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Lipopolysaccharide (LPS) down-regulates CD4 expression in primary human macrophages through induction of endogenous tumour necrosis factor (TNF) and IL-1β

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SUMMARY

The regulation of CD4 expression on macrophages and its role in immune cell interactions remain obscure. In contrast with primary lymphocytes, primary macrophages express only low amounts of surface CD4, which is regulated differentially for example by adherence *in vitro*. We report that addition of LPS for 1–5 days to human blood monocyte tissue culture-derived macrophages (TCDM) down-regulates both surface CD4 expression and total cellular CD4 antigen content as measured by flow cytometry and Western blot analysis. TNF-α and IL-1β, proinflammatory cytokines which are both induced by LPS, also down-regulate surface and total CD4 expression in TCDM. This down-regulation of CD4 expression by LPS, TNF-α, and IL-1β occurs at the level of transcription. The decreased macrophage CD4 expression induced by LPS was blocked by MoAbs directed against human TNF-α and IL-1β, demonstrating that LPS acts on CD4 expression through induction of endogenous TNF-α and IL-1β. Conversely, neither LPS nor TNF-α and IL-1β were able to modulate surface CD4 expression on quiescent or phytohaemagglutinin (PHA)-activated lymphocytes. Of other cytokines and growth factors tested, Th2 cytokines (IL-4, IL-10, IL-13), chemokines (MCP-1, MIP-1α, RANTES), and macrophage colony-stimulating factor did not alter CD4 expression in primary macrophages; granulocyte-monocyte colony-stimulating factor and the prototypal Th1 cytokine interferon-gamma (IFN-γ) modulated surface CD4 expression only after prolonged treatment (5 days). Our results show that LPS, TNF-α and IL-1β selectively down-regulate CD4 expression in primary human macrophages, and that decreased CD4 expression induced by LPS results from endogenous secretion of TNF-α and IL-1β by the macrophages.

Keywords lipopolysaccharide CD4 macrophages tumour necrosis factor IL-1β

INTRODUCTION

The CD4 molecule plays a central role in immune functions and AIDS pathophysiology. CD4 is a glycoprotein, member of the immunoglobulin superfamily, which is expressed on thymocytes, T helper lymphocytes, and monocytes/macrophages [1,2]. The CD4 molecule is involved in T lymphocyte maturation [3] and in mature CD4$^+$ T cell activation [4], but its role in monocyte/macrophage immune functions is poorly defined. The extracellular domain of the CD4 molecule interacts with MHC class II molecules and is the main receptor for HIV on T lymphocytes and mononuclear phagocytes [5–7].

Modulation of the CD4 molecule is important in regard to thymocyte signaling [8] and intercellular adhesion [9]. CD4 modulation pathways have been investigated mainly in lymphoid cells, in which CD4 down-regulation occurs during antigenic stimulation [10] and after phorbol myristate acetate (PMA) treatment. PMA induces CD4 internalization [11] and a transient decrease in CD4 mRNA levels which is caused by both reduced transcriptional initiation and destabilization of mRNA molecules [12,13]. In AIDS, the surface CD4 decrease observed in HIV-infected lymphocytes has been correlated with inhibition of CD4 gene expression [14], intracellular sequestration of CD4–gp120 complexes [15–17], and the action of viral genes [18,19]. Protein *nef* can induce internalization of cell surface CD4 without affecting CD4 transcript or protein levels [20], and protein *vpu* causes degradation of CD4 molecules that have been blocked in the endoplasmic reticulum [21].

In contrast to T helper lymphocytes and some monocytoid cell lines [22], primary human macrophages express low plasma

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membrane CD4 levels [23,24]. Down-regulation of surface CD4 expression by phorbol esters and adherence has been reported in monocytic cell lines and primary macrophages in the absence of modulation of CD4 mRNA and total CD4 protein levels [25,26]. Internalization of CD4 might in part explain the surface CD4 decrease observed in these cells since $p56^{lck}$ tyrosine protein kinase, which forms complexes with CD4 in lymphocytic cells and consequently reduces CD4 endocytosis [27,28], is not present in monocytic cells and primary monocytes/macrophages. Down-regulation of surface CD4 expression in peripheral blood monocytes has also been reported in HIV-infected patients at the terminal stage of the disease, although its mechanism has not been explained [29].

LPS, which is derived from the cell wall of Gram-negative bacteria and plays a major role in pathogenesis of septic shock, interacts with a number of immune cells, not least macrophages. LPS primes macrophages through CD14 receptors for plasma LPS-binding protein to produce three groups of powerful mediators: the proinflammatory cytokines (TNF, IL-1, IL-6, IL-8), reactive oxygen and nitrogen intermediates, and a number of arachidonic acid metabolites, all of which are involved in inflammatory responses and antibacterial host defence.

Taking into account that CD4 interacts with MHC class II molecules and that LPS down-regulates surface MHC class II on macrophages through induction of IL-10 [30], we investigated the modulation of CD4 expression in macrophages by LPS.

