ CD45RA+ T cell clones became CD45RO+ and then gradually reacquired CD45RA expression. This period was extended when peptide-pulsed DCs were used as stimulators compared to the use of anti CD3/CD28 or other APCs

suggesting that professional stimulation delayed CD45RA+ reacquisition [98].

A number of experimental observations support the notion that CD28– T cells are derived from CD28+ T cells. Allogeneic, phytohaemagglutinin or anti-CD3 stimulation of CD8+ CD28+ T cells gives rise to a CD8+ CD28– T cell population [71]; this was accelerated by the presence of type I IFN [99]. Loss of CD28 has also been demonstrated for cord blood cells cultured in IL-12 and IL-15 [100], naïve cells maintained in IL-15 (reversed by IL-21) [101] and CD8+ CD28+ cells propagated using soluble anti-CD3 antibody and IL-2 (reversed by IL-4) [102]. Taken together these studies provide a strong indication that CD8+ CD45RA+ CD28– T cells develop in conditions of antigen absence in a particular cytokine environment, although it is unclear why this occurs to a greater extent during HCMV infection compared to other chronic viral infections.

CD8+ CD45RA+ CD28– T cells are CCR7–, however, antigenic stimulation induces CCR7 re-expression [98]; this has also been demonstrated for HCMV-specific CD45RA+ CCR7– CD27– T cells following HCMV antigen re-stimulation [54]. The CCR7 upregulation has also been shown to be functional for migration to CCL19 and CCL21 [55].

What is the capacity of CD8+ CD45RA+ CD28– T cells to proliferate in response to antigen?

Whether CD8+ T cells that lack CD28 expression have a defect in proliferation has been controversial and this is critical to know, given the predominance of HCMV-specific CD45RA+ CD28– T cells in many individuals. Non-specific stimulation using anti-CD3, anti-CD2, PHA or PMA + ionomycin has shown CD28– T cells have a lesser capacity for proliferation than CD28+ T cells [70, 103–106]. However, this has not been replicated in other studies using plate-bound anti-CD3 stimulation [107] or autologous PHA-stimulated blast cells as stimulators in HIV-infected subjects [108]. CD28– and CD28+ CD8+ T cells have also been shown to have a similar cloning efficiency, however, greater numbers of progeny were derived from CD28+ precursors [70].

A number of studies have suggested that CD8+ CD45RA+ CD28– T cells have a proliferation defect. This has been shown using monocyte-derived DCs and combinations of IL-7 and IL-15 [96] and non-specific anti-CD3 stimulation [97], anti CD3 and CD28 as well as HCMV peptide stimulation [109] although these studies only followed the cells for 3 days poststimulation.

In contrast, many other studies dispute that there is a proliferation defect in this population; highly purified CD8+ CD45RA+ CD28– CCR7– T cells have been

shown to proliferate in response to HCMV peptide [18]. Proliferation of all HCMV tetramer-staining cells in long-term carriers suggests that these revertant memory cells can proliferate perfectly well [54]. EBV-specific CD8+ CD45RA+ T cells are also able to proliferate in response to peptide stimulation and have longer telomeres and were more resistant to apoptosis as compared to EBV-specific central memory T cells [110] and CD8+ CD45RA+ CCR7– T cells respond to anti-CD3/CD28 [98] and anti-CD2 stimulation (with a reliance on exogenous IL-2 or IL-15) [67].

We have recently shown that the cloning efficiency of CD8+ CD45RA+ CD28– T cells is not dissimilar to that of HCMV-specific central memory CD8+ CD45RO+ T cells, which are believed to be fully functional. CD8+ CD45RA+ CD28– T cells do require professional antigen presentation, as autologous fibroblasts expressing HCMV peptides are unable to activate these memory cells. We have investigated the additional signals they need for activation and proliferation. They exhibit transient re-expression of CD28 and early upregulation of the costimulatory molecules 4-1BB and ICOS. However, only antibody blocking of 4-1BBL limits proliferation of CD8+ CD28– T cells. Autologous fibroblasts transfected with 4-1BBL are able to support antigen-driven proliferation of CD45RA+ CD28– T cells whilst those expressing ICOSL or CD80 and CD86 (the ligands for CD28) do not. In agreement with others, activated CD28– CD45RA+ T cells transiently re-express the chemokine receptor CCR7 (lymph node homing) and in addition, express CXCR3 and CCR5 (inflamed peripheral tissue homing) later [111]. This is an intriguing observation

as it may endow a dual capacity to provide immediate effector function and to migrate to draining lymph nodes (Fig. 2). We concluded that HCMV-specific, CD45RA+ CD28– T cells are not terminally differentiated, able to proliferate and acquire heightened effector function following restimulation. While these observations defined an important costimulation pathway, costimulation via other molecules may also be important.

It has been demonstrated that whilst HCMV-specific CD8+ CD28– T cells do not produce IL-2 and require its provision for growth, transduction of these cells to express CD28 restores their ability to produce IL-2, sufficient to support their proliferation [112]. Therefore, it seems likely CD8+ CD45RA+ CD28– T cells have strict activation requirements that have only been provided in certain studies. This may be a requirement for a certain strength of signal, use of particular costimulatory pathway(s) and/or reliance on provision of exogenous cytokines such as IL-2. The inhibitory killer cell lectin-like receptor G1 (KLRG1) has previously been suggested to identify CD8+ T cells that have reduced proliferative response to polyclonal stimulation [61]. However, we have noted in many donors, a large proportion of the HCMV-specific cells express KLRG1; an example in two separate donors is shown in Fig. 3, where >95% of the multimer-positive CD8+ T cells in PBMC expressed KLRG1+. Importantly, when stimulated with peptide antigen, these KLRG1+ virus-specific cells showed no apparent proliferation defect and this suggests that appropriate costimulation may be able to “rescue” cells which otherwise exhibit reduced proliferation (Fig. 3).

