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Regulation of CD8+ T Cell Responses to Infection With Parasitic Protozoa

Kimberly A. Jordan[1] and **Christopher A. Hunter**
Department of Pathobiology, University of Pennsylvania, 380 South University Ave, Philadelphia, PA 19104, USA

Introduction

There are over 10,000 species of parasitic protozoa, a subset of which can cause considerable disease in humans. Here we examine in detail the complex immune response generated during infection with a subset of these parasites: *Trypanosoma cruzi*, *Leishmania sp*, *Toxoplasma gondii*, and *Plasmodium sp*. For these organisms, their capacity to replicate inside host cells means that the ability of $CD8^+$ T cells to recognize infected cells and respond with either cytolysis or the production of cytokines is critical for protection. While these particular species perhaps represent the most studied parasites in terms of understanding how T cells function during infection, it is clear that the lessons learned from this body of work are also relevant to the other protozoa known to induce a $CD8^+$ T cell response. Nevertheless, despite major advances in defining the critical role of $CD8^+$ T cells for long-term resistance to many parasites, there remains a paucity of vaccines for use in humans.

This review will highlight some of the key studies that established that $CD8^+$ T cells play a major role in protective immunity to protozoa, the factors that promote the generation as well as maintenance of the $CD8^+$ T cell response during these infections, and draw attention to some of the gaps in our knowledge. Moreover, the development of new tools, including MHC Class I tetramer reagents, TCR transgenic mice, and genetically modified parasites, has provided a better appreciation of how parasite specific $CD8^+$ T cell responses are initiated and new insights into their phenotypic plasticity (Frickel et al., 2008; Garg et al., 1997; Hafalla et al., 2007; Kumar and Tarleton, 2001; Kwok et al., 2003; Martin et al., 2006; Miyakoda et al., 2008; Padilla et al., 2007; Pepper et al., 2004; Rodrigues et al., 1991). A greater understanding of the generation of the cellular immune response to these parasites will create new opportunities to develop effective vaccines for these organisms.

Public Health Impact of Parasitic Protozoa

Parasitic protozoa continue to pose major threats to human health and animals of veterinary importance. In addition, with the growing numbers of immunocompromised individuals, either as a consequence of acquired immunodeficiencies or because of specific treatments designed to suppress the immune system, there is a long list of normally asymptomatic or quiescent protozoal infections that can cause significant clinical disease (Ferreira and Borges, 2002). For

[1]Corresponding author. 380 South University Avenue, Philadelphia, PA 19104-4539. (phone) 215-573-5139 (fax) 215-746-2295; kajordan@mail.med.upenn.edu .

example, while most humans are capable of mounting appropriate T cell responses required to control *T. gondii*, the decline in $CD4^+$ T cell numbers and loss of $CD8^+$ T cell function seen during AIDS can result in reactivation of latent *T. gondii* and the development of life threatening toxoplasmic encephalitis (Luft et al., 1984). *T. cruzi* and *Leishmania sp*., both organisms are normally associated with the inability to clear chronic infection, can also cause severe acute disease, or recrudesce as a consequence of HIV infection (Ferreira and Borges, 2002). The heaviest burden of morbidity and mortality following infection with *Plasmodium sp*. is found in children but there is also considerable disease associated with infection in adults. Further, despite having detectable $CD8^+$ T cell responses to malaria antigens (Doolan et al., 1997; Plebanski et al., 1997; Sedegah et al., 1992), people in endemic areas suffer re-infection with *Plasmodium* many times over their lifetime (Good, 2005).

