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Ox40L–Ox40 pathway plays distinct roles in regulating Th2 responses but does not determine outcome of cutaneous leishmaniasis caused by *Leishmania mexicana* and *Leishmania major*

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Abstract

Ox40 ligand (Ox40L)–Ox40 pathway has been shown to enhance Th2 responses and play a role in pathogenesis of cutaneous leishmaniasis (CL) caused by *Leishmania major*. Using *Ox40l*$^{-/-}$ BALB/c mice we analyzed the role of this pathway in determining the outcome to CL caused by *L. mexicana* and compared to *L. major*. Contrary to our expectations, *Ox40l*$^{-/-}$ mice were highly susceptible to both *L. major* (LV39) and *L. mexicana* (M379) and developed large non-healing lesions containing parasites comparable to *Ox40l*$^{+/+}$ BALB/c mice. Interestingly, upon *in vitro* stimulation with *Leishmania* antigen (LmAg), the lymph node cells from *L. major* infected *Ox40l*$^{-/-}$ mice produced significantly less IL-4 and IL-10 compared to *Ox40l*$^{+/+}$ mice. *L. mexicana* infected *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice did not show any difference in the production of IL-4 and IL-10. No difference was noted in the amount of Th1 cytokines IFN-γ and IL-12 produced by *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice infected with either parasite. These results indicate that the Ox40L–Ox40 pathway promotes Th2 bias only in *L. major* infection but not *L. mexicana* infection and this pathway is not critical for susceptibility to CL.

Graphical abstract

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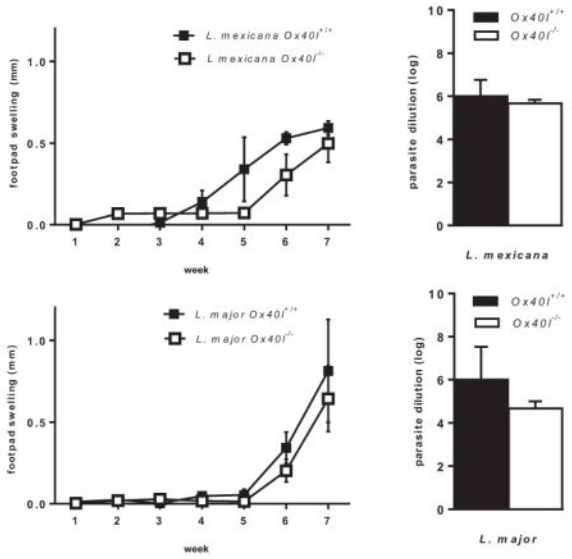

Keywords

Ox40L–Ox40; *L. major*; *L. mexicana*; Cutaneous leishmaniasis

1. Introduction

1.1. Cutaneous leishmaniasis (CL)

CL manifests as localized cutaneous lesions at the site of infection with the parasite *Leishmania* (Herwaldt, 1999). Nearly 1.5 million new cases of CL are detected annually (Alvar et al., 2012). *L. major* and *L. mexicana* are common agents of CL in the Old World and the New World respectively (Herwaldt, 1999). In the mammalian host, *Leishmania* primarily survives and replicates within host macrophages, and clinical outcomes depend on whether macrophages are fully activated to clear the parasite. CD4+ Th1 cells produce IFN-γ which activates inducible nitric oxide synthase (iNOS) in macrophages, leading to the production of leishmanicidal nitric oxide (NO) (Scott, 1991). IL-12, another Th1 promoting cytokine produced by macrophages and dendritic cells (DCs), indirectly contributes to host immunity by inducing IFN-γ production in NK cells (Stamm et al., 1999). On the other hand, IL-4 produced by Th2 cells inhibits IL-12 mediated Th1 activation. IL-10, an anti-inflammatory cytokine, indirectly enhances Th2 responses by suppressing IL-12, IFN-γ and NO production by *Leishmania* infected cells. IL-4 and IL-10 thus act to favor parasite persistence and establishment of chronic CL (Chatelain et al., 1999a, 1999b).

1.2. Ox40 ligand (Ox40L)–Ox40 co-stimulation

During antigen presentation, co-stimulatory molecules on antigen presenting cells (APCs) also activate naive CD4+T cells via specific receptors which are critical in influencing differentiation of T cells into Th1 or Th2 lineages (Sharpe and Freeman, 2002). The co-stimulatory molecule, Ox40L is expressed by DCs, macrophages and B cells and signals via its receptor Ox40 which is a protein of the tumor necrosis factor (TNF) receptor super

family expressed on activated T cells. Ox40L–Ox40 co-stimulation leads to activation of TNF receptor associated factor (TRAF) 2, 3 and 5. This pathway has been shown to prolong the survival of effector CD4+Th cells via expression of anti-apoptotic factors Bcl-2 and Bcl-$_{XL}$ as well as contributes to generation of memory T cells (Croft, 2010).

