

Inhibition of Human Monocyte Adhesion by Interleukin-4

By Michael J. Elliott, Jennifer R. Gamble, Linda S. Park, Mathew A. Vadas, and Angel F. Lopez

The adhesion of monocytes to vascular surfaces is central to inflammation and atherogenesis; however, very little is known about regulatory factors that can prevent these processes. Here we report the inhibition of human monocyte adhesion to human endothelial layers and plastic by interleukin-4 (IL-4), a T-cell-derived glycoprotein with pleiotropic activities. The inhibitory effects of IL-4 were seen with basal and cytokine-stimulated monocyte adhesion, were apparent at low concentration, and were abolished by inactivating IL-4. No direct toxic effect of IL-4 on monocytes was detected. Inhibition of adhesion was accompanied by small

increases in monocyte surface expression of the leukocyte-functional antigen group of adhesion structures, suggesting that absolute levels of expression may be less important than the functional status of such molecules in the regulation of monocyte adhesion. In addition, inhibition by IL-4 of cytokine-stimulated monocyte adhesion was not associated with changes in the surface expression of cytokine receptors. These results suggest a role for IL-4 in the regulation of monocyte adhesion, and may provide for a common mechanism for the inhibitory effects of IL-4 on monocyte function. © 1991 by The American Society of Hematology.

MONOCYTES ARE blood cells involved in chronic inflammation and atherogenesis. For both processes migration of these cells from blood into tissues is essential. In the case of fat-induced atherogenesis, monocyte adhesion to localized patches of endothelium is the first recognizable process.^{1,2} In chronic inflammation a necessary step for the migration of monocytes into the tissues is the adhesion of these cells to endothelial cells. The adhesion process has become better understood in the last few years; adhesion for monocytes can be induced in endothelial cells by cytokines such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α)³ (J. Gamble, unpublished) and recently the hematopoietic growth factors granulocyte-macrophage colony-stimulating factor (GM-CSF)^{4,5} and IL-3⁵ have been shown to act on monocytes, stimulating their adherence to endothelium and other surfaces.

Given the importance of the adhesive process it seems likely that mechanisms exist that act as negative regulators of adhesion and ensure the uninterrupted circulation of monocytes. In this report we demonstrate that the T-cell-derived glycoprotein IL-4 inhibits basal monocyte adhesion as well as GM-CSF- and IL-3-stimulated adhesion, and we attempt to define the mechanisms by which such inhibition might occur. The anti-adhesive property of IL-4 has therapeutic potential and may help explain some of the anti-inflammatory effects of IL-4.

MATERIALS AND METHODS

Cytokines. The human cytokines used were recombinant and purified to greater than 99% as specified by the suppliers. IL-4 from yeast was obtained from Immunex Corporation (Seattle, WA) while IL-3 from *Escherichia coli* and GM-CSF from COS cells were kind gifts from Dr S Clark (Genetics Institute, Cambridge, MA). The two latter cytokines will be referred to collectively as 'CSF.' All cytokines contained undetectable (<10 pg/mL) levels of lipopolysaccharide (LPS) by Limulus Amoebocyte Lysate Assay (Whittaker Bioproducts Inc, Walkersville, MD) at the maximum concentrations used in these experiments (6,000 pmol/L).

Monoclonal antibodies (MoAb). Purified mouse MoAb 60.3 to the leukocyte functional antigen (LFA) family common β chain (CD18) and 60.1 to the α chain of the MAC-1 complex (CD11b) were kind gifts from Dr P Beatty (Fred Hutchinson Cancer Research Center, Seattle, WA). MoAb TS 1/22 (α chain of the LFA-1 complex, CD11a) and TS 1/18 (common β chain) were kind gifts from Dr T Springer (Harvard Medical School, Boston, MA).⁶ MoAb MO-1 (α chain of MAC-1 complex, CD11b) and 150/95 (α

chain of p150/95 complex, CD11c) were purchased from Coulter Immunology (Hiialeah, FL).