While alterations in immune status can profoundly alter the outcome of infection, changes in the parasite population structure can also have a significant impact on the ability of these organisms to cause disease. This is illustrated by recent reports that have noted atypical strains of *T. gondii* associated with ocular disease or acute lethality in immunocompetent individuals (Demar et al., 2007; Grigg et al., 2001; Khan et al., 2006). Similarly, outbreaks of acute Chagas disease in normal human populations, believed to have been transmitted orally, have recently been identified in Brazil (Nobrega et al., 2009; Steindel et al., 2008; Valente et al., 2009). Another example is the *P. knowlesi* strain of malaria, which infects macaques in Southeast Asia, but which has emerged as a threat to humans (Cox-Singh et al., 2008; Cox-Singh and Singh, 2008). The development of drug resistance is also a major problem, and this has been well-described in the case of malaria (Anderson, 2009; Khatoon et al., 2009; Pearce et al., 2009; Schonfeld et al., 2007; Schunk et al., 2006). Moreover, drug-resistant strains of *T. cruzi*, *T. gondii* and *Leishmania* have also emerged (Aspinall et al., 2002; Robello et al., 1997; Ubeda et al., 2008; Wilkinson et al., 2008) and this has accentuated the need for a better understanding of how the immune system can be used to limit infection.

Characterization of parasite specific $CD8^+$ T cell responses in humans

While patients with defects in T cell mediated immunity illustrate the role of T cells in resistance to multiple intracellular parasites, numerous studies have characterized the T cell responses to these infections in humans. This work has focused on the ability of different T cell populations to make IFN-γ or lyse infected cells, two main effector functions of $CD8^+$ T cells. For example, *T. gondii*-specific $CD4^+$ as well as $CD8^+$ T cells have been cloned from infected humans (Khan et al., 1990; Montoya et al., 1996; Purner et al., 1996), and another study noted a correlation of HLA haplotype with susceptibility to toxoplasmic encephalitis (Suzuki et al., 1996). Parasite-specific $CD8^+$ T cells have also been isolated from the peripheral blood of *T. cruzi*-infected patients (Brodskyn et al., 1996; Wizel et al., 1998), and two other studies reported low frequencies of positive IFN-γ responses to predicted HLA-A2 $CD8^+$ T cell epitopes (Fonseca et al., 2005; Laucella et al., 2004). $CD8^+$ T cell responses to malaria circumsporozoite (CS) protein have also been identified following human infection (Braga et al., 2002; Doolan et al., 1993; Plebanski et al., 1997; Suphavilai et al., 2004), and this protein is a component of several candidate vaccines against malaria (Nardin et al., 2004; Oliveira et al., 2005; Wang et al., 2004). Further, even in the case of *Leishmania* where resistance is mainly mediated by $CD4^+$ T cells, parasite-specific $CD8^+$ T cells have been identified following human infection (Antonelli et al., 2004; Barral-Netto et al., 1995; Da-Cruz et al., 1994).

Defining the role of $CD8^+$ T cells in animal models of parasitic infection

While the approaches discussed above highlight the presence of $CD8^+$ T cell responses to parasites in humans, it is the development of experimental models that allowed the use of antibody depletion, adoptive transfers or knockout mice, that has clarified the role of this T

cell subset in resistance to multiple infections. Some of the first evidence that an endogenous $CD8^+$ T cell population was critical for resistance to a parasite was demonstrated when depletion of $CD8^+$ T cells led to increased susceptibility to primary challenge with *T. cruzi*, associated with greatly increased parasite burdens (Tarleton, 1990). Subsequent studies showed that depletion of $CD8^+$ T cells during the chronic stage resulted in the exacerbation of inflammation in the heart, the site of chronic *T. cruzi* infection, as well as higher parasite burden (Tarleton et al., 1994). Furthermore, β2-m–deficient mice, which lack the ability to express stable MHC class I molecules on the cell surface and therefore have minimal development of Class I-restricted $CD8^+$ T cells, rapidly succumb to *T. cruzi*, thereby confirming the importance of $CD8^+$ T cells in protection against this organism (Tarleton et al., 1992). This conclusion was complemented by studies in which the adoptive transfer of *T. cruzi*-specific $CD8^+$ T cells protected mice against parasite challenge (Wizel et al., 1997).