Earlier studies using *in vitro* models indicated that Ox40L–Ox40 interactions led to generation of Th2 responses during antigen presentation. While a number of disease models supported a Th2 response enhancing role (Jember et al., 2001; Tsukada et al., 2000; Yoshioka et al., 2000), other models have contradicted this role (Ishii et al., 2003; Zubairi et al., 2004). Activation of the Ox40 pathway has been shown to promote Th1 responses and contribute to parasite killing during *L. donovani* infection of mice (Zubairi et al., 2004). On the other hand, studies of *L. major* infection in *Ox40l* transgenic BALB/c mice, which overexpress OX40L mainly on T cells and display constitutive Ox40L–Ox40 interaction, showed increased parasite burdens and elevated Th2 responses (Ishii et al., 2003). Further *Ox40l* gene deficient BALB/c mice were shown to be more resistant to *L. major* infection than WT BALB/c mice and this was associated with a significant reduction in the production of Th2 cytokines (Ishii et al., 2003). However, the role of Ox40L–Ox40 interactions during *L. mexicana* infection has not been examined. Murine models have shown that immunological mechanisms governing resistance or susceptibility to CL are different between *L. major* and *L. mexicana* (Alexander and Kaye, 1985; McMahon-Pratt and Alexander, 2004).

To further examine the role of Ox40L–Ox40 interactions in CL, we examined host immune responses of wild type (*Ox40l*$^{+/+}$) and *Ox40l* gene deficient (*Ox40l*$^{-/-}$) mice to infection by *L. mexicana* and compared this with similar infection using *L. major* (LV39). Our results suggest that pathogen derived virulence factors could affect the role of the Ox40L–Ox40 pathway in determining disease outcomes of CL.

2. Materials and methods

2.1. Mice

Female *Ox40l*$^{+/+}$ BALB/c mice were purchased from Harlan laboratory. *Ox40l*$^{-/-}$ BALB/c mice were provided by Dr. Arlene Sharpe (Brigham and Women's Hospital) which were then bred and maintained at Ohio State University's Animal facility in accordance with University Laboratory Animal Resource (ULAR) guidelines. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Ohio State University.

2.2. Parasites and infections

Two million *L. mexicana* (M379) and *L. major* (LV39) metacyclic promastigotes recovered from animal stocks were injected s.c. into hind left footpad of age and sex matched *Ox40l*$^{+/+}$ and *Ox40l*$^{-/-}$ mice in 50 μl volume. Five mice per group were used in the study and 3 independent sets of such experiments were performed. Mice were monitored weekly for thickness of infected and uninfected contra-lateral footpads using a dial gauge micrometer (Mitutoyo). Difference in thickness of infected hind left footpad compared with the uninfected contralateral footpad is expressed as mean footpad swelling.

2.3. Quantification of parasite load

Infected footpads from mice sacrificed 7 weeks p.i. were cut and ground in 5 ml Schneider's media (Gibco) supplemented with 10% fetal calf serum (FCS) (Gibco), 100 U/ml penicillin (Gibco) and 100 μg/ ml streptomycin (Gibco) over 70 μm nylon cell strainer (BD Falcon). The suspension was then centrifuged at 3000 rpm and resuspended in 400 μl of Schneider's media. Limiting dilution assay was performed in duplicate as described previously (Rosas et al., 2005). Parasite viability was recorded after 4–7 days of incubation at 25 °C. The highest log dilution with viable parasites was reported.

2.4. Histopathology

Infected footpads from one representative mouse in each group were fixed in 10% formalin for at least 4 days. Fixed tissues were sent to Ohio State University's Department of Comparative Pathology for routine H&E staining.

2.5. T cell proliferation assay and cytokine estimation

Popliteal lymph nodes were collected from infected mice, 7 weeks p.i. and single cell suspension was prepared in 5 ml RPMI 1640 (Gibco) supplemented with 10% FCS (Gibco), 100 U/ml penicillin (Gibco), 100 μg/ml streptomycin (Gibco), 10 μM HEPES (Gibco) and 500 μl beta-mercaptoethanol (Gibco). T cell proliferation was performed as previously described (Rosas et al., 2005). Supernatants were collected 72 hours after incubation and cytokine ELISA was performed according to manufacturer instructions. Cytokine clones used were IL-4: 11B11-capture, BVD6-24G2-detection (BD Biosciences), IL-10: JES5-16E3-capture, JES5-2A5-detection (Biolegend), IL-12: C18.2-capture, C17.8-detection (Biolegend), IFN-γ: R4-6A2-capture, XMG1.2-detection (Biolegend).

2.6. Antibody ELISA

Tail vein bleeding was done at weeks 2, 4 and 6 p.i., according to ULAR's guidelines. Serum was obtained by centrifuging blood at 5000 rpm for 10 minutes and stored at −20 °C. *L. mexicana* and *L. major* specific IgG1 and IgG2a were detected by ELISA as previously described (Rosas et al., 2005) using HRP conjugated anti IgG1 and IgG2a antibodies and Streptavidin AKP (BD Pharmingen).

2.7. Statistical analysis

All data presented were obtained from 3 independent experiments. Unpaired Student's *t* test was performed to compare statistical significance in footpad swelling, parasite load and cytokine concentration results. *p* value below 0.05 was considered significant. Mann–Whitney U prime test was used to compare antibody titers.

