

# 1 Exhaustion and Inflation at Antipodes of T-cell Responses 2 to Chronic Virus Infection

3 Luka Cicin-Sain<sup>1,2,3</sup>, Ramon Arens<sup>4</sup>

4 <sup>1</sup>Department of Vaccinology and Applied Microbiology, Helmholtz Centre for Infection  
5 Research, Braunschweig, Germany

6 <sup>2</sup>Institute for Virology, Medical School Hannover, Hannover, Germany

7 <sup>3</sup>German Center for Infection Research (DZIF), Partner site Hannover/Braunschweig, Germany

8 <sup>4</sup>Department of Immunohematology and Blood Transfusion, Leiden University Medical Center,  
9 Leiden, the Netherlands

10 Correspondence: [Luka.cicin-sain@helmholtz-hzi.de](mailto:Luka.cicin-sain@helmholtz-hzi.de)

11 Keywords: Immune Exhaustion; Memory Inflation; Chronic Virus Infection; Viral Latency; Viral  
12 Persistence; CD8 T-cell

## 13 **Abstract**

14 Viruses that have coevolved with their host establish chronic infections that are well tolerated by  
15 the host. Other viruses, that are partly adapted to their host, may induce chronic infections where  
16 persistent replication and viral antigen expression occur. The former induce highly functional and  
17 resilient CD8 T-cell responses called memory inflation. The latter induce dysfunctional and  
18 exhausted responses. The reasons compelling T-cell responses towards inflationary or exhausted  
19 responses are only partly understood. In this review we compare the two conditions and describe  
20 mechanistic similarities and differences. We also provide a list of potential reasons why  
21 exhaustion or inflation occur in different virus infections. We propose that T cell mediated  
22 transcriptional repression of viral gene expression provides a critical feature of inflation that  
23 allows peaceful virus and host coexistence. The virus is controlled, but its genome is not  
24 eradicated. If this mechanism is not available, as in the case of RNA viruses, the virus and the  
25 host are compelled to an arms race. If virus proliferation and spread proceed uncontrolled for too  
26 long, T cells are forced to strike a balance between viral control and tissue destruction, losing  
27 antiviral potency and facilitating virus persistence.

28 **Virus chronicity as hallmark of adaptation**

29 Viruses critically depend on the host for their survival and reproduction. This lifestyle compels  
30 the viruses to a continuous dance on a knife-edge. On one hand, they are relentlessly hunted by  
31 the immune system; they may propagate only if they avoid detection or outrun the host defenses.  
32 Yet, even if they succeed at overwhelming the immunity, this is likely to result in disease and  
33 death of the host, and thus ultimately in the demise of the virus.

34 Natural selection has forced viruses to either aggressively transmit through large and diverse  
35 populations of hosts [1], or to undergo co-evolution with defined host species and coexist over  
36 long-periods of time minimizing the harm to the host [2]. The first strategy is manifest in viruses  
37 that rapidly evolve to cause infections across various species (such as influenza virus), or in  
38 vector-carried viruses whose transmission is not hindered by severe disease that immobilizes the  
39 host (such as arboviruses) [1]. The outcome of such infections may be the resolution of the  
40 disease and the clearance of infection, or disease progression until death. Viral survival is  
41 achieved by their rapid spread to other hosts. On the other hand, viruses that are well adapted to  
42 the host typically have milder disease courses and establish a détente with the host immune  
43 system. This allows viral persistence in absence of overt disease for long periods, but requires  
44 viral adaptation to the specific immune system of the host. These outcomes are common in  
45 herpesvirus infections, where the persistence of viral genomes in host cells is achieved by  
46 silencing the transcription of most of their genes. The latently infected host is typically healthy  
47 and unaware of the presence of the latent virus in the body, yet the virus may reactivate if the  
48 host becomes severely ill [3], providing a chance to the virus to spread from the dying host.

49 Wide varieties of outcomes are possible between these two extremes. Among those intermediate  
50 outcomes, are the clinically relevant persistent virus infections. Human immunodeficiency virus

51 (HIV), hepatitis C virus (HCV), and to a lesser extent hepatitis B virus (HBV) cause only mild  
52 direct cytopathic effects and thus may persistently proliferate in hosts, driving chronic and  
53 progressive diseases that are fatal unless treated. These remain a major public health burden  
54 worldwide, despite tremendous advances in therapeutic options against HCV and HIV. For  
55 instance, a therapy clearing HIV from the body is still missing. Therefore, there is a growing  
56 population of patients worldwide who undergo combined retroviral therapy over numerous years,  
57 where HIV persistence encumbers the immune system, thus increasing the risk of inflammatory  
58 conditions and cancer, as well as accelerating the onset of immune aging [4, 5].

59 Since virus chronicity requires adaptation to the host, clinically relevant chronic viruses are  
60 highly adapted to humans and cannot be efficiently studied in animal models *in vivo*. Therefore,  
61 experimental models of immune responses to persistent or latent infections have relied on viruses  
62 that naturally infect animals, and in particular mice. The most common mouse models of chronic  
63 virus infection are based on the infection of mice with lymphocytic choriomeningitis virus  
64 (LCMV) clone 13 [6] and with LCMV strain WE [7]. These LCMV models induce a state of  
65 virus-specific T cell ‘exhaustion’ (as will be discussed in detail hereafter), which has remarkable  
66 similarity with the T cell responses to chronic viral infections in humans such as HIV or hepatitis  
67 C virus infections [8]. The immune response to a persistent (latent) herpesvirus has been  
68 extensively studied in the mouse CMV (MCMV) infection model [9], which has striking  
69 resemblance to HCMV infection with respect to the impact on the function and phenotype of the  
70 virus-specific T cells [10], or to T cell responses to other DNA-virus infections, such as  
71 adenoviruses [11, 12].

## 72 **Effects of chronic viral infections on the immune system**

73 Persistent infections with high-level replicating viruses, like HIV, HCV or LCMV induce T-cell  
74 responses that share similar traits in mice and men. Over time, cytokine production and  
75 cytotoxicity in antigen-specific CD8 T-cell populations is lost [8, 13]. This loss of function is not  
76 only associated with poor control of the offending virus, but also with an increase in chronic  
77 inflammation that induces cumulative immune pathology, a propensity for cancer and a  
78 premature onset of immune senescence [14, 15]. Such effects are particularly pronounced in  
79 patients co-infected with HIV and HCV, where HIV co-infection accelerates inflammatory liver  
80 injuries and hepatic decompensation elicited by HCV [16].

81 It is important to note that herpesviruses, such as cytomegalovirus (CMV), have also been  
82 suspected to play a role in immune senescence [17]. Ongoing and intermittent antigenic  
83 stimulation by CMV engages the cellular immune system at times of latency [18, 19], driving  
84 responses of differentiated T cells [20]. However, the scientific consensus has evolved over the  
85 years towards a conclusion that the presence of latent CMV does not necessarily accelerate the  
86 onset of immune aging or impair the immune system in older people [21, 22], but is likely linked  
87 to the strength of the CMV infection and the immune status of the host.

