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REVIEW

Fuel and brake of memory T cell inflation

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Abstract
Memory T cell inflation is a process in which a large number of effector memory T cells accumulates in peripheral tissues. This phenomenon is observed upon certain low level persistent virus infections, but it is most commonly described upon infection with the β-herpesvirus Cytomegalovirus. Due to the induction of this large pool of functional effector CD8 T cells in peripheral tissues, the interest in using CMV-based vaccine vectors for vaccination purposes is rising. However, the exact mechanisms of memory T cell inflation are not yet fully understood. It is clear that repetitive exposure to antigen is a key determinant for memory inflation, and therefore the viral inoculum dose and the subsequent number of viral reactivation events strongly impact on the magnitude of the inflationary T cell pool. In addition, the number of CMV-specific CD8 T cells that is able to sense these reactivation events affects the size of the inflationary T cell pool. In the following, we will discuss factors that either promote or limit T cell inflation from both the virus and host perspective. These factors mostly operate by influencing the amount of available antigen or by affecting the T cell pool that is able to respond to the antigen. Furthermore, we will discuss the recent use of CMV-based vaccines in pre-clinical experimental settings, where these vectors have shown promising results by inducing prolonged effector memory T cell responses to foreign-introduced epitopes and thereby provided protection from subsequent virus or tumour challenges.

Keywords Cytomegalovirus infection · CD8 T cell · Memory inflation

Introduction

Upon exposure to cognate antigen, naïve T cells undergo robust clonal expansion and differentiate into effector cells. Besides this first signal, that is initiated after the T cell receptor recognizes a specific peptide presented in the context of MHC molecules, interactions between costimulatory receptors and ligands (signal 2) and the presence of pro-inflammatory cytokines such as type I IFNs and IL-12 (signal 3) in the environment are critical and influence the outcome of the T cell response. When the antigen has weaned, the majority of clonally expanded T cells are eliminated and a limited number is preserved as memory cells. Acquisition of memory has proven to be beneficial to the host's immune system since an encounter with the same pathogen or similar pathogens sharing common antigens can accelerate their control. Memory T cells are present in higher numbers, in different locations, exhibit faster effector responses and have reduced activation thresholds compared to naïve T cells and therefore respond more rapidly upon re-encounter of cognate antigen. To control a secondary infection, memory T cells can exert immediate effector functions and/or undergo secondary expansion and differentiation into secondary effector cells. Distinct memory subsets have been described based on differences in the expression of surface markers, proliferative capacities, effector functions, migration abilities, their localisation and maintenance requirements. Therefore memory cells can generally be divided into central memory T cells (T_{CM}), effector memory T cells (T_{EM}), tissue-resident memory T cells (T_{RM}) and peripheral memory cells (T_{PM}) [1].

In case of absence or incomplete elimination of the infectious agent from the host, the dynamics of memory formation is strongly altered. Persistent virus infections lead to prolonged exposure to viral antigen. One prominent virus

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leading to a persistent infection with high prevalence in the human population is cytomegalovirus (CMV), a member of the herpesvirus family, which is never completely eradicated from the infected individual after primary infection. Infection with CMV leads first to active viral replication, followed by a state of viral latency, with no or limited viral replication and absent detection of infectious virions. Nevertheless, the virus is able to sporadically reactivate from latency, leading presumably to repetitive low-level viral antigen presentation. This postulated sporadic presence and exposure to antigens impacts the virus-specific immune response, particularly the CD8 T cell response. This is manifested by an atypical accumulation of high numbers and frequencies of virus-specific CD8 T cells in blood and peripheral tissues, collectively termed as 'memory inflation' [2–6]. In contrast to resting T_{CM} cells (KLRG1$^-$, CD127$^+$, CD62L$^+$, CD27$^+$), the majority of inflationary T cells retain an activated T_{EM} phenotype (KLRG1$^+$, CD127$^-$, CD62L$^-$, CD27^{low}) over the individuals' lifetime and exhibit robust effector cell functions. Continuous and repetitive antigenic stimulation of T cells explains their activated phenotype in comparison to non-inflationary CD8 T cells, which do not encounter TCR stimulation during CMV latency. Due to those peculiar T-cell responses, recombinant CMVs have gained considerable interest as vaccine vectors mediating (peripheral) T cell immunity to diverse antigens. Here we will discuss the formation of T cell memory in the context of murine CMV (MCMV) infection. We will emphasize on factors that operate early and late during infection and that either promote or limit memory T cell inflation. In addition, we will discuss the results that have been obtained using CMV-based vaccines and we argue what factors have to be considered for the rational design of CMV-based vaccine vectors.

Factors fueling memory inflation during acute infection: virus perspective

The most critical factor that drives memory inflation is the repetitive exposure to antigen and this is related to the nature of the virus. Recombinant expression of MCMV epitopes in other non-persistent viruses, such as Vaccinia virus, or MCMV epitopes administered as peptide vaccines do not induce inflationary T cell responses [2, 7], and vice versa, when other virally derived or model epitopes are expressed in the CMV genome, inflationary T cell responses can be elicited [8–10]. Yet the induction of memory T cell inflation is dependent on the location and the insert size of the epitope in the CMV genome, with expression in immediate early genes inducing higher accumulation of effector memory T cells in peripheral tissues as compared to expression in the late M45 gene [9, 11]. However, also expression of an epitope within the M45 gene can induce accumulation of effector memory T cell responses and this is related to whether the epitope is expressed on the C- or N-terminus of the M45 gene, with the first allowing for optimal processing by the constitutive proteasome [12]. Furthermore, inflationary T cell responses are less dependent on processing by the immunoproteasome [13], a proteasome configuration that is constitutively expressed in antigen presenting cells. Different epitopes derived from the same viral gene can induce both conventional and inflationary T cell responses [14], further supporting the notion that differences in processing by the proteasome promote conventional and inflationary T cell responses.

Upon infection with a replication-deficient MCMV strain [temperature-sensitive mutant 5 (tsm5), which carries an attenuating mutation within the DNA primase gene], memory inflation was abrogated [15], indicating that some viral replication and/or expression of viral transcripts is required for antigenic stimulation of memory inflation. Whether tsm5 immunization leads to viral latency and reactivation events coupled with viral gene transcription is unclear. Furthermore, the extent of memory inflation relates to the initial infection dose and hence also to the abundance of CMV latent genomes, as infection with a low CMV inoculum dampens memory inflation [16]. Not only the dose but also the route of infection impacts the degree of T cell inflation. The amount of latent viral loads established in the spleen differed between infection routes and the extent of memory inflation correlated to the amount of latent viral genomes in the spleen [17]. Infection of the first target cells of MCMV suffices to drive T cell inflation, as infection with an MCMV mutant that lacks the expression of the glycoprotein L and is therefore unable to spread from cell to cell, induces memory inflation, albeit to a lesser extent than a WT strain [18]. However, this only occurred if the virus was administrated systemically, implying again that the route of infection is impacting memory inflation. Taken from the virus perspective, early factors that fuel memory inflation are the epitope location in the viral genome, the ability to be processed by the constitutive proteasome and the dose and route of infection.