Fig. 2 CD45RA+ CD28– T cells use the 41BB–41BBL co-stimulation pathway and display sequence of chemokine receptor expression at their cell surface. Following contact with antigen CD45RA+ CD28– T cells rapidly up-regulate 41BB expression, which is required to support their proliferation. These cells subsequently re-express

CCR7 and CD62L, which would allow for lymph node migration and on continued proliferation, CCR7 expression is replaced by CXCR3 and CCR5 expression thus allowing homing to inflammatory cytokines from the periphery, such as CCL19 and 21 MIPs, IP-10, RANTES, etc.

Fig. 3 The majority of HCMV pp65-specific T cells express the inhibitory receptor KLRG1, however, this does not prevent proliferation. T cells from two separate donors stained with KLRG1 and either a pp65 HLA-A2 NLV MHC Class I multimer or pp65 peptide HLA-B7 TPR (NLV and TPR represent the first three amino acids of the full length peptides NLVPMVATV and TPRVTGGGAM, respectively) MHC Class I multimer (**a** and **b**). The Cloning efficiency of T cells from the donor represented in **a** was determined by measuring the functional frequency using limiting dilution analysis of each sorted subpopulations of HCMV-specific CD8⁺ T cells, the absolute frequency of HCMV-specific T cells in each subpopulation was determined by HLA-A2 NLV MHC Class I multimers. **c** The cloning efficiency is expressed as the percentage of actual T cells that are functional

T cell replicative history can be assessed by measuring their telomere length. The loss of CD28 expression has been associated with a reduction in telomerase activity [113] and a study comparing telomere length concluded that CD8⁺ CD28⁻ T cells did indeed have shorter telomeres than CD8⁺ CD28⁺ T cells [114]. This may be misleading since the CD8⁺ CD28⁺ T cell population contains many naïve cells, which have not divided. An early study showed that CD45RA⁺ cells had longer telomeres than CD45RO⁺ cells [115], however, separating CD45RA⁺ T cells, which lack CD27 expression (memory cells) showed that their telomere length was similar to CD45RO⁺ mem-

ory cells [116, 117]. A recent study has suggested that CD8⁺ CD28⁻ T cells are able to upregulate telomerase activity but that CD8⁺ CD28⁻ CD27⁻ T cells cannot [118]. Both telomerase activity and proliferation by CD8⁺ CD28⁻ CD27⁻ T cells following anti-CD3 and APC stimulation were restored almost to the level of other CD8⁺ T cells on the addition of IL-2 or IL-15 [118].

Memory inflation and T cell immunosenescence

It is well established that the HCMV-specific memory CD8⁺ T cell frequency is very large, however, it has been noted that elderly HCMV seropositive donors often have strikingly high HCMV-specific memory CD8⁺ T cells frequencies [16]. These cells are often termed to be highly differentiated as they do not express CD27 or CD28 but many express CD57 and the inhibitory NK receptor CD85J [63]. This phenomenon has been termed “memory inflation” and also observed to occur for MCMV-specific T cells in mice [119, 120, 87, 121, 122].

The proportion of CD8⁺ CD45RA⁺ T cells that are antigen experienced rather than naïve is much greater in elderly subjects as compared to the young [123, 124], as might be expected since they could be assumed on average to have been exposed to a larger number of pathogens. These T cells are apoptosis resistant [110] and have a slow rate of disappearance [124].

Of concern is that these large expansions of HCMV-specific T cells may have become dysfunctional. A number of studies have suggested that the proportion of HCMV-specific T cells (as measured by MHC Class I tetramer stains) that are able to produce IFN γ following peptide stimulation is substantially reduced in the elderly [125, 126]. The data presented, only uses a single HCMV derived peptide (HLA A2 NLV-pp65) and as such, we do not know if this is a more global phenomenon or something unusual to this particular peptide and the highly oligoclonal T cells that have been selected to recognize the peptide. Function of these T cells in this context is also solely measured as their ability to produce IFN γ , no assessment of other functional abilities, such as cytotoxicity or proliferative capacity has yet been determined. Given that IFN γ assays are short-term assays and that these types of T cells may require a particular restimulation environment, some caution should be observed and further investigations are warranted before the “dysfunction” in these T cells in the elderly is definitively demonstrated.

A number of investigators have implicated HCMV infection and the size and phenotype of the T cell population directed against the virus in immune dysfunction in the elderly [127, 128]. HCMV seropositivity has been shown to impair the immune response to a co-resident EBV infection

[123]. HCMV seropositivity or a high frequency of CD8+ CD28– T cells in elderly individuals has been correlated with poor humoral responses to influenza vaccination [129–131]. The mechanism for this has been suggested to be the accumulation of oligoclonal expansions of HCMV-specific T cells of the very differentiated phenotype (CD8+ CD45RA+ CD28– and most often CCR7–, CD27–) might “fill up” the CD8+ T cell pool, reducing the size of the naïve pool and subsequently impairing the ability to mount new responses [127, 128].

It has also been noted that the absolute number of CD8+ T cells as well as the number and proportion of differentiated CD45RA+ or CD28– T cells is increased in HCMV seropositive versus seronegative elderly or young individuals [132], so that, the absolute number of naïve cells may not differ dramatically although they are more diluted.

HCMV has now been correlated with a decrease in life expectancy. Longitudinal studies have identified an immune risk phenotype (IRP), which includes HCMV seropositivity, a CD4:CD8 T cell ratio of <1, an increased proportion of differentiated CD8+ T cells and clonal expansions and elevated pro-inflammatory cytokines in blood. This IRP reduces survival over the age of 80 [133–135]. It should be noted that not all HCMV seropositive individuals display the IRP and the reason for this is unknown. There is currently no evidence that HCMV is reactivated more often in elderly individuals. Other studies have showed that the number of clones responding to the NLVPMVATV pp65 epitope correlates with survival and absence of the IRP in the elderly [136].

It therefore remains unclear that HCMV is the causative agent of systemic immunosenescence and that in very elderly subjects, the HCMV-specific T cell population may be dysfunctional but it is clear that these are important questions to answer for the future.