88 It remains unclear why different chronic infections result in diametrically opposing outcomes in  
89 the functionality of responding T cells. Why do some chronic virus infection exhaust the immune  
90 system, while other ones do not? How may we guide the immune reaction to HCV or HIV  
91 towards a functional and protective response? To begin to understand these aspects, one needs to  
92 consider the specificities of the immune response to latent viruses and distinguish them from the  
93 immune exhaustion elicited by productively replicating chronic-persistent infections. These two  
94 scenarios will be described and reasons for their divergent outcomes discussed.

## 95 **Immune exhaustion**

96 Productive viral replication induces chronic inflammatory conditions and exhausts the adaptive  
97 host immune system over time, in particular the CD8 T-cell compartment [19, 23]. While  
98 exhaustion and virus persistence are parts of a vicious cycle, it remains unclear if the inability of  
99 exhausted T cells to clear the virus results in persistent infection, or if viral persistence results in  
100 exhaustion. Either way, these viruses pose major clinical problems, not only due to direct  
101 cytotoxicity, but also due to the long-term immune pathology that they elicit. The paradigmatic  
102 murine LCMV infection models allowed the study of immune responses to chronic persistent  
103 infections in mechanistic detail. Labelling of T cells with peptide-MHC (pMHC) tetramers  
104 revealed that the virus-specific T cells are not lost in chronic LCMV infection. They are merely  
105 hypofunctional cells, designated as exhausted, in functional assays (e.g. cytokine production)  
106 [13]. T cell exhaustion is driven by continuous high-level cognate antigenic triggering, and  
107 eventually exhausted T cells become antigen-addicted for their maintenance [24, 25]. In contrast,  
108 conventional T cells do not rely on their cognate antigen for survival but on IL-7 and/or IL-15-  
109 driven homeostatic self-renewal [26]. Comparison of transcriptional networks in LCMV-specific  
110 CD8 T cells revealed a partial overlap of genes that are activated during acute and chronic  
111 LCMV infection, and a key role for the transcriptional factors T-bet and Eomesodermin  
112 (EOMES) in both conditions [27] (Table 1). Importantly, the CD28-like PD-1 receptor is retained  
113 on exhausted T cells and inhibition of PD-1 with its ligand PD-L1 by monoclonal antibodies  
114 restores the T cell function and enhances the clearance of chronic LCMV infection [28]. PD-1  
115 blockade also restores the function of HIV-1 specific T cells [29, 30]. However, reprogramming  
116 of exhausted T cells into durable memory T cells via blocking PD-1 is limited due to irreversible  
117 epigenetic alterations [31]. Interestingly, responses to LCMV antigens are not reduced in  
118 immunoproteasome deficient mice [32] and antigen presentation on non-hematopoietic cells  
119 substantially expands the pool of responding T cells [33].

120 Besides PD-1 expression, exhausted T cells express a number of other inhibitory receptors  
121 including CTLA-4, LAG3, TIM3, 2B4, CD160 and TIGIT [34]. Moreover, molecules involved in  
122 metabolism like the ectonucleotidase CD39 are also highly expressed [35]. Expression of central-  
123 memory markers such as CD62L and CD127 (IL-7R $\alpha$ ) is absent. Remarkably, the effector cell  
124 marker KLRG1 is not highly expressed [36]. It is important to note that the exhausted state of T  
125 cells is acquired progressively. For example, the loss in cytokine polyfunctionality of exhausted  
126 CD8<sup>+</sup> T cells starts with the loss of IL-2 followed by tumor necrosis factor (TNF) and finally the  
127 capacity to produce interferon-gamma (IFN $\gamma$ ) wanes. The progressive loss of memory CD8<sup>+</sup> T  
128 cell potential is likely associated with the gradual loss of autocrine IL-2 production [37]. Notably,  
129 heterogeneity exists in exhausted T cell populations. Exhausted T cells can be reinvigorated by  
130 blocking PD-1 and other inhibitory receptors, but cells expressing high levels of T-bet and  
131 intermediate PD-1 expression respond better to PD-1 blockade as compared to cells expressing  
132 high levels of PD-1 and EOMES [38, 39]. Either way, this reversion of the exhausted state has  
133 led to clear clinical benefit in chronically infected individuals and cancer patients, arguing that  
134 exhausted cells are essentially dysfunctional. While exhaustion may represent a breakdown of the  
135 equilibrium between the immune system and a persistent virus, it has recently been proposed that  
136 repressed functionality in “exhausted” CD8 T cells serves to limit immune pathogenesis, while  
137 the same CD8 T cells still contribute to immune surveillance of the virus [40]. This idea was  
138 predicated on observations that persistent viruses rapidly replicate in animals lacking CD8 T cells  
139 and that exhausted phenotypes are observed in patients with good outcomes of autoimmune  
140 disease [41]. In that case, exhaustion might be a misnomer, because “exhausted” CD8 T cells  
141 contribute to host survival. Therefore, it is possible that exhaustion is a condition of equilibrium  
142 after all.

### 143 **Memory Inflation**

144 Ongoing antigenic stimulation by latent herpesviruses, in particular by the  $\beta$ -herpesvirus CMV,  
145 also strongly engages the cellular immune system in the chronic phase of infection [18, 42].  
146 However, the functionality of CMV-specific T-cells is maintained into old age [22, 43], and  
147 functional responses to in vivo CMV challenge in immunosenescent non-human primates were  
148 essentially undistinguishable from those in young adult monkeys [44]. T-cell depletions in  
149 experimentally infected animals showed that persistent T-cell responses, and in particular a  
150 functional IFN $\gamma$  response, are crucial for the repression of CMV reactivation from latency [45].  
151 This life-long functionality of T-cell responses is particularly remarkable in light of T-cell  
152 exhaustion in other scenarios of virus persistence [46]. Hence, juxtaposing the processes  
153 underlying T-cell responses to CMV and exhausted responses to persistent viruses may help us to  
154 understand both of these mechanisms. In this respect, it is noteworthy to mention that the  
155 transcriptional signatures of inflationary CD8 T cells are different from conventional or  
156 exhausted T cells with respect to the level of transcription factors such as Blimp1, T-bet and  
157 EOMES (Table 1).

158 It is important to note that Rhesus CMV (RhCMV) based vaccine vectors may elicit highly  
159 unconventional CD8 T-cell responses against epitopes presented on MHC-II [47] or HLA-E  
160 molecules [48]. However, this was shown to occur only in the context of a RhCMV mutant that  
161 was cloned upon extensive in vitro passaging of the virus [49]. This does not seem to represent  
162 the response of human CMV (HCMV)-based vaccines [50], or natural T-cell responses to wild-  
163 type RhCMV infection, which elicits conventional MHC-I restricted CD8 T-cell responses [47,  
164 48]. Therefore, the nature of the RhCMV vector-induced responses will not be discussed further.

