IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Biological Sciences

2011; 7(9):1382-1390

Review

Role of Co-stimulation in Leishmaniasis

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Received: 2011.09.01; Accepted: 2011.10.01; Published: 2011.11.01

Abstract

Leishmania are obligate intracellular parasites that cause a wide spectrum of diseases ranging from cutaneous, mucocutaneous and the visceral kind. Persistence or resolution of leishmaniasis is governed by host immune response. Co-stimulation is an important secondary signal that governs the extent, strength and direction of the immune response that follows. Co-stimulation by CD40, B7 and OX40 family has been shown to influence the outcome following *Leishmania* infection and manipulation of these pathways has shown promise for use in immune therapy of leishmaniasis. In this review, we discuss the roles of CD40, B7 and OX40 co-stimulatory pathways in regulating immunity to *Leishmania* and their implications in the treatment of this disease.

Key words: Leishmaniasis, *Leishmania*, co-stimulatory pathways, immunity

Leishmaniasis

The genus *Leishmania* comprises of obligate intracellular parasites which are released into the bloodstream of their vertebrate host when an infected vector sand fly takes a blood meal thereby injecting promastigote form of the parasite [1]. The promastigotes are taken up by a variety of immune cells early on, like neutrophils and dendritic cells (DCs). The parasites eventually gain entry into their natural host cell, the macrophages, where they differentiate into intracellular amastigotes [2]. Then on, the interplay between host's innate and adaptive immune cells and the resultant cytokines released ultimately skew the immune response towards either disease resolution or persistence. The range of clinical manifestations of leishmaniasis is documented broadly as cutaneous leishmaniasis (CL), caused by *L. major*, *L. tropica* and *L. mexicana*, the mucocutaneous leishmaniasis (MCL), caused by *L. braziliensis* and visceral leishmaniasis (VL), caused by *L. donovani* and *L. infantum* (*chagasi*) [1]. World over, 12 million people are estimated to be infected with leishmaniasis and 350 million people are potentially at risk of acquiring leishmaniasis [3].

Long term immunity to CL is governed by development of an effective adaptive immune response where T helper cells play a crucial role. Induction of T helper phenotype 1 (Th1) is associated with production of proinflammatory cytokines IFN-γ and IL-12 while Th2 cells predominantly produce the immunosuppressive cytokines IL-4 and IL-10. IFN-γ induces production of leishmanicidal nitric oxide (NO) from macrophages [4-6] while IL-12 indirectly aids this pathway by inducing IFN-γ from natural killer (NK) and T cells. IL-4 and IL-10 on the other hand catalyze arginase activity over inducible nitric oxide synthase (iNOS) preventing the production of NO. The disease exacerbating role of Th2 vs. resolving role of Th1 cytokines has been well established in CL models with *L. major*. BALB/c mice are predisposed to generation of Th2 cells following *L. major* infection and hence are highly susceptible while C57BL/6 mice

produce Th1 cells following CL and have a better prognosis [7, 8].

Compared to CL model of leishmaniasis by *L. major*, where a defined disease exacerbating role of Th2 response is noted; in VL, the absence of disease resolving Th1 response is related to the severity of infection [9].

Co-stimulation

Effective long term immunity against antigens requires activation of adaptive arm of immune response mediated by T lymphocytes. Antigen presenting cells (APC) such as DCs, macrophages and B cells present processed antigenic peptides via major histocompatibility complex (MHC) to T cell receptor (TCR) of naïve T cells. The presentation of antigens by APC provides the first signal for T cell activation. This interaction determines the specificity of the immune response. However, antigenic stimulation in itself cannot activate T cells effectively unless a second signal is received by the cells. Ligands on APCs called co-stimulatory molecules interact with specific receptors on T cells providing the second signal which then leads to activation of the antigen stimulated T cell [10, 11].

A number of co-stimulatory pathways are involved in mediating T cell responses. Co-stimulation is critical not only for activation but also inhibition and often acts in a spatial manner wherein certain pathways come into play during priming and others during the effector stages of infection or memory generation [10]. In this review we take a closer look at some of the co-stimulatory pathways involved in the modulation of *Leishmania* infection.