It should be clear from this brief review that we have a detailed knowledge of the frequency, specificity and phenotype of the T cells selected into memory following HCMV infection. Our knowledge of the events determining this selection is considerably less complete. We do not know why HCMV is unusual (but not unique) in driving such a large accumulation of CD45RA+ CD28– memory T cells and it is still unclear, which if not all of the T cells, we can actually measure in long-term HCMV carriers are effective at detecting and preventing viral dissemination. An understanding of the specificities and functional characteristics of T cells that are best at detecting viral reactivation and preventing dissemination is of the utmost importance, if we are to deliver rational vaccine design or adoptive therapy approaches to either prevent infection or suppress viral reactivation. The information being derived from the Rhesus Macaque models of cytomegalovirus infection and

immunity should offer an additional approach to addressing some of these questions.

References

1. Kaech SM, Hemby S, Kersh E, Ahmed R (2002) Molecular and functional profiling of memory CD8 T cell differentiation. *Cell* 111:837–851
2. Kalia V, Sarkar S, Gourley TS, Rouse BT, Ahmed R (2006) Differentiation of memory B and T cells. *Curr Opin Immunol* 18(3):255–264
3. Borysiewicz LK, Morris S, Page JD, Sissons JG (1983) Human cytomegalovirus-specific cytotoxic T lymphocytes: requirements for in vitro generation and specificity. *Eur J Immunol* 13(10):804–809
4. Borysiewicz LK, Hickling JK, Graham S, Sinclair J, Cranage MP, Smith GL, Sissons JG (1988) 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* 168(3):919–931
5. McLaughlin Taylor E, Pande H, Forman SJ, Tanamachi B, Li CR, Zaia JA, Greenberg PD, Riddell SR (1994) Identification of the major late human cytomegalovirus matrix protein pp65 as a target antigen for CD8+ virus-specific cytotoxic T lymphocytes. *J Med Virol* 43(1):103–110 issn: 0146–6615
6. Wills MR, Carmichael AJ, Sissons JGP (2006) Adaptive cellular immunity to human cytomegalovirus. In: Reddehase MJ (eds) *Cytomegaloviruses: molecular biology and immunology*. Caister Academic press, Wymondham, pp 341–366
7. Boppana SB, Britt WJ (1996) Recognition of human cytomegalovirus gene products by HCMV-specific cytotoxic T cells. *Virology* 222(1):293–296
8. 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 (1998) T-cell epitope mapping by flow cytometry. *Nat Med* 4(8):975–978
9. Wills MR, Carmichael AJ, Mynard K, Jin X, Weekes MP, Plachter B, Sissons JG (1996) 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* 70(11):7569–7579
10. Sylwester AW, Mitchell BL, Edgar JB, Taormina C, Pelte C, Ruchti F, Sleath PR, Grabstein KH, Hosken NA, Kern F, Nelson JA, Picker LJ (2005) Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. *J Exp Med* 202(5):673–685
11. Davrinche C (2006) Combat between cytomegalovirus and dendritic cells in T-cell priming. In: M.J. Reddehase (Eds) *Cytomegaloviruses: molecular biology and immunology*. Caister Academic press, Wymondham, pp 367–382
12. Arrode G, Boccaccio C, Lule J, Allart S, Moinard N, Abastado JP, Alam A, Davrinche C (2000) Incoming human cytomegalovirus pp65 (UL83) contained in apoptotic infected fibroblasts is cross-presented to CD8(+) T cells by dendritic cells. *J Virol* 74(21):10018–10024
13. Tabi Z, Moutafsi M, Borysiewicz LK (2001) Human cytomegalovirus pp65- and immediate early 1 antigen-specific HLA class I-restricted cytotoxic T cell responses induced by cross-presentation of viral antigens. *J Immunol* 166(9):5695–5703
14. Weekes MP, Wills MR, Mynard K, Carmichael AJ, Sissons JG (1999) 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* 73(3):2099–2108