Monocyte purification. Monocytes were purified from the peripheral blood of normal donors, obtained from the Adelaide Red Cross Transfusion Service as previously described.⁵ In brief, mononuclear cells were prepared by centrifugation of whole blood on Lymphoprep (Nyegaard, Oslo, Norway) and washed twice in Hanks Balanced Salt Solution without Ca²⁺ or Mg²⁺ (HBSS). Monocytes were purified in a Beckman J-6M/E elutriator (Beckman Instruments Inc, Palo Alto, CA) using the Sanderson chamber, a flow rate of 12 mL/min and a constant rotor speed of 2,050 rpm. Using these methods, monocyte purity as assessed by morphology and nonspecific-esterase staining was always greater than 90% and usually greater than 95%.⁵

Adhesion assay. Adhesion was measured by an isotopic method as previously described.⁵ In brief, purified monocytes (0.5 to 1 \times 10⁸) were incubated for 30 minutes at 37°C with 500 μ Ci ⁵¹Cr in the form of sodium chromate (Amersham Int, Buckinghamshire, England). For measurement of adhesion, 1 to 2.5 \times 10⁵ monocytes were aliquotted per well in 96-well microtitre plates (Nunc, Kamstrup, Denmark) together with stimuli or control medium to a total volume of 100 μ L, and incubated for the indicated periods. At harvest, samples of supernatant were taken to assess spontaneous ⁵¹Cr release (usually <10% of cell-associated radioactivity), wells were washed thrice with RPMI 1640 at 37°C, and residual adherent cells lysed as described.⁵ Lysates were counted in a Packard auto-gamma 5650 (Packard Instrument Company, Meriden, CT) and percent adhesion calculated according to the formula:

$$\% \text{ adhesion} = \frac{\text{residual adherent cpm} \times 100}{\text{total cpm added} - \text{cpm spontaneously released}}$$

Suspension cultures of monocytes. In experiments involving adhesion to endothelium, monocytes were incubated for up to 21

From the Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide, South Australia; and Immunex Corporation, Seattle, WA.

Submitted August 24, 1990; accepted February 8, 1990.

Supported by grants from the National Health and Medical Research Council (NH & MRC) of Australia. M.J.E. was supported by a NH & MRC Medical Postgraduate Scholarship.

Address reprint requests to A.F. Lopez, MD, PhD, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Rd, Adelaide, South Australia, 5000.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1991 by The American Society of Hematology.

0006-4971/91/7712-0005\$3.00/0

hours in suspension before the measurement of adhesion. In these cases, the ^{51}Cr -labeled cells were rotated in polypropylene tubes at 37°C either with or without the addition of stimuli. The cells were then centrifuged from suspension, washed once in RPMI 1640, cell counts performed, and used immediately in adhesion assays as described above. With this procedure we routinely obtained greater than 90% viable cells as judged by Trypan blue exclusion.

Preparation of endothelial monolayers. Human umbilical vein endothelial cells (HUVEC) were obtained by collagenase treatment of umbilical veins. The cells were plated onto gelatin-coated Costar 25-cm² flasks (Costar, Cambridge, MA), and maintained in medium 199 (Cytosystems, Sydney, New South Wales, Australia) with 20% fetal calf serum (FCS), 20 mmol/L HEPES, and added sodium bicarbonate, sodium pyruvate, nonessential amino acids and antibiotics, as previously prescribed.⁷ Cells were used between 4 and 8 days after establishment of cultures. For use in adherence assays, cells were harvested using trypsin-EDTA and replated into the central 60 wells of flat-bottomed gelatin-coated microtitre trays (Nunc) at 2×10^4 cells/well and grown overnight. Purity was assessed by staining with factor VIII and exceeded 99% HUVEC. Confluence of monolayers was assessed visually on the day of use. Immediately before use monolayers were washed twice with RPMI 1640.