165 The ongoing accumulation of antigen-specific CD8 T cells in CMV infection has been first  
166 described in the mouse model [51] and aptly termed **memory inflation** [52] (reviewed in [42]).

167 Data from the murine model closely predicted the nature of CD8 T-cell responses to human CMV  
168 [53, 54]. The inflationary responses accrue over time, but do not constitute an overall expansion  
169 of the primed compartment, whose size remains relatively stable upon infection [55]. Rather,  
170 some antigenic epitopes encoded by MCMV induce dominant inflationary responses and expand  
171 at the expense of other, subdominant, epitopes [56, 57]. The phenotype of inflationary T cells is  
172 effector-memory like, and is characterized by low levels of CD62L and CD127. In contrast to  
173 exhausted T cells, KLRG1 is highly expressed, while PD-1 expression is low [58, 59]. Other  
174 epitopes elicit conventional immune responses, akin to responses observed upon infection with  
175 non-persistent pathogens. These responses are marked by robust expansion of T cells early upon  
176 infection, contraction by day 14 and a shift of phenotypes of antigen-specific T cells towards  
177 central-memory like during the maintenance phase [56, 60]. Therefore, the requirements for  
178 inflation can be studied by comparing conventional or inflationary CD8 T-cell responses in the  
179 context of MCMV infection. In this respect, it should be noted that memory inflation is not  
180 exclusively linked to CMV infection (albeit most pronounced), but is also observed after  
181 infection with certain adenovirus and parvovirus strains [59].

182 Inflationary responses require the presentation of antigenic epitopes on non-hematopoietic cells,  
183 whereas this is dispensable for conventional responses [61, 62]. On the other hand, conventional  
184 responses require processing by the immunoproteasome, yet the constitutive proteasome is  
185 sufficient for the emergence of inflationary responses [63]. We showed recently that moving an  
186 immunoproteasome-dependent MCMV epitope from its native position within the viral protein to  
187 an alternative position where the epitope is available to processing by the constitutive proteasome  
188 resulted in drastic changes in size and quality of responses [64]. The response improved by a  
189 factor of 10, shifted from conventional to inflationary, and was present in mice with impaired  
190 antigen presentation on hematopoietic cells [64]. Taken together, these data demonstrated that

191 antigen processed by the constitutional proteasome in non-hematopoietic cells sustains  
192 inflationary responses during virus latency.

193 So, why do non-hematopoietic cells drive inflationary responses? Although this question is not  
194 conclusively answered, it is likely that this depends on the cells that harbor latent MCMV (and  
195 thus that express viral antigens at times of latency). MCMV transcription proceeds at low levels  
196 during virus latency [65] and endothelial cells were shown to harbor latent virus [66]. The link  
197 between latent transcription and memory inflation was exposed by a study where latent  
198 transcription of viral genes was enhanced in an MCMV mutant lacking a single antigenic epitope  
199 within the IE1 gene [67]. It has been therefore proposed that low levels of sporadic antigenic  
200 expression in latent CMV infection drive CD8 T-cell responses, which in turn limits further viral  
201 transcription, establishing a state of dynamic equilibrium between the virus and the host [53].  
202 This theory, called Immune Sensing theory, has been further corroborated by transgenic MCMVs  
203 expressing foreign epitopes. These epitopes induced forceful inflationary T-cell responses, yet the  
204 inflationary response against endogenous epitopes was significantly diminished [68, 69] at the  
205 expense of effector cell responses, while central memory responses against the same epitopes  
206 were unaffected [70]. The competition of antigenic peptides for inflationary CD8 T-cell  
207 responses was not observed when mice were coinfectd with the mutant and the wild-type  
208 MCMV [69]. This behavior was predicted by the Immune Sensing theory [53], because CD8 T  
209 cells would only be able to compete for epitopes that are expressed within the same latently  
210 infected cell. If viral genes were expressed from different cells, the dominant epitope could not  
211 outcompete the subdominant ones. At a glance, this theory contradicts clinical evidence that the  
212 immunodominant and inflating CD8 T-cell response to the HLA A2:01 restricted HCMV epitope  
213 NLVPMVATV [71, 72] targets a peptide derived from a late HCMV gene (i.e. UL83 (pp65)),  
214 because immune sensing should prevent the expression of late genes. However, HCMV is

215 maintained latent in numerous cell types, including hematopoietic cells [73], yet the  
216 transcriptional activity of UL83 was assessed in fibroblastic cell cultures, rather than primary  
217 human cells bearing latent genomes. Thus, additional evidence is required to understand how  
218 UL83/pp65 immunodominance may fit into the immune sensing theory (or alternatively, how it  
219 may disprove it).

220 Taken together, a model emerges where the non-hematopoietic cells transcribe low levels of  
221 antigen from otherwise latent CMVs, and thus keep poking CD8 T cells. CD8 T cells respond  
222 with IFN $\gamma$  production, which represses viral transcription, and reaffirms the latent state, thus  
223 providing relief to the T cells. The epitopes that induce such responses are processed by the  
224 constitutive proteasome, which means that epitope presentation does not require interferon-  
225 mediated induction of the proteasome. This then implies that viral control is achieved in  
226 conditions of minimal inflammation. Such balance hinges on transcriptional silencing of viral  
227 DNA (**Fig.1**) by cytokines secreted by T cells. Notably, viruses inducing T-cell exhaustion in  
228 chronic infection are typically RNA viruses, and thus may not allow a similar peaceful  
229 coexistence with the host.

### 230 **Potential causes of differences between inflation and exhaustion**

231 CD8 T-cell responses to chronic LCMV and MCMV differ in their functional capacity, but  
232 notably also display numerous similarities. The priming of naïve virus-specific CD8 T cells does  
233 not occur exclusively during primary LCMV or MCMV infection, since novel naïve cells are also  
234 recruited in the chronic phase [58, 74, 75]. T-cell responses to LCMV-encoded antigens [32] or  
235 inflationary MCMV epitopes are still maintained in immunoproteasome deficient mice [63]. The  
236 pool of responding T cells is substantially expanded by antigen-presentation on non-  
237 hematopoietic cells upon MCMV or acute LCMV infection [33, 61], and antigen presentation on

238 the non-hematopoietic cells exacerbates exhaustion in chronic LCMV infection [76]. Likewise,  
239 virus specific cells showing effector phenotypes (KLRG1<sup>+</sup>, CD62L<sup>-</sup>, CD27<sup>-</sup>, CD127<sup>-</sup>) indicative  
240 of recent antigenic encounter, are detected for a long time after either of these infections. The  
241 responding cells seem to depend on continuous TCR stimulation to maintain their pools, as  
242 evidenced in adoptive transfer experiments [25, 58]. Therefore, it stands to reason that antigens  
243 are expressed during LCMV persistence, but also during CMV latency. In light of that, we  
244 consider several models that might explain the difference in T-cell functionality in these two  
245 scenarios. Notably, these propositions are not mutually exclusive, and it is likely that two or more  
246 occur at the same time and are interconnected.

