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Increased adhesion of human monocytes to IL-4-stimulated human venous endothelial cells via CD11/CD18, and very late antigen-4 (VLA-4)/vascular cell adhesion molecule-1 (VCAM-1)-dependent mechanisms

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SUMMARY

Expression of adhesion molecules on endothelial cells (EC) can be up-regulated or induced by cytokines. The aim of the present study was to investigate the effect of IL-4 on both the expression of adhesion molecules on EC and monocyte adhesion to EC. Flow cytometric analysis showed that VCAM-1 expression on EC was up-regulated after stimulation with IL-4 for 24 h, whereas the expression of E-selectin (formerly called endothelial leucocyte adhesion molecule-1 (ELAM-1)) was not enhanced, and that of intercellular adhesion molecule-1 (ICAM-1) only slightly. The adhesion of monocytes to EC increased to maximum values upon stimulation of EC with IL-4 for 24 h. Coating of monocytes with MoAb against the integrin β_2-subunit (CD18) significantly inhibited their adhesion to IL-4-stimulated EC; maximal inhibition was found when monocytes were coated with anti-CD18 MoAb in combination with MoAb against CD49d (the α-chain of VLA-4), whereas no inhibition was found when monocytes were coated only with MoAb against CD49d. Monocyte adhesion was not significantly inhibited when IL-4-stimulated EC were coated with MoAbs against ICAM-1 or VCAM-1 alone or in combination. Adhesion of monocytes was inhibited to a greater extent when in addition to coating of monocytes with MoAb against CD18 the EC were coated with MoAb against VCAM-1. From these results we conclude that monocytes bind to IL-4-stimulated EC via interaction of CD11/CD18 molecules on the monocytes with an as yet unknown endothelial ligand, and interaction of VLA-4 on monocytes with VCAM-1 on EC.

Keywords IL-4 adhesion molecules human monocytes endothelial cells

INTRODUCTION

Monocyte adhesion to endothelial cells (EC) is the first step required for the migration of monocytes from blood to the tissue at sites of inflammation. These monocyte–EC interactions are mediated by adhesion molecules expressed on the surface of leucocytes and EC. Known adhesion molecules on monocytes belong to the integrin superfamily and include VLA-4 (CD49d/CD29), a β_1-integrin molecule, and leucocyte function associated antigen-1 (LFA-1; CD11a/CD18), complement receptor-3 (CR3; CD11b/CD18) and p150,95 (CD11c/CD18), all three members of the subfamily of β_2-integrin molecules [1–3]. Adhesion molecules on EC are P-selectin (granule membrane protein-140; CD62), E-selectin (ELAM-1), intercellular adhesion molecule-1 (ICAM-1; CD54), ICAM-2 and VCAM-1, which are not or moderately expressed on the surface of non-stimulated EC [4,5]. After exposure of EC to cytokines like IL-1α, tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) or various other inflammatory mediators, the expression of these adhesion molecules on the surface of EC, except for ICAM-2, is induced or enhanced to various degrees, depending on the kind of stimulus and the duration of exposure [6,7]. Furthermore, different adhesion molecules on stimulated EC bind different types of leucocytes, e.g. P-selectin and E-selectin on EC preferentially bind granulocytes [6,8–11], whereas ICAM-1 and VCAM-1 on EC bind lymphocytes [6,12,13]. E-selectin [10,14] and VCAM-1 [14] on EC have been shown to bind monocytes, and ICAM-1 on EC is involved in the binding of monocytes after their initial binding [6] via CD14 to EC has taken place [15]. These findings have led to the conclusion that during an inflammatory response the production and release of various kinds of inflammatory mediators determine the type and number of leucocytes that bind to EC and subsequently migrate from the blood to the sites of inflammation.

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IL-4, a T lymphocyte-derived cytokine, stimulates B or T lymphocyte proliferation [16], enhances the expression of MHC class II antigens and IgE receptors (FCεRII; CD23) on human monocytes, B lymphocytes and eosinophils, and induces the production of IgE by B lymphocytes [17,18]. It has been shown recently that atopy is associated with the preferential activation of lymphocytes having a cytokine profile that is similar to the cytokines produced by the murine T helper type 2 subset, i.e. IL-3, IL-4, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [19]. In atopic patients the percentage of T lymphocytes that produce IL-4 is significantly increased [20], suggesting an important role for this cytokine in the pathogenesis of atopic diseases like asthma, dermatitis or rhinitis [19,21]. IL-4 has recently been shown to increase the adhesiveness of EC for lymphocytes, but not for granulocytes [22]. The present study concerns the effect of stimulation of EC with IL-4 on the expression of E-selectin, ICAM-1 and VCAM-1, and the involvement of these adhesion molecules in the binding of monocytes.