3. Results and discussion

3.1. *Ox40l*$^{-/-}$ mice are susceptible to *L. mexicana* infection

Ox40L–Ox40 co-stimulation has been implicated in inducing susceptibility to CL caused by *L. major* (Akiba et al., 2000; Ishii et al., 2003) but mediates resistance to visceral leishmaniasis (Zubairi et al., 2004). Although both *L. major* and *L. mexicana* cause CL,

these parasites are significantly different in their phylogenetic, virulence and immunogenic characteristics (McMahon-Pratt and Alexander, 2004). We therefore analyzed the role of Ox40L–Ox40 co-stimulatory pathway in *L. mexicana* (M379) infection using *Ox40l*$^{+/+}$ and *Ox40l*$^{-/-}$ BALB/c mice. We observed similar progressive footpad swellings in *L. mexicana* infected *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice (Fig. 1A). Seven weeks p.i., infected footpads in *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice further had similar parasite burdens (Fig. 1B). H&E staining of infected footpads also revealed similar inflammatory infiltrate comprising of lymphocytes and parasitized macrophages in both groups of mice (Fig. 1C–F). Our observations indicate that unlike previous studies with *L. major*, susceptibility to *L. mexicana* in BALB/c mice is independent of Ox40L pathway.

Immunological mechanisms governing CL are different between *L. major* and *L. mexicana*. C57BL/6 mice infected with *L. major* develop a robust Th1 response and are resistant to infection but develop chronic CL upon infection with *L. mexicana*. Also, virulence factors which have the ability to modulate host immune responses are markedly different between these two *Leishmania* species. For example, surface lipophosphoglycan is an important virulence factor for *L. major*, but not *L. mexicana* (McMahon-Pratt and Alexander, 2004; Mottram et al., 2004). Cathepsin L-like cysteine protease B (CPB) enzymes have been identified as an important virulence factor in *L. mexicana* but not *L. major* (Buxbaum et al., 2003; Mottram et al., 2004). Moreover, genes involved in immune responses modulating susceptibility/ resistance to *Leishmania* infection are distinct for *L. major* and *L. mexicana* (Alexander and Kaye, 1985). It is therefore not surprising that the roles for co-stimulatory molecules in regulating immunity to *Leishmania* may differ between the two species.

3.2. Ox40L–Ox40 interaction does not affect Th1 or Th2 responses in *L. mexicana* infected BALB/c mice

In *L. major* infected mice, Ox40 co-stimulation is associated with the generation of Th2 responses (Ishii et al., 2003), while in *L. donovani infection* Ox40 stimulation results in Th1 immune response generation (Zubairi et al., 2004). We therefore examined whether Ox40L–Ox40 interaction has any role in enhancing or suppressing either Th1 or Th2 immune responses during *L. mexicana* infection. Cytokine analysis of lymph node cells from *L. mexicana* infected *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice re-stimulated with *Leishmania* antigen (LmAg) showed no differences in production of IL-4, IL-10, IFN-γ and IL-12 (Fig. 2A–D). Further, *L. mexicana* specific serum IgG1 and IgG2a antibodies, indicative of a Th2 and Th1 response respectively (Rosas et al., 2005), were comparable in both *Ox40l*$^{-/-}$and *Ox40l*$^{+/+}$ mice infected with *L. mexicana* (Fig. 2E,F). Our results indicate that Ox40L–Ox40 interaction does not contribute to the development of Th1 nor Th2 immune responses during *L. mexicana* infection of BALB/c mice. This contrasts with the Th2 promoting role of Ox40 co-stimulatory pathway in response to *L. major* infection (Ishii et al., 2003), as well as the enhanced Th1 response and subsequent parasite clearance in *L. donovani* infected mice following OX40 co-stimulation (Zubairi et al., 2004). Although mechanisms behind these observed differences are yet to be fully understood, these results seem to suggest that the immunological consequence of Ox40L–Ox40 interactions depends on prevailing immune conditions elicited by the infecting pathogen (Gupta et al., 2013; Oghumu et al., 2010; Tuladhar et al., 2011).

Ox40L–Ox40 pathway is implicated in prolonging the survival and proliferation of effector T cells (Pippig et al., 1999), and it may be that Ox40 stimulation functions primarily to sustain ongoing T cell activation processes rather than direct T helper differentiation in *Leishmania* infections (Gramaglia et al., 1998). This would explain why different T helper states are enhanced by the Ox40L–Ox40 pathway after infection with different species of *Leishmania*. Further, other pathways including CD40L-CD40, B7-CD28/CTLA4 also play a role in skewing Th1/Th2 responses (Tuladhar et al., 2011), and Ox40L–Ox40 interactions have been shown to exert their effects much later after initial T cell activation (Curry et al., 2004; Gramaglia et al., 1998). It is therefore possible that the effects of Ox40 stimulation during *L. mexicana* infections are minimal, unlike in *L. major* infection. Another factor that may contribute to this difference is the role of parasite virulence factors. While *L. mexicana* virulence is mediated primarily by CPB, this factor has a much less profound role in *L. major* virulence (Mottram et al., 2004). CPB has been shown to be capable of inducing a Th2 response in *L. mexicana* infected BALB/c mice (Buxbaum et al., 2003). The immunomodulatory ability of *L. mexicana* specific virulence factors could potentially override any immune response exerted by Ox40 co-stimulation in mice infected with *L. mexicana* (Buxbaum et al., 2003; McMahon-Pratt and Alexander, 2004; Mottram et al., 2004).