Factors fueling memory inflation during acute infection: host perspective

Although memory inflation occurs after CMV replication is controlled in most peripheral tissues, factors that are operating during the acute phase of infection impact the outcome of the inflationary T cell response. The priming of T cells that pursue inflationary kinetics is mainly restricted to cross-presenting dendritic cells [19–22] and initial T cell expansion is highly dependent on costimulatory signals mediated via CD27 and CD28 [23–25]. Despite the reduction of

MCMV-specific T cells in the acute phase of infection when these costimulatory signals are impaired, the inflationary T cell pool does not seem to be hampered in magnitude in the absence of CD28-signaling, but is hampered when CD27-signaling is abrogated [23, 24]. These differences might be explained by the increased viral load in the absence of CD28-costimulation due to severely diminished MCMV-specific CD4 T cell responses [26, 27], that subsequently enhances inflationary T cell accumulation [24]. Costimulatory signals mediated by OX40 and 4-1BB are important for accumulation of inflationary T cells as well [28, 29]. OX40-mediated signals also impact MCMV-specific CD4 T cell responses. Therefore, the importance of this pathway might be operating indirectly via CD4 T cells as these helper cells also have been implicated to provide critical signals to inflationary T cells [30, 31]. The majority of inflationary T cells are poor producers of IL-2, however, inflation was completely abrogated in mice that lacked CD25 expression on CD8 T cells, the high affinity IL-2 receptor [32], showing that memory inflation heavily relies on IL-2 mediated signals. When exactly IL-2-signals are critical is unknown as well as the source of IL-2. Conceivably, the IL-2 could be provided by CD4 T cells or it could be produced in an autocrine manner, and is therefore restricted to only a small subset of IL-2-producing inflationary T cells [33].

It has been shown that the majority of cells giving rise to the inflationary T cell pool are primed during the acute phase of infection [34]. The T cells that are initially recruited into the pool of inflationary cells are mainly of high avidity (manuscript submitted). We recently observed a higher degree of memory inflation when we experimentally increased the precursor frequency of naïve MCMV-specific CD8 T cells that induce inflationary T cell responses before infection (manuscript submitted) (Fig. 1a). Furthermore, these naïve cells had to be recruited within the first few days of infection, as MCMV-specific cells that were transferred at day 7 post MCMV infection, were barely recruited into the inflationary T cell pool, which might be related to diminished antigen presentation at this time point. Thus, the amount of T cells with specificity for inflationary epitopes that are recruited early into the response during priming is indicative for the level of memory inflation. Taken together, these findings highlight that signals operating early during acute MCMV infection impact the outcome of memory inflation.

Factors fueling memory inflation in the latent phase of infection: virus perspective

One key feature that drives memory inflation is the sporadic reactivation of latent virus that gives rise to low levels of viral transcription. After resolution of acute MCMV infection in most organs, active viral replication is only found in the salivary glands. CD4 T cells are critical to control MCMV replication in the salivary gland [35, 36]. However, removal of the salivary glands does not impair memory inflation [37], it is rather the tissues where the virus establishes latency that are important drivers for this process. This conclusion comes from a series of observations; when antigen presentation is abrogated in non-hematopoietic cells, memory T cell inflation is completely abolished and in this situation T cells that normally follow an inflationary kinetics undergo a classical expansion/contraction type of response and do not display an activated phenotype [38]. Moreover, the majority of latent viral genomes are found in non-hematopoietic cells [39, 40] and these cells also rely on the constitutive proteasome for the generation of peptides that can be presented in MHC class I molecules. Additionally, no memory inflation is observed when antigen presentation is only restricted to professional APCs [20]. The identity and location of these non-hematopoietic cells that present viral antigen during latency remains to be revealed. CMV-specific T cells with specificity for inflationary epitopes divide at a higher rate in the lymph node than anywhere else, suggesting that antigen recognition could occur at this site [38, 41]. However, it has also been proposed that viral reactivation events, likely within vascular endothelial cells, are sensed by CMV-specific CD8 T cells in the circulation [41].

A high CMV infection dose also results in more latent viral reservoirs and viral reactivation events [16, 42]. As memory inflation is driven by presence of antigen, the number of viral reactivation events most likely contributes to the degree of memory inflation (Fig. 1a). This is difficult to examine, since these reactivation events are rather sporadic and do not necessarily lead to production of infectious virions, making detection challenging. Hosts have to be severely immunocompromised before full viral reactivation occurs [43]. If full CMV reactivation occurs in immune competent hosts, it is likely rapidly controlled by the presence of CMV-specific antibodies, and the high number of CMV-specific inflationary effector-like T cells. This is also explained by the immune sensing hypothesis; as soon as viral reactivation takes place, it is terminated by the inflationary T cells [44]. It is interesting to note that upon partial depletion of a specific inflationary T cell population by a saporin-conjugated tetramer-based

