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Toxoplasma: Immunity and Pathogenesis

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Abstract

Toxoplasma gondii infection induces a strong immunity in the host. Although the response is manifested by innate response during early infection, adaptive immunity is critical for long-term protection. Amongst the adaptive immune response CD4 T cells play an important helper role for CD8 T cells which are the primary effector cells responsible for controlling the infection. Notwithstanding the induction of robust CD8 T immunity during acute infection, the parasite is not eradicated. One of the reasons for this is the functional exhaustion of CD8 T cells during latent infection. Recent studies from our laboratory have reported that primary cause of CD8 T cell exhaustion is compromised CD4 T cell help during latent toxoplasmosis. CD8 T cell dysfunctionality is preceded by CD4 exhaustion and effector immunity is severely compromised

Summary:

Prevention of loss of CD4 T cell function can be important therapeutic strategy for controlling chronic toxoplasmosis and preventing reactivation of latent infection.

Keywords

Toxoplasma; CD8 T cells; CD4 T cells; IL-12; IL-21; Exhaustion

Introduction:

Toxoplasma gondii (*T. gondii*) is an intracellular protozoan parasite of medical importance. The Centers for Disease Control and Prevention (CDC) has reported that, in the United States, *T. gondii* infection is an important foodborne illness and affects more than 60 million people. *T. gondii* is found worldwide with a prevalence rate of 10 to 20% in United States and over 50% in parts of Europe and Africa [1-3]. Toxoplasma encephalitis (TE), due to reactivation of latent *T. gondii* infection, is still among the most prevalent central nervous system infection in patients with AIDS and can be fatal [4]. In a recent study, the worst neurocognitive disorders in HIV infected individuals were reported to be associated with

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Conflict of Interest

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latent toxoplasmosis [5]. Other serious complications of *T. gondii* infection, such as myocarditis, are also seen in HIV-infected patient population [6]. First time *Toxoplasma gondii* infection of pregnant women can cause congenital diseases in fetuses and newborn children [7].

Protective immune responses against *T. gondii*:

Like many other intracellular pathogens, innate immune response acts as a first line of defense against *T. gondii* infection [8]. Infected hosts need to detect the invasion so as to prevent its dissemination [9]. Role of TLRs that recognize microbial components called pathogen-associated molecular patterns (PAMPs) has been demonstrated [10]. Furthermore, innate immune components like macrophages, dendritic cells and NK cells during the early stages of acute infection play an important role in restricting parasite multiplication [11]. With regards to the innate immunity, IL-12 producing dendritic cells play a critical role not only in stimulating the innate immune response [12], but also in shaping the adaptive immunity, which is essential for ultimate control of parasite infection [13]. Among the T cell subsets, the CD4 population, due to their ability to produce cytokines like IFN γ , are important for controlling the early stage of the acute infection [14], while the CD8 T cell subset is critical for the long-term protection and to keep the infection in a chronic state [15]. The protective role of CD8 T cells against *T. gondii* infection was demonstrated in mice immunized with the major membrane protein (SAG-1) that developed a strong cytolytic response [16]. Subsequently, the same group reported that antigen-specific CD8 T cell clone protected the naïve animals against a lethal infection [17]. Moreover, the important role of CD8 T cells in the protection against *T. gondii* infection was also demonstrated by other laboratories. In these studies, CD8 CTL (cytotoxic T lymphocytes) generated against a vaccine strain of the parasite protected the animals against a lethal challenge with a virulent strain of the parasite [18]. In a report conducted by different group, the importance of CD8 T cells in controlling toxoplasma cyst burden was demonstrated [19]. Similarly, other groups have confirmed the dominant role of CD8 T cells in long-term immunity against *Toxoplasma gondii* infection, that is important to keep the chronic infection under control [20] [21] [22]. The mechanism of CD8 T cell mediated protection during the late stages of the acute infection can be attributed to their ability to produce IFN γ [17], which plays a pivotal role in immune-protection against *T. gondii* infection [23]. However, during chronic toxoplasmosis perforin dependent cytotoxic ability of CD8 T cell population is involved in restricting the parasite to chronic state [24].

