

Cytokine Profile and Immunomodulation in Asymptomatic Human T-Lymphotropic Virus Type 1-Infected Blood Donors

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Summary: The modulation of the immune response has been used as therapy for clinical disorders associated with human T-lymphotropic virus type 1 (HTLV-1) infection. In this study, the cytokine profile was evaluated in 26 asymptomatic HTLV-1 blood donors. Additionally, both the cell responsible for producing interferon- γ (IFN- γ) and the role of exogenous interleukin (IL)-10 in downregulating IFN- γ production were studied. Cytokine levels were determined in supernatants of unstimulated lymphocyte cultures by enzyme-linked immunosorbent assay. The levels of IFN- γ , tumor necrosis factor- α , IL-5, and IL-10 were higher in supernatants of the lymphocyte cultures taken from HTLV-1-infected donors than in those taken from healthy subjects. Although depletion of CD8⁺ T cells and natural killer cells did not affect IFN- γ production, depletion of CD4⁺ T cells significantly decreased IFN- γ production. Furthermore, at a concentration of 2 ng/ml, IL-10 had only a minimum effect on IFN- γ production, although at high concentrations (100 ng/ml), IL-10 decreased IFN- γ production by 50% in HTLV-1-infected individuals. These data indicate that both T helper 1 and T helper 2 cytokines are elevated in HTLV-1 infection and that IL-10 in high concentrations modulates IFN- γ production in these patients. **Key Words:** Cellular immunity in HTLV-1—Cytokines in HTLV-1—HTLV-1—Immune response in HTLV-1—Immunomodulation in HTLV-1.

The immunologic response in human T-lymphotropic virus type 1 (HTLV-1) infection is characterized by spontaneous T-cell proliferation with increasing secretion of interleukin (IL)-2 and expression of the IL-2 receptor (1-3). Abnormalities in the response have been shown in patients with HTLV-1-associated myelopathy (tropical spastic paraparesis) compared with asymptomatic HTLV-1-positive individuals, including elevated levels of cytokines such as tumor necrosis factor- α (TNF- α), IL-6, and IL-2 in sera and cerebrospinal fluid (4,5). Both CD4⁺ and CD8⁺ T cells are infected by HTLV-1 (6,7). Although only a small percentage of in-

fectured individuals develop clinical manifestations associated with HTLV-1, the prevalence of this retrovirus is high in endemic areas such as Salvador, Bahia, Brazil, where 1.35% of blood donors are infected with HTLV-1 (8). Although high levels of IL-2 and interferon- γ (IFN- γ) have been documented in supernatants of lymphocyte cultures from asymptomatic HTLV-1 carriers (3), little is known about the secretion of T helper (Th) 2 cytokines or the ability of cytokines and cytokine antagonists to modulate the lymphocyte function in the course of this viral infection. The major aim of the current study was to evaluate the cytokine profile in asymptomatic subjects infected with HTLV-1, to determine which cells are secreting IFN- γ in such patients, and to evaluate the ability of IL-10 to downregulate IFN- γ production in patients infected with HTLV-1.

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MATERIALS AND METHODS

Patients

The subjects ($n = 26$) were recruited in the study referrals of HTLV-1-positive donors found at two blood banks (Hemocentro da Bahia and Serviço de Transfusão de Sangue) located in Salvador, Bahia, Brazil. The subjects (Group 1) were given a questionnaire to determine the route of transmission and clinical manifestations of HTLV-1 infection. A physical examination was carried out in these patients. A group of 15 medical students and healthy employees with negative HTLV-1 serology was also studied (Group 2). These two groups were comparable apart from sex ratio, age, and nutritional status. Informed consent was obtained, and human experimentation guidelines of the Hospital Universitário Prof. Edgard Santos were followed in the conduct of this clinical research.

Serologic Tests

All sera were screened for HTLV-1/2 antibodies by enzyme-linked immunosorbent assay (ELISA) (Cambridge Biotech Corporation, Worcester, MA, U.S.A.). Repeatedly reactive samples were subjected to Western blot analysis to distinguish between HTLV-1 and HTLV-2 using HTLV blot 2.4 (Genelabs, Singapore) according to the manufacturer's instructions. Additionally, all sera were screened for HIV, syphilis, hepatitis B, and hepatitis C.

