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Mucosal Leishmaniasis Patients Display an Activated Inflammatory T-cell Phenotype Associated with a Nonbalanced Monocyte Population

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Abstract

Leishmania braziliensis is a parasite that can induce at least two clinical forms of leishmaniasis in humans: cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML). In humans, the specific mechanisms that determine which form will develop following infection are not well established. In this study, peripheral blood mononuclear cells from 17 CL and 9 ML patients were compared both *ex vivo* and after culture with soluble leishmania antigen (SLA). Patients with ML presented a higher frequency of activated T cells as measured by *ex vivo* frequencies of $CD4^+CD69^+$, $CD4^+CD28^-$, $CD4^+CD62L^-$ and $CD8^+CD69^+$ than those with CL. Moreover, after stimulation with SLA, patients with ML presented a higher frequency of TNF-α-producing $CD4^+$ and $CD14^+$ cells than CL individuals. While CL patients displayed a positive correlation between the frequency of IL-10 and TNF-α-producing monocytes, the ML patients did not. This lack of a positive correlation between IL-10-producing and TNF-α-producing monocytes in ML patients could lead to a less controlled inflammatory response *in vivo*. These results corroborate with a model of an exacerbated, unregulated, immune response in ML patients and point to key immunomodulatory leucocyte populations and cytokine networks that may be involved in the development of immunopathology in ML patients.

Introduction

Cutaneous leishmaniasis (CL) is characterized by single or multiple well-defined ulcerated dermal lesions. It is the most common form of disease in endemic areas affected by dermatotropic strains of *Leishmania*. About 3% of patients with CL develop mucosal leishmaniasis (ML), months or years after the cutaneous lesions. These patients may present with disfiguration and serious morbidity due to destruction of the oral and nasopharyngeal cavities [1, 2]. The exact mechanisms involved in the formation of the ML clinical form are not understood; however, previous studies from Bacellar *et al.* have shown that ML patients display higher levels of inflammatory cytokines (TNF-α and IFN-γ) and lower IL-10 production as compared with CL patients [3]. Moreover, ML patients show weak modulation by IL-10 and TGF-β [3]. Others have reported that ML patients demonstrated increased IFN-γ as compared with CL patients which is maintained after cure [4].

The existence of an ongoing control of the inflammatory immune response in human CL was shown recently by Antonelli *et al.* where a positive correlation among soluble leishmania antigen (SLA)-specific IFN-γ- and IL-10-producing, or TNF-α- and IL-10-producing, lymphocytes was demonstrated. Moreover, a higher frequency of parasite-specific (anti-SLA or anti-LACK) IL-10-producing lymphocytes was correlated with a lower frequency of TNF-α-producing monocytes demonstrating an antigen-specific delivery of IL-10 for induction of negative regulation of monocyte activity [5]. Some aspects of the human immune response differ from those of mice – for instance, IL-10 is not a Th2-specific cytokine and is produced by several T-cell types, as well as by activated monocytes and macrophages [6]. Accordingly, IL-10 has been enhanced by neutralization of the T helper type 1 (Th1)- and type 2 (Th2)-inducing cytokines, such as IL-4, IL-12 and IFN-γ, influencing T-cell differentiation,

APC functioning and T-cell activation [6–9]. However, further studies of the role of IL-10 in ML disease are necessary.

In mice, it is well established that the Th1 phenotype is important for the outcome of the *Leishmania major* infection [10, 11]. In resistant mice, IFN-γ and TNF-α are keys in activating macrophages to kill *Leishmania* [12]. Susceptibility to *L. major* in mice is associated with Th2 phenotype and the production of IL-10 that impairs efficient macrophage activation and, thus, killing the pathogens [13]. In humans, the mechanisms of susceptibility and resistance are complex due to the balance between pathogenic and protective responses, a complex genetic background, and the diverse immunologic history of the infected individuals. Despite this, it is clear that the most severe clinical forms of leishmaniasis are associated with poor Th1 responses. Visceral and diffuse CL are both associated with high numbers of parasite in the tissue and display a deficient Th1 response [14, 15]. However, the high inflammatory response, production of IFN-γ and TNF-α and the low parasitism associated with ML all suggest that the immune response could be involved in tissue damage [16].

To understand cellular mechanisms that act *in vivo* during the course of leishmaniasis, we performed a comparative analysis using flow cytometry between individuals infected with *Leishmania braziliensis* presenting with either CL or ML disease. An understanding of human immunoregulation and cooperation between inhibitory and inflammatory cytokines in ML will clarify how, and why, some patients develop a more severe clinical form of leishmaniasis. Moreover, determination of the mechanisms involved in the human disease may help in the development of vaccines and/or immunotherapy of human leishmaniasis.

