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CD40 AND THE IMMUNE RESPONSE TO PARASITIC INFECTIONS

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Abstract

The interaction between CD40 and CD154 regulates many aspects of cellular and humoral immunity. The CD40 — CD154 pathway is important for resistance against a variety of parasites. Studies done with these pathogens have provided important insight into the various mechanisms by which this pathway enhances host protection, mechanisms by which pathogens subvert CD40 signaling, conditions in which the CD40 — CD154 pathway promotes disease and on modulation of this pathway for immunotherapy.

Keywords

Protozoa; helminth; cytokine; autophagy

Introduction

CD40 is a member of the TNF receptor superfamily that is expressed on antigen presenting cells (APC) and various non-hematopoietic cells [1-4]. Its counter receptor CD154 (CD40 ligand) is expressed primarily on activated CD4⁺ T cells [5,6]. The interaction between CD40 and CD154 controls many aspects of cellular and humoral immunity including T cell-mediated activation of dendritic cells (DC) and monocyte/macrophages, T cell priming, proliferation of B cells, Ig synthesis, isotype switch, and germinal center formation [2,7-12].

The relevance of CD40 in humans was established by the discovery that the congenital immunodeficiency called X-linked hyper IgM syndrome (X-HIM) is caused by the lack of functional CD154 [13]. Although patients with this syndrome exhibit defects in humoral immunity, the most important clinical feature of this immunodeficiency is an increased incidence of infections with opportunistic pathogens that include parasites such as *Cryptosporidium parvum* and *Toxoplasma gondii* [14-17]. Studies using parasites provided important insight into the role of CD40 in regulation of cell-mediated immunity as well as likely explanations for the susceptibility to opportunistic infections observed in patients with X-HIM [17].

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Toxoplasma gondii

Toxoplasma gondii is an obligate intracellular protozoan of worldwide distribution. The parasite exists in three forms during its life cycle: 1) the tachyzoite, the form of the parasite that infects almost any nucleated cell; 2) the tissue cyst that appears to persist in tissues of the infected host for life; and 3) the oocyst that forms during the sexual cycle that takes place exclusively in the intestine of felines. Infection in humans follows ingestion of poorly cooked meat that contains tissue cysts, or ingesting food or water contaminated with oocysts.

Acute infection with *T. gondii* is characterized by dissemination of tachyzoites throughout the body. T cell-mediated immunity and the production of cytokines, notably IFN- γ and IL-12, are critical for control of the infection [18,19]. However, the organism successfully evades eradication. The chronic phase of infection that ensues is characterized by the disappearance of tachyzoites and the formation of tissue cysts (primarily in the central nervous system and skeletal muscle).

T. gondii infection is usually asymptomatic in immunocompetent humans. The development of disorders of cell-mediated immunity results in reactivation of the chronic infection that typically manifests as toxoplasmic encephalitis [20,21]. The other scenario where *T. gondii* infection is of clinical relevance is when the infection is acquired congenitally.

Role of CD40 — CD154 signaling in regulation of type 1 cytokine response during *T. gondii* infection—

The fact that patients with X-HIM are susceptible to toxoplasmic encephalitis and disseminated toxoplasmosis [16,17,22,23] plus the demonstration that CD154^{-/-} mice infected with *T. gondii* develop encephalitis provide clear evidence of the relevance of the CD40 — CD154 pathway in resistance against *T. gondii*. Studies in humans revealed that this pathway regulates IL-12 and IFN- γ production during the interaction between human T cells and *T. gondii*-infected APC [17,24]. Interestingly, *T. gondii* induces profound phenotypic changes in APC that influence type 1 cytokine production. Infection with viable *T. gondii* but not phagocytosis of killed parasites or incubation with *T. gondii* lysate antigens induces activation of purified human monocytes and immature monocyte-derived DC characterized by up-regulation of MHC class II, CD40, CD80, and CD86 [17,25-27] (Figure 1). Human monocyte-derived DC that contain intracellular tachyzoites do not produce IL-12 p70 unless they receive CD40 stimulation from T cells [26] (Figure 1). Interestingly, IL-12 p70 production is only observed if monocyte-derived DC are infected with viable tachyzoites and not if these cells phagocytose killed parasites or if they are exposed to *T. gondii* lysates [26,28]. Studies using mouse DC revealed that production of IL-12 p70 appears to depend on *T. gondii*-induced IL-12 p40 production and CD40 upregulation, while CD40 stimulation of DC results in balanced production of IL-12 p35 [29]. The studies using human and mouse cells indicate that the response of DC to *T. gondii* together with CD40 stimulation of these cells allow the immune system to induce IL-12 p70 production in situations where such a response would be appropriate [26,29,30].

CD40 - CD154 interaction between human T cells and *T. gondii*-infected APC results in IL-12 production that in turn, drives secretion of IFN- γ [17,26] (Figure 1). However, experiments using neutralizing anti-IL-12 mAb indicated that *T. gondii*-infected APC also induce IFN- γ production independently of IL-12 [26]. This IL-12-independent production of IFN- γ may be caused by direct co-stimulation to T cells since it is ablated by simultaneous neutralization of the CD40-CD154 and CD80/CD86-CD28 pathways [26] (Figure 1). Of relevance, *in vivo* studies in mice demonstrated that pathogen-specific CD4⁺ T cells of the Th1 phenotype develop in animals deficient in IL-12 [31]. In addition, blockade of both CD154 and CD28 pathways is required for effective inhibition of IFN- γ production in a mouse model of toxoplasmosis [32].

While the role of CD40 - CD154 signaling for control of IL-12 production has also been reported in *T. gondii*—infected CD154^{-/-} mice [33], other animal studies revealed the existence of CD40-independent IL-12 secretion in response to *T. gondii* [34]. *T. gondii* lysates induce IL-12 production by mouse CD8 α ⁺ DC in a CD40-independent manner [34]. IL-12 production in response to soluble antigens was explained by *T. gondii* profilin-like protein that binds to TLR11 [35] and in part by *T. gondii* cyclophilin 18, a molecule released by tachyzoites that binds to CCR5 [36,37] (Figure 2). While this pathway induces IL-12 production in mice, it is not known whether the same can be said for humans. *T. gondii* lysates antigens do not appear to cause IL-12 production by human dendritic cells [28]. Moreover, TLR11 is represented in humans only by a pseudogene [38] that contains a stop codon that would prevent protein expression [39].

Studies in patients with defective CD40 - CD154 signaling support the relevance of this pathway in the regulation of type 1 cytokine response in humans. Toxoplasmic encephalitis and disseminated toxoplasmosis have been reported in patients with X-HIM [16,17,22,23]. Peripheral blood mononuclear cells (PBMC) and T cells from X-HIM patients secrete markedly decreased amounts of IFN- γ in response to *T. gondii* compared to healthy controls [17]. Similarly, PBMC from patients with X-HIM fail to secrete or secrete low amounts of IL-12 after incubation with *T. gondii* [17]. In contrast, IL-12 production in response to *Staphylococcus aureus* Cowan I strain plus IFN- γ is similar to that in control subjects [17]. Studies using recombinant CD154 trimer further confirmed the relevance of CD154 signaling for regulation of cytokine synthesis. CD154 trimer restores IL-12 secretion in response to *T. gondii* by PBMC from patients with X-HIM, and through this mechanism, it normalizes IFN- γ production in response to the parasite [17]. In addition to regulation of type 1 cytokine production, studies in patients with X-HIM indicate that CD154 appears to be crucial for *in vivo* priming of human T cells against *T. gondii* [17]. Taken together, the studies using *T. gondii* identified defective type 1 cytokine response and impaired T cell priming as likely explanations for susceptibility to opportunistic infections in X-HIM patients.

CD40 — CD154 signaling is also relevant to HIV-1⁺ patients. Studies using antigenic stimulation by pathogens including *T. gondii* as well as polyclonal T cell stimulation revealed that CD4⁺ T cells from these individuals exhibit lower levels of CD154 [40-43]. This defect appears to contribute to impaired type 1 cytokine response associated with HIV-1 infection [40,41]. The explanation for diminished CD154 expression in HIV-1 infection likely lies on the fact that the CD40 — CD154 interaction induces bi-directional signaling: CD40 not only induces activation of APC but CD40 also regulates CD154 expression. Many studies reported that CD40 decreases CD154 expression [44-46]. Enhanced susceptibility to CD40-mediated regulation likely explains why expression of CD154 during T cell — APC interaction is diminished in CD4⁺ T cells from HIV-1⁺ patients [42]. Studies that used *T. gondii* support a model whereby CD4⁺ T cells from these patients have an altered set point that controls the level of CD154 expression and thus, the strength of CD40-induced activation of APC [42]. Diminished CD40 stimulation would particularly affect IL-12 production since secretion of this cytokine appears to require strong CD40 stimulation [47].

CD40 is a novel regulator of autophagy—While CD40 regulates type 1 cytokine production in response to *T. gondii*, studies in CD154^{-/-} mice pointed to additional mechanisms of resistance controlled by the CD40-CD154 pathway [33]. Indeed, CD40 stimulation of humans and mouse macrophages results in killing of the parasite [48-52]. This effect does not require the presence of IFN- γ and occurs independently of NOS2, the immune related GTPase (IRG) LRG-47, IGTP and IRG-47, the oxidative pathway and tryptophan degradation [48, 49,52]. These findings raised the possibility that CD40 stimulation might be inducing killing of *T. gondii* by inducing lysosomal degradation.