MATERIALS AND METHODS

Isolation and cultivation of primary human macrophages

Human monocytes were isolated from healthy donors as described [31]; in short, Ficoll–Hypaque (Pharmacia, Uppsala, Sweden)-isolated peripheral blood mononuclear cells (PBMC) were incubated for 1 h on 2% gelatin-coated plates. Adherent tissue culture-derived macrophages (TCDM), > 94% $CD14^{+}$ by flow cytometry (Becton Dickinson, Mountain View, CA), were cultivated in serum-free medium (X-Vivo; Whittaker M.A. Bioproducts, Walkersville, MD) for 48 h before transfer to 50-mm Petri dishes (Bibby Sterilin Ltd, Stone, UK) at a density of 2×10^6 cells in 3 ml total volume.

Preparation of resting and phytohaemagglutinin A-activated lymphocytes

Non-adherent cells, > 90% of which were peripheral blood lymphocytes (PBL), were harvested after Ficoll–Hypaque isolation and adherence as described above. PBL were cultivated either in serum-free medium alone or supplemented for 48 h with phytohaemagglutinin A (PHA; 5 μg/ml; Wellcome Diagnostic, Beckenham, UK) before addition of human recombinant (hr) IL-2 (50 NIH/BRMP U/ml; Pharmacia).

Cytokine treatment

hrTNF-α (10 ng/ml; Sigma, Poole, UK) and 100 U/ml hIL-1β (Sigma) (endotoxin < 10 EU/ml by Limulus amoebocyte lysate assay for both cytokines) were added to cultures for 1–5 days. To inhibit the effects of recombinant and endogenous TNF-α, 1·3 μg mouse IgG1 anti-human TNF-α MoAb (kindly provided by Dr M. Feldmann, Kennedy Institute of Rheumatology, London, UK) was mixed for 30 min at room temperature with 10 ng/ml hrTNF-α or 100 ng/ml LPS and then added to 2×10^6 TCDM in 3 ml final volume. To inhibit the effects of both recombinant and endogenous IL-1, 660 U/ml rabbit IgG anti-human IL-1 MoAb (Calbiochem, Nottingham, UK) were mixed as above with 100 U/ml hrIL-1β or 100 ng/ml LPS and added to TCDM. Heat inactivation of hrTNF-α and hrIL-1β was performed at 70°C for 30 min. hrIL-4 (10 ng/ml; Immunex, Seattle, WA), 10 ng/ml hrIL-10 (Dnax, Palo Alto, CA), 10 ng/ml hrIL-13 (Sanofi, Labège, France), 20 ng/ml hr interferon-gamma (IFN-γ; Boehringer, Mannheim, Germany), 100 U/ml hr macrophage colony-stimulating factor (M-CSF; Sandoz, Basle, Switzerland), 300 U/ml hr granulocyte-monocyte colony-stimulating factor (GM-CSF; Sandoz), 100 ng/ml hr monocyte chemoattractant protein-1 (MCP-1; Genentech Inc, San Francisco, CA), 100 ng/ml hr macrophage inflammatory protein-1 alpha (MIP-1α; Genentech Inc), 100 ng/ml hr RANTES (Genentech Inc) were added for 1–5 days to primary TCDM and quiescent or PHA-activated PBL.

LPS treatment

LPS (phenol extract from *Escherichia coli*; Sigma) was added to cell (TCDM or PBL) cultures for 1–5 days. The concentrations used ranged from 0·01 to 1 μg/ml.

Cell viability after cytokine and LPS treatment was > 95% as measured with the colorimetric reaction (MTT) based on the capacity of mitochondrial dehydrogenase of living cells to reduce 3-(4,5-dimethylthiazol-2yl)–2.5 diphenyl tetrazolium bromide into formazan. The quantity of formazan produced and measured at an optical density of 540 nm was correlated with the number of living cells [32].

Detection of TNF-α and IL-1β in culture supernatants by ELISA

Supernatants of human macrophage cultures were harvested and frozen at − 80°C. The levels of TNF-α and IL-1β were determined by ELISA (Quantikine, R & D Systems, Abingdon, UK).

Flow cytometry

Cells fixed in 3% paraformaldehyde in PBS were quenched with 10% v/v goat serum in PBS, labelled, and analysed by flow cytometry. Surface CD4 was detected by using $F(ab')_2$ fragments of mouse anti-human CD4 (MoAb 4120 (IgG1) from Dr Q. Sattentau provided by the MRC AIDS Directed Programme, ADP318) followed by FITC-conjugated goat anti-mouse IgG (Fab-specific) (Sigma). Specific fluorescence was assessed by comparison with cells stained only with the FITC-conjugated antibody (control). Intracellular staining was performed as above, but reagents were supplemented with 0·1% saponin during washing and with 0·3% saponin during labelling. Relative antigen density was calculated as follows:

$$\frac{(\text{geometric mean (cytokine or LPS)} - \text{geometric mean (control)})}{(\text{geometric mean (mock)} - \text{geometric mean (control)})} \times 100$$

For the detection of surface LFA-1 a mouse IgG1 directed against CD11a (Sigma) was used. The characterization of the macrophage population was confirmed by using mouse anti-huCD68 IgG1 (Dako Ltd, Cambridge, UK) and mouse anti-huCD14 IgG2 (Sigma) MoAbs.

