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Leishmania (*Leishmania*) *chagasi* infection alters the expression of cell adhesion and costimulatory molecules on human monocyte and macrophage☆

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Abstract

The initial steps of *Leishmania* infection in humans are largely unknown. There is limited information on the *Leishmania* infected human monocytes, the first cells that the parasite lives in, particularly related to costimulatory molecules. We show here that *Leishmania (L.) chagasi* infection avoids inducing proinflammatory molecules and has striking down modulating effects on human monocytes or macrophages. It does not induce CD54, interleukin (IL)-12 or tumour necrosis factor-α, potent proinflammatory cytokines and down modulates CD11b expression in monocytes. Lipopolysaccharide stimulated IL-12 (p40) levels, CD54 and HLA-DR expression are diminished in infected monocytes as well as interferon-γ stimulated HLA-DR and HLA-ABC expression in infected macrophages. There is a negative correlation between CD54 and CD86 expression in both monocytes and macrophages. The depressed expression of class I and II molecules, absence of key proinflammatory cytokines and impaired expression of costimulatory molecules induced by *L. chagasi* could leave the immune system, at least in its initial phases in anergy or ignorance.

Keywords: Costimulation; CD54; CD11b; CD86; Cytokines; *Leishmania*

1. Introduction

The early steps of *Leishmania* infection are of utmost importance in determining whether a Th1 predominance, leading to resistance, or a Th2 advantage, related to susceptibility, occurs (Reiner and Locksley, 1995). Recently arrived monocytes are probably the first permissive human cells to phagocyte *Leishmania* in the blood pool (de Almeida, 2002) formed by the sand fly bites (Ribeiro, 1987) followed by the scarce residual macrophages present in the normal human skin (Milon et al., 1995; Urmacher, 1997). Human neutrophils are short-lived cells involved in clearing the parasite (Chang, 1981) but not in sustaining the parasite growth. Monocytes and macrophages could play distinct roles in *Leishmania* infection (Cervia et al., 1993; Milon et al., 1995).

Inside the macrophage, the parasite has several strategies for evading the host immunological system (Reiner, 1994; Buates and Matlashewski, 2001). One of these strategies is the down modulation or not induction of molecules involved directly or indirectly in antigen presentation. For example, *Leishmania (L.) donovani* infection has been shown to down modulate the expression of MHC I and II molecules (Reiner et al., 1987). Studies in mice are controversial, concerning the ability of *Leishmania* infection to induce changes in the expression pattern of costimulatory molecules of monocyte/macrophage. No changes on costimulatory molecules expression were found on macrophages from the resistant C57BL/6 mice upon infection with *L. donovani* (Saha et al., 1995) or with *Leishmania (L.) major* (von Stebut et al., 1998). *Leishmania donovani* infection of macrophages from susceptible BALB/c mouse marginally increases CD54 (Saha et al., 1995) but fails to trigger CD80 (Kaye et al., 1994). *Leishmania major* was also not able to change the low level of CD80 expression and the basal level of CD86 expression in human macrophages (Brodskyn et al., 2001).

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The pattern of expression of costimulatory molecules has been implicated in driving the immune system to a Th1 or Th2 response leading to secretion of cytokines that could activate the macrophage and arrest the infection or burst it (Kaye, 1995; Hunter and Reiner, 2000). These initial steps of *Leishmania* infection in humans are largely unknown, and there is limited information on the response of the *Leishmania* infected human monocytes, particularly related to costimulatory molecules. Herein, we report the expression of costimulatory molecules, cytokines as well as HLA, on human blood monocytes and on in vitro differentiated macrophages upon infection with *Leishmania (L.) chagasi*.

2. Materials and methods

2.1. The parasite

Leishmania chagasi (MHOM/BR/90/Ba 307) was isolated from a spleen of a patient with visceral leishmaniasis. The isolate was inoculated in the spleen of a Syrian hamster (*Mesocricetus auratus*), seeded in Schneider's insect culture medium (Sigma) plus 10% of foetal calf blood serum at 25°C. In its first subculture, several samples of the parasite were collected and frozen in liquid nitrogen. Stocks were thawed and cultivated in same conditions until the tenth in vitro subcultures. The parasites were collected at stationary growth phase for in vitro infection of human monocytes and macrophages or heat killed (70°C, 10 min) for control experiments.

