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Memory T cells in cutaneous leishmaniasis

Nelson D. Glennie and Phillip Scott*

Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

Leishmania causes a spectrum of diseases that range from self-healing to fatal infections. Control of leishmania is dependent upon generating CD4⁺ Th1 cells that produce IFN γ , leading to macrophage activation and killing of the intracellular parasites. Following resolution of the disease, short-lived effector T cells, as well as long-lived central memory T cells and skin resident memory T cells, are retained and able to mediate immunity to a secondary infection. However, there is no vaccine for leishmaniasis, and the drugs used to treat the disease can be toxic and ineffective. While a live infection generates immunity, a successful vaccine will depend upon generating memory T cells that can be maintained without the continued presence of parasites. Since both central memory and skin resident memory T cells are long-lived, they may be the appropriate targets for a leishmaniasis vaccine.

Keywords

Leishmania; Memory T cells; Vaccine

1. Introduction

Leishmaniasis, caused by several different intracellular protozoan parasites, remains a serious global public health problem, and is characterized by diverse clinical entities ranging from self-healing cutaneous lesions, to severe, disfiguring chronic lesions, to fatal disease. Drug treatment for leishmaniasis is notoriously poor and, despite decades of research, there is no vaccine for this disease. Antibodies play no protective role, and indeed may exacerbate disease. Instead, IFN γ produced by CD4⁺ T cells is required to activate infected macrophages to kill the parasites, and the inability to develop a successful vaccine is largely due to a lack of knowledge on how to generate long-lived memory CD4⁺ T cells [1].

The life cycle of leishmania is relatively simple [1]. The parasites are transmitted by sand flies that harbor the flagellated promastigote form of the parasite. When feeding, sand flies deposit promastigotes in the skin, and phagocytic cells rapidly take up the parasites. While the initial cells that become infected include neutrophils, macrophages, and monocytes, it is primarily within macrophages that the parasites survive and replicate as non-flagellated amastigotes. Surprisingly, unlike other intracellular parasites, such as toxoplasma or

*Corresponding author at: Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Room 310B Hill Pavilion, 380 South University Avenue, Philadelphia, PA 19104-4539, USA. pscott@vet.upenn.edu (P. Scott).

Trypanosoma cruzi, leishmania survives and replicates within the phagolysosome, having evolved the ability to survive in an environment meant to destroy most microbes. After several rounds of replication, the amastigotes rupture the infected cell and invade other neighboring macrophages. The parasites only differentiate back into promastigotes when taken up by sand flies, and thus a successful vaccine needs to be directed at antigens expressed by amastigotes.

Existing vaccines primarily depend upon generating protective antibodies [2,3]. Given that antibodies are not protective in leishmaniasis, a trait shared by other diseases without vaccines such as tuberculosis and leprosy, it has been more challenging to induce long-term protection by immunization. Many experimental vaccines have been developed and large human vaccine trials have been performed over the last 25 years, and yet no human leishmaniasis vaccine exists [4,5]. It has been suggested that a major problem is that long-term memory T cells fail to develop in leishmaniasis, and thus any vaccine developed will require continual boosting to maintain resistance [6]. In this review we will consider the type of immune responses that need to be generated for protection, and discuss the current understanding of memory T cells in leishmaniasis.

2. Protective mechanisms in cutaneous leishmaniasis

The production of reactive oxygen species (ROS) during phagocytosis, called the respiratory burst, is an innate response that allows macrophages and neutrophils to kill microbes before the development of an adaptive response. Those microbes that survive innate killing become pathogens that require a greater response for elimination, which can be induced by the action of IFN γ . This IFN γ response leads to increased production of ROS, as well as activation of inducible nitric oxide synthase, leading to the release of nitric oxide (NO). Leishmania parasites are killed by both ROS and NO, although NO may be more important in the mouse than in the human [7], and different myeloid cells may be better able to kill by one mechanism than the other. For example, inflammatory monocytes exhibit a high level of ROS and can kill leishmania without being activated, while macrophages require IFN γ activation for killing [7,8]. Under some circumstances neutrophils cooperate with macrophages to kill the parasites [9], although in other situations neutrophils appear to inhibit parasite control [10]. Furthermore, while it is clear that NO is required for the ability of mouse macrophages to kill leishmania, the role of NO in human cells remains controversial [7,11,12]. Nonetheless, in both mice and humans IFN γ is absolutely required to control the parasites sufficiently and control the disease.

