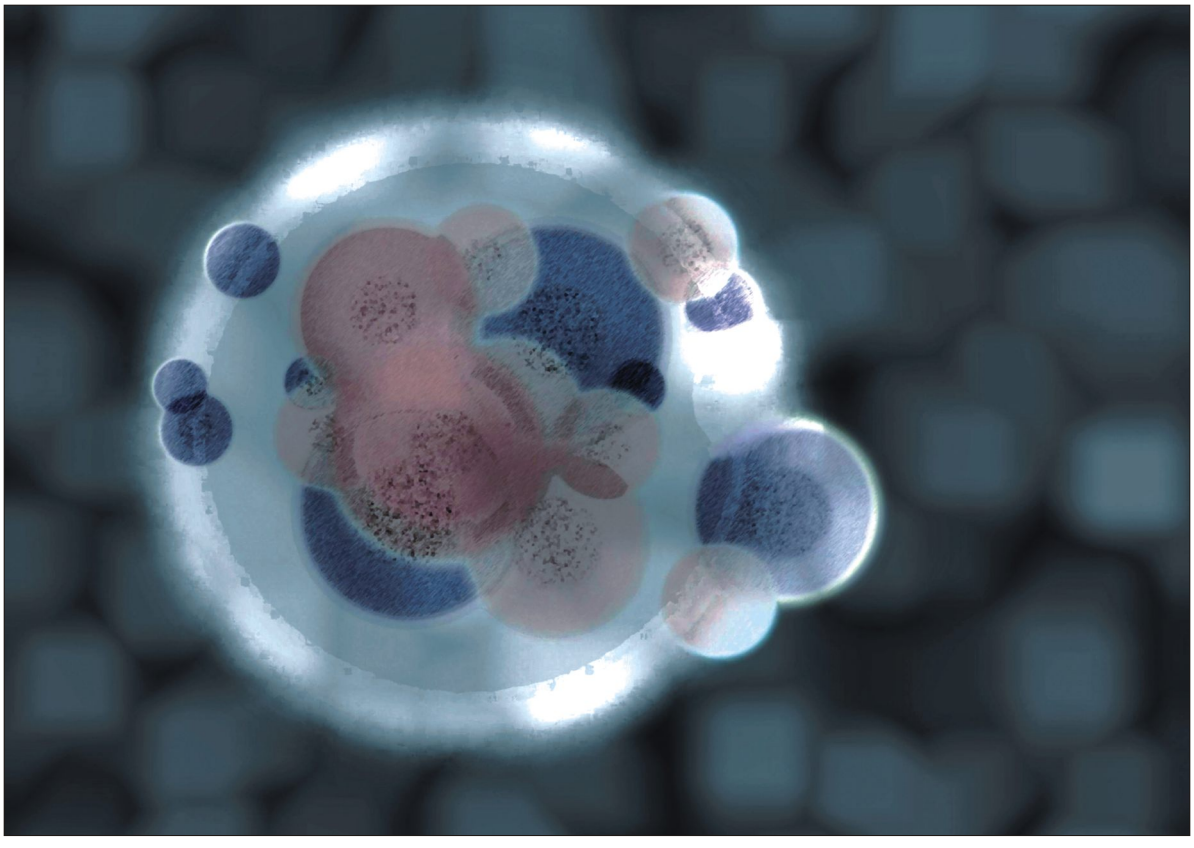

Journal of Biological Sciences

ISSN 1727-3048

science alert

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2019.1.6

Review Article
Dual Role of CD40 Receptor Signaling in Host Protection and Diseases Progression Against *Leishmania* Infection

