Interaction between T cells and antigen-presenting cells (APCs) results in the engagement of surface molecules that regulate the immune response. CD40 and its counter-receptor CD154 (CD40 ligand) are surface molecules pivotal to this cell-to-cell communication. CD40 is a surface glycoprotein expressed on professional APCs and on non-hematopoietic cells [1, 2]. In contrast, CD154 is expressed primarily on activated CD4+ T cells [1, 2].

Early studies demonstrated the importance of CD40-CD154 interaction for the regulation of thymus-dependent humoral immune responses [1, 2]. More recent data indicated that CD40-CD154 signaling is also central to T cell–mediated activation of monocyte/macrophages and dendritic cells (DCs). Ligation of CD40 on these APCs stimulates cytokine secretion, induces/up-regulates expression of costimulatory molecules, activates DC to cross-prime CD8+ T cells, enhances production of nitric oxide (NO) by murine macrophages, induces monocyte tumorici-dal activity, and protects APCs from apoptosis [1, 2]. Of potential relevance to the initiation and maintenance of Th1 type responses was the demonstration that T cells regulate interleukin (IL)-12 production in a CD154-dependent manner. Incubation of APCs with either anti-CD3–activated T cells, allogeneic T cells, or T cell clones plus appropriate soluble antigen resulted in CD40/CD154-dependent IL-12 production [3–5].

The studies described above suggested that CD40-CD154 interaction might be important for in vivo control of infections with intracellular pathogens. Indeed, treatment with anti-CD154 monoclonal antibody (MAb) decreased the ability of mice to clear Pneumocystis carinii [6], and CD154-deficient (CD154−/−) mice had increased susceptibility to Leishmania infection [7, 8]. Reduced resistance to Leishmania was attributed to defective production of IL-12, interferon (IFN)-γ, lympho-toxin–tumor necrosis factor, and NO [7, 8]. However, subsequent studies cast doubt on the relevance of CD154 signaling for control of infections with intracellular pathogens since CD154−/− mice infected with Mycobacterium tuberculosis and Histoplasma capsulatum generated a protective IL-12/IFN-γ (type-1) immune response [9, 10].

The relevance of CD154 in humans was established with the discovery that a congenital immunodeficiency called X-linked hyper IgM syndrome (X-HIM) is caused by the lack of functional CD154. Patients with this syndrome exhibit defects in humoral immunity compatible with the role of CD154 in regulation of B cell function. However, the most important clinical feature of this immunodeficiency is an increased incidence of infections with opportunistic pathogens (P. carinii, Cryptosporidium parvum, mycobacteria, Cryptococcus neoformans, Toxoplasma gondii, H. capsulatum, and cytomegalovirus [CMV]) [11]. These results prompted studies of the role of CD40-CD154 interaction in regulation of cell-mediated immunity (CMI) against opportunistic pathogens in humans.
T. gondii–specific CD8+ T cells (an important effector arm in control of infection), in the following, I will expand on the role of CD154 in regulation of IL-12/IFN-γ production in response to the parasite.

Viable and killed tachyzoites of T. gondii have contrasting effects on cytokine production by unprimed T cells (T. gondii–seronegative donors). While monocytes that contain either viable or killed T. gondii trigger T cell proliferation, only monocytes or DCs infected with viable parasites induce unprimed T cells to produce high levels of IFN-γ [15–17]. This T cell response is associated with profound phenotypic changes on T. gondii–infected monocytes and DCs. Infection with viable T. gondii but not phagocytosis of killed parasites induces activation of purified monocytes and immature DCs; the activation is characterized by up-regulation of HLA-DR and of the costimulatory ligands CD40, CD80, and CD86 [15–17] (figure 1). These results suggested that these costimulatory ligands might be crucial for regulation of IFN-γ production by T cells. Thus, studies examined if signals between CD40 and CD154 and between CD80/CD86 and their counter-receptor CD28 (a major costimulatory molecule for T cells) modulate cytokine secretion in response to T. gondii.