Materials and methods

Patients. Peripheral blood mononuclear cells (PBMC) were analysed from 17 individuals with CL and nine individuals with ML. All CL patients had an initial ulcerated lesion with an onset of 15–180 days as reported by the patient. All ML patients reported a past cured cutaneous lesion, with elapsed times since the first cutaneous lesion between 30 days and 20 years. All individuals were residents of the same endemic area of *L. braziliensis,* Corte de Pedra, in the state of Bahia, Brazil, and agreed to participate in the study following informed consent. All individuals received treatment whether they chose to participate in this study or not. Diagnosis of leishmaniasis was based on dermatological findings, and either positive parasitological examinations or positive skin test for *Leishmania* antigens or both. The blood was drawn immediately before any treatment was initiated. Ethical clearance was obtained from the Federal University of Bahia.

In vitro cultures. PBMC were obtained by separation over Ficoll (Amersham Biosciences, Uppsala, Sweden). A fraction of these cells was submitted to antigen-specific stimulation *in vitro* at 1×10^6 cells/ml in RPMI plus 5% human AB sera. Approximately 200,000 cells were incubated with 10 μg/ml of SLA from L. braziliensis, and after approximately 16 h of culture, 1 μl/ml of brefeldin A (1 mg/ml stock) (Sigma Chemical Co., St. Louis, MO, USA) was added to impair cytokine secretion. After 4 h of incubation, these cells were stained for surface markers and intracellular cytokines as described below.

Ex vivo staining to determine cell profile. After Ficoll PBMC separation, 200,000 cells were incubated with fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- or cychrome (Cy)-labelled antibody solutions for 20 min at 4 °C. After staining, preparations were washed with 0.1% sodium azide PBS, fixed with 200 μl of 2% formaldehyde (Sigma Chemical Co.) in Dubecco's phosphate buffer solution (PBS) (Sigma Chemical Co.) and kept at 4 °C until data were acquired using a FACSCalibur (Becton Dickinson, San Jose, CA, USA). Nonspecific immunoglobulins with the same fluorochrome were used as negative controls. The monoclonal antibodies used were anti-CD4-FITC, anti-CD28-FITC, anti-CD8-FITC, anti-CD14-FITC and anti-CD69-FITC; anti-CD28-PE, anti-CD8-PE, anti-CD4-PE and anti-CD62L-PE; anti-CD8-Cy and anti-CD4-Cy; all obtained from Caltag Laboratories, San Francisco, CA, USA.

Cell surface and single-cell cytoplasmic staining. Surface and intracellular staining were performed as described by Bottrel *et al.* [17]. Briefly, cell cultures were stained for surface markers using FITC-labelled anti-CD4 (Caltag Laboratories) and anti-CD14 (Caltag Laboratories) and Cy-labelled anti-CD8 (Caltag Laboratories) monoclonal antibodies by incubation for 20 min with antibody solutions, followed by washes and fixation using 2% formaldehyde solution. These cells were permeabilized and further stained with PE-labelled anti-IFN-γ, anti-TNF-α or anti-IL-10 (Caltag Laboratories) monoclonal antibodies in a 0.5% saponin solution in PBS. After 15 min, preparations were washed with permeabilization solution and resuspended in PBS and read using a FACSCalibur (Becton Dickinson). In all cases, at least 30,000 gated events were acquired assuring the reliability of positive populations.

Flow cytometry data analysis and statistical analysis. PBMC were analysed for their surface markers and intracellular patterns in a number of ways using the program Cell Quest (Becton Dickinson). The frequencies of positive cells were analysed in using either a lymphocyte gate (R1) or a monocyte gate (R2). All quadrants were set according to the negative population and isotype controls. The subregions were determined making use of confirmed positioning of mononuclear cells based on size and

