Pathogen-Specific Induction of CD154 Is Impaired in CD4+ T Cells from Human Immunodeficiency Virus–Infected Patients

Carlos S. Subauste, Matthew Wessendarp, Jose-Andres C. Portillio, Rosa M. Andrade, Linda M. Hinds, Francisco J. Gomez, A. George Smulian, Peter A. Grubbs, and Lisa A. Haglund

The pathogenesis of immunodeficiency associated with human immunodeficiency virus (HIV) infection remains incompletely understood. CD154, a molecule that is expressed primarily on activated CD4+ T cells, is pivotal for regulation of cell-mediated and humoral immunity and is crucial for control of many opportunistic infections. We investigated whether CD4+ T cells from HIV-infected patients exhibit defective induction of CD154 in response to opportunistic pathogens. Incubation of purified human CD4+ T cells with monocytes plus antigenic preparations of either Candida albicans, cytomegalovirus, or Toxoplasma gondii resulted in induction of CD154. Expression of CD154 in response to these pathogens was impaired in CD4+ T cells from HIV-infected patients. This defect correlated with decreased production of interleukin (IL)-12 and interferon (IFN)-γ in response to T. gondii. Recombinant CD154 partially restored secretion of IL-12 and IFN-γ in response to T. gondii in cells from HIV-infected patients. Together, defective induction of CD154 is likely to contribute to impaired cell-mediated immunity against opportunistic pathogens in HIV-infected patients.

Before undergoing CD4+ T cell depletion, human immunodeficiency virus (HIV)–infected patients exhibit defects in cell-mediated immunity (CMI) that include impaired in vitro production of interleukin (IL)-2, IL-12, and interferon (IFN)-γ and decreased lymphocyte proliferation [1–3]. These defects are important because they are associated with progression of HIV infection [4], and they are likely to contribute to susceptibility to opportunistic infections. Although antiretroviral therapy controls viral replication and increases CD4+ T cell counts [5], it does not fully restore immune function [6–9]. Hence, a better understanding of the mechanisms by which HIV disrupts CMI may lead to adjunctive approaches to improve immune reconstitution.

CD154, a molecule that is expressed primarily on activated CD4+ T cells, is pivotal for regulation of both cellular and humoral immunity. Through its interaction with CD40, CD154 regulates activation of antigen-presenting cells, priming of CD4+ and CD8+ T cells, stimulation of the production of IL-12 and IFN-γ, B-cell proliferation, and immunoglobulin synthesis [10–12]. The role that CD154 plays in controlling fundamental aspects of the immune response is likely to explain why subjects with a congenital defect in CD154 signaling (X-linked Hyper IgM syndrome [X-HIM]) are susceptible to infections with Pneumocystis carinii, Cryptosporidium parvum, Mycobacteria, Toxoplasma gondii, Cryptococcus neoformans, Histoplasma capsulatum, and cytomegalovirus (CMV) [13, 14].

By use of polyclonal stimulation, it has been found that expression of CD154 is impaired on CD4+ T cells from HIV-infected patients [9, 15, 16]. However, studies of induction of CD154 in response to opportunistic pathogens more directly address whether CD154 plays a role in the increased susceptibility to these pathogens, which are associated with progressive HIV infection. Here, we report that induction of CD154 in response to microbial pathogens is impaired in CD4+ T cells from...
HIV-infected patients, and we present data that suggest that this defect plays a role in impaired in vitro production of IL-12 and IFN-γ in response to opportunistic pathogens.

SUBJECTS, MATERIALS, AND METHODS

HIV-infected patients and control subjects. Blood samples were obtained from 35 HIV-infected patients who were monitored at the Infectious Diseases Center at the University of Cincinnati and the Cincinnati Veterans Affairs Medical Center; all but 7 were treated with combination antiretroviral therapy. Plasma virus load was measured by reverse-transcriptase polymerase chain reaction (Amplicor). Blood samples were obtained from 16 healthy volunteers who served as control subjects; CMV-seropositive subjects were used to test in vitro responses to CMV. T. gondii-seropositive subjects and T. gondii-seronegative subjects were used to examine in vitro responses to T. gondii. Informed consent was obtained from all subjects, and the human-experimentation guidelines of the US Department of Health and Human Services and of the University of Cincinnati were followed.