Immunofluorescence. Monocytes were cultured in suspension for 18 hours with or without 600 pmol/L IL-4. The cells were then centrifuged at 100g for 5 minutes, and resuspended in RPMI with 0.2% sodium azide (NaN_3) and 5% FCS. Cell suspensions were cooled to 4°C and all subsequent steps were performed at this temperature in 5-mL polypropylene tubes (Disposable Products, Adelaide, South Australia). Fifty-microliter aliquots of monocyte suspensions were added to 50 μL of a 1:20 dilution of normal rabbit serum to block Fc-mediated binding. Following 15 minutes of incubation, a further 50 μL of medium containing the primary staining MoAb at appropriate dilution was added and allowed to bind for 20 minutes. The cells were then centrifuged at 100g for 5 minutes in washing medium consisting of phosphate-buffered saline (PBS) with 0.2% NaN_3 and 5% FCS. The cell pellets were resuspended in 50 μL of medium containing a 1:40 dilution of a fluoresceine-isothiocyanate-labeled sheep antimouse antibody [$\text{F}(\text{ab}')_2$ fragments; Silenus Laboratories, Hawthorn, Victoria, Australia] and incubated for 20 minutes. After a final wash, the cells were fixed in a solution of PBS with 0.2% NaN_3 , 2% glucose, and 1% formaldehyde and subjected to flow cytometry on a Coulter Epics V. Antibody binding was measured by green fluorescence, with at least 10,000 cells counted per point. The co-efficient of variation for the flow cytometer was 2% to 3%.

Radioligand binding. Binding of ^{125}I -IL-3 and ^{125}I -GM-CSF to monocytes was measured after preincubation of the cells in suspension culture with or without 600 pmol/L IL-4. The N-terminal-modified CSF used, the methods of iodination and radioligand characterization, and the details of the binding assay have been previously described.⁸ Binding was performed at 4°C for 18 hours, under which conditions internalization of radioligand does not occur.⁸ Nonspecific binding was defined as that seen in the presence of a 100-fold excess molar concentration of homologous nonlabeled ligand. Specific binding was calculated as total binding minus nonspecific.

Maintenance of LPS-free conditions. All laboratory glassware was washed using E-Toxa-Clean (Sigma) and heated to 160°C for 3 hours to reduce LPS contamination. Plastic disposable laboratoryware was used where possible. RPMI 1640 was obtained from the Commonwealth Serum Laboratories (Melbourne, Victoria, Australia) and contained less than 10 pg/mL LPS. Batches of FCS were screened by limulus amoebocyte lysate assay and selected for low LPS levels. At the maximum concentrations of FCS used in these

experiments (10%) the media contained less than 40 pg/mL LPS. All other reagents used contained undetectable levels of LPS at the maximal concentrations used in these experiments.

Statistics. Results for pooled experiments were expressed as the mean \pm SEM. Results in individual experiments were expressed as the mean \pm SD of four to seven replicates per point. Significance was determined using the Student's unpaired *t*-test or Wilcoxon's matched-pairs test.

RESULTS

Inhibition of adhesion to HUVEC and plastic. Pooled data from three experiments measuring adhesion to HUVEC and 10 experiments measuring adhesion to plastic are shown in Fig 1. HUVEC experiments were conducted following monocyte culture for 21 hours in suspension, while plastic experiments were performed with culture in the microtitre wells for the same period. Each experiment included IL-3- and GM-CSF-stimulated groups, as well as control groups, with and without IL-4. In most experiments, IL-3 or GM-CSF were used at a final concentration of 60 pmol/L, and IL-4 at 600 pmol/L. IL-4 inhibited basal monocyte adhesion to HUVEC by an average of 53%, while IL-3- and GM-CSF-stimulated adhesion were reduced by an average of 33% and 47%, respectively. Inhibition of adhesion to plastic was also seen, with average reductions of 45%, 31%, and 30% for basal, IL-3-stimulated, and GM-CSF-stimulated adhesion, respectively. Analysis of the pooled HUVEC data using the unpaired *t*-test showed that the IL-4-induced inhibition of adhesion was highly significant ($P < .001$). Similarly, analysis of the plastic data by the Wilcoxon's matched-pairs test showed that all three groups were significantly ($P < .01$) inhibited by IL-4.