2.2. Isolation of monocytes and parasite infection

Monocyte isolation was performed as previously described (de Almeida et al., 2000) with slight modifications. Briefly, a two-step procedure with single gradients in each step was used. Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats (obtained from normal blood donors) by centrifugation over a Ficoll–Hypaque (400 × *g*, 35 min, 25–35°C). The PBMC were washed three times with phosphate buffered sodium (PBS)/citrate (1.49 mM $Na_2H_2PO_4$; 9.15 mM Na_2HPO_4; 139.97 mM NaCl, 13 mM $C_6H_5Na_3O_7{\cdot}2H_2O$; pH 7.2, 100 × *g*,15 min, 25–35°C) to avoid platelet contamination. Around 10^8 PBMC were then incubated (15–30 min, 37°C) in 15 ml of a solution formed by nine parts of RPMI 1640 medium (Sigma) supplemented by 2 mM L-glutamine, 15 mM HEPES, 10% human serum and 50 μg/ml gentamicin and one part of trisodium citrate ($C_6H_5Na_3O_7{\cdot}2H_2O$) 3.8% (w/v) in 50 ml conical polypropylene tubes with loose lids in a CO_2 incubator. This suspension was then layered on top of a 15 ml Percoll gradient [1:1 (v:v) isosmotic Percoll plus PBS/Citrate] and centrifuged (400 × *g*, 35 min, 25–35°C). The percentage of monocytes recovered on top of the gradient was higher than 90% with more than 90% viable cells (de Almeida et al., 2000). Cells were washed twice in PBS before cultivation on cell culture medium. Some experiments were done using monocytes purified by adherence using a previous established protocol (Wahl and Smith, 1994), in this case, the percentage of contaminant lymphocytes was 20–30%. Monocyte percentage was determined using morphology and peroxidase activity detection in cytospin preparations, height and granularity characteristics in light scattering, and in some samples double staining with fluorescein isothiocyanate (FITC) and r-phycoerythrin (PE)-conjugated mouse anti-human mAbs: CD14 (PE) and CD3 (FITC). Monocytes were cultivated at 1×10^6 ml in 1 ml RPMI 1640 medium (Sigma) with 10% human AB serum, 2 mM L-glutamine, 50 μg/ml gentamicin and 15 mM HEPES in 24-well plates in a 5% CO_2 incubator at 37°C. The monocytes were immediately infected (10:1 parasite/human cell ratio) or left to differentiate to macrophages for 7 days, in this case, half of the cell culture medium was replenished with new complete medium each 48 h. Macrophages were also infected at a 10:1 parasite/human cell ratio. Both monocytes and macrophages were also incubated with lipopolysaccharide (LPS)-free latex beads (Sigma) at 10:1 bead/human cell ratio or with heat killed promastigotes (only done with macrophages) at the same 10:1 cell ratio. Two hours after the infection, the wells were gently washed with RPMI 1640 to remove non-infective parasites or non-ingested particles. New medium was then replenished. At this time, cells were also stimulated with LPS (10 ng/ml, Sigma) or recombinant human IFNγ (Roussel–Uclaf, 10–100 U/ml). After 48 h in a 5% CO_2 incubator at 37°C, cell free supernatant was collected and cells scrapped for flow cytometry analysis. In our hands, 90% of the monocytes or macrophages were infected after 48 h.

2.3. Flow cytometry analysis

Surface markers were analysed by direct immunofluorescence staining using the following FITC- or PE-conjugated mouse anti-human mAbs: CD11b (PE), CD54 (PE), CD80 (FITC), HLA-ABC (FITC), HLA-DR (PE), CD14 (PE), CD3 (FITC) purchased from PharMingen. The biotin-conjugated mouse anti-human mAbs CD49e, CD86 purchased from PharMingen were detected with indirect immunofluorescence using the biotin–streptavidin system. The mouse isotype controls $IgG_{1,K}$ (PE, FITC), $IgG_{2a,k}$ (PE) were also purchased from PharMingen. Briefly, the scrapped cells were washed in cold PBS/bovine serum albumin (BSA) 1%/N_3Na 0.1%. The cell pellet was suspended in PBS/human serum (v:v, 200 μl; 5×10^6 cells/ml) for 15 min at 4°C. To 20 μl of this suspension were mixed a previous titrated amount of mAbs and left 30 min at 4°C in the dark. The cell suspension was then washed in cold PBS. The cells for direct immunofluorescence were suspended in 400 μl of cold PBS and ready to be analysed. The pellets of cells labelled with biotin-conjugated antibodies were suspended in 20 μl of PBS and added 3 μl of streptavidin-PE (Becton Dickinson) for

15 min at 4°C. These cells were then washed and suspended in 400 μl of cold PBS. The fluorescence was analysed by FACScan (Becton Dickinson). Dead cells and residual lymphocytes were excluded according to their forward and side scatter characteristics. The mean fluorescence intensity of each experimental condition was measured excluding the fluorescence of the respective isotype controls, and the streptavidin non-specific binding for biotin labelled antibodies. Five thousand events were analysed for each sample using the Cell Quest software (Becton Dickinson). Relative fluorescence (RF) was defined as the mean fluorescence intensity of each experimental condition relative to the mean fluorescence intensity of its control considered as 100%, thus RF was calculated dividing the numerical value of mean fluorescence intensity of each experimental condition by the numerical value of its control and then multiplying this result by 100.

2.4. Cytokine detection

Tumour necrosis factor (TNF)-α, IL-10 and IL-12 (p40) determination was done by commercial sandwich immunoassay (DuoSET ELISA; Genzime diagnostics) according to the manufacturer's instructions.

2.5. Statistical analysis

Data were analysed using de GraphPad Prism version 3.00 for Windows. Comparisons of multiple groups with control group were performed with one-way analysis of variance (ANOVA) using the Dunnet's post-test and the comparison between previously selected pairs of groups using the Bonferroni's method (GraphPad software). The level of significance adopted was $P < 0.05$.

3. Results

3.1. Pattern of expression of surface molecules on infected cells

With the exception of very low CD80 cell positivity (measured only on macrophages) the frequency of cell positivity to markers here measured was higher than 90% in human monocytes and between 60 and 90% in human macrophages (data not shown). When human monocytes were infected by *L. chagasi*, CD11b expression was diminished (mean RF = 63.35 ± 19.09 SD; $P < 0.01$, Fig. 1A, B). There was also a trend towards reduced expression of CD54 (eight out of nine blood donors) and increased expression of CD86 (four out of six blood donors) but the differences were not statistically significant. Infection of human macrophages by *L. chagasi* lead to a decreased expression HLA-DR (mean RF = 74.46 ± 25.08 SD; $P < 0.05$) and an increased CD86 expression (mean RF = 120.2 ± 19.96 SD; $P < 0.05$, Fig. 1C, D). No changes were observed in the expression of CD54 (mean RF = 92.75), CD49e (mean RF = 101.9), HLA-ABC (mean RF = 97.03) or CD11b (mean RF = 107.6) as shown in Fig. 1C. Interestingly, upon infection there was a negative correlation between CD54 and CD86 expression both on monocytes (Fig. 2A, $r = -0.9222$, $P < 0.01$) and macrophages (Fig. 2B, $r = -0.7478$, $P < 0.05$).