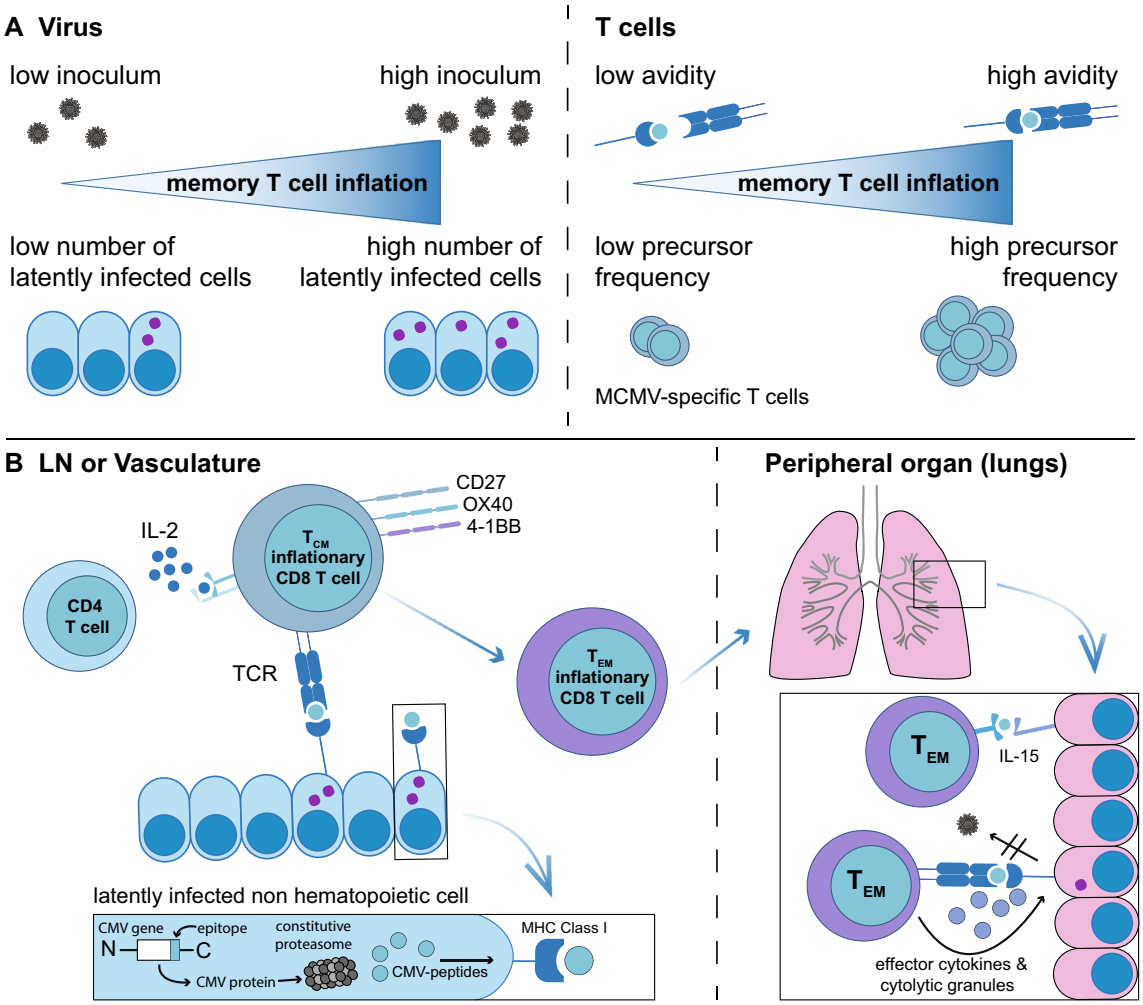

Fig. 1 Factors involved in memory T cell inflation. **a** The initial viral inoculum dose and the number of latently infected cells impact the degree of memory inflation. From the host perspective, a higher precursor frequency of MCMV-specific T cells and T cells with a high avidity contribute to a higher degree of memory inflation. **b** The underlying mechanism of memory T cell inflation. In latently infected non-hematopoietic cells, viral proteins are processed by the constitutive proteasome, and peptides that are efficiently processed by the constitutive proteasome are more abundantly presented by non-hematopoietic cells undergoing viral reactivation events. In response to this antigen encounter, proliferation-competent T_{CM} cells with inflationary specificity recognize viral peptides presented on latently infected, virus reactivating non-hematopoietic cells, either in the lymph node or in niches close to the vasculature. This leads to the proliferation of the T_{CM} cells and the differentiation into effector memory cells that will move to peripheral tissues where they are able to control viral reactivation events by exerting their effector functions. Furthermore, in the periphery, inflationary T cells sense IL-15-mediated signals that enhance their survival. Other factors that are critical for memory inflation are CD4 T cell help, IL-2 signals, and costimulatory signals mediated via CD27, OX40 and 4-1BB

method, in which saporin is internalized in the CMV-specific cells and subsequently leads to the inactivation of a ribonuclease protein that then induces cell death, this population bounced back to even higher levels than before depletion [45], implying that there is a tight equilibrium between viral reactivation and the immune surveillance by inflationary T cells. Moreover, more viral transcripts were found when MCMV lacked an immunodominant epitope driving memory CD8 T cell inflation [46], showing that inflationary T cells have a protective function by keeping the number of viral reactivation events in check.

Factors fueling memory inflation in the latent phase of infection: host perspective

The inflationary pool of CMV-specific CD8 T cells is remarkably stable in numbers over time in peripheral tissues [47], yet the half-life of individual inflationary T cells is estimated to be around 8–10 weeks in the periphery and 6–8 weeks in the circulation [34, 47]. The half-life of inflationary T cells is comparable in naïve and latently MCMV infected hosts, suggesting that their maintenance and decay

occurs in an antigen-independent manner [34, 47]. To maintain the inflationary pool at a constant level, there has to be a continuous resupply of cells that fuel the pool of peripheral inflationary cells. Previous studies have indicated that naïve T cells can be recruited to the inflationary T cell pool in latent MCMV infection [34]. However this contribution is rather limited, as thymectomized mice do not show impaired memory T cell inflation [37]. Furthermore, memory inflation is also observed for adoptively transferred mature CD8 T cells with specificity for inflationary epitopes, precluding incorporation of new naïve CD8 T cells into this response [38]. Although the majority of the inflationary T cell pool exhibits an effector memory T cell phenotype, a small subset of CD8 cells with specificity for inflationary epitopes that is highly enriched in the lymph nodes has more characteristics of central memory T cells in both humans and mice [38, 48]. Consistent with previous studies [49], adoptive transfer experiments of this T_{CM} subset showed that these cells have a markedly increased proliferation potential as compared to the T_{EM} subset [38, 50]. Thus, it is likely that this population gives rise to a progeny of effector cells that relocates to peripheral tissues to maintain the inflationary T cell pool (Fig. 1b). Supporting this notion, we recently demonstrated that the number of inflationary cells with a T_{CM} phenotype relates to the degree of memory inflation (manuscript submitted). As these T_{CM} cells are mostly enriched in the lymph node, it is conceivable that viral reactivation events are also sensed at this site. There are studies that contradict this hypothesis, since interfering with lymph node egress did not alter the maintenance of the inflationary T cell pool in the periphery [41]. In these experiments, however, lymph node egress was only blocked for 5 weeks, and as the half-life for inflationary T cells in the periphery is estimated to be around 8–10 weeks, longer blockade of lymph node exit might be required to see an effect on the peripheral inflationary T cell population. Recently, it was also postulated that an intermediate population of cells exists in the linear differentiation path from a T_{CM} cell to effector cell that could play a role in memory T cell inflation. These cells express CX3CR1 (also termed peripheral memory CD8 T cells, T_{PM}) and retain a proliferative and self-renewal capacity [51–53]. Thus, the question still remains, where and how exactly viral reactivation events are being sensed that lead to reactivation of T_{CM} or potentially T_{PM} cells with specificities for inflationary epitopes and consequently to their proliferation and differentiation into effector-like cells that home to peripheral organs. CD70-mediated signals have been implicated to also play a role in the maintenance of the inflationary T cell pool in the chronic phase of infection [23]. However, as the inflationary T cell pool is characterized by a low cell surface expression of CD27 [10, 23, 34], these signals likely act on the small subset of T_{CM} cells that retain the expression of this molecule. The importance of IL-2 signaling on fueling the inflationary T cell pool might also be restricted to this T_{CM} population, as IL-2 production is restricted to this small subset of cells. Recently it was shown that the IL-2 producing cells within an antigen-specific population also have a superior expansion potential, and specifically autocrine IL-2 production is critical for the secondary expansion [33, 54]. Accordingly, it seems that IL-2 signaling is important during the restimulation process itself and not for maintenance of inflationary CD8 T cells in peripheral tissues, as IL-2 neutralization did not affect the maintenance of the peripheral pool of inflationary cells. Instead IL-15 signaling proved to be critical for the survival of inflationary CD8 T cells in peripheral tissues such as the lung [47].