Notwithstanding the strong CD8 T cell response induced during *T. gondii* infection, to date, a sterile immunity has not been achieved and this persistent infection continues to pose a constant risk of re-activation in infected individuals. Why robust CD8 T cell immunity fails to cause total eradication of the parasite is an important question. In mice susceptible to toxoplasmic encephalitis, it has been observed that although the animals develop a vigorous CD8 T cell effector immunity, the memory response is severely compromised [25]. In this review, we will discuss the available information related to the development of a long-term CD8 T cell immunity against *T. gondii* infection and the factors involved in the maintenance of this response.

Development of CD8 T cell immunity and maintenance of their functionality:

CD8 T cell response is induced by their interaction with antigen-presenting cells which is followed by presentation of peptide. [26]. It has been demonstrated that outcome of infection can be influenced by the differences in MHC haplotype [27, 28]. These findings accentuate the critical role of CD8 T cell immunity in the protective immunity against the *T.gondii* infection. Studies performed using transgenic mice expressing human MHC class I genes have emphasized allelic dependence for the restricting toxoplasma infection. [27]. Thus identification of *T.gondii* specific epitope(s) will be highly beneficial in discerning of antigens responsible for induction of CD8 T cell immune response against the parasite. Notwithstanding the fact that number of antigens and secreted proteins (e.g. dense granules, microneme and rhoptry) have been categorized as T cell antigens, whether epitopes present in these antigens comprise of dominant *T. gondii*-specific CD8 T cell response in the infected mice remains to be determined. [29, 30, 28, 31, 32]. Till date most of the CD8 epitopes have been identified by BALB/C (H-2 L) mice, H-2K (C57BL/6) restricted CD8 T cell epitope derived from Tgd057 (a protein of unknown function) has been reported[33]. Unlike viral infections, *T.gondii* is a complex parasite and task of identifying T cell epitopes is a daunting process. However, studies related to identification of multiple CD8 T cell epitopes will prove to be a significant advancement in the field that will determine their specificity in terms of stage-specific recognition. This information will be highly essential in designing CD8-based vaccines against various stages of *Toxoplasma* infection.

The importance of CD8 T cells in controlling chronic toxoplasmosis was demonstrated by antibody depletion studies. In these experiments, treatment of chronically infected mice with anti-CD8 antibody led to the reactivation of the latent infection [34, 35]. However, the biggest challenge in restricting the infection to the chronic phase, is the fact that CD8 T cells need to be maintained in a functional state. Studies conducted in an encephalitis model have demonstrated that during the later stages of chronic infection, CD8 T cells exhibit a graded increase in the expression of inhibitory receptor PD-1 resulting in their dysfunctionality/exhaustion [35]. CD8 T-cell exhaustion has been reported in several other chronic viral infections, like LCMV, HBV, HCV, HIV, and Simian Immunodeficiency Virus, which are characterized by high levels of persisting viremia ([36, 37]. Similar to these viral infections, blockade of the PD-1-PDL-1 pathway in mice carrying chronic toxoplasma infection reinvigorates the suboptimal CD8 T-cell response, resulting in the control of parasites reactivation and prevention of mortality [35]. Interestingly, in continuation of these studies, it was observed that PD-1 is preferentially expressed on polyfunctional memory CD8 T cells, which leads to their loss of functionality and renders them susceptible to apoptosis [25]. The selective dysfunctionality in memory CD8 population could be an impediment for the development of a robust CD8 T cell response needed for long-term protection against the infection.