The CD40 family

The CD40 receptor is a Type I transmembrane receptor phosphorylated glycoprotein that consists of an extracellular carboxy terminal segment with 22 cysteine residues, homologous to other members of the tumor necrosis receptor family (TNRF). It is found ubiquitously on the surface of monocytes, macrophages, DCs as well as non-immune cells such as epithelial cells and platelets [12-14]. CD40L, the ligand for CD40 receptor protein is a Type II transmembrane protein that exists as a homotrimer. CD40L is primarily present on the surface of activated mature T cells as well as non resting T cells. It is mostly present on CD4+ T cells although a small population of CD8+ T cells also possesses this antigen. Other immune cells such as eosinophils, basophils and mast cells are also known to possess CD40L while it is only expressed under certain conditions by B cells, DCs, NK cells, monocytes and macrophages [12].

CD40:CD40L signaling

CD40:CD40L interaction results in the activation of protein tyrosine kinases (like lyn, syk and Jak3), phosphoinositide-3-kinase and phospholipase CG2. Other studies have also discovered the interplay of serine/threonine kinases like JNK/SAPK (c-jun amino terminal kinase/stress activated protein kinase), p38MAPK (mitogen-activated protein kinase) and ERK (extracellular signal regulated mitogen-activated protein kinase) [15-17]. Further, certain cytoplasmic adaptor proteins called TRAFs (TNF-R associated factors) are recruited to the cytoplasmic tail of CD40 and this process is enhanced by receptor oligomerization. All TRAF proteins, with the exception of TRAF 1, have zinc finger domain that are predicted to aid in binding DNA or enhance protein-protein interaction. This allows the TRAF proteins to participate in the activation of a variety of protein kinases, like the MAP kinase family (JNK, ERK and p38MAPK) [15, 16]. The existence of CD40 receptors on distinct 'signalosomes' have been shown in human monocyte-derived DCs where the CD40 receptor is constitutively associated with cholesterol and sphingolipid rich microdomains in the plasma membrane called lipid rafts or membrane rafts. This ordered liquid structure provides a platform for CD40 signaling events. There is evidence to support that CD40 receptor engagement by antibody treatment or association with CD40L results in phosphorylation of Lyn tyrosine kinase which is associated with the membrane rafts. Further, the presence of Syk tyrosine kinase specifically in the detergent-soluble fractions of membrane extract compared to the presence of CD40 receptor and Lyn tyrosine kinase in the detergent-resistant fractions indicates a possible spatial and temporal regulation of CD40 signaling cascade where membrane bound Lyn undergoes phosphorylation first followed by Syk. Other proteins that were found to constitute the CD40 signalosome were TRAF 2 and TRAF 3 and were recruited to the CD40 cytoplasmic tail following receptor engagement [15].

Role of CD40:CD40L interactions in leishmaniasis

Early studies on CD40:CD40L interactions have identified key role of this interaction in the establishment of effective humoral response, with particular involvement in immunoglobulin (Ig) isotype class switching, memory cell maturation and germinal center formation. Recent studies have begun to shed light on the importance of CD40:CD40L interactions in cell mediated immunity [12-14]. CD40 ligation mediates upregulation of various co-stimulatory mole-

cules, B7-1 and B7-2 in APCs and CD58 in DCs [18], apoptosis regulation in CD4+ T cells [19] and production of various cytokines amongst which IL-12 production by DCs [20] is important during *Leishmania* infection [21, 22]. With particular relevance to *Leishmania* infections, CD40 interactions are involved in macrophage activation, IL-12 production in DCs and CD4+ T cell priming.

The protective role of CD40 has been demonstrated in footpad infection model on CD40 deficient mice infected with *L. major*. The $CD40^{-/-}$ mice presented with rapid systemic development of leishmaniasis compared to the susceptible BALB/c control mice. A polarized Th2 response was noted in the $CD40^{-/-}$ mice as indicated by excessive IL-4 levels (at values similar to a Th2 skewed response in BALB/c mice) and lower levels of IFN-γ production by cells of the draining lymph nodes [23]. A similar pattern of low IFN-γ, tumor necrosis factor (TNF), and NO production and high IL-4 production was seen in CD40L deficient mice infected with *L. mexicana* [24]. This is indicative of impaired T cell mediated macrophage activation which results in high parasite tissue burden. Although, it has been shown that IFN-γ is a potent activator of macrophage activity [25, 26], infected macrophages treated with IFN-γ alone are unable to clear parasites effectively. The addition of anti-CD40 antibody (Ab) was found to be an important priming signal for macrophages resulting in lower parasite count when used in conjunction with IFN-γ treatment as compared to the use of either treatment separately. Hence, CD40:CD40L interaction may be important in vivo for macrophage activation and parasite clearance [23].

