Brief Definitive Report

CD40 Signaling Is Impaired in \textit{L. major}-infected Macrophages and Is Rescued by a p38MAPK Activator Establishing a Host-protective Memory T Cell Response

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Abstract

Leishmania, a protozoan parasite, lives and multiplies as amastigote within macrophages. It is proposed that the macrophage expressed CD40 interacts with CD40 ligand on T cells to induce IFN-\(\gamma\), a Th1-type cytokine that restricts the amastigote growth. Here, we demonstrate that CD40 cross-linking early after infection resulted in inducible nitric oxide synthetase type-2 (iNOS2) induction and iNOS2-dependent amastigote elimination. Although CD40 expression remained unaltered on \textit{L. major}-infected macrophages, delay in the treatment of macrophages or of mice with anti-CD40 antibody resulted in significant reduction in iNOS2 expression and leishmanicidal function suggesting impaired CD40 signaling in \textit{Leishmania} infection. The inhibition of CD40-induced iNOS2 expression by SB203580, a p38-mitogen activated protein kinase (p38MAPK)-specific inhibitor, and the reversal of the inhibition by anisomycin, a p38MAPK activator, suggested a crucial role of p38MAPK in CD40 signaling. Indeed, the CD40-induced p38MAPK phosphorylation, iNOS2 expression and anti-leishmanial function were impaired in \textit{Leishmania}-infected macrophages but were restored by anisomycin. Anisomycin’s effects were reversed by SB203580 emphasizing the role of p38MAPK in CD40-induced iNOS2-dependent leishmanicidal function. Anisomycin administration in \textit{L. major}-infected BALB/c mice resulted in significant reduction in the parasite load and established a host-protective Th1-type memory response. Also implicated in these findings is a scientific rationale to define novel anti-parasite drug targets and to bypass the problem of drug resistance.

Key words: Leishmaniasis • immune evasion mechanism • CD40 signaling • p38MAPK • T cell memory

Introduction

Macrophages are the primary target cells for \textit{Leishmania major}, the causative agent of the disease cutaneous leishmaniasis. As activated macrophages eliminate the intracellular amastigotes (1) and play a crucial role in the initiation and regulation of host-protective anti-leishmanial T cell response, the parasite deploys different immune evasion strategies to survive within the macrophages (2). Categorically, the strategies include direct interference with IFN-\(\gamma\)-mediated macrophage activation (3) and indirect interference with macrophage activation by skewing the T-helper (Th) cells to Th2 type, the disease-promoting Th subset that suppresses the host-protective Th1 subset (4). It was proposed that the interaction between CD40, a costimulatory molecule expressed on macrophages, B cells, and dendritic cells (5), and its ligand, CD40 ligand (CD154) on T cells (6) results in Th subset skewing. The CD40-deficient mice develop a Th2-skewed response and are susceptible to \textit{Leishmania} infection (7). The susceptibility to \textit{Leishmania} infection can be prevented by IL-12 administration in these mice suggesting that CD40–CD154 interaction is required for the production of IL-12, which polarizes the Th cells to Th1 type (7, 8). Thus, the host-protective function of CD40 was attributed to setting a Th1 bias (7–9). However, these reports (7–9) neither explain the mechanism of the failure of CD40 to prevent the progression of the disease in susceptible mouse strains nor do they implicate modulation of CD40 effector functions in macrophages as the parasite’s immune evasion strategy. Therefore, we tested whether or not CD40 signaling alone results in \textit{Leishmania} killing in macrophages. We have also studied the regulation of CD40...
signaling in *Leishmania*-infected macrophages to explore the nature of host-pathogen interaction and finally, based on these experiments; we have tested whether or not a pharmacological manipulation of CD40 signaling results in a host-protective immune response.