Induction of expression of CD154. Purified CD4+ T cells and monocytes were obtained from peripheral blood mononuclear cells (PBMCs) by negative depletion, by use of immunomagnetic beads, as described elsewhere [17]. Populations of CD4+ T cells were >90% (mean ± SD, 95.22 ± 0.58%) CD3+ CD4+ when obtained from HIV-infected patients and >95% (mean ± SD, 97.88 ± 0.40%) CD3+ CD4+ when obtained from control subjects. Purity of monocyte populations was similar (>92% CD14+) in both groups. CD4+ T cells (5 × 10⁵ cells/mL) and autologous monocytes (1.25 × 10⁵ cells/mL) were cultured in 96-well plates containing complete medium (CM) consisting of RPMI 1640 medium plus 10% human AB serum (Gemini Bio-Products). CD4+ T cells were stimulated with antigenic preparations, by addition of nonstimulated CD4+ T cells, revealed that the lower limit of detection of antigen-specific CD69+ CD154+ CD4+ T cells is 0.05%. No significant induction (<0.05%) of CD69+ CD154+ T cells was noted when CD4+ T cells were stimulated in the absence of microbial antigens.

Induction of production of IL-12 and IFN-γ. To study cytokine secretion in response to T. gondii, CD4+ T cells (3 × 10⁶ cells/mL for IL-12 and 5 × 10⁵ cells/mL for IFN-γ) were incubated with monocytes in 96-well Teflon plates at a ratio of 6 CD4+ T cells:1 monocyte. Monocytes were left either uninfected or infected with T. gondii, as reported elsewhere [17]. For evaluation of antigen-specific CD4+ T cell responses by flow cytometry, CD4+ T cells (5 × 10⁵ cells/mL) and monocytes (1.25 × 10⁵ cells/mL) were cultured in 96-well plates in CM plus anti-CD28 and anti-CD49d MAbs (1 µg/mL), with either CMV antigen (10 µg/mL; Advanced Biotechnologies) or control antigen. After the antigens had been added, brefeldin A (Sigma) was added at a concentration of 10 µg/mL 2 h; after 12 h of in vitro culture, cells were obtained for flow cytometric analysis.

Flow cytometry. Cells were stained with anti-CD3 fluorescein isothiocyanate (FITC), anti-CD4 peridinin chlorophyll protein (PerCP), anti-CD69 allophycocyanin (APC), anti-CD154 phycoerythrin (PE), and appropriate-isotype control MAbs (BD Pharmingen). Expression of CD69+ and CD154+ cells were calculated by subtracting values obtained with microbial antigens from values obtained with either CM or control lysates.

For detection of intracellular IFN-γ, cells were first stained with anti-CD3-APC (BD Pharmingen) and anti-CD4-PerCP. Cells were fixed and permeabilized by use of IntraPrep permeabilization reagent (Coulter-Immunotech), following the manufacturer’s instructions. Thereafter, cells were stained with anti–IFN-γ–FITC, anti–CD69–PE, or the appropriate-isotype control MAbs (BD Pharmingen). After fixation with 1% paraformaldehyde, cells were analyzed by use of a FACScalibur flow cytometer (BD Pharmingen). Expression of CD69 and CD154 were analyzed in 2.5 × 10⁴ CD3+ CD4+ T cells/well. Data are shown as mean of triplicate wells. Percentages of CD69+ and CD154+ cells were calculated by subtracting values obtained with microbial antigens from values obtained with either CM or control lysate.

Cytokine ELISA. Concentrations of IL-12 p40 (R&D Systems) and IFN-γ (Endogen) were measured by ELISA in supernatants obtained at 24 and 72 h, respectively. The lower limit of detection for both assays was 39 pg/mL. Data are shown as the mean ± SEM of triplicate wells.

Statistical analysis. Analysis of variance was used to compare groups of HIV-infected patients and control subjects. Student’s t test was used to calculate the significance of the effects of neutralizing MAbs. Linear-regression analysis was performed to determine the relationship between expression of CD154 and production of IL-12 and IFN-γ.