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Abstract

In striking contrast to previous observation of two counteracting cells like Th1 and Th2 or two counteracting receptors like CD28 and CTLA4 expressed on the same cell, regulate homeostatic functioning of a physiological process, CD40, a single receptor triggers counteracting effector functions through kinases in reciprocally signaling modules to serve as a bifunctional switch. CD40 differentially activates these modules as a function of stimulus strength and *Leishmania* infection. With increasing CD40 stimulation, CD40 relocates to cholesterol-rich membrane microdomains called raft. *Leishmania*, a protozoan parasite impairs the relocation by depleting membrane cholesterol, as cholesterol supplementation in *Leishmania*-infected macrophages restores CD40 migration to raft. Cholesterol depletion by *Leishmania* infection or β-methyl cyclodextrin treatment does not abrogate CD40 signaling but switches it from p38MAP kinase module to ERK-1/2 module, modulating effector functions accordingly. The mechanism of signal switching is through differential recruitment of TRAF proteins and membrane associated kinases to detergent-resistant membrane microdomains called membrane raft and detergent-soluble membrane microdomains called membrane non-raft. *Leishmania* infection and β-methyl cyclodextrin impair whereas cholesterol supplementation restores the CD40 signaling. This review, discussed the different reported functions of CD40-CD40-L interaction and its detailed signaling and importance in *Leishmania* infection.

Key words: *Leishmania*, macrophages, ERk1/2, p38, detergent resistant, CD40, signaling

Received: May 28, 2018 **Accepted:** September 04, 2018 **Published:** December 15, 2018

Citation: Abdul Aziz A. Bin Dukhyil, 2019. Dual role of CD40 receptor signaling in host protection and diseases progression against *Leishmania* infection. J. Biol. Sci., 19: 1-6.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

CD40 receptor is a member of tumor necrosis factor receptor (TNFR) family[1]. It is expresses by Antigen Presenting Cells (APCs) including B cells, dendritic cells and macrophages. It is also expressed by many non-hematopoietic cells including epithelial cells, fibroblasts and endothelial cells in response to inflammatory signals. CD40 binds with its ligand, CD40-L also known as CD154. It also belongs to TNFR family. It is expressed on activated CD4 and CD8 T cells. CD154 is also expressed by platelets, monocytes and B cells[2]. CD40 ligand is secreted also from activated platelets and lymphocytes known as soluble CD154 (sCD154). The idea of central role of CD40 in thymus dependent B cell activation came into existence upon the investigation of patients suffering from X-linked hyper-IgM syndrome (HIGM) had mutations in their CD154 gene[3,4]. Initially CD40 was studied in B cell activation, differentiation, proliferation, immunoglobulin class switching and B cell memory generation. Both HIGM patients and CD40/CD154 deficient mice exhibited problem in naive $CD4^+$ T cell priming. This interaction is also important in dendritic cell mediated cross-priming of cytotoxic-T cell ($CD8^+$). Intervention with CD40-CD154 through deficient mice or blocking antibodies showed positive effects in experimental models of infectious diseases, transplantation and autoimmunity[3,5,6]. Interaction between APCs expressed CD40 and its T cell expressed ligand CD154 enhances cytokine production mainly IL-12 which plays pivotal role to induce T cells to produce interferon-γ (IFN-γ) to mount Th1 response to fight against intracellular pathogens[7,8]. It is reported that CD40 and CD40-L deficient mice were susceptible to *Leishmania* infection[9]. Administration of IL-12 cytokine in these knockout mice inhibited the growth of parasite and reduced the disease progression[10,11]. CD40-CD40-L interaction induces the formation of several inflammatory molecules including Nitric Oxide (NO) which kills the amastigotes inside macrophages[9,12].