**Materials and Methods**

*Leishmania major* and *Infection of Animals*. BALB/c mice were infected subcutaneously with $2 \times 10^6$ *Leishmania major* (MHOM/Su73/5ASKH) promastigotes in saline (25 µl). The disease was scored by measuring the footpad swelling by a digital micrometer (Mitituyo) and by limiting dilution analysis of parasite burden (10). In some experiments, BALB/c mice were primed with $5 \times 10^5$ promastigotes with or without anisomycin.

Reagents. AMT, aminosynicyn, and SB203580 were procured from Sigma-Aldrich. The FITC and PE-labeled anti-CD40 and control isotypes, the purified anti-inducible nitric oxide synthase type-2 (iNOS2) antibodies and the cytokine Opt-EIA kits were procured from BD Biosciences. Anti-CD40 antibody for control isotypes, the purified anti-inducible nitric oxide synthase type-2 (iNOS2) antibodies and the cytokine Opt-EIA kits from Sigma-Aldrich. The FITC and PE-labeled anti-CD40 antibody or anisomycin was used to probe for phospho-p38AMPK and reprobe for dephospho-p38MAPK-specific antibodies. The same blot was used to probe for phospho-p38AMPK and reprobe for dephospho-p38MAPK-specific antibodies. Similar protocols were followed for detecting the phospho- and dephospho-p38-mitogen-activated protein kinase (p38MAPK) in differently treated macrophages using phospho or dephospho-p38MAPK-specific antibodies. The same blot was used to probe for phospho-p38AMPK and reprobe for dephospho-p38MAPK.

*p38MAPK Activation Ameliorates Leishmania Infection of Macrophages.* Thioglycolate-elicited peritoneal macrophages from BALB/c mice were infected with *Leishmania* promastigotes at a ratio of 1:10 for 6 h (10). The extracellular parasites were washed out and the macrophages were cultured with or without anti-CD40 antibody and anisomycin or SB203580 treatment at the indicated doses for 72 h. The macrophages were then fixed, Giemsa-stained, and counted to calculate the number of amastigotes per 100 macrophages (10).

**CD40-induced Nitrite Production by Leishmania-infected and Uninfected Macrophages.** BALB/c-derived peritoneal macrophages ($5 \times 10^5$ cells/ml in IMDM), uninfected or *Leishmania*-infected, were treated with the anti-CD40 antibody (10 µg/ml) for 48 h. The culture supernatants were collected and the protein contents were measured by Griess reagent (ICN Biomedicals) following manufacturer’s instructions. The nitrocellulose filter was washed, blocked with 5% nonreactive bands were visualized by the enhanced chemiluminescence system (Amersham Biosciences). Each sample was blotted with anti-β-actin antibody (Santa Cruz Biotechnology, Inc.) to ensure equal input of protein samples onto the gel.

Similar protocols were followed for detecting the phospho- and dephospho-p38-mitogen-activated protein kinase (p38MAPK) in differently treated macrophages using phospho or dephospho-p38MAPK-specific antibodies. The same blot was used to probe for phospho-p38AMPK and reprobe for dephospho-p38MAPK.

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**Results**

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CD40 persists in late-infected macrophages. Therefore, the macrophages were infected for 12 to 72 h before they were treated with the anti-CD40 antibody for another 24 h. It was observed that the anti-CD40 antibody treatment resulted in a 67% decrease in the number of amastigotes per 100 12-h infected macrophages as compared with the 22–38% decrease in 24 to 72 h infected macrophages (Fig. 1 D) implying a significant impairment in CD40-induced anti-leishmanial function in macrophages parasitized for more than 12 h. The decrease in anti-leishmanial function was possibly due to impaired CD40-induced iNOS2 expression (Fig. 1 E), as confirmed by the analysis in the exponential phase of PCR amplification (Fig. 1 F). Corroborating to the RT-PCR data, the CD40-induced nitrite production was significantly reduced in the late-infected macrophages (Fig. 1 G).