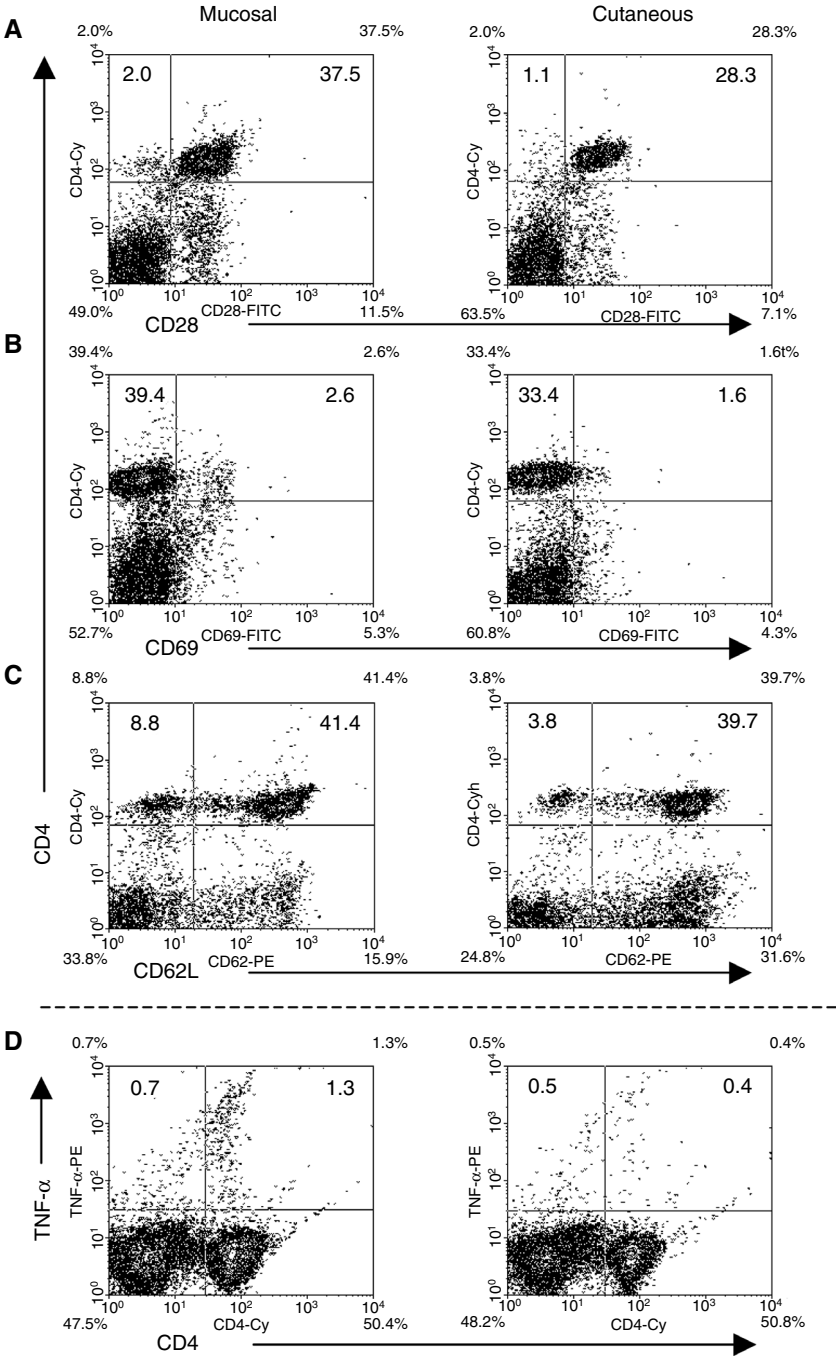

Figure 1 Representative flow cytometry profiles of mucosal and cutaneous leishmaniasis PBMC. (A–C) Dot plots and population frequencies for the indicated cell populations following *ex vivo* evaluation of cell surface markers for costimulatory activation, or homing molecules from mucosal or cutaneous leishmaniasis patients. (D) Dot plots for TNF-α production by lymphocytes following 20 h of culture with SLA as described in the section entitled 'Materials and methods'. Cy, cychrome; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PBMC, peripheral blood mononuclear cells.

granularity profiles, and with specific markers for T cells and monocytes. Analyses were performed comparing subpopulations between CL and ML patients (Fig. 1A–D). Individual data files were analysed blindly and decoded at the time the numbers were transferred to the spreadsheet for statistical analysis. The JMP 5 statistical program, from SAS Institute Inc. (Cary, NC, USA), was used to analyse the samples. Correlation analyses were performed by Spearman's test, and differences in mean were performed using Student's t-test. The results were considered significant when the P-value was ≤ 0.05.

Results

ML patients display increased T-cell activation as compared with CL patients

To compare T-cell activation between ML and CL patients, the expression of total CD4 and CD8 T cells, as well as the expression of CD69 (early activation marker), CD62L (a homing molecule) and CD28 (a costimulation molecule), was determined, *ex vivo* (Fig. 1A–C). ML patients demonstrated a higher frequency of $CD4^+$