Cell Preparation and Culture

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized peripheral blood by a Ficoll-Hypaque gradient as previously described (9). To evaluate the role of PBMC subpopulations in the modulation of the immune response, macrophages were depleted by their ability to adhere to a plastic surface, and nonadherent cells were fractionated using the Mini Macs System (Milteny Biotec, Sunnyvale, CA, U.S.A.) as previously described (10). The Mini Macs System uses T-cell subpopulation (CD4 or CD8)-specific monoclonal antibodies for positive selection as well as for negative selection. For natural killer (NK) cell depletion, PBMCs were incubated with beads coated with antibodies against CD3, CD4, CD19, and CD33. The positively selected cells correspond to the NK depleted cell population. Cell populations were analyzed by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA, U.S.A.) using specific monoclonal anti-CD4, anti-CD8, anti-CD3, and anti-CD56. After CD4 and CD8 depletion, the remaining populations contained less than 10% of these cells. The purity of the CD4 or CD8 cells was 99%. NK depleted PBMCs showed only 0.39% of CD56⁺/CD3⁻ cells.

Macrophages were depleted by adherence on Petri dishes (11). Briefly, 10^7 PBMCs in RPMI supplemented with 20% AB serum were incubated in 100 × 15-mm plastic Petri dishes (Falcon Labware, Oxnoid, CA, U.S.A.) for 2 hours at 37°C. After a second depletion, nonadherent cells were recovered, and the extent of macrophage depletion was determined by the esterase staining (number of esterase-positive cells before depletion [26% ± 3%] and after depletion [2% ± 0.7%]).

Cytokine Determination

Cytokine levels (TNF- α , IFN- γ , IL-5, and IL-10) in supernatants of the different subpopulations of cells were measured by ELISA. Briefly, the whole PBMCs and the different cell subpopulations were adjusted to 3×10^6 per milliliter in RPMI 1640 (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% AB plus serum containing 100 U of

penicillin/G and 10 μ g/ml of streptomycin. The cells were kept unstimulated. All cultures were incubated at 37°C in 5% CO₂ for 72 hours. Supernatants were collected and stored at -20°C. In some experiments, supernatants were collected 6, 24, and 48 hours after incubation. IFN- γ , TNF- α , IL-5, and IL-10 levels were measured by the ELISA sandwich technique, and the results were expressed as picograms per milliliter based on a standard curve generated using recombinant cytokines. To evaluate the ability of IL-10 in modulating IFN- γ production, exogenous IL-10 in concentrations ranging from 2 to 100 ng/ml was added to unstimulated cultures of PBMCs of HTLV-1-infected subjects. As controls, PBMCs from 5 individuals not infected with HTLV-1 were stimulated with purified protein derivative (of tuberculin) (PPD) (2 μ g/ml) and IL-10 (concentrations of 2 and 20 ng/ml) added to the cultures. After 72 hours at 37°C, 5% CO₂ IFN- γ was measured in the supernatants of the cultures.

RESULTS

Spontaneous secretion of high levels of IFN- γ has been observed in HTLV-1-infected subjects (12). With the aim of determining whether subjects with HTLV-1 secrete high levels of other cytokines in addition to high levels of IFN- γ , the levels of IL-5, IL-10, and TNF- γ were measured in the supernatants of lymphocyte cultures. The cytokine profile in the supernatants of lymphocyte cultures from 26 blood donors infected with HTLV-1 (Group 1) and 15 controls (Group 2) is shown in Figure 1. The mean \pm SD of IFN- γ levels in patients infected with HTLV-1 was 1128 ± 1037 pg/ml (range: 0–2966 pg/ml), and the mean \pm SD in supernatants of healthy subjects was 1 ± 4 pg/ml ($p < .01$). High concentrations of IFN- γ in lymphocyte supernatants of asymptomatic HTLV-1 individuals could be observed as soon as 6 hours after incubation, although higher levels were observed in cultures incubated for 48 and 72 hours. The mean level of IL-5 in the group infected with HTLV-1 was 220 ± 287 pg/ml (range: 0–1126 pg/ml), which was higher ($p < .01$) than that observed in subjects not infected with HTLV-1 (2 ± 2 pg/ml). There was also a significant difference between the levels of TNF- α and IL-10 in these two groups. The TNF- α (213 ± 202 pg/ml) and IL-10 (139 ± 124 pg/ml) levels in patients infected with HTLV-1 were higher ($p < .01$) than those observed in the control group (60 ± 63 pg/ml and 2.6 ± 10 pg/ml, respectively). Of the 26 subjects with HTLV-1-positive serologic tests, 3 had positive serology for syphilis and 2 had positive serology for hepatitis B virus. All subjects (Groups 1 and 2) had negative serology for HIV.

