Published in final edited form as:

Curr Opin Immunol. 2010 June ; 22(3): 348–354. doi:10.1016/j.coi.2010.03.004.

OPPOSING POSITIVE AND NEGATIVE REGULATION OF T CELL ACTIVITY DURING VIRAL PERSISTENCE

Laura M. Fahey and David G. Brooks

Department of Microbiology, Immunology and Molecular Genetics and the UCLA AIDS Institute, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California 90095

SUMMARY

Vigorous T cell responses are critical for the control of viral infections. However, in some instances antiviral T cell responses are suppressed resulting in viral persistence. The loss of T cell function is regulated by a variety of host-based immunosuppressive factors that directly inhibit antiviral immunity and prevent viral clearance. Nevertheless, residual levels of T cell activity are actively sustained to exert an important degree of control over persistent virus replication. How T cells are differentially regulated in response to persistent infection and the positive and negative signals that result in these divergent functional responses are just now beginning to come to light. Unraveling this complex dual counter-regulation of T cell responses during persistent virus infection will provide valuable insight toward the development of therapies to overcome immune suppression and stimulate T cell responses to eliminate persistent viral replication. In this review we will highlight this emerging field and discuss the complex interplay between immune-modulatory factors that suppress and sustain antiviral immunity to control and in some instances eliminate persistent viral replication.

IMMUNE INDUCTION

The majority of viral infections stimulate strong T cell responses that clear infection. Following viral infection, professional antigen-presenting cells [APC: B cells, macrophages and most notably dendritic cells (DC)] present viral peptides to T cells in a process termed priming. The combination of stimulating APC populations, the composition and level of stimulatory/inhibitory molecules displayed and the type of cytokines encountered during priming program T cell responses. In most situations, the initial priming induces a robust CD8 cytolytic T-lymphocyte (CTL) response that is responsible for killing virally infected cells and clearing infection (reviewed in [1,2]). Simultaneously, antiviral CD4 T cells proliferate and produce immune-modulatory and antiviral cytokines that direct and “help” the immune response to promote effective CD8 T cell and B cell development (reviewed in [3]). Together, these complex interactions and effector mechanisms are successful in purging the majority of viral infections.

In response to persistent viral infections, antiviral CD4 and CD8 T cells are either physically deleted or persist in a “non-functional” (exhausted) state, characterized by the inability to

© 2010 Elsevier Ltd. All rights reserved.

Corresponding Author: David G. Brooks, UCLA MIMG, University of California, Los Angeles, Los Angeles, CA 90095, Phone: 310-983-1073, Fax: 310-206-5321, dbrooks@microbio.ucla.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

proliferate, produce key antiviral and immune stimulating cytokines (e.g. IL-2, TNF α , IFN γ) or lyse infected cells [4–7]. This multiparameter loss of T cell function directly facilitates persistence [8–10]. T cell exhaustion is observed during a diverse range of persistent virus infections, including HIV, HCV, HBV in humans and lymphocytic choriomeningitis virus (LCMV) infection in rodents (reviewed in [11]), suggesting that aside from virus-encoded immune evasion strategies, common and conserved host-based suppressive mechanisms also inhibit T cell activity. As a result, similar therapeutic approaches to neutralize host immunosuppressive factors may be able to be implemented to restore T cell function and treat a wide range of persistent viral infections. Unlike these persistent virus infections characterized by sustained viremia, persistent viruses predominantly characterized by prolonged periods of latency (such as CMV and other herpes viruses) are often associated with functional T cell responses that rapidly control viral replication upon reactivation. In a novel twist, the sustained functional T cell responses by herpes viruses may ultimately provide protection against bacterial infections and potentially be effective to generate functional T cell responses to persistent viruses that otherwise induce T cell exhaustion [12,13].