MATERIALS AND METHODS

Human endothelial cells

Human venous EC were isolated from umbilical cords by 0·1% collagenase digestion as described previously [3,23]. The EC were cultured in plastic culture dishes (Falcon; Becton Dickinson UK Ltd, Plymouth, UK) coated with 0·5% gelatin (Difco Labs, Detroit, MI) in medium 199 (GIBCO Labs, Grand Island, NY) supplemented with 20% heat-inactivated pooled human serum, 1 mM L-glutamine (Flow Labs, Irvine, UK), 0·1 mg/ml endothelial cell growth factor isolated from bovine hypothalamus [24], 5 U/ml heparin, 0·1 mg/ml streptomycin (Biochemie GmbH, Vienna, Austria), 100 U/ml penicillin G (Gist-Brocades, Delft, The Netherlands) and 100 U/ml amphotericin-B (Squibb B.V., Rijswijk, The Netherlands) in a 5% CO_2-incubator at 37°C. At confluency the EC were detached with 0·05% (w/v) trypsin (Difco) and 0·01% (w/v) EDTA (Sigma, St Louis, MO) and subcultured on 0·5% gelatin-coated flat-bottomed 96-well tissue culture plates (Nunclon; Nunc, Roskilde, Denmark). For these experiments confluent monolayers of first- or second-passage cultures of EC were used.

Human monocytes

Human monocytes were isolated from the buffy coat of 500 ml peripheral venous blood from healthy donors by centrifugation on a Ficoll–Isopaque (Pharmacia, Uppsala, Sweden) gradient for 20 min at 650 ***g*** at 18°C. Monocytes were further purified by countercurrent centrifugal elutriation in a Beckman J2-21 centrifuge using a JE-6 rotor and a standard chamber (Beckman Instruments, Inc., Palo Alto, CA). Monocyte-enriched preparations contained more than 85% monocytes, and the viability was more than 95% as assessed by trypan-blue exclusion.

Cytokines and MoAbs

All cytokines were used as recombinant human proteins. IL-4 and TNF-α were a generous gift of Dr J. E. de Vries (DNAX, Palo Alto, CA), IL-1α was kindly provided by Dr P. Lomedico (Hoffmann-La Roche, Nutley, NJ), and IFN-γ was given by Dr P. H. van der Meide (Institute of Applied Radiobiology and Immunology, TNO, Rijswijk, The Netherlands). Anti-VCAM-1 MoAb 4B9 (IgG1) [13] was kindly provided by Dr J. M. Harlan (Department of Medicine, University of Washington, Seattle, WA), anti-E-selectin MoAb H18/7 (IgG2a) [25] was a gift from Dr M. Bevilacqua (Department of Pathology, Brigham and Women's Hospital, Boston, MA), and anti-ICAM-1 (CD54) MoAb RR1/1 (IgG1) [26] was donated by Dr T. A. Springer (Dana Farber Cancer Institute, Boston, MA). Anti-VLA-4α-subunit (CD49d) MoAbs HP1/3 (IgG3) and HP1/2 (IgG1) [27] were given by Dr F. Sánchez-Madrid (Department of Immunology, Hospital de la Princesa, Madrid, Spain) and anti-β_2-integrin (CD18) MoAb IB4 (IgG2a) [28] was obtained as a supernatant of the IB4 cell line (ATCC, Rockville, MD). Neutralizing MoAb against human IL-4 was a gift from Dr L. A. Aarden (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands).