In 5 subjects studied, IFN- γ was 1870 ± 687 pg/ml in unseparated mononuclear cells. To determine the source of IFN- γ , the mononuclear cell population was first depleted of CD4⁺ T cells, and a separate group of cells was then depleted of CD8⁺ T cells. Finally, a third group was depleted of NK cells, and the levels of IFN- γ were

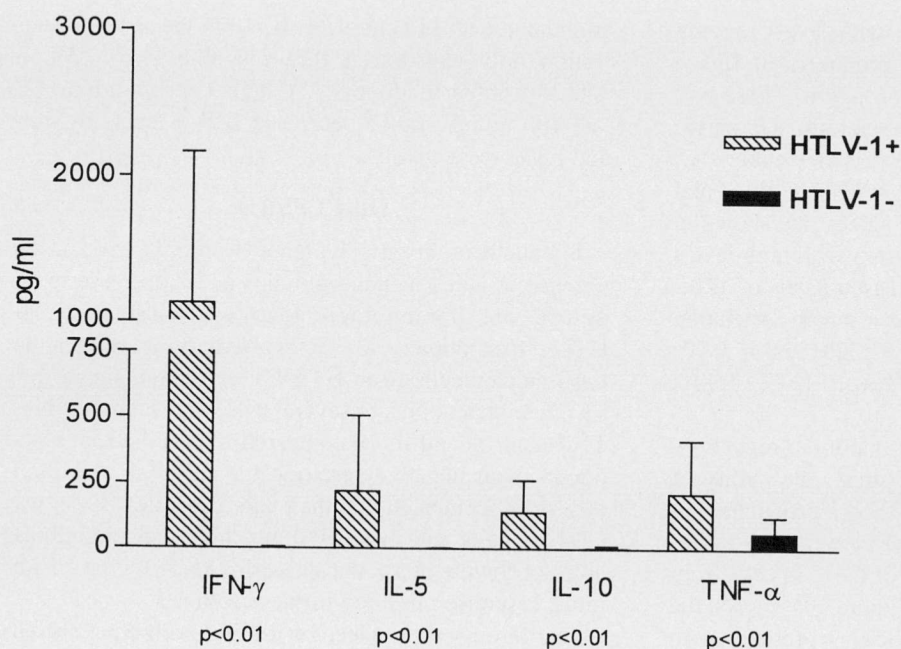


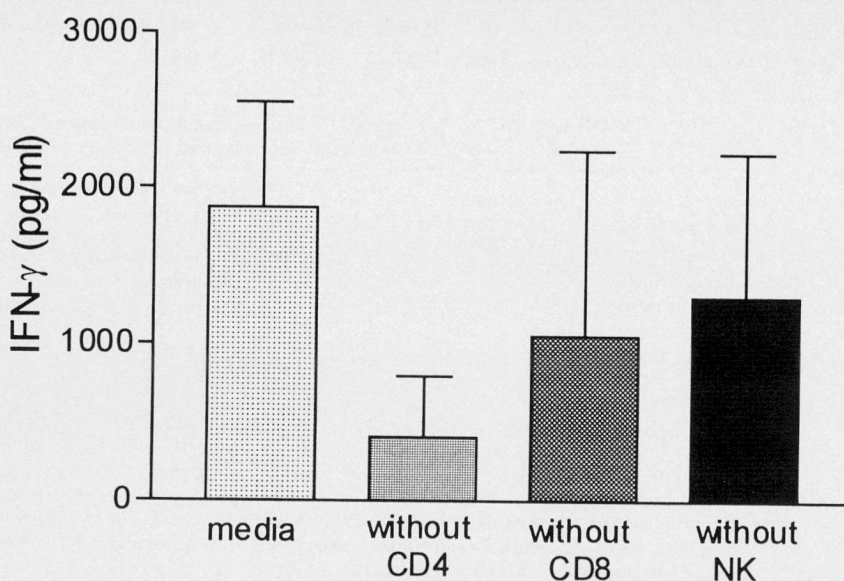
FIG. 1. Cytokine profile (IFN- γ , IL-5, IL-10, and TNF- α) in supernatants of unstimulated mononuclear cells in 26 blood donors with positive serology for human T-lymphotropic virus type 1 (HTLV-1) and 15 healthy subjects not infected with HTLV-1.