15. Khan N, Cobbold M, Keenan R, Moss PA (2002) Comparative analysis of CD8+ T cell responses against human cytomegalovirus proteins pp65 and immediate early 1 shows similarities in precursor frequency, oligoclonality, and phenotype. *J Infect Dis* 185(8):1025–1034
16. Khan N, Shariff N, Cobbold M, Bruton R, Ainsworth JA, Sinclair AJ, Nayak L, Moss PA (2002) Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals. *J Immunol* 169(4):1984–1992
17. Wills MR, Carmichael AJ, Weekes MP, Mynard K, Okecha G, Hicks R, Sissons JG (1999) Human virus-specific CD8+ CTL clones revert from CD45ROhigh to CD45RAhigh in vivo: CD45RAhighCD8+ T cells comprise both naive and memory cells. *J Immunol* 162(12):7080–7087
18. Wills MR, Okecha G, Weekes MP, Gandhi MK, Sissons PJ, Carmichael AJ (2002) Identification of naive or antigen-experienced human CD8(+) T cells by expression of costimulation and chemokine receptors: analysis of the human cytomegalovirus-specific CD8(+) T cell response. *J Immunol* 168(11):5455–5464
19. Gillespie GM, Wills MR, Appay V, O'Callaghan C, Murphy M, Smith N, Sissons P, Rowland-Jones S, Bell JI, Moss PA (2000) Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors. *J Virol* 74(17):8140–8150
20. Riddell SR, Watanabe KS, Goodrich JM, Li CR, Agha ME, Greenberg PD (1992) Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science* 257(5067):238–241
21. Peggs KS, Verfuert S, Pizzey A, Khan N, Guiver M, Moss PA, Mackinnon S (2003) Adoptive cellular therapy for early cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines. *Lancet* 362(9393):1375–1377
22. Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, Riddell SR (1995) Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med* 333(16):1038–1044
23. Cwynarski K, Ainsworth J, Cobbold M, Wagner S, Mahendra P, Apperley J, Goldman J, Craddock C, Moss PA (2001) Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. *Blood* 97(5):1232–1240
24. Einsele H, Roosnek E, Rufer N, Sinzger C, Riegler S, Löffler J, Grigoleit U, Moris A, Rammensee HG, Kanz L, Kleihauer A, Frank F, Jahn G, Hebart H (2002) Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood* 99(11):3916–3922
25. Reddehase MJ, Mutter W, Munch K, Buhning HJ, Koszinowski UH (1987) CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. *J Virol* 61(10):3102–3108
26. Podlech J, Holtappels R, Pahl-Seibert MF, Steffens HP, Reddehase MJ (2000) Murine model of interstitial cytomegalovirus pneumonia in syngeneic bone marrow transplantation: persistence of protective pulmonary CD8-T-cell infiltrates after clearance of acute infection. *J Virol* 74(16):7496–7507
27. Polic B, Hengel H, Krmpotic A, Trgovcich J, Pavic I, Luccaroni P, Jonjic S, Koszinowski UH (1998) Hierarchical and redundant lymphocyte subset control precludes cytomegalovirus replication during latent infection. *J Exp Med* 188(6):1047–1054
28. Barnes PD, Grundy JE (1992) Down-regulation of the class I HLA heterodimer and beta 2-microglobulin on the surface of cells infected with cytomegalovirus. *J Gen Virol* 73(Pt 9):2395–2403
29. Wiertz EJ, Tortorella D, Bogoy M, Yu J, Mothes W, Jones TR, Rapoport TA, Ploegh HL (1996) Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction [see comments]. *Nature* 384(6608):432–438
30. Wiertz EJ, Jones TR, Sun L, Bogoy M, Geuze HJ, Ploegh HL (1996) The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell* 84(5):769–779
31. Jones TR, Hanson LK, Sun L, Slater JS, Stenberg RM, Campbell AE (1995) Multiple independent loci within the human cytomegalovirus unique short region down-regulate expression of major histocompatibility complex class I heavy chains. *J Virol* 69(8):4830–4841
32. Jones TR, Wiertz EJ, Sun L, Fish KN, Nelson JA, Ploegh HL (1996) Human cytomegalovirus US3 impairs transport and maturation of major histocompatibility complex class I heavy chains. *Proc Natl Acad Sci USA* 93(21):11327–11333
33. Lehner PJ, Karttunen JT, Wilkinson GW, Cresswell P (1997) The human cytomegalovirus US6 glycoprotein inhibits transporter associated with antigen processing-dependent peptide translocation. *Proc Natl Acad Sci USA* 94(13):6904–6909
34. Ahn K, Gruhler A, Galocha B, Jones TR, Wiertz EJ, Ploegh HL, Peterson PA, Yang Y, Fruh K (1997) The ER-luminal domain of the HCMV glycoprotein US6 inhibits peptide translocation by TAP. *Immunity* 6(5):613–621
35. Gold MC, Munks MW, Wagner M, McMahon CW, Kelly A, Kavanagh DG, Slifka MK, Koszinowski UH, Raulet DH, Hill AB (2004) Murine cytomegalovirus interference with antigen presentation has little effect on the size or the effector memory phenotype of the CD8 T cell response. *J Immunol* 172(11):6944–6953
36. Khan N, Bruton R, Taylor GS, Cobbold M, Jones TR, Rickinson AB, Moss PA (2005) Identification of cytomegalovirus-specific cytotoxic T lymphocytes in vitro is greatly enhanced by the use of recombinant virus lacking the US2 to US11 region or modified vaccinia virus Ankara expressing individual viral genes. *J Virol* 79(5):2869–2879
37. Holtappels R, Podlech J, Pahl-Seibert MF, Julch M, Thomas D, Simon CO, Wagner M, Reddehase MJ (2004) Cytomegalovirus misleads its host by priming of CD8 T cells specific for an epitope not presented in infected tissues. *J Exp Med* 199(1):131–136
38. Kern F, Surel IP, Faulhaber N, Frommel C, Schneider-Mergener J, Schonemann C, Reinke P, Volk HD (1999) Target structures of the CD8(+)-T-cell response to human cytomegalovirus: the 72-kilodalton major immediate-early protein revisited. *J Virol* 73(10):8179–8184
39. Besold K, Frankenberg N, Pepperl-Klindworth S, Kuball J, Theobald M, Hahn G, Plachter B (2007) Processing and MHC class I presentation of human cytomegalovirus pp65-derived peptides persist despite gpUS2–11-mediated immune evasion. *J Gen Virol* 88(Pt 5):1429–1439
40. Manley TJ, Luy L, Jones T, Boeckh M, Mutimer H, Riddell SR (2004) Immune evasion proteins of human cytomegalovirus do not prevent a diverse CD8+ cytotoxic T-cell response in natural infection. *Blood* 104(4):1075–1082
41. Gamadia LE, Remmerswaal EB, Weel JF, Bemelman F, van Lier RA, Ten Berge IJ (2003) Primary immune responses to human CMV: a critical role for IFN-gamma-producing CD4+ T cells in protection against CMV disease. *Blood* 101(7):2686–2692
42. Reddehase MJ, Jonjic S, Weiland F, Mutter W, Koszinowski UH (1988) Adoptive immunotherapy of murine cytomegalovirus adenitis in the immunocompromised host: Cd4-helper-independent antiviral function of Cd8-positive memory lymphocytes-T derived from latently infected donors. *J Virol* 62(3):1061–1065
43. Kern F, Bunde T, Faulhaber N, Kiecker F, Khatamzas E, Rudawski IM, Pruss A, Gratama JW, Volkmer-Engert R, Ewert R, Reinke P, Volk HD, Picker LJ (2002) Cytomegalovirus (CMV) phosphoprotein 65 makes a large contribution to shaping the T