Monocyte–EC adherence assay

The binding of monocytes to non-stimulated or cytokine-stimulated EC was determined as follows. Confluent monolayers of EC grown on 96-well plates were incubated with culture medium alone or supplemented with the various cytokines at the indicated concentrations for various incubation periods. Subsequently EC were washed once with warm (37°C) culture medium, and 1×10^5 monocytes were added to each well and incubated with EC for 30 min at 37°C. The non-adherent monocytes were then removed by three washes with warm PBS, and the number of EC-bound monocytes was determined using a modification of the myeloperoxidase (MPO) assay [29]. Briefly, 0·5% (w/v) hexadecyltrimethyl-ammoniumbromide (HTAB) (Sigma) in modified PBS (without Ca^{2+} and Mg^{2+}; pH 6·0) was added to the wells for 30 min to lyse all cells, which resulted in the release of MPO from the monocytes. The amount of MPO in the lysate, which reflects the number of monocytes bound to EC, was measured spectrophotometrically at 450 nm after the addition of 0·2 mg/ml dianisidinedihydrochloride (Sigma) and 0·4 mM H_2O_2 in modified PBS for 15 min using an ELISA-reader (Titertek; Flow).

The number of EC-bound monocytes was also determined microscopically as described previously [3]. Briefly, EC were cultured on glass coverslips which were precoated with 0·5% gelatin. When a confluent monolayer of EC was formed the medium was removed and suspensions with increasing numbers of monocytes were added and allowed to adhere for 30 min at 37°C. The non-adherent monocytes were removed by washing the coverslips with warm PBS, and the cells were fixed and stained with Giemsa stain. The number of adherent monocytes was counted in 25 high power fields and the total number of monocytes bound per well was calculated.

Assessment of inhibition of monocyte–EC interaction

To study the role of adhesion molecules on EC or monocytes in the monocyte–EC interaction, both cell types were incubated with MoAbs against the various adhesion molecules before the adherence assay. Confluent monolayers of non-stimulated or cytokine-stimulated EC on 96-well culture plates were incubated with culture medium containing saturating concentrations of MoAb (10–20 μg/ml immunoglobulin) for 30 min at 37°C. Monocytes were incubated with 10–20 μg/ml MoAb in medium 199 plus 5% heat-inactivated human AB serum for 30 min on ice. The adhesion assay was performed as described above. Control EC or monocytes were cells treated with medium without MoAbs.

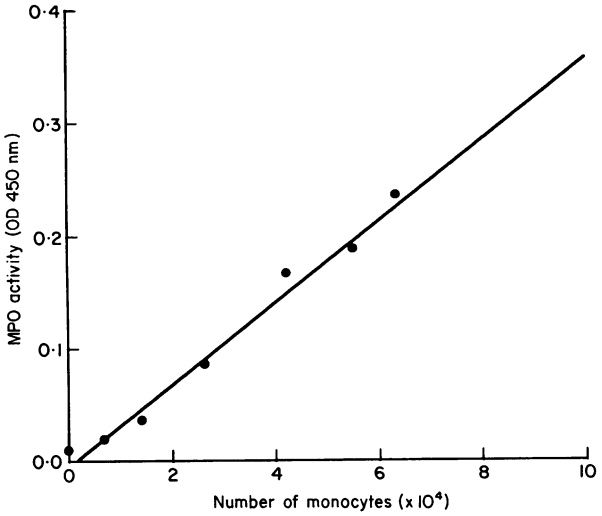

Fig. 1. Correlation between myeloperoxidase (MPO) activity of monocytes and their number adherent to endothelial cells (EC). The correlation coefficient is $r^2 = 0{\cdot}957$.

FACS analysis

The expression of E-selectin, ICAM-1 and VCAM-1 on cytokine-stimulated EC was measured by FACS analysis. Briefly, EC stimulated with 16 U/ml IL-4, 5 U/ml IL-1α or 500 U/ml IFN-γ for 4 h or 24 h were harvested with 0·05% trypsin and 0·01% (w/v) EDTA and washed once with PBS. About 3×10^6/ml EC were incubated with 10–20 μg/ml MoAb H18/7, RR1/1 or 4B9 in PBS supplemented with 5% heat-inactivated human AB serum on ice for 30 min, washed with cold PBS, incubated with 1:100 diluted FITC-conjugated F(ab) sheep anti-mouse immunoglobulin MoAb (Nordic Immunologic Laboratories, Tilburg, The Netherlands) in PBS, plus 5% heat-inactivated human AB serum on ice for 30 min and washed once with cold PBS. The proportion of EC stained with the MoAbs under study was analysed and quantified by flow cytometry using a fluorescence-activated cell sorter (FACStar; Becton Dickinson, Mountain View, CA). Control cells treated with FITC-conjugated MoAb only were included to determine background fluorescence.