Tachyzoites of *T. gondii* reside within a parasitophorous vacuole that allows parasite survival by preventing the release of the contents of endosomes/lysosomes into the parasitophorous vacuole. [53-55]. Until recently, the dogma was that the lack of fusion between the parasitophorous vacuole and the late endosomes/lysosomes was irreversible and was established at the time of active invasion [54,55]. However, through CD40, cell-mediated immunity changes the non-fusogenicity of the parasitophorous vacuole and targets it for lysosomal degradation. CD40 causes fusion of parasite-containing vacuoles with late endosomes and lysosomes [49,56]. Pharmacologic and genetic approaches that block different components of the endosomal/lysosomal pathway demonstrated that CD40 induces killing of *T. gondii* through vacuole-lysosome fusion [49]. These studies uncovered a new paradigm where interaction between CD154 on T cells and CD40 expressed on macrophages leads to the killing of an intracellular pathogen via the induction of vacuole-lysosomal fusion [49,56]. The fact that *in vivo* CD40 stimulation induces macrophage toxoplasmicidal activity and reduces the parasite load [52] strongly suggests that vacuole-lysosome fusion induced by CD40 contributes to host protection.

CD40 re-directs the parasitophorous vacuole to the endosomal/lysosomal compartment through macroautophagy, a pathway to lysosomal degradation that is different from the classical phago-lysosomal fusion [49]. Macroautophagy, usually referred to as autophagy, is a process that directs cytoplasmic material and organelles to lysosomes [57,58]. During autophagy an isolation membrane encircles portions of cytosol and organelles leading to the formation of autophagosomes. This structure fuses with late endosomes and lysosomes resulting in lysosomal degradation of its contents. Autophagy is a homeostatic mechanism in response to cellular stress such as nutrient deprivation or the accumulation of damaged organelles [57-60]. However, autophagy can also function as an innate mechanism that leads to lysosomal degradation of pathogens such as *Mycobacterium tuberculosis*, *Streptococcus pyogenes*, *Listeria monocytogenes* and *Salmonella enterica* [61-64]. Adaptive immunity also activates autophagy as anti-microbial strategy. IFN- γ appears to kill *M. tuberculosis* via autophagy [61]. Studies using *T. gondii* demonstrated that autophagy can be activated by adaptive immunity to induce pathogen killing. CD40 stimulation of macrophages causes recruitment of the highly specific autophagy marker LC3 (Atg8) around the parasitophorous vacuole [49]. This is followed by fusion with late endosomes/lysosomes [49]. Moreover, knockdown of the autophagy molecule Beclin 1 revealed that autophagy is required for fusion of the parasitophorous vacuole with late endosomes/lysosomes and for killing of *T. gondii* by CD40-activated macrophages [49].

Autophagic killing of *T. gondii* requires TRAF6-dependent synergy between CD40 and TNF- α . The TRAF6 binding site plays a dual role in the autophagic killing of *T. gondii*: it enhances autocrine production of TNF- α [65] and TRAF6 signals downstream of CD40 cooperate with TNF- α to mediate activate autophagy and lysosomal degradation of the parasite [49,56] (Figure 3A).

The CD40 - CD154 pathway is key for control of toxoplasmic encephalitis likely through autophagy. CD154^{-/-} mice develop this disease despite upregulation of IFN- γ [33] indicating that the CD40 - CD154 pathway likely promotes protection against encephalitis by activating mechanisms of host resistance that act independently of IFN- γ and its downstream effector molecules. Indeed, autophagic killing induced by CD40 occurs independently of IFN- γ , NOS2 and IRG. A model can be proposed whereby the IFN- γ /TNF- α -induced NOS2 pathway is essential but not sufficient resistance against cerebral toxoplasmosis [33,66], and full control of toxoplasmosis would require CD40-induced autophagic killing of *T. gondii* [67] (Figure 3B).

The immune response against *T. gondii* in mice does not fully mimic that in humans. In addition to differences described above (TLR11 expression), the relative importance of IFN- γ and downstream effector molecules is likely different in humans and mice. Mice infected with *T. gondii* require STAT-1, IRGs (LRG-47, IGTP and IIGP1) and NOS2 for survival during different phases of infection [68-75]. In marked contrast, children with an autosomal dominant defect in IFN- γ R1 that causes a deletion in the STAT-1 binding site are not susceptible to toxoplasmosis [76], IRG in humans have been reduced to a truncated gene *IRGM* and *IRGC* that lack an IFN-inducible element [77], and NOS2 in humans is more tightly regulated than in rodents and the production of nitric oxide appears to be weaker in humans than in mice [78]. CD40-induced autophagic killing of *T. gondii* may promote resistance against *T. gondii* in humans because CD40 induces killing of *T. gondii* independently of IFN- γ , STAT1, NOS2 and IRG [48,49,52,56]. In addition to patients with X-HIGM, defects in the CD40 pathway may explain susceptibility to cerebral and/or ocular toxoplasmosis in newborns and HIV-1⁺ patients. Several groups reported impaired expression of CD154 on neonatal CD4⁺ T cells [79-83] and reduced levels of CD40 on DC [83]. Defective CD154 induction is particularly more pronounced in preterm babies [83], a finding relevant to toxoplasmosis in newborns since this is an infection acquired prior to birth. As described above, HIV-1⁺ patients can have defective CD154 induction on their CD4⁺ T cells.

Role of CD40 signaling in immunopathology during *T. gondii* infection—Although type 1 cytokine response is central to resistance against *T. gondii*, uncontrolled cytokine production leads to immunopathology. Indeed, IL-10^{-/-} mice control the parasite and yet exhibit mortality associated with high production of IL-12, TNF- α , and IFN- γ production and liver damage [84]. Similarly, C57BL/6 mice develop acute inflammatory ileitis after oral infection with *T. gondii*, a process that is dependent on CD4⁺ T cells and IFN- γ [85]. Immunopathology in this model of infection as well as in IL-10^{-/-} mice is inhibited by blockade of CD154 [32, 86], especially in the setting of simultaneous blockade of CD28 [32]. Taken together, like other mediators of host resistance, the CD40 - CD154 pathway plays a role in protection against *T. gondii* and in situations where the infection causes immunopathology.

Cryptosporidium parvum

Cryptosporidium is a protozoan that can cause diarrhea. Humans become infected by ingestion of oocysts that release sporozoites in the intestinal lumen. Sporozoites then infect epithelial cells. Infected individuals shed oocyst in their feces. Thus, humans can become infected by person-to-person contact. Waterborne outbreaks of cryptosporidiosis can also occur. Infection can be asymptomatic or can cause self-limited diarrhea. However, patients with AIDS or other immunodeficiencies can develop severe disease that may involve the biliary tract.

Animal studies revealed that cell-mediated immunity confers protection against *C. parvum*. CD4⁺ T cells and MHC class II are required to control *C. parvum* [87,88]. In addition, IFN- γ and IL-12 mediate protection against the pathogen [89,90]. Although infection leads to antibody production, it appears that humoral immunity is of secondary importance compared to cell-mediated immunity.

The identification of *C. parvum* as an important pathogen in patients with X-HIM syndrome provided clear evidence for the relevance of the CD40-CD154 pathway for resistance against *C. parvum* [91]. Indeed, both CD40^{-/-} and CD154^{-/-} mice are unable to control *C. parvum* [92]. While parasite shedding in the stools disappears by 4 weeks in control mice, CD40^{-/-} and CD154^{-/-} mice show unabated parasite shedding for at least 8 weeks [92]. Moreover, *C. parvum* is detected in the gut, gallbladder and biliary tree of CD40^{-/-} and CD154^{-/-} mice and causes cholangitis, a feature of cryptosporidiosis in immunodeficient patients [92]. Further evidence of the importance of CD154 came from studies in SCID mice [92]. These animals

are susceptible to *C. parvum*. While administration of spleen cells from wild type mice induces control of the parasite, spleen cells from CD154^{-/-} are unable to confer protection [92].

Studies in HepG2 cells, a hepatocellular carcinoma cell line with features of biliary epithelial cells, revealed that CD40 stimulation induces anti-microbial activity against *C. parvum* [91]. This effect may be explained by induction of apoptosis of infected cells [92]. However, the *in vivo* relevance of this mechanism is unclear. Experiments with bone marrow chimeras showed that expression of CD40 on bone marrow cells is sufficient to induce resistance against *C. parvum* while CD40 expression on non-hematopoietic cells is unable to confer protection [93].

Leishmania

Leishmania is an obligate intracellular protozoan that causes disease that is endemic in the tropics, subtropics and southern Europe. Female phlebotomine sandflies release promastigotes into the skin while probing for a blood meal. The promastigote infects primarily macrophages and DC. The amastigote form of Leishmania replicates within phagolysosomes. The host cell is eventually destroyed and the parasites that are released infect other host cells.

Leishmania can cause cutaneous, mucosal and visceral disease. There are a variety of species of Leishmania that are of clinical relevance in humans. Some examples include: *Leishmania major*, a cause of cutaneous disease; *Leishmania amazoniensis* and *Leishmania tropica*, both causes of cutaneous leishmaniasis (less commonly visceral disease); and *Leishmania donovani*, a cause of visceral leishmaniasis (less commonly cutaneous disease).

Leishmania provides a classical model system to study the role of cytokines in determining resistance or susceptibility to a pathogen. A mouse model of cutaneous leishmaniasis after infection with *L. major* allowed to demonstrate that strains of mice that develop preferential production of IL-12 and activation of IFN- γ -producing Th1 cells are able to control the infection [94]. In contrast, progressive disease occurs in mice that respond by preferential activation of Th2 cells that produce IL-4 [94].

