Cellular Immunology 309 (2016) 32–36

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Research paper

Pathways leading to interleukin-12 production and protective immunity in cutaneous leishmaniasis

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ARTICLE INFO

Article history:
Received 10 April 2016
Revised 27 June 2016
Accepted 28 June 2016
Available online 29 June 2016

Keywords:
IL-12
Macrophage antigen 1
CD40
CD40 ligand
Leishmania major
Infection
Th1 response
Immunity

ABSTRACT

Leishmaniasis affects millions of people worldwide and continues to pose public health problem. There is extensive evidence supporting the critical role for IL-12 in initiating and maintaining protective immune response to *Leishmania* infection. Although gene deletion studies show that CD40-CD40L interaction is an important pathway for IL-12 production by antigen-presenting cells and subsequent development of protective immunity in cutaneous leishmaniasis, several studies have uncovered other pathways that could also lead to IL-12 production and immunity in the absence of intact CD40-CD40L signaling. Here, we review the literature on the role of IL-12 in the induction and maintenance of protective T cell-mediated immunity in cutaneous leishmaniasis and the different pathways leading to IL-12 production by antigen-presenting cells following *Leishmania major* infection.

1. Introduction

Leishmaniasis is a spectrum of diseases caused by different species of intracellular protozoan parasites belonging to the genus *Leishmania*. Recent estimates show that the annual incidence of leishmaniasis is around 1.5 to 2 million with about 12 million people currently afflicted with the disease and 350 million people at risk of infection world-wide [1]. A recent report suggests that the real disease incidence and prevalence may be significantly higher due to underreporting [2]. Apart from patients in endemic regions, leishmaniasis is also frequently diagnosed in travellers, soldiers or immigrants [2]. For example, leishmaniasis has been diagnosed in British [3], American [4,5] and Canadian [6,7] soldiers returning from active military duties in endemic countries including Afghanistan and Iraq. Interestingly, leishmaniasis have also been diagnosed in people with no travel history to endemic regions in the United States of America, thus suggesting an enzootic transmission due to previously undescribed vector-host interaction [8].

The development of protective immunity against leishmaniasis requires cooperation between the innate and adaptive arms of the immune system. However, since *Leishmania* are obligate intracellular parasites, activation of optimal cell-mediated immunity is critical for disease control. Indeed, studies show that the induction and maintenance of $CD4^+$ Th1 immune response is important for effective clearance of *Leishmania* parasites [9]. The induction of an early IFN-γ-producing Th1 cells is initiated by interleukin 12 (IL-12) produced by infected dendritic cells. This IFN-γ produced by $CD4^+$ Th1 cells is critical for activating infected macrophages to produce nitric oxide (NO) that is responsible for killing *Leishmania* parasites within infected cells [10]. Gene deletion and blocking antibody experiments clearly show that IL-12 is required for the development of effective Th1 response and immunity against leishmaniasis [11–13]. Several pathways responsible for this early IL-12 production by dendritic cells have been described. Here, we review the literature and provide an overview of the different pathways leading to IL-12 production and immunity in cutaneous leishmaniasis.

2. Cutaneous leishmaniasis and interleukin 12 (IL-12)

Initially identified for its ability to stimulate natural killer cells, IL-12 is a heterodimeric cytokine made up of two subunits: a 40 and 35 kDa chains. Several mouse studies highlighted the important role of IL-12 in immunity to cutaneous leishmaniasis. Resistant C3H mice infected with *Leishmania major* showed an early increase in numbers of IL-12 p40-producing cells in the lymph nodes [11]. This was associated with enhanced NK activity

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http://dx.doi.org/10.1016/j.cellimm.2016.06.004
0008-8749/

and IFN-γ production by lymph node cells. Interestingly, *in vivo* blockade of IL-12 activity led to loss of this early IFN-γ response [11], suggesting a critical role for this cytokine in IFN-γ production. In line with this, treatment of susceptible BALB/c mice strain with rIL-12 at time of infection led to robust IFN-γ production by $CD4^+$ T cells (Th1 response) and complete resolution of cutaneous lesions [12,13]. In contrast, neutralization of IL-12 with anti-IL-12 monoclonal antibody or deletion of IL-12 gene in the usually resistant mouse strains led to susceptibility (non-healing cutaneous lesion and uncontrolled parasite proliferation) to *L. major* infection [14,15]. However, another study reported that the resistant C3H mice strain treated with anti-IL-12 monoclonal antibody still maintained a population of $CD4^+$ T cells that expressed IL-12 receptor beta 1 and 2 and were able to produce levels of IFN-γ comparable to untreated controls [15]. This discrepancy could be due to the use of different resistant mouse strains (C57BL/6 vs. C3H) in these studies. Furthermore, there is also the possibility that anti-IL-12 monoclonal antibody did not completely abrogate IL-12 activity in the treated C3H mice.

The difference in disease outcome in the susceptible BALB/c and resistant C57BL/6 mice strains following infection with *L. major* seems to be due to differences in their ability to regulate IL-12 receptor expression and signaling [15].

