The skin homing receptor cutaneous leucocyte-associated antigen (CLA) is up-regulated by Leishmania antigens in T lymphocytes during active cutaneous leishmaniasis

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Introduction

Cutaneous leishmaniasis (CL) is caused by many Leishmania species, but differences in clinical, diagnostic and therapeutic aspects of the disease points to intrinsic characteristics of the parasite influencing the pathogenesis [1]. CL due to Leishmania (Viannia) braziliensis is the most common clinical form of leishmaniasis observed in Brazilian patients, characterized by a single or a few localized skin lesions [2]. The skin lesions are characterized by a chronic granulomatous inflammatory infiltrate consisting mainly of lymphocytes, plasmocytes and histiocytes [3]. Memory T lymphocytes predominate in lesions [4], but the proportions of T CD4+ and T CD8+ cells are variable [5,6], suggesting that these T cell subsets can have distinct requirements for their recruitment to lesions.

Several reports have suggested that a huge influx of T cells takes place in lesions [4,7,8], as not only is a higher frequency observed of local lymphocytes responding to L. braziliensis in comparison with blood [9], but also an increased proportion of T CD4+ and T CD8+ cells is reported in older lesions [5,6]. Although the effector T cell response mounted in response to the parasite can lead to a cure, it also seems to be responsible for tissue damage [10,11]. However, even considering the importance of lymphocyte traffic to the formation of inflammatory infiltrates, few reports have addressed the mechanisms underlying the cell-homing to leishmaniasis lesions [12–15].

Lymphocyte migration is a complex process involving many molecules that direct cells specifically to an appropriate compartment. Selectins and integrins are families of adhesion molecules that not only mediate the migration process towards lymphoid organs (p.ex. CD62L), but also direct cells to inflamed tissues through the endothelial epithelium [16]. For access to the skin compartment, T cells utilize a skin ‘code’ named the cutaneous leucocyte-associated antigen receptor (CLA). CLA is a member of the selectin family that is expressed in memory T cells and binds

Summary

The cutaneous leucocyte-associated antigen receptor (CLA) can direct Leishmania-specific T lymphocytes towards inflamed skin lesions. Homing receptors [CLA, lymphocyte-associated antigen 1 (LFA-1) or CD62L] were analysed in lymphocytes from blood and cutaneous leishmaniasis (CL) lesions. CL patients with active lesions (A-CL) presented lower levels of T lymphocytes expressing the CLA+ phenotype (T CD4+ = 10·4% ± 7·5% and T CD8+ = 5·8% ± 3·4%) than did healthy subjects (HS) (T CD4+ = 19·3% ± 13·1% and T CD8+ = 21·6% ± 8·8%), notably in T CD8+ (P < 0·001). In clinically cured patients these percentages returned to levels observed in HS. Leishmanial antigens up-regulated CLA in T cells (CLA+ in T CD4+ = 33·3% ± 14·1%; CLA+ in T CD8+ = 22·4% ± 9·4%) from A-CL but not from HS. An enrichment of CLA+ cells was observed in lesions (CLA+ in T CD4+ = 45·9% ± 22·5%; CLA+ in T CD8+ = 46·4% ± 16·1%) in comparison with blood (CLA+ in T CD4+ = 10·4% ± 7·5%; CLA+ in T CD8+ = 5·8% ± 3·4%). Conversely, LFA-1 was highly expressed in CD8+ T cells and augmented in CD4+ T from peripheral blood of A-CL patients. In contrast, CD62L was not affected. These results suggest that Leishmania antigens can modulate molecules responsible for migration to skin lesions, potentially influencing the cell composition of inflammatory infiltrate of leishmaniasis or even the severity of the disease.