CD40 — CD154 interaction and the regulation of type 1 cytokine response in Leishmania infection

Three simultaneous studies revealed the central role of the CD40 — CD154 pathway for protection against Leishmania. CD154^{-/-} or CD40^{-/-} mice on a resistant background (C57BL/6) developed ulcerating cutaneous lesions after infection with promastigotes of *L. major* or *L. amazoniensis* [95-97]. The central role of CD154 in host resistance was supported by the demonstration that treatment of infected CD154^{-/-} mice with recombinant CD154 afforded partial protection against *L. major* [95]. CD154^{-/-} and CD40^{-/-} mice had a profound defect in the induction of a Th1 type response [95-97]. T cells from CD154^{-/-} mice were unable to induce of IL-12 production when stimulated with Leishmania-infected macrophages [95]. Moreover, this defect in IL-12 production appears to be central to enhanced susceptibility to infection because *in vivo* treatment with IL-12 increased IFN- γ production and prevented disease progression [95]. CD40 appears to require NF- κ B2 to induce IL-12 production [98]. CD154 is also required not only for protection against cutaneous leishmaniasis but also against visceral leishmaniasis. CD154^{-/-} mice are susceptible to *L. donovani* [99], and the lack of CD154 promotes replication of residual *L. donovani* that survive after chemotherapy [100].

The importance of CD40 signaling for resistance against Leishmania was confirmed by studies using wild-type mice. Administration of anti-CD154 mAb to C57BL/6 mice infected with *L. major* resulted in impaired IL-12 production and worsened cutaneous lesions [101,102]. These studies indicate that CD40 signaling is required to maintain control of *L. major*. The CD40 —

CD154 pathway controls type 1 cytokine response not only in mice but also in humans. Incubation of human peripheral blood lymphocytes with autologous macrophages infected with *L. major* resulted in IFN- γ and IL-12 that were inhibited by addition of an anti-CD154 mAb [103].

The fact that Leishmania reside within macrophages suggested that these cells might be the source of IL-12 required to control the infection. However, Leishmania inhibits IL-12 production by macrophages [104]. Thus, investigators turned their attention to DC. Indeed, these cells produce IL-12 p40 in response to *L. donovani* and *L. major* [105,106]. Importantly, production of bioactive IL-12 p70 is dependent on the CD40 — CD154 pathway. Human monocyte-derived DC infected with *L. major* produce high levels of IL-12 p70 when stimulated with CD154 [107]. Moreover, *L. major*-infected monocyte-derived DC from patients with cutaneous leishmaniasis induced IFN- γ production by autologous T cells in a CD154-dependent manner [107]. These results suggest that CD40 signaling in skin DC at the site of infection may contribute to the development of a protective immune response [107]. CD40 stimulation also induces IL-12 production by DC infected with *L. amazoniensis* [108]. However, this response was detected only in DC from a resistant strain of mice (C3H/HeJ). In contrast, DC from a susceptible strain (BALB/c) produced IL-4 in response to CD40 stimulation [108].

The ability of Leishmania-infected dendritic cells to produce IL-12 in response to CD40 stimulation may provide an explanation for the different clinical presentation of leishmaniasis: localized versus disseminated disease and healing versus non-healing skin lesions. In this regard, while monocyte-derived DC infected with *L. major* produce IL-12 p70 in response to CD40 stimulation, DC infected with *L. donovani* and *L. tropica* fail to do so [109]. However, it appears that the inability of *L. donovani* to prime DC for IL-12 p70 production may be dependent on the form of parasite used. In contrast to human monocyte-derived DC infected with promastigotes of *L. donovani*, DC infected with *L. donovani* amastigotes upregulated IL-12 p70 production in response to CD40 stimulation [110].

While the CD40 — CD154 pathway is important for induction of type 1 cytokine response and resistance against Leishmania, this pathway may not always be required for protection. CD154^{-/-} mice that are infected with a low dose *L. major* promastigotes or that are also CD28^{-/-} can acquire and maintain resistance against the pathogen [102,111]. Treatment of infected CD154^{-/-} mice with TRANCE receptor fusion protein revealed that IL-12 production and the induction of a Th1-type response is mediated by the TRANCE — RANK pathway in the absence of CD154 [112]. These findings likely represent an example of the development of compensatory mechanisms of host protection in knockout mice.

The CD40 — CD154 pathway and the induction of macrophage anti-Leishmania activity

In addition to conferring host resistance by promoting induction of a type 1 cytokine response, the CD40 — CD154 pathway likely enhances protection by also activating anti-Leishmania activity in macrophages. Draining lymph nodes from CD154^{-/-} mice infected with *L. amazoniensis* have an impaired ability to induce nitric oxide (an important mediator of anti-Leishmania activity) by infected macrophages [96]. In addition, macrophages that receive CD40 stimulation plus IFN- γ acquire leishmanicidal activity [97]. CD40 also cooperates with other molecules to trigger killing of Leishmania by macrophages. Activated T cells induce anti-Leishmania activity in macrophages through cooperation between CD40 and CD11a (LFA-1) [113]. These two molecules stimulate nitric oxide production and thus, induce killing of *L. major* [113]. This process occurs independently of TNF receptors providing a potential explanation for the ability of TNFR1/2^{-/-} mice to control *L. major* [113].

Manipulation of CD40 signaling by Leishmania

The evidence that strains of mice susceptible to *L. major* have decreased production of IL-12 plus the central role of the CD40 - CD154 pathway in induction of this cytokine led to the proposal that defects in this pathway may be important in explaining susceptibility to infection [101]. CD154 expression was similar in susceptible and resistant strains of mice infected with *L. major* [101]. Thus, it was proposed that there might be a defect in CD40 signaling in Leishmania infection [101]. Indeed, macrophages from BALB/c mice infected with *L. major* exhibit defective induction of NOS2 in response to CD40 stimulation [114]. This defect appeared to be due to diminished p38 MAPK signaling [114]. Anisomycin, a p38 MAPK stimulator, restored NOS2 expression and anti-Leishmania activity of macrophages [114]. Moreover, *in vivo* treatment of BALB/c mice with anisomycin led to reduced parasite burden and induction of a Th1-type memory response [114].

The effects of Leishmania on CD40 signaling vary in macrophages from resistant (C57BL/6) versus susceptible (BALB/c) mice [115]. While CD40 stimulation induced anti-*L. major* activity in macrophages from C57BL/6 mice, macrophages from BALB/c exhibited increased parasite replication [115]. This discrepant outcome appears to be explained by the induction of IL-10 in CD40-stimulated macrophages from BALB/c mice [115]. Relevant to these findings, *L. major* has been reported to skew intracellular signaling in CD40-stimulated macrophages from BALB/c mice [47]. Weak CD40 signals induce ERK-1/2 signaling and IL-10 expression, whereas stronger signals induce p38 MAPK signaling and IL-12 p40 expression [47]. *L. major* skews CD40 signaling toward ERK-1/2 and IL-10 production in macrophages from BALB/c mice [47]. As a result, there is inhibition of p38 MAPK activation, of expression of NOS2 and of IL-12 production [47] (Figure 4). ERK-1/2 inhibition or IL-10 neutralization restores CD40-induced p38MAPK activation and parasite killing in macrophages [47]. Whether Leishmania fails to skew intracellular signaling in macrophages from C57BL/6 mice remains to be determined.

A recent study suggests that *L. major* influences signaling downstream of CD40 through its ability to deplete cholesterol [116]. Pharmacologic depletion of cholesterol or infection with *L. major* impairs the assembly of CD40 signalosomes that contain TRAF2, TRAF3 TRAF6 and Lyn, and as a result, cause inhibition in IL-12 production [116]. In contrast, cholesterol depletion or *L. major* infection promote the assembly of CD40 signalosomes that contain TRAF6 and Syk, leading to production of IL-10 [116]. However, in an apparent paradox, it has previously been demonstrated that CD40 triggers IL-12 production through the TRAF6 binding site and not the TRAF2,3 binding site [117].

Manipulation of CD40 signaling as immunotherapy

Stimulation of CD40 — CD154 signaling enhances resistance against Leishmania. Mice of a susceptible background (BALB/c) infected with *L. major* developed a type 1 cytokine response and acquire protection against the infection if they are treated with an agonistic anti-CD40 mAb [118]. BALB/c and C57BL/6 infected with *L. donovani* exhibited a reduction in parasite burden in the liver, increased IFN- γ production and enhanced mononuclear cell recruitment as well as granuloma formation after treatment with agonistic anti-CD40 mAb [99]. Anti-CD40 mAb potentiated the effect of pentavalent antimony, a chemotherapeutic agent for visceral leishmaniasis [99]. These results suggest that approaches to stimulate CD40 signaling may have therapeutic application for the treatment of leishmaniasis. However, the induction of an undesirable pro-inflammatory response raises concern about utilizing generalized stimulation of CD40 signaling.

The IL-12-inducing activity of CD154 suggested that this molecule could be used as an adjuvant to vaccination to preferentially induce a type 1 cytokine response. Indeed, vaccination

of susceptible BALB/c mice with soluble Leishmania antigen plus a plasmid that encodes CD154 enhanced antigen-specific IFN- γ production and induced protection against *L. major* [119]. Likewise, vaccination of susceptible BALB/c mice with soluble Leishmania lysate antigens plus L929 cells that express CD154 induced a type 1 cytokine response and protected mice against challenge infection with *L. major* [120]. Similar effects were observed when mice were immunized with L292 cells that express both CD154 and the Leishmania antigen gp63, or with immunization with *L. major* promastigotes adsorbed with recombinant CD154 [120]. Another approach to stimulate CD40 signaling during immunization has been to utilize transgenic parasites that express CD154. Compared to wild-type parasites, infection with *L. major* promastigotes that express the extracellular domain of CD154 caused mild disease in susceptible BALB/c mice and afforded resistance against challenge infection to C57BL/6 mice [121].