CD40-CD154 and CD80/CD86-CD28 are critical for T cell production of IFN-γ in response to T. gondii. Blockade of these pathways during the incubation of unprimed human T cells with T. gondii–infected monocytes or DCs inhibits IFN-γ secretion [15–17]. Moreover, IL-12 production by T. gondii–infected DCs is controlled by CD40-CD154 signaling since secretion of this cytokine is inhibited by an anti-CD154 MAb [16, 17, 19]. In turn, IL-12 mediates T cell production of IFN-γ inasmuch as neutralization of IL-12 inhibits IFN-γ production [17] (figure 1). Although the relevance of CD154 for control of IL-12 production has been confirmed in T. gondii–infected CD154−/− mice [20], other animal studies have reported the existence of CD154-independent IL-12 secretion in response to T. gondii–soluble antigens [21].

These results and those from studies in murine models indicate that IL-12 is pivotal for regulation of IFN-γ production in response to T. gondii [13]. However, neutralization of IL-12 only partially inhibits in vitro secretion of IFN-γ by T cells

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**Figure 1.** Antigen presenting cell (APC)–T cell cognate interaction is crucial for production of interleukin (IL)-12 and interferon (IFN)-γ in response to Toxoplasma gondii. Infection of either human immature dendritic cells (iDC) or monocytes (Mo) with viable tachyzoites of T. gondii induces induction/up-regulation of CD80 and up-regulation of CD40, CD86, and major histocompatibility complex (MHC) class II molecules. T cells activated through their T cell receptor acquire expression of CD154. Infected APCs 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.
stimulated with *T. gondii*–infected DCs [17]. Thus, there are both IL-12–dependent and –independent mechanisms of IFN-γ production by human T cells. Of relevance is the evidence of IL-12–independent in vivo secretion of IFN-γ in mice infected with the ts-4 strain of *T. gondii* [22].

Additional support for the importance of T cell–APC cognate interaction came from the demonstration that CD28 and CD154 signaling regulate IL-12–independent production of IFN-γ by unprimed human T cells [17]. Blockade of CD80/CD86-CD28 and to a lesser extent of CD40-CD154 interactions almost completely inhibits IL-12–independent secretion of IFN-γ and to a lesser extent of CD40-CD154 interactions almost completely inhibits IL-12–independent secretion of IFN-γ. These results suggest that CD28 and CD154 also regulate IFN-γ production through direct T cell costimulation as reported in other models [1, 2, 23]. Taken together, cognate interaction between T cells and *T. gondii*–infected APCs controls IFN-γ secretion through CD154-dependent release of IL-12 and through CD28 and CD154 signaling (probable direct T cell stimulation) that can act in concert with IL-12 or mediate IFN-γ production even in the absence of IL-12 (figure 1). These results explain why simultaneous blockade of CD28 and CD154 signaling results in additive inhibition of IFN-γ secretion in response to *T. gondii*. Further studies are needed to determine if cognate signals between T cells and *T. gondii*–infected APCs regulate production of IL-18, a cytokine that also controls IFN-γ secretion.

Only viable *T. gondii* induce changes in infected APCs, which in turn are pivotal for influencing cytokine response. This pathogen–APC–T cell interaction results in IL-12/IFN-γ production in situations where a type-1 cytokine response would be appropriate (infection with viable tachyzoites), whereas no such response is triggered when encountering non-viable parasites. These data are compatible with the “danger” model, in which distinction between noxious and harmless stimuli is made by APCs through up-regulation of costimulatory ligands [24]. Our results suggest that the immune system regulates cytokine response against *T. gondii* through modulation of CD154 and CD28 signaling. Killed *T. gondii* induce low or undetectable T cell production of IFN-γ due to the absence of IL-12 p70 secretion (a result of the lack of CD40-CD154 pathway activation) and to the lower levels of CD28- and CD154-dependent costimulation (i.e., lower APC expression of CD40, CD80, and CD86) [15–17].