Statistical analysis

Results were evaluated statistically by means of the Mann–Whitney *U*-test or the Wilcoxon matched pairs test.

RESULTS

Evaluation of the used method; MPO assay

In earlier studies the adherence of monocytes to EC was counted microscopically [3]. In the present study the MPO activity of monocytes was used to quantify the monocyte–EC interactions. To validate this method for the quantification of the binding of monocytes to EC we studied the correlation between the number of monocytes adherent to EC measured by the MPO assay and the number of bound monocytes which were counted microscopically, which showed a linear correlation (Fig. 1).

Effect of IL-4 on the adhesiveness of EC for monocytes

Incubation of EC with IL-4 for 24 h caused an increased adhesiveness of EC for monocytes which was dependent on the concentration of IL-4 (Fig. 2a). Whereas incubation of EC with as little as 0·4 U/ml promoted monocyte binding, the maximal increase in monocyte binding, i.e. about two-fold, was observed when EC were stimulated with more than 3·4 U/ml IL-4. Stimulation of EC with 16 U/ml IL-4 for various intervals caused a time-dependent increase in their adhesiveness for monocytes (Fig. 2b). Increased binding of monocytes already occurred within 1 h and reached a maximum after 1–2 days of exposure of the EC to IL-4. The increase in monocyte binding to IL-4-stimulated EC could be blocked with MoAb directed against IL-4 (data not shown).

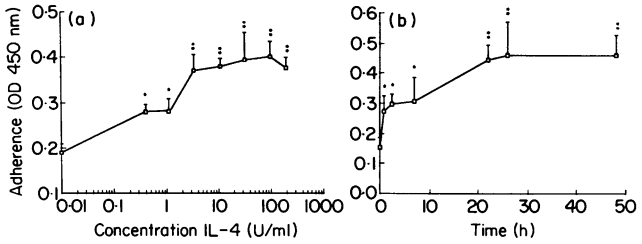

Fig. 2. Dose and time dependence of the IL-4-induced increase in monocyte binding to endothelial cells (EC). Monolayers of human venous EC were incubated with varying concentrations of IL-4 for 24 h at 37°C (a) or with 16 U/ml IL-4 for different intervals at 37°C (b). Next, the adherence of monocytes was assessed by means of the myeloperoxidase (MPO) assay. Values represent the mean ± s.d. of one representative experiment out of three performed in triplicate wells. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$ *versus* control.

Expression of E-selectin, ICAM-1 and VCAM-1 on IL-4-stimulated EC

The expression of various adhesion molecules on EC stimulated with 16 U/ml IL-4 for 4 h or 24 h was determined and compared with the expression of these molecules on non-stimulated EC, and on EC stimulated with IL-1α or IFN-γ. The results of a representative experiment are given in Fig. 3 and Table 1. Flow cytometric analysis revealed that stimulation of EC with IL-4 for 4 h did not induce expression of E-selectin or ICAM-1, and only slightly increased VCAM-1 expression above basal levels. Stimulation of EC with IL-4 for 24 h markedly increased VCAM-1 expression on EC, whereas E-selectin expression was not increased and ICAM-1 expression only moderately. Stimulation of EC with IL-1α transiently induced E-selectin after 4 h, while the maximum increase in ICAM-1 and VCAM-1 expression on EC was found after 24 h of stimulation. Incubation of EC with IFN-γ for 4 h was without effect, whereas incubation for 24 h almost exclusively increased ICAM-1 expression. Similar expression of various adhesion molecules was found when EC were detached mechanically after incubation with 0·2% EDTA (data not shown), indicating that trypsin treatment does not affect the expression of these adhesion molecules or the epitopes to which specific MoAbs bind.

Effect of the stimulation of EC with IL-4 in combination with IFN-γ, IL-1α or TNF-α on the adhesiveness for monocytes

Since stimulation of EC with IL-1α, TNF-α or IFN-γ induced the expression of different adhesion molecules on the surface of EC than stimulation with IL-4 (Fig. 3), and enhanced the adherence of monocytes [30], the effect of the incubation of EC with IL-4 in combination with these cytokines on the binding of