Trypanosoma

Trypanosomes are protozoa that can cause Chagas' disease in the Americas (*Trypanosoma cruzi*) or african trypanosomiasis (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*). Infection in humans is transmitted by hematophagous insects. These insects release infective trypomastigotes that enter through disruptions of the skin or mucosa. Trypomastigotes disseminate throughout the blood and infect a variety of cells leading to the intracellular replicative form called the amastigote. Infection with *T. cruzi* in BALB/c mice mimics disease as it occurs in humans: an acute phase characterized by parasitemia followed by a chronic where the parasite is not detectable in the blood but is present in peripheral tissues. IL-12, IFN- γ , TNF- α and nitric oxide have been implicated in resistance against *T. cruzi* [122-125].

CD40 induces anti-microbial activity against *T. cruzi*. Peritoneal macrophages treated with supernatants of spleen cells stimulated with CD154-expressing fibroblasts prevent infection with *T. cruzi* [126]. This effect is mediated by nitric oxide and the production of this radical requires IL-12, IFN- γ and TNF- α [126]. Moreover, CD40 stimulation of IFN- γ -primed macrophages also induces anti-microbial activity against *T. cruzi* [126]. The relevance of the cascade CD40 - IL-12 - IFN- γ is supported by *in vivo* experiments where mice that received CD154-expressing fibroblast exhibited reduced parasitemia and increased survival [126].

Two additional approaches of *in vivo* modulation of CD40 — CD154 signaling support the relevance of this pathway for induction of protection against *T. cruzi*. In one of them, parasites were genetically engineered to express host CD154 [127]. Infection of mice with CD154-expressing *T. cruzi* trypomastigotes led to low parasitemia, survival and resistance to challenge infection [127]. These effects were associated by evidence of preserved cell mediated immunity as assessed by lymphocyte proliferation and production of IFN- γ [127]. In the second approach, mice were treated with small trivalent molecules that bind CD40 and mimic the effects of soluble CD154 (miniCD40L) [128]. Mice treated with miniCD40L exhibited a decrease in parasitemia and in mortality that were accompanied by prevention of the immunosuppression that typically follows *T. cruzi* infection [128]. MiniCD40L-treated mice were protected from depletion of CD8⁺ T cells as well as impairment in lymphocyte proliferation and IFN- γ production [128]. These studies demonstrated that rationally designed CD154 mimetics can be effective *in vivo* [128].

CD40 appears to also promote resistance against trypanosoma also through stimulation of humoral immunity. African trypanosomes such as *T. congolense* are an important cause of disease in livestock. The extracellular nature of this pathogen likely explains the important role of humoral immunity in host protection. Experiments where SCID mice were reconstituted with bovine fetal liver/thymus/lymph nodes (SCID-bo) examined the role of CD40 in resistance

against *T. congolense* [129]. SCID-bo mice infected with *T. congolense* were treated with a suboptimal dose of the trypanocidal agent Berenil [129]. Co-administration of a stimulatory anti- CD40 mAb led to a modest increase in survival, increased in the population of CD5⁺ B cells and appeared to also increase parasite-specific antibody response [129].

Plasmodium

Malaria is a major health problem in developing tropical countries. Malaria in humans is caused by 4 species of Plasmodium (*Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium ovale*). Infection is transmitted by an anopheles mosquito that releases sporozoites into the bloodstream. This form of the parasite infects hepatocytes where intracellular replication of merozoites takes place. Merozoites are released into the bloodstream and infect red cells where they become trophozoites. Malaria is a common cause of fever and anemia in tropical countries. *P. falciparum* can cause severe malaria characterized by decreased mental status (cerebral malaria) and multi-organ failure.

P. berghei provides an animal model of severe malaria. Infection in susceptible strains of mice is characterized by breakdown of the blood-brain barrier, hemorrhages and sequestration of macrophages and platelets in cortical venules, neurological abnormalities and death [130]. TNF- α is a major mediator of severe malaria in mice [131]. Studies using CD40^{-/-} and CD154^{-/-} mice reported that the CD40 - CD154 pathway contributes to death in malaria. While parasitemia is unaffected in CD40^{-/-} and CD154^{-/-} infected with *P. berghei*, these animals exhibited decreased mortality [130]. Genetic disruption of the CD40 - CD154 pathway also protected mice against blood-brain barrier breakdown and against sequestration of macrophages in brain venules [130]. These beneficial effects are not due to diminished TNF- α levels. Although CD40^{-/-} mice exhibited diminished CD54 mRNA levels, this decrease is modest and it is uncertain if it can explain diminished macrophage sequestration [130]. Taken together, these findings indicate that the CD40 — CD154 pathway plays an essential role in the pathogenesis of severe malaria.

Susceptibility to severe *P. falciparum* malaria has been associated with polymorphisms of genes such as glucose 6 phosphate dehydrogenase, β -globin, Duffy and HLA-B53 [132]. An allele of CD154 which has a mutation in the promoter region has been linked to susceptibility to severe malaria [133]. Gambian males that are hemizygous for *CD154-726C* exhibit significant protection against cerebral malaria and severe malarial anemia [133]. These results suggest that future studies should further characterize the role of *CD154* polymorphisms in susceptibility to infections.

Helminths

Helminths are multicellular organisms that are the cause of some of the most prevalent infections in the world. They include trematodes (schistosomes), nematodes (roundworms) and cestodes (tape worms). Chronic infection caused by the trematode *Schistosoma mansoni* can cause intestinal and liver disease. Humans become infected by cercaria released from infected snails. The parasite migrates through veins and lymphatics as a schistosomula. The adult female worm reaches veins of the intestine where it releases eggs. These eggs can be excreted via the feces, or can remain within the host either in the intestine or in the liver. Eggs trigger formation of granulomas and are responsible for most disease manifestations.

Chronic disease cause by helminths such as schistosomes typically induces a Th2-type immune responses associated with the production of IL-4, IL-13, IL-5, IgE and eosinophila [134]. Antigens secreted by the eggs are responsible for inducing a Th2 response. This cytokine response is required for host survival but is also responsible for the induction of complications of the infection such as hepatic fibrosis [135,136].

Studies in a mouse model of schistosomiasis revealed that CD40 - CD154 interaction controls Th2 responses against a pathogen. CD154^{-/-} mice infected with *S. mansoni* exhibit a defect in production of Th2-associated cytokines [137]. This defect is of functional relevance because CD154^{-/-} mice have increased mortality as well as worsened histopathology in the lung after infection with *S. mansoni* [137]. Thus, the CD40 - CD154 pathway is required for the development of an appropriate immune response [137]. Studies with DC further support the relevance of CD40 in the immune response against *S. mansoni*. *In vivo* administration of bone marrow derived DC pulsed with schistosome egg antigen (SEA) results in induction of a Th2 response [138]. This response is blunted when mice receive DC that lack CD40 [138]. The likely explanation for the importance of this receptor for induction of an immune response against schistosome is that the CD40 - CD154 pathway is required for *in vivo* activation of dendritic cells. Dendritic cells incubated with SEA do not upregulate costimulatory ligands or MHC class II molecules [138]. However, animals infected with *S. mansoni* exhibit evidence of *in vivo* activation of DC, a process that requires CD154 [139].

Administration of radiation-attenuated *S. mansoni* larvae induces protective immunity associated with a Th1 type response [140]. The CD40 - CD154 pathway is also important for the generation of this immune response. In mice immunized with radiation-attenuated larvae, production of IL-12 p40 by DC, T cell priming and production of IFN- γ are dependent on the presence of CD154 [141]. Moreover, the CD40 - CD154 pathway is essential for the development of protective immunity since CD154^{-/-} mice vaccinated with radiation-attenuated larvae are not resistant to challenge infection [142]. Interestingly, the protective effect of CD154 was not mediated exclusively by the induction of a Th1 response since administration of IL-12 to vaccinated CD154^{-/-} mice restored Th1 response but failed to confer protection [142]. It is not clear whether the protective role of CD154 is also mediated by antibody production.

Increased intestinal muscle contractility leads to expulsion of nematodes [143]. This response is dependent on STAT6, IL-4 and IL-13 [144]. Moreover, T cell infiltration of intestinal muscle layers and well as expression of IL4 and IL-13 at this site has been reported [144,145]. Studies in mice infected with the nematode *Trichinella spiralis* revealed that production of IL-4, IL-13, IgG1, IgE, mucosal MCP-1 and goblet cell response are impaired in mice that lack CD154 [146]. As a result, there is diminished intestinal muscle contractility and impaired parasite expulsion [146]. Thus, the CD40 - CD154 pathway acts as an upstream regulator of the induction of Th2 response and expulsion of a nematode. CD40 has also been found to be important for the induction of a Th2 response against the cestode *Taenia crassiceps* [147].

Conclusions

Studies done with parasites have helped establish the central role of the CD40 — CD154 pathway in the regulation of the immune response against infections. These studies revealed that CD40 — CD154 signaling activates anti-microbial mechanisms that are dependent not only on type 1 cytokine response but also on IFN- γ -independent autophagy as well as on stimulation of protective type 2 cytokine response in the case of helminths. Work using models of parasitic infections has provided likely explanations for the susceptibility to opportunistic infections in patients with defective CD40 — CD154 signaling. Recent studies are beginning to uncover how pathogens can skew CD40 signaling to impair protective cell-mediated immunity and on the likely role of polymorphisms of CD154 in determining susceptibility to infection. Finally, models of parasitic infections have contributed to the identification of modulation of CD40 — CD154 signaling as an approach to enhance the effectiveness of vaccines and, perhaps as an avenue to develop new treatment modalities against various infections.