Leishmaniasis is a vector born disease caused by protozoan parasite, *Leishmania* spp., belonging to the family Trypanosomatidae[13]. Leishmaniasis has been found to be a major global health problem in 89 different countries throughout the world affecting 1.2-2 million people every year[14]. The WHO has recognized this vector borne disease as one of the Neglected Tropical Diseases (NTD)[15]. *Leishmania* invades human macrophages and being transmitted by infected sandflies viz ; *Phlebotomus* and *Lutzomyia*[16,17]. There are 3 forms of leishmaniasis depending upon the parasitic species; visceral, cutaneous and mucocutaneous leishmaniasis[18,19]. *Leishmania donovani* is the causative agent of visceral leishmaniasis, *Leishmania major* is responsible for cutaneous leishmaniasis, manifest as severe skin infection and *Leishmania braziliensis* is responsible for mucocutaneous leishmaniasis[20-22]. *Leishmania* infection causes depletion membrane cholesterol and inhibits CD40 induced phosphorylation of Lyn and p38 kinases, resulting in reduced production of IL-12 whereas enhances CD40-induced phosphorylation of Syk and ERK1/2 kinases, resulting in increased production of IL-10[23]. CD40 induced high IL-12 production causes host-protection and induces *Leishmania* killing inside macrophages whereas enhanced IL-10 production aggravates the infection. CD40 signaling protects the host or progresses the disease was a conundrum. Recent studies solved this issue that higher CD40 stimulation showed protection against *Leishmania* infection whereas low CD40 stimulation helped the parasite to grow and aggravate the infection[23]. This reciprocal mode of signaling not only exists in case of CD40 but there are some more examples in nature. Like immune homeostasis is often maintained by counteracting cells such as Th1 cells mediating delayed-type hypersensitivity whereas Th2 cells suppressing it[23,24]. In other cases, two counteracting receptors on the same cell regulate a physiological effector function such as T cell receptor-triggered T cell proliferation and IL-2 production are potentiated by CD28 but suppressed by CTLA-4 signaling[25,26].

IMMUNE FUNCTIONS OF CD40 SIGNALING

In humoral immune response: Hyper IgM syndrome, an X-linked immunodeficiency disorder is reported due to the mutation in the CD40-L gene which impaired the CD40/CD40-L interaction[3,4]. Such patients suffer from very low or no titer of IgA, IgE or IgG and no B cell memory formation. CD40-CD40-L interaction is required for the proliferation and germinal center formation of B cells *in vivo*[27].

CD40 and CD40-L deficient mice were developed and found that they have similar pathological condition of high level of serum IgM similar to that of patients suffering from hyper IgM syndrome[3,4]. CD40-L expression was found altered in B cell chronic lymphocytic leukemia[28,29].

Further neutralizing or cross-linking studies were performed to study the role of CD40-CD40-L interaction in humoral immune response. Administration of neutralizing antibody against CD40L inhibited the production of autoantibodies in *in vivo* models of experimental autoimmune encephalomyelitis (EAE), collagen induced arthritis and systemic lupus erythematosus (SLE) nephritis. Treatment of agonistic antibody against CD40 receptor resulted in drastic isotype switching against pneumococcal polysaccharides[30].

Co-administration of trimeric CD40-L and leishmanial soluble antigen showed protection against *Leishmania major* infection in mouse model[31]. Thus, CD40-CD40-L interaction has significant role in humoral immune response.

In T-cell immune response: Susceptibility to *Cryptosporidium* and *Pneumocystis infections* to patients suffering from hyper-IgM syndrome suggested the role of CD40-CD40-L interaction in cell mediated immune response. CD40-L deficient mice had suppressed cell mediated immune response against pathogen infections[32,33]. CD40-L knockout mice were found susceptible to *Leishmania* infection[32,34]. It has been proved that CD40-CD40-L interactions plays critical role in T-cell differentiation and effector functions[33,35]. Interaction between CD40, expressed on antigen presenting cells and CD40-L, expressed on T-cells regulate the expression of co-stimulatory molecules expression and cytokine secretion. It also plays important role in T-cell subset generation. It has been observed that CD40-L and CTLA4 have cooperative effects. There is an argument whether lack of this interaction induces tolerance[36]. There are some examples such as immunity against LCMV and VSV virus where T-cell priming was found independent of this interaction[37]. CD40 agonistic antibody administration showed CTL-mediated regression of tumors[38].

In autoimmunity: CD40 mediates T-dependent B-cell response and efficient T-cell priming therefore, it is a possible contender to be implicated in autoimmune diseases. Development of CD40, CD40-L knockout mice and their blocking antibodies made it possible to study the role of CD40 in disease models of autoimmunity, microbial infection and transplantation.