measured. Although depletion of CD8⁺ T cells and NK cells decreased IFN- γ levels by 44% and 30%, respectively, CD4⁺ cell depletion decreased IFN- γ production by 78%. The levels of IFN- γ after CD8⁺ and NK depletion were 1093 \pm 1899 pg/ml and 1303 \pm 921 pg/ml, respectively, although depletion of CD4⁺ T cells reduced IFN- γ levels to 405 \pm 388 pg/ml (Fig. 2). To confirm that CD4⁺ T cells were the predominant cell secreting IFN- γ , CD4⁺ T cells and CD8⁺ T cells in 3 patients were positively selected and cultured separately for 72 hours. In these experiments, although the IFN- γ level in the

unseparated population was 2087 \pm 907 pg/ml, in CD4⁺ and CD8⁺ T cells, it was 2045 \pm 890 and 717 \pm 281 pg/ml, respectively.

Although the levels of IFN- γ in all subjects infected with HTLV-1 were higher than those observed in controls, there was great variability in the levels of this cytokine in subjects with positive serology for HTLV-1. With the aim of determining whether the high or low levels of IFN- γ were related to the concentration of Th2 type cytokines such as IL-5 and IL-10, these subjects were divided in two groups according to IFN- γ level:

FIG. 2. Cell source of interferon- γ (IFN- γ) production in asymptomatic blood donors with positive serology for human T-lymphotropic virus type 1. Data represent mean and SD of IFN- γ in unseparated mononuclear cells and in cell populations depleted of CD4⁺ T cells, CD8⁺ T cells and natural killer cells.



high producers of IFN- γ ($n = 15$) with levels ranging from 712 to 2966 pg/ml and low producers of IFN- γ ($n = 11$) with levels lower than 366 pg/ml. These two groups were comparable apart from sex ratio, age, nutritional status, and serology for hepatitis B, hepatitis C, and syphilis (Table 1). The levels of TNF- α , IL-5, and IL-10 in the high and low IFN- γ producers are shown in Table 1. It was observed that the group with high levels of IFN- γ also had significantly increased levels of TNF- α , IL-5, and IL-10. There was also a direct correlation between IFN- γ and IL-5 levels ($p < .0001$; $r_s = 0.73$) and between IFN- γ and IL-10 levels ($p < .0001$; $r_s = 0.79$).

Interleukin-10 is one of the most important regulatory cytokines for human cells. In this study, the ability of exogenous IL-10 to suppress the IFN- γ production of unstimulated PBMCs from HTLV-1 carriers was investigated. Figure 3A shows the effect of IL-10 in cells from HTLV-1 patients ($n = 5$), and Figure 3B shows the ability of IL-10 to downregulate IFN- γ production in cultures from healthy subjects stimulated with PPD ($n = 4$). IFN- γ production in the supernatants of mononuclear cells from 4 HTLV-1-infected donors was 1525 ± 1043 pg/ml. Addition of 2, 20, and 100 ng/ml of IL-10 reduced IFN- γ production to 1187 ± 1136 , 910 ± 966 , and 671 ± 512 pg/ml, respectively. In contrast, in cells from healthy subjects stimulated with PPD, addition of IL-10 at 2 and 20 ng/ml reduced IFN- γ production from 1096 ± 57 to 77 ± 37 pg/ml and 54 ± 46 pg/ml, respectively. Although IL-10 was able to decrease IFN- γ production to some extent, the suppression mediated by IL-10 in cultures from HTLV-1 asymptomatic subjects was lower ($p < .01$) than that observed in cultures from healthy subjects stimulated with PPD. Although IL-10 at a concentration of 2 ng/ml suppressed IFN- γ production in cultures from healthy subjects

stimulated with PPD by 97%, IL-10 in the same concentration only suppressed IFN- γ synthesis by 23% in HTLV-1-infected donors. At high concentrations (20 and 100 ng/ml), IL-10 decreased IFN- γ levels by 40% and 56%, respectively.