Keywords: cutaneous leucocyte-associated antigen (CLA), homing molecules, L-selectina (CD62L), leishmaniasis lesions, T lymphocytes
to the E-selectin receptor on endothelial cells of inflamed skin [17]. CLA is found in 10–25% of circulating CD3+CD45RO+ T cells, as well as in other blood cells such as neutrophils, monocytes and dendritic cells [18,19]. CLA-E-selectin is the first adhesion step in migration, followed by very late antigen-4 (VLA-4) and lymphocyte-associated antigen 1 (LFA-1) attachment to activated endothelium and further lymphocyte transmigration to inflamed skin [20,21]. CLA has been recognized as one of the most important skin-homing molecules and its expression in activated lymphocytes can affect cell composition of the inflammatory infiltrate. Consequently, CLA has been associated with the pathogenesis or severity of many inflammatory skin diseases, such as atopic dermatitis [22], psoriasis [23] and leprosy [24].

Molecules involved in the adhesion of Leishmania-infected macrophages [25] or recruitment of effector lymphocytes to the skin can potentially influence the magnitude of immunopathological process in response to the infection [6,24,26,27]. In situ analyses have shown high levels of LFA-1α- and LFA-1β-positive cells in the dermis of leishmaniasis patients, although expressed differentially in CL and diffuse cutaneous leishmaniasis [28]. High percentages of activated cells expressing CD62Lhigh, LFA-1α and LFA-1β are found in peripheral blood in comparison to the lymph nodes of CL patients in the early stages of the disease, suggesting that these cells are available for recruitment to lesion sites [15]. The percentage of skin cells presenting CLA is very low, comprising less than one-third of the cells from CL [14,29], which is surprising considering the high percentage of memory T cells in the inflammatory infiltrate [4]. Differences in the expression of migration molecules in CD4+ and CD8+ T cells indicate that there are specific requirements for the homing of these cells to a leishmaniasis lesion [13,15,26]. However, it is not known whether CD4+ or CD8+ T cells express CLA differentially in leishmaniasis lesions and blood.

Considering the importance of CLA in directing activated T cells to skin lesions, we performed flow cytometry studies to investigate their relationship on the frequency of this molecule on T cell subsets in blood and leishmaniasis lesions. Our hypothesis was that leishmanial antigens stimuli can up-regulate CLA, facilitating the homing of Leishmania-specific T lymphocytes towards the inflammatory infiltrate of cutaneous leishmaniasis. We believe that differences in the expression of CLA in T CD4+ or T CD8+ can affect the T cell composition of leishmaniasis lesions.

Materials and methods

Patients

Thirty-one CL patients [22 male and nine female; mean age ± standard deviation (s.d.) 39.4 ± 16 years old] and eight healthy subjects (HS, four male, four female) were studied. Patients had acquired the disease in endemic areas for L. braziliensis infection in Rio de Janeiro, Brazil. The diagnosis of active cutaneous leishmaniasis (A-CL) was confirmed by clinical, parasitological and immunological criteria as described elsewhere [30]. Patients were treated with pentavalent antimonials (N-methyl-glucamine), according to the guidelines of the Brazilian Ministry of Health, and then followed-up. Patients were grouped as follows: active disease (A-CL; n = 16), at the end of therapy (early cured CL; ECCL; n = 8) and 1 year after the end of therapy (late cured CL; LCCL; n = 11). Four patients were follow-up ECCL and LCCL. Blood was drawn after informed consent was obtained from each subject. All procedures were approved by the Ethical Committee of the Fundação Oswaldo Cruz and of the IPEC, Ministério da Saúde, Rio de Janeiro, Brazil.