Early studies to define the role of different lymphocyte populations in resistance to *T. gondii* were dependent on the use of a temperature-sensitive strain that provides protection from subsequent challenges (Suzuki et al., 1988). The transfer of T cells from infected or immunized mice to naïve mice provided protection against a lethal challenge of *T. gondii*, but this was abolished by depletion of $CD8^+$ T cells prior to transfer (Parker et al., 1991; Suzuki and Remington, 1988). Similarly, transfer of $CD8^+$ T cells from chronically infected mice to naïve WT or nude mice was also able to provide protection from *T. gondii* challenge (Parker et al., 1991). However, in the case of β2-m–deficient mice, a potent NK cell response could compensate for the lack of $CD8^+$ T cells in response to *T. gondii*, though mice remained more susceptible than WT mice (Denkers et al., 1993b). Consistent with a protective role for $CD8^+$ T cells, multiple genetic studies revealed that H-2 haplotype profoundly influenced the outcome of this infection (Brown and McLeod, 1990; Suzuki et al., 1994). While the initial studies focused on the ability of $CD8^+$ T cells to protect against acute challenges, with the onset of the AIDS pandemic it became of interest to define which T cell populations were involved in preventing reactivation of *T. gondii* in the CNS. The finding that depletion of $CD8^+$, but not $CD4^+$, T cells during chronic infection led to increased mortality established the importance of these lymphocytes in long term resistance to toxoplasmic encephalitis (Gazzinelli et al., 1992a).

For *Plasmodium*, the ability of this organism to invade and replicate inside erythrocytes, which lack MHC class I expression, ensures little interaction of these infected cells with $CD8^+$ T cells; but hepatocytes also get infected and can present parasite-derived antigens (Bongfen et al., 2007). Studies on the role of $CD8^+$ T cells in resistance to malaria are contradictory: early studies showed that $CD8^+$ T cell depletion had no effect on peak parasite titers but was associated with transient recrudescence of parasites in the blood (Podoba and Stevenson, 1991). However, β2-m-deficient mice have normal resolution of blood-stage malaria infection (van der Heyde et al., 1993). Adoptive transfer of $CD8^+$ T cells could provide protection in some models (Khusmith et al., 1994; Mogil et al., 1987; Rodrigues et al., 1991), but not in others (Vinetz et al., 1990). Irradiated sporozoites, which target hepatocytes, have long been known to induce protective immunity to malaria (Nussenzweig et al., 1967) and multiple studies have shown that while neither $CD4^+$ T cells or antibody is required for this immunity, $CD8^+$ T cells are required for a protective response to the liver stage of this parasite (Doolan and Hoffman, 2000; Erb et al., 1996; Mueller et al., 2007; Romero et al., 2007; Tsuji and Zavala, 2003; Weiss et al., 1988).

$CD4^+$ T cell production of IFN-γ is essential for protection against acute *L. major* and understanding the biology of T helper populations has been the main focus of study in resistance against this parasite (Launois et al., 1998; Moll et al., 1988; Reiner and Locksley, 1995). Much of the evidence does not support a protective role for $CD8^+$ T cells in the control of primary challenge with *Leishmania*, as demonstrated by experiments in mice lacking $CD8^+$ T cells or

MHC-Class I expression where control of infection was not impaired (Huber et al., 1998; Overath and Harbecke, 1993; Wang et al., 1993). In MHC-Class II-deficient mice lacking $CD4^+$ T cells, $CD8^+$ T cells were not found to protect (Erb et al., 1996). However, several reports defined an important role for $CD8^+$ T cells during *Leishmania*, either in memory responses or during low-dose intradermal challenge (Belkaid et al., 2002; Muller et al., 1993; Muller et al., 1994; Rafati et al., 2002; Uzonna et al., 2004). Adaptive immune responses against the parasites discussed herein are complex, and the outcome of the experiment may depend on dose or route of infection, or the strain of mouse that is infected. However, adaptive immune responses, and $CD8^+$ T cells in particular, are clearly important for resistance to the parasites described in this review.