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References

1. Clark EA, Ledbetter J. Activation of human B cells mediated through two distinct cell surface differentiation antigens, Bp35 and Bp50. *Proc. Natl. Acad. Sci. USA* 1986;83:4494–4498. [PubMed: 3487090]
2. Alderson MR, Armitage RJ, Tough TW, Strockbine L, Fanslow WC, Spriggs MK. CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by the ligand for CD40. *J. Exp. Med* 1993;178:669–674. [PubMed: 7688031]
3. Hollenbaugh D, Mischel-Petty N, Edwards CP, et al. Expression of functional CD40 by vascular endothelial cells. *J. Exp. Med* 1995;182:33–40. [PubMed: 7540655]
4. Schriever F, Freedman AS, Freeman G, et al. Isolated human follicular dendritic cells display a unique antigenic phenotype. *J. Exp. Med* 1989;169:2043–2058. [PubMed: 2471772]
5. Armitage RJ, Fanslow WC, Strockbine L, et al. Molecular and biological characterization of a murine ligand for CD40. *Nature* 1992;357:80–82. [PubMed: 1374165]
6. Hollenbaugh D, Grosmaire LS, Kullas CD, et al. The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. *EMBO J* 1992;11:4313–4321. [PubMed: 1385114]
7. Caux C, Massacrier C, Banbervliet B, et al. Activation of human dendritic cells through CD40 cross-linking. *J. Exp. Med* 1994;180:1263–1271. [PubMed: 7523569]
8. Kiener PA, Moran-Davis P, Rankin BM, Wahl AF, Aruffo A, Hollenbaugh D. Stimulation of CD40 with purified soluble gp39 induces proinflammatory responses in human monocytes. *J. Immunol* 1995;155:4917–4925. [PubMed: 7594496]
9. Ridge JP, di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a CD4⁺ T-helper and a T-killer cell. *Nature* 1998;393:474–478. [PubMed: 9624003]
10. Bennett SRM, Carbone FR, Karamalis F, Flavell RA, Miller JFAP, Heath WR. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 1998;393:478–480. [PubMed: 9624004]
11. Schoenberger SP, Toes REM, van der Voort EIH, Offringa R, Melief CJM. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 1998;393:480–483. [PubMed: 9624005]
12. Durie FH, Foy TM, Masters SR, Laman JD, Noelle RJ. The role of CD40 in the regulation of humoral and cell-mediated immunity. *Immunol. Today* 1994;15:406–411. [PubMed: 7524518]
13. Aruffo A, Farrington M, Hollenbaugh D, Li X, Milatovich A, Nonoyama S. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell* 1993;72:291–300. [PubMed: 7678782]
14. Levy J, Espanol-Boren T, Thomas C, et al. Clinical spectrum of X-linked hyper-IgM syndrome. *J. Pediatr* 1997;131:47–54. [PubMed: 9255191]
15. Winkelstein JA, Marino MC, Ochs HD, et al. The X-linked Hyper-IgM syndrome. Clinical and immunologic features of 79 patients. *Medicine* 2003;82:373–384. [PubMed: 14663287]
16. Leiva LE, Junprasert J, Hollenbaugh D, Sorensen RU. Central nervous system toxoplasmosis with an increased proportion of circulating $\gamma\delta$ T cells in a patient with hyper IgM syndrome. *J. Clin. Immunol* 1998;18:283–290. [PubMed: 9710745]
17. Subauste CS, Wessendarp M, Sorensen RU, Leiva L. CD40 - CD40 ligand interaction is central to cell-mediated immunity against *Toxoplasma gondii*: Patients with hyper IgM syndrome have a defective type-I immune response which can be restored by soluble CD40L trimer. *J. Immunol* 1999;162:6690–6700. [PubMed: 10352287]
18. Suzuki Y, Orellana MA, Schreiber RD, Remington JS. Interferon- γ : the major mediator of resistance against *Toxoplasma gondii*. *Science* 1988;240:516–518. [PubMed: 3128869]

19. Gazzinelli RT, Hieny S, Wynn TA, Wolf S, Sher A. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon γ by an intracellular parasite and induces resistance in T-cell-deficient hosts. *Proc. Natl. Acad. Sci. USA* 1993;90:6115–6119. [PubMed: 8100999]
20. Navia BA, Petito CK, Gold JW, Cho ES, Jordan BD, Price RW. Cerebral toxoplasmosis complicating the acquired immune deficiency syndrome: clinical and neuropathological findings in 27 patients. *Ann. Neurol* 1986;19:224–238. [PubMed: 3963767]
21. Israelski DM, Remington JS. Toxoplasmosis in the non-AIDS immunocompromised host. *Curr. Clin. Top. Infect. Dis* 1993;13:322–356. [PubMed: 8397917]
22. Tsuge I, Matsuoka H, Nakagawa A, et al. Necrotizing toxoplasmic encephalitis in a child with the X-linked hyper-IgM syndrome. *Eur. J. Pediatr* 1998;157:735–737. [PubMed: 9776532]
23. Yong PF, Post FA, Gilmour KC, et al. Cerebral toxoplasmosis in a middle-aged man as first presentation of primary immunodeficiency due to a hypomorphic mutation in the CD40 ligand gene. *J. Clin. Pathol* 2008;61:1220–1222. [PubMed: 18955577]
24. Seguin R, Kasper LH. Sensitized lymphocytes and CD40 ligation augment interleukin-12 production by human dendritic cells in response to *Toxoplasma gondii*. *J. Infect. Dis* 1999;179:467–474. [PubMed: 9878033]
25. Subauste CS, de Waal Malefyt R, Fuh F. Role of CD80 (B7.1) and CD86 (B7.2) in the immune response to an intracellular pathogen. *J. Immunol* 1998;160:1831–1840. [PubMed: 9469444]
26. Subauste CS, Wessendarp M. Human dendritic cells discriminate between viable and killed *Toxoplasma gondii* tachyzoites: Dendritic cell activation after infection with viable parasites results in CD28 and CD40 Ligand signaling that controls IL-12-dependent and -independent T cell production of IFN- γ . *J. Immunol* 2000;165:1498–1505. [PubMed: 10903756]
27. Wei S, Marches F, Borvak J, et al. *Toxoplasma gondii*-infected human myeloid dendritic cells induce T-lymphocyte dysfunction and contact-dependent apoptosis. *Infect. Immun* 2002;70:1750–1760. [PubMed: 11895936]
28. Semnani RT, Sabzevari H, Iyer R, Nutman TB. Filarial antigens impair the function of human dendritic cells during differentiation. *Infect. Immun* 2001;69:5813–5822. [PubMed: 11500459]
29. Schulz O, Edwards AD, Schito M, et al. CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells in vivo requires a microbial priming signal. *Immunity* 2000;13:453–462. [PubMed: 11070164]
30. Subauste CS. CD154 and type-1 cytokine response: From Hyper IgM syndrome to Human Immunodeficiency Virus infection. *J. Infect. Dis* 2002;185:S83–S89. [PubMed: 11865444]
31. Jankovic D, Kulberg MC, Hieny S, Caspar P, Collazo CM, Sher A. In the absence of IL-12, CD4⁺ T cell response to intracellular pathogens fails to default to a Th2 pattern and are host protective in an IL-10^{-/-} setting. *Immunity* 2002;16:429–439. [PubMed: 11911827]
32. Villegas EN, Wille U, Craig L, et al. Blockade of costimulation prevents infection-induced immunopathology in interleukin-10-deficient mice. *Infect. Immun* 2000;68:2837–2844. [PubMed: 10768980]
33. Reichmann G, Walker W, Villegas EN, et al. The CD40/CD40 ligand interaction is required for resistance to toxoplasmic encephalitis. *Infect. Immun* 2000;68:1312–1318. [PubMed: 10678943]
34. e Sousa, C Reis; Hieny, S.; Scharton-Kersten, T., et al. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J. Exp. Med* 1997;186:1819–1829. [PubMed: 9382881]
35. Yarovinsky F, Zhang D, Andersen JF, et al. TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* 2005;308:1626–1629. [PubMed: 15860593]
36. Aliberti J, e Sousa C Reis, Schito M, et al. CCR5 provides a signal for microbial induced production of IL-12 by CD8 α^+ dendritic cells. *Nature Immunology* 2000;1:83–87. [PubMed: 10881180]
37. Aliberti J, Valenzuela JG, Carruthers VB, et al. Molecular mimicry of a CCR5 binding-domain in the microbial activation of dendritic cells. *Nat. Immunol* 2003;4:485–490. [PubMed: 12665855]
38. Roach JC, Glusman G, Rowen L, et al. The evolution of vertebrate Toll-like receptors. *Proc. Natl. Acad. Sci. USA* 2005;102:9577–9582. [PubMed: 15976025]
39. Zhang D, Zhang G, Hayden MS, et al. Atoll-like receptor that prevents infection by uropathogenic bacteria. *Science* 2004;303:1522–1526. [PubMed: 15001781]

