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TITLE:
CD8+ T Cells in Leishmania Infections: Friends or Foes?

ABSTRACT:
Host protection against several intracellular pathogens requires the induction of CD8(+) T cell responses. CD8(+) T cells are potent effector cells that can produce high amounts of pro-inflammatory cytokines and kill infected target cells efficiently. However, a protective role for CD8(+) T cells during Leishmania infections is still controversial and largely depends on the infection model. In this review, we discuss the role of CD8(+) T cells during various types of Leishmania infections, following vaccination, and as potential immunotherapeutic targets.

Introduction:
CD8+ T cells play a major role in protective immunity to a wide variety of pathogens, including viruses, bacteria, and protozoan parasites. However, the protective role of CD8+ T cells during Leishmania infections has been controversial, mainly because of the discrepancy among infections with different Leishmania species. Different Leishmania species have different tropisms and their diversity is reflected in the various clinical manifestations they induce. Hence, it is not surprising that the contribution of CD8+ T cells to the immune response against the parasite depends on the clinical form and the species that is causing it. Here, we discuss the literature on the contribution of CD8+ T cells to the immune response against Leishmania, taking into account the various clinical forms and experimental models.

CD8@!start@+@!end@ T Cell Functions:
CD8+ T cells recognize peptides that are presented in the context of major histocompatibility complex (MHC) class I molecules via the T cell receptor (TCR). Although peptides presented via MHCI mainly derive from endogenous antigens, various exogenous cell-associated antigens have also been shown to be uploaded onto the MHCI pathway, by a process referred to as cross-presentation. Leishmania antigens were also shown to be cross-presented (Bertholet et al., 2006). During in vivo infections, cross-presentation of Leishmania antigens may result from several internalization pathways, such as direct infection, receptor-mediated uptake (Woelbing et al., 2006), or internalization of apoptotic vesicles (Winau et al., 2006). Thus far, two different processing pathways have been proposed. An early work demonstrated that a surface antigen of L. amazonensis was processed in a proteasome-dependent manner within the cytosol (Kima et al., 1997). In contrast, a more recent study showed that cross-presentation of secreted leishmanial antigens is confined to an intraphagosomal processing pathway that is TAP- and proteasome-independent (Bertholet et al., 2006).
After activation, antigen-specific CD8+ T cells differentiate into effector cells and acquire the capacity to kill target cells, and produce several cytokines and chemokines (Kaech et al., 2002; Harty and Badovinac, 2008). Among the various CD8+ T cell subsets, Tc1 were shown to play a major role in the fight against several protozoan parasites (Jordan and Hunter, 2010). The hallmark of this subset is the production of IFN-γ and TNF, and cytotoxic capacity (Woodland and Dutton, 2003). The precise mechanism underlying cytotoxic T lymphocyte (CTL) killing of microbes is still poorly understood. CTLs can exert cytotoxicity through various mechanisms: via exocytosis of lytic granula containing perforin, granzyme A/B, and/or granulysin; through the interaction between FasL and Fas expressed on targets cells; via TNF; or via TRAIL (Trapani and Smyth, 2002). A study has also shown that reactivated memory CD8+ T cells efficiently killed Listeria monocytogenes via a mechanism mediated by CCL3 and involving the induction of radical oxygen intermediates (Narni-Mancinelli et al., 2007). Direct killing of extracellular pathogen by CTLs has also been described. For example, CTLs can mediate killing of Mycobacterium tuberculosis through the release of anti-bacterial products (Stenger et al., 1998; Canaday et al., 2001). However, direct killing of Schistosoma mansoni (Ellner et al., 1982) and Entamoeba histolytica (Salata et al., 1987) is thought to be contact-dependent. CTLs have also been reported to directly kill extracellular Toxoplasma gondii (Khan et al., 1990). Interestingly, killing in this case appeared to be antigen-specific. To date there is no evidence that CD8+ T cells can mediate protection against Leishmania parasites through their cytotoxic activity. However, since CTLs