3.3. *Ox40l*$^{-/-}$ mice display decreased Th2 responses than Ox40l$^{+/+}$ mice but are equally susceptible to a virulent strain of *L. major*

Previous studies indicated that blockade of Ox40L–Ox40 co-stimulation in *L. major* infection results in decreased IL-4 and IL-10 levels and led to abrogation of CL (Akiba et al., 2000; Ishii et al., 2003). Similar to these reports, we observed significantly reduced IL-4 and IL-10 production by *L. major* infected *Ox40l*$^{-/-}$ mice compared to *Ox40l*$^{+/+}$ mice upon stimulation of lymph node cells with LmAg (Fig 3A,B). *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice did not exhibit any differences in IFN-γ and IL-12 production (Fig. 3C,D). Further, production of serum IgG1 (Fig. 3E) and IgG2a (Fig. 3F) were similar between *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice infected with *L. major*.

However, contrary to previously published results, we observed comparable footpad swelling (Fig. 3G) and parasite loads (Fig. 3H) in *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice infected with *L. major* (LV39). H&E staining of infected footpads also revealed similar degree of inflammation in *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ mice (Fig. 3I–L). The difference in the outcome of *L. major* infection observed between our study and previous studies may possibly be a result of the different strains of *L. major* used. Previous studies used the *L. major* strain (MHOM/SU/73/5ASKH) while our current study was performed using *L. major* strain LV39 (MRHO/SU/59/P). A number of studies have demonstrated that different strains of *L. major* parasites vary in their pathogenicity and in the induction of immune responses which can alter the outcome of infection (Alimohammadian et al., 2010; Asadpour et al., 2013). Taken together, these results suggest that pathogen derived factors could affect the role of the OX40L–OX40 pathway in determining disease outcomes of CL.

It is surprising that although Th2 cytokine responses were reduced in *L. major* infected *Ox40L*$^{-/-}$ mice compared to *Ox40L*$^{+/+}$ mice, lesion sizes and antigen specific IgG1 and

IgG2a antibodies were comparable between both groups of mice. Although it is generally accepted that the Th2 cytokine IL-4 contributes to the generation of antigen specific IgG1 antibodies, other cytokines (Bryson et al., 2008) and parasite derived factors (Buxbaum, 2013; Mohammadi et al., 2006) have been shown to affect IgG subclass distribution. Moreover, levels of IgG1 and IgG2a antibodies do not always correlate with disease outcome (Fossati-Jimack et al., 2000). For example increased levels of IgG2a antibodies have been shown to contribute to disease exacerbation of CL in *PD-L2*$^{-/-}$ mice due to increased binding of opsonized parasites to FcγR and suppression of protective immune responses (Liang et al., 2006), which contrasts with the protective immune responses associated with elevated IgG2a levels in WT mice. Taken together our results suggest that IL-4 independent mechanisms affect the generation of antigen specific IgG isotypes in OX40L deficient mice during CL caused by *L. major*.

4. Conclusions

This is the first report that examines the effect of Ox40L deficiency in CL caused by *L. mexicana*. Our study demonstrates that the Ox40L–Ox40 pathway does not contribute to Th1 nor Th2 bias and subsequently disease outcome of *L. mexicana* infection. Our study also shows that this co-stimulatory pathway promotes Th2 responses, but is not required for susceptibility to CL caused by *L. major* strain LV39.

Acknowledgments

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Abbreviations

CL	cutaneous leishmaniasis
CPB	cysteine protease B
FCS	fetal calf serum
LmAg	*Leishmania* antigen
Ox40L	Ox40 ligand
p.i	post infection
TNFR	tumor necrosis factor receptor

References

Akiba H, Miyahira Y, Atsuta M, Takeda K, Nohara C, Futagawa T, et al. Critical contribution of OX40 ligand to T helper cell type 2 differentiation in experimental leishmaniasis. J Exp Med. 2000; 191:375–380. [PubMed: 10637281]

Alexander J, Kaye PM. Immunoregulatory pathways in murine leishmaniasis: different regulatory control during Leishmania mexicana mexicana and Leishmania major infections. Clin Exp Immunol. 1985; 61:674–682. [PubMed: 3907906]

Alimohammadian MH, Darabi H, Ajdary S, Khaze V, Torkabadi E. Genotypically distinct strains of Leishmania major display diverse clinical and immunological patterns in BALB/c mice. Infect Genet Evol. 2010; 10:969–975. [PubMed: 20601170]

Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE. 2012; 7:e35671. [PubMed: 22693548]