IL-12, an important cytokine for generating Th1 response [22] during *Leishmania* infections [27] has recently been shown in CL and VL models to be predominantly produced by DCs and not macrophages [27-30]. Though a decrease in production of IL-12 in CD40 deficient mice was not directly related to the absence of CD40:CD40L interaction [23], a study on IL-12 production in DCs infected with *L. major* has shown that this event is dependent on CD40 priming by activated CD40+ T cells. Human monocyte-derived DCs infected with *L. major* upregulate the expression of costimulatory molecules like B7-2 and CD40 on their cell surface. However, the production of IL-12p70 does not occur unless the cells have a secondary priming signal from the CD40:CD40L interaction. The direct role of CD40:CD40L interaction in the T cell-DC interface was established by the reactivation of T cells, isolated from *L. major* infected patients, by *L. major* infected DCs, either in the presence or absence of anti CD40L Ab [28]. IL-12 production is also affected during defective CD4+ T cell priming exemplified by low IL-12 production by splenocytes from $CD40L^{-/-}$ mice compared to wildtype (WT) mice [31].

Activation of CD4+ T cells is important for production of IFN-γ which in turn activates macrophages to produce IL-12, dictating a Th1 immune response [32]. In a study of *L. major* infection on $CD40L^{-/-}$ mice, the mice developed severe ulcerating lesions which were significantly bigger than susceptible BALB/c control mice unlike the study with *L. major* infected $CD40^{-/-}$ mice, where a lack of initial footpad swelling was observed [23, 31]. The inability of T cells to undergo priming was evident by low IFN-γ levels in in vitro cytokine profiles of the draining lymph node cells. The specific defect in the T cells from $CD40L^{-/-}$ mice was evident when peritoneal macrophages obtained from $CD40L^{-/-}$ mice when treated with soluble CD40L and IFN- γ, showed IL-12 production [31].

CD40:CD40L interaction has also been shown to be critical in clearing VL. $CD40L^{-/-}$ C57BL/6 mice did not develop granuloma at 4 weeks post infection and granulomas that developed at 8 weeks were heavily infiltrated with parasites. Treatment with agonist anti-CD40 monoclonal antibody (MAb) in *L. donovani* infected BALB/c mice resulted in greater than 50% reduction in liver parasite burden, high serum IFN-γ activity while antimony treatment in infected $CD40L^{-/-}$ mice, resulted in no disease resolution. This, along with the lack of granuloma formation points to insufficient antigen activated CD4+ T cell and DC development. Further, anti CD40 MAb treatment resulted in development of mature granulomas; these were characterized by unique parasitized foci encased with mononuclear cells (morphologically lymphocytes) which was not visualized in the IgG treated control mice (both infected C57BL/6 and BALB/c mice) [33].

IL-10 is an important cytokine that is implicated in the sustained persistence and exacerbation of leishmaniasis [34-36]. What makes the understanding of disease resolution complicated from the perspective of CD40 co-stimulation is the fact that both IL-12 and IL-10 are produced upon CD40 receptor engagement [28, 37]. The balance between the two cytokines can determine disease resolution or persistence in a given model of infection [38]. CD40 stimulation in macrophages causes IL-12 production via p38 MAP kinase pathway [39, 40]. CD40 primed macrophages can also produce IL-10 by ERK1/2 activation of the MAP kinase family [39, 41]. Interestingly, the strength of CD40 cross linking determines amount of IL-10 and IL-12 produced by macrophages, as shown previously [39]. Uninfected macrophages show higher produc-