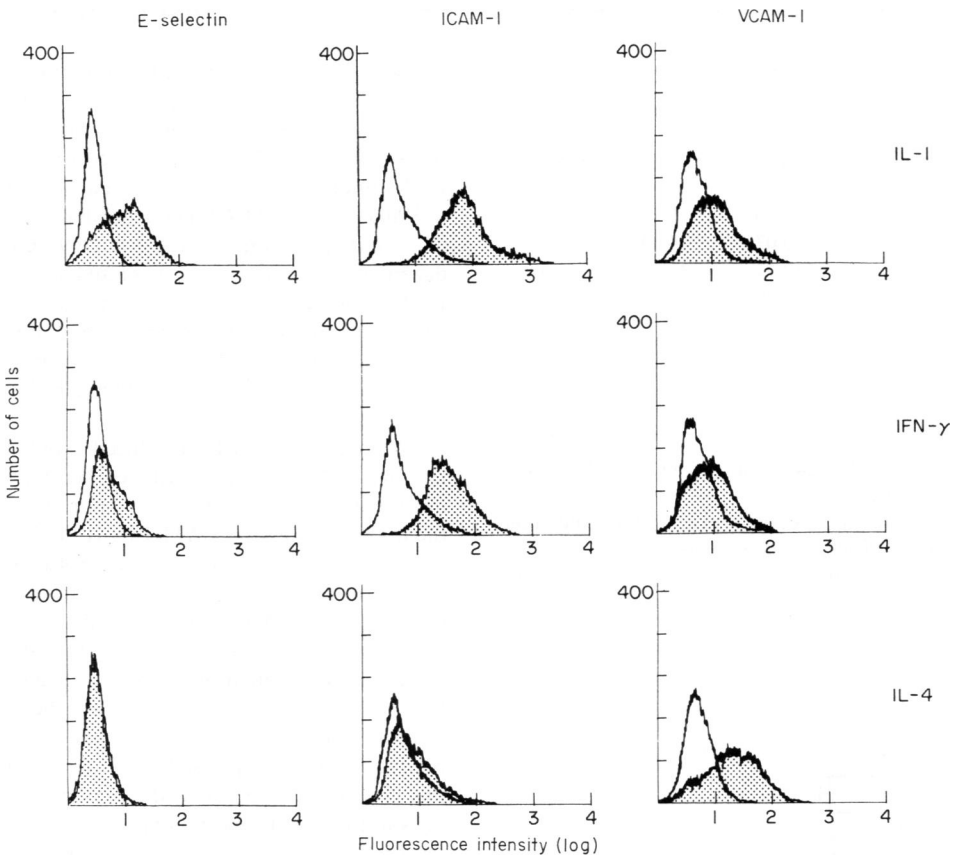

Fig. 3. FACS analysis of antigen expression on endothelial cells (EC). Monolayers of EC were incubated with medium alone, 16 U/ml IL-4, 5 U/ml IL-1α or 500 U/ml IFN-γ for 4 h or 24 h at 37°C. Next, suspensions of the EC, which were incubated with the various cytokines for 4 h, were incubated with MoAb 18/7 to determine E-selectin expression; EC which were stimulated with the various cytokines for 24 h were incubated with MoAb RR1/1 or MoAb 4B9 to determine intercellular adhesion molecule-1 (ICAM-1) and VCAM-1 expression, respectively. the cells were prepared for fluorescence analysis and the relative number of EC with a specific fluorescence intensity was analysed with the FACStar. The expression of E-selectin, ICAM-1 and VCAM-1 on unstimulated EC (□) was determined and compared with E-selectin expression on EC stimulated with the various cytokines for 4 h (▤) or ICAM-1 and VCAM-1 expression on EC stimulated with the various cytokines for 24 h (▤). Background fluorescence was established by incubation of EC with the FITC-conjugated MoAb only.

monocytes to EC was studied. Stimulation of EC with 3 U/ml IL-4 and 50 U/ml IFN-γ for 24 h caused an increased binding of monocytes, whereas incubation with 3 U/ml IL-4 and 0·5 U/ml IL-1α or 50 U/ml TNF-α for 24 h did not enhance the adhesiveness of EC for monocytes compared with incubation with each cytokine alone (Fig. 4).