RESULTS

Induction of CD154 in response to microbial antigens is impaired in CD4+ T cells from HIV-infected patients. Antigen-
presenting cells, especially B cells, cause CD40-mediated down-regulation of CD154 [18]. Thus, purified CD4+ T cells plus monocytes were used in the present study to avoid diminished expression of CD154 caused by an increase in antigen-presenting cell:CD4+ T cell ratios in HIV-infected patients with low CD4+ T cell counts. CD4+ T cells and monocytes were incubated with lysates of *C. albicans*, CMV, and *T. gondii*, control lysate, or *T. gondii* tachyzoites. Antigenic stimulation was conducted in the presence of MAbs against CD28 and CD49d because provision of dual exogenous costimulation is required for detection, by flow cytometry, of maximal responder frequencies in CD4+ T cell populations [19]. In the case of *T. gondii*, CD4+ T cells from both *T. gondii*-seronegative subjects and *T. gondii*-seropositive subjects were examined, since naive CD4+ T cells from seronegative adults and newborns proliferate in response to *T. gondii* [17].

Activation of CD3+ CD4+ T cells after antigenic stimulation was determined by induction of CD69. Stimulation with *T. gondii* tachyzoites, *C. albicans*, or CMV lysates induced CD69 on CD4+ T cells (figure 1). Similar results were obtained with *T. gondii* lysate (data not shown). The percentages of CD69+ T cells obtained after stimulation with *C. albicans* and CMV were similar in HIV-infected patients and control subjects (*P* > .1). Stimulation of CD3+ CD4+ T cells after antigenic stimulation was determined by induction of CD69. Stimulation with *T. gondii* tachyzoites, *C. albicans*, or CMV lysates induced CD69 on CD4+ T cells (figure 1). Similar results were obtained with *T. gondii* lysate (data not shown). The percentages of CD69+ T cells obtained after stimulation with *C. albicans* and CMV were similar in HIV-infected patients and control subjects (*P* > .1). Stimulation of CD4+ T cells from *T. gondii*-seronegative subjects, with *T. gondii*, resulted in significantly lower percentages of CD69+ T cells only in HIV-infected patients with CD4+ T cell counts <200 cells/µL, compared with those for control subjects (*P* = .04). In *T. gondii*-seropositive subjects, induction of CD69 after incubation with *T. gondii* was similar in HIV-infected patients and control subjects (*P* > .1), although few *T. gondii*-seropositive subjects were identified in the present study. Together, except for presumably primary T cell response to *T. gondii* (*T. gondii*-seronegative subjects) in subjects with CD4+ T cell counts <200 cells/µL, induction of CD69 in response to microbial antigens was similar in HIV-infected patients and control subjects.

The level of expression of CD154 on CD4+ T cells directly influences production of type-1 cytokines and the generation of cytotoxic T lymphocyte activity and correlates with improved protection during viral infection [20]. Thus, we examined the levels of expression of CD154 (mean fluorescence intensity [MFI]) on CD69+ CD4+ T cells. In contrast to induction of CD69, expression of CD154 in response to *C. albicans* was impaired in the 3 groups of HIV-infected patients (*P* < .0002; figure 2). Induction of CD154 in response to CMV was defective in the groups of HIV-infected patients with CD4+ T cell counts <500 cells/µL (*P* < .004). Stimulation of CD4+ T cells

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**Figure 1.** Induction of CD69 in response to microbial antigens in CD4+ T cells from human immunodeficiency virus (HIV)-infected patients and control subjects. CD4+ T cells were incubated with monocytes plus either *Candida albicans*, cytomegalovirus (CMV), or *Toxoplasma gondii* antigenic preparations. HIV-infected patients were classified on the basis of their CD4+ T cell count (<200, 200–500, or >500 cells/µL). Percentage of CD69+ cells was calculated as described in Subjects, Materials, and Methods. White circles are superimposed when values are similar. HIV+, HIV positive; HIV−, HIV negative.
Figure 2. Induction of CD154 in response to microbial antigens in CD4+ T cells from human immunodeficiency virus (HIV)–infected patients. CD4+ T cells from HIV-infected patients and control subjects were stimulated with monocytes plus either Candida albicans, cytomegalovirus (CMV), or Toxoplasma gondii antigenic preparations. HIV-infected patients were classified on the basis of their CD4+ T cell count (<200, 200–500, or >500 cells/µL). Expression of CD154 (mean fluorescence intensity [MFI]) on CD69+ CD4+ T cells was assessed by flow cytometry. White circles are superimposed when values are similar. HIV+, HIV positive; HIV−, HIV negative.