have been observed in various mouse models and also in human patients, a possible protective role for Leishmania-specific cytotoxic T cells should not be excluded.
In addition to killing and releasing cytokines and chemokines, recent studies have ascribed a novel regulatory role for CD8+ T cells (Sun et al., 2009; Palmer et al., 2010; Trandem et al., 2011). Regulatory CD8+ T cells represent a transient state of effector CD8+ T cells (Trandem et al., 2011), which is possibly induced by potent TCR stimulation, which promotes the production of the immunosuppressive cytokine IL-10 (Zhang and Bevan, 2011). Not only do these cells produce IL-10, but they are also excellent killers and produce normal to higher amounts of IFN-γ and TNF (Sun et al., 2009; Palmer et al., 2010; Trandem et al., 2011). The main function of these cells is thought to lie in the prevention of immunopathology during infection without affecting the kinetics of pathogen clearance. IL-10-producing CD8+ T cells have also been observed in human patients infected with L. guyanensis (Bourreau et al., 2007) and in patients suffering from post-kala-azar dermal leishmaniasis (PKDL; Ganguly et al., 2008). The role of regulatory CD8+ T cells in the immune response against parasitic infections is still unknown.

CD8@!start@+@!end@ T Cells in Experimental Cutaneous Leishmaniasis:
The role of CD8+ T cells in the immunity to L. major has always been controversial. Early studies in BALB/c mice reported that CD8+ T cells were the main mediators of protection following CD4+ T cell depletion in mice infected with L. major (Titus et al., 1987; Hill et al., 1989; Muller et al., 1991). Interestingly, depletion of CD4+ T cells was rendering susceptible BALB/c mice resistant to L. major infection. The results obtained using the CD4+ T cell depletion model suggested that CD8+ T cells could potentially control L. major infection in mice. A few years later, experiments in β2-microgobulin-deficient mice contradicted these findings and revealed that CD8+ T cells were not essential in mediating protection in L. major-infected BALB/c mice (Wang et al., 1993). Moreover, a study in CD8+ T cell-deficient mice demonstrated that Cd8−/− mice were able to control L. major infection for at least 1 year, suggesting that CD8+ T cells were not required for long-lasting immunity (Huber et al., 1998). The contribution of CD8+ T cells in the control of primary L. major infection became less important also because of the strong evidence that Th1 cells were the primary cells involved in mediating protection against cutaneous leishmaniasis (Reiner and Locksley, 1995; Louis et al., 1998; Sacks and Noben-Trauth, 2002). Several studies have demonstrated that Th1 cells producing IFN-γ were essential in controlling L. major infection, and that failure to develop a Th1 response resulted in susceptibility to the diseases. Hence, a consensus was reached in that if a mouse generates Th2 responses, this will lead to susceptibility; in contrast, Th1 responses were successfully controlling infection without the help of CD8+ T cells.
This paradigm was later challenged when new findings arose from a more natural model of infection, where 100 metacyclic promastigotes were inoculated intradermally in the ears of C57BL/6 mice. In this model, Cd8−/− and CD8+ T cell-depleted mice fail to control L. major infection, and CD8+ T cells were thought to be necessary for supporting Th1 responses (Belkaid et al., 2002). The discrepancy between the findings in the low- and the high-parasite dose model was clarified by another work that compared the requirements of CD8+ T cells in both systems (Uzonna et al., 2004). Interestingly, in the low infection model CD8+ T cells producing IFN-γ were essential for modulating CD4+ T cell responses toward a Th1 response. In contrast, C57BL/6 mice inoculated with a high L. major dose did not require CD8+ T cell help to generate protective Th1 responses. The CD8+ T cell requirement for optimal IFN-γ production by Th1 cells was also proposed in a high-dose L. major infection model in BALB/c mice (Herath et al., 2003). Moreover, CD8+ T cell-derived IFN-γ was reported to contribute to the induction of nitric oxide production in macrophages during experimental cutaneous leishmaniasis (Stefani et al., 1994).
Although the role of CD8+ T cells-derived IFN-γ has been clarified, little is known about the involvement of cytotoxic CD8+ T cells in cutaneous leishmaniasis. In a low-dose model of L. major infection, CD8+ T cell responses were shown not only to be protective, but also to mediate pathology (Belkaid et al., 2002). Hence, it is possible that CTLs may be involved in the ulceration of skin lesions through tissue disruption. This suggests that perhaps two types of CD8+ T cell effectors are generated during L. major infection: antigen-specific CD8+ T cells that produce IFN-γ but lack cytotoxic activity; and CTLs that are potent killers but produce little to no IFN-γ and promote pathology.
Although the role of CD8+ T cells during primary immune responses is controversial, these cells appear to play a prominent role in protecting mice from a secondary challenge (Muller et al., 1993, 1994). Indeed, antigen-specific CD8+ T cells were expanding up to 50-fold in the spleen and lymph nodes of reinfected BALB/c mice (Muller et al., 1994). This expansion correlated with a