Western blot

Adherent macrophage cultures were washed and lysed. Protein concentrations in cell lysates were determined by the BCA method (Pierce, Rockford, IL). Cell lysates, prepared in 150 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 10 mM NaN_3, 1 mM phenylmethylsulphonylfluoride, 5 mM iodoacetamide, and 1% v/v Nonidet P40, were electrophoresed in a 10% SDS–PAGE under non-reducing conditions and then electroblotted onto nitrocellulose. Before probing, blots were blocked in PBS with 3% w/v dried milk, and 0·1% v/v Tween 20; anti-CD4 $F(ab')_2$ antibody (MoAb Q4120) binding was detected by chemiluminescence (ECL; Amersham, Aylesbury, UK).

RNA extraction, reverse transcription and amplification of cDNA by polymerase chain reaction

Total cellular RNA was extracted from 2×10^6 TCDM using 1 ml RNAzol (Cinna/Biotex, Houston, TX) with 20 μg *E. coli* rRNA (Boehringer) as carrier. Reverse transcription was performed in a volume of 50 μl containing 800 U of M-MLV reverse transcriptase (GIBCO-BRL, Paisley, UK), 2·5 mM dNTP, 5 μg oligo dT_{12-18}, and 31 U RNase inhibitor (all from Pharmacia) for 1 h at 37°C. Polymerase chain reaction (PCR) amplification was performed using an MJ Research programmable thermal controller (Genetic Research Instrumentation Ltd, Felsted, UK) operating the following temperature steps: 60 s at 94°C, then 30 cycles (30 s denaturation at 94°C; 30 s annealing at 60°C; 60 s extension time at 72°C). $MgCl_2$ concentrations used for huCD4 and human β-actin cDNA amplification were 2·0 and 2·5 mM, respectively. The following primer pairs were used: huCD4 (5′ primer, GCTAGGCATCTTCTTCTGTG; 3′ primer, CTGCTACATTCATCTGGTCC; size of amplified fragment, 223 bp); human β-actin (5′ primer, GATGCAGAAGGAGATCACTG; 3′ primer, AGTCATAGTCCGCCTAGAAG; size of amplified fragment, 205 bp), all synthesized by Dr K. Gould (Sir William Dunn School of Pathology, Oxford, UK). PCR products were separated by 2% agarose electrophoresis and visualized by ethidium bromide staining. A 123-bp DNA ladder (GIBCO) was used for molecular weight determination.

RESULTS

TCDM were treated with LPS, hrTNF-α, and hrIL-1β for 1–5 days. Surface and total CD4 expression was determined by flow cytometry and Western blot analysis, using $F(ab')_2$ anti-CD4 MoAb, to avoid Fc-dependent interactions. LPS treatment (100 ng/ml) resulted in significant time-dependent decrease in specific plasma membrane CD4 expression, with 59% (± 8%) and 18% (± 9%) residual expression after 1 and 5 days, respectively, relative to untreated culture controls (Fig. 1a,b). LPS-induced surface CD4 down-regulation was dose-dependent; the residual surface CD4 expression on TCDM after 1 day of treatment ranged from 70% to 30% as LPS concentrations were increased from 10 ng/ml to 1 μg/ml (data not shown). Human rTNF-α (10 ng/ml) treatment of TCDM resulted in 54% (± 10%) and 20% (± 9%) surface CD4 residual expression after 1 and 5 days of treatment, respectively (Fig. 1c,d). Levels of surface CD4 dropped to 62% (± 8%) and 32% (± 6%) after hrIL-1β (100 U/ml) treatment for 1 and 5 days, respectively (Fig. 1e,f). In agreement with surface CD4 down-regulation, LPS (100 ng/ml), hrTNF-α (10 ng/ml), or hrIL-1β (100 U/ml) treatment for 1 day reduced total CD4 levels, with residual expression of 46% (± 9%), 30% (± 7%), and 59% (± 5%), respectively, as shown by flow cytometry of saponin-permeabilized TCDM (Fig. 2a). Down-regulation of total CD4 was confirmed by Western blotting of human TCDM treated for 3 days by LPS, hrTNF-α, and hrIL-1β (Fig. 2b).

To determine if the inhibition of CD4 protein synthesis by LPS, hrTNF-α, and hrIL-1β occurs at the level of transcription we performed reverse transcription (RT)-PCR analysis of CD4 message. The CD4 mRNA levels were reduced after treatment of TCDM for 1 day with LPS (100 ng/ml), hrTNF-α (10 ng/ml), and hrIL-1β (100 U/ml) (Fig. 3), indicating that inhibition