Role of CD4 T cells in CD8 T cell functionality during chronic toxoplasmosis:

T. gondii induces a strong CD4 T cell response that is a major source of IFN γ both during acute, as well as chronic infection [14, 34], and is similar to what has also been observed in other infections [38]. Earlier studies, including those from our laboratory, have demonstrated the importance of CD4 T cells for the maintenance of the CD8 T cell responses against intracellular pathogens [39-41]. It is believed that CD8 T cells play an important effector role during *T. gondii* infection, while the CD4 T cell subset provides the essential help needed for their maintenance. It has been reported that depletion of both CD4 and CD8 T cell populations results in the reactivation of latent toxoplasmosis and, as a consequence, the animals susceptible to TE succumb to the infection [41]. Similarly, in the human population, the emergence of severe toxoplasmosis is concomitant with a decline in CD4 T cell numbers in patients infected with HIV [42]. Although depleted CD4 numbers in HIV patients cause increased susceptibility to TE, most cases of toxoplasmosis in HIV patients occur during the late stage of HIV infection (advanced AIDS), when a deficiency in CD8 T cells due to the decreased CD4 population is also evident [42]. Overall, the depleted CD4 population during the late stages of HIV infection compromises the CD8 T cell immunity against the chronic (latent) toxoplasmosis leading to reactivation of the infection.

As stated above, several studies conducted in our laboratory have demonstrated severe CD8 T cell dysfunctionality during chronic toxoplasma infection [25, 35, 43]. In recent publications, we reported that the dysfunction observed in the CD8 T cell population from chronically infected mice is due to inadequate help caused by CD4 exhaustion [44, 45]. This was established by our findings which showed that transfer of antigen-specific non-exhausted CD4 T cells to chronically infected mice reversed CD8 dysfunctionality and prevented reactivation of latent infection [44]. **A recent report emphasized that a durable CD4 T cell response is more efficient in promoting a robust CD8 T cell immunity against tumors than targeting the CD8 T cells directly** [46]. It is believed that the “strong cytokine producing help” from CD4 T cells facilitates the maintenance of CD8 functionality [47, 48]. Thus, the underlying causes for CD4 T cell dysfunction during chronic toxoplasmosis need to be investigated to better understand how to keep the latent infection under control. Moreover, understanding the mechanism(s) by which CD4 T cells ensures the maintenance of a robust CD8 T cell immunity will provide important insights into the development of new therapeutic strategies that would allow the maintenance of functional CD8 T cell memory, therefore preventing TE in infected immunosuppressed patients with latent *T. gondii* infection.

CD4 T cell exhaustion during chronic toxoplasmosis:

The important question that needed attention is why CD4 T cells during chronic toxoplasmosis become exhausted/dysfunctional? Exhausted CD4 population expressed elevated levels of multiple inhibitory receptors concomitant with the reduced functionality and up-regulation of Blimp-1, a transcription factor. Although Blimp-1 has been intrinsically linked with CD8 T cell exhaustion [49], studies from our laboratory have demonstrated that

ablation of this transcription factor in CD4 T cells reverses their exhaustion status and allows them to provide essential help to CD8 T cells. This restores long-term CD8 functionality and prevents reactivation of the latent infection [44]. Recent studies from our laboratory have demonstrated that ablation of Blimp-1 on CD4 T cells causes an upregulation of positive co-stimulatory molecules especially 4-1BB (fig 1A) and to some extent OX-40 (fig 1B). Apart from the TCR signal and cytokine milieu, one of the most important factors governing the CD4 response is the balance between **positive signals from costimulatory receptors and negative signals from inhibitory receptors** [50]. It has been postulated that the interplay between signals from co-stimulatory molecules and inhibitory receptors play an important role in T cell activation, differentiation, effector function, and exhaustion [50]. In this regard, it is important to report that as stated in a recent review, a robust therapeutic response against infectious diseases or cancer requires not only blocking the inhibitory receptors, but also stepping targeting the appropriate T cell co-stimulatory molecules to promote the expansion and functionality of T cells [51]. Significantly, treatment of infected animals with a 4-1BB agonist increased the ability of these animals to control parasite multiplication (fig 2) and also increased the ability of infected animals to release IL-21 (Khan *et al.* unpublished studies), a cytokine produced predominantly by CD4 T cells [52] and known to be important for the maintenance of CD8 T cell functionality [53]. Molecular mechanism(s) involved in Blimp-1/4-1BB mediated CD4 T cell dysfunction during chronic toxoplasmosis that leads to CD8 exhaustion in chronically infected animals needs further investigation.