Although generally assumed that the loss of T cell function is bad (which it is in terms of the ability to prevent viral persistence), it is likely exhaustion is a mechanism to prevent excessive immunopathology and mortality when antigen persists after a certain period of time. For example, using the mouse model of persistent LCMV infection, in situations that T cells do not initially exhaust or their numbers are therapeutically increased early following viral infection enhanced immunopathology and in many cases rapid death occurs [14,15]. Conversely, blockade of these factors or administration of the same therapies during the chronic phase of infection (once T cells have exhausted and contracted) elevates the number and functional capacity of virus-specific T cells without associated immunopathology or mortality [14,16•]. This difference in mortality observed at the two phases of persistent infection may be due to a variety of factors, including decreased levels of infected cells as viral loads plateau, alterations in the T cell effector mechanisms that control virus replication and/or the decrease in the absolute number of virus-specific T cells as infection progresses. Therapeutically this difference has two important implications (1) early therapeutic interventions to prevent exhaustion and increase the size of the virus-specific T cell population may increase morbidity and should be cautiously approached and (2) a negative impact of early treatment does not necessarily indicate a similar effect during the chronic phase of infection.

NEGATIVE REGULATION / IMMUNOSUPPRESSION DURING VIRAL PERSISTENCE

It has long been established that persistent viral infections are associated with immunosuppression and loss of T cell activity. The landmark finding that PD-1:PD-L1 interactions regulate T cell exhaustion during persistent LCMV infection and could be blocked to restore T cell activity [14] defined the dominant role of negative regulatory receptors during viral persistence. The suppressive role of PD-1 was rapidly extended to show that in vitro blockade of PD-1 restored HIV, HCV and HBV specific T cell responses (reviewed in [17]). Excitingly, a recent study by Velu et al. demonstrated that in vivo PD-1 blockade in SIV infected macaques enhanced T cell (and interestingly B cell) function leading to long-term control of virus replication [16•]. By extension, PD-1 blockade may also be effective to control HIV replication in vivo thereby setting the foundation for future restorative therapies in humans. In addition to PD-1, IL-10 has been identified as a critical immunosuppressive factor up-regulated during viral infection. We and others have found that the early production of IL-10 following persistent LCMV infection induces T cell exhaustion and directly leads to viral persistence [10,18]. Similar to PD-1, elevated IL-10 levels are also observed during HIV, HCV and HBV infections and correlate with diminished T cell activity and increased virus replication

(reviewed in [19]), suggesting IL-10 also suppresses immunity during these persistent infections.

It has become clear that multiple inhibitory factors (e.g., PD-1, IL-10, CTLA-4, Tim-3, Lag-3, TGF β) actively and simultaneously suppress antiviral T cell activity during viral persistence [20–23]. The level and composition of inhibitory receptors on a specific T cell correlates with its extent of exhaustion and the ability to restore function to the cell [20,24]. Mechanistically, these inhibitory factors appear to suppress antigen specific T cell proliferation, since blockade of these factors induces T cell proliferation but only modestly enhances T cell cytokine production from individual exhausted cells- although the increased proliferation increases the amount of functional cytokine producing cells [20,25]. Further, different inhibitory factors may regulate distinct aspects of T cell exhaustion [20,26], suggesting that exhaustion is a process resulting from multiple, converging signals. Thus, by blocking distinct inhibitory factors it may be possible to restore desired antiviral functions while limiting those that cause excessive immunopathology or autoimmunity.

Since many of these same inhibitory factors are also initially increased during acutely resolved infections, it appears that this is a normal response to immune activation. Yet, the question remains as to how virus replication triggers their expression and why their expression is down-regulated in acutely cleared infections and sustained during persistent infections. In instances when virus is cleared acutely, virus-specific T cells rapidly outnumber infected cells, whereas in the face of what will become a persistent infection, virus-specific T cells are rapidly outnumbered (and presumably overwhelmed) by infected cells [27]. Two recent studies suggest that the increased MHC:TcR itself potentiates CD8 T cell exhaustion [28,29]. Consistent with this mechanism, T cells present in sites of active virus replication exhibit increased levels of inhibitory factors and severe functional exhaustion [30,31]. The higher levels of antigen may additionally increase interaction with cells simultaneously expressing inhibitory factors thereby suppressing T cell function. The sustained expression of virus replication may trigger rheostat-like molecules that modulate expression of inhibitory factors. When virus replication is rapidly controlled, the initial expression of inhibitory factors is not sufficient to inhibit T cell responses. On the other hand, high amounts of continued virus replication may progressively increase inhibitory factor expression until a critical threshold is reached that induces T cell exhaustion. It will be important to determine the interplay between virus replication and inhibitory factor expression and how the immune environment “senses” and translates levels of virus replication into suppressive signals that limit T cell activity.