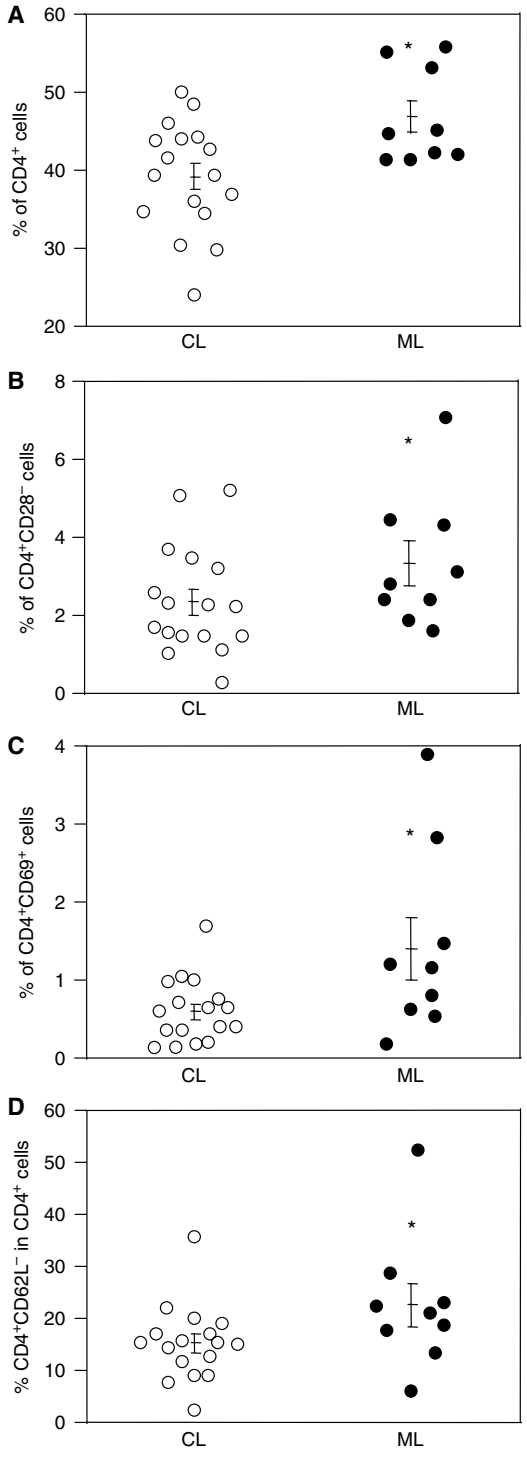

Figure 2 ML patients have a higher frequency of activated T cells than CL patients in PBMC upon *ex vivo* evaluation. (A) Percentage of total $CD4^+$ T cells; (B) frequency of $CD4^+CD28^-$ T cells; (C) frequency of $CD4^+CD69^+$ T cells; (D) frequency of $CD4^+CD62L^-$ T cells. The bars show the mean with SD. All differences were statistically significant (*), with a $P < 0.05$ using Student's *t*-test. The (*n*) was 17 CL patients and nine ML patients. CL, cutaneous leishmaniasis; ML, mucosal leishmaniasis; PBMC, peripheral blood mononuclear cells.

T cells (Fig. 2A), but not of $CD8^+$ T cells, as compared with CL patients. These results are mirrored by a higher frequency of $CD4^+CD28^-$ T cells in ML when compared with CL (Fig. 2B). Analysis of T-cell activation demonstrated that ML patients show an increased frequency of $CD4^+$ $CD69^+$ T cells compared with CL patients (Fig. 2C). Moreover, ML patients displayed a higher frequency of the $CD4^+$ T cells displaying the $CD62L^-$ phenotype, a marker that is often used as an indicator of previous T-cell activation [18], as compared with CL patients (Fig. 2D), while no difference was seen in the frequency of $CD8^+CD62L^-$ T cells. The overall percentage of $CD8^+$ T cells between ML and CL individuals was equivalent (data not shown), while the frequency of activated $CD8^+$ T cells measured through CD69 expression was increased in the ML patients as compared to that in the CL individuals (data not shown).

The balance of $CD4^+$ T-cell subpopulations differ in ML and CL patients

To gain a better picture of possible differences in the balance of T-cell subpopulations between ML and CL patients, correlation analyses were performed between total T-cell population frequencies and subpopulation frequencies. Both clinical forms presented positive correlations between the frequency of total $CD4^+$ versus $CD4^+CD28^-$ T cells (Fig. 3A,B). By contrast, while CL patients showed a positive correlation between $CD4^+$ versus $CD4^+CD62L^+$ T cells (Fig. 3C), ML patients showed a positive correlation between higher frequencies of $CD4^+$ T cells and $CD4^+CD62L^-$ T cells (Fig. 3D). Finally, CL and ML patients showed positive correlations between the frequencies of total CD8+ T cells versus $CD8^+CD28^-$ T cells (Fig. 3E,F) and versus $CD8^+CD62L^+$ T cells (Fig. 3G,H). No correlation was observed between the frequencies of total $CD8^+$ T cells and $CD8^+CD62L^-$ in $CD8^+$ T cells in ML patients, in contrast to the $CD4^+$ T-cell population (Fig. 3).

Thus, while $CD4^+$ T cells from CL individuals demonstrate a positive correlation with increased frequencies of $CD62L^+$ T cells, ML individuals showed a correlation with $CD62L^-$ T cells indicating a bias towards previously activated T cells in this clinical form.

ML patients display a higher frequency of antigen-specific TNF-α-producing cells than CL patients

Given that IFN-γ and TNF-α are important cytokines involved in *Leishmania* elimination, and the fact that previous findings had demonstrated a higher production of these cytokines in ML than in CL patients by ELISA, the frequency of T cells producing IFN-γ and TNF-α was compared between the two groups following SLA