Overall conclusions:

T. gondii induces a vigorous innate and adaptive immune response manifested by a strong T cell immunity. As stated earlier, amongst the T cells, CD8 T cells play a predominant role in effector immunity and the CD4 T cell subset provides an essential helper role needed for the maintenance of the former [34, 41]. There is a consensus that CD8 T cells are critical for the long-term protection against *T. gondii* infection, especially during the chronic phase of the disease [20, 35]. Effectively, although the infection generates a robust CD8 T cell effector response during the acute infection, long-term immunity is compromised due to exhaustion of memory cells which leads to reduced functionality [47]. Recent studies from our laboratory have demonstrated this to be a sequela of CD4 T cell exhaustion that deprives CD8 T cells of essential help. Studies from our laboratory have demonstrated that CD4 T cell dysfunction can be attributed to up-regulation of Blimp-1, that leads to imbalance between inhibitory receptors (like PD-1, LAG-3, 2B4) and positive costimulatory molecules (like 4-1BB) [44]. This compromises CD4 T cell response (like cytokine production: IL-21) and deprives CD8 T cells the help needed for maintenance of their functionality (IFN γ production and cytotoxicity) (figure 3). Overall, agonist for costimulatory molecules (like 4-1BB) which are being used in clinical trials for cancer therapy may be used as an adjuvant in vaccines against infectious diseases when CD8 T cells are involved in host protection.

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References:

Recently published papers of particular interest have been highlighted as:

● Of importance

●● Of major importance

1. Luft BJ and Castro KG, An overview of the problem of toxoplasmosis and pneumocystosis in AIDS in the USA: implication for future therapeutic trials. *Eur J Clin Microbiol Infect Dis*, 1991 10(3): p. 178–81. [PubMed: 2060523]
2. Clumeck N and Hermans P, New therapeutic approaches in the acquired immune deficiency syndrome. *Antibiot Chemother* (1971), 1991 43: p. 235–56. [PubMed: 1883219]
3. Zumla A, et al., Toxoplasma serology in Zambian and Ugandan patients infected with the human immunodeficiency virus. *Trans R Soc Trop Med Hyg*, 1991 85(2): p. 227–9. [PubMed: 1887478]
4. Montoya JG and Liesenfeld O, Toxoplasmosis. *Lancet*, 2004 363(9425): p. 1965–76. [PubMed: 15194258]
5. Bharti AR, et al., Latent Toxoplasma Infection and Higher Toxoplasma gondii Immunoglobulin G Levels Are Associated With Worse Neurocognitive Functioning in HIV-Infected Adults. *Clin Infect Dis*, 2016.
6. Cuervo G, et al., Toxoplasma myocarditis: a rare but serious complication in an HIV-infected late presenter. *AIDS*, 2016 30(14): p. 2253–4. [PubMed: 27574795]
7. Kravetz JD and Federman DG, Prevention of toxoplasmosis in pregnancy: knowledge of risk factors. *Infect Dis Obstet Gynecol*, 2005 13(3): p. 161–5. [PubMed: 16126501]
8. Gazzinelli RT, et al., Innate resistance against Toxoplasma gondii: an evolutionary tale of mice, cats, and men. *Cell Host Microbe*, 2014 15(2): p. 132–8. [PubMed: 24528860]
9. Sasai M, Pradipta A, and Yamamoto M, Host immune responses to Toxoplasma gondii. *Int Immunol*, 2018 30(3): p. 113–119. [PubMed: 29408976]
10. Sasai M and Yamamoto M, Pathogen recognition receptors: ligands and signaling pathways by Toll-like receptors. *Int Rev Immunol*, 2013 32(2): p. 116–33. [PubMed: 23570313]
11. Yarovsky F, Innate immunity to Toxoplasma gondii infection. *Nat Rev Immunol*, 2014 14(2): p. 109–21. [PubMed: 24457485]
12. Denkers EY, et al., Neutrophils, dendritic cells and Toxoplasma. *Int J Parasitol*, 2004 34(3): p. 411–21. [PubMed: 15003500]
13. Shah S, et al., An extrafollicular pathway for the generation of effector CD8(+) T cells driven by the proinflammatory cytokine, IL-12. *Elife*, 2015 4.
14. Liesenfeld O, et al., Association of CD4+ T cell-dependent, interferon-gamma-mediated necrosis of the small intestine with genetic susceptibility of mice to peroral infection with Toxoplasma gondii. *J Exp Med*, 1996 184(2): p. 597–607. [PubMed: 8760813]
15. Hwang S and Khan IA, CD8+ T cell immunity in an encephalitis model of Toxoplasma gondii infection. *Semin Immunopathol*, 2015 37(3): p. 271–9. [PubMed: 25944514]
16. Khan IA, Smith KA, and Kasper LH, Induction of antigen-specific parasitocidal cytotoxic T cell splenocytes by a major membrane protein (P30) of Toxoplasma gondii. *J Immunol*, 1988 141(10): p. 3600–5. [PubMed: 2460541]
17. Khan IA, Ely KH, and Kasper LH, Antigen-specific CD8+ T cell clone protects against acute Toxoplasma gondii infection in mice. *J Immunol*, 1994 152(4): p. 1856–60. [PubMed: 7907106]
18. Gazzinelli RT, et al., Synergistic role of CD4+ and CD8+ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated Toxoplasma gondii vaccine. *J Immunol*, 1991 146(1): p. 286–92. [PubMed: 1670604]
19. Brown CR and McLeod R, Class I MHC genes and CD8+ T cells determine cyst number in Toxoplasma gondii infection. *J Immunol*, 1990 145(10): p. 3438–41. [PubMed: 2121825]
20. Suzuki Y, et al., Removal of Toxoplasma gondii cysts from the brain by perforin-mediated activity of CD8+ T cells. *Am J Pathol*, 2010 176(4): p. 1607–13. [PubMed: 20167872]