tion of IL-10 at CD40 Ab concentrations of 2μg/ml, peaking at 4μg/ml and falling at about 8μg/ml. However, there is a steady increase of IL-12p40 levels from 2μg/ml to 16μg/ml concentrations of CD40 Ab. This indicates that at low CD40 cross linking, more IL-10 is produced while higher CD40 cross linking shifts the balance towards IL-12 production in uninfected macrophages. However, surprisingly, this pattern is not seen in macrophages infected with *L. major*. With increasing parasite to macrophage ratio at an intermediate concentration of CD40 Ab (3μg/ml), there is increasing production of IL-10 while a downward trend for IL-12 production [39]. Apart from the strength of CD40 cross linking to provide host protective immune response, the relative expression levels of CD40 also play a role in modulating immune responses. Recent studies have shown that differential levels of CD40 expression on DCs can act to develop specific subsets of T cells. At lower CD40 expression levels, as seen in DCs isolated from $CD40^{+/-}$ background, there was selective development of regulatory T cells (Tregs) which dampen inflammatory responses. However, at higher expression of CD40, which is seen in DCs isolated from $CD40^{+/+}$ background, there was development of effector T cells that facilitated parasite clearance and reduced susceptibility to *L. donovani* infection. These results indicate that CD40 signaling does not contribute solely to proinflammatory immune processes (like T cell priming, macrophage activation and IL-12 production), but is also responsible for modulating immune responses by IL-10 production and Treg cell development [42].

Studies have also shown that *Leishmania* is actively involved in elevating disease exacerbating cytokine levels by manipulating CD40 signaling cascade. This is seen in macrophages infected with *L. major* which predominately show high ERK1/2 phosphorylation and thus high IL-10 production [39]. Other studies have shown that *L. major* causes depletion of membrane cholesterol on macrophage surface and selective decrease in p38MAP kinase phosphorylation and increase in ERK1/2 phosphorylation. Hence, this is an immune evasion strategy where *L. major* actively downregulates the production of IL-12 in the macrophage and alters the CD 40 signalosome to enhance IL-10 production and thus parasite survival [43].

The B7 family

This family comprises of molecules of Ig superfamily and is characterized by an extracellular IgV like and IgC like domains [10, 44]. B7 molecules are expressed by APCs such as DCs, macrophages, B cells and also T cells [45]. B7-2(CD86) is constitutively expressed and rapidly upregulated following activation while B7-1(CD80) is induced after B7-2 expression [44, 46]. B7-1/B7-2 have dual specificity for receptors CD28 and cytotoxic T lymphocyte antigen-4 (CTLA-4/CD152) on T cells. CD28 and CTLA-4 are also members of the Ig superfamily. The extracellular IgV like domain on CD28 and CTLA-4 have an MYPPPY motif on the complementarity determining region 3 (CDR3) like region. This motif is involved in binding to B7-1 and B7-2 [10, 47-49]. CD28 is constitutively expressed by T cells while CTLA-4 is induced rapidly by activated T cells [10, 44]. CTLA-4 has higher binding affinity to B7-1/B7-2 than CD28 [44]. These factors establish a hierarchy during T cell activation stages such that B7 signals via CD28 during the initial stage of antigenic stimulation. The activation then induces CTLA-4 expression that can suppress T cell activation and establishes immune homeostasis. The latter role of CTLA-4 is mediated perhaps by its ability to outcompete CD28 for binding and also by its ability to dampen major players of the CD28 signaling pathway [50].

B7:CD28/CTLA-4 signal transduction

Interaction between B7 and CD28 activates tyrosine phosphorylation of YMNM motif in the intracellular signaling domain of CD28. This motif interacts with phosphatidylinositol-3 kinase (PI3K) and Grb2 [51]. The downstream effects of this signaling, results in upregulation of intrinsic survival and anti apoptotic factor Bcl-X_L [52, 53], stabilization and transcription of IL-2 mRNA and progression of T cell into cell cycle. CD28 signaling is thus responsible for T cell proliferation, differentiation, survival and IL-2 synthesis [52].

CTLA-4 signal transduction on the other hand recruits Src homology 2 domain containing phosphatase 2 (SHP-2) [51] which dampens the activating effects of CD28 perhaps via dephosphorylation of TCRζ [51, 54]. B7: CTLA-4 signaling is also associated with generation of indoleamine-2,3-dioxygenase (IDO) from DCs triggered by IFN-γ. IDO degrades tryptophan into kynurenines and causes apoptosis of T cells [44, 55, 56]. CTLA-4 signal transduction has also been implicated in autocrine induction of transforming growth factor β (TGFβ), an immunosuppressive cytokine from T cells [6]. The T cell inhibiting effect of CTLA-4 is likely due to CD28 signal usurping role of CTLA-4 by outcompeting with the ligand and also the inhibitory pathways activated by it [57].