Adhesion molecules involved in monocyte binding to IL-4-stimulated EC

Since VCAM-1 and to a lesser extent ICAM-1 are expressed on EC after 24 h stimulation with IL-4, the relative contributions of these adhesion molecules and their respective ligands CD49d/CD29 and CD11/CD18 on monocytes to the increased binding of monocytes to EC were determined. Monolayers of non-stimulated or IL-4-stimulated EC were incubated with anti-VCAM-1 MoAb, anti-ICAM-1 MoAb, the combination of anti-VCAM-1 and anti-ICAM-1 MoAbs or plain medium, and the adhesion of non-coated monocytes or monocytes coated with anti-CD18 MoAb was determined. Incubation of EC stimulated with IL-4 for 24 h with either anti-VCAM-1 or anti-ICAM-1 MoAb alone or in combination did not significantly ($P > 0{\cdot}05$) inhibit the binding of non-coated monocytes (Fig. 5). Differences in MPO activity in monocytes derived from different donors account for the large s.d. found between the experiments. The binding of monocytes to IL-4-stimulated EC signifcantly decreased ($P < 0{\cdot}001$) when the monocytes were coated with MoAb against CD18, and was even lower ($P < 0{\cdot}001$) when in addition the IL-4-stimulated EC were coated with anti-VCAM-1 MoAb (Fig. 5). The adhesion of anti-CD18 MoAb-coated monocytes to IL-4-stimulated EC was not further inhibited when EC were coated simultaneously with anti-ICAM-1 MoAb (data not shown). No further reduction in the binding of anti-CD18 MoAb-coated monocytes was found when in addition to anti-VCAM-1 EC were also coated with anti-ICAM-1 MoAb (Fig. 5). Thus both CD11/CD18 molecules on monocytes and VCAM-1 on EC mediate monocyte adhesion to IL-4-stimulated EC. The contribution of VCAM-1-mediated adhesion was confirmed by the use of MoAb against CD49d, the principal ligand for VCAM-1 on monocytes. The degree of inhibition of monocyte binding to IL-4-stimulated EC was

Table 1. FACS analysis of antigen expression on endothelial cells (EC) after incubation with different cytokines

Incubation EC*		Mean fluorescence intensity†		
Cytokine	Incubation time (h)	E-selectin‡	ICAM-1‡	VCAM-1‡
—	—	1·51	5·78	2·95
rIL-4	4	0·07	4·90	4·50
rIL-1α	4	12·52	44·21	11·49
rIFN-γ	4	0·00	4·33	0·00
rIL-4	24	0·81	9·98	30·47
rIL-1α	24	3·48	110·49	14·43
rIFN-γ	24	1·58	51·76	9·49

* EC were stimulated for 4 h or 24 h with 16 U/ml IL-4, 5 U/ml IL-1 or 500 U/ml IFN-γ at 37°C.

† Mean fluorescence intensity was expressed in arbitrary units after subtraction of the values of background fluorescence.

‡ MoAbs were used in optimal concentrations: H18/7 anti-E-selectin (20 μg/ml IgG2a), RR1/1 anti-intercellular adhesion molecule-1 (ICAM-1) (10 μg/ml IgG1) and 4B9 anti-VCAM-1 (10 μg/ml IgG1).

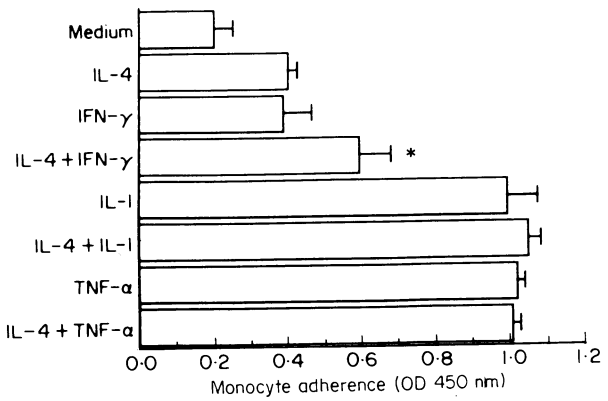

Fig. 4. Effect of the stimulation of endothelial cells (EC) with IL-4 in combination with other cytokines. EC were incubated with culture medium alone or with 3 U/ml IL-4, 50 U/ml IFN-γ, 50 U/ml tumour necrosis factor-alpha (TNF-α) or 0·5 U/ml IL-1α alone or in combination for 24 h at 37°C. Values represent the mean ± s.d. of one representative experiment out of three performed in triplicate wells. *$P < 0{\cdot}05$ *versus* IL-4 alone.

maximal, i.e. 77·6 ± 15·9% ($n = 6$), when monocytes were coated with both anti-CD18 MoAb and anti-CD49d MoAb, which was significantly ($P < 0{\cdot}001$) more than the inhibition of binding found when monocytes were coated with anti-CD18 MoAb alone (Fig. 6). No extra inhibitory effect was found when monocytes coated with both anti-CD18 and anti-CD49d MoAb were added to IL-4-stimulated EC which had been coated with anti-VCAM-1 MoAb (data not shown). Coating of monocytes with anti-CD49d MoAb alone was without effect (Fig. 6).