Recent reports confirm the relevance of CD40-CD154 interaction for regulation of CMI against intracellular pathogens. Thus, CD40-CD154 interaction is pivotal for in vitro secretion of IL-12 and IFN-γ in response to *C. neoformans* and *Leishmania major* in humans [25, 26], and mice deficient in CD154 or treated with anti-CD154 MAb cannot control infections with *C. parvum, Mycobacterium avium, T. gondii, or Salmonella dublin* [20, 27–29]. It remains to be determined why CD154−/− mice develop a normal IFN-γ response when infected with certain intracellular pathogens [9, 10, 20]. The demonstration that these mice develop compensatory mechanisms of resistance against an intracellular pathogen [29] indicates that studies in these animals should be interpreted with caution.

**Patients with X-HIM Have Defective Type-1 Cytokine Response**

Production of IFN-γ and IL-12 by cells from X-HIM patients has been examined to confirm the clinical relevance of CD154 signaling in the regulation of CMI against opportunistic pathogens. Compared with peripheral blood mononuclear cells (PBMC) and T cells in healthy controls, such cells from X-HIM patients secrete markedly decreased amounts of IFN-γ in response to *T. gondii* (figure 2A) [16]. This defect is not caused by an intrinsic inability to produce this cytokine since IFN-γ secretion in response to phorbol myristate acetate plus iono-

![Figure 2](image-url) **Figure 2.** Patients with X-HIM (a congenital immunodeficiency) have defective in vitro production of interferon (IFN)-γ and interleukin (IL)-12 in response to *Toxoplasma gondii*; production is restored by CD154 trimer. Peripheral blood mononuclear cells (PBMC) from healthy *T. gondii*–seronegative controls and from patients with X-HIM syndrome (A, 1 × 10^6/mL; B, 2 × 10^5/mL) were incubated with *T. gondii* tachyzoites (Tg) (A, 1 × 10^6/mL; B, 5 × 10^4/mL) in the absence or presence of CD154 trimer (1 μg/mL). IFN-γ and IL-12 p40 were measured by ELISA in supernatants collected at 72 and 24 h, respectively. Neither IFN-γ nor IL-12 were detected in supernatants obtained from uninfected PBMC incubated with CD154 trimer. Modified from [16].
Production of IL-12 in response to an opportunistic pathogen is also impaired in patients with X-HIM. In contrast to PBMC from healthy controls, PBMC from patients with X-HIM fail to secrete or secrete low amounts of IL-12 after incubation with *T. gondii* (figure 2B) [16]. In contrast, IL-12 production in response to *Staphylococcus aureus* Cowan I strain plus IFN-γ is similar to that in control subjects [16]. The low amounts of IL-12 occasionally secreted in response to *T. gondii* by PBMC from patients with X-HIM suggest the presence of additional factors besides CD154 that regulate IL-12 production in response to the parasite. In this regard, intravenous administration of a preparation of *T. gondii*–soluble antigens results in a transient, CD154-independent production of IL-12 by murine DCs [21]. X-HIM patients also have defective IFN-γ/IL-12 production in response to polyclonal stimulation [30].

To further determine the relevance of CD154 signaling for regulation of cytokine synthesis, the effects of recombinant CD154 trimer on IL-12 and IFN-γ production in response to *T. gondii* have been examined. 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 (figure 2) [16]. These data suggest that CD154 trimer may be useful for immunotherapy in patients with X-HIM.

Toxoplasmonic encephalitis and disseminated toxoplasmosis have been reported in patients with X-HIM [11, 31]. Studies of in vitro reactivity of T cells from patients with X-HIM who developed toxoplasmonic encephalitis strongly suggest that CD154 is crucial for in vivo T cell priming against *T. gondii*. T cells from *T. gondii*–seropositive (chronically infected) and seronegative healthy volunteers proliferate and secrete IFN-γ when stimulated with *T. gondii*–infected PBMC [14, 16]. However, only T cells from chronically infected individuals exhibit in vitro reactivity to low concentrations of *T. gondii*–infected cells [14, 16]. T cells from a patient with X-HIM chronically infected with *T. gondii* proliferated only to a high concentration of *T. gondii*–infected PBMC [16]. In addition, T cells from this patient produced markedly reduced amounts of IFN-γ even after incubation with high concentrations of *T. gondii*–infected PBMC [16]. Together, these results indicate that despite prior exposure to *T. gondii* in vivo, T cells from the patient with X-HIM react in a manner similar to T cells isolated from seronegative individuals.