Although the size of the inflationary T cell pool is quite stable over time, its composition is highly dynamic [34]. Competition and antigen availability on the level of the APC are processes that favor the selection of one clone over the other [9, 55, 56]. In elderly humans a contribution of low-avidity T cells to the inflationary T cell pool has been observed [57]. This is in contrast to what we saw in our MCMV experiments (manuscript submitted), where we adoptively transferred either low- or high-avidity TCR transgenic T cells into mice before MCMV infection and found that only high-avidity T cells were recruited into the inflationary response. One has to keep in mind that the time resolution between humans and mice is completely different, and the inflationary T cell response in humans can last for decades. Moreover, in our experimental setup we adoptively transferred TCR transgenic T cells, thereby also the precursor frequency will be increased and the subsequent pool of T_{CM} cells with inflationary specificity will be higher. A longer observation period might be needed before the pool of high-avidity T_{CM} cells is emptied and low-avidity MCMV-specific T_{CM} cells start to be recruited. Transferring lower numbers of TCR transgenic T cells or longitudinal avidity analysis of an endogenous CMV-specific inflationary response might be options to address this issue.

The brake of memory T cell inflation

Our immune system is equipped with several mechanisms that can dampen immune responses to prevent immune-mediated pathology. These mechanisms include the activation and induction of regulatory T cells, the tight regulation of co-inhibitory receptor expression and the production of anti-inflammatory cytokines such as IL-10. As CMV induces a low-level persistent infection, it is likely that (some) of these factors are operating along the course of infection. It was shown recently that depletion of regulatory T cells enhanced inflationary T cell responses during latent MCMV infection [58]. Furthermore, IL-10 restricts the accumulation of MCMV-specific CD8 T cells [59].

Recently, we also investigated factors that limit memory inflation. We speculated that inflationary T cells might compete for survival niches in the periphery, such as for instance provision of IL-15 in the lungs [47]. Using an approach where we transferred male MCMV-specific $M38_{316–323}$ TCR transgenic CD8 T cells (Maxi cells) [38] into female recipients, we were able to generate a population of M38-specific CD8 T cells that consisted of 50% of transferred Maxi cells and of 50% of endogenous M38-specific CD8 T cells. After 10–14 days the male Maxi cells were rejected in the female hosts, thereby depleting 50% of the M38-specific CD8 T cell population and potentially creating new space for endogenous MCMV-specific T cells to catch up. However, we observed that this vacant niche was not refilled, indicating that "space" in peripheral tissues is not a limiting factor for memory T cell inflation (manuscript submitted). These results would be more consistent with a dominant role of the number/frequency of CD8 T cells with specificity for inflationary epitopes that are able to respond to TCR stimulation by proliferation, differentiation into effector-like cells and migration into peripheral tissues—most likely T_{CM} cells which were also depleted by 50% using the experimental condition described above.

If we think of other factors that limit memory inflation, these factors are in fact similar to the factors that fuel inflation. The amount of viral reactivation events is one dominant parameter, as antigen presentation is required to drive inflation. A limiting factor from the host side would be the supply of T_{CM} cells able to sense these viral reactivation events. Whether these cells would directly differentiate into effector progeny or via an intermediate $CX3CR1^+$ cell subset remains to be explored. The number of T_{CM} cells that establishes after resolution of acute CMV infection is related to the precursor frequency, the number of T cells that are recruited into the response and the timing at which the CD8 T cells are activated with respect to the onset of infection (manuscript submitted). Recently, we performed experiments where we adoptively transferred naïve TCR transgenic MCMV-specific T cells at different time points relative to MCMV infection, thereby changing the ratio of cells that adopt an effector or memory precursor phenotype. Although there was a marked difference in the peak clonal expansion of these cells, with late-transferred cells (relative to the infection) exhibiting much lower peak expansion than early-transferred cells, the level of memory inflation was similar in these mice and this degree of memory inflation was correlated to the amount of $KLRG1^-$ cells that were found in the acute phase of infection (manuscript submitted). This suggests that by modulating the number of cells that are recruited into the response in the acute phase of infection, and in particular the cells that adapt a $KLRG1^-$ phenotype, the level of memory inflation can be modulated. As cytokines such as IL-12 and type I IFNs drive effector cell formation [60–62], modulating the level of these pro-inflammatory cytokines in the environment in the acute phase of infection might be a way to change the number of $KLRG1^+$ and $KLRG1^-$ cells and thereby impact the degree of memory inflation.

CMV-based vaccine vectors

Due to the induction of a large pool of effector memory T cells in peripheral tissues, CMV-based vaccines have gained considerable interest for T cell-based vaccination purposes. The activation of these cells upon antigen re-encounter is fast, as these cells are already at the location where pathogens enter the body and therefore these cells can immediately exert their effector functions and rapidly control the infection. The reactivation of T_{CM} cells is more time-consuming since antigen first has to be transported to secondary lymphoid tissues. Moreover, upon activation, the T cells have to expand, differentiate and migrate to the site of infection. Inflationary T cells are in close contact with the circulation [41, 47, 63, 64], and therefore have the ability to continuously seed peripheral tissues. The protective capacity of inflationary T cells is directly correlated with the total number of effector memory T cells that are present in the non-lymphoid tissue (manuscript submitted) [8, 65]. Tissue-resident memory T cells (T_{RM}) are another subset of memory cells that are situated in peripheral tissues [66]; however, these cells are disconnected from the circulation. In the lung and salivary gland tissues, for instance, T_{RM} cells are confined to epithelial structures [67], whereas inflationary cells are in close proximity to endothelial cells [41, 47]. It will be interesting to examine if this difference in localization has an impact on the relative protective capacity of these memory T cell subsets [68].