Administration of agonistic antibody against CD40-L in NOD mice belated the insulin-dependent type-1 diabetes[6]. It is reported recently that TCR (T-cell receptor) and CD40 mediated activation of NOD-T cells increased the expression of CD40 on T-cells and inhibited CTLA4 expression[6]. Role of CD40 in Inflammatory Bowel Disease (IBD) was also studied in human and found that its expression on different cell types induces the secretion of IL-12, IL-23 and IL-6 which increases the inflammation and aggravates the disease in susceptible patients[6]. In one study, IBD patient's administration of anti-CD40 monoclonal antibody decreased the severity of the disease in 77% cases[6]. *In vivo* treatment of blocking antibody against CD40-L in disease models of Lupus Nephritis, Collagen-induced arthritis and EAE reduced disease severity. It was observed that SLE patients had increased expression of CD40-L on T lymphocytes[6].

In transplant acceptance and rejection: It is well documented that CD40 plays a significant role in recognition of antigenic peptide by naive T-cells therefore; intervention in CD40-CD40-L interaction had been extensively studied to increase the survival of transplanted allografts including skin, pancreatic islets and heart aorta in rodent, nonprimate and primate experimental models[39,40]. Administration of antibody against CD40-L delayed the of graft rejection which was further enhanced if donor splenocytes or CTLA4 antibody is co-administered[39]. Further studies proved that co-administration of anti-CD40 and anti-CD28 enhanced the acceptance of skin and cardiac allograft[39]. In a rhesus monkey model of kidney, it was observed that administration of anti-CD40-L with or without CTLA4 antibody enhanced the survival[40]. The current developments in CD40-CD40-L based treatment options may open new window for the treatment of several patients suffering from organ failure.

CD40 signaling in *Leishmania* infection: It has been shown in macrophages that, CD40 serves as a bifunctional switch whereby it regulates counteracting effector functions by reciprocal signaling through p38MAPK and ERK-1/2 kinase modules[23]. At higher doses of CD40 stimulation, kinases in the p38MAPK module were activated whereas the kinases in the ERK-1/2 module were inhibited. *Leishmania* infection of macrophages reversed this profile of activation of the reciprocal kinase modules. Inhibition of syk, a kinase in the ERK-1/2 module or p38MAPK activation ameliorated *Leishmania* infection[23] (Fig. 1).

It is reported that CD40 signaling is originate only from membrane raft, disruption of which by β-methyl cyclodextrin (b-MCD)-induced cholesterol depletion abrogated the signaling whereas in other finding it was reported that membrane cholesterol played critical role in unique CD40 signaling. It was reported that cholesterol depletion by b-MCD inhibited CD40-induced p38MAPK but augmented ERK-1/2 phosphorylation and reduced CD40-induced IL-12 but enhanced IL-10 expressions. In the same finding it was suggested that raft disruption by cholesterol depletion rather switched the signaling between two modules than abrogating it completely. Increasing doses of CD40 stimulation CD40 translocates it to raft but *Leishmania* impairs its translocation by membrane cholesterol depletion. Membrane cholesterol depletion with β-methylcyclodextrin impaired CD40 relocation in raft whereas cholesterol replenishment restored CD40 in raft in both b-MCD-treated and *Leishmania*-infected macrophages (Fig. 1). b-MCD treatment and *Leishmania* infection to macrophages up-regulated IL-10 but down-regulated IL-12 and iNOS2 expressions[23]. It is well studied that