- cell repertoire in CMV-exposed individuals. *J Infect Dis* 185(12):1709–1716
44. Davignon JL, Clement D, Alriquet J, Michelson S, Davrinche C (1995) Analysis of the proliferative T cell response to human cytomegalovirus major immediate-early protein (IE1): phenotype, frequency and variability. *Scand J Immunol* 41(3):247–255
 45. Beninga J, Kropff B, Mach M (1995) Comparative analysis of fourteen individual human cytomegalovirus proteins for helper T cell response. *J Gen Virol* 76(Pt 1):153–160
 46. Davignon JL, Castanie P, Yorke JA, Gautier N, Clement D, Davrinche C (1996) Anti-human cytomegalovirus activity of cytokines produced by CD4+ T-cell clones specifically activated by IE1 peptides in vitro. *J Virol* 70(4):2162–2169
 47. Elkington R, Shoukry NH, Walker S, Crough T, Fazou C, Kaur A, Walker CM, Khanna R (2004) Cross-reactive recognition of human and primate cytomegalovirus sequences by human CD4 cytotoxic T lymphocytes specific for glycoprotein B and H. *Eur J Immunol* 34(11):3216–3226
 48. Weekes MP, Wills MR, Sissons JG, Carmichael AJ (2004) Long-term stable expanded human CD4+ T cell clones specific for human cytomegalovirus are distributed in both CD45RAhigh and CD45ROhigh populations. *J Immunol* 173(9):5843–5851
 49. Bitmansour AD, Douek DC, Maino VC, Picker LJ (2002) Direct ex vivo analysis of human CD4(+) memory T cell activation requirements at the single clonotype level. *J Immunol* 169(3):1207–1218
 50. Rentenaar RJ, Gamadia LE, van DerHoek N, van Diepen FN, Boom R, Weel JF, Wertheim-van Dillen PM, van Lier RA, ten Berge IJ (2000) Development of virus-specific CD4(+) T cells during primary cytomegalovirus infection. *J Clin Invest* 105(4):541–548
 51. van Leeuwen EM, Remmerswaal EB, Vossen MT, Rowshani AT, Wertheim-van Dillen PM, van Lier RA, ten Berge IJ (2004) Emergence of a CD4+CD28– granzyme B+, cytomegalovirus-specific T cell subset after recovery of primary cytomegalovirus infection. *J Immunol* 173(3):1834–1841
 52. Miller DM, Cebulla CM, Rahill BM, Sedmak DD (2001) Cytomegalovirus and transcriptional down-regulation of major histocompatibility complex class II expression. *Semin Immunol* 13(1):11–18
 53. Kuijpers TW, Vossen MT, Gent MR, Davin JC, Roos MT, Wertheim-van Dillen PM, Weel JF, Baars PA, van Lier RA (2003) Frequencies of circulating cytolytic, CD45RA+CD27–, CD8+ T lymphocytes depend on infection with CMV. *J Immunol* 170(8):4342–4348
 54. van Leeuwen EM, Gamadia LE, Baars PA, Remmerswaal EB, ten Berge IJ, van Lier RA (2002) Proliferation requirements of cytomegalovirus-specific, effector-type human CD8+ T cells. *J Immunol* 169(10):5838–5843
 55. van Leeuwen E, van Buul JD, Remmerswaal EBM, Hordijk PL, ten Berge IJ, van Lier RA (2005) Functional re-expression of CCR7 on CMV-specific CD8+ T cells upon antigenic stimulation. *Int Immunol* 17:713–719
 56. Appay V, Dunbar PR, Callan M, Klennerman P, Gillespie GM, Papagno L, Ogg GS, King A, Lechner F, Spina CA, Little S, Havlir DV, Richman DD, Gruener N, Pape G, Waters A, Easterbrook P, Salio M, Cerundolo V, McMichael AJ, Rowland-Jones SL (2002) Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. *Nat Med* 8(4):379–85
 57. Kern F, Khatamzas E, Sural I, Frommel C, Reinke P, Waldrop SL, Picker LJ, Volk HD (1999) Distribution of human CMV-specific memory T cells among the CD8pos. Subsets defined by CD57, CD27, and CD45 isoforms. *Eur J Immunol* 29(9):2908–2915
 58. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, Casazza JP, Kuruppu J, Migueles SA, Connors M, Roederer M, Douek DC, Koup RA (2003) Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* 101(7):2711–2720
 59. Pittet MJ, Speiser DE, Valmori D, Cerottini JC, Romero P (2000) Cutting edge: cytolytic effector function in human circulating CD8+ T cells closely correlates with CD56 surface expression. *J Immunol* 164(3):1148–1152
 60. Speiser DE, Colonna M, Ayyoub M, Cella M, Pittet MJ, Batard P, Valmori D, Guillaume P, Lienard D, Cerottini JC, Romero P (2001) The activatory receptor 2B4 is expressed in vivo by human CD8+ effector alpha beta T cells. *J Immunol* 167(11):6165–6170
 61. Voehringer D, Koschella M, Pircher H (2002) Lack of proliferative capacity of human effector and memory T cells expressing killer cell lectinlike receptor G1 (KLRG1). *Blood* 100(10):3698–3702
 62. Ibegbu CC, Xu YX, Harris W, Maggio D, Miller JD, Kourtis AP (2005) Expression of killer cell lectin-like receptor G1 on antigen-specific human CD8+ T lymphocytes during active, latent, and resolved infection and its relation with CD57. *J Immunol* 174(10):6088–6094
 63. Northfield J, Lucas M, Jones H, Young NT, Klennerman P (2005) Does memory improve with age? CD85j (ILT-2/LIR-1) expression on CD8+ T cells correlates with “memory inflation” in human cytomegalovirus infection. *Immunol Cell Biol* 83:182–188
 64. Antrobus RD, Khan N, Hislop AD, Montamat D-Sicotte, Garner LI, Rickinson AB, Moss PA, Willcox BE (2005) Virus-specific cytotoxic T lymphocytes differentially express cell-surface leukocyte immunoglobulin-like receptor-1, an inhibitory receptor for class I major histocompatibility complex molecules. *J Infect Dis* 191(11):1842–1853
 65. Gandhi MK, Wills MR, Okecha G, Day EK, Hicks R, Marcus RE, Sissons JG, Carmichael AJ (2003) Late diversification in the clonal composition of human cytomegalovirus-specific CD8+ T cells following allogeneic hemopoietic stem cell transplantation. *Blood* 102(9):3427–3438
 66. van Leeuwen EM, Koning JJ, Remmerswaal EB, van Baarle D, van Lier RA, ten Berge IJ (2006) Differential usage of cellular niches by cytomegalovirus versus EBV- and influenza virus-specific CD8+ T cells. *J Immunol* 177(8):4998–5005
 67. Hamann D, Baars PA, Rep MH, Hooibrink B, Kerkhof-Garde SR, Klein MR, van Lier RA (1997) Phenotypic and functional separation of memory and effector human CD8+ T cells. *J Exp Med* 186(9):1407–1418
 68. Chan KS, Kaur A (2007) Flow cytometric detection of degranulation reveals phenotypic heterogeneity of degranulating CMV-specific CD8+ T lymphocytes in rhesus macaques. *J Immunol Methods* 325(1–2):20–34
 69. Vallejo AN (2005) CD28 extinction in human T cells: altered functions and the program of T-cell senescence. *Immunol Rev* 205:158–169
 70. Azuma M, Phillips JH, Lanier LL (1993) CD28– T lymphocytes. Antigenic and functional properties. *J Immunol* 150(4):1147–1159
 71. Posnett DN, Edinger JW, Manavalan JS, Irwin C, Marodon G (1999) Differentiation of human CD8 T cells: implications for in vivo persistence of CD8+ CD28– cytotoxic effector clones. *Int Immunol* 11(2):229–241
 72. Fagnoni FF, Vescovini R, Mazzola M, Bologna G, Nigro E, Lavagetto G, Franceschi C, Passeri M, Sansoni P (1996) Expansion of cytotoxic CD8+ CD28– T cells in healthy ageing people, including centenarians. *Immunology* 88(4):501–507
 73. Effros RB, Boucher N, Porter V, Zhu X, Spaulding C, Walford RL, Kronenberg M, Cohen D, Schachter F (1994) Decline in CD28+ T cells in centenarians and in long-term T cell cultures: a possible cause for both in vivo and in vitro immunosenescence. *Exp Gerontol* 29(6):601–609