3.2. Latex beads and heat killed Leishmania promastigotes controls

On human monocytes, phagocytosis of latex beads (Fig. 2C, black bars), compared to uninfected controls (broken line), did not change HLA-DR or CD11b expression, but was significantly different when compared to infected controls (+, $P < 0.001$ for both molecules). On human macrophages, phagocytosis of latex beads (Fig. 2D, black bars) increase HLA-DR expression when compared to uninfected control (*, $P < 0.001$) and also when compared to infected control (+, $P < 0.001$). Phagocytosis of latex beads (black bars) did not change the levels of CD86 expression on human macrophages compared to uninfected control (broken line), but was significantly different when compared to infected control (+, $P < 0.05$). On human macrophages, phagocytosis of heat killed leishmania (hatched bars, Fig. 2D) increased the expression of CD54 and HLA-DR compared to uninfected controls (*, $P < 0.05$ for CD54; *, $P < 0.001$ for HLA-DR) and also when compared to infected controls (+, $P < 0.05$ for CD54; +, $P < 0.001$ for HLA-DR). Heat killed experiments were not done with human monocytes. These results suggest that promastigotes must be viable to induce the set of effects observed, chiefly the down modulatory effects that were not observed in latex or heat killed experiments in both monocytes or macrophages.

3.3. LPS-induced stimulation on uninfected monocyte/macrophages

LPS-stimulated uninfected monocytes and macrophages (Fig. 3, black bars) over express, compared to uninfected controls (broken line, Fig. 3A, C), CD54 (*, $P < 0.001$ for monocytes, Fig. 3A, B; *, $P < 0.001$ for macrophages, Fig. 3C) and CD86 (*, $P < 0.01$ for monocytes, Fig. 3A; *, $P < 0.05$ for macrophages; Fig. 3C). LPS addition increased also CD49e expression (*, $P < 0.01$; Fig. 3A) in human uninfected monocytes, and HLA-DR in human uninfected macrophages (*, $P < 0.05$; Fig. 3C, D).

3.4. Effect of infection on LPS-induced monocyte/macrophage stimulation

Infection had a powerful depressant effect on LPS-induced CD54 expression of human monocytes ($P < 0.001$) (Fig. 3A, B) and macrophages ($P < 0.001$) (Fig. 3C). As shown in Fig. 3C, D, *L. chagasi* infection diminishes HLA-

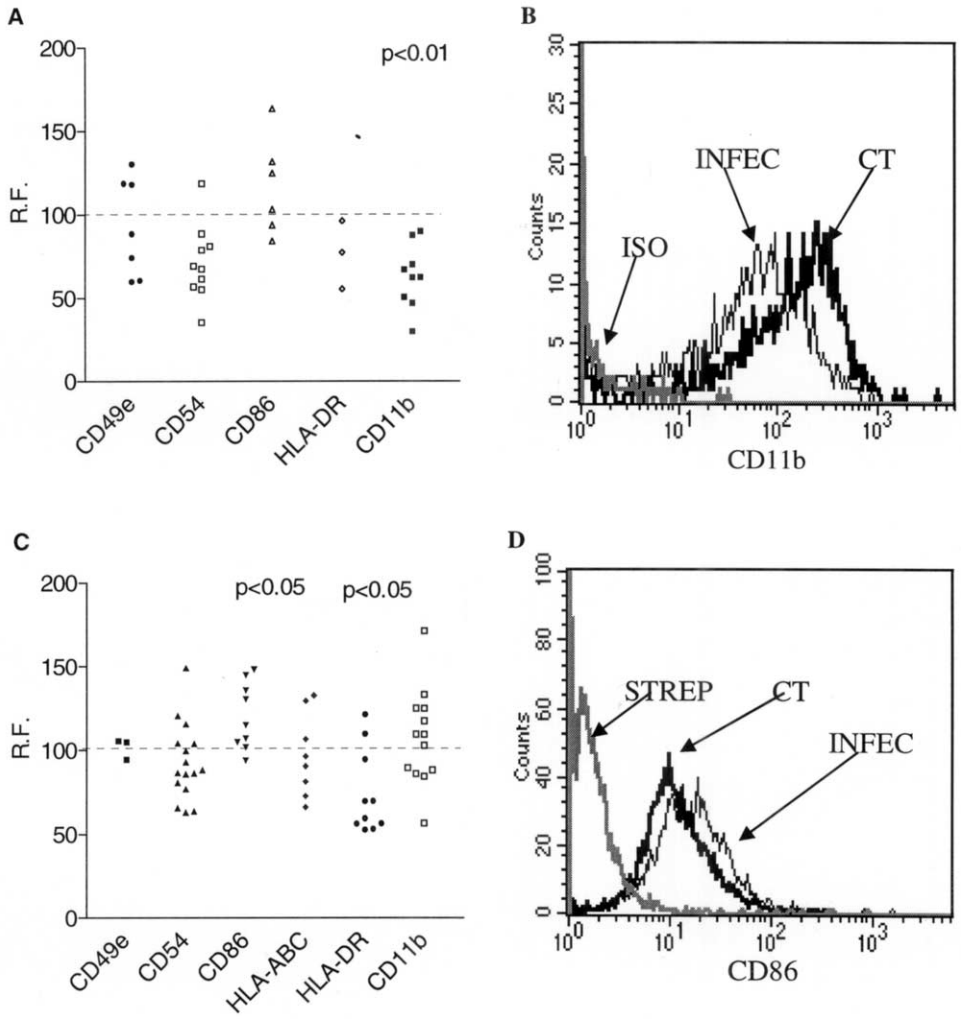

Fig. 1. Pattern of expression of surface molecules on infected cells. Relative fluorescence was calculated dividing the numerical value of mean fluorescence intensity of each experimental condition by the numerical value of its control and then multiplying this result by 100. Human monocytes (A) and macrophages (C) were infected with *L. chagasi*. The broken line shows the control level. (A) *L. chagasi infection* decrease CD11b expression on human monocytes, $P < 0.01$. (C) CD86 expression is increased on infected macrophages, ($P < 0.05$) and HLA-DR expression decreased, ($P < 0.05$). Representative histograms are shown for monocytes, CD11b (B) and macrophages, CD86 (D): infected cells (INFEC), uninfected cells (CT), isotype control (ISO) and streptavidin–r-phycoerythrin (STREP). Error bars mean 1 SD.