DISCUSSION

Spontaneous in vitro lymphocyte proliferation in the absence of an exogenous stimulus and a decrease in apoptosis are immunologic features characteristic of HTLV-1 infection (1,13). In this study, it was shown that unstimulated cells from HTLV-1 asymptomatic carriers secrete high amounts of several cytokines such as IFN- γ , TNF- α , IL-5, and IL-10 compared with cells from blood donors with negative serology for HTLV-1. Additionally, we documented that the major source of IFN- γ was CD4⁺ T cells and that IL-10 at a high concentration is able to downregulate the exacerbated Th1 type of immune response observed in these subjects.

Interferon- γ is produced by different cell types such as CD4⁺ and CD8⁺ T cells and NK cells. Although it has been shown that HTLV-1 infects predominantly T cells (7), the source of the high production of this cytokine in HTLV-1-infected subjects is not clear. This study shows that although depletion of CD8⁺ and NK cells decreases IFN- γ production to some extent, in the absence of CD4⁺ T cells, mononuclear cells produce only small amounts of IFN- γ . These data indicate that although NK and CD8⁺ T cells produce IFN- γ , the CD4⁺ T cells are the major source of IFN- γ in HTLV-1-infected subjects. The important role of the CD4⁺ T cells in the production of IFN- γ in such patients was confirmed when cells were stimulated after positive selection. In this case, the production of IFN- γ by CD4⁺ T cells was similar to the level of this cytokine produced in unseparated mononuclear cells.

TABLE 1. TNF- α , IL-5, and IL-10 levels in asymptomatic human T-lymphotropic virus type 1-infected individuals with high and low production of IFN- γ ^a

Variables	HTLV-1 individuals with high production of IFN- γ ($n = 15$)	HTLV-1 individuals with low production of IFN- γ ($n = 11$)	<i>p</i> value
IFN- γ	1900 \pm 635	75 \pm 107	< .0001
TNF- α	291 \pm 191	107 \pm 171	.005
IL-5	332 \pm 333	69 \pm 86	.001
IL-10	216 \pm 105	32 \pm 39	< .0001
Age	35 \pm 9	36 \pm 9	
Gender (M/F)	4/1	4.5/1	
Stool examination	3/15 (S. mansoni)	2/11 (S. mansoni)	
Positive serology for hepatitis B and C	1/15	1/11	
Positive serology for syphilis	1/15	2/11	

^a Data represent mean and SD of IFN- γ levels in supernatant of unstimulated cultures.

HTLV-1, human T-lymphotropic virus type-1; IFN- γ , interferon- γ ; TNF- α , tumor necrosis factor- α ; IL, interleukin; M, male; F, female.