21. Feliu V, et al., Location of the CD8 T cell epitope within the antigenic precursor determines immunogenicity and protection against the *Toxoplasma gondii* parasite. *PLoS Pathog*, 2013 9(6): p. e1003449. [PubMed: 23818852]
22. Bhadra R, Cobb DA, and Khan IA, Donor CD8+ T cells prevent *Toxoplasma gondii* de-encystation but fail to rescue the exhausted endogenous CD8+ T cell population. *Infect Immun*, 2013 81(9): p. 3414–25. [PubMed: 23817617]
23. Suzuki Y, et al., Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science*, 1988 240(4851): p. 516–8. [PubMed: 3128869]
24. Denkers EY, et al., Perforin-mediated cytotoxicity plays a limited role in host resistance to *Toxoplasma gondii*. *J Immunol*, 1997 159(4): p. 1903–8. [PubMed: 9257855]
25. Bhadra R, Gigley JP, and Khan IA, PD-1-mediated attrition of polyfunctional memory CD8+ T cells in chronic toxoplasma infection. *J Infect Dis*, 2012 206(1): p. 125–34. [PubMed: 22539813]
26. Xia D, Hao S, and Xiang J, CD8+ cytotoxic T-APC stimulate central memory CD8+ T cell responses via acquired peptide-MHC class I complexes and CD80 costimulation, and IL-2 secretion. *J Immunol*, 2006 177(5): p. 2976–84. [PubMed: 16920933]
27. Brown CR, et al., Effects of human class I transgenes on *Toxoplasma gondii* cyst formation. *J Immunol*, 1994 152(9): p. 4537–41. [PubMed: 8157968]
28. Deckert-Schluter M, et al., *Toxoplasma* encephalitis in congenic B10 and BALB mice: impact of genetic factors on the immune response. *Infect Immun*, 1994 62(1): p. 221–8. [PubMed: 8262631]
29. Hisczynska-Sawicka E, et al., Comparison of immune response in sheep immunized with DNA vaccine encoding *Toxoplasma gondii* GRA7 antigen in different adjuvant formulations. *Exp Parasitol*, 2010 124(4): p. 365–72. [PubMed: 19962376]
30. Beghetto E, et al., A combination of antigenic regions of *Toxoplasma gondii* microneme proteins induces protective immunity against oral infection with parasite cysts. *J Infect Dis*, 2005 191(4): p. 637–45. [PubMed: 15655789]
31. Scorza T, et al., A GRA1 DNA vaccine primes cytolytic CD8(+) T cells to control acute *Toxoplasma gondii* infection. *Infect Immun*, 2003 71(1): p. 309–16. [PubMed: 12496180]
32. Blanchard N, et al., Immunodominant, protective response to the parasite *Toxoplasma gondii* requires antigen processing in the endoplasmic reticulum. *Nat Immunol*, 2008 9(8): p. 937–44. [PubMed: 18587399]
33. Wilson DC, et al., Differential regulation of effector- and central-memory responses to *Toxoplasma gondii* Infection by IL-12 revealed by tracking of Tgd057-specific CD8+ T cells. *PLoS Pathog*, 2010 6(3): p. e1000815. [PubMed: 20333242]
34. Gazzinelli R, et al., Simultaneous depletion of CD4+ and CD8+ T lymphocytes is required to reactivate chronic infection with *Toxoplasma gondii*. *J Immunol*, 1992 149(1): p. 175–80. [PubMed: 1351500]
35. Bhadra R, et al., Control of *Toxoplasma* reactivation by rescue of dysfunctional CD8+ T-cell response via PD-1-PDL-1 blockade. *Proc Natl Acad Sci U S A*, 2011 108(22): p. 9196–201. [PubMed: 21576466]
36. Mueller SN and 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(21): p. 8623–8. [PubMed: 19433785]
37. Shin H and Wherry EJ, CD8 T cell dysfunction during chronic viral infection. *Curr Opin Immunol*, 2007 19(4): p. 408–15. [PubMed: 17656078]
38. Green AM, Difazio R, and Flynn JL, IFN-gamma from CD4 T cells is essential for host survival and enhances CD8 T cell function during *Mycobacterium tuberculosis* infection. *J Immunol*, 2013 190(1): p. 270–7. [PubMed: 23233724]
39. Williams MA, et al., Developing and maintaining protective CD8+ memory T cells. *Immunol Rev*, 2006 211: p. 146–53. [PubMed: 16824124]
40. Carvalho LH, et al., IL-4-secreting CD4+ T cells are crucial to the development of CD8+ T-cell responses against malaria liver stages. *Nat Med*, 2002 8(2): p. 166–70. [PubMed: 11821901]
41. Casciotti L, et al., CD8(+)-T-cell immunity against *Toxoplasma gondii* can be induced but not maintained in mice lacking conventional CD4(+) T cells. *Infect Immun*, 2002 70(2): p. 434–43. [PubMed: 11796568]