The roles played by these molecules have been highlighted in a variety of studies. CTLA-4 deficient mice develop lymphoproliferative disorders and are

highly susceptible to autoimmune disorders [50]. This highlights the crucial role of CTLA-4 in maintaining peripheral immunity and tolerance. CD28 blockade leads to prolonged allograft and xenograft survival [53]. Blocking of B7:CD28 pathway has been found to be beneficial in autoimmune disease, asthma, allergy and transplantation models [44, 47, 58]. CTLA-4 Ig a known inhibitor of this pathway has been tested in clinical trials for treatment of rheumatoid arthritis [59]. Blocking of B7:CTLA-4 signaling has been implicated in enhancing tumor rejection [44, 56].

Role of B7 co-stimulation in leishmaniasis

Induction of either Th1 or Th2 phenotypes is governed by a number of factors amongst which cytokine milieu at the time of antigenic stimulation is one of the most critical [60]. A number of studies have indicated that co-stimulatory molecules B7-1/B7-2 can differentially influence the development of Th1 or Th2 cells. An in vitro experimental allergic encephalomyelitis model indicates a Th1 inducing role of B7-1 and a Th2 inducing role of B7-2 [60]. B7-2 however was shown to have Th1 inducing role in non-obese diabetic mice model [61] and in murine allergy and helminthic infections it was found to be associated with Th2 induction [62]. Studies in *L. major* CL implicate B7-2 in development of Th2 pathway and hence susceptibility while B7-1 did not influence CL [63].

Use of CTLA-4 Ig, an antibody that binds with B7 ligands and essentially prevents B7:CD28/CTLA4 pathway or administration of B7-2 MAb has been shown to negatively affect Th2 cell development [63-65]. Both treatments led to reduction in parasite burden in otherwise susceptible BALB/c mice. The protective effects correlated with a decrease in IL-4 in CTLA4-Ig treatment while B7-2 MAb treatment also resulted in a significant decrease in IL-10 along with IL-4. These treatments, however, had no effect on cytokine profiles of C57BL/6 mice infected in a similar manner though reduction in parasite load was evident [63, 64]. Treatment with B7-1 MAb also had no significant effect thus eluding to a critical role of only B7-2 in Th2 induction in *L. major* infection [63, 65].

When *L. major* CL on CD28 gene deficient mice was studied, parasite burden and disease progression in the $CD28^{-/-}$ mice was comparable with that of similarly infected WT mice in BALB/c strain while $CD28^{-/-}$ C57BL/6 were able to heal lesion as well as their WT counterparts. The $CD28^{-/-}$ BALB/c mice in these experiments were fully able to mount a Th2 response indicating that CD28 is not necessary for generation of Th2 response [66]. This experiment used high infection dose model, and the ability to mount a Th2 response even in the absence of CD28 was seen to be independent of CTLA-4 signal transduction as $CD28^{-/-}$ mice administered CTLA-4 Ig still mounted a Th2 response [65]. Interestingly, another study that compared CL in BALB/c $CD28^{-/-}$ and $B7\text{-}1^{-/-}$/ $B7\text{-}2^{-/-}$ double knockout (KO) mice also resulted in dissimilar phenotypes. Unlike the $CD28^{-/-}$ or $B7\text{-}1^{-/-}$ mice which were both susceptible to *L. major*, the $B7\text{-}2^{-/-}$ and $B7\text{-}1^{-/-}$/$B7\text{-}2^{-/-}$ mice were resistant to CL. These studies also highlighted a critical role of B7-2 in directing Th2 immune response in *L. major* CL as interpreted from low IL-4 levels in the resistant mice [67]. The difference in the phenotype of $CD28^{-/-}$ mice from $B7\text{-}1^{-/-}$/$B7\text{-}2^{-/-}$ mice may be due to a compensatory co-stimulatory pathway or B7 signaling via alternate ligands in the $CD28^{-/-}$ mice [65]. This difference may also be attributable to parasite dose, as a study using lower parasite inoculum (750 promastigotes) in a similar infection study was shown to be associated with resolution of CL by the $CD28^{-/-}$ mice in an IFN-γ dependent fashion [68].