Many cell types can produce IFN γ , including NK cells, ILCs, $\gamma\delta$ T cells, CD4 T cells and CD8 T cells. However, while all IFN γ producing cells may contribute to an effective response, only CD4⁺ T cells can provide resistance to leishmania infection in reconstituted RAG mice [13,14]. The critical attribute of these cells appears to be their MHC restriction, since it was recently found that adoptive transfer of Class II restricted, but double negative (CD4⁻, CD8⁻) T cells, also provides resistance to leishmania [15]. Nevertheless, while not protective by themselves, other IFN γ producing cells can play important roles in augmenting protection. NK cells (and possibly other innate lymphoid cells) accelerate the development of a Th1 response when CD4⁺ T cells are being primed in the draining lymph

nodes [16–18]. Similarly, following a low dose challenge, IFN γ from CD8 T cells promotes the CD4+ Th1 response [19].

It is important to note that control of the parasites and the development of disease are often dissociated. This is most evident in *Leishmania braziliensis* patients with mucosal disease, where the severe lesions are chronic and debilitating, but contain few parasites. Conversely, while RAG mice are unable to control parasite replication, they develop only minor swelling at the site of infection [13,14,20]. Adoptive transfer of CD4+ T cells to these immunocompromised mice is sufficient to allow for parasite control, but may lead to the development of larger lesions, although these lesions generally resolve, depending on the leishmania species [13,14,20]. On the other hand, when RAG mice are reconstituted with CD8+ T cells they not only fail to control the parasites, but also develop a severe inflammatory response, as well as the development of metastatic lesions [13,14]. This is a surprising result, since under certain circumstances CD8 T cells can promote protection [19,21,22]. It turns out that whether CD8 T cells promote resistance or increased disease relates directly to whether they primarily produce IFN γ or are cytolytic, as increased cytolytic activity promotes a pathologic inflammatory response [14,23].

Although there is a shared pathway for resistance to leishmania that requires IFN γ , there are many different strains and species of the parasites, and these can induce distinct immune responses, and have different sensitivities to activated macrophage killing. This is particularly true of species in South America, which can produce chronic infections in mice normally resistant to *Leishmania major* [24]. One example is *Leishmania amazonensis*, which induces a chronic disease in C57BL/6 mice [25]. *L. amazonensis* parasites induce a weaker CD4+ Th1 response than *L. major* infection, and are also able to resist killing by activated macrophages that can kill *L. major* [26]. Even within the same leishmania species, different strains can lead to diverse outcomes following infection [27]. From this, one might conclude that vaccines and the memory T cells they generate might be effective against one species or strain of leishmania, but may be less effective against another. On the other hand, several studies have shown cross-protection between species, providing some evidence that a single vaccine may work for different leishmania parasites, and even protect against the visceral form of the disease [28,29].

3. Circulating memory T cells in cutaneous leishmaniasis

Following resolution of a primary infection mice are highly resistant to reinfection with leishmania, a fact that led to the belief that it would be relatively straight-forward to develop a vaccine for leishmaniasis. This resistance is primarily dependent upon CD4+ T cells, although immune mice also contain a population of IFN γ -producing CD8+ T cells that contribute to immunity, since depletion of either CD4+ or CD8+ T cells in immune mice decreases resistance to reinfection [21,22]. The strong resistance observed in healed mice is dependent in part on the persistence of a low number of parasites [30,31]. Thus, the few parasites that are left maintain a pool of effector CD4+ T cells that can rapidly respond to a challenge. This raises the question of whether memory T cells develop during a leishmania infection, and if so what type of memory T cells contribute to protection.

Memory T cells have classically been divided into two subsets, central memory (T_{CM}) and effector memory (T_{EM}) cells, based on surface marker expression, tissue tropism, proliferative capacity, and effector function [32]. Central memory T cells express CD62L and CCR7, which allow them to efficiently traffic through the blood and lymph nodes. Upon restimulation, T_{CM} cells rapidly proliferate and thus provide a pool of differentiated, antigen-specific cells to combat a secondary infection. In contrast, T_{EM} cells lack CD62L and CCR7, circulate through blood and non-lymphoid tissues, and exhibit effector functions, such as cytokine production and cytotoxicity, upon restimulation. T_{EM} cells are often distinguished from closely related T effector cells (T_{Eff}) by their ability to persist after antigen is cleared, but can also be identified by IL-7R expression on CD8 T cells [33], and additionally by the absence of Ly6C expression on CD4 T cells [34].