To determine whether IL-4 might inhibit adhesion at earlier time points, experiments were performed in which

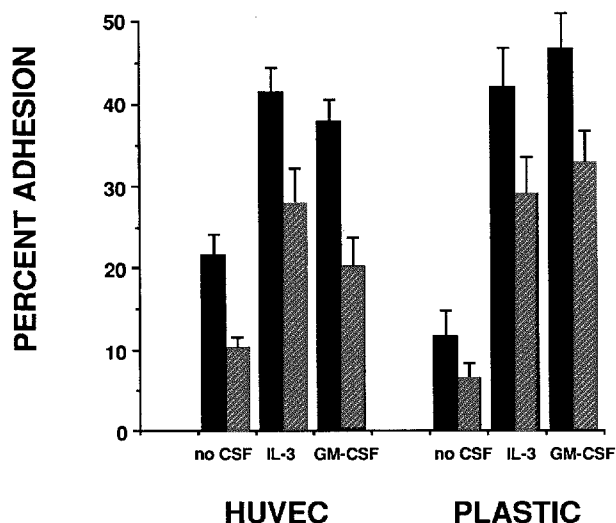


Fig 1. Inhibition of monocyte adhesion to HUVEC and to plastic. ^{51}Cr -labeled monocytes were preincubated in suspension (HUVEC experiments) or in microtitre wells for 21 hours, before measurement of adhesion. CSF (60 pmol/L) and/or IL-4 (600 pmol/L) were included from the onset of culture. Each column represents the mean \pm SEM of three (HUVEC) or ten (plastic) separate experiments performed in the absence (■) or presence (▨) of IL-4.

Table 1. Time Course of IL-4-Indicated Inhibition of Adhesion

Stimulus	Time (h)			
	1	2	11	17
No CSF	50.5 ± 14.3*	29.2 ± 4.2	19.2 ± 2.3	7.4 ± 1.3
+IL-4	52.7 ± 6.7	29.6 ± 4.3	16.4 ± 0.6 (<i>P</i> = .019)†	8.2 ± 2.1 (<i>P</i> = .48)
IL-3	54.9 ± 5.6	28.1 ± 4.8	23.1 ± 2.4	25.1 ± 4.6
IL-3 + IL-4	59.9 ± 5.5	30.2 ± 0.7	18.3 ± 2.7 (<i>P</i> = .007)	13.6 ± 1.1 (<i>P</i> < .0001)
GM-CSF	64.4 ± 8.2	29.1 ± 3.7	22.9 ± 3.2	27.1 ± 3.2
GM-CSF + IL-4	56.8 ± 7.0	32.3 ± 5.8	17.6 ± 3.1 (<i>P</i> = .021)	14.4 ± 1.5 (<i>P</i> < .0001)

⁵¹Cr-labeled monocytes were incubated in plastic microtitre wells with IL-3 or GM-CSF (final concentration 6,000 pmol/L) or control medium, with or without IL-4 (final concentration 4,000 pmol/L). Adhesion was measured at the indicated times as described in Materials and Methods.

*Percent adhesion, mean ± SD of seven replicates per point.

†Significance, compared with no IL-4, by Student's *t*-test.

monocyte adhesion was measured at several different times during the course of the assay. The results of such an experiment are shown in Table 1, where adhesion at 1 and 2 hours of culture is not inhibited by IL-4. By 11 hours of culture, IL-4-treated cells show small decreases in adhesiveness compared with controls (*P* ≤ .021), and by 17 hours, IL-3- and GM-CSF-treated cells show highly significant inhibition of adhesion by IL-4 (*P* < .0001). IL-4 did not inhibit control-treated cell adhesion in this experiment, possibly due to the very low level of adhesion seen in this group (Table 1). Experiments during the first hour of

culture, during which time IL-3 and GM-CSF enhance monocyte adhesion,⁵ did not show a significant inhibitory effect of IL-4 (data not shown).