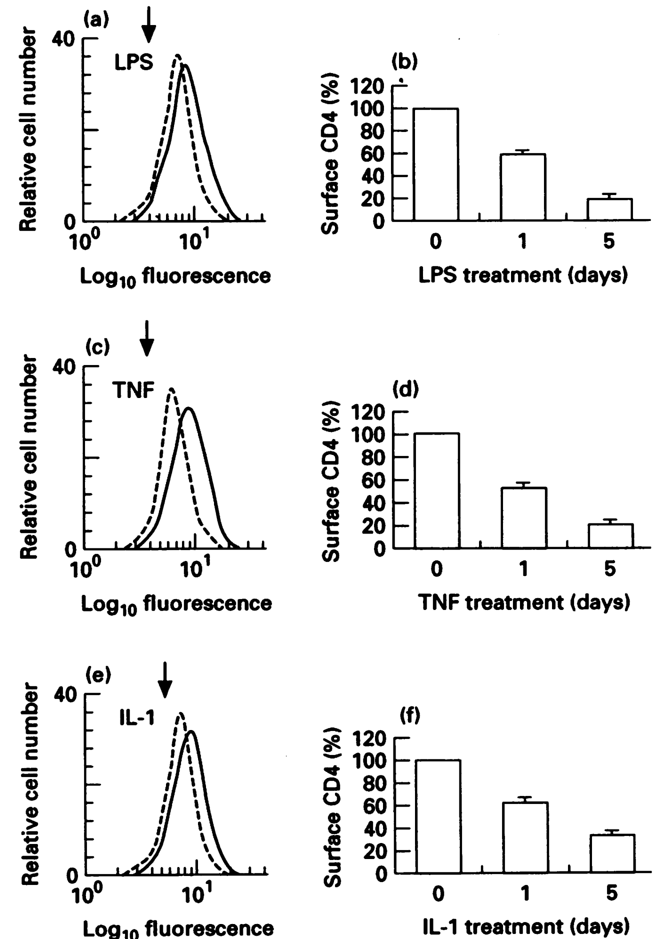

Fig. 1. LPS, hrTNF-α, and hrIL-1β down-regulate surface CD4 on human tissue culture-derived macrophages (TCDM). Three-day-old TCDM were treated with 100 ng/ml LPS, 10 ng/ml hrTNF-α, or 100 U/ml hrIL-1β for 1 and 5 days, fixed as described in Materials and Methods and analysed together by flow cytometry. Fixed TCDM were first gated on the basis of forward scatter and side scatter; all gated cells were TCDM as confirmed by anti-CD14 MoAb labelling (data not shown). Fluorescence profiles (at 1 day) and histograms (at 1 and 5 days) show surface CD4 levels in LPS- (a,b), hr TNF-α- (c,d), and hrIL-1β- (e,f) treated *versus* control cells cultured for similar periods. Arrows indicate fluorescence of TCDM incubated with the FITC-conjugated antibody alone. The data reported in the histograms are representative of at least three independent experiments.

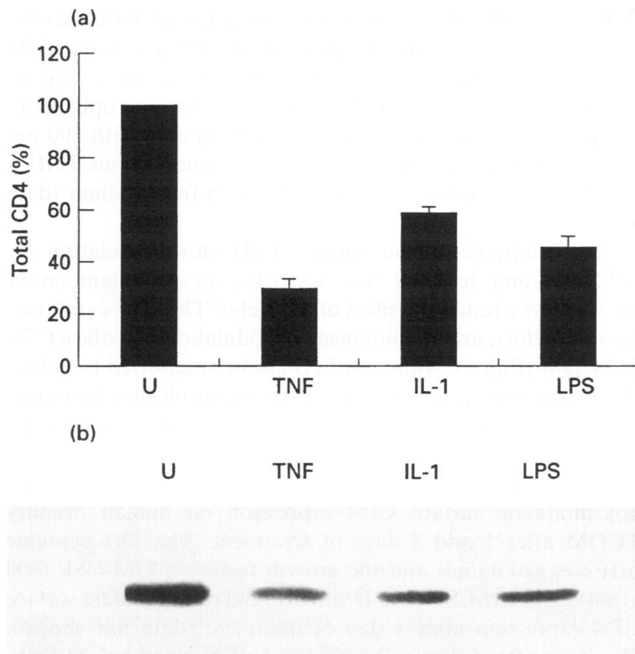

Fig. 2. LPS, hrTNF-α, and hrIL-1β down-regulate total CD4 in human tissue culture-derived macrophages (TCDM). (a) Three-day-old TCDM were treated with 100 ng/ml LPS, 10 ng/ml hrTNF-α, or 100 U/ml IL-1β for 1 day and analysed by flow cytometry. Fixed TCDM were saponin-permeabilized and gated on the basis of forward scatter and side scatter; all gated cells were TCDM as confirmed by anti-CD68 MoAb labelling (data not shown). Histogram shows total CD4 levels in LPS-, hrTNF-α-, and hrIL-1β-treated *versus* control cells (U). All data are representative of three independent experiments. (b) Western blot-detected total CD4 in TCDM after 3 days of treatment with 100 ng/ml LPS, 10 ng/ml hrTNF-α, 100 U/ml hrIL-1β *versus* untreated control cells (U); 20 μg of cellular protein were loaded per lane.