Asadpour A, Riazi-Rad F, Khaze V, Ajdary S, Alimohammadian MH. Distinct strains of Leishmania major induce different cytokine mRNA expression in draining lymph node of BALB/c mice. Parasite Immunol. 2013; 35:42–50. [PubMed: 23106526]

Bryson KJ, Wei XQ, Alexander J. Interleukin-18 enhances a Th2 biased response and susceptibility to Leishmania mexicana in BALB/c mice. Microbes Infect. 2008; 10:834–839. [PubMed: 18538609]

Buxbaum LU. Leishmania mexicana infection induces IgG to parasite surface glycoinositol phospholipids that can induce IL-10 in mice and humans. PLoS Negl Trop Dis. 2013; 7:e2224. [PubMed: 23675550]

Buxbaum LU, Denise H, Coombs GH, Alexander J, Mottram JC, Scott P. Cysteine protease B of Leishmania mexicana inhibits host Th1 responses and protective immunity. J Immunol. 2003; 171:3711–3717. [PubMed: 14500670]

Chatelain R, Mauze S, Coffman RL. Experimental Leishmania major infection in mice: role of IL-10. Parasite Immunol. 1999a; 21:211–218. [PubMed: 10320618]

Chatelain R, Mauze S, Varkila K, Coffman RL. Leishmania major infection in interleukin-4 and interferon-gamma depleted mice. Parasite Immunol. 1999b; 21:423–431. [PubMed: 10417676]

Croft M. Control of immunity by the TNFR-related molecule OX40 (CD134). Annu Rev Immunol. 2010; 28:57–78. [PubMed: 20307208]

Curry AJ, Chikwe J, Smith XG, Cai M, Schwarz H, Bradley JA, et al. OX40 (CD134) blockade inhibits the co-stimulatory cascade and promotes heart allograft survival. Transplantation. 2004; 78:807–814. [PubMed: 15385798]

Fossati-Jimack L, Ioan-Facsinay A, Reininger L, Chicheportiche Y, Watanabe N, Saito T, et al. Markedly different pathogenicity of four immunoglobulin G isotype-switch variants of an antierythrocyte autoantibody is based on their capacity to interact in vivo with the low-affinity Fcgamma receptor III. J Exp Med. 2000; 191:1293–1302. [PubMed: 10770797]

Gramaglia I, Weinberg AD, Lemon M, Croft M. Ox-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T cell responses. J Immunol. 1998; 161:6510–6517. [PubMed: 9862675]

Gupta G, Oghumu S, Satoskar AR. Mechanisms of immune evasion in leishmaniasis. Adv Appl Microbiol. 2013; 82:155–184. [PubMed: 23415155]

Herwaldt BL. Leishmaniasis. Lancet. 1999; 354:1191–1199. [PubMed: 10513726]

Ishii N, Ndhlovu LC, Murata K, Sato T, Kamanaka M, Sugamura K. OX40 (CD134) and OX40 ligand interaction plays an adjuvant role during in vivo Th2 responses. Eur J Immunol. 2003; 33:2372–2381. [PubMed: 12938213]

Jember AG, Zuberi R, Liu FT, Croft M. Development of allergic inflammation in a murine model of asthma is dependent on the costimulatory receptor OX40. J Exp Med. 2001; 193:387–392. [PubMed: 11157058]

Liang SC, Greenwald RJ, Latchman YE, Rosas L, Satoskar A, Freeman GJ, et al. PD-L1 and PD-L2 have distinct roles in regulating host immunity to cutaneous leishmaniasis. Eur J Immunol. 2006; 36:58–64. [PubMed: 16358363]

McMahon-Pratt D, Alexander J. Does the Leishmania major paradigm of pathogenesis and protection hold for New World cutaneous leishmaniases or the visceral disease? Immunol Rev. 2004; 201:206–224. [PubMed: 15361243]

Mohammadi MR, Zeinali M, Ardestani SK, Kariminia A. Identification of novel Leishmania major antigens that elicit IgG2a response in resistant and susceptible mice. Korean J Parasitol. 2006; 44:43–48. [PubMed: 16514281]

Mottram JC, Coombs GH, Alexander J. Cysteine peptidases as virulence factors of Leishmania. Curr Opin Microbiol. 2004; 7:375–381. [PubMed: 15358255]

Oghumu S, Lezama-Davila CM, Isaac-Marquez AP, Satoskar AR. Role of chemokines in regulation of immunity against leishmaniasis. Exp Parasitol. 2010; 126:389–396. [PubMed: 20206625]

Pippig SD, Pena-Rossi C, Long J, Godfrey WR, Fowell DJ, Reiner SL, et al. Robust B cell immunity but impaired T cell proliferation in the absence of CD134 (OX40). J Immunol. 1999; 163:6520–6529. [PubMed: 10586044]

Rosas LE, Keiser T, Barbi J, Satoskar AA, Septer A, Kaczmarek J, et al. Genetic background influences immune responses and disease outcome of cutaneous L. mexicana infection in mice. Int Immunol. 2005; 17:1347–1357. [PubMed: 16141242]