POSITIVE REGULATION DURING PERSISTENT VIRAL INFECTION

T cell responses are clearly inhibited during persistent virus infections. However, T cells still retain some level of residual function and exert control over virus replication during chronic infections. This is most apparent in the situation of HIV infection wherein progressive loss of T cells leads to increased virus titers, but is also observed in a variety of experimental models. For example, experimental deletion of CD4 T cells prior to LCMV or SIV infection enhances CD8 T cell exhaustion leading to increased virus replication [32–34]. Similarly, the magnitude and the breadth of the T cell response correlates with long-term control of HCV infection and maintenance of this response is necessary to prevent viral recrudescence [35]. Thus, in addition to suppressive factors, positive stimulatory signals are also implemented to sustain residual T cell function during viral persistence (Figure 1).

Multiple costimulatory interactions are important for inducing and shaping the primary response, but may also function to sustain responses during periods of prolonged virus infection (reviewed in [17]). Key pathways involved in priming T cell responses such as CD28/B7 interactions, CD40L and 4-1BB have all been implicated in sustaining T cell immunity during

viral persistence [36–39]. However, many of these studies utilized mice genetically deficient in these molecules making it difficult to differentiate ineffective priming versus long-term sustaining of T cells. Williams et al. simultaneously blocked the CD28 and CD40L pathways during an established persistent infection and observed a rapid decrease in LCMV-specific CD8 T cells and increase in virus replication, indicating that prolonged costimulation is either directly or indirectly required to sustain CD8 T cell control of persistent infection [40]. Similarly, B7-1/B7-2 stimulation is required to sustain adoptively transferred memory T cells to resolve persistent LCMV infection [41,42], again highlighting the need for enhanced/sustained stimulatory signals in the face of prolonged virus replication.

Unlike blocking inhibitory factors, which rapidly restores T cell function, therapies to amplify positive stimulation may be less effective due to the constraints imposed by the suppressive environment during persistent infection. Therapeutic vaccines are generally poor inducers of T cell function whereas these same vaccine agents are often immunogenic when given prior to infection. Two recent publications provide insight into this enigma and show that one reason for the failure of therapeutic vaccines is that they fail to neutralize the immunosuppressive environment [43•,44•]. Therapeutic vaccination in the presence of IL-10 or PD-L1 blocking antibodies allowed otherwise ineffective vaccines to now stimulate robust T cell responses that controlled infection. Then again certain factors may be better equipped to overcome the suppressive environment. Administration of high doses of IL-2 during persistent LCMV infection increased virus-specific CD8 T cells and reduced virus replication [45]. Further studies are needed to differentiate the role of these and other stimulatory pathways and the mechanisms that perpetuate/limit their activity during viral persistence.

CD4 T CELL HELP

The need for CD4 T cell help during viral infection has long been recognized and many aspects of immune exhaustion during persistent viral infection are associated with functions performed by CD4 T cells; e.g., programming memory T cell development [46–49], stimulating B cell differentiation and directing the production of antiviral antibodies [3]. Additionally, signals provided by CD4 T cells are required throughout viral persistence to sustain CD8 T cells and in the case of LCMV, to ultimately resolve persistent infection [33,34]. Following HCV infection, resolution of infection and resistance to re-infection is associated with robust CD4 T cell responses that sustain CD8 T cell activity [9,35,50]. Subsequent loss of CD4 T cell activity after initial control of HCV or LCMV allows the virus to re-emerge [35,51]. Despite their important impact, a defined mechanism of CD4 T cell help during persistent viral infections had remained elusive.