substantial production of IFN-γ, which is thought to be essential for controlling Leishmania infections. These observations have major implications for vaccine design.
In summary, during experimental cutaneous leishmaniasis, CD8+ T cells are necessary to support protective Th1 responses through IFN-γ production, but they are also involved in the development of immunopathology. Further investigations are needed to better identify various subtypes of CD8+ T cells that arise during cutaneous leishmaniasis.

CD8@!start@+@!end@ T Cells in Experimental Visceral Leishmaniasis:
In contrast to the cutaneous models, CD8+ T cells have always been thought to play a major role in experimental visceral leishmaniasis (VL). Over 20 years ago, Stern et al. (1988) demonstrated for the first time that CD8+ T cells significantly contribute to the formation of granulomas in the liver of L. donovani-infected mice. Indeed, CD8+ T cell depletion resulted in impaired granuloma formation and exacerbation of liver disease. In agreement with these results, Kaye et al. (1992) also reported a delayed onset and a decrease of the liver granulomatous response in non-obese, diabetic mice expressing transgenic I–E molecules, suggesting that antigen-specific CD8+ T cells are required for proper granuloma formation. CD8+ T cells appear to participate in controlling parasite growth in the spleen as well, since CD8+ T cell depletion during chronic VL significantly increased splenic parasite burden (Stäger, unpublished). This observation was underscored by the fact that adoptive transfer of antigen-specific CD8+ T cells during chronic L. donovani infection resulted in 90% reduction in the splenic parasite burden (Polley et al., 2006). Moreover, therapeutic vaccination aimed at reactivating CD8+ T cells during chronic VL ensued in the control of parasite growth in the spleen (Joshi et al., 2009).
Interestingly, CD8+ T cells do not only participate in primary responses to L. donovani, but are also the major mediators of resistance upon reinfection (Stern et al., 1988). Indeed, protection was abrogated following CD8+ T cell but not CD4+ T cell depletion.
A prominent function for CD8+ T cells was also described in L. infantum-infected mice. Using an intradermal infection model, Ahmed et al. (2003) demonstrated that CD8+ T cells contribute to parasite clearance in the skin of L. infantum-infected mice. Another study also showed that CD8+ T cells purified from L. infantum-infected mice expressed IFN-γ and TNF, and displayed considerable cytotoxic activity against cells expressing Leishmania antigens (Tsagozis et al., 2003). Interestingly, killing of infected target cells was mediated by both the perforin and Fas/FasL pathways (Tsagozis et al., 2003). The Fas/FasL pathway has also been implicated in the defense against L. donovani (Alexander et al., 2001). Indeed, gld and lpr mice, which lack a functional Fas/FasL pathway, were shown to be more susceptible to L. donovani.
Additionally to the classical cytotoxic pathways, a novel counterregulatory function for a subset of cytotoxic CD8+ T cells has recently been proposed in the L. donovani infection model (Martin et al., 2010). In this study, CD3+CD8+CD40+ T cells are shown to suppress regulatory T cells via CD40/CD40L interaction during the early stages of infection in BALB/c mice. CD40 signals through Ras, PI3K, and protein kinase C, leading to the induction of granzyme and perforin, and ultimately to the killing of Tregs.
CD8+ T cells may not be merely participating in the primary immune response by secreting IFN-γ and possibly killing infected target cells and/or Tregs, but they could also be involved in the recruitment of inflammatory cells and in the maintenance of granulomas. Indeed, a study using L. infantum demonstrated that CD8+ T cells expressed RANTES and MIP-1α (Tsagozis et al., 2003), two chemokines that are involved in the recruitment of T cells at the inflammatory site and in the formation and maintenance of granulomas (Mackay, 2001). The authors proposed that CD8+ T cells may thus be involved in granuloma formation. This hypothesis is in agreement with the depletion data (Stern et al., 1988), showing that depletion of CD8+ T cells results in impaired granuloma formation and ultimately in disease exacerbation.
Despite the documented evidence that CD8+ T cells strongly participate in the immune response to L. donovani and L. infantum, our recent findings suggest that L. donovani induces defective antigen-specific CD8+ T cell responses (Joshi et al., 2009). Interestingly, mice infected with L. donovani generate CD8+ T cell responses with limited clonal expansion. The extension of the clonal expansion is thought to be correlated with the effectiveness in eliminating pathogens. It was calculated that a naïve CD8+ T cell may go through 19 cell divisions in the first week after pathogen inoculation (Badovinac et al., 2007). Massive clonal expansions have not only been observed during viral infections, but also following the injection of irradiated Plasmodium berghei sporozoites (Sano et al., 2001). During L. donovani infection, CD8+ T cells underwent at least 8–9 rounds of division, but failed to accumulate in the spleen (Joshi et al., 2009). Moreover, only 10% of CD8+ T cells during clonal expansion expressed markers typically associated with end-