Scott P. IFN-gamma modulates the early development of Th1 and Th2 responses in a murine model of cutaneous leishmaniasis. J Immunol. 1991; 147:3149–3155. [PubMed: 1833466]

Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol. 2002; 2:116–126. [PubMed: 11910893]

Stamm LM, Satoskar AA, Ghosh SK, David JR, Satoskar AR. STAT-4 mediated IL-12 signaling pathway is critical for the development of protective immunity in cutaneous leishmaniasis. Eur J Immunol. 1999; 29:2524–2529. [PubMed: 10458767]

Tsukada N, Akiba H, Kobata T, Aizawa Y, Yagita H, Okumura K. Blockade of CD134 (OX40)-CD134L interaction ameliorates lethal acute graft-versus-host disease in a murine model of allogeneic bone marrow transplantation. Blood. 2000; 95:2434–2439. [PubMed: 10733518]

Tuladhar R, Natarajan G, Satoskar AR. Role of co-stimulation in Leishmaniasis. Int J Biol Sci. 2011; 7:1382–1390. [PubMed: 22110389]

Yoshioka T, Nakajima A, Akiba H, Ishiwata T, Asano G, Yoshino S, et al. Contribution of OX40/OX40 ligand interaction to the pathogenesis of rheumatoid arthritis. Eur J Immunol. 2000; 30:2815–2823. [PubMed: 11069062]

Zubairi S, Sanos SL, Hill S, Kaye PM. Immunotherapy with OX40L-Fc or anti-CTLA-4 enhances local tissue responses and killing of Leishmania donovani. Eur J Immunol. 2004; 34:1433–1440. [PubMed: 15114677]

HIGHLIGHTS

- First study to examine the role of Ox40L–Ox40 in *L. mexicana* infection.
- Ox40L pathway does not induce Th2 responses in experimental *L. mexicana* infection.
- Ox40L–Ox40 co-stimulation is not critical for susceptibility to *L. mexicana*.
- Ox40L–Ox40 promotes Th2 response but does not affect outcome of *L. major* infection.

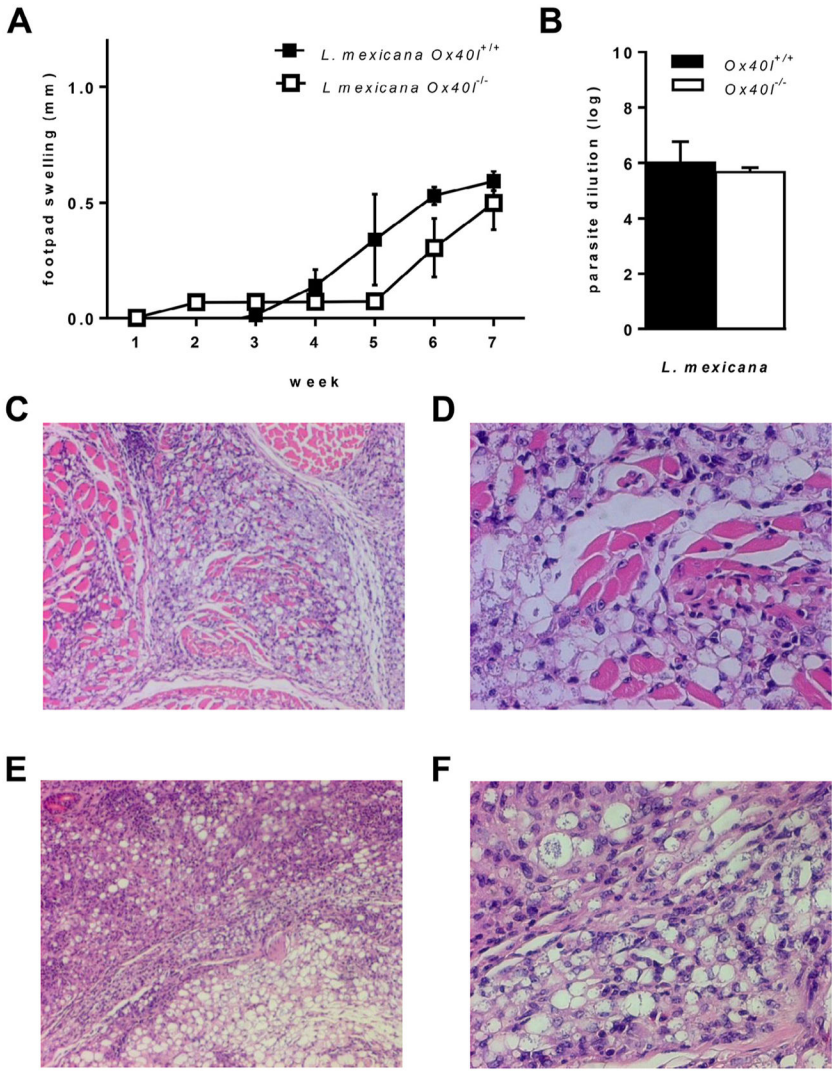

Fig. 1.