Memory CD4 and CD8 T cells share many defining features, but important distinctions have been identified between the two, specifically in lineage development and recall function. After viral infection, CD8 T cells appear to follow a temporally regulated pathway of differentiation from effector, to effector memory, to central memory cells [35], though there may be some heterogeneity in how these populations arise [36]. During recall, CD8 T cells can produce cytokines such as $IFN\gamma$, but are best known for their cytotoxic activity. In contrast, CD4 memory T cell generation is thought to be more plastic and highly heterogeneous, varying based on factors such as the nature of the pathogen, amount of antigen exposure, and the cytokine milieu [37–40]. Activated memory CD4 cells also perform a wide variety of functions, largely mediated by their ability to produce a diverse array of cytokines. Because CD4 T cells are the predominant mediator of protection against leishmaniasis [41], this review will focus on the memory CD4 T cell subsets required for resistance.

During a primary infection, effector T cells proliferate in the draining lymph nodes, and migrate to the site of infection to mediate protection (Fig. 1). Some of these effector T cells remain once the infection resolves, as a population of short-lived $Ly6C^+$ effector cells have been well characterized [42]. These cells closely resemble phenotypically the CD4 T_{Eff} cells generated after viral infection [34], produce $IFN\gamma$ at a high frequency, and can transfer protection, but they fail to persist in the absence of antigen. However, a population of long-lived $CD62L^+$ T_{CM} cells have also been identified [43]. These cells proliferate rapidly in response to restimulation, produce IL-2, and do not require persistent antigen for survival. Intriguingly, $CD62L^+$ memory cells can also transfer resistance, but this protection is delayed compared to $CD62L^-$ cells. This result indicates that T_{CM} cells are important, but may first need to differentiate into new T_{Eff} cells to provide immunity (Fig. 2).

Since leishmania is a chronic infection, T_{CM} cells must be generated while the parasite is still present. In fact, antigen-specific $CD62L^+CCR7^+$ cells can be identified within the first couple weeks of infection, indicating that subsets of T_{CM} and T_{Eff} are generated concurrently. This cell fate decision appears to be affected by the timing of antigen experience and the number of proliferative cycles a cell has gone through [44]. The role for T_{EM} cells in leishmania infection is less defined, and may not be particularly relevant in a chronic infection. Nonetheless, there is evidence that leishmania infection generates a population of antigen experienced $CD62L^- IL-7R^+Ly6C^-$ cells [44], though their role in

protection remains unclear as they may not be able to migrate to the infection site or proliferate effectively [42].

4. Resident memory T cells in cutaneous leishmaniasis

Tissue resident memory (T_{RM}) T cells have been established as a third population of memory T cells that are critical for protection against a number of pathogens. T_{RM} cells occupy barrier surfaces such as the lung, gut, and skin [45–47], and phenotypically resemble T_{EM} cells in that they are low for CD62L and CCR7 expression [48]. Global analysis has revealed that T_{RM} indeed have a unique gene signature, displaying a wide array of migration, adhesion, and activation markers [49,50].

T_{RM} cells have been shown to increase protection against a variety of pathogens, in a number of tissues, and in a variety of different ways. Many of the initial studies were done on CD8 memory cells, but it is becoming clear that there are a variety of roles for CD4 T_{RM} cells as well. In the skin, herpes simplex virus-specific CD8 T_{RM} cells provide enhanced local protection [51]. Moreover, skin infection with modified vaccinia virus generates CD8 T_{RM} cells that are globally distributed throughout the skin and provide protection superior to that of circulating memory [52]. CD8 T_{RM} generated by lymphatic choriomeningitis virus infection in the female reproductive tract robustly protect against rechallenge by recruiting circulating T_{Eff} more rapidly [53], and can also increase local humoral responses, dendritic cell maturation, and natural killer cell activation [54].

At steady state many CD4 T cells circulate through tissues such as the skin, but CD4 cells also form skin resident populations, particularly after inflammation [55,56]. CD4 T_{RM} cells in the lung provide increase protection against influenza [57], and appear to do so by orchestrating the establishment of local CD8 cells [58]. Furthermore, CD4 cells in the vaginal mucosa can interact with local macrophages to protect against herpes simplex virus-2 [59]. However, CD4 T_{RM} cells are not always beneficial and have also been implicated in psoriasis [60] and asthma [61].

T_{RM} cells have now been shown to play a critical role in protection against leishmaniasis [62]. T_{RM} cells are generated early in infection, and appear to infiltrate the skin globally, as they can be detected in many sites distal to the primary site of infection. Importantly, these cells are long lived and establish residence in the absence of persistent parasites, as demonstrated by their ability to remain in skin when grafted onto a naive host [62], or after parabiotic surgery (Glennie & Scott, unpublished data). Upon restimulation, these cells produce IFN γ which results in a more rapid recruitment of T_{Eff} cells to the skin in a CXCR3 dependent manner, which in turn results in better parasite control. In concert with circulating T cells, T_{RM} cells play a critical role in promoting optimal immunity to infection. Moreover, it is possible that the rapid production of cytokines by T_{RM} in the skin may promote additional protective responses even in the absence of T_{Eff} cells. These potential responses, such as the direct activation of infected cells, maturation of dendritic cells, or recruitment of phagocytes should continue to be explored.