Concentration dependence for IL-4 inhibition. IL-4 inhibited both IL-3-stimulated and unstimulated adhesion, and showed activity at concentrations as low as 20 pmol/L (Fig 2). Maximal inhibition of unstimulated adhesion, or adhesion stimulated by low concentrations of IL-3, was seen with 60 pmol/L IL-4. In contrast, when higher concentrations of IL-3 were used to stimulate adhesion (60 pmol/L, 600 pmol/L), concentrations of IL-4 of at least 600 pmol/L were

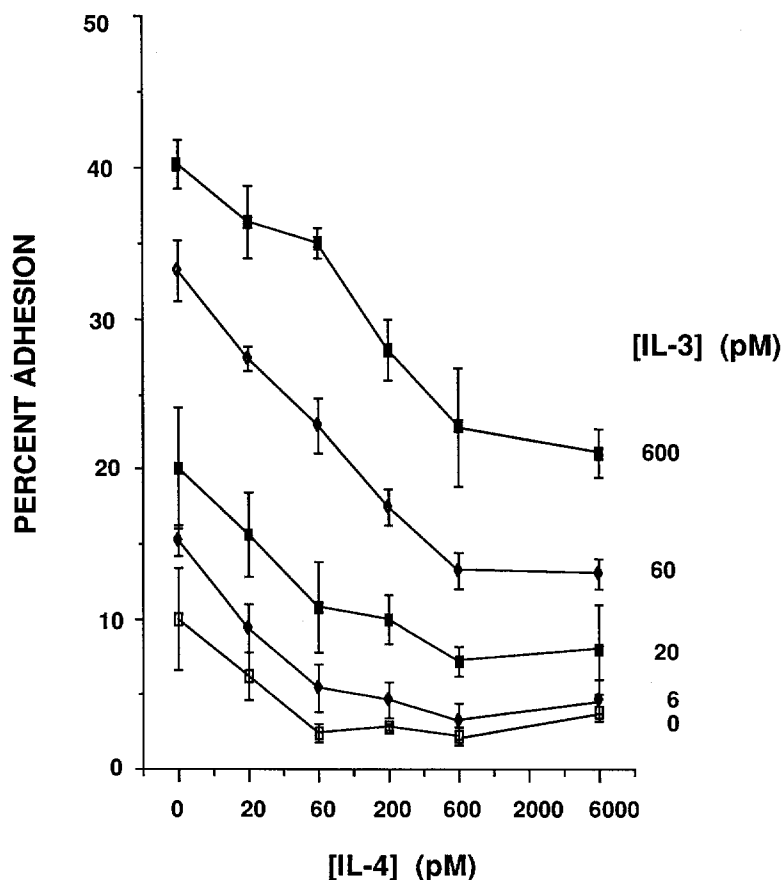


Fig 2. Inhibition of IL-3-stimulated monocyte adhesion to plastic by varying concentrations of IL-4. ⁵¹Cr-labeled monocytes were incubated for 21 hours in microtitre wells with varying concentrations of IL-3 and IL-4. Each point represents the mean ± SD of six replicates.

required to achieve maximal inhibition. Maximal inhibition by IL-4 ranged from 79% (6 pmol/L IL-3) to 48% (600 pmol/L IL-3). Similar results were observed with IL-4 inhibition of GM-CSF-stimulated monocyte adhesion (data not shown), where inhibition ranged from 80% (6 pmol/L GM-CSF) to 37% (600 pmol/L GM-CSF).

Heat inactivation of IL-4. Although the preparation of IL-4 used in these experiments was greater than 99% pure (by amino acid analysis) and was free of detectable LPS or sodium azide, it was sought to further confirm IL-4 as the inhibitory agent by taking advantage of its heat-lability. To this end, the IL-4 diluted in HBSS, and HBSS alone were boiled for 45 minutes before their inclusion in adhesion assays, and compared with identical samples maintained at room temperature. IL-3- and GM-CSF-stimulated adhesion were significantly inhibited by unboiled IL-4 ($P = .01$, $P = .001$, respectively). In contrast, adhesion in the presence of boiled IL-4 did not differ significantly from that seen with boiled HBSS ($P > .05$), establishing that the inhibitory molecule was heat-labile (Fig 3A). Further evidence against the presence of a toxic contaminant within the IL-4 preparation was provided by cell viability studies. The spontaneous release of ^{51}Cr from radiolabeled monocytes following overnight culture was less than 15% of total radioactivity for both IL-4-treated and untreated groups, with no significant differences seen between the groups ($P > .05$) (Fig 3B). These findings were seen despite the clear inhibitory activity of IL-4 on the adhesive status of the same monocyte cultures (Fig 3B). In addition, cell survival and viability by Trypan-blue exclusion exceeded 90% for all treatment groups after overnight suspension culture.