74. Effros RB, Allsopp R, Chiu CP, Hausner MA, Hirji K, Wang L, Harley CB, Villeponteau B, West MD, Giorgi JV (1996) Shortened telomeres in the expanded CD28[−]CD8⁺ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. *Aids* 10(8):F17–F22
75. Hazzan M, Labalette M, Noel C, Lelievre G, Dessaint JP (1997) Recall response to cytomegalovirus in allograft recipients: mobilization of CD57⁺, CD28⁺ cells before expansion of CD57⁺, CD28[−] cells within the CD8⁺ T lymphocyte compartment. *Transplantation* 63(5):693–698
76. Weekes MP, Carmichael AJ, Wills MR, Mynard K, Sissons JG (1999) Human CD28[−]CD8⁺ T cells contain greatly expanded functional virus-specific memory CTL clones. *J Immunol* 162(12):7569–7577
77. Day EK, Carmichael AJ, Ten Berge IJ, Waller EC, Sissons JG, Wills MR (2007) Rapid CD8⁺ T Cell repertoire focusing and selection of high-affinity clones into memory following primary infection with a persistent human virus: human cytomegalovirus. *J Immunol* 179(5):3203–3213
78. van Leeuwen EM, de Bree GJ, ten Berge IJ, van Lier RA (2006) Human virus-specific CD8⁺ T cells: diversity specialists. *Immunol Rev* 211:225–235
79. Marchant A, Appay V, Van Der Sande M, Dulphy N, Liesnard C, Kidd M, Kaye S, Ojuola O, Gillespie GM, Vargas Cuero AL, Cerundolo V, Callan M, McAdam KP, Rowland-Jones SL, Donner C, McMichael AJ, Whittle H (2003) Mature CD8(+) T lymphocyte response to viral infection during fetal life. *J Clin Invest* 111(11):1747–1755
80. Miles DJ, vander Sande M, Jeffries D, Kaye S, Ismaili J, Ojuola O, Sanneh M, Touray ES, Waight P, Rowland-Jones S, Whittle H, Marchant A (2007) Cytomegalovirus infection in Gambian infants leads to profound CD8 T-cell differentiation. *J Virol* 81(11):5766–5776
81. van Leeuwen EM, de Bree GJ, Remmerswaal EB, Yong SL, Tesselar K, ten Berge IJ, van Lier RA (2005) IL-7 receptor alpha chain expression distinguishes functional subsets of virus-specific human CD8⁺ T cells. *Blood* 106(6):2091–2098
82. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R (2003) Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat Immunol* 4(12):1191–1198
83. Huster KM, Busch V, Schiemann M, Linkemann K, Kerksiek KM, Wagner H, Busch DH (2004) Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct CD8⁺ memory T cell subsets. *Proc Natl Acad Sci USA* 101(15):5610–5615
84. Weekes MP, Wills MR, Mynard K, Hicks R, Sissons JG, Carmichael AJ (1999) Large clonal expansions of human virus-specific memory cytotoxic T lymphocytes within the CD57⁺ CD28[−]CD8⁺ T-cell population. *Immunology* 98(3):443–449
85. Sourdive DJ, Murali K, Krishna, Altman JD, Zajac AJ, Whitmire JK, Pannetier C, Kourilsky P, Evavold B, Sette A, Ahmed R (1998) Conserved T cell repertoire in primary and memory CD8 T cell responses to an acute viral infection. *J Exp Med* 188:71–79
86. Blattman JN, Sourdive DJ, Murali-Krishna K, Ahmed R, Altman JD (2000) Evolution of the T cell repertoire during primary, memory, and recall responses to viral infection. *J Immunol* 165(11):6081–6090
87. Karrer U, Sierro S, Wagner M, Oxenius A, Hengel H, Koszinowski UH, Phillips RE, Klenerman P (2003) Memory inflation: continuous accumulation of antiviral CD8⁺ T cells over time. *J Immunol* 170(4):2022–2029
88. Pahl-Seibert MF, Juelch M, Podlech J, Thomas D, Deegen P, Reddehase MJ, Holtappels R (2005) Highly protective in vivo function of cytomegalovirus IE1 epitope-specific memory CD8 T cells purified by T-cell receptor-based cell sorting. *J Virol* 79(9):5400–5413
89. Trautmann L, Rimbert M, Echasserieu K, Saulquin X, Neveu B, Dechanet J, Cerundolo V, Bonneville M (2005) Selection of T cell clones expressing high-affinity public TCRs within human cytomegalovirus-specific CD8 T cell responses. *J Immunol* 175(9):6123–6132
90. Lawson TM, Man S, Wang EY, Williams S, Amos N, Gillespie GM, Moss PA, Borysiewicz LK (2001) Functional differences between influenza A-specific cytotoxic T lymphocyte clones expressing dominant and subdominant TCR. *Int Immunol* 13(11):1383–1390
91. Fujii Y, Okumura M, Inada K, Nakahara K (1992) Reversal of CD45R isoform switching in CD8⁺ T cells. *Cell Immunol* 139(1):176–184
92. Michie CA, McLean A, Alcock C, Beverley PC (1992) Lifespan of human lymphocyte subsets defined by CD45 isoforms. *Nature* 360(6401):264–265
93. Sparshott SM, Bell EB (1994) Membrane CD45R isoform exchange on CD4 T cells is rapid, frequent and dynamic in vivo. *Eur J Immunol* 24(11):2573–2578
94. Bell EB, Sparshott SM (1990) Interconversion of CD45R subsets of CD4 T cells in vivo. *Nature* 348(6297):163–166
95. Monteiro M, Evaristo C, Legrand A, Nicoletti A, Rocha B (2007) Cartography of gene expression in CD8 single cells: novel CCR7[−] subsets suggest differentiation independent of CD45RA expression. *Blood* 109(7):2863–2870
96. Geginat G, Lanzavecchia A, Sallusto F (2003) Proliferation and differentiation potential of human CD8⁺ memory T cell subsets in response to antigen or homeostatic cytokines. *Blood* 101:4260–4266
97. Dunne PJ, Belaramani L, Fletcher JM, Fernandez S de Mattos, Lawrenz M, Soares MV, Rustin MH, Lam EW, Salmon M, Akbar AN (2005) Quiescence and functional reprogramming of Epstein-Barr virus (EBV)-specific CD8⁺ T cells during persistent infection. *Blood* 106(2):558–565
98. Carrasco J, Godelaine D, Van Pel A, Boon T, vander Bruggen P (2006) CD45RA on human CD8 T cells is sensitive to the time elapsed since the last antigenic stimulation. *Blood* 108(9):2897–2905
99. Borthwick NJ, Lowdell M, Salmon M, Akbar AN (2000) Loss of CD28 expression on CD8(+) T cells is induced by IL-2 receptor gamma chain signalling cytokines and type I IFN, and increases susceptibility to activation-induced apoptosis. *Int Immunol* 12(7):1005–1013
100. Kim YJ, Brutkiewicz RR, Broxmeyer HE (2002) Role of 4–1BB (CD137) in the functional activation of cord blood CD28(−)CD8(+) T cells. *Blood* 100(9):3253–3260
101. Alves NL, Arosa FA, van Lier RA (2005) IL-21 sustains CD28 expression on IL-15-activated human naive CD8⁺ T cells. *J Immunol* 175(2):755–762
102. Labalette M, Leteurtre E, Thumerelle C, Grutzmacher C, Tourville B, Dessaint JP (1999) Peripheral human CD8(+)CD28(+) T lymphocytes give rise to CD28(−) progeny, but IL-4 prevents loss of CD28 expression. *Int Immunol* 11(8):1327–1336
103. Scheuring UJ, Sabzevari H, Theofilopoulos AN (2002) Proliferative arrest and cell cycle regulation in CD8(+)CD28(−) versus CD8(+)CD28(+) T cells. *Hum Immunol* 63(11):1000–1009
104. Borthwick NJ, Bofill M, Gombert WM, Akbar AN, Medina E, Sagawa K, Lipman MC, Johnson MA, Janossy G (1994) Lymphocyte activation in HIV-1 infection. II. Functional defects of CD28[−] T cells. *AIDS* 8(4):431–441
105. Lewis DE, Yang L, Luo W, Wang X, Rodgers JR (1999) HIV-specific cytotoxic T lymphocyte precursors exist in a