40. Subauste CS, Wessendarp M, Smulian AG, Frame PT. Role of CD40 ligand signaling in defective type-1 cytokine response in HIV infection. *J. Infect. Dis* 2001;183:1722–1731. [PubMed: 11372024]
41. Subauste CS, Wessendarp M, Portillo J-AC, et al. Pathogen-specific induction of CD154 is impaired in CD4⁺ T cells from HIV-infected individuals. *J. Infect. Dis* 2004;189:61–70. [PubMed: 14702154]
42. Subauste CS, Subauste A, Wessendarp M. Role of CD40-dependent down-regulation of CD154 in impaired induction of CD154 in CD4⁺ T cells from HIV-1-infected patients. *J. Immunol* 2007;178:1645–1653. [PubMed: 17237414]
43. Zhang R, Fichtenbaum CJ, Hildeman DA, Lifson JD, Chougnet C. CD40 ligand dysregulation in HIV: HIV glycoprotein 120 inhibits signaling cascades upstream of CD40 ligand transcription. *J. Immunol* 2004;172:2678–2686. [PubMed: 14764743]
44. Ludwig B, Henn V, Schroeder JM, Graf D, Kroczeck RA. Induction, regulation, and function of soluble TRAP (CD40 ligand) during interaction of primary CD4⁺ CD45RA⁺ T cells with dendritic cells. *Eur. J. Immunol* 1996;26:3137–3143. [PubMed: 8977315]
45. van Kooten C, Gaillard C, Galizzi JP, et al. B cells regulate expression of CD40 ligand on activated T cells by lowering the mRNA level and through the release of soluble CD40. *Eur. J. Immunol* 1994;24:787–792. [PubMed: 7512028]
46. Yellin MJ, Sippel K, Inghirami G, et al. CD40 molecules induce down-modulation and endocytosis of T cell surface T cell-B cell activating molecule/CD40-L. Potential role in regulating helper effector function. *J. Immunol* 1994;152:598–608. [PubMed: 7506727]
47. Mathur RK, Awashti A, Wadhwa P, Ramanamurthy B, Saha B. Reciprocal CD40 signals through p38MAPK and ERK-1/2 induce counteracting immune responses. *Nat. Med* 2004;10:540–544. [PubMed: 15107845]
48. Andrade RM, Portillo J-AC, Wessendarp M, Subauste CS. CD40 signaling in macrophages induces anti-microbial activity against an intracellular pathogen independently of IFN- γ and reactive nitrogen intermediates. *Infect. Immun* 2005;73:3115–3123. [PubMed: 15845519]
49. Andrade RM, Wessendarp M, Gubbels MJ, Striepen B, Subauste CS. CD40 induces macrophage anti-*Toxoplasma gondii* activity by triggering autophagy-dependent fusion of pathogen-containing vacuoles and lysosomes. *J. Clin. Invest* 2006;116:2366–2377. [PubMed: 16955139]
50. Andrade RM, Wessendarp M, Portillo J-AC, et al. TRAF6 signaling downstream of CD40 primes macrophages to acquire anti-microbial activity in response to TNF- α . *J. Immunol* 2005;175:6014–6021. [PubMed: 16237096]
51. Andrade RM, Wessendarp M, Subauste CS. CD154 activates macrophage anti-microbial activity in the absence of IFN- γ through a TNF- α -dependent mechanism. *J. Immunol* 2003;171:6750–6756. [PubMed: 14662879]
52. Subauste CS, Wessendarp M. CD40 restrains the in vivo growth of *Toxoplasma gondii* independently of gamma interferon. *Infect. Immun* 2006;74:1573–1579. [PubMed: 16495528]
53. Lingelbach K, Joiner KA. The parasitophorous vacuole membrane surrounding Plasmodium and Toxoplasma: an unusual compartment in infected cells. *J. Cell Sci* 1998;111:1467–1475. [PubMed: 9580555]
54. Joiner KA, Fuhrman SA, Mietinnen H, Kasper LH, Mellman I. *Toxoplasma gondii*: fusion competence of parasitophorous vacuoles in Fc receptor transfected fibroblasts. *Science* 1990;249:641–646. [PubMed: 2200126]
55. Mordue DG, Sibley LD. Intracellular fate of vacuoles containing *Toxoplasma gondii* is determined at the time of formation and depends on the mechanisms of entry. *J. Immunol* 1997;159:4452–4459. [PubMed: 9379044]
56. Subauste CS, Andrade RM, Wessendarp M. CD40-TRAF6 and autophagy-dependent anti-microbial activity in macrophages. *Autophagy* 2007;3:245–248. [PubMed: 17224624]
57. Mizushima N, Ohsumi Y, Yoshimori T. Autophagosome formation in mammalian cells. *Cell Struct. Funct* 2002;27:421–429. [PubMed: 12576635]
58. Yoshimori T. Autophagy: a regulated bulk degradation process inside cells. *Biochem. Biophys. Res. Comm* 2004;313:453–458. [PubMed: 14684184]
59. Dunn WAJ. Autophagy and related mechanisms of lysosome-mediated protein degradation. *Trends Cell Biol* 1994;110:1923–1933.

60. Lum JJ, DeBerardinis RJ, Thompson CB. Autophagy in metazoans: cell survival in the land of plenty. *Nat. Rev. Mol. Cell Biol* 2005;6:439–448. [PubMed: 15928708]
61. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MC, Deretic V. Autophagy is defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* 2004;119:753–766. [PubMed: 15607973]
62. Nakagawa I, Amano A, Mizushima N, et al. Autophagy defends cells against invading Group A *Streptococcus*. *Science* 2004;306:1037–1040. [PubMed: 15528445]
63. Rich KA, Burkett C, Webster P. Cytoplasmic bacteria can be targets for autophagy. *Cell. Microbiol* 2003;5:455–468. [PubMed: 12814436]
64. Birmingham CL, Smith AC, Bakowski MA, Yoshimori T, Brumell JH. Autophagy controls *Salmonella* infection in response to damage to the *Salmonella*-containing vacuole. *J. Biol. Chem* 2006;281:11374–11383. [PubMed: 16495224]
65. Mukundan L, Bishop GA, Head KZ, Zhang L, Wahl L, Suttles J. TNF receptor-associated factor 6 is an essential mediator of CD40-activated proinflammatory pathways in monocytes and macrophages. *J. Immunol* 2005;174:1081–1090. [PubMed: 15634933]
66. Yap GS, Scharton-Kersten T, Charest H, Sher A. Decreased resistance of TNF receptor p55- and p75-deficient mice to chronic toxoplasmosis despite normal activation of inducible nitric oxide synthase in vivo. *J. Immunol* 1998;160:1340–1345. [PubMed: 9570552]
67. Subauste CS. Autophagy and immunity against *Toxoplasma gondii*. *Curr. Topics Microbiol. Immunol.* 2009In press
68. Gavriulescu LC, Butcher BA, del Rio L, Taylor GA, Denkers EY. STAT1 is essential for antimicrobial effector function but dispensable for gamma interferon production during *Toxoplasma gondii* infection. *Infect. Immun* 2004;72:1257–1264. [PubMed: 14977926]
69. Lieberman LA, Banica M, Reiner SL, Hunter CA. STAT1 plays a critical role in the regulation of antimicrobial effector mechanisms, but not in the development of Th1-type responses during toxoplasmosis. *J. Immunol* 2004;172:457–463. [PubMed: 14688355]
70. Collazo CM, Yap G, Hieny S, et al. The function of gamma interferon-inducible GTP-binding protein IGTP in host resistance to *Toxoplasma gondii* is Stat1 dependent and requires expression in both hematopoietic and nonhematopoietic cellular compartments. *Infect. Immun* 2002;70:6933–6939. [PubMed: 12438372]
71. Collazo CM, Yap GS, Sempowski GD, et al. Inactivation of LRG-47 and IRG-47 reveals a family of interferon γ -inducible genes with essential, pathogen-specific roles in resistance to infection. *J. Exp. Med* 2001;194:181–187. [PubMed: 11457893]
72. Martens S, Parvanova I, Zerrahn J, et al. Disruption of *Toxoplasma gondii* parasitophorous vacuoles by the mouse p47-resistance GTPases. *PLoS Pathogens* 2005;1:187–201.
73. Hayashi S, Chan C-C, Gazzinelli RT, Pham NTH, Cheung MK, Roberge FG. Protective role of nitric oxide in ocular toxoplasmosis. *Br. J. Ophthalmol* 1996;80:644–648. [PubMed: 8795379]
74. Hayashi S, Chan CC, Gazzinelli R, Roberge FG. Contribution of nitric oxide to the host parasite equilibrium in toxoplasmosis. *J. Immunol* 1996;156:1476–1481. [PubMed: 8568250]
75. Scharton-Kersten T, Yap G, Magram J, Sher A. Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii*. *J. Exp. Med* 1997;185:1261–1273. [PubMed: 9104813]
76. Janssen R, van Wengen A, Verhard E, et al. Divergent role for TNF- α in IFN- γ -induced killing of *Toxoplasma gondii* and *Salmonella typhimurium* contributes to selective susceptibility of patients with partial IFN- γ receptor 1 deficiency. *J. Immunol* 2002;169:3900–3907. [PubMed: 12244188]
77. Bekpen C, Hunn JP, Rohde C, et al. The interferon-inducible p47 (IRG) GTPases in vertebrates: loss of the cell autonomous resistance mechanism in the human lineage. *Genome Biol* 2005;6:R92. [PubMed: 16277747]
78. Kroncke KD, Fehsel K, Kolb-Bachofen V. Inducible nitric oxide synthase in human disease. *Clin. Exp. Immunol* 1998;113:147–156. [PubMed: 9717962]
79. Nonoyama S, Penix LA, Edwards CP, et al. Diminished expression of CD40 ligand by activated neonatal T cells. *J. Clin. Invest* 1995;95:66–75. [PubMed: 7814647]