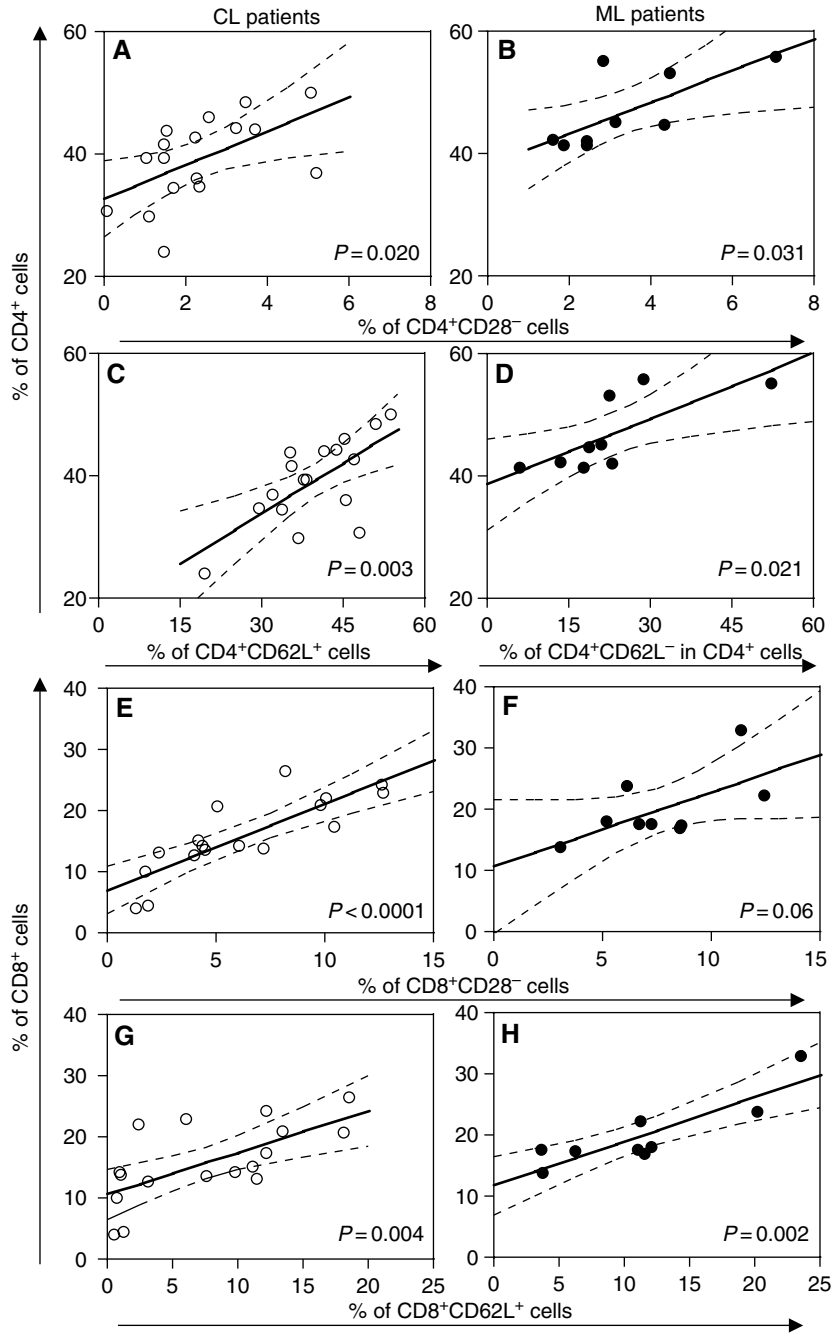

Figure 3 *Ex vivo* phenotype correlations between T-cell subpopulations in CL and ML patients. (A) Correlation between total frequency of $CD4^+$ T cells with $CD4^+CD28^-$ T cells in CL patients; (B) correlation between total frequency of $CD4^+$ T cells with $CD4^+CD28^-$ T cells in ML patients; (C) correlation between total frequency of $CD4^+$ T cells with $CD4^+CD62L^+$ T cells in CL patients; (D) correlation between total frequency of $CD4^+$ T cells with $CD4^+CD62L^-$ in $CD4^+$ T cells in ML patients; (E) correlation between total frequency of $CD8^+$ T cells with $CD8^+CD28^-$ T cells in CL patients; (F) correlation between total frequency of $CD8^+$ T cells with $CD8^+CD28^-$ T cells in ML patients; (G) correlation between total frequency of $CD8^+$ T cells with $CD8^+CD62L^+$ T cells in CL patients; (H) correlation between total frequency of $CD8^+$ T cells with $CD8^+CD62L^+$ T cells in ML patients. Positive correlations were considered significant when the $P < 0.05$ using Spearman's correlation test. The (*n*) used was 17 patients with CL and nine patients with ML. CL, cutaneous leishmaniasis; ML, mucosal leishmaniasis.

stimulation. In humans, T cells can produce IFN-γ, while T cells and monocytes can produce TNF-α. Thus, analysis was performed using different populations: monocytes (R2) and lymphocytes (R1), as described in the section entitled 'Materials and methods' (Fig. 1D). Patients with ML displayed a significantly higher frequency of TNF-α-producing lymphocytes than CL patients (Figs 1D and 4A). Moreover, the frequency of TNF-α-producing $CD4^+$ T cells was also higher in ML patients compared to that in CL patients (Fig. 4B). There were no significant differences between the frequencies of $CD8^+$ T cells producing TNF-α in CL and ML patients (data not shown). Moreover, the frequency of TNF-α-producing $CD14^+$ monocytes in ML patients was significantly higher than that seen in CL patients (Fig. 4C).

There were no significant differences between total IFN-γ produced by lymphocytes from CL patients and that produced by lymphocytes from ML patients. Moreover, differences within $CD4^+$ and $CD8^+$ T-cell populations producing IFN-γ were not seen when comparing cell frequencies from CL and ML patients (data not shown).