Acquisition of mononuclear cells from blood and leishmaniasis lesions

Peripheral mononuclear blood cells (PBMC) were separated by centrifugation over a gradient of Ficoll–Hypaque (Histopaque 1077; Sigma Chemical Company, St Louis, MO, USA). Cells were resuspended in RPMI-1640 supplemented with 10% heat-inactivated human antibody Rh+ serum, 10 mM HEPES, 1.5 mM l-glutamine, 0.04 mM 2-mercaptoethanol, 200 IU of penicillin per ml and 200 μg of streptomycin per ml (RPMI supplemented; Sigma). PBMCs were adjusted to 3 x 10⁶/ml and processed for phenotypical analyses of surface molecules or in vitro stimulation with Leishmania antigens. An incisional biopsy from the skin lesion border was performed for diagnostic purposes, and part of the tissue fragment was separated for cell extraction. Mononuclear cells from leishmaniasis lesions (LeMC) were obtained as described elsewhere [6]. In brief, the skin specimen, stripped of subcutaneous fat, was placed into a tissue sieve, fitted with a 64 μm mesh filter and containing RPMI supplement. The cells were separated mechanically using a stick. The single-cell suspension was washed once and the mononuclear cells separated by centrifugation over a Ficoll–Hypaque gradient (Histopaque 1077; Sigma). LeMCs (10⁶/ml) were resuspended in cold phosphate-buffered saline (PBS) containing 0.01% sodium azide (NaN₃; Sigma) and 10% fetal bovine serum (PBSAz/FBS), and processed for phenotypical analysis.

In vitro stimulation of PBMC with Leishmania braziliensis antigens

PBMC (3 x 10⁶ in a final volume of 2 ml) were cultured in 24-well flat-bottomed plates (Nunc, Roskilde, Denmark) with medium alone or in the presence of 5 x 10⁶ disrupted L. (V.) braziliensis promastigotes (MHOM/BR/75/M2903). Cells were incubated for 5 days at 37°C in a humidified atmosphere of 5% CO₂. L. braziliensis-stimulated T cells were washed and adjusted to 3 x 10⁴/ml in PBSAz/FBS for phenotypical analysis.
Phenotypical characterization of T cell subsets and surface molecules related to migration

Ex-vivo PBMC, Leishmania-stimulated PBMC and ex-vivo LeMC (10^6 cells in 200 μl of PBSA/z/BSA) were incubated for 30 min at 4°C in the presence of 5 μl of fluorescein isothiocyanate (FITC), phycoerythrin (PE) or tandem conjugate phycoerythrin–cyanine 5 (PC5)-labelled monoclonal antibodies. After incubation, the cells were washed in PBSA/z/BSA and resuspended in a fixing solution containing 1% paraformaldehyde in PBS prior to the analysis. Monoclonal antibodies for anti-CD3, -CD4, -CD8 and -CD11a (LFA-1) labelled with PC5, PE or FITC (Immunotech, Beckman Coulter Corporation, Marseille, France); anti-CLA and -CD62L labelled with PE or FITC (Becton Dickinson Bioscience Pharmingen, Franklin Lakes, NJ, USA). Three-colour cytfluorimetry protocols were created for each sample, including: CD3-PC5/CD4-FITC/CD8-PE and CD4, CD8 and one homing molecule (CLA, CD62L or CD11a). For flow cytometry analysis, 10,000 events per sample were acquired in a fluorescence activated cell sorter (FACS-Calibur flow cytometer, Becton Dickinson Bioscience). Surface molecules were analysed for total lymphocytes or in gates defined electronically in T CD4+ and in T CD8+ cell populations using Summit 4.3 software (DakoCytomation, Fort Collins, CO, USA). The total lymphocyte gate (R1) was settled based on size (forward scatter: FSC) and granularity (side-scatter: SSC). Positive cells were defined (or gated) based on the control sample with isotype antibodies. T cell subtypes (CD4+ or CD8+) were defined in lymphocyte gated cells. The frequency of positive CLA, CD62L or CD11a was determined in this positive T cell subpopulation. For Leishmania-stimulated T cell analysis, the lymphocyte gate was expanded and defined to also cover the blast population with higher FSC and SSC. The results were expressed as a percentage of positive cells and mean fluorescence intensity.

Statistical analysis

Statistical analysis was performed by the two-tailed Mann–Whitney and Kruskal–Wallis tests using the GraphPad Prism software version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). The results were expressed as the mean ± s.d. and median.