**Leishmania Infection of Macrophages Does Not Down-regulate CD40 Expression.** The interference with the CD40-induced anti-leishmanial activity might be executed by two different mechanisms: down-regulated CD40 expression resulting in a quantitative decrease in CD40 signaling or impaired CD40 signaling despite unaltered CD40 expression. Therefore, we tested whether or not Leishmania infection results in reduced CD40 expression. We observed that in striking contrast with the deregulated expression of other costimulatory molecules like CD80 or ICAM-1 (14), CD40 expression was not significantly altered on Leishmania-infected macrophages (Fig. 2 A). LPS-induced p38MAPK activity (15), we tested whether or not Leishmania infection resulted in reduced CD40 expression. We observed that in striking contrast with the deregulated expression of other costimulatory molecules like CD80 or ICAM-1 (14), CD40 expression was not significantly altered on Leishmania-infected macrophages (Fig. 2 A). Therefore, the loss of anti-leishmanial function in late-infected macrophages was perhaps due to impaired CD40 signaling.

**p38MAPK Plays an Important Role in CD40 Signaling in Macrophages.** As Leishmania infection is proposed to alter LPS-induced p38MAPK activity (15), we tested whether or not CD40 signals through p38MAPK in macrophages and if it does, whether or not the regulation of p38MAPK activity by its pharmacological inhibitor, SB203580 (16), or activator, anisomycin (17), modulates the CD40-
induced leishmanicidal activity. It was observed that SB203580 prevented CD40-induced p38MAPK phosphorylation and iNOS2 expression while anisomycin induced p38MAPK phosphorylation and iNOS2 expression in a dose-dependent manner (Fig. 2, B and C). Together with these observations, the reversal of SB203580-mediated inhibition of CD40-induced iNOS2 expression by anisomycin (Fig. 2 C) suggested that CD40 signals through p38MAPK in macrophages regulating iNOS2 expression and that anisomycin directly activates p38MAPK, as anisomycin reverses the inhibitory activities of SB203580.

CD40 Signaling Is Impaired in Leishmania-infected Macrophages but Rescued by Anisomycin. We tested whether or not CD40 signaling through p38MAPK is impaired in late-infected (72 h infected) macrophages and if it is, whether or not anisomycin restored CD40 signaling. It was observed that anti-CD40-induced p38MAPK phosphorylation (Fig. 3 A) and iNOS2 expression (Fig. 3 B) were impaired in the late-infected macrophages. Treatment of these macrophages with anti-CD40 antibody either at the beginning of infection (CD40-E) with or without SB203580 (2 μg/ml) or 72 h after infection (CD40-L) with or without anisomycin (Aniso) rescued the CD40-induced anti-leishmanial effect (CD40-L+An) significantly (*, P < 0.01).

![Figure 2](image1.png)  
**Figure 2.** CD40 signals through p38MAPK in macrophages and its expression remain unchanged in Leishmania-infected macrophages. BALB/c-derived macrophages were cultured uninfected or Leishmania-infected for 72 h. The macrophages were stained with anti-CD40-PE antibody and CD40 expression was analyzed with a FACSVantage™ flow cytometer. CD40 expression by RT-PCR was performed as described in Materials and Methods. (B) Anisomycin (Aniso), a p38MAPK activator, and CD40 cross-linking induces p38MAPK phosphorylation and SB203580 (SB), an inhibitor of p38MAPK, inhibits the CD40-triggered p38MAPK phosphorylation. Macrophages were stimulated with anti-CD40 (2 μg/ml) alone or with the indicated doses of SB203580 or anisomycin for 15 min. Cell extracts were prepared and Western blot was performed to detect phospho- and dephospho-p38MAPK as described earlier. (C) SB203580 inhibits while anisomycin induces the CD40-triggered iNOS2 expression. RNA was isolated from BALB/c macrophages, which were stimulated with anti-CD40 antibody alone (2 μg/ml) or with the indicated doses of SB203580 or anisomycin for 3 h and RT-PCR for iNOS2 message was performed as described earlier. The data shown is from one of three individual experiments.