Antigen Presentation During Parasite Infection

Naïve $CD8^+$ T cells are activated following exposure to their cognate antigen in the context of MHC I on the surface of antigen-presenting cells (APC). It was previously believed that only cell-associated endogenous antigens were presented by MHC Class I molecules. More recently, it has been appreciated that during transplant rejection as well as during viral or parasite infection that professional APC can sample and display exogenously-derived proteins on MHC I in the process of cross-presentation (Rock and Shen, 2005). Several studies, using a model of transient dendritic cell (DC) depletion, indicated that this pathway was critically required for generation of antigen-specific $CD8^+$ T cells in mice infected with *Listeria monocytogenes* and *P. yoelii* (Jung et al., 2002; Liu et al., 2006). For malaria, transgenic parasites expressing the model antigen ovalbumin were used to show that Transporter Associated with Antigen Processing (TAP)-dependent cross-presentation of antigen begins shortly following infection, after APC travel to the skin draining LN (Miyakoda et al., 2008). TAP was not required for $CD8^+$ T cell priming during *L. major*, nor was TAP required for resistance to infection, indicating that cross-priming occurs in a TAP-independent manner during this infection (Bertholet et al., 2006). In contrast, TAP was required to induce proliferation of T cells with *T. gondii*-infected DC (Bertholet et al., 2006) and another component of this TAP-dependent pathway, the ER-associated aminopeptidase, has also been implicated in antigen presentation and resistance to *T. gondii* (Blanchard et al., 2008). There is evidence in favor of (John et al., 2009), as well as against (Dzierszinski et al., 2007; Goldszmid et al., 2009; Gubbels et al., 2005), cross-presentation of antigen to $CD8^+$ T cells during toxoplasmosis. Distinguishing which pathways are involved in these events may not just be of academic interest as they may determine the type of pathogen antigens that are presented to $CD8^+$ T cells and therefore influence the generation of vaccine-mediated immunity.

The protozoan parasites that are the focus of this review all have relatively large microbial genomes, and this has complicated the discovery of relevant $CD8^+$ T cell epitopes that would allow antigen processing and presentation to be studied more easily. Nevertheless, extensive efforts by many different groups have lead to the sequencing and annotation of the genomes of each of these organisms (El-Sayed et al., 2005; Gardner et al., 2002; Ivens et al., 2005; Kissinger et al., 2003), and formed the basis for the discovery of the endogenous $CD8^+$ T cell epitopes from *T. cruzi*, *T. gondii*, and *Plasmodium* shown in Table I. One common theme has emerged from these studies, as well as from earlier reports using parasites that expressed model antigens: there appears to be preferential processing and presentation of antigens that are either secreted, or those located on the cell surface of these intracellular microbes (Bertholet et al., 2005; Garg et al., 1997; Gubbels et al., 2005; Pepper et al., 2004; Wilson et al., 2010). Therefore, targeting immune responses against surface-derived proteins would most likely be an effective vaccine approach.

Antigen recognition during parasite infection is further complicated by the distinct developmental stages associated with initiation of infection, development of disease and latency. For instance, *T. gondii* has been shown to express different antigens, depending on the stage of infection, with some antigens being expressed only during the tachyzoite stage, while others are not expressed until the parasite has encysted in the brain and muscle tissue in its bradyzoite form (Kim and Boothroyd, 2005; Kwok et al., 2003; Lutjen et al., 2006). Though changes in antigen expression can also occur during *T. cruzi* infection (Araya et al., 1994), an immunodominant $CD8^+$ T cell epitope located in a trans-sialidase gene has been described which accounts for up to 30% of the antigen-specific $CD8^+$ T cell response in certain inbred mouse strains (Martin et al., 2006). Many studies of antigen presentation during malaria infection have focused on the circumsporozite (CS) protein, which contains an immunodominant epitope, though work is on-going in order to identify other endogenous $CD8^+$ epitopes from malaria parasites (Bongfen et al., 2007; Kumar et al., 2006; Plebanski et al., 2005) as well as those from the *Leishmania* genome (Herrera-Najera et al., 2009).