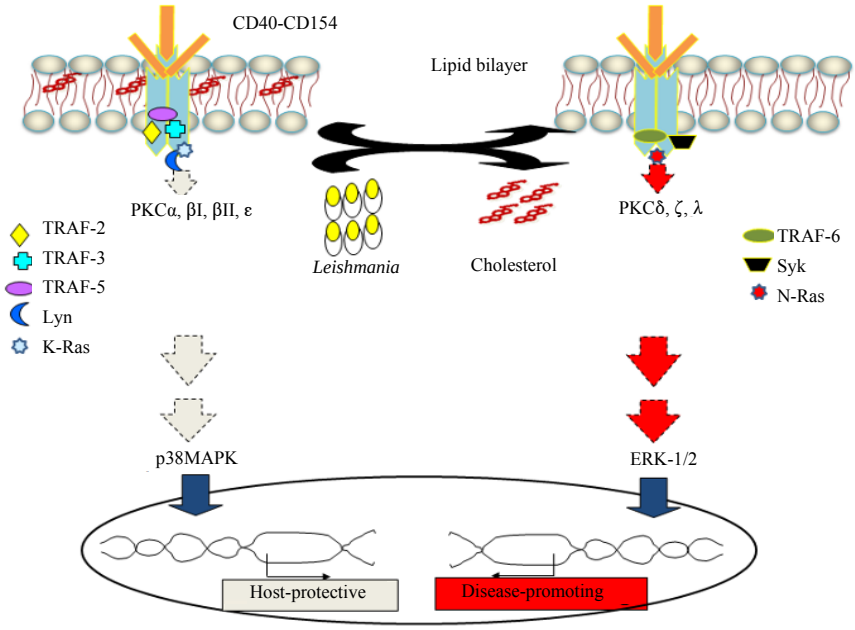

Fig. 1: Model explaining the current scenario of CD40 signaling

CD40 does not have any kinase activity in its cytoplasmic domain but it activates kinases by recruiting through TNF-a receptor associated factors (TRAFs)[23]. Upon higher stimulation CD40 recruits TRAF-2, TRAF-3, TRAF-5 but upon low stimulation and in *Leishmania* infected macrophages it recruits TRAF-6. CD40 also recruits Syk and Lyn kinases in different domains depending upon the strength of stimulation and *Leishmania* infection. It has also been studied that at higher doses of stimulation CD40 activated PKCα, βI, βII and ε whereas at lower doses of stimulation it activated PKCδ, ζ and λ. *Leishmania* infection enhanced CD40 induced PKCδ, ζ and λ but suppressed PKCα, βI, βII and ε. CD40 stimulation also activated Ras isoforms in dose depended manner[41-43]. It activated N-Ras at low doses but K-Ras and H-Ras higher doses. *Leishmania* activated the CD40 induced N-Ras activation whereas suppressed K-Ras activation in macrophages *in vitro*[42]. Dual roles played by CD40 in the models of *Leishmania* infection and prostate tumor may be because of its reciprocal association with TRAF-3, TRAF-6, PKC isoforms and Ras isoform[42,43]. Treatment of anti-CD40 and CD40-L showed protection from *Leishmania* infection. This protection was further enhanced by co-administration of anti-CD40 antibody with mevalonolactone[23] (Fig. 1).

CONCLUSION

The possible molecular mechanism of switching of CD40 signaling from ERK-1/2 to p38MAPK modules may involve increased CD40 clustering via conformational changes in the transmembrane domain. Such conformational alterations may result in hydrophobicity mismatch between the transmembrane domain of CD40 and the surrounding lipids resulting in receptor partitioning into different membrane domains. Cholesterol depletion by *Leishmania* may affect this partitioning excluding CD40. How *Leishmania* depletes cholesterol is unknown but it possibly interferes with trafficking of intracellular cholesterol or cholesterol biosynthesis by regulating the expression and activity of the rate-limiting enzyme, HMG-CoA reductase or by exchanging host cholesterol for its ergosterol, which differs from cholesterol in various biophysical parameters.

SIGNIFICANCE STATEMENT

This study discovers the dual role of CD40 signaling in host-protection and diseases-progression against *Leishmania* infection. At weak activation CD40 helps in growth of parasite on the other hand at high activation it helps in clearance of parasite. *Leishmania* removes membrane cholesterol for its survival. This removal does not suppress all the pathways of CD40 signaling but activates some of them. This study has lots of breath and scope and will help the researchers to uncover the critical role of CD40 signaling in other infectious diseases and autoimmune disorders.

ACKNOWLEDGMENT

I am highly thankful for Deanship of Scientific Research Majmaah University, Al Majmaah, KSA, for support.

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