![Figure 3](image2.png)  
**Figure 3.** CD40-induced p38MAPK phosphorylation, iNOS2 induction, and anti-leishmanial function are impaired during Leishmania infection but restored by anisomycin. The uninfected and the *L. major*–infected macrophages, treated as indicated, were lysed to obtain protein, for studying p38MAPK phosphorylation (A) or RNA, for studying iNOS2 expression (B), as described in Materials and Methods. In some experiments (C), macrophages were treated with anti-CD40 antibody either at the beginning of infection (CD40-E) with or without SB203580 (2 μg/ml) or 72 h after infection (CD40-L) with or without anisomycin. Macrophages were fixed, stained and counted to record the number amastigotes per 100 macrophages. The data (mean ± SD) showed that anisomycin rescued the CD40-induced anti-leishmanial effect (CD40-L+An) significantly (*, P < 0.01).

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phages with anisomycin alone or in combination with anti-CD40 resulted in the restoration of p38MAPK phosphorylation and iNOS2 expression (Fig. 3, A and B). Similarly, CD40-induced anti-leishmanial function was significantly reduced in late-infected macrophages but rescued by anisomycin (P < 0.01; Fig. 3 C). Therefore, these results indicate that Leishmania interrupts the CD40 signaling by inhibiting p38MAPK phosphorylation. As anisomycin does not kill promastigotes (data not shown) and, as predicted by docking simulation, fits into p38MAPK (unpublished data), it is suggested that the anti-leishmanial effect of anisomycin is through p38MAPK.

Anisomycin Retains Its Anti-leishmanial Activity In Vivo and Establishes a Host-protective Memory T Cell Response. As anisomycin rescued the CD40 signaling defect in vitro, we administered this drug in Leishmania-infected BALB/c mice to test its therapeutic potential. Four groups of L. major-infected mice were either left untreated or treated with anti-CD40 antibody alone from the beginning or 7 d after infection or with the antibody plus anisomycin 7 d after infection. It was observed that the delay in beginning the treatment with anti-CD40 antibody alone failed to reduce the infection but when treated in combination with anisomycin, the disease, as assessed by foot-pad swelling and parasite load, was significantly less severe (P < 0.001) compared with controls (Fig. 4, A and B). A comparison between the cytokine profiles (Fig. 4, A and B). A comparison between the cytokine profiles (Fig. 4, A and B) of infected mice treated with anisomycin alone or in combination with anti-CD40 antibody or saline demonstrated that IFN-γ production significantly (P < 0.001), decreased IL-4 and increased IFN-γ production significantly (P < 0.01).

Figure 4. Anisomycin treatment results in significant decrease in infection (A and B) and is associated with a Th1-type response (C). BALB/c mice were infected with L. major promastigotes (2 × 10^6) and were treated either at the beginning of infection with saline (Control) or anti-CD40 (CD40-E) or 7 d after infection with anti-CD40 antibody alone (CD40-L) or in combination with anisomycin (Aniso). The footpad swelling was measured weekly (A) and the parasite load was measured on day 35 post-infection (B). CD4+ T cells from lymph nodes of naïve or infected mice treated with saline, anti-CD40 antibody or anisomycin were stimulated in vitro with anti-CD3 + anti-CD28 for 36 h. The cell culture supernatants were assayed for IL-4 and IFN-γ (C). The data (mean ± SD) represent one of three individual experiments. Anti-CD40 or anisomycin treatment reduced infection significantly (*, P < 0.001), decreased IL-4 and increased IFN-γ production significantly (P < 0.01).

Although we do not know whether or not anisomycin treatment in vivo established the host-protective memory by working on T cells or macrophages or both, nevertheless, our results suggest that p38MAPK plays an important role in establishing host-protective memory T cell response.