Anti-Parasitic Effector Mechanisms Mediated by $CD8^+$ T Cells

With the recognition that $CD8^+$ T cells play a role in limiting the replication of many different parasites, the next goal became to define how these lymphocytes mediated protection. IFN-γ is made by $CD4^+$ and $CD8^+$ T cells as well as NK cells, and has been demonstrated to be crucial for a protective response to numerous intracellular parasites by studies using neutralizing antibody to IFN-γ or mice deficient in its production (Scharton-Kersten et al., 1996; Schofield et al., 1987; Suzuki et al., 1989; Suzuki et al., 1988; Torrico et al., 1991). Evidence that production of this cytokine and subsequent protection against these parasitic diseases is dependent on $CD8^+$ T cells was demonstrated by showing that treatment of infected mice with anti-CD8 antibodies resulted in reduced production of IFN-γ and loss of IFN-γ-mediated protection (Gazzinelli et al., 1991; Schofield et al., 1987; Shirahata et al., 1994; Tarleton, 1990; Weiss et al., 1988). Further, $CD8^+$ T cells are also known to produce the pro-inflammatory cytokines IL-17 and TNF-α, and while both of these cytokines are associated with resistance to *T. gondii*, relatively little is known about the contribution of $CD8^+$ T cells as a source of these factors during parasitic infections (Johnson, 1992; Kelly et al., 2005; Stumhofer et al., 2006).

$CD8^+$ T cells can also mediate perforin-dependent cytotoxicity against target cells that present the correct peptide in the context of MHC on their cell surface. Early reports with *T. gondii* showed that $CD8^+$ T cells isolated from immunized or infected mice were capable of lysing infected cells or targets pulsed with parasite antigens (Denkers et al., 1993a; Hakim et al., 1991; Khan et al., 1991; Subauste et al., 1991). These studies frequently relied on the *in vitro* expansion of rare T cell populations and the use of chromium release assays to demonstrate lytic activity. More recently, based on the development of *in vivo* cytotoxicity assays (Barber et al., 2003), cytotoxic $CD8^+$ T cells have been detected *in vivo* during murine toxoplasmosis (Jordan et al., 2009), *T. cruzi* (Martin et al., 2006) and the blood stage of *P. berghei* (Lundie et al., 2008). However, the contribution of CD8-mediated cytolysis to resistance *in vivo* was uncertain until perforin-deficient mice became available. Initial studies with these mice revealed that they were less susceptible to infection than CD8-deficient mice, and could generate a protective $CD8^+$ T cell response, but showed increased susceptibility to chronic toxoplasmosis indicating that the ability to recognize and lyse infected cells was required for optimal resistance (Denkers et al., 1993b; Denkers et al., 1997). In contrast, the cytolytic effector function of $CD8^+$ T cells during malaria does not protect the host, but rather through perforin-mediated lysis of endothelial cells in the brain contributes to the pathology associated with cerebral malaria (Nitcheu et al., 2003; Potter et al., 2006).

Induction of CD8+ T Cell Responses

Many factors influence the generation of $CD8^+$ T cell responses, including cytokines such as IL-2 and IL-12, which contribute to T cell expansion, survival, and the acquisition of effector function. $CD4^+$ T cell help is important in the generation of effector $CD8^+$ T cells following immunization with replication-deficient *T. gondii* (Jordan et al., 2009), but not during the acute stage of infection with a replicating strain of *T. gondii* (Lutjen et al., 2006). Work from two other groups found that $CD4^+$ T cells were required to maintain optimal $CD8^+$ T cell responses to *T. gondii* during chronic infection (Casciotti et al., 2002; Gazzinelli et al., 1992b). Following infection with irradiated malaria sporozoites, $CD4^+$ T cell help was critical in the development of the $CD8^+$ tetramer population (Carvalho et al., 2002), and in other malaria immunization models $CD4^+$ T cell help also enhanced the CTL response (Valmori et al., 1994; Widmann et al., 1992). In contrast, antigen-specific $CD8^+$ T cells specific for subdominant epitopes can develop during *T. cruzi* in the absence of $CD4^+$ T cell help, but responses to dominant $CD8^+$ T cell epitopes were $CD4^+$ T cell dependent (Padilla et al., 2007). The seemingly disparate requirements for $CD4^+$ T cell help might be explained by the inflammatory environment where priming occurs. When inflammation is limited, $CD4^+$ T cells can have a significant role in promoting $CD8^+$ T cell expansion through activating DC or providing growth factors such as IL-2 (Rajasagi et al., 2009; Wilson and Livingstone, 2008).