Monocyte adherence to non-stimulated EC was markedly inhibited by anti-CD18 MoAb (73·7 ± 18·9% inhibition; $P < 0{\cdot}001$; $n = 6$). Addition of either anti-CD49d MoAb to monocytes or anti-ICAM-1 and/or anti-VCAM-1 MoAb to non-stimulated EC was without effect (Figs 5 and 6).

DISCUSSION

The present results show that incubation of human venous EC with IL-4 induces the expression of VCAM-1 on EC and increases the adhesiveness of EC for human monocytes. Incubation with specific MoAbs indicated that monocyte binding to IL-4-stimulated EC involves at least two adhesion mechanisms: a β_2-integrin (CD11/CD18)-dependent and ICAM-1-independent mechanism, and a VLA-4 (CD49d/CD29) and VCAM-1-dependent mechanism. The increased binding of lymphocytes to IL-4-stimulated EC is also controlled by a CD11a/CD18-dependent and ICAM-1-independent pathway [31]. Apparently an as yet unknown molecule expressed on IL-4-stimulated EC is involved in the CD11/CD18-dependent binding of monocytes and lymphocytes.

We observed that IL-4 induced expression of VCAM-1 on EC, which is consistent with earlier findings [32], although divergent results have also been reported [33,34]. Together with the up-regulation of VCAM-1 by IL-4, the adhesiveness of EC for monocytes is found to be increased in a time- and dose-dependent fashion. Monocytes can adhere via VLA-4 (CD49d/CD29) to VCAM-1, as has been demonstrated in VCAM-1-transfected chinese hamster ovary (CHO) cells [14]. In the present study a similar binding of monocytes to IL-4-stimulated EC has been found according to the inhibition with the respective MoAbs. Although monocytes have been shown to bind to E-selectin expressed on cytokine-stimulated EC [14,30], this is unlikely to occur in the present experiments since IL-4 does not induce E-selectin expression on EC, as has also been reported earlier [35]. ICAM-1 is not involved in the CD11/CD18-dependent binding of monocytes to IL-4-stimulated EC, since the expression of ICAM-1, which is considered to be the receptor for CD11a/CD18 [26] and CD11b/CD18 [36], was very low on IL-4-stimulated EC, and anti-ICAM-1 MoAb did not inhibit the increased monocyte binding to IL-4-stimulated EC. The failure of the anti-ICAM-1 MoAb (RR1/1) to inhibit monocyte binding can not be explained by binding of the MoAb to monocytes via its Fc domain, which would mask the inhibitory effect, since we found that anti-VCAM-1 MoAb (4B9), which has the same isotype, in combination with anti-CD18 MoAb, inhibited the adhesion of monocytes to IL-4-stimulated EC.

Since IL-4 selectively up-regulated VCAM-1 on EC and IFN-γ almost exclusively up-regulated ICAM-1 on EC, the increase in the binding of monocytes to EC stimulated with both IL-4 and IFN-γ might be due to the induction of two different adhesion molecules on EC, and thus two different ligand–receptor interactions. The lack of an increased stimulatory effect on monocyte binding when EC were incubated with IL-4 together with IL-1α or TNF-α can be explained by the fact that both ICAM-1 and VCAM-1 are already expressed on EC stimulated with IL-1α (this study) or TNF-α [6,14]. Hence no additional molecules on the EC become available for monocyte binding.