This and other *in vivo* observations of the importance of IL-12 in the induction of potent Th1 immune response and resistance to *L. major* prompted studies to test whether it can be used as a vaccine adjuvant to promote Th1 immunity in experimental leishmaniasis. BALB/c mice vaccinated with soluble *Leishmania* antigen (SLA) with rIL-12 were protected against virulent *L. major* challenge. This protection was associated with early induction of IFN-γ-producing NK cells (0–2 weeks post-infection) that was followed by activation of IFN-γ-producing $CD4^+$ T cells later during the infection (>2 weeks) [16]. However, vaccination of Vervet monkeys with killed *L. major* and rIL-12 did not elicit protection against virulent *L. major* challenge despite the induction of strong cell-mediated immune response [17]. This observation suggests that while the effect of IL-12 at inducing enhanced cellular response may be universal, its ability to induce protection may be species dependent.

Although IL-12 is mostly known for its ability to initiate Th1 response and resistance to *L. major* infection, studies also show that it plays a critical role in the maintenance of this immunity. For instance, *L. major*-infected IL-12 deficient mice on the C57BL/6 background treated with rIL-12 heal their lesion but will spontaneously reactivate disease when treatment is stopped, suggesting that continuous IL-12 is required for maintenance of Th1 cell-mediated immunity to the infection [14]. It was later confirmed that IL-12 is indeed required for the maintenance of established cell-mediated immunity against *Leishmania major* [18]. Although the critical role of IL-12 in resistance to experimental cutaneous leishmaniasis is unequivocal, its role in humans is not very clear. Interestingly, humans with active CL lesions produce more IL-12 compared to individuals that have healed their lesions [19,20]. This could represent an attempt by the host immune system to fight the infection and more studies are required to definitively determine the role of IL-12 in human leishmaniasis.

3. Pathways to IL-12 production in cutaneous leishmaniasis

3.1. CD40-CD40L interaction

The CD40-CD40L pathway is a well-characterized co-stimulatory pathway that is important for optimal T and B cell responses and the development of humoral and cell-mediated immunity [21]. CD40 is a type I transmembrane protein that was initially described in B cells where it was shown to induce B cell proliferation and antibody isotype switching [22,23]. It is constitutively expressed or induced on several immune and non-immune cells including basophils, dendritic cells, B cells, epithelial cells, macrophages, endothelial cells, smooth muscle cells and fibroblasts. The ligand for CD40 is CD40 Ligand (CD40L) [24], which is an inducible type II transmembrane protein expressed on activated $CD4^+$ and $CD8^+$ T cells, B cells, epithelial cells, eosinophils, monocytes, macrophages, and NK cells under inflammatory conditions [25]. CD40L also exists in a soluble form (sCD40L) that mediates biological activity similar to the transmembrane form [26].

The interaction of CD40 and CD40L is critical for the production of IL-12p70 [27]; an important cytokine for the induction and maintenance of cell-mediated immunity. As such, studies have been carried out to determine the role of CD40-CD40L pathway in IL-12 production and subsequent immunity in cutaneous leishmaniasis. Gene deletion and antibody studies show that the usually resistant C57BL/6 mice deficient in either CD40L or CD40 gene expression are highly susceptible to *L. major* [28,29] and *Leishmania amazonensis* [30] infections. Furthermore, injection of CD40L antagonists led to susceptibility, which was associated with reduced IL-12 production [31]. Also blockade of CD40-CD40L interaction in human PBMCs stimulated with or without *Leishmania* antigen led to reduced IFN-γ production [32]. In contrast, treatment of *L. major*-infected usually susceptible BALB/c mice with CD40 agonistic antibody results in resolution of cutaneous lesions [33]. Interestingly, some studies suggest that CD40-CD40L interaction is dispensable for IL-12 production and protection against *L. major* [34,35]. Also in patients with CL due to *Leishmania braziliensis*, blocking CD40-CD40L interaction did not affect IFN-γ production by PBMCs [36]. Taken together, the above studies indicate that the role of CD40-CD40L interaction in IL-12 production and resistance to *L. major* infection remains unclear. The discrepancies in the role of CD40 and CD40L in resistance could be due to differences in experimental approaches, including parasite strain and dose, site of infection and methods of assessment of immune response and protection. In a recent study, we attempted to clarify the role of CD40-CD40L interaction in immunity to experimental CL caused by *L. major*. In line with previously published data, we showed that both CD40 and CD40L KO mice were highly susceptible to *L. major* infection and the susceptibility was associated with impaired IL-12 production by dendritic cells from infected mice [37]. However, treatment of both infected CD40 and CD40L KO mice with exogenous IL-12 lead to different outcomes: complete healing with no lesion reactivation and resistance to secondary challenge in CD40 KO mice and only a short-term lesion resolution and disease reactivation in the CD40L KO mice few weeks after cessation of rIL-12 treatment [37]. Disease reactivation in CD40L KO mice was associated with enhanced Th2 and reduced Th1 responses in contrast to the CD40 KO mice that maintained a sustained Th1 response [37]. The finding of striking differences in immune response and disease outcome between infected CD40 and CD40L KO mice following cessation of rIL-12 treatment indicates the existence of an alternative and/or redundant pathway for IL-12 production in CD40 KO mice that is absent in CD40L KO mice. It is also in agreement with previous finding by Padigel et al. [34] that showed that CD40-CD40L pathway is not the sole pathway for IL-12 production in leishmaniasis.

3.2. LIGHT, lymphotoxin beta (LTβ) and herpes virus entry mediator (HVEM) pathways

LIGHT (Homologous to lymphotoxin, exhibits inducible expression, competes with HSV glycoprotein D for HVEM, a receptor expressed on T cells) is expressed on different immune cells such as activated T cells, monocytes, granulocytes and immature dendritic cells [38–40]. It binds to two receptors; LTβR and HVEM in