5. Implications for the development of a cutaneous leishmaniasis vaccine

Cutaneous leishmaniasis poses a conundrum for vaccine development. On the one hand, control of a primary infection is highly protective, a prospect that bodes well for any potential vaccine. On the other hand, the parasite is never fully cleared, and forcing elimination of the parasite reduces immunity to reinfection [30,31]. While a live vaccine that provides persistent antigen might provide protection, it could be difficult to deliver such a vaccine due to ethical and logistical concerns. Encouragingly, it is not required that a cutaneous leishmaniasis vaccine provides sterile immunity, as it is apparent that low levels of parasites can be maintained without pathology. Therefore, the goal of a cutaneous leishmaniasis vaccine might not be to completely eliminate the parasites but instead reduce the parasite load below a certain threshold, and in particular decrease the incidence of the most pathologic outcomes of disease such as mucosal, disseminated, and diffuse cutaneous leishmaniasis.

Circulating CD4 T_{Eff} cells are critical mediators of immunity and thus will be important to target in a vaccine. Unfortunately, these T cells require parasites to persist, and therefore maintaining them will demand a strategy that supplies continuous antigen either through a live-attenuated pathogen, frequent boosts, or some other persistent delivery platform. The development of a safe live-attenuated parasite that maintains T_{Eff}, T_{CM}, and T_{RM} may be the simplest path to an effective vaccine, but has proven difficult to develop. Another barrier to the generation of a CD4 T cell vaccine has been the lack of well-defined immunodominant CD4 epitopes, as most immunization strategies have required complex polypeptide constructs to induce responses [4,63]. The recent identification of a protein, phosphoenolpyruvate carboxykinase, that reacts with approximately 20% of *L. major*-specific CD4 T cells and has broad cross-reactivity may help resolve this need, and will also be an invaluable tool for assessing CD4 responses [29]. CD8 T cells have been shown to be protective in some vaccine and mouse models [64], but also might be problematic as CD8 cytotoxic lymphocytes are associated with increased pathology in *Leishmania braziliensis* [23,65].

Fortunately, there are at least two populations of memory T cells that are maintained in the absence of persistent parasites: T_{CM} and T_{RM} cells. CCR7⁺ CD4 memory cells that show increased proliferative capacity in response to leishmania antigen have been identified in cutaneous leishmaniasis patients that have healed [66], suggesting that T_{CM} cells are generated by infection in humans and may be a good target in a vaccine.

T_{RM} have never been targeted in a leishmania vaccine, and may be the missing link for optimal vaccine-induced protection. Indeed, when challenged by sand fly bite instead of needle injection, only immune mice and not vaccinated mice are protected against *L. major* [67]. Since immune mice contain a population of T_{RM} cells, this result may demonstrate the importance of T_{RM} for a vaccine. Further, since all species of the parasite are transmitted through the skin by sand fly bite, T_{RM} cells may be able to provide significant cross-protection, even against the visceral form of the disease. Skin-scratification has been shown to induce increased proliferation of T cells in skin-draining lymph nodes, as well as increased homing to peripheral tissue [68], suggesting that a delivery strategy involving

epidermal abrasion may generate T_{RM} cells more effectively than other delivery methods. Since T_{RM} cells can respond rapidly, a vaccine that combines the induction of T_{RM} cells and circulating T_{CM} responses may offer the best hope of an effective vaccine for cutaneous leishmaniasis.

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Fig. 1.

T cell responses following infection with leishmania. Leishmania are deposited in the skin by infected sand flies and rapidly invade phagocytic cells, including dendritic cells (DC). DCs migrate from the skin to the draining lymph node (1) where they stimulate naïve T cells, which proliferate and differentiate into effector T (Teff) cells (2) or central memory (Tcm) cells (3). Teff cells leave the lymph nodes and migrate to the site of infection (4), where they produce IFN γ leading to parasite control. Some of the Teff cells will also migrate through distant skin sites, where some will become resident memory T (Trm) cells (5).

Fig. 2.

T cell responses following a secondary infection with leishmania. When mice that have resolved a primary infection are challenged with leishmania, Trm cells are activated (1) and produce cytokines and chemokines that lead to recruitment of Teff cells from the blood (2), as well as other cells including inflammatory monocytes (iMO) (3). DCs also migrate from the skin to the draining lymph node (4) where they stimulate Tcm cells, which proliferate and differentiate into effector T (Teff) cells (5), thus rapidly increasing the population of Teff cells that are able to control the infection.