Figure 5. Priming of BALB/c mice with ultra-low dose of L. major plus anisomycin establishes a host-protective Th1-type memory response. (A) BALB/c mice, primed with 5 × 10^2 Leishmania promastigotes alone (Circle) or with anisomycin (inverted triangle), showed no demonstrable infection after 6 wk (Primary). The mice were then challenged with L. major promastigotes (2 × 10^6). The net footpad swelling in the control (open bar) or anisomycin-treated (hatched bar) mice was measured 6 wk after infection (Secondary). Limiting dilution assay assessed parasite load in the footpad (Inset). Values are expressed in mean ± SD. Anisomycin priming reduced the infection significantly (*, P < 0.001). (B) CD4+ T cells from popliteal lymph nodes of the control (open bar) or anisomycin-treated (hatched bar) mice were stimulated with anti-CD3 + anti-CD28 for 36 h. IL-4 and IFN-γ were measured in the supernatants by ELISA. The data (mean ± SD) representing one of three experiments shows that anisomycin priming reduced IL-4 production but increased IFN-γ production significantly (*, P < 0.005).
Discussion

Successful invasion and survival of a parasite within an immunocompetent host depends on its ability to adapt itself to the anti-parasitic microenvironment and to subvert the host’s immune response (2). Our data reveals a novel fact that *Leishmania* impairs the CD40 effector functions by interrupting the CD40 signaling through p38MAPK in macrophages. The impairment in p38MAPK in *Leishmania*-infected macrophages could be due to the inhibition of PI-3 kinase (6), ras-raf-rac system (18), or protein kinase C (19) that are known to be involved in CD40 signaling and p38MAPK phosphorylation. The signaling intermediates upstream of p38MAPK could be impaired by the parasite directly, where a parasite product interacts with one or more of these proteins (20) to inhibit phosphorylation or increase dephosphorylation (21) or, indirectly, by inducing a host-derived factor that may act in an autocrine manner to incapacitate the CD40 signaling. For example, a selective impairment of LPS-activated protein kinase C isotypes in *Leishmania donovani*-infected macrophages by leishmanial lipophosphoglycan and host-derived IL-10 is reported (19). We have observed that IL-10 impairs CD40-activated iNOS2 expression and anti-leishmanial function (unpublished data). As CD40-CD40 ligand (CD154) interaction generates CD40 signals that play important roles in modulating Th1-dependent anti-leishmanial response (7–9), we have studied the effect of *Leishmania* infection on CD40 signaling in macrophages and the involvement of p38MAPK in the establishment of anti-leishmanial immune response. Our observations indicate that as an immune evasion strategy and for establishing infection, *Leishmania* inhibits CD40-activated p38MAPK signaling. On the other hand, anisomycin’s ability to restore CD40 signaling and eliminate amastigotes not only highlights the susceptibility of amastigotes to killing after p38MAPK activation but also suggests a potential use of anisomycin as an anti-leishmanial drug.

The two most important problems in designing an anti-parasitic drug are defining the target and the development of drug-resistance by the parasite. The current study suggests a strategy that addresses both the problems. First, since a parasite impairs the functions of the host cell proteins employed to eliminate the parasite, a drug target can in principle be identified by tracking the host cell proteins impaired by the parasite. An activator of such a crucial host cell protein, like anisomycin for the p38MAPK in the present report, may therefore be considered as a drug candidate. Second, the parasite biomolecules, which are absent in the host, are considered as drug targets. As these drugs inhibit the activities of the target parasite molecules, they exert a selection pressure on the parasite resulting in the emergence of drug resistance (22). As anisomycin controls the parasite by restoring the host immune system, the emergence of drug-resistance can be avoided. Furthermore, the amelioration of the disease is accompanied by Th1-predominance suggesting that the anisomycin treatment generates a bystander host-protective Th cell memory rendering the host relatively resistant to reinfection.

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References