differentiated effector cells, such as KLRG1, PD-1 and Fas (Bankoti and Stäger, unpublished). The cause of this limited expansion is yet unknown and may depend on several factors. One of the possible explanations is limited antigen availability that may result from poor antigen-processing and presentation. Processing of Leishmania antigens is thought to be confined to a TAP-independent, intraphagosomal pathway that is less efficient and requires higher amounts of antigen than the endoplasmic reticulum-based, TAP-dependent cross-presentation pathway (Bertholet et al., 2006). Furthermore, the major surface protease of Leishmania, gp63, was shown to cleave epitopes within the parasitophorous vacuole, further reducing antigen availability (Garcia et al., 1997). Hence, Leishmania antigens may be poorly presented and this poor presentation may not be enough to induce and sustain a massive clonal expansion of antigen-specific CD8+ T cells.

Nonetheless, antigen may be suddenly available in large amounts later on during L. donovani infection, since CD8+ T cells undergo a second round of activation, become dysfunctional, and ultimately die by “exhaustion” (Joshi et al., 2009); high antigen levels have been described as a cause of CD8+ T cell “exhaustion” during chronic viral infections (Mueller and Ahmed, 2009). Further research is needed to clarify the mechanisms involved in CD8+ T cell exhaustion during chronic VL.

In conclusion, CD8+ T cells are required to control parasite growth during experimental VL and reactivation of these responses results in a dramatic reduction in parasite burden. Therefore, immune interventions that target CD8+ T cell responses may have great therapeutic potential against VL.

CD8@!start@+@!end@ T Cells and Human Leishmania Infections:

The role of CD8+ T cells in human leishmaniasis patients is still unclear and seems to depend on the various species of parasites and the disease they cause.

Few studies have been conducted with human VL patients. However, most of the studies ascribe a protective role for CD8+ T cells, in agreement with results obtained from experimental models. Indeed, the control of L. infantum infection was shown not only to be associated with IFN-γ-producing CD4+ T cells, but also with CD8+ T cells (Mary et al., 1999). Interestingly, during active VL, CD8+ T cells are less responsive to stimulation and a greater percentage stains positive for Annexin V compared to healthy controls (Clarencio et al., 2009). These observations correlate very well with what we observed in mice experimentally infected with L. donovani, where CD8+ T cells became increasingly dysfunctional during chronic infection and died by exhaustion (Joshi et al., 2009). Whether human CD8+ T cells also display signs of exhaustion during active VL still remains to be tested.