80. Durandy A, de Saint Basile G, Lisowska-Groszpiere B, et al. Undetectable CD40 ligand expression on T cells and low B cell responses to CD40 binding agonists in human newborns. *J. Immunol* 1995;154:1560–1568. [PubMed: 7530739]
81. Julien P, Cron RQ, Dabbagh K, et al. Decreased CD154 expression by neonatal CD4⁺ T cells is due to limitations in both proximal and distal events of T cell activation. *Int. Immunol* 2003;15:1461–1472. [PubMed: 14645155]
82. Han P, McDonald T, Hodge G. Potential immaturity of the T-cell and antigen-presenting cell interaction in cord blood with particular emphasis on the CD40-CD40 ligand costimulatory pathway. *Immunology* 2004;113:26–34. [PubMed: 15312133]
83. Kaur K, Chowdhury S, Greenspan NS, Schreiber JR. Decreased expression of tumor necrosis factor family receptors involved in humoral immune responses in preterm neonates. *Blood* 2007;110:2948–2954. [PubMed: 17634409]
84. Gazzinelli RT, Wysocka M, Hieny S, et al. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on CD4⁺ T cells and accompanied by overproduction of IL-12, IFN- γ , and TNF- α . *J. Immunol* 1996;157:798–805. [PubMed: 8752931]
85. Liesenfeld O, Kosek J, Remington JS, Suzuki Y. Association of CD4⁺ T cell-dependent, interferon- γ -mediated necrosis of the small intestine with genetic susceptibility of mice to peroral infection with *Toxoplasma gondii*. *J. Exp. Med* 1996;184:597–607. [PubMed: 8760813]
86. Li W, Buzoni-Gatel D, Debbabi H, et al. CD40/CD154 ligation is required for the development of acute ileitis following oral infection with an intracellular pathogen in mice. *Gastroenterology* 2002;122:762–773. [PubMed: 11875009]
87. McDonald V, Bancroft GJ. Mechanisms of innate and acquired resistance to *Cryptosporidium parvum* infection in SCID mice. *Parasite Immunol* 1994;16:315–320. [PubMed: 7970868]
88. Aguirre SA, Mason PH, Perryman LE. Susceptibility of major histocompatibility complex (MHC) class I-and MHC class II-deficient mice to *Cryptosporidium parvum* infection. *Infect. Immun* 1994;62:697–699. [PubMed: 7905464]
89. Chiu JH, Hu CP, Lui WY, Lo SC, Chang CM. Requirements for CD4⁺ cells and gamma interferon in resolution of established *Cryptosporidium parvum* infection in mice. *Infect. Immun* 1990;61:3928–3932.
90. Urban JF, Fayer R, Chen S, Gause W, Gately MK, Finkelman FD. IL-12 protects immunocompetent and immunodeficient neonatal mice against infection with *Cryptosporidium parvum*. *J. Immunol* 1996;156:263–268. [PubMed: 8598471]
91. Hayward AR, Levy J, Facchetti F, et al. Cholangiopathy and tumors of the pancreas, liver, and biliary tree in boys with X-linked immunodeficiency with hyper-IgM. *J. Immunol* 1997;158:977–983. [PubMed: 8993019]
92. Cosyns M, Tsirkin S, Jones M, Flavell R, Kikutani H, Hayward AR. Requirement for CD40-CD40 ligand interaction for elimination of *Cryptosporidium parvum* from mice. *Infect. Immun* 1998;66:603–607. [PubMed: 9453615]
93. Hayward AR, Cosyns M, Jones M, Ponnuraj EM. Marrow-derived CD40 positive cells are required for mice to clear a *Cryptosporidium parvum* infection. *Infect. Immun* 2001;69:1630–1634. [PubMed: 11179337]
94. Reiner SL, Locksley RM. The regulation of immunity to *Leishmania major*. *Annu. Rev. Immunol* 1995;13:151–177. [PubMed: 7612219]
95. Campbell KA, Ovendale PJ, Kennedy MK, Fanslow WC, Reed SG, Maliszewski CR. CD40 ligand is required for protective cell-mediated immunity to *Leishmania major*. *Immunity* 1996;4:283–289. [PubMed: 8624818]
96. Soong L, Xu JC, Grewal IS, et al. Disruption of CD40-CD40 ligand interactions results in an enhanced susceptibility to *Leishmania amazoniensis* infection. *Immunity* 1996;4:263–273. [PubMed: 8624816]
97. Kamanaka M, Yu P, Yasui T, et al. Protective role of CD40 in *Leishmania major* infection at two distinct phases of cell-mediated immunity. *Immunity* 1996;4:275–281. [PubMed: 8624817]

98. Speirs KM, Caamano J, Goldschmidt MH, Hunter CA, P. S. NF- κ B2 is required for optimal CD40-induced IL-12 production but dispensable for Th1 cell differentiation. *J. Immunol* 2002;168:4406–4413. [PubMed: 11970983]
99. Murray BW, Lu CM, Brooks EB, Ficht RE, DeVecchio JL, Heinzel FP. Modulation of T-cell costimulation as immunotherapy or immunochemotherapy in experimental visceral leishmaniasis. *Infect. Immun* 2003;71:6453–6462. [PubMed: 14573667]
100. Murray BW. Prevention of relapse after chemotherapy in a chronic intracellular infection: mechanisms in experimental visceral leishmaniasis. *J. Immunol* 2005;174:4916–4923. [PubMed: 15814719]
101. Heinzel FP, Rerko RM, Hujer AM. Underproduction of interleukin-12 in susceptible mice during progressive leishmaniasis is due to decreased CD40 activity. *Cell. Immunol* 1998;184:129–142. [PubMed: 9630839]
102. Padigel UM, Perrin PJ, Farrell JP. The development of a Th1-type response and resistance to *Leishmania major* infection in the absence of CD40-CD40L costimulation. *J. Immunol* 2001;167:5874–5879. [PubMed: 11698463]
103. Brodskyn CI, DeKrey GK, Titus RG. Influence of costimulatory molecules on immune response to *Leishmania major* by human cells in vitro. *Infect. Immun* 2001;69:665–672. [PubMed: 11159953]
104. Carrera L, Gazzinelli RT, Badolato R, et al. *Leishmania* promastigotes selectively inhibit interleukin 12 in bone marrow-derived macrophages from susceptible and resistant mice. *J. Exp. Med* 1996;183:515–526. [PubMed: 8627163]
105. Gorak PM, Engwerda CR, Kaye PM. Dendritic cells, but not macrophages, produce IL-12 immediately following *Leishmania donovani* infection. *Eur. J. Immunol* 1998;28:687–695. [PubMed: 9521079]
106. Konecny P, Stagg AJ, Jenbbari H, English N, Davidson RN, Knight SC. Murine dendritic cells internalize *Leishmania major* promastigotes, produce IL-12 p40 and stimulate primary T cell proliferation *in vitro*. *Eur. J. Immunol* 1999;29:1803–1811. [PubMed: 10382742]
107. Marovich MA, McDowell MA, Thomas EK, Nutman TB. IL-12p70 production by *Leishmania major*-harboring human dendritic cells is a CD40/CD40 ligand-dependent process. *J. Immunol* 2000;164:5858–5865. [PubMed: 10820265]
108. Qi H, Popov V, Soong L. *Leishmania amazoniensis*-dendritic cell interactions in vitro and the priming of parasite-specific CD4⁺ T cells in vivo. *J. Immunol* 2001;167:4534–4542. [PubMed: 11591781]
109. McDowell MA, Marovich M, Lira R, Braun M, Sacks D. *Leishmania* priming of human dendritic cells for CD40 ligand-induced interleukin-12p70 secretion is strain and species dependent. *Infect. Immun* 2002;70:3994–4001. [PubMed: 12117904]
110. Ghosh M, Mandal L, Maitra S, et al. *Leishmania donovani* infection of human myeloid dendritic cells leads to a Th1 response in CD4⁺ T cells from healthy donors and patients with Kala-Azar. *J. Infect. Dis* 2006;184:294–301. [PubMed: 16826476]
111. Padigel UM, Farrell JP. CD40-CD40 ligand costimulation is not required for initiation and maintenance of a Th1-type response to *Leishmania major* infection. *Infect. Immun* 2003;71:1389–1395. [PubMed: 12595456]
112. Padigel UM, Kim N, Choi Y, Farrell JP. TRANCE-RANK costimulation is required for IL-12 production and the initiation of a Th1-type response to *Leishmania major* infection in CD40L-deficient mice. *J. Immunol* 2003;171:5437–5441. [PubMed: 14607948]
113. Nashleanas M, Scott P. Activated T cells induce macrophages to produce NO and control *Leishmania major* in the absence of tumor necrosis factor receptor p55. *Infect. Immun* 2000;68:1428–1434. [PubMed: 10678956]
114. Awashti A, Mathur R, Khan A, et al. CD40 signaling is impaired in *L. major*-infected macrophages and is rescued by a p38MAPK activator establishing a host-protective memory T cell response. *J. Exp. Med* 2003;197:1037–1043. [PubMed: 12695487]
115. Nunes MP, Cysne-Finkelstein L, Monteiro BC, de Souza DM, Gomes NA, DosReis GA. CD40 signaling induces reciprocal outcomes in *Leishmania*-infected macrophages; roles of host genotype and cytokine milieu. *Microbes and Infect* 2005;7:78–85. [PubMed: 15716074]