Contrary to above observations, a study using anti CTLA-4 Ab was found to exaggerate the disease phenotype in *L. major* infected BALB/c mice concomitant with increased production of IL-4 and IL-13. An interesting aspect of this study was that even $IL\text{-}4^{-/-}$ BALB/c mice which are otherwise resistant to CL developed lesions upon treatment with CTLA-4 Ab. The CTLA-4 Ab treated mice were also noted to produce more IL-13. IL-13 is capable of inhibiting the activity of iNOS. This study thus seems to indicate that other Th2 responses apart from IL-4 may also be at play [21].

In vitro studies using human cells where *L. major* infected macrophages were used to prime peripheral blood leukocytes indicated a decrease in IFN-γ and IL-5 upon blocking of the B7 pathway with CTLA4-Ig or anti CD86 [69]. In a similar experiment where peripheral blood mononuclear cells from CL patients were treated with *Leishmania* antigen, a decrease was noted in the production of TNF-α, IL-10 and IFN-γ on blocking B7-CD28/CTLA-4 using CTLA-4 Ig. While more studies are required in human model to better understand the modulatory role of this pathway, it does seem this pathway especially B7-2 is important in shaping immune response to *Leishmania* [70].

Studies done in VL model have also suggested a modulatory role of B7 co-stimulation in the outcome of infection. BALB/c mice infected with *L. chagasi* when treated with CTLA-4 MAb resulted not only in restoration of splenic T cell proliferative ability but also led to restoration of IL-2 and IFN- γ synthesis comparable with the WT cells. In the same study, blockade of B7-1 but not B7-2 was shown to enhance T cell response. Further CTLA-4 MAb treatment of

parasite infected splenic macrophages led to significant clearance of parasite when activated with anti CD-3 in an in vitro study [9]. Anti CTLA-4 has also been shown to be effective in treatment of VL caused by *L. donovani* [33, 71]. In both BALB/c mice and C57BL/6 mice infected with *L. donovani*, administration of anti CTLA-4 MAb led to better granuloma formation [72]. In this study B7-2: CTLA-4 blockade led to clearance of parasite from liver [71] rather than B7-1: CTLA-4 as with the *L. chagasi* model [9]. Anti CTLA-4 treatment in *L. donovani* infected mice model was further noted to enhance the anti leishmanicidal activity of sodium stibogluconate [33, 72].

While most of the studies on the role of B7 family co-stimulation have focused on B7-1 and B7-2, some studies have looked at other B7 pathways as well. Inducible co-stimulatory protein (ICOS), a CD28 homolog, is induced in T cells upon TCR stimulation. ICOS signals via its ligand B7-H2/B7RP-1 [73, 74] and has been implicated in inducing Th2 immune response in allergic disease models. An ICOS-/- 129S4/SvJae mouse when infected with L. mexicana, another agent of CL, was found to produce delayed and smaller lesions and less inflammation. The KO mice were also defective in Ig class switching and production of both IL-4 and IFN-γ was impaired in this system denoting ICOS is essential in *L. mexicana* CL for both Th1 and Th2 responses [73]. However, in the *L. major* model of CL, antibody blockade of the receptor for ICOS or ICOS deficient mice led to better prognosis of infected BALB/c mice indicating a Th2 inducing role of the pathway [74].

These studies account for a definite role of B7 family of co-stimulatory molecules in modulating the outcome of leishmaniasis caused by various species of the parasite. The blockade of certain molecules or their receptors presents an attractive tool to manage this disease**.** However, due to the complex nature of co-stimulation and the obvious differences in the pathway in different species calls for careful and detailed study before these pathway can be manipulated for treatment.