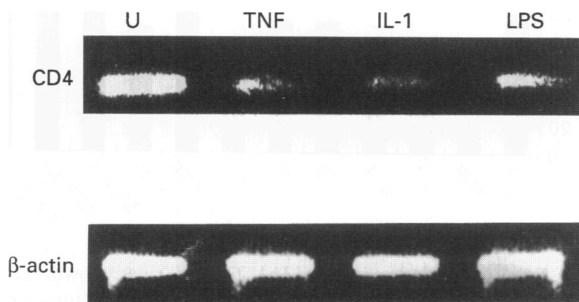

Fig. 3. LPS, hrTNF-α, and hrIL-1β down-regulate CD4 mRNA in human tissue culture-derived macrophages (TCDM). Three-day-old TCDM were untreated (U) or treated for 24 h with 100 ng/ml LPS, 10 ng/ml hrTNF-α, or 100 U/ml IL-1β. RNA was extracted from 2×10^6 cells with 20 μg *Escherichia coli* rRNA as carrier, reverse transcribed, and the cDNA amplified as described in Materials and Methods. The polymerase chain reaction (PCR) products for human CD4 (223 bp) and for control β-actin (205 bp) were visualized on a 2% agarose gel.

of CD4 expression occurred at transcription. Approximately three-fold inhibition of the CD4 mRNA in LPS-treated TCDM *versus* untreated control cells was demonstrated by serial cDNA titration (data not shown). Inhibition of CD4 gene expression in TCDM was more efficient after hrTNF-α or hrIL-1β treatment than after LPS. We performed control experiments (Fig. 4a,b) showing that both hrTNF-α and hrIL-1β preparations used were heat-labile and did not contain endotoxin, which is heat-resistant. Neutralizing MoAbs against either TNF-α or IL-1β blocked the activity of these cytokines in regard to CD4 modulation in TCDM. The addition of MoAb directed against TNF-α or IL-1β to the TCDM resulted in surface CD4 expression levels higher than those observed in untreated TCDM, suggesting endogenous secretion of TNF-α and IL-1β in untreated TCDM. The expression of plasma membrane LFA-1 on TCDM was not affected by LPS (100 ng/ml), hrTNF-α (10 ng/ml), and hrIL-1β (100 U/ml) (data not shown).

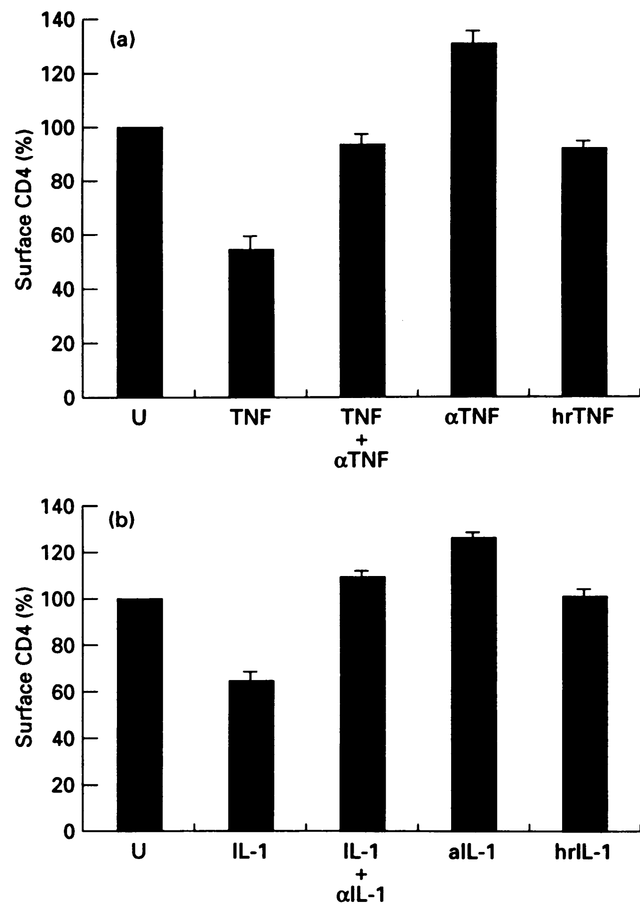

Fig. 4. HrTNF-α and hrIL-1β down-regulate surface CD4 expression in human tissue culture-derived macrophages (TCDM). Three-day-old TCDM were treated for 1 day with: (a) 10 ng/ml hrTNF-α, 10 ng/ml hrTNF-α mixed with 1·3 μg/ml anti-TNF-α MoAb, 1·3 μg/ml anti-TNF-α MoAb, or 10 ng/ml heat-inactivated hrTNF-α; (b) 100 U/ml hrIL-1β, 100 U/ml hrIL-1β mixed with 660 U/ml anti-IL-1 MoAb, 660 U/ml anti-IL-1 MoAb, or 100 U/ml heat-inactivated hrIL-1β. Surface CD4 expression was analysed by flow cytometry in treated *versus* untreated TCDM (U). All data re representative of two independent experiments.