ABC and HLA-DR expression of LPS stimulated macrophages ($P < 0.001$ for each pair). Infection did not change CD86 and CD49e expression of LPS-stimulated macrophages or monocytes. Effect of LPS-stimulated HLA-ABC and HLA-DR expression was not evaluated in infected monocytes.

3.5. IFNγ induced stimulation on uninfected monocyte/macrophages

IFNγ-stimulated uninfected monocytes and macrophages (Fig. 4, black bars), compared to uninfected controls (broken line, Fig. 4A, C), over express CD54 (*, $P < 0.01$ for monocytes, Fig. 4A; *, $P < 0.001$ for macrophages, Fig. 4C). IFNγ increased also the expression of HLA-BC and HLA-DR in uninfected macrophages (*, $P < 0.001$ for each molecule, Fig. 4C). Interestingly, IFNγ reduced the expression of CD49e in both monocytes and macrophages (*, $P < 0.05$ for monocytes, Fig. 4A; *, $P < 0.001$ for macrophages, Fig. 4C).

3.6. Effect of infection on IFNγ induced monocyte/macrophage stimulation

As shown in Fig. 4, IFNγ stimulated infected cells (hatched bars), when compared to IFNγ stimulated uninfected cells (black bars), overexpress CD86 on both monocytes (A, B) and macrophages (C, D), ($P < 0.05$ and $P < 0.001$, respectively), with IFNγ increasing the stimulatory effect of infection on CD86 expression of macrophages (+, $P < 0.001$). *Leishmania chagasi* infection did not change the CD49e expression inhibition of IFNγ stimulated human macrophages or monocytes. Infection did not reduce the stimulatory action of IFNγ on CD54