From T. gondii–seronegative subjects, with T. gondii, revealed that induction of CD154 was impaired in all groups of HIV-infected patients (P < .01). In T. gondii–seropositive subjects, T. gondii–mediated induction of CD154 appeared to be impaired only in HIV-infected patients with CD4+ T cell counts ≤500 cells/µL (P = .05). Together, antigen-specific induction of CD154 is defective in CD4+ T cells from most HIV-infected patients.

We determined whether there was an association between plasma virus load and the level of expression of CD154, after antigenic stimulation. HIV-infected patients were classified on the basis of whether plasma virus load was detectable (figure 3). After stimulation with C. albicans, both groups of HIV-infected patients exhibited significantly lower levels of expression of CD154 than control subjects (P < .0001). Induction of CD154 in response to CMV was impaired only in the group of HIV-infected patients with detectable plasma virus loads (P < .0001). T. gondii–mediated induction of CD154 was impaired in both groups of T. gondii–seronegative, HIV-infected patients (P < .04). T. gondii–seropositive subjects with detectable plasma virus load had impaired induction of CD154 in response to T. gondii (P = .037). No significant difference in expression of CD154 was noted among subjects with detectable virus loads when they were further stratified on the basis of their virus load (50–500 copies/mL, followed by log increase of up to >50,000 copies/mL; data not shown). Thus, expression of CD154 in response to CMV and T. gondii in seropositive subjects is defective in subjects with detectable plasma virus loads, whereas induction of CD154 in response to C. albicans and T. gondii in seronegative subjects is impaired even in subjects with undetectable plasma virus loads.

To confirm the defect in induction of CD154 that is associated with HIV infection, we determined the percentage of CD4+ T cells that became CD69+ CD154+ after antigenic stimulation. Figure 4 shows that the percentage of CD69+ CD154+ T cells induced by C. albicans was significantly impaired in all groups of HIV-infected patients (P < .05). The percentages of CD69+ CD154+ T cells after stimulation with CMV or T. gondii (seropositive and seronegative subjects) were significantly decreased in the groups of HIV-infected patients with CD4+ T cell counts <500 cells/µL (P < .05). These results contrast with the reported increase in the percentage of CMV-reactive CD4+ T cells (detected by expression of intracellular cytokines) in HIV-infected patients [21]. Thus, we compared induction of CD154 and
in intracellular IFN-γ in response to CMV. Whereas 0.35% ± 0.11% of the CD4+ T cells from control subjects (n = 6) became CD69+ IFN-γ+ after stimulation with CMV, 0.87% ± 0.20% of CD4+ T cells from HIV-infected patients expressed IFN-γ (n = 3; CD4+ T cell count range, 229–385 cells/μL; plasma virus load range, 19,800–448,611 copies/mL). In contrast, in parallel studies, the mean ± SD percentages of CD69+ CD154+ cells were 0.48% ± 0.2% for control subjects and 0.02% ± 0.01% for HIV-infected patients (P = .01). Together, these data indicate that, in the presence of increased CMV-reactive CD4+ T cell populations, there is defective induction of CD154 in response to CMV, as assessed by intracellular cytokine production.

Defective induction of CD154 in CD4+ T cells in HIV-infected patients correlates with impaired secretion of IL-12 and IFN-γ in response to an opportunistic pathogen. We previously reported that in vitro production of IL-12 by human PBMCs incubated with *T. gondii* and the production of IFN-γ by unseparated T cells incubated with *T. gondii*-infected monocytes are partially dependent on CD154 [22]. These results were confirmed by use of purified CD4+ T cells and *T. gondii*-infected, seronegative control subjects. The addition of a neutralizing anti-CD154 MAb resulted in inhibition of production of IL-12 and IFN-γ (n = 3; mean ± SD inhibition, 93.50% ± 2.09% for IL-12 and 72.51% ± 8.02% for IFN-γ; P < .02) (figure 5A and 5B). Moreover, blockade of CD154 also inhibited the production of IL-12 and IFN-γ by cells from *T. gondii*-seropositive subjects (n = 3; mean ± SD inhibition, 87.12% ± 6.17% for IL-12 and 56.98% ± 3.56% for IFN-γ; P < .02) (figure 5C and 5D). Thus, secretion of type-1 cytokines during recall and presumably primary responses to an opportunistic pathogen are dependent on CD154.