Three publications recently identified IL-21 as a key mechanism of CD4 T cell help to sustain CD8 T cell responses during persistent LCMV infection [52••,53••,54••]. Deletion of IL-21 activity did not hamper the initial T cell responses to persistent infection or to acutely cleared viral infections. However, in the absence of direct IL-21 signaling LCMV-specific CD8 T cells were physically deleted as persistent infection progressed and those CD8 T cells that remained exhibited a more severe functional exhaustion. The effect on CD8 T cells in the absence of IL-21 mediated help was similar to that observed in CD4-deficient situations despite the sustained presence of virus-specific CD4 T cells. Decreased IL-21 expression also correlates with loss of CD4 T cells and AIDS progression during HIV infection [55], suggesting a similar IL-21 mediated mechanism of CD4 T cell help. Interestingly, LCMV-specific CD8 T cells that could not respond to IL-2 also suffered a more severe exhaustion during persistent infection [56]. Although IL-2 expression is rapidly lost by both CD4 and CD8 T cells during persistent infection [5,57,58], CD4 T cells produce IL-2 early following infection with persistent LCMV and low levels of IL-2 producing CD4 T cells are observed as infection progresses [52••,57]. Thus, early IL-2 signals may institute a long term developmental program that sustains CD8

T cells during viral persistence as is observed for memory T cell development [59]. Alternatively, IL-2 and IL-21 may work in concert through persistent infection to sustain CD8 T cells, similar to the way multiple inhibitory factors work together to suppress CD8 T cell immunity. It will ultimately be important to define how and when CD4 T cells deliver these helper signals to modulate CD8 T cell activity and control persistent infection.

ALTERED T CELL DIFFERENTIATION

The differential T cell activity observed during persistent versus acute viral infections likely stems from an altered transcriptional program. To explore the relationship between transcriptional variations and T cell function in exhausted versus effector CD8 T cells, Wherry et al. compared gene expression during acute and chronic LCMV infection [60]. CD8 T cell responses to chronic and acute LCMV infection are initially similar; however, chronic infection rapidly induced a gene expression program distinct from functional effector or memory CD8 T cells, including dramatic changes in signal transduction factors, metabolic pathways, chemokines and the expression of chemokine receptors [60]. Interestingly, gene expression by exhausted CD8 T cells was most similar to the effector responses observed during acute infection (compared with naïve or memory responses), indicating that viral persistence drives a more differentiated form of effector responses and consistent with the need for prolonged control of virus replication. Extending these studies, Shin et al. identified Blimp1 as a key transcription factor responsible for driving terminal effector CD8 T cell differentiation and inhibitory receptor expression during viral persistent [26••]. In the absence of Blimp1, the expression of multiple inhibitory receptors was down-regulated on CD8 T cells. However, it was only in the presence of intermediate levels of Blimp1 that CTL activity and cytokine production were balanced to control virus replication, suggesting that Blimp1 is acting as a transcriptional rheostat regulating CD8 T cell function. In separate studies, a relationship between Blimp1 and terminal differentiation of effector memory responses to acutely cleared influenza and LCMV infections was observed, further substantiating the role of Blimp1 in driving terminal differentiation of effector CD8 T cells [61,62]. How CD4 helper T cell differentiation is impacted by persistent viral infection and how these changes affect other immune parameters is not yet known, but their progressive development into IL-21 producing cells [52••], indicates that their developmental program is also affected by prolonged virus replication. It will be fascinating to further uncover the epigenetic and transcriptional regulation of CD4 and CD8 T cell exhaustion and identify how the antigenic environment (e.g., the molecular signals and cellular interactions) potentiates these alterations. Collectively, these findings illustrate the complex regulation and relationship between functional and exhausted T cells and highlight that exhaustion is not a loss of function but instead is a tightly regulated developmental program.

CONCLUSION

T cell function is tightly regulated during persistent viral infection through a complex interplay of positive and negative immune-modulating factors. It is likely that each factor does not contribute equally to suppress or sustain T cell responses; but rather it is the compilation of these newly identified factors (and likely many others yet undiscovered) that sustain immune balance during persistent infection. Possibly the variations in the level and/or impact of these factors between individuals may explain inherent differences in the ability of each person to control the same viral infection and differing responses to antiviral/ immunostimulatory therapies. Furthermore, viral clearance is a multi-factorial process; B cells, NK cells, and other immune cells work in concert along with T cells to resolve viral infections. Thus, understanding this balance and delineating how different positive and negative immune modulators are sustained, regulate T cell exhaustion and control persistent viral replication will further aid in

the development of antiviral immunotherapeutic strategies to eliminate persistent viral infections.