CMV-based vectors have been used successfully in experimental settings. Recombinant MCMVs expressing viral epitopes from lymphocytic choriomeningitis virus (LCMV) or influenza A virus were used as vaccine vectors to induce inflationary responses to those foreign viral antigens in mice [8]. The inflationary CD8 T cells towards these epitopes were able to protect the hosts from peripheral infection with recombinant vaccinia viruses encoding similar epitopes and from systemic LCMV challenge [8]. More recently, a recombinant MCMV virus expressing a CD8 T cell epitope from the nucleoprotein from Zaire ebolavirus (ZEBOV) mediated protection against lethal ebolavirus (EBOV) infection [69, 70]. Also MCMV encoding RSV-epitopes provided protection from a subsequent RSV challenge; however, this protection was due to the induction of T_{RM} cells in the lung mucosa after intranasal administration of the vaccine vector [71, 72]. It has been shown that influenza-induced T_{RM} cells in the lung tissue wane over time [73, 74] and this is partly because

effector memory T cells lose the ability to home to the lungs over time [74]. This decay in T_{RM} cells leads for instance to the loss of heterosubtypic immunity to Influenza viruses. However, intranasal administration with a CMV-based vaccine has been shown to generate a population of T_{RM} cells that does not decline in time, and the maintenance of this T_{RM} population was dependent on memory inflation [71, 72]. The exact mechanism how inflationary T cells give rise to T_{RM} cells remains to be determined, but this is likely due to continuous production of T_{RM} cells and therefore similar as to what has been described for CMV-specific T_{RM} cells in the salivary glands [63, 64].

CMV-based vectors were also used as vaccines in primates. Recombinant rhesus CMV (RhCMV) expressing simian immunodeficiency virus (SIV)-derived Gag, Rev-Tat-Nef and Env epitopes (RhCMV-SIV) induced specific CD4 and CD8 T cell responses independently of previous RhCMV infection. These SIV-specific T cells displayed an effector memory phenotype and appeared to be long-lived, resembling inflationary T cell responses described in mice and humans [75]. Half of the vaccinated animals were able to control SIV infection and were protected for more than 1 year. This control of SIV correlated strongly with a robust SIV-specific CD8 T cell response. In contrast, macaques vaccinated with DNA/Ad5 were not protected from pathogenic SIV infection and developed progressive infection, despite lower viral plasma loads compared to unvaccinated macaques [76]. Subsequent studies have illustrated that regardless of the route of SIV challenge, RhCMV-SIV-vaccinated macaques were able to control highly pathogenic SIV infection [77]. Surprisingly, a large fraction of vaccine-induced SIV-specific CD8 T cells was restricted by antigen presentation by MHC-II or non-classical MHC-E molecules, violating the classical T cell recognition paradigm [78].

CMV vectors were also tested in the context of anti-tumour immunity. In a murine model of prostate cancer, vaccination with MCMV/PSA_{65-73} vector led to effective anti-tumour CD8 T-cell responses with delayed growth of PSA-expressing tumours [79]. This was also observed in a melanoma setting where CMV-based vaccines prolonged the survival of tumour-bearing mice in a therapeutic immunization setting, as well as in a prophylactic vaccination setting due to the induction of tumour-specific CD8 T cell responses [80]. Moreover, intra-tumoral injection of the CMV-vector improved therapeutic efficacy by delaying tumour growth and improving overall survival [81]. Recently, it was shown that CMV-based vectors encoding an HPV16 E7 epitope also protected mice in a prophylactic manner from a tumour expressing the E7 protein. Strikingly, the T cell response had to reach a defined threshold before protection was observed [11]. Taken together, there is a growing body of evidence that CMV-based T cell vaccines are of enormous potential to provide protection against infectious agents and tumours.

Not only do CMV-specific CD8 T cells increase in frequency over time, but also CMV-specific antibodies are accumulating in the serum of infected hosts [26]. Interestingly, CMV-based vectors encoding foreign antigens can also induce long-lasting antibody responses, as is shown for an MCMV-vector encoding tetanus toxin fragments [82]. Furthermore, a recombinant RhCMV vector expressing Ebola virus glycoprotein was able to provide protective immunity in 80% of rhesus macaques from a lethal EBOV infection. Vaccination was associated with high levels of GP-specific antibodies, but with no detectable GP-directed cellular immunity [83]. Moreover, CMV vectors encoding the melanoma antigen TRP-2, provided protection from a melanoma tumour in an antibody-dependent manner [84, 85]. Thus, CMV-based vectors are not only promising to induce a large pool of peripheral CD8 T cells, but they can also induce strong ongoing humoral responses.

Concluding remarks

The success of CMV-based vaccines is due to the large accumulation of functional effector memory T cells in peripheral tissues, therefore understanding the mechanism of memory inflation is pivotal to optimize the efficacy of these vaccines. When designing CMV-based vaccines the location of the epitope in the viral genome, the ability to be processed by the constitutive proteasome and the avidity of the epitope are important factors to consider [9, 12, 65]. Furthermore, the dose and the route of administration impact on memory inflation, and this should therefore be optimized for vaccine delivery [16, 17]. We have shown that the number of T cells that are recruited into the response sets the limit for memory inflation, and that the number of T_{CM} cells fuel the inflationary T cell population. Modulation of these subsets might be another way to enhance CMV-based vaccines.