- CD28—CD8+ T cell subset and increase with loss of CD4 T cells. *AIDS* 13(9):1029–1033
106. Brinchmann JE, Dobloug JH, Heger BH, Haaheim LL, Sannes M, Egeland T (1994) Expression of costimulatory molecule CD28 on T cells in human immunodeficiency virus type 1 infection: functional and clinical correlations. *J Infect Dis* 169(4):730–738
 107. Vingerhoets JH, Vanham GL, Kestens LL, Penne GG, Colebunders RL, Vandenbruaene MJ, Goeman J, Gigase PL, De Boer M, Ceuppens JL (1995) Increased cytolytic T lymphocyte activity and decreased B7 responsiveness are associated with CD28 down-regulation on CD8+ T cells from HIV-infected subjects. *Clin Exp Immunol* 100(3):425–433
 108. Fiorentino S, Dalod M, Olive D, Guillet JG, Gomard E (1996) Predominant involvement of CD8+CD28– lymphocytes in human immunodeficiency virus-specific cytotoxic activity. *J Virol* 70(3):2022–2026
 109. Champagne P, Ogg GS, King AS, Knabenhans C, Ellefsen K, Nobile M, Appay V, Rizzardi GP, Fleury S, Lipp M, Forster R, Rowland-Jones S, Sekaly RP, McMichael AJ, Pantaleo G (2001) Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature* 410(6824):106–111
 110. Dunne PJ, Faint JM, Gudgeon NH, Fletcher JM, Plunkett FJ, Soares MV, Hislop AD, Annels NE, Rickinson AB, Salmon M, Akbar AN (2002) Epstein-Barr virus-specific CD8(+) T cells that re-express CD45RA are apoptosis-resistant memory cells that retain replicative potential. *Blood* 100(3):933–940
 111. Waller EC, McKinney N, Hicks R, Carmichael AJ, Sissons JG, Wills MR (2007) Differential costimulation through CD137 (4-1BB) restores proliferation of human virus-specific “effector memory” (CD28 CD45RAHI) CD8+ T cells. *Blood* 110(13):4360–4366
 112. Topp MS, Riddell SR, Akatsuka Y, Jensen MC, Blattman JN, Greenberg PD (2003) Restoration of CD28 expression in CD28—CD8+ memory effector T cells reconstitutes antigen-induced IL-2 production. *J Exp Med* 198(6):947–955
 113. Valenzuela HF, Effros RB (2002) Divergent telomerase and CD28 expression patterns in human CD4 and CD8 T cells following repeated encounters with the same antigenic stimulus. *Clin Immunol* 105(2):117–125
 114. Monteiro J, Batliwalla F, Ostrer H, Gregersen PK (1996) Shortened telomeres in clonally expanded CD28—CD8+ T cells imply a replicative history that is distinct from their CD28+CD8+ counterparts. *J Immunol* 156(10):3587–3590
 115. Weng NP, Levine BL, June CH, Hodes RJ (1995) Human naive and memory T lymphocytes differ in telomeric length and replicative potential. *Proc Natl Acad Sci USA* 92(24):11091–11094
 116. Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, Schulzer M, Lansdorp PM (1999) Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. *J Exp Med* 190(2):157–167
 117. Hamann D, Kostense S, Wolthers KC, Otto SA, Baars PA, Miedema F, van Lier RA (1999) Evidence that human CD8+CD45RA+CD27– cells are induced by antigen and evolve through extensive rounds of division. *Int Immunol* 11(7):1027–1033
 118. Plunkett FJ, Franzese O, Finney HM, Fletcher JM, Belaramani LL, Salmon M, Dokal I, Webster D, Lawson AD, Akbar AN (2007) The loss of telomerase activity in highly differentiated CD8+CD28—CD27– T cells is associated with decreased Akt (Ser473) phosphorylation. *J Immunol* 178(12):7710–7719
 119. Holtappels R, Pahl-Seibert MF, Thomas D, Reddehase MJ (2000) Enrichment of immediate-early 1 (m123/pp89) peptide-specific CD8 T cells in a pulmonary CD62L(lo) memory-effector cell pool during latent murine cytomegalovirus infection of the lungs. *J Virol* 74(24):11495–11503
 120. Holtappels R, Thomas D, Podlech J, Reddehase MJ (2002) Two antigenic peptides from genes m123 and m164 of murine cytomegalovirus quantitatively dominate CD8 T-cell memory in the H-2d haplotype. *J Virol* 76(1):151–164