Footpad infection of *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ BALB/c mice with *L. mexicana*. (Results are representative of three independent experiments.) (A) Lesion size is expressed as mean footpad swelling (millimeters) ± SE (*n* = 15 mice per group). (B) Parasite load is expressed as the largest log dilution that yields viable parasite upon culture for 4–7 days and is expressed as mean log dilution ± SE (*n* = 12 mice per group). (C–F) Histopathological examination of infected footpads after H&E staining (*n* = 3 mice per group). *L. mexicana* infected *Ox40l*$^{+/+}$ mice, 10× (C) and 40× (D) and *L. mexicana* infected *Ox40l*$^{-/-}$ mice, 10× (E) and 40× (F).

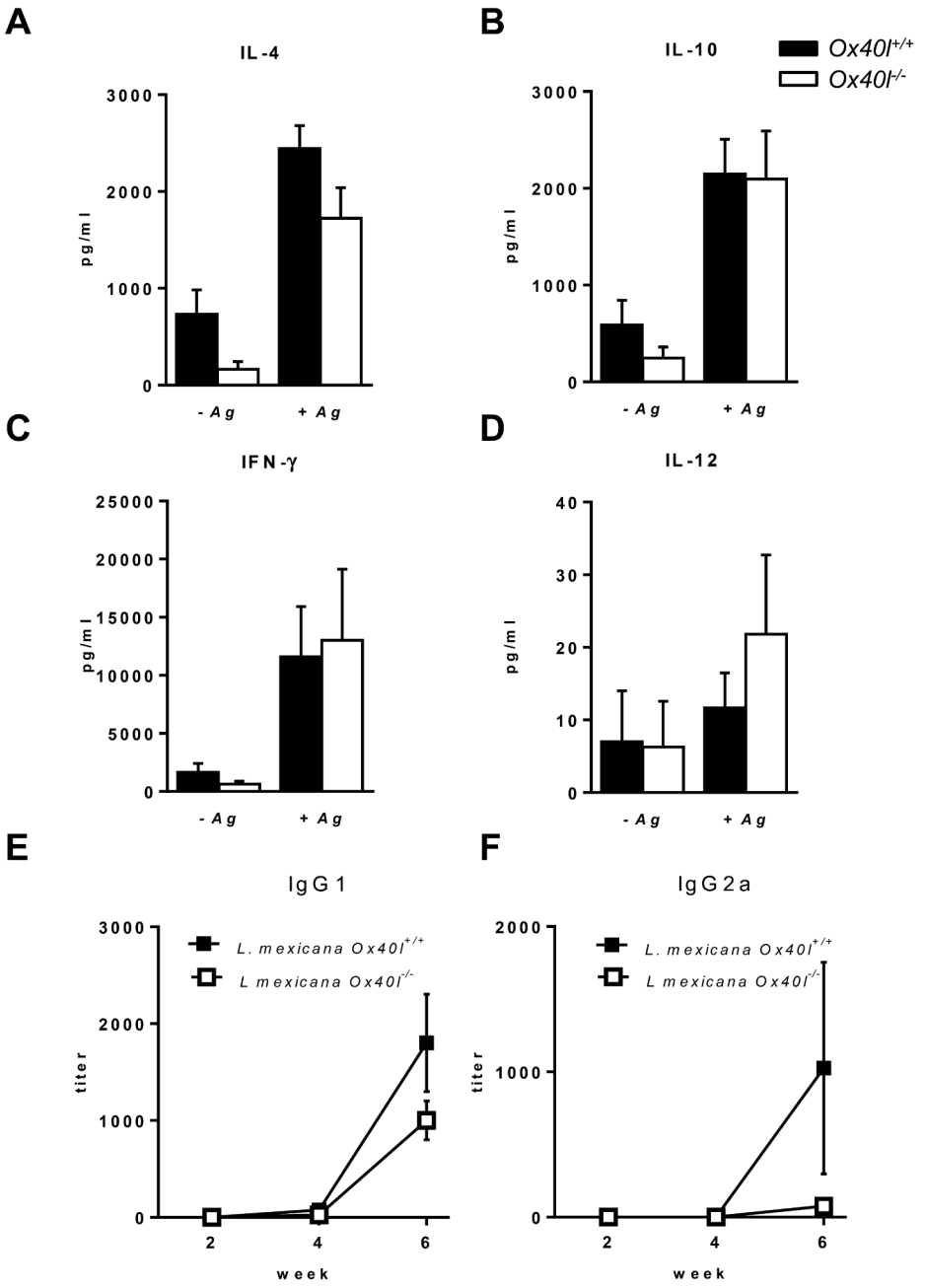

Fig. 2.

Cytokines and antibodies in *Ox40l*$^{+/+}$ and *Ox40l*$^{-/-}$ mice infected with *L. mexicana*. IL-4 (A), IL-10 (B), IFN-γ (C) and IL-12 (D) concentrations measured by ELISA are expressed as mean concentration (picogram) ± SE (*n* = 15 mice per group). Serum antibody was tested for IgG1 (E) and IgG2a (F) specific for *L. mexicana* by ELISA. Results are represented as mean antibody titer ± SE (*n* = 15 mice per group).