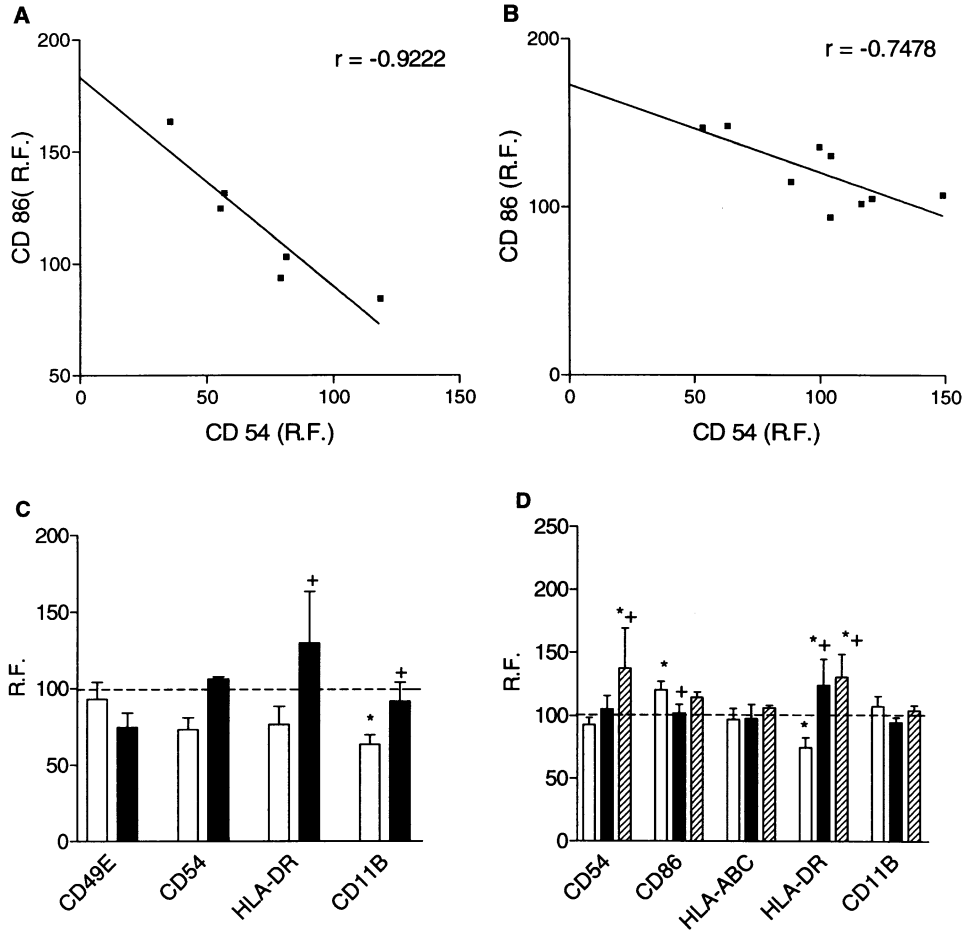

Fig. 2. (A,B) Correlation between relative fluorescence of CD54 and CD86 of *L. chagasi* infected human monocytes (A) or macrophages (B). (C,D) Pattern of expression of surface molecules of monocytes (C) or macrophages (D) that phagocytosed latex beads (black bars), heat killed *Leishmania* promastigotes (hatched bars) or live promastigotes (white bars). (A,B) r is the correlation coefficient, $P < 0.01$ for monocytes or macrophages. (C) Phagocytosis of latex beads (black bars) compared with uninfected controls (broken line), did not change HLA-DR or decrease CD11b expression, but these expressions were significantly higher when compared with infected cells (+, $P < 0.001$ for both molecules). Each black bar represents the mean of three (CD54 and CD11B) and two (CD49e and HLA-DR) experiments. (D) Phagocytosis of latex beads (black bar), contrary to infected cells (white bar), increase HLA-DR expression when compared with uninfected control (*, $P < 0.001$). Phagocytosis of latex beads (black bars) did not change the levels of CD86 expression on human macrophages compared to uninfected control, but was significantly different when compared to infected control (+, $P < 0.05$). Phagocytosis of heat killed *Leishmania* promastigotes (hatched bar) increased the expression of CD54 and HLA-DR compared with uninfected controls (broken line, *, $P < 0.05$ for CD54; *, $P < 0.001$ for HLA-DR) and also when compared with infected controls (+, $P < 0.05$ for CD54; +, $P < 0.001$ for HLA-DR). Each black bar represents, except for CD86 (three experiments), the mean of four experiments. Each hatched bar means the mean of three (CD86), four (CD86, HLA-ABC) and five experiments (HLA-DR and CD11b). The symbol (*) means a significant difference compared with respective uninfected cells (broken line), and (+) means a significant difference compared with respective infected cells (white bars). See details in the text. Error bars mean 1SD.

expression of macrophages (Fig. 4C) but did it on monocytes (Fig. 4A) ($P < 0.01$), indeed infection abolishes it as there is no significant difference between IFNγ plus infection (hatched bar) and infected control group (white bar). Inhibitory effect of infection on IFNγ stimulation was also found on expression of HLA-ABC ($P < 0.001$) and HLA-DR ($P < 0.01$) on human macrophages (Fig. 4C). Effect of infection on IFNγ stimulation was not measured for HLA-ABC and HLA-DR in human monocytes.

3.7. Cytokine production

Leishmania chagasi infection per se did not induce IL-10, IL-12 (p40, data no shown for macrophages) or TNF-α production in either monocytes or macrophages, as shown in Fig. 5A, B for TNF-α in monocytes and macrophages and in Fig. 5C for IL-10 and Fig. 5D for IL-12 (p40) in monocytes. Phagocytosis of latex beads did not induce TNF-α production in human monocytes or macrophages, nor did phagocytosis of heat killed *Leishmania* promastigotes in human macrophages (data not shown). Both cell populations were able to produce TNF-α (Fig. 5A,B), IL-10 or IL-12 (p40, Fig. 5C,D) upon LPS stimulation (10 ng/ml). Additionally *L. chagasi* infection did not diminish TNF-α production by LPS-stimulated human monocytes and macrophages (Fig. 5A,B), or IL-10 in monocytes (not done on macrophages), but diminished IL-12 (p40) production by LPS-stimulated human monocytes (mean of

391.7 pg/ml ± 527.6 SD; $n = 5$, LPS vs. LPS-infection, $P < 0.05$, Fig. 5D, not done on macrophages).

4. Discussion

A direct relationship between costimulatory molecules, particularly CD80 and CD86 and a Th1 or Th2 response in established leishmaniasis models is not clear (Murphy et al., 1997; Hunter and Reiner, 2000). For instance, blocking of CD86 in mice and human systems has given contradictory results (Brown et al., 1996; Brodskyn et al., 2001; Murphy et al., 1997). A possible explanation for these results is that a more complex picture could be involved in driving lymphocytes to Th1 or Th2 response (Kim et al., 1999). Whereas CD80 or CD86 expression leads to high IL-4 and IL-10 production by naive CD4 T cells, co-expression of CD54 plus CD80 or CD86 resulted in decreased IL-4 and IL-10 production (Luksch et al., 1999). Additionally, co-stimulation by CD80 and CD54, but not by CD80 or CD54 alone, leads to rapid TNF-α cytotoxicity, tumour rejection and generation of memory T cells (Nishio and Podack, 1996). Moreover, both B7 and CD54 may co-regulate activation-driven maturation of T cells (Damle et al., 1992). We report here for the first time that a parasite infection leads to increased CD86 expression simultaneously to decreased CD54 expression in both monocytes and macrophages. In human lepromatous leprosy, characterised by marked immunosuppression, high CD54 with low B7 expression has been shown (Agrewala et al., 1998). It is possible that avoidance of simultaneous expression of these two molecules, and consequently optimal costimulatory activity (Damle et al., 1992; Kim et al., 1999; Camacho et al., 2001) is an escape mechanism exploited by *L. chagasi*