CMV can super-infect individuals, despite pre-existing immunity. This is important for the potential use of CMV-based vaccines, as the majority of the world's population is CMV-positive. Experimental studies have shown that also antigen-specific CD8 T cells to the newly encoded antigens in the vector can be elicited upon a superinfection [80], and new inflationary responses can develop despite pre-existing ones [86], although, the strength of the T cell response elicited by the vaccine depends on the magnitude of the pre-existing T cell response [11]. CMV infection, however, can be detrimental in certain risk groups, such as immunocompromised individuals and pregnant women who are CMV-negative. Furthermore, CMV infection has been associated with immune senescence, although these studies are rather contradicting [87] and might depend on the initial viral inoculum dose [88]. Nevertheless, HCMV vaccine vectors should be attenuated before they can be safely used in

humans. In a recent study it was shown that administration of replication-incompetent adenoviruses in mice led to the accumulation CD8 T cells exhibiting a transcriptional profile reminiscent of CMV-specific inflationary CD8 T cells [89, 90]. The use of these adenovirus vectors provides a safer alternative as compared to replication-competent CMV vectors, but it remains to be tested if these vectors also elicit inflationary responses in humans. Also some differences between inflationary T cell responses induced by these vectors or elicited by CMV have been described, for instance IL-21 is required for memory inflation induced by adenoviral vectors but not for memory inflation induced by CMV [91]. Thus, it remains to be investigated if the mechanistic understanding of memory inflation we have now, can be directly translated to the adenoviral vectors. Another alternative is the use of spread-deficient CMV-vectors, as these viruses are attenuated but still induce memory T cell inflation [18]. Moreover, memory inflation by these vectors could be enhanced by exogenous administration of recombinant IL-33 [92]. Additional attenuation of the virus might be achieved by deleting immune-evasion genes from the viral genome or by recombinant expression of NKG2D ligands [93, 94]. As most of this detailed knowledge is based on experimental animal studies, future studies are necessary to test if this is applicable for HCMV-based vaccine vectors as well.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Martin MD, Badovinac VP (2018) Defining memory CD8 T cell. Front Immunol 9:2692
2. Karrer U, Sierro S, Wagner M et al (2003) Memory inflation: continuous accumulation of antiviral CD8 + T cells over time. J Immunol 170(7):2022–2029 **(*This article contains an errata: Karrer U, Sierro S, Wagner M et al (2003) Memory inflation: continuous accumulation of antiviral CD8 + T cells over time. J Immunol 171(7) 3895)**
3. Klenerman P, Oxenius A (2016) T cell responses to cytomegalovirus. Nat Rev Immunol 16:367–377
4. O'hara GA, Welten SP, Klenerman P et al (2012) Memory T cell inflation: understanding cause and effect. Trends Immunol 33:84–90
5. Holtappels R, Pahl-Seibert MF, Thomas D et al (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 74:11495–11503
6. Holtappels R, Thomas D, Podlech J et al (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 76:151–164
7. Panagioti E, Redeker A, Van Duikeren S et al (2016) The breadth of synthetic long peptide vaccine-induced CD8 + T cell responses determines the efficacy against mouse cytomegalovirus infection. PLoS Pathog 12:e1005895
8. Karrer U, Wagner M, Sierro S et al (2004) Expansion of protective CD8 + T-cell responses driven by recombinant cytomegaloviruses. J Virol 78:2255–2264
9. Dekhtiarenko I, Jarvis MA, Ruzsics Z et al (2013) The context of gene expression defines the immunodominance hierarchy of cytomegalovirus antigens. J Immunol 190:3399–3409
10. Sierro S, Rothkopf R, Klenerman P (2005) Evolution of diverse antiviral CD8 + T cell populations after murine cytomegalovirus infection. Eur J Immunol 35:1113–1123
11. Beyranvand Nejad E, Ratts RB, Panagioti E et al (2019) Demarcated thresholds of tumor-specific CD8 T cells elicited by MCMV-based vaccine vectors provide robust correlates of protection. J Immunother Cancer 7:25
12. Dekhtiarenko I, Ratts RB, Blatnik R et al (2016) Peptide processing is critical for T-cell memory inflation and may be optimized to improve immune protection by CMV-based vaccine vectors. PLoS Pathog 12:e1006072
13. Hutchinson S, Sims S, O'hara G et al (2011) A dominant role for the immunoproteasome in CD8 + T cell responses to murine cytomegalovirus. PLoS One 6:e14646
14. Munks MW, Cho KS, Pinto AK et al (2006) Four distinct patterns of memory CD8 T cell responses to chronic murine cytomegalovirus infection. J Immunol 177:450–458
15. Beswick M, Pachnio A, Al-Ali A et al (2013) An attenuated temperature-sensitive strain of cytomegalovirus (tsm5) establishes immunity without development of CD8(+) T cell memory inflation. J Med Virol 85:1968–1974
16. Redeker A, Welten SP, Arens R (2014) Viral inoculum dose impacts memory T-cell inflation. Eur J Immunol 44:1046–1057
17. Oduro JD, Redeker A, Lemmermann NA et al (2016) Murine cytomegalovirus (CMV) infection via the intranasal route offers a robust model of immunity upon mucosal CMV infection. J Gen Virol 97:185–195
18. Snyder CM, Cho KS, Bonnett EL et al (2011) Sustained CD8 + T cell memory inflation after infection with a single-cycle cytomegalovirus. PLoS Pathog 7:e1002295
19. Torti N, Walton SM, Murphy KM et al (2011) Batf3 transcription factor-dependent DC subsets in murine CMV infection: differential impact on T-cell priming and memory inflation. Eur J Immunol 41:2612–2618
20. Seckert CK, Schader SI, Ebert S et al (2011) Antigen-presenting cells of haematopoietic origin prime cytomegalovirus-specific CD8 T-cells but are not sufficient for driving memory inflation during viral latency. J Gen Virol 92:1994–2005
21. Busche A, Jirmo AC, Welten SP et al (2013) Priming of CD8+ T cells against cytomegalovirus-encoded antigens is dominated by cross-presentation. J Immunol 190:2767–2777
22. Snyder CM, Allan JE, Bonnett EL et al (2010) Cross-presentation of a spread-defective MCMV is sufficient to prime the majority of virus-specific CD8+ T cells. PLoS One 5:e9681
23. Welten SP, Redeker A, Franken KL et al (2013) CD27-CD70 costimulation controls T cell immunity during acute and persistent cytomegalovirus infection. J Virol 87:6851–6865
24. Arens R, Loewendorf A, Redeker A et al (2011) Differential B7-CD28 costimulatory requirements for stable and inflationary mouse cytomegalovirus-specific memory CD8 T cell populations. J Immunol 186:3874–3881