#### 247 *Antigen persistence vs. intermittence*

248 It has been proposed that virus replication and thus antigenic stimulation causes immune  
249 exhaustion while intermittent virus replication with limited periods of antigen presence would  
250 retain functional T-cell responses [19]. It is important to consider that herpesviruses typically  
251 cause productive infections that lyse the infected cells [77]. Therefore, antigen expression during  
252 CMV latency is bound to be a result of intermittent and recurring transcriptional events [78],  
253 rather than an ongoing and continuous production. On the other hand, the direct cytopathic effect  
254 of hepatitis viruses is typically low [79], implying that productive hepatitis infections may  
255 simmer continuously. A similar persistence of antigenic expression was described in LCMV  
256 variants associated with immune exhaustion [80]. Hence, MCMV and LCMV infections may  
257 induce different kinds of T-cell responses due to the intermittent antigen expression in MCMV  
258 infection, which differs from continuous and sustained presence of an antigen in LCMV  
259 infection. However, this explanation is essentially based on correlative evidence. Therefore, it  
260 remains unclear if forced persistence of an antigen in the context of an MCMV infection would

261 also drive the exhaustion of cognate T cells. Furthermore, exhaustion and inflation alone do not  
262 explain why some viruses replicate persistently, whereas other ones only intermittently.

### 263 *Antigen abundance vs. scarcity*

264 Another proposed explanation for the onset of exhaustion is that strong viral replication during  
265 the onset of virus infection promotes viral persistence and drives exhaustion [80]. [81]. This idea  
266 fits with experimental evidence that the abundance and availability of LCMV antigen defines the  
267 extent of exhaustion [76, 82]. The conditions of primary MCMV infection also define the latent  
268 virus load [83] and the size of the inflationary response [84], but the amount of antigen remains  
269 overall low since virus replication is essentially silenced [85], and limited virus antigen is present  
270 in conditions of MCMV inflation [65, 78]. Consequently, persistent antigen leading to exhaustion  
271 is much more abundant than the antigen driving inflationary phenotypes. On the other hand, this  
272 interpretation is likely to be too simplistic, because it would imply that a low-dose infection with  
273 LCMV or hepatitis C would result in inflationary responses, but clinical and experimental  
274 evidence argues that low dose infection with these viruses results in virus clearance and  
275 conventional responses.

### 276 *Cellular niche*

277 It has also been proposed that the cellular niche of viral replication predisposes the immune  
278 response towards exhaustion [86]. Both MCMV and LCMV persistently stimulate CD8 T-cell  
279 responses by antigen expression in non-hematopoietic cells (see above), but MCMV is latent in  
280 liver endothelial cells [66], while LCMV persists in fibroblastic reticular cells [87]. However, it is  
281 unclear if either virus is restricted to these cell types during the chronic phase of infection, or may

282 be found in other ones as well. Furthermore, a mechanism explaining the link between virus  
283 tropism for defined cell types and T-cell responses has not been established.

284 *T cell costimulation and inflammatory cytokines*

285 We compared the priming of CD8 T-cell responses upon primary LCMV or MCMV infection  
286 and observed a clear difference in costimulatory signal (“signal 2”) requirement [88]. We  
287 analyzed responses to the LCMV epitope KAVYNFATC (GP<sup>33</sup>) upon infection with LCMV or a  
288 recombinant MCMV expressing the same epitope and showed that co-stimulation by CD80/86 is  
289 required for priming against GP<sup>33</sup> when expressed by MCMV, but highly redundant with other  
290 signal 2 co-receptors in the context of LCMV infection. On the other hand, LCMV infection  
291 induced much stronger type I IFN responses. Soluble cytokines may co-stimulate T cells during  
292 priming (“signal 3”) and priming against LCMV-encoded epitopes depended strongly on type I  
293 IFN-dependent signal 3 [88]. CD8 T-cells lacking type I IFN receptors are susceptible to NK-  
294 cell mediated apoptosis in chronic LCMV infection [89, 90], implying that IFN- $\alpha/\beta$  provides a  
295 critical survival signal to these cells. However, it remains unclear if CD8 T-cell survival depends  
296 on strong type I IFN responses to LCMV, or if tonic IFN responses would be sufficient. While  
297 high levels of type I IFN promote T-cell priming [88] in acute LCMV infection, they were also  
298 shown to support the onset of chronic virus infection [91, 92]. IL-12 and type I IFN responses are  
299 more balanced during MCMV infection, but type I IFN responses push MCMV into latency [93],  
300 implying that interferon induces the chronic state in both infections and that exhaustion or  
301 inflation pathways might be defined during the priming stage of T cells. While we observed  
302 clearly different priming requirements in MCMV and acute LCMV infections [88], a comparison  
303 of T-cell responses to GP<sup>33</sup> expressed by LCMV or the recombinant MCMV during chronic

304 infection has not yet been performed. Therefore, further studies are required to address this  
305 question conclusively.

306 *CD4 T-cell help*

307 In LCMV infection, the progressive exhaustion of CD8 T cells is accelerated by the lack of CD4  
308 T cells [94, 95]. Moreover, high dose infection has been shown to result in the deletion of  
309 activated CD4 T-cells by activated NK cells, thereby promoting exhaustion [96]. Taken together,  
310 a relative lack of CD4 T cells promotes exhaustion. In MCMV infection, the development of  
311 inflationary CD8<sup>+</sup> T cells depends on the presence of CD4 T cells [97]. This feature affects only  
312 some inflationary epitopes [98], although this requirement was much stricter upon infection with  
313 a viral mutant that is poorly controlled by NK cells [99]. CD4 T cells may affect virus replication  
314 directly by releasing antiviral cytokines or indirectly by affecting CD8 T cells or B cells. For  
315 instance, interleukin 10 (IL-10), a cytokine that is frequently released by regulatory CD4 T cells  
316 affects memory inflation, which was much more pronounced in IL-10 deficient mice [100]. On  
317 the other hand, the modulation of T cell activity by regulatory CD4 T cells exerts pleiotropic and  
318 organ specific effects in the spleen and salivary glands [101]. Therefore, these phenomena are  
319 complex and complicated, and the net effect of CD4 T cells remains incompletely understood.

320

321 *The common cytokine receptor gamma chain ( $\gamma_c$ ) cytokines IL-2, IL-7, and IL-15.*

322 The common  $\gamma_c$  cytokine family members have crucial roles in T-cell survival, proliferation and  
323 differentiation [102]. IL-2 signaling involving CD25 (IL-2R $\alpha$ : forming together with CD122 and  
324 CD132 the high-affinity IL-2R complex) is important for the maintenance of both exhausted and  
325 inflationary CD8<sup>+</sup> T cells [103]. Since the percentage of cells producing autocrine IL-2 within the

326 inflationary T cell populations correlates to their expansion of the inflationary pool [104], the  
327 induction of autocrine IL-2 appears to be critical for inflationary expansions. Remarkably, the  
328 expression of CD122, the IL-2R $\beta$  chain, which is also shared by IL-15, is differentially  
329 expressed. Inflationary CD8<sup>+</sup> T cells have low CD122 levels [58, 60], while exhausted cells  
330 maintain CD122 expression. The increased expression of CD122 marks the exhausted state, and  
331 signaling via CD122 upon IL-2 and IL-15 binding is likely directly involved via upregulation of  
332 inhibitory receptors [105]. Both exhausted and inflationary T cells have low CD127 (IL-7R $\alpha$ )  
333 expression. However, long-term IL-7 treatment during the contraction phase of chronic LCMV  
334 infection enhances the magnitude and functionality of specific CD8<sup>+</sup> T cells [106, 107].