LPS (100 ng/ml), hrTNF-α (10 ng/ml), or hrIL-1β (100 U/ml) treatment for 1–5 days did not change surface CD4 expression on quiescent or PHA-activated lymphocytes (data not shown), indicating that CD4 down-regulation by LPS, hrTNF-α, and hrIL-1β was selective for macrophages. Other cytokines tested (IL-4, IL-10, IL-13, IFN-γ, M-CSF, GM-CSF, MCP-1, MIP-1α, RANTES) also did not modulate surface CD4 expression on quiescent or PHA-activated lymphocytes (data not shown).

LPS down-regulation of surface CD4 was inhibited by MoAbs directed against TNF-α and IL-1β (Fig. 5). When added separately, anti-TNF-α and anti-IL-1 MoAbs inhibited the down-regulation of CD4 expression on TCDM induced by 100 ng/ml LPS. Moreover, the combination of both MoAbs in the presence of LPS resulted in enhanced surface CD4 expression to 130% (± 2%) of control levels, suggesting an endogenous secretion of TNF-α and IL-1β by macrophages in culture. In agreement with these data, treatment with 100 ng/ml LPS for 24 h induced the synthesis of endogenous TNF-α and IL-1β by TCDM cultivated in serum-free medium (data not shown).

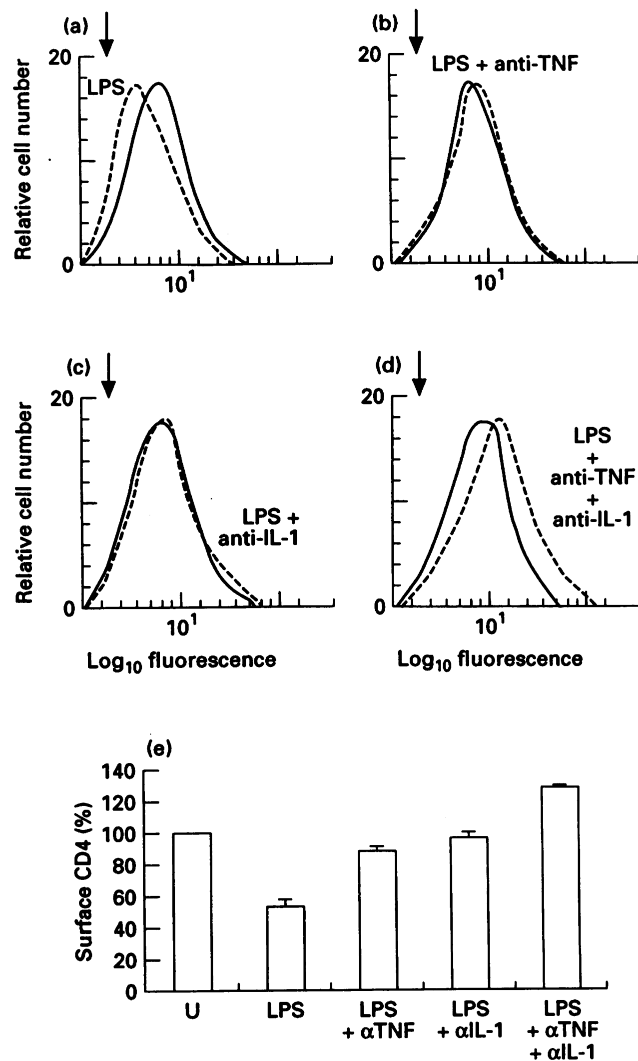

Fig. 5. Neutralization of endogenous TNF-α and IL-1β blocks down-regulation of CD4 by LPS. Three-day-old tissue culture-derived macrophages (TCDM) were untreated or treated for 1 day with 100 ng/ml LPS alone (a), LPS mixed with 1·3 μg/ml anti-TNF-α MoAb (b), LPS mixed with 660 U/ml anti-IL-1 MoAb (c), LPS mixed with both 1·3 μg/ml anti-TNF-α MoAb and 660 U/ml anti-IL-1 MoAb (d). Flow cytometry analysis was performed; fluorescence profiles (a–d) and histogram (e) show surface CD4 levels. Arrows indicate fluorescence of TCDM incubated with the FITC-conjugated antibody alone. The histogram summarizes results from three independent experiments.

To determine if the surface CD4 down-regulation by hrTNF-α and hrIL-1β was restricted to proinflammatory cytokines, we tested the effect of a panel of Th1, Th2 cytokines, growth factors, and chemokines on modulation of surface CD4 in TCDM (Fig. 6). Although LPS has been reported to induce IL-10 synthesis in human monocytes/macrophages, treatment with hrIL-10 (10 ng/ml) for 1 or 5 days did not alter surface CD4 expression on primary human TCDM. The other Th2 cytokines tested, hrIL-4 (10 ng/ml) and hrIL-13 (10 ng/ml), did not modulate surface CD4 expression on human primary TCDM after 1 and 5 days of treatment. The Th1 cytokine hrIFN-γ (20 ng/ml) and the growth factors hrGM-CSF (300 U/ml) and hrM-CSF (100 U/ml) also did not modulate surface CD4 expression after 1 day of treatment (data not shown). However, after 5 days of treatment, hrIFN-γ and hrGM-CSF, respectively, down-regulated (40%) and up-regulated (165%) surface CD4 in TCDM. Human rM-CSF (100 U/ml) and the chemokines hrMCP-1 (100 ng/ml), hrMIP-1α (100 ng/ml), and hrRANTES (100 ng/ml) did not modulate surface CD4 expression on human primary TCDM after 5 days of treatment.