Another study investigating CD8+ T cell responses in patients infected with L. chagasi has revealed that the frequency of CD18+CD45RO+ CD8+ T cells is significantly decreased in the spleen of patients with active VL (Clarencio et al., 2009). In contrast, CD18+ CD8+ T cells seem to be retained in the bone marrow of VL patients. CD18, or integrin β-2, is the β subunit of LFA-1 (CD11a), CD11b, CD11c, and CD11d. In humans, lack of CD18 causes leukocyte adhesion deficiency, a disorder characterized by lack of leukocyte extravasation from blood into the tissue (Bunting et al., 2002). With exception of the fact that CD18+ cells appear in the granulomas of dogs with asymptomatic VL (Sanchez et al., 2004), very little is known about the function of CD18+ CD8+ T cells during VL and whether cells lacking CD18 expression have similar migratory capacity and effector functions to their CD18+ counterparts.

Not only is the frequency of CD18+ CD8+ T cells reduced in L. chagasi patients, but also, the level of circulating memory T cells is significantly decreased during active VL (Hailu et al., 2005; Clarencio et al., 2009). This observation is in agreement with our findings in the experimental model of VL, where the majority of the CD8+ T cells displayed an effector phenotype during chronic infection (Joshi et al., 2009).

Although CD8+ T cells positively correlate with cure of VL patients, one report suggested that these cells may contribute to the immunopathogenesis of PKDL (Ganguly et al., 2008). Indeed patients suffering from PKDL showed a significant increase in the percentage of CD8+ T cells producing IL-10, which disappeared after cure (Ganguly et al., 2008). IL-10-secreting CD8+ T cells are thought to play a regulatory role in different viral infection models. This CD8+ T cell subset was shown to display great cytotoxicity and produce granzyme B, IFN-γ, and TNF (Sun et al., 2009; Palmer et al., 2010; Trandem et al., 2011). IL-10+ CD8 T cells seem to represent a transient and reversible state of CD8+ effector T cell differentiation. Its primary function is to balance pathogen clearance with bystander tissue damage (Zhang and Bevan, 2011). Interestingly, in viral model, this subset disappears after the infection is cleared. Hence, it is possible that the IL-10-