IL-12 was demonstrated to augment vaccine-induced responses to *L. major* and was thought to act as an adjuvant in that system (Afonso et al., 1994). Since that time it has been recognized that IL-12 can profoundly influence the generation, phenotype, and effector function of $CD8^+$ T cells generated in response to *T.* gondii (Wilson et al., 2008), *T. cruzi* (Katae et al., 2002) and *Plasmodium* infection (Doolan and Hoffman, 1999), as well as playing a role during bacterial infection (Badovinac and Harty, 2007). Increasingly, it has also been demonstrated that while IL-12 can act as an adjuvant during the primary response to infection, its presence negatively affects the generation of $CD8^+$ T cell memory responses (Joshi et al., 2007; Pearce and Shen, 2007; Takemoto et al., 2006). The role that IL-12 and other cytokines play in effector versus memory differentiation during parasite infection is still being unraveled, and it is likely that factors including duration of antigen exposure, cytokine milieu and priming environment will influence these events. The ability of a vaccine to induce $CD4^+$ T cell help as well as cytokines that promote T cell differentiation, effector function, and memory formation must therefore be taken into consideration during vaccine design.

Regulation of Immune Responses During Chronic Infection

Parasite infection can induce mixed cytokine responses that differ based on mouse strain and may be linked with susceptibility or resistance to disease (Liesenfeld et al., 1996; Reiner and Locksley, 1995; Roggero et al., 2002; Zhang and Tarleton, 1996). While control of the parasites discussed within this review is generally dependent on robust Th1-polarized immune responses, these must be carefully controlled to prevent pathology in the host. In many studies mentioned above, pathological effects are mediated by $CD4^+$ T cells rather than $CD8^+$ T cells, one prominent exception being in the case of cerebral malaria where CTL caused damage to the brain via perforin-dependent mechanisms (Nitcheu et al., 2003). Immunoregulatory cytokines such as IL-10 and IL-27 are an important mechanism to limit these pro-inflammatory adaptive immune responses during chronic infection. For instance, $IL\text{-}10^{-/-}$ mice have increased susceptibility to *T. gondii, P. chabaudi chabaudi* and *T. cruzi* driven in part by overproduction of IFN-γ and TNF-α (Gazzinelli et al., 1996; Hunter et al., 1997; Li et al., 1999; Wilson et al., 2005). During experimental leishmaniasis IL-10 can either promote protection or the development of non-healing lesions, depending on the strain of parasite (Anderson et al., 2005; Kane and Mosser, 2001). Another more recently described cytokine, IL-27, has also been show to downregulate the inflammatory responses induced during *T.*

gondii. This cytokine is important in limiting host pathology induced by IFN-γ (Villarino et al., 2003) as well as IL-17 (Stumhofer et al., 2006). IL-27 signaling also limits pro-inflammatory cytokine responses during *T. cruzi* (Hamano et al., 2003) while its role in *L. major* is more complex (Artis et al., 2004; Yoshida et al., 2001). Overall, these studies point to an important balance of the immune system as it attempts to combat invading pathogens, especially in the context of chronic infection.