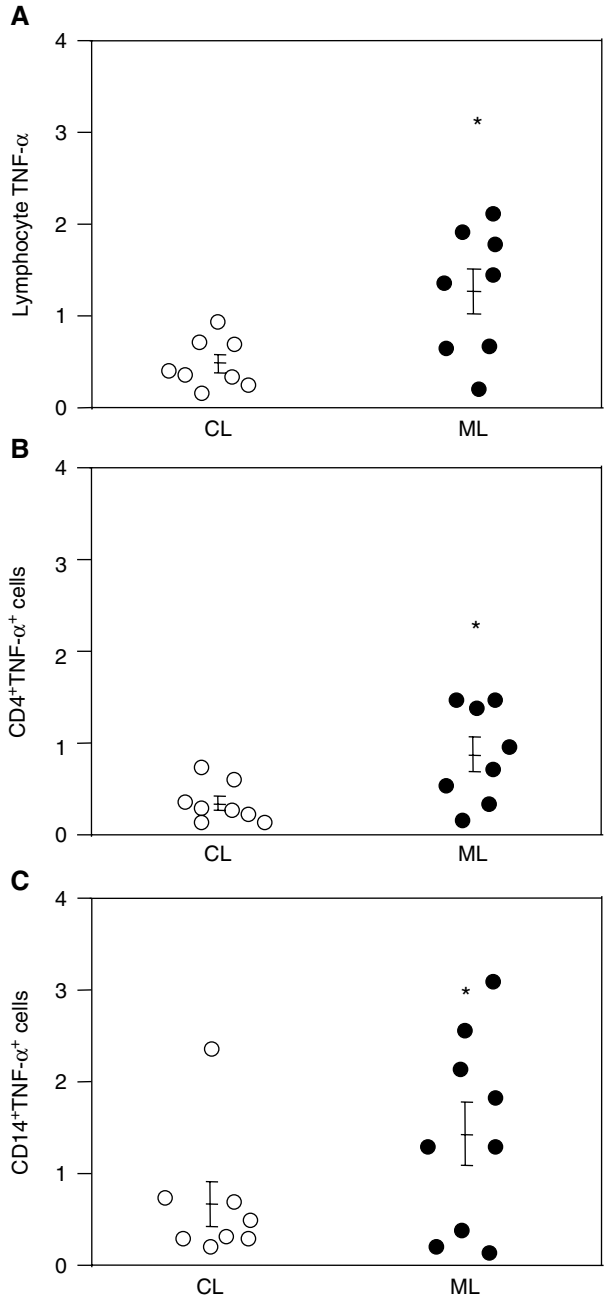

Figure 4 ML patients show an increase of TNF-α-producing cells as compared with CL patients after SLA overnight stimulation. (A) Total frequency of TNF-α-producing lymphocytes; (B) percentage of TNF-α-producing $CD4^+$ T cells; (C) total frequency of TNF-α-producing $CD14^+$ monocytes. The bars represent the mean with SD. Statistical analyses were performed using Student's *t*-test, and differences were considered significant with $P < 0.05$ (*). The (*n*) used was eight patients with CL and nine patients with ML for A–B; eight patients with CL and nine with ML for C. CL, cutaneous leishmaniasis; ML, mucosal leishmaniasis; SLA, soluble leishmania antigen.

Both ML and CL patients display cooperation between inflammatory cytokine-producing $CD4^+$ T-cell subpopulations

Correlation analyses were performed between inflammatory cytokine-producing cell populations from both ML and CL patients to generate models for immunoregulation. Previous studies by us using this approach determined that there was a strong coregulation of inflammatory cytokine-producing cell populations from CL individuals [5].

After SLA activation, both CL and ML patients displayed a positive correlation between the total frequency of IFN-γ-producing lymphocytes and that of TNF-α-producing lymphocytes (Fig. 5A and 5B, respectively). Moreover, a positive correlation was seen within the $CD4^+$ T-cell population in both CL and ML patients (Fig. 5C and 5D, respectively). However, neither CL nor ML patients demonstrated a positive correlation among $CD8^+$ T cells producing IFN-γ and TNF-α (data not shown).

Monocytes from ML patients do not show coregulation of TNF-α- and IL-10-producing populations as seen in CL patients

Given the importance of endogenously produced, antigen-induced IL-10 in the control of inflammatory immune responses and monocyte activity in human CL [5], correlation analyses were performed between IL-10-producing and TNF-α-producing cell populations in both CL and ML individuals.

As previously shown by us, the CL individuals demonstrated a positive correlation between the frequency of TNF-α- and IL-10-producing monocytes (Fig. 5E), while the ML patients did not demonstrate positive correlation (Fig. 5F).

Discussion

The complex interactions between cell subpopulations and cytokines that lead to the development of protective versus pathologic immune responses in human disease are an area of intense study. The elucidation of the cell populations and cytokines involved in the immune response against *Leishmania* in human leishmaniasis becomes critical when considering immune-based treatments and/or vaccine development. Recent reports have hypothesized that ML is caused, in part, by an exacerbated host immune response characterized by an unregulated Th1 response [1, 2]. This is supported by findings that point to a higher production of TNF-α and IFN-γ in these patients [3], as well as studies demonstrating the coordinate regulation of inflammatory cytokines by IL-10 in human CL patients [5].