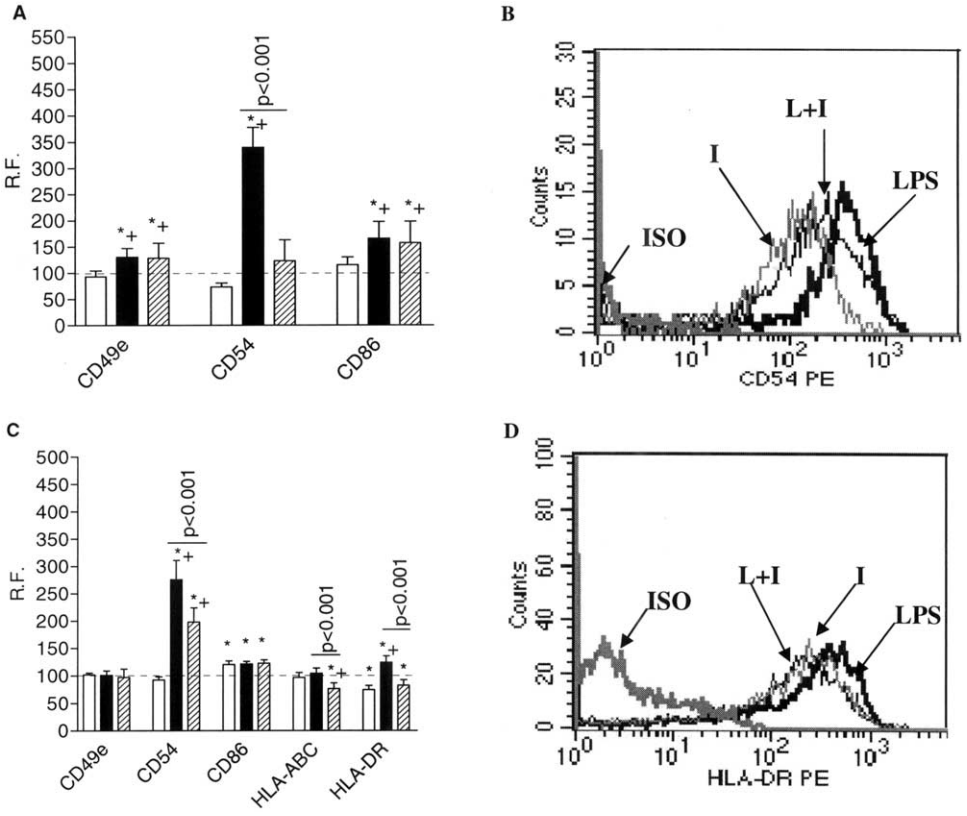

Fig. 3. Effect of infection on lipopolysaccharide-induced monocyte/macrophage stimulation. Relative fluorescence was calculated dividing the numerical value of mean fluorescence intensity of each experimental condition by the numerical value of its control and then multiplying this result by 100. Relative fluorescence of infected monocytes (A) or macrophages (C) stimulated with lipopolysaccharide 10 ng/ml for 48 h (hatched bars) were compared with lipopolysaccharide alone (black bars) or infection (white bars). See details in text. The broken line shows the control level (cells without stimulation). (A) Infection counteracted the stimulatory effect of LPS on CD54 expression of human monocytes, $P < 0.001$. Each bar represents the mean of four (CD86), five (CD49e) and six experiments (CD54). (C) Infection counteracted the stimulatory effect of lipopolysaccharide on CD54 ($P < 0.001$) and HLA-DR ($P < 0.001$) expression of human macrophages. LPS stimulated and infected cells had a lower HLA-ABC expression than LPS stimulation alone ($P < 0.001$) or infection (+, $P < 0.01$). Each bar represents the mean of three (CD49e), six (CD54, HLA-ABC and HLA-DR) and eight experiments (CD86). Representative histograms are shown for monocytes, CD54 (B) and macrophages, HLA-DR (D): infected cells (I), LPS stimulation alone (LPS), LPS plus infection (L + I) and isotype control (ISO). The symbol (*) means a significant difference compared with respective uninfected cells (broken line), and (+) means a significant difference compared with respective infected cells (white bars). See details in the text. Comparisons between other specific groups are shown by a short horizontal line over these groups with the level of significance above the line. Error bar means 1SD.

