Figure S1. Monocytes isolation and expression of CD11b and CD86
A 20 ml peripheral blood sample was obtained from volunteers using sodium heparin (Roche, Brazil) as an anticoagulant. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque (GE, USA) density gradient centrifugation, and then washed three times with saline solution. The cells were maintained in medium RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (Gibco Life Technologies, Rockville, MD), 50 μM β-mercaptoethanol, 60 mg/ml penicillin and 100 mg/ml streptomycin (Sigma), pH 7.4. For fresh monocytes (day 0), PBMC were seeded at a concentration of 1 × 10^7 cells per well for 2h in 12-well plates. Afterwards, nonadherent cells were removed by extensive washing. In order to determine CD11b and CD86, expression in monocytes cells were collected and incubated for 10 min with 5% PBS+ fetal calf serum solution. Cells were stained for 30 min at 4°C with PE conjugated anti-CD86, FITC conjugated anti-CD11b (all from BD Biosciences, USA) monoclonal antibodies. After the incubation period, cells were washed with PBS and analyzed by flow cytometry (FACScan – Becton & Dickinson, USA). Ten thousand cells were acquired based on forward and side scatter; also, data analyses were performed using WinMDI 2.9 software.
Figure S2. Cells were incubated with IL-4 + GM-CSF (50 ng/ml), in the presence or absence of neutralizing anti–TNF-α (1 µg/mL), for 5 days at 37°C in a 5% CO2 atmosphere. The neutralizing antibody was added in the first day and in the third day of culture. After this period, CD14 and CD1a expression were analyzed by flow cytometry. Representative histograms of 5 (CTR) or 6 (HTLV+) independent experiments showing CD14 and CD1a (A) expression patterns, DCs (IL-4 + GM-CSF), DCs+anti-TNF (IL-4 + GM-CSF + neutralizing anti–TNF-α). TNF-α (20 ng/ml) inducing CD83 (B) expression was used as a control for TNF-α activity and neutralization. The percentage of positively stained cells (R1) and their mean fluorescence intensity are also shown.
Figure S3. Cells were incubated with IL-4 + GM-CSF (50 ng/ml), for 5 days at 37°C in a 5% CO2 atmosphere. After this period, cells were incubated in the presence or absence of TNF-α for 48h at 37°C in a 5% CO2 atmosphere. Following, the supernatants were collected and assayed for TGF-β and IL-10 production by ELISA. Data in bars are expressed as pg/ml of TGF-β (A), pg/ml of IL-10 (B). White bars represent the supernatant obtained from culture of cells derived from non-infected individuals (CTR; n=5), and grey bars represent the supernatant obtained from culture of cells derived from HTLV-1 infected donors (n=5). Data are means ± SD.