DISCUSSION

Both function and modulation of MHC class II molecule by cytokines have been studied extensively in primary macrophages. In contrast, the role of CD4 molecule which is the physiological ligand of MHC class II, and its modulation by cytokines in macrophages are largely unexplored. In this study

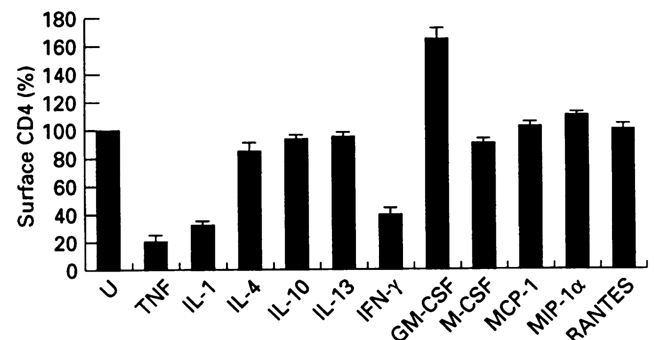

Fig. 6. Effects of proinflammatory, Th1, Th2 cytokines, growth factors, and chemokines on surface CD4 modulation in tissue culture-derived macrophages (TCDM). Three-day-old TCDM were untreated (U) or treated for 5 days with hrTNF-α (10 ng/ml), hrIL-1β (100 U/ml), hrIL-4 (10 ng/ml), hrIL-10 (10 ng/ml), hrIL-13 (10 ng/ml), hrIFN-γ (20 ng/ml), hr granulocyte-macrophage colony-stimulating factor (GM-CSF; 300 U/ml), hrM-CSF (100 U/ml), hr monocyte chemoattractant protein-1 (MCP-1; 100 ng/ml), hr macrophage inflammatory protein-1 alpha (MIP-1α; 100 ng/ml), and RANTES (100 ng/ml), fixed as described in Materials and Methods, and analysed by flow cytometry for surface CD4 expression. Results were reproduced in duplicate experiments.

we tested the effect of LPS and a panel of recombinant human cytokines on CD4 expression in human primary macrophages. We show that LPS and the proinflammatory cytokines, TNF-α and IL-1β, down-regulate both surface and total CD4 expression in primary human macrophages at the level of transcription. The CD4 down-regulation induced by LPS is mediated through the synthesis of endogenous TNF-α and IL-1β, but not through IL-10, which is also produced by LPS-activated macrophages, and is known to down-regulate MHC class II expression in LPS-treated human primary monocytes [30]. In contrast to GM-CSF and IFN-γ which, respectively, up-regulate and down-regulate CD4 in macrophages [33,34], modulation by Th2 cytokines (IL-4, IL-10, IL-13), chemokines (MCP-1, MIP-1α, RANTES) and M-CSF was not evident.

LPS down-regulated both surface and total CD4 protein in primary macrophages at the level of transcription. The down-regulation of CD4 protein in LPS-treated macrophages occurred through the action of endogenous TNF-α and/or IL-1β production, as demonstrated by the abolition of CD4 down-regulation in LPS-treated macrophages by MoAbs directed against TNF-α and IL-1β. The observation that down-regulation of both CD4 protein and message was less efficient after LPS treatment than after TNF-α treatment might be explained by rapid internalization of TNF receptors by macrophages in response to LPS [35]. In macrophages treated with LPS, TNF-α, or IL-1β, the CD4 mRNA levels could be decreased as a result of either inhibition of gene expression or the lack of CD4 mRNA stability, although we did not attempt to distinguish between these possibilities. Inhibition of CD4 gene expression by LPS or proinflammatory cytokines might result from an inhibition of the enhancer or promoter of the CD4 gene [36,37] or activation of its silencer [38,39], all of which have been described so far in lymphocytes, but not in macrophages. Although our results account for a transcriptional CD4 regulation in macrophages by LPS, TNF-α, and IL-1β, we cannot exclude additional actions at post-transcriptional levels. For example, phorbol ester has been reported to diminish surface CD4 expression in monocytic cell lines through an endocytosis-mediated pathway [25]. Similarly, adherence of monocytes to tissue culture plastic diminishes surface CD4 levels as early as 1 h after their isolation from peripheral blood by a post-transcriptional mechanism; neither total CD4 expression nor CD4 mRNA levels are modified by adherence [26]. IFN-γ, which enhances the secretion of TNF-α and IL-1β by the macrophages, down-regulated surface CD4 expression on primary macrophages only after the addition of the cytokine for 5 days [33]; this action could be mediated through the induction of endogenous TNF-α and IL-1β secretion. IL-10 was not able to down-regulate surface CD4 expression on macrophages, as reported previously [40]. This suggests that, in contrast to MHC class II DR expression which is down-regulated by IL-10 in LPS-treated human monocytes [30], CD4 decrease by LPS is not mediated through IL-10 but through proinflammatory cytokines. The CD4 down-regulation by LPS, TNF-α, and IL-1β has been observed in primary human macrophages, but never in quiescent or PHA-activated primary human lymphocytes, suggesting that the mechanism of CD4 modulation by both proinflammatory cytokines and LPS is restricted to macrophages.