producing CD8+ T cells in PKDL patients are actually killing parasites and protecting patients from tissue damage, rather than suppressing protective responses. Further studies are needed in order to define the nature of these cells.
CD8+ T cells also actively participate in the immune response to cutaneous infections in human. As observed in the low-dose model in mice (Belkaid et al., 2002; Uzonna et al., 2004), L. major also induces Th1 and CD8+ T cells in human patients and both responses are associated with disease resolution (Nateghi Rostami et al., 2010). CD8+ T cells were not only observed in large numbers in the lesions of L. major patients during the acute phase, but also during the healing process (Da-Cruz et al., 1994, 2002, 2005; Gaafar et al., 1999). The exact role of CD8+ T cells in L. major infections in humans is not yet known. A major correlate of protection appears to be the high amounts of IFN-γ produced by CD8+ T cells after restimulation (Nateghi Rostami et al., 2010). In vitro studies have also demonstrated that Leishmania-specific CTLs are generated upon co-culturing human naïve T cells with antigens from L. amazonensis promastigotes and IL-12 (Russo et al., 1999), or with L. major parasites (Da Conceicao-Silva et al., 1994). Moreover, increased granzyme B activity was also found in patients with an active infection and was associated with a good prognosis (Bousoffara et al., 2004). In this study, in vitro cytotoxicity by peripheral blood lymphocytes on L. major-infected macrophages appeared to be mediated by granzyme B, suggesting that CTL activity may be involved in controlling parasite growth. It is possible, though, that the cytotoxic activity not only contributes to disease clearance, but also to the development of skin ulceration, as observed in L. major-infected mice (Belkaid et al., 2002).
A strong CD8+ T cell expansion has also been observed in L. mexicana patients during the healing process (Salaiza-Suazo et al., 1999). Interestingly, lesions of patients with localized cutaneous leishmaniasis (LCL) harbor a higher number of CD8+ T cells compared to patients with diffuse cutaneous leishmaniasis (DCL; Hernandez-Ruiz et al., 2010). As already observed in VL patients, CD8+ T cells in DCL patients, unlike LCL patients, show a reduced capacity to respond to antigen-specific stimulation during active infection. In fact, these cells displayed low cytotoxicity and only produced little IFN-γ upon stimulation, therefore showing typical signs of functional exhaustion (Hernandez-Ruiz et al., 2010). Strikingly, effector functions could be restored in vitro after stimulation with TLR2 agonists, highlighting the potential therapeutic benefit of the revival of CD8+ T cell functions in DCL patients.
In contrast to the cutaneous and visceral forms of leishmaniasis – where CD8+ T cells seem to correlate with cure and contribute to the immune response – in mucocutaneous infections (ML) CD8+ T cells seem to be implicated in the pathogenesis of the disease. Indeed, high numbers of cytotoxic CD8+ T cells were observed in ML patients (Barral-Netto et al., 1995; Brodskyn et al., 1997). Moreover, the recruitment of granzyme A+ CD8+ T cells is associated with lesion progression (Faria et al., 2009), suggesting that CTLs may contribute to immunopathology. The development of ML is not only associated with the presence of CTL, but also with a high frequency of activated CD4+ T cells, an extreme IFN-γ and TNF production, and a reduced control of inflammation due to low expression of the IL-10 receptor (Gaze et al., 2006; Faria et al., 2009). Furthermore, IL-17-secreting CD4+ and CD8+ T cells were also found in ML patients (Boaventura et al., 2010). Consequently, neutrophils, which are typically recruited during a TH17-mediated inflammatory response, were also detected in necrotic and perinecrotic areas (Boaventura et al., 2010). This suggests that neutrophils, together with CTLs, may be involved in tissue injury and in the development of immunopathology.
Taken together, the literature shows that CD8+ T cells actively participate in the fight against most Leishmania infections in humans and their presence correlates with cure. In contrast, CD8+ T cells in ML patients contribute to disease exacerbation.

CD8@!start@+@!end@ T Cells and Their Role in Future Vaccine Development:
Vaccination of humans with heat-killed Leishmania or recombinant parasite proteins has so far failed to induce long-term immunity and only recovery from natural or experimental infection has provided proper protection. Several trials have analyzed the protective effect of autoclaved L. major plus Bacillus-Calmette–Guérin (BCG) versus BCG alone assessing the cumulative incidence of cutaneous leishmaniasis caused by L. tropica (Sharifi et al., 1998) or L. major (Momeni et al., 1999), or of VL (Khalil et al., 2000) caused by L. donovani. Although no trial showed a significant effect on disease incidence, the vaccination induced skin test conversion and provided limited protection. Additionally, a study showed that immunization of Colombian soldiers with three doses of L. amazonensis alone was non-protective (Velez et al., 2005).
In the human disease, there is evidence that mixed T helper cytokine profiles are present, while healing and protection against reinfection are associated with dominant Th1 and CD8+ T cells.

These findings suggest that it is the cytokine balance that activates or suppresses activation of macrophages harboring Leishmania parasites. This, in turn, determines the outcome of the infection. Thus treatments or antigen/adjuvant formulations that can alter the type of T helper response may change the course of disease (Da-Cruz et al., 2002; Rogers and Titus, 2004; Mohajery, 2007). For this purpose, different vaccination strategies have been examined in animal models including leishmanization (Modabber, 1990), killed parasite (Grimaldi, 1995), live attenuated parasite (Titus et al., 1995), and subsequently, subunit vaccines composed of recombinant or native proteins from different stages of the parasite's life cycle, and DNA vaccines (Skeiky et al., 1998; Webb et al., 1998; Stager et al., 2000; Bottrel et al., 2001; Campos-Neto et al., 2001; Rafati et al., 2001; Coler et al., 2002). The latter two strategies encompass candidates such as gp63, gp46, LACK, CPB, CPA, Kmp11, LmsTI1, TSA, LeIF, HASPB1, and LPG, and have shown promising results in murine models. Nonetheless, only Leish111f (a recombinant fusion protein of LmsTI1, TSA, and LeIF) progressed through phase I and II clinical trials (Llanos-Cuentas et al., 2010; Chakravarty et al., 2011).