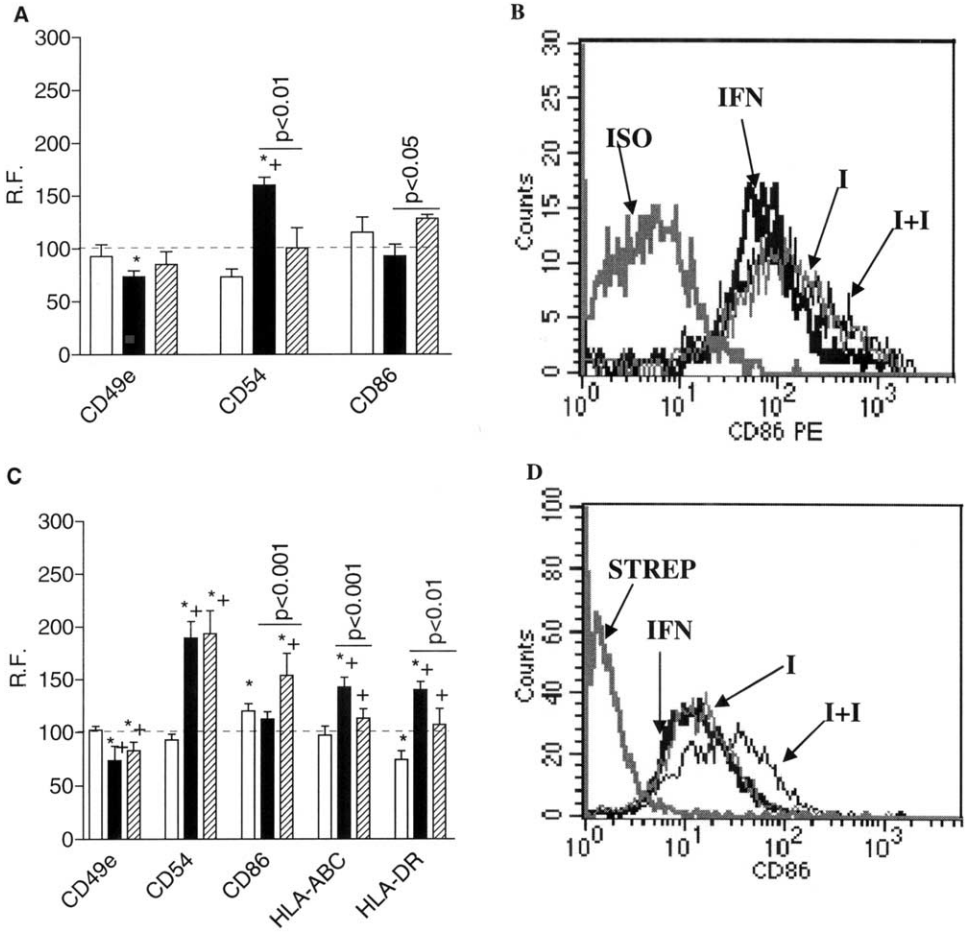

Fig. 4. Effect of infection on IFNγ-induced monocyte/macrophage stimulation. Relative fluorescence was calculated dividing the numerical value of mean fluorescence intensity of each experimental condition by the numerical value of its control and then multiplying this result by 100. Relative fluorescence of infected monocytes (A) or macrophages (C) stimulated with 100 U/ml of interferon-γ for 48 h. (hatched bars) were compared with interferon-γ stimulation alone (black bars) or infection (white bars). See details in text. The broken line shows the control level (cells without stimulation). (A) Infection counteracts the stimulatory effect of interferon-γ on CD54 expression of human monocytes, $P < 0.01$. Although the levels of CD86 expression were higher in interferon-γ stimulated and infected cells compared with interferon-γ stimulated cells alone ($P < 0.05$), there was no significant difference between interferon-γ plus infection and infection alone. Each bar represents the mean of four (CD86), five (CD49e) and six experiments (CD54). (C) Infection counteracts the stimulatory effect of interferon-γ on HLA-ABC and HLA-DR expression of human macrophages, $P < 0.001$ and $P < 0.01$, respectively. Interferon-γ increased the stimulatory effect of infection on CD86 expression (hatched bar, +, $P < 0.001$). Each bar represents the mean of three (CD49e), six (CD 54, HLA-ABC and HLA-DR) and eight experiments (CD86). Representative histograms are shown for monocytes, CD86 (B) and macrophages, CD86 (D): infected cells (I), interferon-γ stimulation alone (IFN), interferon-γ stimulation plus infection (I + I), isotype control (ISO) and streptavidin–r-phycoerythrin (STREP). The symbol (*) means a significant difference compared with respective uninfected cells (broken line), and (+) means a significant difference compared with respective infected cells (white bars). See details in the text. Comparisons between other specific groups are shown by a short horizontal line over these groups with the level of significance above the line. Error bars mean 1SD.

and *Mycobacterium leprae*. On the other hand, *Trypanosoma cruzi* infected mouse macrophages, having upregulated CD86 expression levels and maintained high basal levels of CD54 expression, showed strong costimulatory activity towards the Th1 side of the immune response (Frosch et al., 1997). Reinforcing this point, a *Leishmania* antigen, capable of upregulating both CD54 and B7 in human macrophages and monocyte derived dendritic cells, induced IL-12 production and Th1-type T cell response in PBMC (Probst et al., 1997). Coherently, IFNγ treated infected macrophages were able to upregulate CD54 and CD86 expression, and clinical trials have shown successful IFNγ treatment of human visceral leishmaniasis (Badaro and Johnson, 1993).

CD54 (ICAM-1) binds LFA-1 on lymphocytes (Dustin and Springer, 1991), and CD54 expression induces IFNγ by co-stimulated T cells (Kim et al., 1999), conversely, as we show here, IFNγ was able to increase CD54 expression on both monocytes and macrophages. On the contrary, *L. chagasi* infection had a tendency to decrease CD54 expression in monocytes besides down modulating CD54 expression in LPS or IFNγ stimulated monocytes. Thus, the probably *L. chagasi* lowering of the strength of monocyte adhesion to lymphocyte, could impair IFNγ production,