Acknowledgments

Our work was supported by the UCLA Center for AIDS Research, the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA and grants from the National Institutes of Health (AI082975, AI085043 to D.G.B.).

REFERENCES

1. Zinkernagel RM. Lymphocytic choriomeningitis virus and immunology. *Curr Top Microbiol Immunol* 2002;263:1–5. [PubMed: 11987811]
2. Williams MA, Bevan MJ. Effector and Memory CTL Differentiation. *Annu Rev Immunol*. 2006
3. Castellino F, Germain RN. Cooperation between CD4+ and CD8+ T cells: when, where, and how. *Annu Rev Immunol* 2006;24:519–540. [PubMed: 16551258]
4. Gallimore A, Glithero A, Godkin A, Tissot AC, Pluckthun A, Elliott T, Hengartner H, Zinkernagel R. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I peptide complexes. *J Exp Med* 1998;187:1383–1393. [PubMed: 9565631]
5. Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J Virol* 2003;77:4911–4927. [PubMed: 12663797]
6. Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R. Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med* 1998;188:2205–2213. [PubMed: 9858507]
7. Brooks DG, McGavern DB, Oldstone MB. Reprogramming of antiviral T cells prevents inactivation and restores T cell activity during persistent viral infection. *J Clin Invest* 2006;116:1675–1685. [PubMed: 16710479]
8. Oldstone MB, Blount P, Southern PJ, Lampert PW. Cytoimmunotherapy for persistent virus infection reveals a unique clearance pattern from the central nervous system. *Nature* 1986;321:239–243. [PubMed: 3086743]
9. Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001;194:1395–1406. [PubMed: 11714747]
10. Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence in vivo. *Nat Med* 2006;12:1301–1309. [PubMed: 17041596]
11. Klenerman P, Hill A. T cells and viral persistence: lessons from diverse infections. *Nat Immunol* 2005;6:873–879. [PubMed: 16116467]
12. Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS, Miller VL, Virgin HW. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 2007;447:326–329. [PubMed: 17507983]
13. Hansen SG, Vieville C, Whizin N, Coyne-Johnson L, Siess DC, Drummond DD, Legasse AW, Axthelm MK, Oswald K, Trubey CM, et al. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat Med* 2009;15:293–299. [PubMed: 19219024]
14. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2005;439:682–687. [PubMed: 16382236]
15. Brooks DG. Unpublished observations.
16. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, Vanderford TH, Chennareddi L, Silvestri G, Freeman GJ, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. *Nature*. 2008 •• This study demonstrates that in vivo PD-1 blockade during chronic SIV infection results in enhanced virus-specific CD8 T cell and B cell immunity, leading to a reduction of plasma viral load.

Importantly, the enhanced immune response correlated to prolonged survival of SIV-infected macaques.