Nowadays, it is clear that CD8+ T cells play an important role in the mechanisms for cure of and resistance to Leishmania infection, either by production of IFN-γ and activation of macrophages, or by direct killing of parasitized macrophages, or a combination of both effects. CD8+ T cells have been associated with protection against Leishmania reinfection in murine models; however, the induction of these T cell subsets in humans seems to be also related to the healing process. Today, there are several reports about different leishmanial antigens eliciting CTL responses such as P8, gp46 (Colmenares et al., 2003), HASPB1 (Stager et al., 2000), Kmp11 (Basu et al., 2007), CPB (Rafati et al., 2002), nucleosomal histones (Iborra et al., 2004), LmaCIN (Farajnia et al., 2005), LmsTI1, and TSA (Coler et al., 2002).

The essential point to be considered in vaccine design for a heterogeneous population, such as that of humans, is the HLA polymorphism. Effective vaccination against a complex parasitic infection such as Leishmania would require a multivalent vaccine composed of several antigens to enhance the possibility of covering a good number of MHC types. This is possible either through recombinant fusion proteins encompassing the whole antigen or through vaccines composed of peptides from different antigens (Campos-Neto et al., 2001; Rafati et al., 2001; Mendez et al., 2002). The latter strategy, called polytope vaccine, is finding its way in vaccinology because of its extraordinary properties, especially the ability to direct the immune response toward the induction of CTLs (Sbai et al., 2001; Schirmbeck et al., 2003; Robinson and Amara, 2005).

As CTL responses play a pivotal role in defense against viruses and tumor cells, polytope vaccines have found their way in these fields but there is still no report on leishmaniasis even it has been shown that CTLs could be very important in protection and long-lasting resistance to infection. Recently, we took advantage of the potential of immunoinformatics tools to screen for L. major epitopes that could be presented in HLA A2, which is the most prevalent HLA supertype in the Iranian population. In vitro stimulation to recall memory CD8+ T cells from Leishmania-infected individuals and intracellular cytokine assays for IFN-γ-producing cells confirmed that HLA A2 positive individuals that recovered from an L. major infection successfully generated CD8+ T cell responses against peptides derived from LmsTI1 and LPG-3 (Seyed et al., 2011). Furthermore, Walden and co-workers have mapped the T cell epitopes from kinetoplastid membrane protein of L. major (Kmp11) via classical mapping for different human HLA class I alleles (Basu et al., 2007). Gazzinelli and co-workers have studied CD8+ T cell responses against the Leishmania A2 antigen and mapped the CD8+ T cell epitopes in BALB/c mice (Resende et al., 2008). Laouini and co-workers (Guerfali et al., 2009) and Dumonteil and co-workers (Dumonteil, 2009) started genome-wide screenings for novel epitopes. Using a combination of T cell epitope prediction tools, they successfully validated such epitopes in both BALB/c and C57BL/6 mice.

Conclusion:

Although understudied, CD8+ T cells appear to play an important role in the immune response to most Leishmania infections. Pilot studies in the murine model of VL have also demonstrated that adoptive transfer of antigen-specific CD8+ T cells (Polley et al., 2006) or reactivation of CD8+ T cell responses through a therapeutic vaccine (Joshi et al., 2009) results in the control of parasite growth. A better understanding of the mode of activation, the specificity, and effector functions of the various CD8+ T cell subsets generated during Leishmania infections could ameliorate the design of vaccines and of novel therapeutic interventions.

Conflict of Interest Statement:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.