335 *Ag transcriptional repression vs. repression by killing of infected cells*

336 Type I and II IFNs play an important role in the control of MCMV infection; in IFN- $\gamma$ R<sup>-/-</sup> mice  
337 MCMV replicates persistently [108]. Similarly, type I IFN represses viral gene expression by  
338 upregulating nuclear domain 10 (ND10) proteins in a reversible process [93]. While type I and  
339 type II IFN signaling was shown to limit LCMV replication as well [109, 110], there is no  
340 evidence that this repression may be transient. The IFN induced silencing of DNA viruses can  
341 affect any episomal DNA within the cell nucleus and silence their transcription [93]. It is  
342 therefore reasonable to assume that IFN- $\gamma$  signaling may silence the transcription of DNA viruses  
343 and represses the expression of antigens upon T-cell activation. Interestingly, immune exhaustion  
344 is typically induced by RNA viruses, where IFN-dependent silencing of DNA transcription  
345 cannot limit antigenic expression. In that case, the ability to limit virus persistence by  
346 transcriptional repression, rather than by cytotoxicity may present a critical hallmark of  
347 inflationary responses, distinguishing it from events in immune exhaustion. Therefore, we  
348 hypothesize that the cytotoxic activity of T cells may compel persistent RNA viruses to an arms

349 race, where they rapidly replicate to achieve escape velocity from T-cell  
350 proliferation upon activation is uniquely rapid with a 2-hour cell cycle time [111]. However, a  
351 replicating virus gives rise to thousands of infectious particles per lytic cycle, spurring drastic  
352 expansions of responsive CD8 T-cell clones. If T cells are unable to limit the spread of a rapidly  
353 replicating virus, cytotoxicity itself will become detrimental to the host. We postulate that such  
354 potential for immune pathology is sensed during the immune response and that the immune  
355 exhaustion program sets in to protect the host. While this hypothesis is consistent with published  
356 data, further detailed studies will be required to validate our prediction.

### 357 **Concluding remarks**

358 The characterization of exhausted *versus* inflationary T-cell responses in chronic viral infections  
359 is advancing in great detail. Available evidence indicates that both inflation and exhaustion are  
360 conditions of equilibrium between the host and the persisting virus, yet their clinical outcomes  
361 are vastly different, because they depend on distinct cellular and molecular mechanisms. While  
362 thorough understanding of the underlying mechanisms leading to these divergent cellular states  
363 remains lacking, the targeting of inhibitory pathways of exhausted T cells has significantly  
364 innovated immunotherapy of chronic infection and cancer, and exploiting of inflationary  
365 responses to improve vaccines has great potential. Addressing the outstanding questions (see  
366 Outstanding Questions Box) will allow manipulations of the antigenic supply and costimulatory  
367 molecules that will allow the induction of optimal and protective T-cell responses.

### 368 **Acknowledgments**

369 This work was supported by the ERC-POC grant VIVAVE and the DFG grant (SFB900 TP B2)  
370 to LCS, and a Dutch Cancer Society grant (KWF UL2015-7817) awarded to RA.

371 **Figure legends**

372 **Figure 1:** Model of T-cell mediated control of viral infections by IFN signaling or cytotoxicity.  
373 Antigenic peptides presented on MHC-I molecules (pMHC-I) are recognized by inflationary or  
374 exhausted CD8 T-cells. Cytokines regulating the transcription of viral genes may repress gene  
375 expression in the case of DNA viruses whose genomes are maintained in the cell nucleus. This  
376 non-lethal control is not available to RNA viruses, which are controlled by cytotoxic mechanisms  
377 (e.g. perforin and GzB). Therefore, they are unable to establish an equilibrium with the host at the  
378 level of single infected cells. This in turn compels RNA viruses to rapid proliferation to  
379 overcome host control. We propose that the difference in the surface receptor expression on  
380 inflationary and exhausted CD8 T-cells may be a result of continuous stimulation with large  
381 amounts of antigen, as opposed to the intermittent exposure to low-levels of antigen.

382

Table 1. Comparison of viral-specific CD8 T-cell populations <sup>a</sup>			
	Central-memory	Inflationary	Exhausted
Homeostatic proliferation	++	-	-
Antigen-dependence	-	++	++
2e Expansion capacity	++	+/-	-
Cytokine polyfunctionality	++	+ (low % IL-2)	-
Lymphoid homing markers			
CD62L	++	-	-
CCR7	++	-	-
Cytokine receptors			
CD122	++	-	+
CD127	++	+/-	-
NK cell receptors			
KLRG1	-	++	-
Costimulatory receptors			
CD28	+	-	-
CD27	++	-	-
Inhibitory receptors			
PD-1/TIM3/LAG3/ etc.	-	-	++
Transcription factors			
T-bet	-	+	+/-
EOMES	-	+/-	+
Blimp-1	+/-	+/-	+