25. Welten SP, Redeker A, Franken KL et al (2015) The viral context instructs the redundancy of costimulatory pathways in driving CD8(+) T cell expansion. Elife 4:e07486
26. Welten SPM, Redeker A, Toes REM et al (2016) Viral persistence induces antibody inflation without altering antibody avidity. J Virol 90:4402–4411
27. Arens R, Loewendorf A, Her MJ et al (2011) B7-mediated costimulation of CD4 T cells constrains cytomegalovirus persistence. J Virol 85:390–396
28. Humphreys IR, Loewendorf A, De Trez C et al (2007) OX40 costimulation promotes persistence of cytomegalovirus-specific CD8 T Cells: A CD4-dependent mechanism. J Immunol 179:2195–2202
29. Humphreys IR, Lee SW, Jones M et al (2010) Biphasic role of 4-1BB in the regulation of mouse cytomegalovirus-specific CD8(+) T cells. Eur J Immunol 40:2762–2768
30. Snyder CM, Loewendorf A, Bonnett EL et al (2009) CD4+ T cell help has an epitope-dependent impact on CD8+ T cell memory inflation during murine cytomegalovirus infection. J Immunol 183:3932–3941
31. Walton SM, Torti N, Mandaric S et al (2011) T-cell help permits memory CD8(+) T-cell inflation during cytomegalovirus latency. Eur J Immunol 41:2248–2259
32. Bachmann MF, Wolint P, Walton S et al (2007) Differential role of IL-2R signaling for CD8+ T cell responses in acute and chronic viral infections. Eur J Immunol 37:1502–1512
33. Redeker A, Welten SP, Baert MR et al (2015) The quantity of autocrine IL-2 governs the expansion potential of CD8+ T Cells. J Immunol 195:4792–4801
34. Snyder CM, Cho KS, Bonnett EL et al (2008) Memory inflation during chronic viral infection is maintained by continuous production of short-lived, functional T cells. Immunity 29:650–659
35. Jonjic S, Mutter W, Weiland F et al (1989) Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes. J Exp Med 169:1199–1212
36. Walton SM, Mandaric S, Torti N et al (2011) Absence of cross-presenting cells in the salivary gland and viral immune evasion confine cytomegalovirus immune control to effector CD4 T cells. PLoS Pathog 7:e1002214
37. Loewendorf AI, Arens R, Purton JF et al (2011) Dissecting the requirements for maintenance of the CMV-specific memory T-cell pool. Viral Immunol 24:351–355
38. Torti N, Walton SM, Brocker T et al (2011) Non-hematopoietic cells in lymph nodes drive memory CD8 T cell inflation during murine cytomegalovirus infection. PLoS Pathog 7:e1002313
39. Mercer JA, Wiley CA, Spector DH (1988) Pathogenesis of murine cytomegalovirus infection: identification of infected cells in the spleen during acute and latent infections. J Virol 62:987–997
40. Seckert CK, Renzaho A, Tervo HM et al (2009) Liver sinusoidal endothelial cells are a site of murine cytomegalovirus latency and reactivation. J Virol 83:8869–8884
41. Smith CJ, Turula H, Snyder CM (2014) Systemic hematogenous maintenance of memory inflation by MCMV infection. PLoS Pathog 10:e1004233
42. Reddehase MJ, Balthesen M, Rapp M et al (1994) The conditions of primary infection define the load of latent viral genome in organs and the risk of recurrent cytomegalovirus disease. J Exp Med 179:185–193
43. Polic B, Hengel H, Krmpotic A et al (1998) Hierarchical and redundant lymphocyte subset control precludes cytomegalovirus replication during latent infection. J Exp Med 188:1047–1054
44. Reddehase MJ, Simon CO, Seckert CK et al (2008) Murine model of cytomegalovirus latency and reactivation. Curr Top Microbiol 325:315–331
45. Sims S, Bolinger B, Klenerman P (2015) Increasing inflationary T-cell responses following transient depletion of MCMV-specific memory T cells. Eur J Immunol 45:113–118
46. Simon CO, Holtappels R, Tervo HM et al (2006) CD8 T cells control cytomegalovirus latency by epitope-specific sensing of transcriptional reactivation. J Virol 80:10436–10456
47. Baumann NS, Torti N, Welten SPM et al (2018) Tissue maintenance of CMV-specific inflationary memory T cells by IL-15. PLoS Pathog 14:e1006993
48. Remmerswaal EB, Havenith SH, Idu MM et al (2012) Human virus-specific effector-type T cells accumulate in blood but not in lymph nodes. Blood 119:1702–1712
49. Bachmann MF, Wolint P, Schwarz K et al (2005) Functional properties and lineage relationship of CD8+ T cell subsets identified by expression of IL-7 receptor alpha and CD62L. J Immunol 175:4686–4696
50. Quinn M, Turula H, Tandon M et al (2015) Memory T cells specific for murine cytomegalovirus re-emerge after multiple challenges and recapitulate immunity in various adoptive transfer scenarios. J Immunol 194:1726–1736
51. Gerlach C, Moseman EA, Loughhead SM et al (2016) The chemokine receptor CX3CR1 defines three antigen-experienced CD8 T cell subsets with distinct roles in immune surveillance and homeostasis. Immunity 45:1270–1284
52. Gordon CL, Lee LN, Swadling L et al (2018) Induction and maintenance of CX3CR1-intermediate peripheral memory CD8(+) T cells by persistent viruses and vaccines. Cell Rep 23:768–782
53. Klenerman P (2018) The (gradual) rise of memory inflation. Immunol Rev 283:99–112
54. Feau S, Arens R, Togher S et al (2011) Autocrine IL-2 is required for secondary population expansion of CD8(+) memory T cells. Nat Immunol 12:908–913
55. Farrington LA, Smith TA, Grey F et al (2013) Competition for antigen at the level of the APC is a major determinant of immunodominance during memory inflation in murine cytomegalovirus infection. J Immunol 190:3410–3416
56. Turula H, Smith CJ, Grey F et al (2013) Competition between T cells maintains clonal dominance during memory inflation induced by MCMV. Eur J Immunol 43:1252–1263
57. Griffiths SJ, Riddell NE, Masters J et al (2013) Age-associated increase of low-avidity cytomegalovirus-specific CD8+ T cells that re-express CD45RA. J Immunol 190:5363–5372
58. Almanan M, Raynor J, Sholl A et al (2017) Tissue-specific control of latent CMV reactivation by regulatory T cells. PLoS Pathog 13:e1006507
59. Jones M, Ladell K, Wynn KK et al (2010) IL-10 restricts memory T cell inflation during cytomegalovirus infection. J Immunol 185:3583–3592
60. Wiesel M, Crouse J, Bedenikovic G et al (2012) Type-I IFN drives the differentiation of short-lived effector CD8+ T cells in vivo. Eur J Immunol 42:320–329
61. Joshi NS, Cui W, Chandele A et al (2007) Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. Immunity 27:281–295
62. Cui W, Joshi NS, Jiang A et al (2009) Effects of Signal 3 during CD8 T cell priming: bystander production of IL-12 enhances effector T cell expansion but promotes terminal differentiation. Vaccine 27:2177–2187
63. Thom JT, Weber TC, Walton SM et al (2015) The salivary gland acts as a sink for tissue-resident memory CD8(+) T cells, facilitating protection from local cytomegalovirus infection. Cell Rep 13:1125–1136
64. Smith CJ, Caldeira-Dantas S, Turula H et al (2015) Murine CMV infection induces the continuous production of mucosal resident T cells. Cell Rep 13:1137–1148