42. Shearer GM, et al., A model for the selective loss of major histocompatibility complex self-restricted T cell immune responses during the development of acquired immune deficiency syndrome (AIDS). *J Immunol*, 1986 137(8): p. 2514–21. [PubMed: 3489767]
43. Gigley JP, et al., T cell exhaustion in protozoan disease. *Trends Parasitol*, 2021 28(9): p. 377–84.
- 44 ●. Hwang S, et al., Blimp-1-mediated CD4 T cell exhaustion causes CD8 T cell dysfunction during chronic toxoplasmosis. *J Exp Med*, 2016 213(9): p. 1799–818. [PubMed: 27481131] These studies for the first time reported BLIMP-1 mediated CD4 T cell exhaustion during chronic *T.gondii* infection.
- 45 ●. Moretto MM, Hwang S, and Khan IA, Downregulated IL-21 Response and T Follicular Helper Cell Exhaustion Correlate with Compromised CD8 T Cell Immunity during Chronic Toxoplasmosis. *Front Immunol*, 2017 8: p. 1436. [PubMed: 29163509] In these studies for the first time exhaustion of FTH population during chronic infection was reported.
- 46 ●. Melssen M and Slingluff CL Jr., Vaccines targeting helper T cells for cancer immunotherapy. *Curr Opin Immunol*, 2017 47: p. 85–92. [PubMed: 28755541] It has been emphasized that CD4 T cells play a critical helper role in the strategies involving cancer immunotherapy.,
47. Bhadra R, Gigley JP, and Khan IA, Cutting edge: CD40-CD40 ligand pathway plays a critical CD8-intrinsic and -extrinsic role during rescue of exhausted CD8 T cells. *J Immunol*, 2011 187(9): p. 4421–5. [PubMed: 21949017]
48. Phan-Lai V, et al., The Antitumor Efficacy of IL2/IL21-Cultured Polyfunctional Neu-Specific T Cells Is TNFalpha/IL17 Dependent. *Clin Cancer Res*, 2016 22(9): p. 2207–16. [PubMed: 26660518]
49. Shin H, et al., A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. *Immunity*, 2009 31(2): p. 309–20. [PubMed: 19664943]
50. Chen L and Flies DB, Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol*, 2013 13(4): p. 227–42. [PubMed: 23470321]
51. Linch SN, McNamara MJ, and Redmond WL, OX40 Agonists and Combination Immunotherapy: Putting the Pedal to the Metal. *Front Oncol*, 2015 5: p. 34. [PubMed: 25763356]
52. Miles B, Miller SM, and Connick E, CD4 T Follicular Helper and Regulatory Cell Dynamics and Function in HIV Infection. *Front Immunol*, 2016 7: p. 659. [PubMed: 28082992]
53. Yi JS, Du M, and Zajac AJ, A vital role for interleukin-21 in the control of a chronic viral infection. *Science*, 2009 324(5934): p. 1572–6. [PubMed: 19443735]

Figure 1.

A. Left: 4-1BB expression on CD4 T cells from CD4Cre Prdm1flox (bold line) and control littermates (gray filled) infected 6 weeks earlier. **Right:** Expression of MFI. White bar: control littermate. Black bar: CD4CrePrdm1flox mice.

B. BLIMP-1cKO and wild type (littermates) mice were infected with 10 cysts of *T. gondii*. At week 6 p.i. antigen specific CD4 T cells from the spleens were evaluated for expression of costimulatory molecules by flow cytometry. The data is presented as percent increase in the expression of MFI on CD4 T cells from BLIMP-1cKO versus WT littermates. The experiment was performed twice.

Figure2.

A. Survival of 4-1BB agonist (3H3) treated mice. C57BL/6 infected mice were treated with 100ug of 4-1BB agonist or control antibody for two weeks (once weekly from week 3-5) p.i. There were 5 mice per group and survival was monitored till 125-day p.i. **B.** At week 8p.i. percentage of *T. gondii* infected cells in the tissues was measured by flow cytometry.

Figure 3:

Acute *T. gondii* infection evokes a strong IL-12 release, especially by dendritic cell population. The cytokine polarizes CD4 TH1 response and CD8 T cell effector immunity. Functional CD4 T cells produce IL-2 and IL-21 which is critical for the maintenance of long term CD8 T cell immunity. However, when the infection becomes chronic, increased BLIMP-1 expression in CD4 T cells causes an upregulation of inhibitory receptor molecules (such as: LAG 3, PD-1 and 2B4) on their surface. This leads to CD4 T cell exhaustion/dysfunctionality which results in decreased cytokine (IL-2/IL-21) production by these cells. This compromises the essential help needed for CD8 maintenance leading to their exhaustion and decreased polyfunctionality.