17. Crawford A, Wherry EJ. The diversity of costimulatory and inhibitory receptor pathways and the regulation of antiviral T cell responses. *Curr Opin Immunol*. 2009
18. Ejrnaes M, Filippi CM, Martinic MM, Ling EM, Togher LM, Crotty S, von Herrath MG. Resolution of a chronic viral infection after interleukin-10 receptor blockade. *J Exp Med* 2006;203:2461–2472. [PubMed: 17030951]
19. Blackburn S, Wherry EJ. IL-10, T cell exhaustion and viral persistence. *Trends in Microbiology* 2007;15:143–146. [PubMed: 17336072]
20. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, Betts MR, Freeman GJ, Vignali DA, Wherry EJ. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009;10:29–37. [PubMed: 19043418] • This paper identified that co-expression of multiple inhibitory receptors (i.e., PD1, LAG3) act in concert to regulate CD8 T cell exhaustion and the severity of infection. It was also found that the pattern of inhibitory receptor co-expression correlates to the extent of exhaustion, indicating that a multi-targeted therapeutic strategy may provide added benefit.
21. Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, Long BR, Wong JC, Satkunarajah M, Schweneker M, Chapman JM, et al. Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. *J Exp Med* 2008;205:2763–2779. [PubMed: 19001139]
22. Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, Miura T, Palmer S, Brockman M, Rathod A, Piechocka-Trocha A, et al. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. *Nat Immunol* 2007;8:1246–1254. [PubMed: 17906628]
23. Tinoco R, Alcalde V, Yang Y, Sauer K, Zuniga EI. Cell-intrinsic transforming growth factor-beta signaling mediates virus-specific CD8+ T cell deletion and viral persistence in vivo. *Immunity* 2009;31:145–157. [PubMed: 19604493]
24. Blackburn SD, Shin H, Freeman GJ, Wherry EJ. Selective expansion of a subset of exhausted CD8 T cells by alphaPD-L1 blockade. *Proc Natl Acad Sci U S A* 2008;105:15016–15021. [PubMed: 18809920]
25. Brooks DG, Ha SJ, Elsaesser H, Sharpe AH, Freeman GJ, Oldstone MB. IL-10 and PD-L1 operate through distinct pathways to suppress T-cell activity during persistent viral infection. *Proc Natl Acad Sci U S A* 2008;105:20428–20433. [PubMed: 19075244] • This study found that the immunosuppressive factors, IL-10 and PD-L1 function through distinct pathways. Consequently, simultaneous blockade of both factors was more effective in inducing T cell immunity and viral clearance than inhibition of either target alone. These results imply that antiviral restorative approaches may be most effective when targeting multiple immunosuppressive factors simultaneously.
26. Shin H, Blackburn SD, Intlekofer AM, Kao C, Angelosanto JM, Reiner SL, Wherry EJ. A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. *Immunity* 2009;31:309–320. [PubMed: 19664943] • This study was the first to identify a specific transcription factor that regulates CD8 T cell exhaustion during persistent infection.
27. Li Q, Skinner PJ, Ha SJ, Duan L, Mattila TL, Hage A, White C, Barber DL, O'Mara L, Southern PJ, et al. Visualizing antigen-specific and infected cells in situ predicts outcomes in early viral infection. *Science* 2009;323:1726–1729. [PubMed: 19325114]
28. Bucks CM, Norton JA, Boesteanu AC, Mueller YM, Katsikis PD. Chronic antigen stimulation alone is sufficient to drive CD8+ T cell exhaustion. *J Immunol* 2009;182:6697–6708. [PubMed: 19454664]
29. Mueller SN, Ahmed R. High antigen levels are the cause of T cell exhaustion during chronic viral infection. *Proc Natl Acad Sci U S A* 2009;106:8623–8628. [PubMed: 19433785]
30. Nakamoto N, Kaplan DE, Coleclough J, Li Y, Valiga ME, Kaminski M, Shaked A, Olthoff K, Gostick E, Price DA, et al. Functional restoration of HCV-specific CD8 T cells by PD-1 blockade is defined by PD-1 expression and compartmentalization. *Gastroenterology* 2008;134:1927–1937. 1937 e1921–1922. [PubMed: 18549878]