Results

CLA, CD62L and LFA-1 molecules in blood T lymphocytes from cutaneous leishmaniasis patients and healthy volunteers

To determine whether leishmaniasis alters the expression of homing molecules in circulating lymphocytes, CLA (skin homing), CD62Lhigh (lymph node homing) and CD11a (LFA-1, adhesion molecule to inflamed endothelium) phenotypes were evaluated ex vivo in the total lymphocyte population and in CD4+ or in CD8+ T cell subpopulations. For the CLA phenotype, CL patients were evaluated with the active disease (A-CL), at the end of therapy (ECCL) and long-term clinically cured (LCCCL).

In A-CL patients, the CLA+ phenotype was significantly higher among T CD4+ (10.4% ± 7.5%, median = 9.6%) than T CD8+ cells (5.8% ± 3.4%, median = 5.2%), but this difference was not observed in HS (T CD4+: 19.3% ± 13.1%, median = 14.6%; T CD8+: 21.6% ± 8.8%, median = 22.1%) (Fig. 1). Moreover, the CLA+ phenotype was significantly lower in A-CL patients for both T cell subsets in comparison with HS (Fig. 1). This is notably a more expressive decrease of CD8+ T cells displaying CLA molecules (P < 0.001, Fig. 1). These low levels of T cells presenting CLA were maintained even at the end of therapy (ECCL) for both T cell subsets (Fig. 4a and b). An increase in the percentage of T CD4+CLA+ (P < 0.01) was seen only long-term after therapy, when the proportions of these cells were comparable to HS. Although LCCL showed an increase of CD8+ T cells displaying the CLA+ phenotype, these levels were still lower than those observed in HS (P < 0.01; Fig. 4b). These results show that CD8+ rather than CD4+ T cells presenting CLA are reduced in blood during active leishmaniasis. These lower levels could be caused by the down-modulation of CLA in T cells or the migration of CLA+ T lymphocytes to the lesions.

A-CL and HS showed a similar mean percentage of the CD62Lhigh phenotype not only among CD4+ T cells (A-CL = 6.6% ± 5.5%, median = 5.3%; HS = 6.8% ± 2.8%, median = 7.5%), but also in CD8+ T cells (A-CL = 8.2% ± 5%, median = 7.9%; HS = 8.6% ± 4.9%, median = 7.8%). Indeed, no significant difference regarding the CD62Lhigh phenotype was observed among T cell subsets.
Higher percentages of the CD11a (LFA-1) phenotype were detected among total lymphocyte population in A-CL (60.8% ± 19.3%, median = 56.1%) in comparison with HS (45.3% ± 10.6%, median = 46.1%). This difference was detected markedly among CD4+ T cells, as CD4+CD11a+ T cells were detected more in A-CL (51.3% ± 21.3%, median = 45.1%) than in HS (30.1% ± 11.8%, median = 30.9%). By contrast, similar levels of CD8+ T cells displaying CD11a were seen in both A-CL (80.1% ± 13.4%, median = 78.6%) and HS (75.8% ± 18.6%, median = 83.6%). However, up-regulation of CD11a+ was observed only in CD4+ T and not in CD8+ T cells during infection, reinforcing the idea that the disease is related to a differential homing profile in these two lymphocyte populations (data not shown).

**T lymphocytes displaying CLA and CD11a, but not CD62L molecules, are more numerous in cutaneous leishmaniasis lesions than in blood**

Considering that the presence of CLA on cell surfaces should facilitate lymphocyte migration to leishmaniasis lesions, we evaluated whether an enrichment of CLA+ T cells was set in the lesion inflammatory infiltrate in comparison with blood. The two compartments were compared individually for each patient.