<sup>a</sup> - absent or low, +/- intermediate, + high, ++ prominent

417

418 **References**

- 419 1. Ewald, P.W. (1996) Guarding against the most dangerous emerging pathogens. *Emerg Infect Dis* 2 (4),  
420 245-57.
- 421 2. Alizon, S. et al. (2009) Virulence evolution and the trade-off hypothesis: history, current state of affairs  
422 and the future. *J Evol Biol* 22 (2), 245-59.
- 423 3. Limaye, A.P. et al. (2008) Cytomegalovirus reactivation in critically ill immunocompetent patients.  
424 *Jama* 300 (4), 413-22.
- 425 4. Appay, V. et al. (2007) Accelerated immune senescence and HIV-1 infection. *Exp Gerontol* 42 (5), 432-  
426 7.
- 427 5. Effros, R.B. et al. (2008) Workshop on HIV Infection and Aging: What Is Known and Future Research  
428 Directions. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of*  
429 *America* 47 (4), 542-553.
- 430 6. Ahmed, R. and Oldstone, M.B. (1988) Organ-specific selection of viral variants during chronic infection.  
431 *J Exp Med* 167 (5), 1719-24.
- 432 7. Rivers, T.M. and McNair Scott, T.F. (1935) MENINGITIS IN MAN CAUSED BY A FILTERABLE VIRUS.  
433 *Science* 81 (2105), 439-440.
- 434 8. Gruener, N.H. et al. (2001) Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with  
435 hepatitis C virus. *J Virol* 75 (12), 5550-8.
- 436 9. Reddehase, M.J. et al. (2008) Murine model of cytomegalovirus latency and reactivation. *Curr Top*  
437 *Microbiol Immunol* 325, 315-31.
- 438 10. Hertoghs, K.M. et al. (2010) Molecular profiling of cytomegalovirus-induced human CD8+ T cell  
439 differentiation. *J Clin Invest* 120 (11), 4077-90.
- 440 11. Bolinger, B. et al. (2015) Adenoviral Vector Vaccination Induces a Conserved Program of CD8(+) T Cell  
441 Memory Differentiation in Mouse and Man. *Cell Rep* 13 (8), 1578-88.
- 442 12. Colston, J.M. et al. (2016) Modification of Antigen Impacts on Memory Quality after Adenovirus  
443 Vaccination. *J Immunol* 196 (8), 3354-63.
- 444 13. Zajac, A.J. et al. (1998) Viral immune evasion due to persistence of activated T cells without effector  
445 function. *J Exp Med* 188 (12), 2205-13.
- 446 14. Tu, T. et al. (2017) Chronic viral hepatitis and its association with liver cancer. *Biol Chem* 398 (8), 817-  
447 837.
- 448 15. Naggie, S. (2017) Hepatitis C Virus, Inflammation, and Cellular Aging: Turning Back Time. *Top Antivir*  
449 *Med* 25 (1), 3-6.
- 450 16. Lo Re, V., 3rd et al. (2014) Hepatic decompensation in antiretroviral-treated patients co-infected with  
451 HIV and hepatitis C virus compared with hepatitis C virus-monoinfected patients: a cohort study. *Ann*  
452 *Intern Med* 160 (6), 369-79.
- 453 17. Pawelec, G. et al. (2004) Is immunosenescence infectious? *Trends Immunol* 25 (8), 406-10.
- 454 18. Sylwester, A.W. et al. (2005) Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells  
455 dominate the memory compartments of exposed subjects. *J Exp Med* 202 (5), 673-85.
- 456 19. Nikolich-Zugich, J. (2008) Ageing and life-long maintenance of T-cell subsets in the face of latent  
457 persistent infections. *Nat Rev Immunol* 8 (7), 512-22.
- 458 20. Appay, V. et al. (2002) Memory CD8+ T cells vary in differentiation phenotype in different persistent  
459 virus infections. *Nat Med* 8 (4), 379-85.
- 460 21. Arens, R. et al. (2015) 5(th) International Workshop on CMV and Immunosenescence - A shadow of  
461 cytomegalovirus infection on immunological memory. *Eur J Immunol* 45 (4), 954-7.
- 462 22. Jackson, S.E. et al. (2017) Latent Cytomegalovirus (CMV) Infection Does Not Detrimentally Alter T Cell  
463 Responses in the Healthy Old, But Increased Latent CMV Carriage Is Related to Expanded CMV-Specific T  
464 Cells. *Frontiers in Immunology* 8 (733).

- 465 23. Virgin, H.W. et al. (2009) Redefining Chronic Viral Infection. *Cell* 138 (1), 30-50.
- 466 24. Shin, H. et al. (2007) Viral antigen and extensive division maintain virus-specific CD8 T cells during  
467 chronic infection. *J Exp Med* 204 (4), 941-9.
- 468 25. Wherry, E.J. et al. (2004) Antigen-independent memory CD8 T cells do not develop during chronic  
469 viral infection. *Proc Natl Acad Sci U S A* 101 (45), 16004-9.
- 470 26. Surh, C.D. and Sprent, J. (2008) Homeostasis of naive and memory T cells. *Immunity* 29 (6), 848-62.
- 471 27. Doering, T.A. et al. (2012) Network analysis reveals centrally connected genes and pathways involved  
472 in CD8+ T cell exhaustion versus memory. *Immunity* 37 (6), 1130-44.
- 473 28. Barber, D.L. et al. (2006) Restoring function in exhausted CD8 T cells during chronic viral infection.  
474 *Nature* 439 (7077), 682-7.
- 475 29. Day, C.L. et al. (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and  
476 disease progression. *Nature* 443 (7109), 350-4.
- 477 30. Trautmann, L. et al. (2006) Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to  
478 reversible immune dysfunction. *Nat Med* 12 (10), 1198-202.
- 479 31. Pauken, K.E. et al. (2016) Epigenetic stability of exhausted T cells limits durability of reinvigoration by  
480 PD-1 blockade. *Science* 354 (6316), 1160-1165.
- 481 32. Nussbaum, A.K. et al. (2005) Immunoproteasome-deficient mice mount largely normal CD8+ T cell  
482 responses to lymphocytic choriomeningitis virus infection and DNA vaccination. *J Immunol* 175 (2), 1153-  
483 60.
- 484 33. Thomas, S. et al. (2007) Antigen presentation by nonhemopoietic cells amplifies clonal expansion of  
485 effector CD8 T cells in a pathogen-specific manner. *J Immunol* 178 (9), 5802-11.
- 486 34. Wherry, E.J. and Kurachi, M. (2015) Molecular and cellular insights into T cell exhaustion. *Nat Rev*  
487 *Immunol* 15 (8), 486-99.
- 488 35. Gupta, P.K. et al. (2015) CD39 Expression Identifies Terminally Exhausted CD8+ T Cells. *PLoS Pathog*  
489 11 (10), e1005177.
- 490 36. Wherry, E.J. et al. (2007) Molecular signature of CD8+ T cell exhaustion during chronic viral infection.  
491 *Immunity* 27 (4), 670-84.
- 492 37. Angelosanto, J.M. et al. (2012) Progressive loss of memory T cell potential and commitment to  
493 exhaustion during chronic viral infection. *J Virol* 86 (15), 8161-70.
- 494 38. Blackburn, S.D. et al. (2008) Selective expansion of a subset of exhausted CD8 T cells by alphaPD-L1  
495 blockade. *Proc Natl Acad Sci U S A* 105 (39), 15016-21.
- 496 39. Paley, M.A. et al. (2012) Progenitor and terminal subsets of CD8+ T cells cooperate to contain chronic  
497 viral infection. *Science* 338 (6111), 1220-5.
- 498 40. Zehn, D. et al. (2016) Immune-surveillance through exhausted effector T-cells. *Curr Opin Virol* 16, 49-  
499 54.
- 500 41. McKinney, E.F. et al. (2015) T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity  
501 and infection. *Nature* 523 (7562), 612-6.
- 502 42. Klenerman, P. and Oxenius, A. (2016) T cell responses to cytomegalovirus. *Nat Rev Immunol* 16 (6),  
503 367-77.
- 504 43. Lelic, A. et al. (2012) The polyfunctionality of human memory CD8+ T cells elicited by acute and  
505 chronic virus infections is not influenced by age. *PLoS Pathog* 8 (12), e1003076.
- 506 44. Cicin-Sain, L. et al. (2011) Cytomegalovirus-specific T cell immunity is maintained in  
507 immunosenescent rhesus macaques. *J Immunol* 187 (4), 1722-32.
- 508 45. Polic, B. et al. (1998) Hierarchical and redundant lymphocyte subset control precludes  
509 cytomegalovirus replication during latent infection. *J Exp Med* 188 (6), 1047-54.
- 510 46. Taddei, T.H. et al. (2016) HIV, Aging, and Viral Coinfections: Taking the Long View. *Curr HIV/AIDS Rep*  
511 13 (5), 269-78.
- 512 47. Hansen, S.G. et al. (2013) Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms.  
513 *Science* 340 (6135), 1237874.