Several cell subpopulations are involved in the immune response against *Leishmania* including lymphocytes and monocytes that are activated by inflammatory cytokines to produce free radicals involved in killing intracellular parasites [19]. Lymphocyte and monocyte subpopulations can be identified by a number of surface markers related to activation, homing and costimulation, as well as through

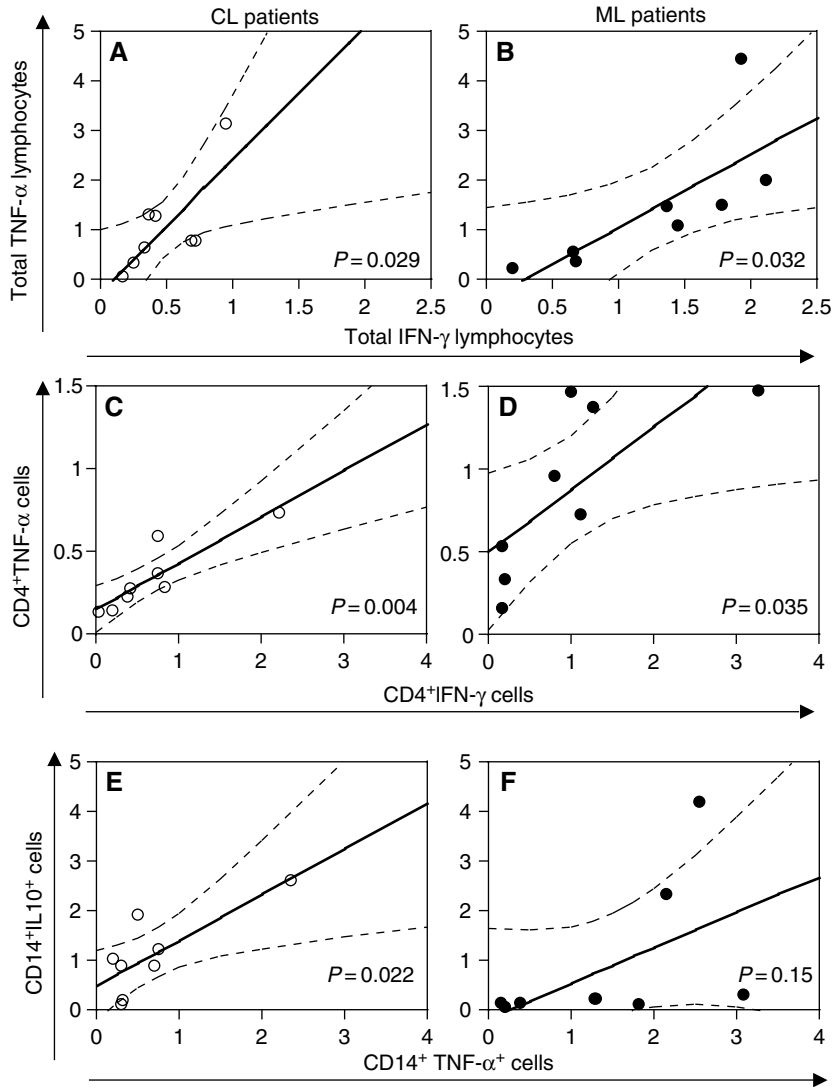

Figure 5 Correlation between cytokine-producing cell populations following SLA stimulation. (A and B) Correlation between total IFN-γ versus total TNF-α both from lymphocytes in CL and ML, respectively; (C and D) correlation between IFN-γ-producing $CD4^+$ T cells versus TNF-α-producing $CD4^+$ T cells in CL and ML, respectively; (E) correlation between IL-10-producing $CD14^+$ monocytes versus TNF-α-producing $CD14^+$ monocytes in CL patients; (F) correlation between IL-10-producing $CD14^+$ monocytes versus TNF-α-producing $CD14^+$ monocytes in ML patients. PBMC were stimulated with SLA overnight as described in the section entitled 'Materials and methods'. Correlations were considered significant when the $P < 0.05$ using the Spearman's correlation test (*). The (*n*) used was eight patients with CL and eight with ML. CL, cutaneous leishmaniasis; ML, mucosal leishmaniasis; PBMC, peripheral blood mononuclear cells; SLA, soluble leishmania antigen.

the production of specific cytokines. Through a comparative analysis of the relationships between lymphocyte subpopulations in different clinical forms of leishmaniasis, this work aims to clarify the possible mechanisms of immunoregulation of the immune response in infected individuals.

CD62L was first demonstrated to be involved in the homing of T cells to the lymph nodes; however, after passage through the node, they can lose this molecule upon activation [18]. However, it was also demonstrated that half of human T cells expressing an activated phenotype continue to express CD62L [18]. Moreover, in CL caused by *Leishmania guyanensis*, it was demonstrated that IFN-γ-producing $CD8^+$ T cells are $CD62L^-$, while LACK-reactive IL-10-producing $CD4^+$ T cells are $CD62L^+$ [20]. Costa *et al.* recently demonstrated that in CL patients, stimulation with SLA induced an increase of $CD4^+CD62L^+$ T cells [21]. Thus, the literature data – together with the findings in the current work that ML patients display a higher frequency of $CD4^+CD62L^-$ T cells within the CD4 T-cell population – may demonstrate an exacerbated inflammatory immune response in ML patients. Thus, the strong correlation between increased frequency of total $CD4^+$ T cells and the frequency of $CD4^+CD62L^-$ T cells within the $CD4^+$ T-cell compartment only in the ML patients (Fig. 3D) indicates a homeostasis in ML patients that may be related to the pathology seen in these patients. Further studies designed to investigate these populations in the lesions are underway.