In the total lymphocyte population of LeMC, 25.8% were CLA-positive cells. The frequency of T lymphocytes displaying CLA molecules was much higher in lesions than in blood for both T CD4+ (lesion = 45.9% ± 22.5%, median = 38.47%; blood = 10.4% ± 7.5%, median = 9.6%; P < 0.01) (Fig. 2a) and T CD8+ cells (lesion = 46.4% ± 16.1%, median = 51.1%; blood = 5.8% ± 3.4%, median = 5.2%; P < 0.001) (Fig. 2b). Although the frequency of CLA+ in lesions was similar in both T subsets, the enrichment of cells displaying this CLA in relation to blood was more expressive among T CD8+ than in T CD4+. In a similar manner, the frequency of CD11a on lymphocytes was also higher in lesions than in blood, but this enrichment in lesions was due to T CD4+ (lesion = 76.9% ± 22%, median = 79.3%; blood = 51.3% ± 21.7%, median = 45.1%) and not to T CD8+ cells (lesion = 80.9% ± 26.9, median = 92.6%; blood = 80.1% ± 13.4, median = 78.6%). As observed in blood, the frequency of T CD8+ cells displaying CD11a was also higher than T CD4+.

Opposite results were obtained for the CD62Lhigh molecule, which showed a lower frequency in lesions for both T cell subsets (T CD4+ = 1.8% ± 0.7%, median = 1.8%, P < 0.05; and T CD8+ = 2.7% ± 1.5%, median = 1.9%, P < 0.01) in comparison with blood (Fig. 2c and d). These results show that lymphocytes displaying CLA and CD11a are enriched in lesions, suggesting that these molecules could contribute to the homing of T lymphocytes to the CL inflammatory infiltrate.

**Leishmania antigens stimuli increase the frequency of T lymphocytes displaying CLA, but reduce CD62Lhigh**

T cell activation by *Leishmania* could play a role in modulating the expression of homing molecules on lymphocyte surfaces. Thus, we decided to analyse whether CLA or CD62Lhigh are up-regulated under the parasite stimuli and also if these molecules are expressed differently in T CD4+ or T CD8+. For those purposes, *Leishmania*-stimulated PBMC were compared individually with *ex vivo* PBMC cells in A-CL and HS.

In A-CL patients, *in vitro* *Leishmania* antigen stimulation led to a significant increase (P < 0.05) in the frequency of both subtypes of T cells displaying CLA in comparison with *ex vivo* cells (Fig. 3a and b). It is worthy of mention that antigen stimulation did not affect the frequency of lymphocytes expressing CLA from HS (Fig. 3c and d), showing that *Leishmania* antigens *per se* do not up-regulate CLA in non-activated specific lymphocytes. A fourfold increase in the percentage of cells expressing CLA after parasite stimuli was observed for both T CD4+ and T CD8+ cells (Fig. 3a and b). Only two patients failed to exhibit an increase of CLA+ on *Leishmania*-reactive T CD4+; however, the frequency of CLA+ in T CD4+ (33.3% ± 14.1%, median = 27.9%) after antigen stimulation was higher than in T CD8+ (22.4% ± 9.4%, median = 23.5%), maintaining the relationship observed in *ex vivo* cells (CLA+ in CD4+ = 10.4% ± 7.5%, median = 9.6%; CLA+ in CD8+ = 5.8% ± 3.4%, median = 5.2%) (Fig. 3). A significant decrease in CLA expression on *Leishmania*-reactive T CD4+ was observed in ECCL and also in LCCL in comparison with A-CL. However, after therapy (ECCL) the percentage of CLA expression among *Leishmania*-reactive T CD8+ cells was still high and only decreased more than 1 year after treatment (LCCL) (P < 0.01; Fig. 4c and d). Only after long-term therapy did the percentages of *Leishmania*-reactive T CD4+ and T CD8+ presenting CLA return to the levels observed in control HS.

These data show that during the pathological process there is an up-regulation of molecules driving T cells to inflamed skin. The frequency of CD62Lhigh was not affected in T CD4+ (8.4% ± 5.1%, median = 8.0%) or T CD8+ (5.5% ± 4.5%, median = 4.5%) *Leishmania*-reactive cells in relation to *ex vivo* cells (data cited above).