514 48. Hansen, S.G. et al. (2016) Broadly targeted CD8(+) T cell responses restricted by major  
515 histocompatibility complex E. *Science* 351 (6274), 714-20.

516 49. Chang, W.L. and Barry, P.A. (2003) Cloning of the full-length rhesus cytomegalovirus genome as an  
517 infectious and self-excisable bacterial artificial chromosome for analysis of viral pathogenesis. *J Virol* 77  
518 (9), 5073-83.

519 50. Murray, S.E. et al. (2017) Fibroblast-adapted human CMV vaccines elicit predominantly conventional  
520 CD8 T cell responses in humans. *J Exp Med* 214 (7), 1889-1899.

521 51. Holtappels, R. et al. (2000) Enrichment of immediate-early 1 (m123/pp89) peptide-specific CD8 T  
522 cells in a pulmonary CD62L(lo) memory-effector cell pool during latent murine cytomegalovirus infection  
523 of the lungs. *J Virol* 74 (24), 11495-503.

524 52. Karrer, U. et al. (2003) Memory inflation: continuous accumulation of antiviral CD8+ T cells over time.  
525 *J Immunol* 170 (4), 2022-9.

526 53. Seckert, C.K. et al. (2012) Viral latency drives 'memory inflation': a unifying hypothesis linking two  
527 hallmarks of cytomegalovirus infection. *Med Microbiol Immunol* 201 (4), 551-66.

528 54. Alexandre, Y.O. et al. (2014) Deciphering the role of DC subsets in MCMV infection to better  
529 understand immune protection against viral infections. *Front Microbiol* 5, 378.

530 55. Cicin-Sain, L. et al. (2012) Cytomegalovirus infection impairs immune responses and accentuates T-  
531 cell pool changes observed in mice with aging. *PLoS Pathog* 8 (8), e1002849.

532 56. Munks, M.W. et al. (2006) Four distinct patterns of memory CD8 T cell responses to chronic murine  
533 cytomegalovirus infection. *J Immunol* 177 (1), 450-8.

534 57. Munks, M.W. et al. (2006) Genome-wide analysis reveals a highly diverse CD8 T cell response to  
535 murine cytomegalovirus. *J Immunol* 176 (6), 3760-6.

536 58. Snyder, C.M. et al. (2008) Memory Inflation during Chronic Viral Infection Is Maintained by  
537 Continuous Production of Short-Lived, Functional T Cells. *Immunity* 29 (4), 650-659.

538 59. O'Hara, G.A. et al. (2012) Memory T cell inflation: understanding cause and effect. *Trends Immunol*  
539 33 (2), 84-90.

540 60. Sierro, S. et al. (2005) Evolution of diverse antiviral CD8+ T cell populations after murine  
541 cytomegalovirus infection. *Eur J Immunol* 35 (4), 1113-23.

542 61. Torti, N. et al. (2011) Non-hematopoietic cells in lymph nodes drive memory CD8 T cell inflation  
543 during murine cytomegalovirus infection. *PLoS Pathog* 7 (10), e1002313.

544 62. Seckert, C.K. et al. (2011) Antigen-presenting cells of haematopoietic origin prime cytomegalovirus-  
545 specific CD8 T-cells but are not sufficient for driving memory inflation during viral latency. *Journal of*  
546 *General Virology* 92 (9), 1994-2005.

547 63. Hutchinson, S. et al. (2011) A dominant role for the immunoproteasome in CD8+ T cell responses to  
548 murine cytomegalovirus. *PLoS One* 6 (2), e14646.

549 64. Dekhtiarenko, I. et al. (2016) Peptide Processing Is Critical for T-Cell Memory Inflation and May Be  
550 Optimized to Improve Immune Protection by CMV-Based Vaccine Vectors. *PLoS Pathog* 12 (12),  
551 e1006072.

552 65. Kurz, S.K. et al. (1999) Focal transcriptional activity of murine cytomegalovirus during latency in the  
553 lungs. *J Virol* 73 (1), 482-94.

554 66. Seckert, C.K. et al. (2009) Liver sinusoidal endothelial cells are a site of murine cytomegalovirus  
555 latency and reactivation. *J Virol* 83 (17), 8869-84.

556 67. Simon, C.O. et al. (2006) CD8 T cells control cytomegalovirus latency by epitope-specific sensing of  
557 transcriptional reactivation. *J Virol* 80 (21), 10436-56.

558 68. Dekhtiarenko, I. et al. (2013) The context of gene expression defines the immunodominance  
559 hierarchy of cytomegalovirus antigens. *J Immunol* 190 (7), 3399-409.

560 69. Farrington, L.A. et al. (2013) Competition for antigen at the level of the APC is a major determinant of  
561 immunodominance during memory inflation in murine cytomegalovirus infection. *J Immunol* 190 (7),  
562 3410-6.

563 70. Borkner, L. et al. (2017) Immune Protection by a Cytomegalovirus Vaccine Vector Expressing a Single  
564 Low-Avidity Epitope. *J Immunol* 199 (5), 1737-1747.

565 71. Boppana, S.B. and Britt, W.J. (1996) Recognition of human cytomegalovirus gene products by HCMV-  
566 specific cytotoxic T cells. *Virology* 222 (1), 293-6.

567 72. Komatsu, H. et al. (2003) Population analysis of antiviral T cell responses using MHC class I-peptide  
568 tetramers. *Clin Exp Immunol* 134 (1), 9-12.

569 73. Hahn, G. et al. (1998) Cytomegalovirus remains latent in a common precursor of dendritic and  
570 myeloid cells. *Proceedings of the National Academy of Sciences* 95 (7), 3937-3942.

571 74. Kemball, C.C. et al. (2005) Late priming and variability of epitope-specific CD8+ T cell responses  
572 during a persistent virus infection. *J Immunol* 174 (12), 7950-60.

573 75. Vezys, V. et al. (2006) Continuous recruitment of naive T cells contributes to heterogeneity of  
574 antiviral CD8 T cells during persistent infection. *J Exp Med* 203 (10), 2263-9.

575 76. Richter, K. et al. (2012) Antigen amount dictates CD8+ T-cell exhaustion during chronic viral infection  
576 irrespective of the type of antigen presenting cell. *Eur J Immunol* 42 (9), 2290-304.

577 77. Prober, C.G. (2012) 204 - Herpes Simplex Virus A2 - Long, Sarah S. In *Principles and Practice of*  
578 *Pediatric Infectious Diseases (Fourth Edition)*, pp. 1026-1035.e3, Content Repository Only!