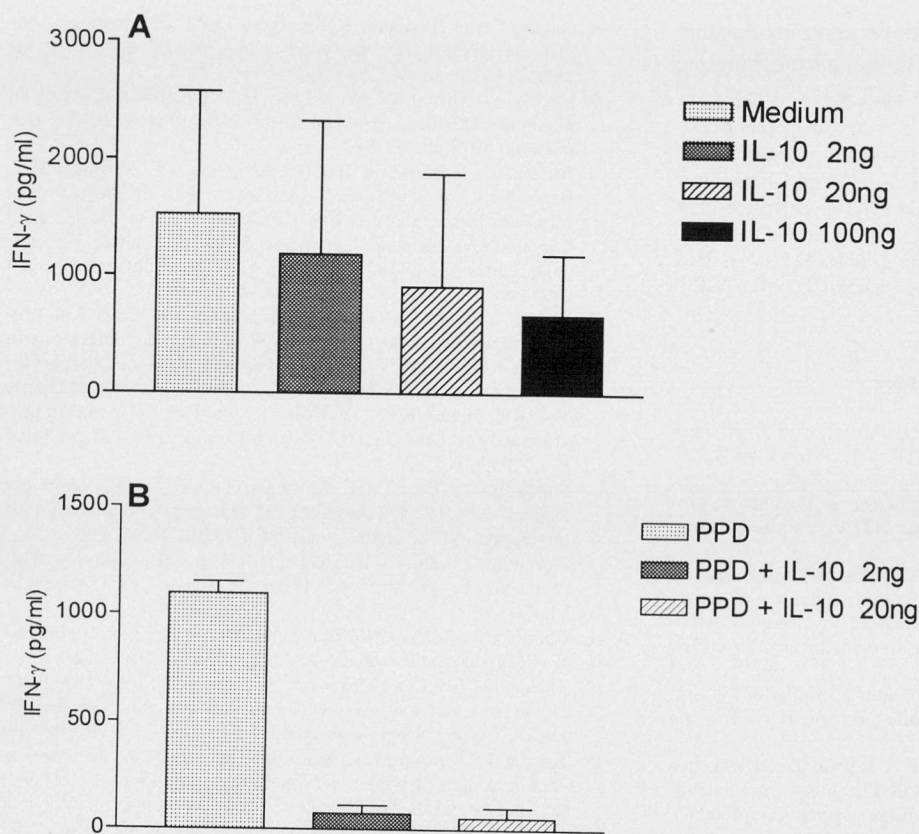


FIG. 3. Reduced ability of IL-10 to modulate IFN- γ production in HTLV-1-infected blood donors and healthy subjects. **(A)** Unstimulated cells from HTLV-1 blood donors ($n = 5$) were cultured for 72 hours in the presence of IL-10 in concentrations of 2, 20, and 100 ng/ml. **(B)** Healthy subjects' mononuclear cells ($n = 4$) stimulated with PPD were cultured in the presence of 2 and 20 ng/ml of IL-10. Data represent mean and SD of IFN- γ .

Although IFN- γ is a Th1 type cytokine, IL-5 and IL-10 are secreted by Th2 cells. It has been shown that as a regulatory mechanism of the immune response, cytokines secreted by Th1 cells may downregulate Th2 cells and vice versa. For instance, IL-4 and IL-10 may downregulate IFN- γ response (14,15), and IFN- γ decreases the secretion of Th2 cytokines (16). Cytokines are not usually detectable in supernatants of unstimulated cultures. In this report, we extend previous observations of high IFN- γ production in unstimulated cultures of HTLV-1-infected donors, showing that other cytokines such as TNF- α , IL-5, and IL-10 are also increased in cell supernatants of HTLV-1-infected subjects. The observation of a direct correlation between IFN- γ and IL-5 levels and between IFN- γ and IL-10 levels suggests that this viral infection, although inducing predominantly IFN- γ production, causes other cytokines to be secreted by unstimulated cells. Previous studies have shown that T-cell clones from HTLV-1-infected donors may be of the Th1 or Th2 type and that Th2 clones tend to secrete IFN- γ rather than IL-4 after in vitro proliferation (17). These observations support our finding of an elevation of IFN- γ , IL-5, and IL-10 in supernatants of cultures and may be one of the explanations why IFN- γ is much more elevated than the other cytokines. Alternatively, the in-

creasing Th2 cytokine levels in cell supernatants from HTLV-1-infected subjects may represent an attempt by the host to downregulate IFN- γ production and lymphocyte proliferation. Taking advantage of the fact that IFN- γ levels were quite variable in the HTLV-1-infected subjects, we classified these individuals in two groups: high and low producers of IFN- γ . The observation that IL-10 and IL-5 levels were higher in the subgroup that had high IFN- γ production than in the subgroup that had low IFN- γ production indicates that IL-10 may come from the same cell that is secreting IFN- γ (e.g., Th0-like cell) or that Th2 cells are also expanded in an attempt to downregulate T-cell activation.

The increased T-cell activation and uncontrolled lymphocyte proliferation in HTLV-1-infected subjects has been associated with the development of diseases (5,18,19). Although the ability of IL-10 to downregulate a Th1 type of immune response in HTLV-1-infected subjects was not as impressive as that observed in cells from healthy subjects stimulated with PPD, IL-10 at higher concentrations was able to downregulate IFN- γ in these patients. Attempts to downregulate T-cell activation in HTLV-1 infection may benefit patients with disease. The observation that exogenous IL-10 at a high concentration is able to decrease IFN- γ production in

HTLV-1-infected subjects suggests to some extent that this cytokine may be one tool to modulate the immune response in subjects infected with HTLV-1.

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