**Discussion**

In this study we observed a decrease in the percentage of CLA+ cells in PBMC of CL patients. Post-therapy, this percentage was increased to comparable levels for HS. In parallel, we observed a high frequency of positive CLA and LFA-1 cells and low frequency of CD62Lhigh in lesions. In active patients, specific T cells up-regulate the CLA expression but not CD62Lhigh, while in HS this pattern was not observed. Thus, it is possible that parasites can influence the modulation of homing molecules during the clinical course of
Fig. 2. Ex vivo analysis of cutaneous leucocyte-associated antigen (CLA) and CD62L in blood and lesions T cell subsets of lymphocytes from cutaneous leishmaniasis patients. The percentage of CLA (a, b) and CD62L ($^{\text{high}}$) (c, d) positive cells were analysed in T CD4$^+$ (▲) or in T CD8$^+$ (△) lymphocytes. Each point represents one individual. The lines represent results obtained simultaneously in a same patient. *$P<0.05$; **$P<0.01$; ***$P<0.001$. (e) Double-staining analysis of T CD4$^+$ and T CD8$^+$ plus anti-CLA or anti-CD62L from leishmaniasis lesions showed by dot plot graphs.
Modulation of homing molecules on lymphocytes can influence the function and migration pattern of effector cells. During active leishmaniasis CLA and LFA-1, but not CD62L<sup>high</sup>, were affected in different ways on circulating CD4<sup>+</sup> or CD8<sup>+</sup> T cells. While both T cell subsets had a decreased percentage of CLA-positive cells, heightened levels of LFA-1 were expressed highly in lymphocytes in comparison with HS. However, these molecules were modulated differently on CD4<sup>+</sup> or CD8<sup>+</sup> T cell subsets. Although A-CL presented lower proportions of CLA<sup>+</sup> cells in comparison to HS, a decreased tendency was most evident for T CD8<sup>+</sup> than for T CD4<sup>+</sup>. On the other hand, even considering that the vast majority of T CD8<sup>+</sup> circulating lymphocytes are LFA-1<sup>+</sup>, we observed an increase of T CD4<sup>+</sup> cells expressing these molecules in CL patients. This fact can indicate that the ability of T CD4<sup>+</sup> cells to interact with the inflamed endothelium via LFA-1 is greater during active leishmaniasis. Conversely, in psoriasis, a disease also characterized by an influx of T lymphocytes to skin inflammatory infiltrate, CLA but not LFA-1 is up-regulated in T cells [23,31]. Additionally, high levels of blood CD8<sup>+</sup> CLA<sup>+</sup> T cells are related closely to the severity of the psoriasis [23]. Thus, expression of adhesion molecules seems to be dependent upon the nature of the stimuli triggering the lymphocytes.

**Fig. 3.** Cutaneous leucocyte-associated antigen (CLA) but not CD62L molecules are up-regulated by *Leishmania*-antigens. CLA<sup>+</sup> expression in ex-vivo and *Leishmania*-stimulated T CD4<sup>+</sup> (A) or T CD8<sup>+</sup> (△) from cutaneous leishmaniasis patients (a, b) or healthy subjects (c, d). Each point represents one individual. ***<i>P</i> < 0.001.

**Fig. 4.** Cutaneous leucocyte-associated antigen (CLA) molecules are differently expressed on CD4<sup>+</sup> and CD8<sup>+</sup> T cells during the clinical course of cutaneous leishmaniasis. Patients were evaluated during active cutaneous leishmaniasis (A-CL), after the end of therapy (early cured, ECCL), and 1 year after the end of therapy (late cured, LCCL). Healthy subjects (HS) were used as control negative cases. The percentage of cells presenting CLA molecules in blood lymphocytes was verified for both T CD4<sup>+</sup> (▲) and T CD8<sup>+</sup> (△) either ex-vivo (a, b) and after *in vitro* stimulation with *Leishmania braziliensis* antigens (Lb-Ag) (c,d). Each point represents one individual and bars represent the median of the results. *<i>P</i> < 0.05; **<i>P</i> < 0.01; ***<i>P</i> < 0.001; n.s.: not significant.
CLA in human leishmaniasis