CD4 modulation in primary human macrophages might have significance in both immunological mechanisms and HIV pathophysiology. Macrophage CD4 modulation could play a role in inflammatory and infectious processes. The Gram-negative and some Gram-positive bacteria induce the synthesis of TNF-α and IL-1β by the macrophages. On the one hand TNF-α and IL-1β increase the amount of MHC class II molecules present on the macrophage surface [41] and might favour antigen presentation to CD4$^+$ T cells that themselves exhibit stable surface CD4 levels. On the other hand, the drop of surface CD4 on the macrophages might diminish CD4–MHC class II interactions [42] occurring between adjacent macrophages. Thus the secretion of proinflammatory cytokines TNF-α and IL-1β might diminish macrophage–macrophage interactions and favour antigen presentation to T helper cells. These phenomena could occur concomitantly with the stimulation of inducible microbicidal mechanisms by the macrophage (NO secretion and respiratory burst) and might optimize the efficiency of both non-specific and specific immune defences.

We have found that TNF-α down-regulates total CD4 expression in the U937 monocytic cell line (data not shown). Phorbol ester-induced differentiation of this cell line to a more macrophage-like phenotype is correlated with decreased CD4 expression [25]. Our results suggest that reduced CD4 expression and differentiation are unlikely to be linked phenomena, in that the growth factors GM-CSF and M-CSF which stimulate maturation of monocytes/macrophages did not down-regulate surface CD4; GM-CSF even increasing surface CD4 expression after 5 days of treatment, as reported previously [34].

TNF-α, IL-1β, IFN-γ, and LPS activate the macrophages in parallel with down-regulation of CD4. In contrast the Th2 cytokines which either deactivate the macrophages (IL-4, IL-10) or alter their activation state (IL-13) [43] did not modulate surface CD4 in primary TCDM. The chemokines (MCP-1, MIP-1α, RANTES) do not directly modulate macrophage CD4 in the inflammatory focus, but through increasing macrophage recruitment could allow macrophage CD4 down-regulation by the proinflammatory cytokines. All these data taken together suggest that the down-regulation of CD4 expression in primary macrophages in an inflammatory focus is strongly related to the effect of proinflammatory cytokines rather than to the state of maturation or activation of the macrophages.

CD4 is the main receptor for HIV on human primary macrophages [6]. Its modulation might interfere with CD4–envelope glycoprotein interactions and possibly modulate both syncytia formation and virus entry. TNF-α, IL-1β, and LPS down-regulate CD4 in macrophages, while their action on HIV-1 replication is opposite. Both TNF-α and IL-1β stimulate HIV-1 replication in macrophages through the activation of the NF-κB [44], while LPS inhibits replication by blocking the viral transcription [45]. The Th2 cytokines (IL-10, IL-13) inhibit HIV-1 replication in primary macrophages [40,46] without modulating surface CD4 expression. Both growth factors GM-CSF and M-CSF stimulate HIV-1 replication in primary macrophages [47], while their action on surface CD4 is different. These data suggest that there is no direct correlation between surface CD4 levels on macrophages and HIV replication.

TNF-α which is known to stimulate HIV-1 replication in primary macrophages induces the formation of a higher number of syncytia than in untreated infected control macro-

phages. Moreover, the addition of anti-TNF-α MoAb to TNF-α-stimulated HIV-infected macrophage culture, while involving decreased HIV growth, induces the formation of a higher number of syncytia than that observed in TNF-α-treated cultures (data not shown). All these data taken together suggest that the down-regulation of surface CD4 by TNF-α, IL-1β, and LPS does not directly influence HIV-1 replication in primary macrophages; rather, the down-regulation of surface CD4 could modulate the course of infection indirectly via its effects on syncytia formation.

High levels of TNF-α have been detected in plasma from AIDS patients [48], and an induction of TNF-α and IL-1 in monocytes isolated from AIDS patients has been observed [49]. A decrease in CD4 surface expression of monocytes isolated from AIDS patients has also been reported [29]. The CD4 down-regulation in monocytes of HIV-1-infected patients has been described both in infected and uninfected cells [50], suggesting that, in contrast to lymphocyte CD4 depletion which is mediated through viral genes, cytokines produced by HIV-infected macrophages such as TNF-α and IL-1β [51,52] may be able to down-regulate CD4 levels in monocytes/macrophages of AIDS patients.

In conclusion, our study indicates that LPS and the pro-inflammatory cytokines TNF-α and IL-1β down-regulate CD4 expression in primary human macrophages *in vitro*. Further experiments are needed *in vivo* to specify the implications of the macrophage CD4 decrease in regard to both inflammatory processes and AIDS pathophysiology.

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