Several indicators of lymphocyte activation such as the higher frequency of $CD4^+CD69^+$, $CD8^+CD69^+$ and $CD4^+CD28^-$ T cells in the ML as compared to CL patients support previous work, demonstrating that ML patients display a hyperactivated state as compared with CL individuals [3, 22, 23]. This increased frequency was also seen within the $CD4^+$ T-cell population indicating an increased commitment of the $CD4^+$ T cells towards an activated state (Fig. 2) (data not shown). The increase in

the $CD4^+CD28^-$ T-cell population also indicates a bias in chronically activated T cells and may indicate a subpopulation of T cells capable of promiscuous cell lyses and independent of costimulation [24]. Recently, our group demonstrated, in Chagas' disease, an increased frequency of $CD4^+CD28^-$ and $CD8^+CD28^-$ T cells [25]. Moreover, a positive correlation between $CD4^+CD28^-$ T cells with total TNF-α in cardiac patients and with IL-10 in indeterminate chagasic patients was seen [26]. The fact that Chagas' disease is also an inflammatory disease caused by an intracellular trypanosomatid may account for some of the similar aspects in the cellular mechanisms seen in the two diseases.

Inflammatory cytokines play a critical role in activation of monocytes for killing intracellular pathogens but when uncontrolled may lead to the formation of pathogenic immune reactions leading to excessive tissue damage. Thus, the present study was designed to gain insight into these cellular mechanisms of immunoregulation in human leishmaniasis. The study results demonstrated a clear increase in the frequency of total leucocytes producing TNF-α following stimulation with SLA in the ML patients as compared to CL (Fig. 4A). This increase was accounted for a higher frequency of both $CD4^+$ T cells (Fig. 4B) and $CD14^+$ monocytes (Fig. 4C). These studies corroborate with others from Bacellar *et al.* demonstrating higher levels of secreted TNF-α [3]. Interestingly, when analysing the frequency of IFN-γ-producing cells in ML and CL patients, no significant difference was seen. However, this is in contrast to what was seen for secreted cytokines [3]. This difference may be due to the presence of a high cytokine-secreting population in ML, which is not present at a higher frequency, but produces higher quantities of the cytokine.

In both CL and ML patients, there was a clear correlation between the frequency of antigen-specific TNF-α- and IFN-γ-producing total lymphocytes (Fig. 5A,B). Also, a positive correlation was seen within the $CD4^+$ T-cell population demonstrating a coregulation of the frequencies of TNF-α- and IFN-γ-producing antigen-specific $CD4^+$ T cells (Fig. 5C,D). Interestingly, the same correlation was not seen for the $CD8^+$ T-cell population (data not shown). The lack of a coregulation of cytokine-producing cells in the $CD8^+$ T-cell population suggests that the CD8 population may differentiate into cytotoxic effector cells with little cytokine production. Finally, it may also reflect past findings that $CD4^+$ T cells rather than $CD8^+$ T cells are the major source of IFN-γ- and TNF-α-producing cells in CL [17, 27].

In attempts to determine the role of IL-10-producing cells in the control of the inflammatory response in CL and ML, correlative analysis was performed between IL-10-producing subpopulations and TNF-α-producing populations. The earlier study by Antonelli *et al.* demonstrated a positive correlation between TNF-α-producing monocytes and IL-10-producing monocytes in CL patients [5]. This same correlation was seen again here for the CL patients (Fig. 5E), but interestingly, the ML patients did not show this same coregulation (Fig. 5F). The lack of regulatory IL-10-producing monocytes increasing together with the TNF-α-producing monocytes could, in part, contribute to an exacerbated inflammatory response in ML patients. By contrast, ML patients demonstrated a positive correlation between IL-10-producing $CD8^+$ T cells and TNF-α-producing $CD8^+$ T cells (data not shown). Thus, these distinct immunoregulatory networks could help explain the different outcomes in CL and ML disease. In CL, a controlled inflammatory immune response may lead to destruction of the parasite, followed by control of the inflammatory immune response. By contrast, in ML patients, this balance mechanism is not present in the monocyte population and thus, may be less effective at controlling the inflammation. Interestingly, even in CL disease, a higher frequency of inflammatory cytokine-producing lymphocytes is associated with larger lesions indicating their pathogenic potential [28].

Summarizing, the current study identifies key differences in the activation state of the immune response in CL and ML as well as points to a lack of the coregulated production of IL-10 and TNF-α in the monocyte population in ML patients indicating a possible defect in the immunoregulation of inflammatory responses in these individuals.

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