Studies in mice demonstrated that CD154 is required for in vivo priming of T cells to protein antigens, and this effect is mediated by CD154-dependent induction of the costimulatory ligand CD80 [1, 2]. The fact that in the case of *T. gondii* infection, induction/up-regulation of CD80 and CD86 on infected APCs is CD154 independent suggests that CD154 is acting through a different mechanism to promote T cell priming against the parasite. In summary, it is likely that defective IL-12/IFN-γ production and T cell priming explain the susceptibility of X-HIM patients to opportunistic pathogens, such as *T. gondii*. These results also suggest that modulation of CD154 signaling may be of use in other conditions associated with impaired type-1 cytokine response.

**CD4+ T Cells from Human Immunodeficiency Virus (HIV)–Infected Patients Have Defective CD154 Induction That Contributes to Impaired In Vitro Type-1 Cytokine Response**

Prior to the decrease in CD4 cell counts, HIV-infected individuals exhibit defects in CMI, including decreased in vitro production of IL-2, IL-12, and IFN-γ and impaired lymphocyte proliferation [32–34]. Impaired cellular immunity is associated with progression of HIV infection [35], and worsening of this immune deficiency ultimately contributes to development of opportunistic infections. Given the central role of IL-12 in regulation of CMI, studies have addressed the mechanisms for decreased IL-12 production associated with HIV [32, 36, 37]. However, these studies reported contrasting results, so the pathogenesis of this defect is not fully understood.

Recent work has addressed whether defective CD154 signaling explains impaired type-1 cytokine response in HIV-infected patients. As an initial step, the effects of CD154 trimer on IL-12 production in response to opportunistic pathogens were examined. Regardless of CD4 cell count, PBMC from HIV-infected patients (both *T. gondii* seropositive and seronegative) secrete significantly lower amounts of IL-12 p40 than those from controls after incubation with *T. gondii* [38] (figure 3A). This defect occurs even in patients receiving antiretroviral therapy. CD154 trimer preferentially enhances IL-12 p40 production in HIV-infected individuals compared with controls. Thus, IL-12 secretion in the presence of CD154 trimer is no longer different between controls and HIV-infected patients regardless of CD4 cell count. Moreover, CD154 trimer corrects defective production of bioactive IL-12 p70 in response to *T. gondii* in HIV-infected patients [38]. CD154 trimer also causes a remarkable increase in IL-12 p40 production in response to CMV [38]. In the presence of CD154 trimer, PBMC from HIV-infected patients and controls secrete similar amounts of IL-12 p40 after incubation with CMV [38]. These results are not due to a nonspecific effect of CD154 trimer since this molecule does not restore defective IL-12 production in response to *S. aureus* Cowan I strain, a stimulus that triggers early production of IL-12 (24 h) in a CD154-independent manner [38].

By enhancing IL-12 secretion, CD154 trimer restores in vitro production of IFN-γ in response to opportunistic pathogens in HIV-infected patients [38]. Regardless of CD4 cell count,
T cells from these patients (even those receiving antiretroviral therapy) secrete lower amounts of IFN-γ than controls in response to PBMC infected with either CMV or T. gondii [38] (figure 3B). CD154 trimer preferentially enhances IFN-γ production in HIV-infected patients. In the presence of CD154 trimer, IFN-γ secretion in patients with CD4 cell counts $200/\mu L$ is similar to that in controls. The percentage of lymphocytes that become CD154+ after phorbol myristate acetate/ionomycin stimulation is decreased in HIV-infected patients [39]. It was proposed that this defect is caused simply by a decrease in CD4+ T cells rather than by a specific defect in CD154 expression [39]. A recent report indicates that CD154 induction is markedly reduced only in patients with CD4 cell counts $<200/\mu L$ [40]. However, while CD154 induction is an early event during T cell–APC interaction, this study assessed CD154 expression during late stages of T cell activation and used P815 cells rather than human APCs as accessory cells.