31. Blattman JN, Wherry EJ, Ha SJ, van der Most RG, Ahmed R. Impact of epitope escape on PD-1 expression and CD8 T-cell exhaustion during chronic infection. *J Virol* 2009;83:4386–4394. [PubMed: 19211743]
32. Vaccari M, Mattapallil J, Song K, Tsai WP, Hryniewicz A, Venzon D, Zanetti M, Reimann KA, Roederer M, Franchini G. Reduced protection from simian immunodeficiency virus SIVmac251 infection afforded by memory CD8+ T cells induced by vaccination during CD4+ T-cell deficiency. *J Virol* 2008;82:9629–9638. [PubMed: 18667509]
33. Battegay M, Moskophidis D, Rahemtulla A, Hengartner H, Mak TW, Zinkernagel RM. Enhanced establishment of a virus carrier state in adult CD4+ T-cell-deficient mice. *J Virol* 1994;68:4700–4704. [PubMed: 7911534]
34. Matloubian M, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T cell responses during chronic viral infection. *J Virol* 1994;68:8056–8063. [PubMed: 7966595]
35. Gerlach JT, Diepolder HM, Jung MC, Gruener NH, Schraut WW, Zachoval R, Hoffmann R, Schirren CA, Santantonio T, Pape GR. Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T-cell response in acute hepatitis C. *Gastroenterology* 1999;117:933–941. [PubMed: 10500077]
36. Fuse S, Zhang W, Usherwood EJ. Control of memory CD8+ T cell differentiation by CD80/CD86-CD28 costimulation and restoration by IL-2 during the recall response. *J Immunol* 2008;180:1148–1157. [PubMed: 18178855]
37. Fuse S, Bellfy S, Yagita H, Usherwood EJ. CD8+ T cell dysfunction and increase in murine gammaherpesvirus latent viral burden in the absence of 4-1BB ligand. *J Immunol* 2007;178:5227–5236. [PubMed: 17404306]
38. Whitmire JK, Flavell RA, Grewal IS, Larsen CP, Pearson TC, Ahmed R. CD40-CD40 ligand costimulation is required for generating antiviral CD4 T cell responses but is dispensable for CD8 T cell responses. *J Immunol* 1999;163:3194–3201. [PubMed: 10477587]
39. Kemball CC, Lee ED, Szomolanyi-Tsuda E, Pearson TC, Larsen CP, Lukacher AE. Costimulation requirements for antiviral CD8+ T cells differ for acute and persistent phases of polyoma virus infection. *J Immunol* 2006;176:1814–1824. [PubMed: 16424212]
40. Williams MA, Onami TM, Adams AB, Durham MM, Pearson TC, Ahmed R, Larsen CP. Cutting edge: persistent viral infection prevents tolerance induction and escapes immune control following CD28/CD40 blockade-based regimen. *J Immunol* 2002;169:5387–5391. [PubMed: 12421910]
41. Garidou L, Heydari S, Truong P, Brooks DG, McGavern DB. Therapeutic memory T cells require costimulation for effective clearance of a persistent viral infection. *J Virol* 2009;83:8905–8915. [PubMed: 19553326]
42. Lauterbach H, Zuniga EI, Truong P, Oldstone MB, McGavern DB. Adoptive immunotherapy induces CNS dendritic cell recruitment and antigen presentation during clearance of a persistent viral infection. *J Exp Med* 2006;203:1963–1975. [PubMed: 16847068]
43. Brooks DG, Lee AM, Elsaesser H, McGavern DB, Oldstone MB. IL-10 blockade facilitates DNA vaccine-induced T cell responses and enhances clearance of persistent virus infection. *J Exp Med* 2008;205:533–541. [PubMed: 18332180] • See annotation to Ref. [44•]
44. Ha SJ, Mueller SN, Wherry EJ, Barber DL, Aubert RD, Sharpe AH, Freeman GJ, Ahmed R. Enhancing therapeutic vaccination by blocking PD-1-mediated inhibitory signals during chronic infection. *J Exp Med* 2008;205:543–555. [PubMed: 18332181] • Together with Ref. [43•], this study demonstrates that blocking inhibitory signals such as PD-1 or IL-10 is a means of improving ineffective therapeutic vaccination strategies to treat persistent infections. These studies highlight the critical role of the immunosuppressive environment in limiting T cell stimulating vaccines and define new a new approach to enhance current therapeutic strategies.
45. Blattman JN, Grayson JM, Wherry EJ, Kaech SM, Smith KA, Ahmed R. Therapeutic use of IL-2 to enhance antiviral T-cell responses in vivo. *Nat Med* 2003;9:540–547. [PubMed: 12692546]
46. Dooks H, Wolslegel K, Lin P, Abbas AK. Interleukin-2 enhances CD4+ T cell memory by promoting the generation of IL-7R{alpha}-expressing cells. *J Exp Med*. 2007
47. Janssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. *Nature* 2003;421:852–856. [PubMed: 12594515]

48. Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science* 2003;300:337–339. [PubMed: 12690201]
49. Sun JC, Bevan MJ. Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science* 2003;300:339–342. [PubMed: 12690202]
50. Grakoui A, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghayeb J, Murthy KK, Rice CM, Walker CM. HCV persistence and immune evasion in the absence of memory T cell help. *Science* 2003;302:659–662. [PubMed: 14576438]
51. Ou R, Zhou S, Huang L, Moskophidis D. Critical role for alpha/beta and gamma interferons in persistence of lymphocytic choriomeningitis virus by clonal exhaustion of cytotoxic T cells. *J Virol* 2001;75:8407–8423. [PubMed: 11507186]
52. Elsaesser H, Sauer K, Brooks DG. IL-21 is required to control chronic viral infection. *Science* 2009;324:1569–1572. [PubMed: 19423777] •• Along with Refs. [53••] and [54••] this study first identified IL-21 as a novel and critical component of CD4 T cell help during persistent viral infection. It was demonstrated that CD8 T cells require IL-21 signaling to avoid deletion and sustain residual levels of antiviral function needed to resolve persistent LCMV infection.
53. Yi JS, Du M, Zajac AJ. A vital role for interleukin-21 in the control of a chronic viral infection. *Science* 2009;324:1572–1576. [PubMed: 19443735] •• See annotation to Ref. [52••]
54. Frohlich A, Kisielow J, Schmitz I, Freigang S, Shamshiev AT, Weber J, Marsland BJ, Oxenius A, Kopf M. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. *Science* 2009;324:1576–1580. [PubMed: 19478140] •• See annotation to Ref. [52••]
55. Iannello A, Boulassel MR, Samarani S, Debbeche O, Tremblay C, Toma E, Routy JP, Ahmad A. Dynamics and Consequences of IL-21 Production in HIV-Infected Individuals: A Longitudinal and Cross-Sectional Study. *J Immunol*. 2009
56. Bachmann MF, Wolint P, Walton S, Schwarz K, Oxenius A. Differential role of IL-2R signaling for CD8+ T cell responses in acute and chronic viral infections. *Eur J Immunol* 2007;37:1502–1512. [PubMed: 17492805]
57. Brooks DG, Teyton L, Oldstone MB, McGavern DB. Intrinsic functional dysregulation of CD4 T cells occurs rapidly following persistent viral infection. *Journal of Virology* 2005;79:10514–10527. [PubMed: 16051844]
58. Fuller MJ, Zajac AJ. Ablation of CD8 and CD4 T cell responses by high viral loads. *J Immunol* 2003;170:477–486. [PubMed: 12496434]
59. Williams MA, Tyznik AJ, Bevan MJ. Interleukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells. *Nature* 2006;441:890–893. [PubMed: 16778891]
60. Wherry EJ, Ha SJ, Kaech SM, Haining WN, Sarkar S, Kalia V, Subramaniam S, Blattman JN, Barber DL, Ahmed R. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* 2007;27:670–684. [PubMed: 17950003]
61. Kallies A, Xin A, Belz GT, Nutt SL. Blimp-1 transcription factor is required for the differentiation of effector CD8(+) T cells and memory responses. *Immunity* 2009;31:283–295. [PubMed: 19664942]
62. Rutishauser RL, Martins GA, Kalachikov S, Chande A, Parish IA, Meffre E, Jacob J, Calame K, Kaech SM. Transcriptional repressor Blimp-1 promotes CD8(+) T cell terminal differentiation and represses the acquisition of central memory T cell properties. *Immunity* 2009;31:296–308. [PubMed: 19664941]

Figure 1.

The balance between positive and negative immune regulators during persistent infections. (A) Throughout a persistent viral infection both positive (e.g., IL-21, IL-2, CD4 T cell help, B cells and antibodies) and negative (e.g., PD-1, Lag3, Tim3, CTLA4, IL-10 and TGF- β) regulatory factors maintain equilibrium within the immune system. This fine balance is critical in sustaining T cell responses while preventing lethal immunopathology. Disrupting the equilibrium between the positive and negative factors leads to dramatic shifts in the immune response. (B) Blocking of negative regulatory factors restores T cell activity leading to enhanced control and in some cases clearance of persistent infections. (C) Conversely, inhibition of positive regulators leads to increased T cell exhaustion and/or deletion,

consequently resulting in the failure to control persistent infections. This is clearly a simplified explanation of a complex situation and it will be important to figure out precisely how this balance between positive and negative regulatory factors results in differential virologic outcomes.