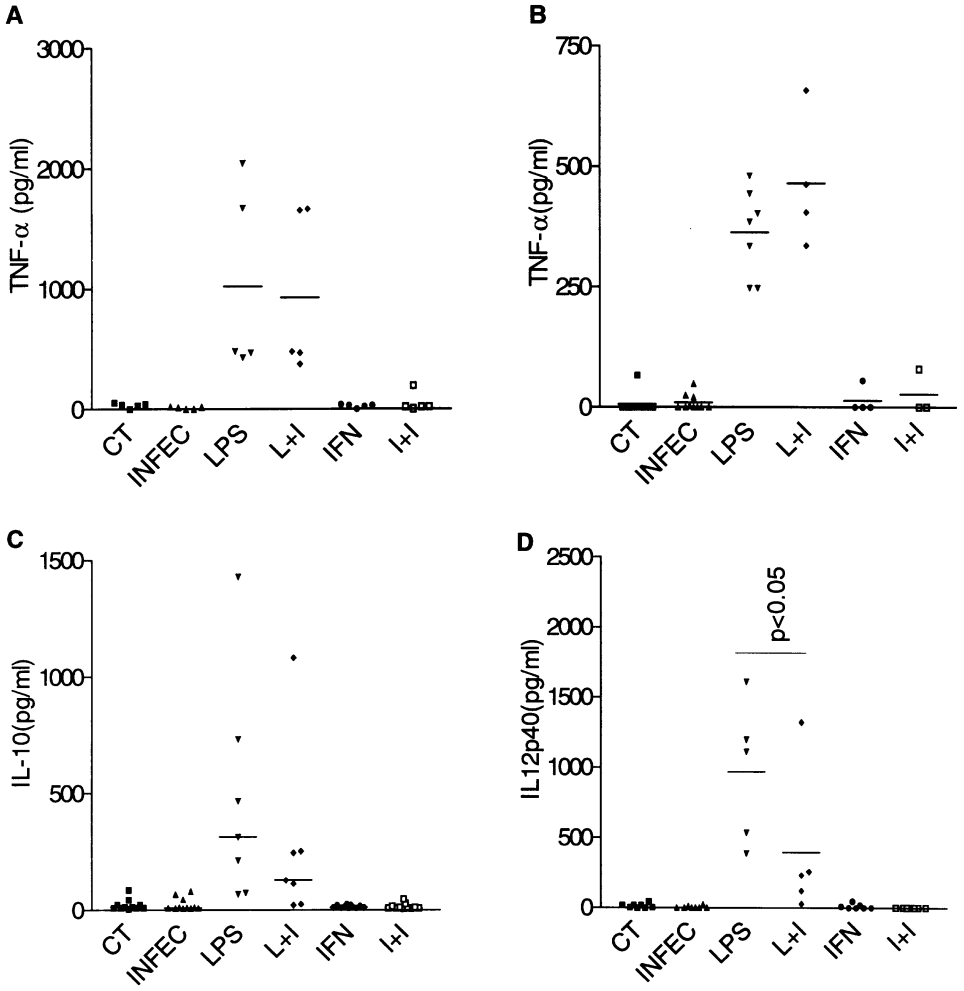

Fig. 5. TNF-α (A,B), IL-10 (C) and IL-12 (D) levels in culture supernatants of uninfected and infected *L. chagasi* human monocytes (A,C,D) and macrophages (B) under several conditions. CT, uninfected cells; INFEC, infected cells; LPS, LPS stimulation alone (10 ng/ml); L + I, LPS (10 ng/ml) stimulation after 2 h of infection; IFN, interferon-γ (10–100 U/ml) stimulation alone; I + I, interferon-γ (10–100 U/ml) stimulation after 2 h of infection. (C) Infection diminished interleukin-12 (p40) production by lipopolysaccharide-stimulated human monocytes (LPS vs. LPS-infection, $P < 0.05$).

increase the antigen dose required for T cell co-stimulation, and influence the Th1/Th2 balance (Luksch et al., 1999).

We report, for the first time, that CD11b has its expression diminished by *Leishmania* infected human monocytes but not macrophages, as recently shown for mice LFA-1 and MAC-1 α chains gene expression in *L. donovani* infected mouse macrophages (Buates and Matlashewski, 2001). The parasite inhibition of CD11b expression could be a previously unreported escape mechanism, as CD11b has recently been shown to play a significant role in LPS induced IL-12 secretion, and CD11b deficient macrophages had diminished nuclear translocation of nuclear factor κB (NF-κB) and reduced mitogen-activated protein kinase (MAPK) phosphorylation (Perera et al., 2001). Noteworthy, CD18 deficient mice macrophages produce less NO and have impaired leishmanicidal activity (Schonlau et al., 2000). In addition, CD11b has been shown to be involved in platelet activating factor synthesis by human monocytes (Elstad et al., 1994). Thus, diminished CD11b expression might interfere with production of essential host protecting agents, platelet-activating factor (Lonardoni et al., 2000) and IL-12 (Reiner et al., 1994). Such a situation would be particularly relevant at the initial phases of *Leishmania* infection when monocytes are the first and most numerous parasited antigen presenting cells.

Others and we have found that *Leishmania* infection does not induce TNF-α or IL-12 production in human monocytes (Reiner et al., 1990; Ghalib et al., 1995; Sartori et al., 1997) or macrophages but inhibits IL-12 production by LPS stimulated monocytes. Importantly, IL-12 is a main driving Th1 cytokine in the initial phases of *Leishmania* infection and is able to restore in vitro patients visceral leishmaniasis PBMC Th1 responses to *Leishmania* antigens, notwithstanding the IL-10 presence (Ghalib et al., 1995). Also, in agreement with previous studies using healthy human PBMC (Ghalib et al., 1993; Sartori et al., 1997) instead of visceral leishmaniasis patients PBMC (Ghalib et al., 1993), *Leishmania* infection did not induce IL-10 release in human

monocytes or macrophages. The binding of the parasite to its receptor (CD11b), and the low CD11b expression on human monocytes, could explain most of these findings as it has been shown that binding to human CD11b, or CD11b deficiency, inhibits LPS-induced IL-12 production with unchanged levels of TNF-α (Marth and Kelsall, 1997; Perera et al., 2001) or IL-10 (Marth and Kelsall, 1997). This may seem surprising for, in human disease there is high IL-10 (Gasim et al., 1998) and TNF-α (Barral-Netto et al., 1991) serum levels, and both TNF-α and IL-10 explain most of the aspects of active disease as immune suppression (Carvalho et al., 1994) cachexia and fever (Barral-Netto et al., 1991). On the other hand, infected monocytes or macrophages, under LPS stimulation, are able to produce similar amounts of TNF-α or IL-10 to their uninfected controls. Thus in the evolution to active disease other factors as expansion of IL-10 producing T cell clones, ingestion of IgG-opsonised amastigotes (Kane and Mosser, 2001), the frequent bacterial infections patients have (Andrade et al., 1990) or immune complex-mediated cell activation (Carvalho et al., 1983; Crawford et al., 1985) could explain TNF-α and IL-10 production.

The data presented here are in agreement with observations that *Leishmania* infection causes deactivation of mononuclear phagocytes (Reiner, 1994) as *L. chagasi* fails to induce TNF-α production and impairs IL-12 production besides decreasing CD54 expression, all important proinflammatory molecules (Camacho et al., 2001). Additionally, such an infection has down modulatory effects on LPS or IFNγ stimulation. The evasive behaviour of *L. chagasi* infection in human phagocytic cells also includes lack of CD11b and CD80 induction in human macrophages (data not shown) and down modulation of CD11b on monocytes. It is tempting to speculate that depressed expression of class I and II molecules, absence of key proinflammatory cytokines and not full expression of costimulatory molecules in monocytes or macrophages conceal *Leishmania* leaving the immune system, at least in initial phases, in anergy (Kaye, 1995) or ignorance (de Almeida, 2002). This ignorance may probably be broken when, due to parasite multiplication, the parasite or its antigens reach other cell, as dendritic cells, since in such cells these down regulatory events are not operative (Gorak et al., 1998; von Stebut et al., 1998; Marovich et al., 2000).

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