The facts that secretion of human IL-12 triggered by T. gondii is CD154 dependent [16, 17, 19] and that CD154 trimer restores IL-12 production in response to the parasite in HIV-infected patients strongly suggest that CD154 signaling is defective even in HIV-infected patients with CD4 cell counts $>500/\mu L$. Indeed, this was confirmed by examining T cell activation after incubation of either PBMC or CD4+ T cells plus monocytes with soluble anti-CD3 monoclonal antibody for 10 h. CD154 and CD69 expression were assessed on CD4+ small lymphocytes. Modified from [38]. D. Defective CD154 induction contributes to impaired in vitro production of IL-12 and IFN-γ in HIV-infected patients. APC, antigen-presenting cells.

Figure 3. Role of CD154 in defective type-1 cytokine response in HIV-infected patients. A, CD154 trimer enhances interleukin (IL)-12 production by peripheral blood mononuclear cells (PBMC) from HIV-infected patients in response to opportunistic pathogens. PBMC (2 $\times 10^6$/mL) from Toxoplasma gondii–seropositive donors were incubated for 24 h in complete medium (CM) with T. gondii tachyzoites (5 $\times 10^5$/mL) with or without CD154 trimer (1 $\mu g$/mL). Circles are superimposed when values are similar. B, CD154 trimer enhances interferon (IFN)-γ production by T cells from HIV-infected patients in response to opportunistic pathogens. T cells (1 $\times 10^6$/mL) from cytomegalovirus (CMV)-seropositive donors were incubated for 72 h in CM with γ-irradiated uninfected or CMV-infected PBMC (2 $\times 10^6$/mL) with or without CD154 trimer. C, CD4+ T cells from HIV-infected individuals exhibit defective CD154 induction. PBMC from HIV-infected and control subjects were stimulated with soluble anti-CD3 monoclonal antibody for 10 h. CD154 and CD69 expression were assessed on CD4+ small lymphocytes. Modified from [38]. D, Defective CD154 induction contributes to impaired in vitro production of IL-12 and IFN-γ in HIV-infected patients. APC, antigen-presenting cells.
and memory (CD45RO+) CD4+ T cells from HIV-infected patients [38].

Defective CD154 induction is not caused exclusively by reduced T cell activation since decreased CD154 induction is observed in patients with unimpaired CD69 induction and since the percentage of activated (CD69+) CD4+ T cells that become CD154+ is lower in HIV-infected patients than in controls [38]. Similar results are obtained when T cells are stimulated with a superantigen (staphylococcal enterotoxin B). The mechanisms responsible for defective CD154 induction on CD4+ T cells from HIV-infected patients remain to be identified. Of note is the demonstration that gp120 inhibits anti-CD3-mediated induction of CD154 on CD4+ T cells from healthy individuals [41].

Since CD154 regulates IL-12 secretion during primary and recall immune responses [3, 4, 7, 16, 17, 19], impaired CD154 induction provides an explanation for decreased T cell–dependent IL-12 production in HIV infection (figure 3D). Moreover, since IL-12 is crucial for generation and maintenance of protective immunity against opportunistic pathogens [13, 42], defective CD154 signaling is likely to contribute to susceptibility to opportunistic infections in HIV-infected patients. Of further relevance is the demonstration that CD154 inhibits replication of M. avium in human macrophages and of T. gondii in human and murine macrophages [20, 28] (Subauste CS, unpublished observations).

Antiretroviral therapy does not induce full immune reconstitution [38, 43]. The studies described together with the fact that the level of CD154 expression on CD4+ T cells affects the development of T cell–mediated immunity [44] suggest that impaired CD154 signaling contributes to immunosuppression in HIV infection. Since CD154 induction is defective even in patients receiving antiretroviral therapy, restoration of CD154 signaling may help achieve improved immune reconstitution in HIV-infected patients. Of note is the demonstration that CD154 can either inhibit or promote HIV replication, depending on the experimental conditions [45]. The ultimate effect of CD154 on viral replication may be dependent on the capacity of this molecule to stimulate anti-HIV immunity.

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