 121. Karrer U, Sierro S, Wagner M, Oxenius A, Hengel H, Koszinowski UH, Phillips RE, Klennerman P (2003) Memory inflation: continuous accumulation of antiviral CD8+ T cells over time. *J Immunol* 171(7):3895
 122. Munks MW, Cho KS, Pinto AK, Sierro S, Klennerman P, Hill AB (2006) Four distinct patterns of memory CD8 T cell responses to chronic murine cytomegalovirus infection. *J Immunol* 177(1):450–458
 123. Khan N, Hislop A, Gudgeon N, Cobbold M, Khanna R, Nayak L, Rickinson AB, Moss PA (2004) Herpesvirus-specific CD8 T cell immunity in old age: cytomegalovirus impairs the response to a coresident EBV infection. *J Immunol* 173(12):7481–7489
 124. Wallace DL, Zhang Y, Ghattas H, Worth A, Irvine A, Bennett AR, Griffin GE, Beverley PC, Tough DF, Macallan DC (2004) Direct measurement of T cell subset kinetics in vivo in elderly men and women. *J Immunol* 173(3):1787–1794
 125. Ouyang Q, Wagner WM, Zheng W, Wikby A, Remarque EJ, Pawelec G (2004) Dysfunctional CMV-specific CD8(+) T cells accumulate in the elderly. *Exp Gerontol* 39(4):607–613
 126. Ouyang Q, Wagner WM, Wikby A, Walter S, Aubert G, Dodi AI, Travers P, Pawelec G (2003) Large numbers of dysfunctional CD8+ T lymphocytes bearing receptors for a single dominant CMV epitope in the very old. *J Clin Immunol* 23(4):247–257
 127. Akbar AN, Fletcher JM (2005) Memory T cell homeostasis and senescence during aging. *Curr Opin Immunol* 17(5):480–485
 128. Koch S, Solana R, Dela Rosa O, Pawelec G (2006) Human cytomegalovirus infection and T cell immunosenescence: a mini review. *Mech Ageing Dev* 127(6):538–543
 129. Trzonkowski P, Mysliwska J, Szmít E, Wieckiewicz J, Lukaszuk K, Brydak LB, Machala M, Mysliwski A (2003) Association between cytomegalovirus infection, enhanced proinflammatory response and low level of anti-hemagglutinins during the anti-influenza vaccination—an impact of immunosenescence. *Vaccine* 21(25–26):3826–3836
 130. Saurwein-Teissl M, Lung TL, Marx F, Gschosser C, Asch E, Blascko I, Parson W, Bock G, Schonitzer D, Trannoy E, Grubeck-Loebenstien B (2002) Lack of antibody production following immunization in old age: association with CD8(+)/CD28(–) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. *J Immunol* 168(11):5893–5899
 131. Goronzy JJ, Fulbright JW, Crowson CS, Poland GA, O’Fallon WM, Weyand CM (2001) Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. *J Virol* 75(24):12182–12187
 132. Looney RJ, Falsey A, Campbell D, Torres A, Kolassa J, Brower C, McCann R, Menegus M, McCormick K, Frampton M, Hall W, Abraham GN (1999) Role of cytomegalovirus in the T cell changes seen in elderly individuals. *Clin Immunol* 90(2):213–219
 133. Wikby A, Johansson B, Olsson J, Lofgren S, Nilsson BO, Ferguson F (2002) Expansions of peripheral blood CD8 T-lymphocyte subpopulations and an association with cytomegalovirus seropositivity in the elderly: the Swedish Nona immune study. *Exp Gerontol* 37(2–3):445–453
 134. Wikby A, Ferguson F, Forsey R, Thompson J, Strindhall J, Lofgren S, Nilsson BO, Ernerudh J, Pawelec G, Johansson B (2005) An immune risk phenotype, cognitive impairment, and survival in very late life: impact of allostatic load in Swedish octogenarian

- and nonagenarian humans. *J Gerontol A Biol Sci Med Sci* 60(5):556–565
135. Olsson J, Wikby A, Johansson B, Lofgren S, Nilsson BO, Ferguson FG (2000) Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. *Mech Ageing Dev* 121(1–3):187–201
136. Hadrup SR, Strindhall J, Kollgaard T, Seremet T, Johansson B, Pawelec G, Thor Straten P, Wikby A (2006) Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. *J Immunol* 176(4):2645–2653