T lymphocytes are activated in lymph nodes soon after *Leishmania* infection [15,32], and this activation leads to loss of expression of some molecules associated with permanence in lymph nodes or T cell migration to blood. Proinflammatory mediators, such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α, released as a consequence of tissue injury due to an infected *Phlebotomus* bite, up-regulate adhesion molecules [p. ex. E-selectin, intercellular adhesion molecule-1 (ICAM-1)] on endothelial cells from inflamed tissues [17], which can contribute to cell recruitment towards the inflamed skin compartment. Consequently, parasite-activated T cells are directed by adhesion molecules to affected tissues. It was demonstrated herein that *Leishmania* stimuli led to a fourfold increase of CLA on both CD4\(^+\) and CD8\(^+\) T cells cultured in vitro. Similar results were observed in mycobacterium or streptococcal antigen-stimulated PBMC from patients of leprosy or psoriasis, respectively [24,33]. That effect was observed in association with the immunopathological conditions present during active leishmaniasis, as it was not seen in clinically cured patients. No effect was seen for CD62L in A-CL. Thus, it was shown that *Leishmania* antigens can mediate the up-regulation of skin homing molecules on circulating activated specific T cells, which in addition to increased secretion of chemokines or expression of adhesion molecules in endothelium conjointly enable the cell influx into affected tissues [34,35]. Preliminary results have shown that this effect was related directly to leishmanial antigens, because this profile was not seen in lymphocytes stimulated in vitro with *Toxoplasma gondii* antigens. In this connection, the effect of non-related antigens on expression of homing molecules in *Leishmania* reactive cells is being investigated currently.

In addition to immunohistochemical studies showing positive CLA in lesions [14,29], we demonstrated that the proportion of CLA\(^+\) cells was higher in T CD4\(^+\) than in CD8\(^+\). Although other mononuclear cells than T cells such as monocytes and dendritic cells can express CLA [19], lymphocytes expressing CLA comprised 25% of the lesion cells. This number is much lower than that observed in psoriasis, where CLA-positive cells account for up to 90% of lesion T cells [36]. Several possibilities could explain the low frequency of T cells expressing CLA in CL lesions: (i) that *Leishmania*-specific T cells utilize other molecules (ligands or receptors) than CLA to enter the skin compartment; (ii) that after entering inflamed skin these cells down-regulate CLA expression; and (iii) or even after antigen activation, lesion-proliferating lymphocytes do not express *de novo* CLA.

An enrichment of CLA and LFA-1-positive cells was observed in lesions in comparison to blood. The lower levels of CLA-positive cells in blood can be a consequence of the migration of lymphocytes to a lesion. It is worthy of mention that the increase of cells expressing CLA was more expressive among CD8\(^+\) T cells (14-fold) in comparison to CD4\(^+\) T cells (eightfold), while LFA-1-positive cells were observed markedly among CD4\(^+\) T lymphocytes. Combining the results obtained from blood and lesions open the discussion as to whether these CD4\(^+\) and CD8\(^+\) T cell subsets utilize different molecules preferentially to enter lesions. LFA-1 is already expressed highly in CD8\(^+\) T cells, and CLA up-regulation in *Leishmania*-reactive cells potentially improves its ability to migrate to the skin. On the other hand, homing CD4\(^+\) T cells can be facilitated by up-regulation of both CLA and LFA-1 on *Leishmania*-reactive cells. Interestingly, CLA-positive cells can use both VLA-4/VCAM-1 and LFA-1/ICAM-1 for extravasation on skin surfaces [20], in congruence with the concept that redundant interactions are important to ensure cell migration.

In conclusion, CLA receptors are enriched in T lymphocytes from lesions of leishmaniasis patients, and leishmanial antigens are able to up-regulate these molecules in both CD4\(^+\) and CD8\(^+\) T cells. Functional studies can help understanding of the role of adhesion molecules in the traffic of inflammatory cells between T cell compartments and injured tissues. An intervention in CLA or other receptors proven to be important in pathogenesis can be a useful therapeutic strategy in the treatment of leishmaniasis.

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Disclosure

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