65. Borkner L, Sitnik KM, Dekhtiarenko I et al (2017) Immune protection by a cytomegalovirus vaccine vector expressing a single low-avidity epitope. J Immunol 199:1737–1747
66. Schenkel JM, Masopust D (2014) Tissue-resident memory T cells. Immunity 41:886–897
67. Takamura S (2017) Persistence in temporary lung niches: a survival strategy of lung-resident memory CD8(+) T cells. Viral Immunol 30:438–450
68. Welten SPM, Sandu I, Baumann NS et al (2018) Memory CD8 T cell inflation vs tissue-resident memory T cells: same patrollers, same controllers? Immunol Rev 283:161–175
69. Tsuda Y, Caposio P, Parkins CJ et al (2011) A replicating cytomegalovirus-based vaccine encoding a single Ebola virus nucleoprotein CTL epitope confers protection against Ebola virus. PLoS Negl Trop Dis 5:e1275
70. Tsuda Y, Parkins CJ, Caposio P et al (2015) A cytomegalovirus-based vaccine provides long-lasting protection against lethal Ebola virus challenge after a single dose. Vaccine 33:2261–2266
71. Morabito KM, Ruckwardt TJ, Bar-Haim E et al (2018) Memory inflation drives tissue-resident memory CD8(+) T cell maintenance in the lung after intranasal vaccination with murine cytomegalovirus. Front Immunol 9:1861
72. Morabito KM, Ruckwardt TR, Redwood AJ et al (2017) Intranasal administration of RSV antigen-expressing MCMV elicits robust tissue-resident effector and effector memory CD8+ T cells in the lung. Mucosal Immunol 10:545–554
73. Pizzolla A, Nguyen THO, Smith JM et al (2017) Resident memory CD8(+) T cells in the upper respiratory tract prevent pulmonary influenza virus infection. Sci Immunol. https://doi.org/10.1126/sciimmunol.aam6970
74. Slutter B, Van Braeckel-Budimir N, Abboud G et al (2017) Dynamics of influenza-induced lung-resident memory T cells underlie waning heterosubtypic immunity. Sci Immunol. https://doi.org/10.1126/sciimmunol.aag2031
75. Hansen SG, Vieville C, Whizin N et al (2009) Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. Nat Med 15:293–299
76. Hansen SG, Ford JC, Lewis MS et al (2011) Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature 473:523–527
77. Hansen SG, Piatak M Jr, Ventura AB et al (2013) Immune clearance of highly pathogenic SIV infection. Nature 502:100–104
78. Hansen SG, Wu HL, Burwitz BJ et al (2016) Broadly targeted CD8(+) T cell responses restricted by major histocompatibility complex E. Science 351:714–720
79. Klyushnenkova EN, Kouiavskaia DV, Parkins CJ et al (2012) A cytomegalovirus-based vaccine expressing a single tumor-specific CD8+ T-cell epitope delays tumor growth in a murine model of prostate cancer. J Immunother 35:390–399
80. Qiu Z, Huang H, Grenier JM et al (2015) Cytomegalovirus-based vaccine expressing a modified tumor antigen induces potent tumor-specific CD8(+) T-cell response and protects mice from melanoma. Cancer Immunol Res 3:536–546
81. Erkes DA, Xu G, Daskalakis C et al (2016) Intratumoral infection with murine cytomegalovirus synergizes with PD-L1 blockade to clear melanoma lesions and induce long-term immunity. Mol Ther 24:1444–1455
82. Tierney R, Nakai T, Parkins CJ et al (2012) A single-dose cytomegalovirus-based vaccine encoding tetanus toxin fragment C induces sustained levels of protective tetanus toxin antibodies in mice. Vaccine 30:3047–3052
83. Marzi A, Murphy AA, Feldmann F et al (2016) Cytomegalovirus-based vaccine expressing Ebola virus glycoprotein protects nonhuman primates from Ebola virus infection. Sci Rep 6:21674
84. Xu G, Smith T, Grey F et al (2013) Cytomegalovirus-based cancer vaccines expressing TRP2 induce rejection of melanoma in mice. Biochem Biophys Res Commun 437:287–291
85. Benonisson H, Sow HS, Breukel C et al (2018) FcgammaRI expression on macrophages is required for antibody-mediated tumor protection by cytomegalovirus-based vaccines. Oncotarget 9:29392–29402
86. Lee LN, Bolinger B, Banki Z et al (2017) Adenoviral vaccine induction of CD8+ T cell memory inflation: Impact of co-infection and infection order. PLoS Pathog 13:e1006782
87. Solana R, Tarazona R, Aiello AE et al (2012) CMV and immunosenescence: from basics to clinics. Immun Ageing 9:23
88. Redeker A, Remmerswaal EBM, Van Der Gracht ETI et al (2017) The contribution of cytomegalovirus infection to immune senescence is set by the infectious dose. Front Immunol 8:1953
89. Bolinger B, Sims S, O'hara G et al (2013) A new model for CD8+ T cell memory inflation based upon a recombinant adenoviral vector. J Immunol 190:4162–4174
90. Bolinger B, Sims S, Swadling L et al (2015) Adenoviral vector vaccination induces a conserved program of CD8(+) T cell memory differentiation in mouse and man. Cell Rep 13:1578–1588
91. Gordon CL, Hutchings CL, Highton AJ et al (2018) Memory inflation following adenoviral vaccination depends on IL-21. Vaccine 36:7011–7016
92. Mclaren JE, Clement M, Marsden M et al (2019) IL-33 augments virus-specific memory T cell inflation and potentiates the efficacy of an attenuated cytomegalovirus-based vaccine. J Immunol 202:943–955
93. Trsan T, Vukovic K, Filipovic P et al (2017) Cytomegalovirus vector expressing RAE-1gamma induces enhanced anti-tumor capacity of murine CD8(+) T cells. Eur J Immunol 47:1354–1367
94. Hirsl L, Brizic I, Jenus T et al (2018) Murine CMV expressing the high affinity NKG2D ligand MULT-1: a model for the development of cytomegalovirus-based vaccines. Front Immunol 9:991