579 78. Grzimek, N.K. et al. (2001) Random, asynchronous, and asymmetric transcriptional activity of  
580 enhancer-flanking major immediate-early genes *ie1/3* and *ie2* during murine cytomegalovirus latency in  
581 the lungs. *J Virol* 75 (6), 2692-705.

582 79. Nakamoto, Y. and Kaneko, S. (2003) Mechanisms of viral hepatitis induced liver injury. *Curr Mol Med*  
583 3 (6), 537-44.

584 80. Bergthaler, A. et al. (2010) Viral replicative capacity is the primary determinant of lymphocytic  
585 choriomeningitis virus persistence and immunosuppression. *Proc Natl Acad Sci U S A* 107 (50), 21641-6.

586 81. Tay, S.S. et al. (2014) Antigen expression level threshold tunes the fate of CD8 T cells during primary  
587 hepatic immune responses. *Proc Natl Acad Sci U S A* 111 (25), E2540-9.

588 82. Utzschneider, D.T. et al. (2016) High antigen levels induce an exhausted phenotype in a chronic  
589 infection without impairing T cell expansion and survival. *J Exp Med* 213 (9), 1819-34.

590 83. Reddehase, M.J. et al. (1994) The conditions of primary infection define the load of latent viral  
591 genome in organs and the risk of recurrent cytomegalovirus disease. *J Exp Med* 179 (1), 185-93.

592 84. Redeker, A. et al. (2014) Viral inoculum dose impacts memory T-cell inflation. *Eur J Immunol* 44 (4),  
593 1046-57.

594 85. Pollock, J.L. and Virgin, H.W.t. (1995) Latency, without persistence, of murine cytomegalovirus in the  
595 spleen and kidney. *J Virol* 69 (3), 1762-8.

596 86. Wong, Y.C. et al. (2015) Immune outcomes in the liver: Is CD8 T cell fate determined by the  
597 environment? *J Hepatol* 63 (4), 1005-14.

598 87. Mueller, S.N. et al. (2007) Viral targeting of fibroblastic reticular cells contributes to  
599 immunosuppression and persistence during chronic infection. *Proc Natl Acad Sci U S A* 104 (39), 15430-5.

600 88. Welten, S.P. et al. (2015) The viral context instructs the redundancy of costimulatory pathways in  
601 driving CD8(+) T cell expansion. *Elife* 4. doi: 10.7554/eLife.07486

602 89. Xu, H.C. et al. (2014) Type I interferon protects antiviral CD8+ T cells from NK cell cytotoxicity.  
603 *Immunity* 40 (6), 949-60.

604 90. Crouse, J. et al. (2014) Type I interferons protect T cells against NK cell attack mediated by the  
605 activating receptor NCR1. *Immunity* 40 (6), 961-73.

606 91. Teijaro, J.R. et al. (2013) Persistent LCMV infection is controlled by blockade of type I interferon  
607 signaling. *Science* 340 (6129), 207-11.

608 92. Wilson, E.B. et al. (2013) Blockade of chronic type I interferon signaling to control persistent LCMV  
609 infection. *Science* 340 (6129), 202-7.

610 93. Dag, F. et al. (2014) Reversible silencing of cytomegalovirus genomes by type I interferon governs  
611 virus latency. *PLoS Pathog* 10 (2), e1003962.

- 612 94. Aubert, R.D. et al. (2011) Antigen-specific CD4 T-cell help rescues exhausted CD8 T cells during  
613 chronic viral infection. *Proc Natl Acad Sci U S A* 108 (52), 21182-7.
- 614 95. Matloubian, M. et al. (1994) CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses  
615 during chronic viral infection. *J Virol* 68 (12), 8056-63.
- 616 96. Waggoner, S.N. et al. (2011) Natural killer cells act as rheostats modulating antiviral T cells. *Nature*  
617 481 (7381), 394-8.
- 618 97. Humphreys, I.R. et al. (2007) OX40 costimulation promotes persistence of cytomegalovirus-specific  
619 CD8 T Cells: A CD4-dependent mechanism. *J Immunol* 179 (4), 2195-202.
- 620 98. Snyder, C.M. et al. (2009) CD4+ T cell help has an epitope-dependent impact on CD8+ T cell memory  
621 inflation during murine cytomegalovirus infection. *J Immunol* 183 (6), 3932-41.
- 622 99. Walton, S.M. et al. (2011) T-cell help permits memory CD8(+) T-cell inflation during cytomegalovirus  
623 latency. *Eur J Immunol* 41 (8), 2248-59.
- 624 100. Jones, M. et al. (2010) IL-10 restricts memory T cell inflation during cytomegalovirus infection. *J*  
625 *Immunol* 185 (6), 3583-92.
- 626 101. Almanan, M. et al. (2017) Tissue-specific control of latent CMV reactivation by regulatory T cells.  
627 *PLoS Pathog* 13 (8), e1006507.
- 628 102. Rochman, Y. et al. (2009) New insights into the regulation of T cells by gamma(c) family cytokines.  
629 *Nat Rev Immunol* 9 (7), 480-90.
- 630 103. Bachmann, M.F. et al. (2007) Differential role of IL-2R signaling for CD8+ T cell responses in acute  
631 and chronic viral infections. *Eur J Immunol* 37 (6), 1502-12.
- 632 104. Redeker, A. et al. (2015) The Quantity of Autocrine IL-2 Governs the Expansion Potential of CD8+ T  
633 Cells. *J Immunol* 195 (10), 4792-801.
- 634 105. Beltra, J.C. et al. (2016) IL2Rbeta-dependent signals drive terminal exhaustion and suppress memory  
635 development during chronic viral infection. *Proc Natl Acad Sci U S A* 113 (37), E5444-53.
- 636 106. Nanjappa, S.G. et al. (2011) Immunotherapeutic effects of IL-7 during a chronic viral infection in  
637 mice. *Blood* 117 (19), 5123-32.
- 638 107. Pellegrini, M. et al. (2011) IL-7 engages multiple mechanisms to overcome chronic viral infection  
639 and limit organ pathology. *Cell* 144 (4), 601-13.
- 640 108. Presti, R.M. et al. (1998) Interferon gamma regulates acute and latent murine cytomegalovirus  
641 infection and chronic disease of the great vessels. *J Exp Med* 188 (3), 577-88.
- 642 109. Ou, R. et al. (2001) Critical role for alpha/beta and gamma interferons in persistence of lymphocytic  
643 choriomeningitis virus by clonal exhaustion of cytotoxic T cells. *J Virol* 75 (18), 8407-23.
- 644 110. Moskophidis, D. et al. (1994) Resistance of lymphocytic choriomeningitis virus to alpha/beta  
645 interferon and to gamma interferon. *J Virol* 68 (3), 1951-5.
- 646 111. Yoon, H. et al. (2010) The cell cycle time of CD8+ T cells responding in vivo is controlled by the type  
647 of antigenic stimulus. *PLoS One* 5 (11), e15423.

648