Next, we determined whether there was a correlation between defective induction of CD154 in CD4+ T cells from HIV-infected patients and impaired production of IL-12 and IFN-γ in response to *T. gondii*. Figure 6 shows that production of IL-12 and IFN-γ in response to *T. gondii* were directly correlated with the levels of expression of CD154 on CD69+ CD4+ T cells from *T. gondii*-seronegative, HIV-infected patients (IL-12, R = 0.971 and P < .0001; IFN-γ, R = 0.615 and P = .03). Cells from subjects with CD154 MFIs above the tenth percentile of those of control subjects secreted amounts of IL-12 and IFN-γ that were similar to those secreted by control subjects (P > .2). In contrast, subjects with impaired expression of CD154 (below the tenth percentile of those of control subjects) had decreased in vitro production of IL-12 and IFN-γ (P < .004). These
findings indicate that there is a correlation between decreased expression of CD154 in response to *T. gondii* and impaired production of IL-12 and IFN-γ.

**Recombinant CD154 (rCD154) restores secretion of IL-12 and IFN-γ in response to an opportunistic pathogen.** To further explore the role that CD154 plays in secretion of IL-12 and IFN-γ in response to an opportunistic pathogen, we studied the effects of rCD154 on production of IL-12 and IFN-γ in response to *T. gondii* in *T. gondii*–seronegative subjects. CD4+ T cells were incubated with *T. gondii*–infected monocytes in the presence or absence of rCD154. HIV-infected patients were divided into 2 groups: (1) those with CD154 MFIs in response to *T. gondii* above the tenth percentile of those of control subjects or (2) those with CD154 MFIs in response to *T. gondii* below the tenth percentile of those of control subjects. In agreement with the results of the studies described above, only cells from HIV-infected patients with low levels of CD154 had impaired production of both IL-12 (mean ± SD, 347.99 ± 87.41 pg/mL; *P* = .01) and IFN-γ (mean ± SD, 352.09 ± 109.98 pg/mL; *P* < .0001), compared with those of control subjects (mean ± SD, 1213.15 ± 313.74 for IL-12 and 2831.79 ± 445.80 pg/mL for IFN-γ) (figure 7). The addition of rCD154 to cells from control subjects resulted in 2.9-fold and 2.3-fold increases in concentrations of IL-12 and IFN-γ, respectively (figure 7). Similar degrees of increase in cytokine production were noted in HIV-infected patients with normal levels of expression of CD154 (3.3-fold for IL-12 and 1.9-fold for IFN-γ). In contrast, production of IL-12 and IFN-γ by cells from subjects with low levels of CD154 increased 7.0-fold and 6.7-fold, respectively, after addition of rCD154. Although, in the presence of rCD154, the levels of IL-12 after stimulation with *T. gondii* were, on average, 59% of those observed with cells from control subjects (mean ± SD, 1897.79 ± 365.9 vs. 3198.92 ± 616.67 pg/mL), this difference was no longer significant (*P* = .1). However, the concentrations of IFN-γ in the presence of rCD154 were still significantly lower than those observed with cells from control subjects (mean ± SD, 2122.00 ± 392.63 vs. 5483.5 ± 774.89 pg/mL) (*P* = .002). Together, rCD154 partially restores production of IFN-γ and IL-12 in response to an opportunistic pathogen.

**DISCUSSION**

The expression of CD154 after activation of CD4+ T cells is under tight regulation. In this regard, CD40-bearing B cells cause down-regulation of CD154 [18]. Thus, detection of membrane CD154 during stimulation of antigenic T cells can be difficult in the presence of B cells. Here, we have used a model in which CD4+ T cells were stimulated with microbial antigens in the presence of monocytes and exogenous costimulation. This model allowed us to consistently detect antigen-induced expression of CD154 on CD4+ T cells from control subjects. Therefore, we were able to examine CD154 signaling during the in vitro immune response to opportunistic pathogens and explore the relevance of CD154 in regulation of cytokine secretion in response to these pathogens.