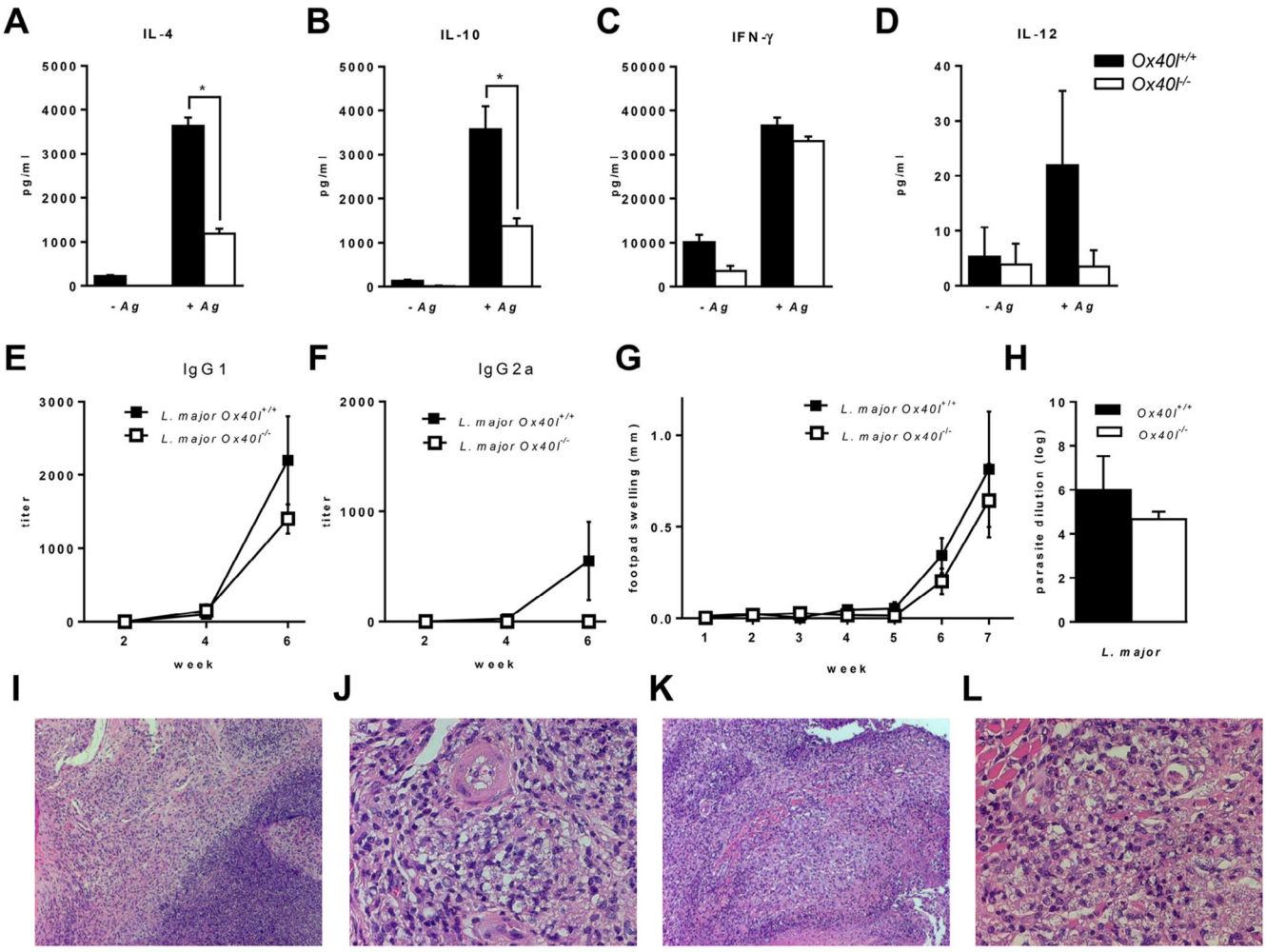

Fig. 3.

Footpad infection of *Ox40l*$^{-/-}$ and *Ox40l*$^{+/+}$ BALB/c mice with *L. major*. (Results are representative of three independent experiments.) IL-4 (A), IL-10 (B), IFN-γ (C) and IL-12 (D) concentrations measured by ELISA are expressed as mean concentration (picogram) ± SE (*n* = 15 mice per group). * denotes $p < 0.05$ calculated by unpaired Student's *t* test. Serum antibody was tested for IgG1 (E) and IgG2a (F) specific for *L. major* by ELISA. Results are represented as mean antibody titer ± SE (*n* = 15 mice per group). (G) Lesion size is expressed as mean footpad swelling (millimeters) ± SE (*n* = 15 mice per group). (H) Parasite burden in infected footpad is expressed as mean log dilution ± SE (*n* = 12 mice per group). (I–L) Histopathological examination of infected footpads by H&E staining (*n* = 3 mice per group). *L. major* infected *Ox40l*$^{+/+}$ mice, 10× (I) and 40× (J) and *L. major* infected *Ox40l*$^{-/-}$ mice, 10× (K) and 40× (L).