IL-4 has become known as a pleiotropic lymphokine with different effects on EC and leucocytes [16–18,37]. It has been reported that incubation of EC with IL-4 resulted in an increased adhesiveness for human lymphocytes, but not for human granulocytes ([22,31] our unpublished observations). Others have shown that IL-4 inhibited the increase in the expression of ICAM-1 and E-selectin on EC induced by IL-1 or

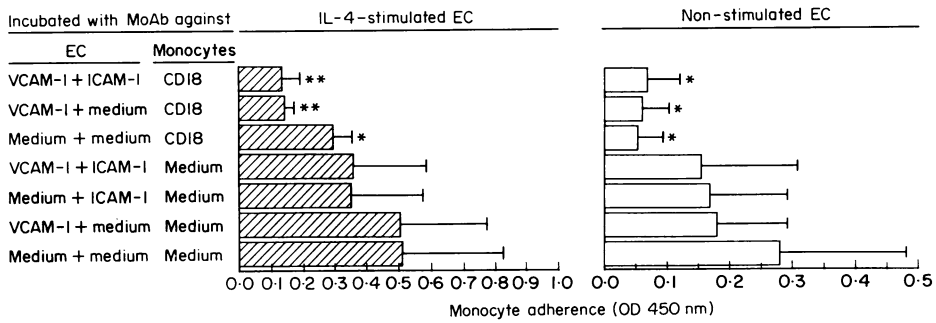

Fig.5. Inhibition of monocyte binding to endothelial cells (EC) stimulated with 16 U/ml IL-4 for 24 h or non-stimulated EC. Monocytes incubated in medium or in medium containing anti-β_2-integrin (CD18) MoAb were added to IL-4-stimulated or non-stimulated EC which had been preincubated with plain medium or medium containing anti-VCAM-1, anti-intercellular adhesion molecule-1 (ICAM-1) MoAb, or a combination of anti-VCAM-1 and anti-ICAM-1 MoAbs. Values represent the mean ± s.d. of three experiments, each performed in quadruplicate wells. $*P < 0{\cdot}001$ *versus* control; $**P < 0{\cdot}001$ *versus* MoAb anti-CD18.

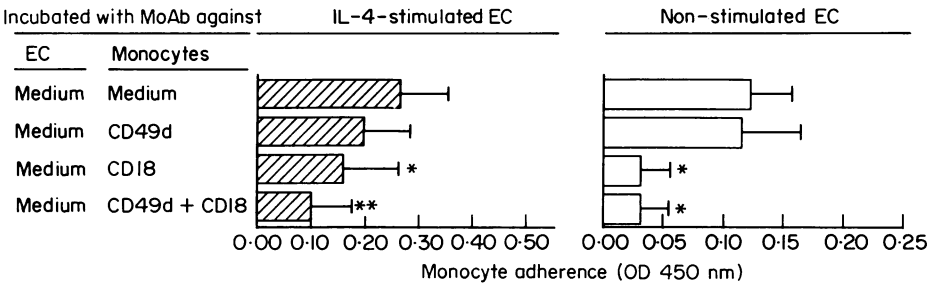

Fig. 6. Inhibition of monocyte binding to endothelial cells (EC) stimulated with 16 U/ml IL-4 for 24 h or non-stimulated EC. Monocytes incubated in medium or in medium containing anti-β_2-integrin (CD18) MoAb, anti-VLA-4α (CD49d) MoAb or a combination anti-CD18 and anti-CD49d MoAbs were added to IL-4-stimulated or non-stimulated EC. Values represent the mean ± s.d. of three experiments, each performed in quadruplicate wells. $*P < 0{\cdot}001$ *versus* control; $**P < 0{\cdot}001$ *versus* MoAb CD18 and $P < 0{\cdot}001$ *versus* MoAb CD49d.

TNF-α [35], which indicates an inhibitory effect of IL-4 on the leucocyte–EC interaction. Incubation of monocytes with IL-4 inhibits monocyte adhesion to EC [38], which is probably mediated via down-regulated expression of the myeloid differentiation antigen CD14 [39,40] on the surface of monocytes. This antigen has been shown to be involved in monocyte adhesion to cytokine-stimulated EC [15].

Whether IL-4 has similar effects *in vivo* as *in vitro* is uncertain. At the site of inflammation T lymphocytes will probably generate locally a high concentration of IL-4, and might thus primarily affect EC at that site. Whether the serum concentration of IL-4 will become high enough to influence the expression of adhesion molecules on leucocytes is unknown as yet. Since monocytes ([14] and this study), lymphocytes [41] and eosinophils [42,43] can adhere via VLA-4 to EC expressing VCAM-1, the preferential recruitment of these cells [44] during the late phase response in atopic diseases like asthma and dermatitis can be regulated by the selective up-regulation of VCAM-1 on EC by IL-4 produced by mast cells [45] and T helper 2-like lymphocytes [19–21,46] at the site of inflammation.

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