We have previously reported that induction of CD154 in response to polyclonal stimulation is defective in CD4+ T cells from HIV-infected patients [9]. Although studies using purified CD4+ T cells and monocytes confirmed the presence of this defect [9], comparison of induction of CD154 in CD4+ T cells from HIV-infected patients and that in control subjects was performed primarily by use of PBMCs. Thus, it is likely that an increase in the antigen-presenting cell:CD4+ T cell ratio contributed to impaired expression of CD154 [9]. Having used constant concentrations of CD4+ T cells and monocytes, we now report that induction of CD154 in response to *C. albicans, T. gondii*, and CMV is defective in CD4+ T cells from most HIV-infected patients. Impaired antigen-specific induction of CD154 was particularly more prevalent in subjects with detectable plasma virus load. In addition, we observed a correlation between defective induction of CD154 and impaired production of IL-12 and IFN-γ in response to *T. gondii*. Finally, the addition of rCD154 partially restored production of IL-12 and IFN-γ in response to *T. gondii*.

The lack of CD154 signaling is associated with increased susceptibility to intracellular pathogens, both in humans and in mice. Indeed, CD40–CD154 is required for control of the pathogens studied here [13, 23–26]. It is likely that a partial defect in expression of CD154 disrupts T cell–dependent in
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Figure 5. Role of CD154 on production of interleukin (IL)–12 and interferon (IFN)–γ. CD4+ T cells and monocytes (Mo) from Toxoplasma gondii-seronegative (A and B) and from T. gondii-seropositive (C and D) subjects were incubated with T. gondii tachyzoites (Tg) in the presence of either a neutralizing anti-CD154 or control monoclonal antibody (mIgG). Concentrations of IL-12 (A and C) and IFN-γ (B and D) were measured by ELISA in cell supernatants. Results are representative of 1 of 3 individual experiments.

vivo control of opportunistic pathogens, since the level of expression of CD154 has profound effects on T cell–mediated immunity [20, 27]. In this regard, a 1.1–2-fold increase in expression of CD154 on activated CD4+ T cells causes a 4–5-fold increase in T cell–dependent antibody production in CD154-transgenic mice [27]. In addition, our demonstration that induction of CD154 in response to C. albicans is impaired at higher CD4+ T cell counts than induction of CD154 during recall responses to CMV and T. gondii may explain why candidiasis occurs at earlier stages of HIV infection than of toxoplasmosis or CMV end-organ disease.

The mechanisms by which defective CD154 signaling increases susceptibility to opportunistic infections are likely multiple. These include decreased production of IL-12 and IFN-γ, impaired macrophage activation, defective priming of CD4+ and CD8+ T cells, and impaired humoral response. Studies of subjects with X-HIM suggest that CD154 is crucial for T cell priming against the opportunistic pathogen T. gondii [22]. CD154 induces antimicrobial activity against Mycobacterium avium and T. gondii in human macrophages [28, 29]. CD154 is crucial for optimal in vitro production of IL-12 and/or IFN-γ in response to T. gondii [22, 30, 31], C. neoformans [32], and Mycobacterium tuberculosis [33]. Having used purified CD4+ T cells and monocytes, we now report that CD154 regulates production of IL-12 and IFN-γ not only during a presumably primary response to T. gondii but also during recall immune responses. That IL-12 is required for maintenance of protective immunity against T. gondii [34] raises the possibility that defective type-1 cytokine response resulting from impaired induction of CD154 decreases resistance against this pathogen.

The present study has revealed that defective induction of CD154 in CD4+ T cells from HIV-infected patients correlates with diminished in vitro secretion of IL-12 and IFN-γ in response to T. gondii. We report that the level of expression of CD154 correlated more strongly with production of IL-12 than with secretion of IFN-γ. This finding is in keeping with our previous demonstration that CD154 regulates production of IFN-γ primarily through enhanced secretion of IL-12 [22, 31]. Our results are consistent with the role of increased levels of expression of CD154 in promoting type-1 cytokine response in an animal model of viral infection [20]. The decreased production of IL-12 and IFN-γ detected in subjects with diminished induction of CD154, together with the fact that rCD154 partially restored type-1 cytokine response in these subjects, strongly suggests that impaired CD154 signaling contributes to defective type-1 cytokine response associated with HIV infection [35]. The reasons why CD154 did not fully normalize secretion of IFN-γ may include the presence of CD154-indepen-
Figure 6. Relationship between expression of CD154 and production of interleukin (IL)–12 and interferon (IFN)–γ in response to Toxoplasma gondii. Concentrations of IL-12 (A) and IFN-γ (B) were measured by ELISA in cell supernatants. Broken lines represents the tenth percentile of CD154 mean fluorescence intensity (MFI) calculated from all *T. gondii*-seronegative control subjects included in the present study. ●, HIV-infected patients; ○, control subjects.