116. Rub A, Dey R, Jadhav M, et al. Cholesterol depletion associated *Leishmania major* infection alters macrophage CD40 signalosome composition and effector function. *Nat. Immunol* 2009;10:273–280. [PubMed: 19198591]
117. Mackey MF, Wang Z, Eichelberg K, Germain RN. Distinct contributions of different CD40 TRAF binding sites to CD154-induced dendritic cell maturation and IL-12 secretion. *Eur. J. Immunol* 2003;33:779–789. [PubMed: 12616498]
118. Ferlin WG, von der Weid T, Cottrez F, Ferrick DA, Coffman RL, Howard MC. The induction of a protective response in *Leishmania major*-infected BALB/c mice with anti-CD40 mAb. *Eur. J. Immunol* 1998;28:525–531. [PubMed: 9521062]
119. Gurunathan S, Irvine KR, Wu CY, et al. CD40 ligand/trimer DNA enhances both humoral and cellular immune responses and induces protective immunity to infectious and tumor challenge. *J. Immunol* 1998;161:4563–4571. [PubMed: 9794383]
120. Chen G, Darrah PA, Mosser DM. Vaccination against the intracellular pathogens *Leishmania major* and *L. amazoniensis* by directing CD40 ligand to macrophages. *Infect. Immun* 2001;69:3255–3263. [PubMed: 11292748]
121. Field AE, Wagage S, Conrad SM, Mosser DM. Reduced pathology following infection with transgenic *Leishmania major* expressing murine CD40 ligand. *Infect. Immun* 2007;75:3140–3149. [PubMed: 17403867]
122. Aliberti JCS, Cardoso MAG, Martins GA, Gazzinelli RT, Vieira LQ, Silva JS. Interleukin-12 mediates resistance to *Trypanosoma cruzi* in mice and is produced by murine macrophages in response to live trypomastigote. *Infect. Immun* 1996;64:1961–1967. [PubMed: 8675294]
123. Cardillo F, Voltarelli JC, Reed SG, Silva JS. Regulation of *Trypanosoma cruzi* infection in mice by gamma interferon and interleukin 10. *Infect. Immun* 1996;64:128–134. [PubMed: 8557330]
124. Abrahamson IA, Coffman RL. *Trypanosoma cruzi*: IL-10, TNF, IFN- γ , and IL-12 regulate innate and acquired immunity to infection. *Exp. Parasitol* 1996;84:231–244. [PubMed: 8932773]
125. Gazzinelli RT, Oswald IP, Hieny S, James SL, Sher A. The microbicidal activity of interferon- γ -treated macrophages against *Trypanosoma cruzi* involves an L-arginine-dependent, nitrogen oxide-mediated mechanism inhibitable by interleukin-10 and transforming growth factor- β . *Eur. J. Immunol* 1992;22:2501–2506. [PubMed: 1396957]
126. Chaussabel D, Jacobs F, de Jonge J, et al. CD40 ligation prevents *Trypanosoma cruzi* infection through interleukin-12 upregulation. *Infect. Immun* 1999;67:1929–1934. [PubMed: 10085038]
127. Chamekh M, Vercruysse V, Habib M, et al. Transfection of *Trypanosoma cruzi* with host CD40 ligand results in improved control of parasite infection. *Infect. Immun* 2005;73:6552–6561. [PubMed: 16177330]
128. Habib M, Rivas M Noval, Chamekh M, et al. Small molecule CD40 ligand mimetics promote control of parasitemia and enhance T cells producing IFN- γ during experimental *Trypanosoma cruzi* infection. *J. Immunol* 2007;178:6700–6704. [PubMed: 17513713]
129. Haas KM, Taylor KA, MacHugh ND, Kreeger JM, Estes DM. Enhancing effects of anti-CD40 treatment of the immune response of SCID-bovine mice to *Trypanosoma congolense* infection. *J. Leuk. Biol* 2001;70:931–940.
130. Piguet PF, Kan CD, Vesin C, Rochat A, Donati Y, Barazzzone C. Role of CD40-CD40L in mouse severe malaria. *Am. J. Pathol* 2001;159:733–742. [PubMed: 11485931]
131. Grau GE, Fajardo LF, Piguet PF, Allet B, Lambert PH, Vasalli P. Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. *Science* 1987;237:1210–1212. [PubMed: 3306918]
132. Hill AV. The immunogenetics of resistance to malaria. *Proc. Assoc. Am. Physicians* 1999;111:373–382. [PubMed: 10519156]
133. Sabeti P, Usen S, Farhadian S, et al. CD40L association with protection from severe malaria. *Genes Immun* 2002;3:286–291. [PubMed: 12140747]
134. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. *Nat. Rev. Immunol* 2002;2:499–511. [PubMed: 12094224]
135. Brunet LR, Finkelman FD, Cheever AW, Kopf MA, Pearce EJ. IL-4 protects against TNF- α -mediated cachexia and death during acute schistosomiasis. *J. Immunol* 1997;159:777. [PubMed: 9218595]

136. Hoffmann KF, Cheever AW, Wynn TA. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J. Immunol* 2000;164:6404.
137. MacDonald AS, Patton EA, La Flamme AC, et al. Impaired Th2 development and increased mortality during *Schistosoma mansoni* infection in the absence of CD40-CD154 interaction. *J. Immunol* 2002;168:4643–4649. [PubMed: 11971013]
138. MacDonald AS, Straw AD, Dalton NM, Pearce EJ. Th2 response induction by dendritic cells: a role for CD40. *J. Immunol* 2002;168:537–540. [PubMed: 11777943]
139. Straw AD, MacDonald AS, Denkers EY, Pearce EJ. CD154 plays a central role in regulating dendritic cell activation during infections that induce Th1 or Th2 responses. *J. Immunol* 2003;170:727–734. [PubMed: 12517934]
140. Vignali DA, Crocker P, Bickle QD, Cobbold S, Waldmann H, Taylor MG. A role for CD4⁺ but not CD8⁺ T cells in immunity to *Schistosoma mansoni* induced by 20 krad-irradiated and Ro 11-3128-terminated infections. *Immunology* 1989;67:466–472. [PubMed: 2570035]
141. Hewitson JP, Jenkins GR, Hamblin PA, Mountford AP. CD40-CD154 interactions are required for the optima; maturation of skin-derived APCs and the induction of helminth-specific IFN- γ but not IL-4. *J. Immunol* 2006;177:3209–3217. [PubMed: 16920960]
142. Hewitson JP, Hamblin PA, Mountford AP. In the absence of CD154, administration of interleukin-12 restores Th1 responses but not protective immunity to *Schistosoma mansoni*. *Infect. Immun* 2007;75:3539–3547. [PubMed: 17485453]
143. Farmer SG. Propulsive activity of the rat small intestine during infection with the nematode *Nippostrongylus brasiliensis*. *Parasite Immunol* 1981;3:227–234. [PubMed: 7301408]
144. Khan WI, Vallance BA, Blennerhasset PA, et al. Critical role for signal transducer and activator of transcription factor 6 in mediating intestinal muscle hypercontractility and worm expulsion in *Trichinella spiralis*-infected mice. *Infect. Immun* 2001;69:838–844. [PubMed: 11159976]
145. Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility disorders. *Gastroenterology* 1996;111:1683–1689. [PubMed: 8942751]
146. Khan WI, Motomura Y, Blennerhasset PA, et al. Disruption of CD40-CD40 ligand pathway inhibits the development of intestinal muscle hypercontractility and protective immunity in nematode infection. *Am. J. Physiol. Gastrointest. Liver Physiol* 2005;288:G15–G22. [PubMed: 15308470]
147. Rodriguez-Sosa M, Satoskar AR, David JR, Terrazas LI. Altered T helper responses in CD40 and interleukin-12 deficient mice reveal a critical role for Th1 responses in eliminating the helminth parasite *Taenia cressiceps*. *Int. J. Parasitol* 2003;33:703–711. [PubMed: 12814650]

Figure 1.

Role of CD40 — CD154 interaction in the induction of a type 1 cytokine response against *T. gondii*. Infection of either human immature dendritic cells (iDC) or monocytes (Mo) with viable tachyzoites of *T. gondii* induces up-regulation of CD40, CD80, CD86, and major histocompatibility complex (MHC) class II molecules. T cells activated through their T cell receptor acquire expression of CD154. Infected antigen presenting cells produce bioactive IL-12 after CD40-CD154 interaction. In turn, IL-12 induces T cells to secrete IFN- γ . T cells can also produce IFN- γ in response to *T. gondii* in the absence of IL-12. This IL-12— independent secretion of IFN- γ requires CD80/CD86-CD28 and, to a lesser extent, CD40-CD154 interaction (presumably resulting in direct T cell stimulation). TCR, T cell receptor. Reprinted with permission (reference 30).

Figure 2.

CD40-independent production of IL-12 in response to *T. gondii*. Profilin and cyclophilin-18 (C-18) secreted by *T. gondii* or present in parasite lysates bind to TLR11 and CCR5 respectively expressed on mouse CD8 α ⁺ dendritic cells (DC). These two pathways cooperate to induce IL-12 production. TLR11 plays a dominant role in stimulation of IL-12 production. This process occurs independently of CD40 signaling.

Figure 3.

Autophagy—dependent killing of *T. gondii* induced by CD40. *A*, *T. gondii*—specific CD4⁺ T cell acquires expression of CD154 after interaction with infected macrophages. CD40—CD154 binding results in recruitment of TRAF6 to the intracytoplasmic tail of CD40 that in turn enhances TNF- α production. TRAF6 signals downstream of CD40 and TNF- α signaling synergize to cause recruitment of autophagosomes to the parasitophorous vacuole that contains the pathogen (*Tg*). This process is dependent on Beclin 1 and Class III PI3K. Subsequent recruitment of Rab7 controls fusion with the lysosomes and killing of *T. gondii*. *B*, Both IFN- γ /TNF- α — induced NOS2 and CD40/TNF- α —induced autophagy appear to be required for control of cerebral toxoplasmosis. Reprinted with permission (reference 67).

Figure 4.

L. major skews CD40 signaling. In response to CD40 stimulation of macrophages from BALB/c mice, *L. major* amastigotes promote ERK1/2 and IL-10 production. This pathway inhibits p38 MAPK activation, the expression of NOS2 and the production of IL-12.