The OX40 family

OX40, a member of Type I membrane glycoprotein has the characteristic 4 cysteine rich domains (three complete and one truncated) of TNFR family of proteins. It is primarily present on activated T cells, including CD4+, CD8+, Th1, Th2, Th17 and Foxp3+ CD4+ Tregs and is not found on naïve T cells or memory cells [75, 76]. OX40 ligand (OX40L) is a Type II membrane protein that consists of an extracellular domain of 132 residues with no site for proteolytic cleavage, indicating that it mainly exists in a membrane bound form [75, 77]. The homotrimeric OX40L interacts with three OX40 monomers for resultant biological effects [77]. OX40L is expressed mainly by professional APCs such as DCs, B cells, Langerhans cells and macrophages upon receiving maturation signals from CD40, inflammatory cytokines like TSLP (thymic stromal lymphopoietin) and Toll-like receptors (TLR2, 4 and 9). However, additional cell types like NK cells, vascular endothelial cells and smooth muscle cells can also express OX40L upon induction [75, 76].

Biological effects of OX40:OX40L interaction

While initial optimal activation of naïve T cells requires co-stimulation via CD28:B7-1/B7-2, CD27:CD70 and CD40L:CD40, OX40:OX40L interaction, provides effector T cells with signals for sustained proliferation, memory CD4+ T cell generation and specific cytokine production [78]. Further, OX40L is expressed on mature DCs and OX40:OX40L interaction enhances production of cytokines such as TNF-α, IL-6, IL-1β and IL-12p40 [79]. The intracellular domain of OX40 binds to TNF-R associated factors, TRAF 2, TRAF 3 and TRAF5. A model of OX40 signaling explains that recruitment of OX40 to lipid rafts occurs upon receptor engagement along with TRAF2, TRAF3and TRAF5, molecules of NF-kB pathway (IKKα, β), p85 subunit of PI3K and Akt which can initiate signaling events [76]. Also, OX40 signaling helps in enhancing expression of the anti-apoptotic Bcl2 family of proteins [76, 80]. OX40 signaling is known to signal survivin, member of IAP (inhibitor of apoptosis) family of proteins, expression in late G1 phase of cell cycle which ultimately mediates T cell proliferation and clonal expansion [80].

OX40:OX40L interactions in *Leishmania* models

The role of OX40:OX40L interactions in Th cell differentiation has long been debated and it is now known that rather than playing a role in directly determining Th1/Th2 differentiation phenotype, OX40:OX40L interaction helps in the expansion of ongoing immune responses whether they are beneficial to the resolution of the disease or not [75, 76]. In a model of VL in C57BL/6 mice, infected with *L. donovani*, the efficacy of OX40L-Fc, a chimeric fusion protein consisting of the extracellular OX40 binding domain of OX40L fused to immunoglobulin Fc region, administration was tested. It was noted that this administration accelerated the development of mature granulomas with lower amastigote population. A

high leishmanicidal activity with the promotion of Th1 cytokine IFN- γ and IL-12p70 was also seen [72]. Interestingly, in a model of CL in Th2 background of BALB/c mice, administration of neutralizing anti-OX40 MAb, helped in reducing lesion development in these mice and reducing Th2 cytokine levels like IL-4, IL-10 and IL-13. However, no visible difference was seen in C57BL/6 mice with this treatment [81]. These results were further elucidated in a study with OX40L transgenic (Tg) mice. Apart from succumbing to non-resolving lesions, the OX40L Tg mice also showed strong Th2 responses of their draining lymph node cells [82]. Taken together, these results suggest that the outcome of OX40:OX40L interactions, depends on the pre-existing immune reactions in the host and manipulating the model can yield different immune responses.

Conclusion

A variety of co-stimulatory pathways contributes to diverse immune reaction against leishmaniasis with variation in response amongst different species of *Leishmania*. CD40:CD40L interaction helps in establishing a protective immune response by IL-12 production, macrophage activation and CD4+ T cell priming, while under certain conditions it promotes suppressive immune response by IL-10 production and Treg development. B7-1/B7-2:CD28/CTLA-4 signaling seems to be critical for Th2 immune response, however a complex relation seems to exist according to the genetic background of the host, the infecting dose and the species of parasite. OX40:OX40L interaction mediates T cell proliferation and clonal expansion following an established Th1/Th2 immune response and thus the outcome of this interaction depends on the pre-existing host immune response. Due to the obvious importance of co-stimulatory pathways in leishmaniasis, further studies that elucidate the complex interactions of co-stimulatory pathways will yield insights into therapeutic strategies against *Leishmania* infection.

Acknowledgements

This work was supported by grants from the National Institutes of Health to ARS.

Conflict of Interests

The authors have declared that no conflict of interest exists.

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