dent production of this cytokine. Indeed, blockade of CD154 signaling partially inhibits secretion of IFN-γ in response to *T. gondii* (the present study and [22]). In addition, it is likely that defective CD154 signaling acts in concert with other mechanisms to explain the decreased type-1 cytokine response associated with HIV infection.

Although controversial, there are reports of impaired activation in T cells from HIV-infected patients [36, 37]. A defect in T cell activation associated with HIV infection may play a role in impaired induction of CD154, since expression of this molecule is dependent on signals through the T cell receptor [38]. However, the lack of association between induction of CD154 and T cell activation, as assessed by expression of CD69, observed in this study suggests that there are other mechanisms responsible for impaired expression of CD154. Interestingly, similar to the results of our studies, CD4C/HIV transgenic mice exhibit defective induction of CD154 but not of CD69 [39].

Exogenous costimulation was provided to optimally identify antigen-specific CD4+ T cell populations [19]. That anti-CD28 MAB stabilizes expression of CD154 [40] may also facilitate the detection of CD154. It is possible that the decrease in expression of CD28 on CD4+ T cells from HIV-infected patients [41] could contribute to impaired induction of CD154. However, expression of CD28 is only modestly diminished and is not associated with defective response to anti-CD28 costimulation [41, 42]. On the contrary, the response of CD4+ T cells from HIV-infected patients to anti-CD28 MAB is either normal or enhanced [42].

The central role that depletion of CD4+ T cells plays in the pathogenesis of immunodeficiency associated with HIV raises the possibility that defective induction of CD154 may be caused by a selective loss of cells capable of expression of CD154 upon activation. We have shown that impaired induction of CMV-specific CD154 takes place in the presence of an expansion of the CMV-reactive CD4+ T cell population, as assessed by intracellular cytokine production. Although this observation does not disprove a selective loss of a subpopulation of CD4+ T cells, it does argue in favor of a specific defect in regulation of induction of CD154.

Antiretroviral therapy causes an improvement in CMI in some HIV-infected patients [43, 44]. Interestingly, expression of CD154 during recall responses to CMV and *T. gondii* was above the tenth percentile of that of control subjects in 54% of the assays performed with cells from HIV-infected patients with undetectable plasma virus loads; in contrast, this was the case in only 6% of the assays performed with cells from subjects with detectable plasma virus loads. However, the decreased expression of CD154 in response to these pathogens in subjects with low levels of detectable plasma virus load and the impaired induction of CD154 in response to *C. albicans* noted even in subjects with undetectable plasma virus load suggest that CD154 is very sensitive to the effects of HIV. Similar to reports

Figure 7. Effect of recombinant CD154 (rCD154) on production of interleukin (IL)–12 and interferon (IFN)–γ in response to Toxoplasma gondii (Tg). CD4+ T cells plus *T. gondii*-infected monocytes were incubated with or without rCD154. Concentrations of CD4+ T cells and monocytes were kept constant for all subjects studied. HIV-infected patients were classified as having either low levels of expression of CD154 (below the tenth percentile of that of control subjects) or normal levels of expression of CD154 (above the tenth percentile of that of control subjects). Concentrations of IL-12 (A) and IFN-γ (B) were measured by ELISA in cell supernatants. White circles are superimposed when values are similar. HIV+, HIV positive; HIV−, HIV negative.
of partial immune reconstitution due to antiretroviral therapy [6–9], CD154 was defective in CD4+ T cells from most HIV-infected patients receiving antiretrovirals. Given the central role of CD154 in regulation of cellular and humoral immunity, identification of the mechanisms by which HIV dysregulates induction of CD154 may lead to adjunctive approaches to enhance immune reconstitution.

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References