$CD8^+$ T Cell Memory in the Setting of Chronic Infection

Memory $CD8^+$ T cell responses that develop following parasite infection have been defined functionally in terms of their ability to protect animals from secondary challenge (Gazzinelli et al., 1991; Muller et al., 1993; Parker et al., 1991; Schofield et al., 1987; Tarleton, 1990; Weiss et al., 1988). However, because many parasites cause persistent infections for the life of the individual, it can be difficult to phenotypically distinguish between chronically activated effectors and bona fide memory cells (Frenkel, 1988; Zhang and Tarleton, 1999). Memory $CD8^+$ T cells induced by acute viral infection differ from what has been seen so far during parasite infection, and are characterized by their expression patterns of $CD62L^+KLRG1^-CD127^{high}$ (Joshi et al., 2007). The phenotype of $CD8^+$ T cells during chronic toxoplasmosis or Chagas disease is that of an effector-memory cell as defined by their phenotype ($CD62L^-$, $KLRG1^+$ and $CD127^{low}$) (Bixby and Tarleton, 2008; Bustamante et al., 2008). Drug-induced clearance of *T. cruzi* caused antigen-specific $CD8^+$ T cells to upregulate their expression of CD62L, CD127 and CCR7 (Bustamante et al., 2008). These data indicate that ongoing antigen exposure was required for these cells to maintain an effector phenotype.

The fact that antigen-specific $CD8^+$ T cells associated with some parasite infections are phenotypically different from viral memory cells, yet are able to protect mice from challenge, suggests that current paradigms for memory responses either need to be modified or recognized as being specific to different pathogens. Some of the same cytokines that have been associated with $CD8^+$ T cell memory in viral and bacterial systems are also important in the development of $CD8^+$ T cell memory during parasite infection; the importance of IL-7 and IL-15 in the survival and homeostatic proliferation of $CD8^+$ T cells has recently been reviewed (Surh et al., 2006). For example, a subset of *T. cruzi*-specific $CD8^+$ T cells was shown to be responsive to the cytokines IL-7 and IL-15 (Bixby and Tarleton, 2008). Thus, although memory cells are not yet as clearly defined as in other model systems, the same cytokines that contribute to the homeostasis of $CD8^+$ T cells in viral models are likely important in maintaining similar populations during chronic parasite infection.

Targeting $CD8^+$ T Cells for Anti-Parasitic Vaccines: What Comes Next?

Despite the advances in understanding the role of $CD8^+$ T cells in immunity to multiple intracellular parasites, there are few vaccines against protozoan parasites. A vaccine to prevent Toxoplasma-induced abortions in cattle is commercially available (Buxton and Innes, 1995), but nothing approved for human use is currently available. The difficulty in developing vaccines against protozoan parasites could in part be attributed to the complicated life cycle of these organisms. Nevertheless, increasing the potency of current candidates, or development of therapeutic vaccines, would be helpful to limit the morbidity and mortality associated with certain parasitic infections. For example, recent work has shown that activation of the NF-κB signaling pathway in DC leads better antigen presentation, an approach that could be used as a generalized adjuvant during vaccination (Andreakos et al., 2006). A better understanding of the transcription factors that regulate the effector and memory potential of $CD8^+$ T cells during parasite infection may provide additional strategies to increase the potency of vaccines. Multiple transcription factors that regulate $CD8^+$ T cell effector functions, such as cytokine production and cytotoxicity, have been described in a range of infection models (Cho et al.,

2009; Intlekofer et al., 2008; Intlekofer et al., 2005; Lieberman et al., 2004; Mason et al., 2004). However, further research is still required to understand how to best apply this information to promote the generation and maintenance of immunity to parasite infection.

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Table I

Recent work in murine infection models of parasitic disease has identified endogenous CD8$^+$ T cell epitopes

CD8$^+$ T Cell Epitopes in *T. gondii*, *T. cruzi* and *P. falciparum*

Parasite	Epitope	Gene family	References
T. cruzi	VDYNFTIV	Trans-sialidase	(Wizel et al., 1997)
	YEIQYVDL	Paraflagellar rod	(Wrightsman et al., 2002)
	ELTMYKQLL	LYT1 (host cell lysis)	(Fralish and Tarleton, 2003)
	ANYKFTLV	Trans-sialidase	(Martin et al., 2006)
T. gondii	SPMNGGYYM	Dense granule protein	(Frickel et al., 2008)
	IPAAAGRFF	Rhoptry protein	(Frickel et al., 2008)
	SVLAFRRL	Putative secreted protein	(Wilson et al., 2010)
Plasmodium	NDDSYIPSAEKI	Circumsporozoite	(Romero et al., 1989)
	SYVPSAEQI	Circumsporozoite	(Rodrigues et al., 1991)