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

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Abstract

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

Virus chronicity as hallmark of adaptation

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

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

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

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

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

Effects of chronic viral infections on the immune system

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

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

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

Immune exhaustion

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

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

Memory Inflation

144 Ongoing antigenic stimulation by latent herpesviruses, in particular by the β-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 IFNγ 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]).

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

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

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

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

Potential causes of differences between inflation and exhaustion

CD8 T-cell responses to chronic LCMV and MCMV differ in their functional capacity, but notably also display numerous similarities. The priming of naïve virus-specific CD8 T cells does not occur exclusively during primary LCMV or MCMV infection, since novel naïve cells are also recruited in the chronic phase [58, 74, 75]. T-cell responses to LCMV-encoded antigens [32] or inflationary MCMV epitopes are still maintained in immunoproteasome deficient mice [63]. The pool of responding T cells is substantially expanded by antigen-presentation on non-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 ($KLRG1^+$, $CD62L^-$, $CD27^-$, $CD127^-$) 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

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

Antigen abundance vs. scarcity

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

Cellular niche

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

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

T cell costimulation and inflammatory cytokines

We compared the priming of CD8 T-cell responses upon primary LCMV or MCMV infection and observed a clear difference in costimulatory signal ("signal 2") requirement [88]. We analyzed responses to the LCMV epitope KAVYNFATC (GP^{33}) upon infection with LCMV or a recombinant MCMV expressing the same epitope and showed that co-stimulation by CD80/86 is required for priming against GP^{33} when expressed by MCMV, but highly redundant with other signal 2 co-receptors in the context of LCMV infection. On the other hand, LCMV infection induced much stronger type I IFN responses. Soluble cytokines may co-stimulate T cells during priming ("signal 3") and priming against LCMV-encoded epitopes depended strongly on type I IFN-dependent signal 3 [88]. CD8 T-cells lacking type I IFN receptors are susceptible to NK-cell mediated apoptosis in chronic LCMV infection [89, 90], implying that IFN-α/β provides a critical survival signal to these cells. However, it remains unclear if CD8 T-cell survival depends on strong type I IFN responses to LCMV, or if tonic IFN responses would be sufficient. While high levels of type I IFN promote T-cell priming [88] in acute LCMV infection, they were also shown to support the onset of chronic virus infection [91, 92]. IL-12 and type I IFN responses are more balanced during MCMV infection, but type I IFN responses push MCMV into latency [93], implying that interferon induces the chronic state in both infections and that exhaustion or inflation pathways might be defined during the priming stage of T cells. While we observed clearly different priming requirements in MCMV and acute LCMV infections [88], a comparison of T-cell responses to GP^{33} 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 $CD8^+$ 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 (γ_c) cytokines IL-2, IL-7, and IL-15.*

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

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

Ag transcriptional repression vs. repression by killing of infected cells

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

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

Concluding remarks

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

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Figure legends

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

Table 1. Comparison of viral-specific CD8 T-cell populations[a]

	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	+/-	+/-	+

[a] - absent or low, +/- intermediate, + high, ++ prominent

References

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

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

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