Monocyte LFA surface expression. Monocyte adhesion to both plastic and endothelium is dependent in part on molecules of the LFA adhesion family.^{5,9-12} To determine the influence of IL-4 on LFA expression, monocytes were cultured overnight in suspension with or without IL-4, and then stained with anti-LFA MoAb as described in Materials and Methods. Instead of a decrease in expression, as might have been predicted, IL-4 induced increases in the expression of CD18 and of the MAC-1 complex, with a lesser increase in expression of p150/95 (Table 2). Similar results were obtained in two experiments, both in terms of levels of expression in unstimulated cells, and in the IL-4-induced increases seen. Parallel adhesion experiments showed approximately 50% inhibition of monocyte adhesion after IL-4 pretreatment (Table 2, legend).

Modulation of CSF receptors by IL-4. To determine whether IL-4 might modulate the expression of CSF receptors, and thereby inhibit the CSF-induced proadhesive effect, experiments were performed in which the specific binding of ^{125}I -IL-3 and ^{125}I -GM-CSF to monocytes was measured after an overnight incubation of the cells with or without IL-4. Pooled data from three such experiments are shown in Table 3, where specific binding is presented both in the absence of competitor and in the presence of heterologous CSF as competitor (IL-3 or GM-CSF) or in the presence of IL-4.

In cells preincubated with medium alone, IL-3 inhibited approximately 70% of ^{125}I -GM-CSF binding, and GM-CSF

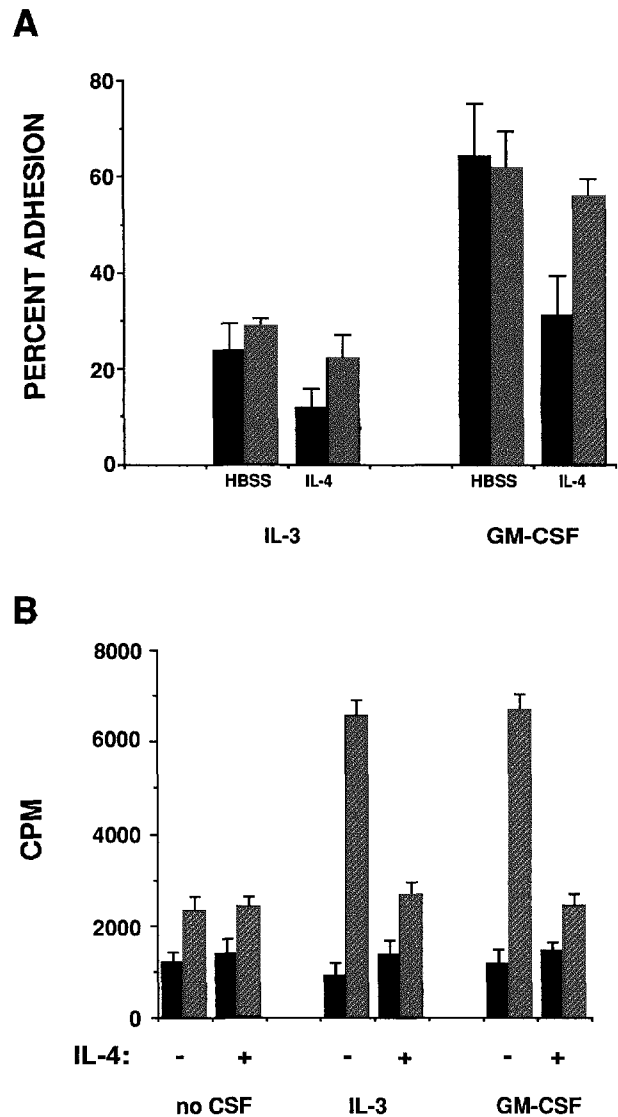


Fig 3. (A) Heat inactivation of IL-4. Monocyte adhesion to plastic was measured after 21 hours of culture with 60 pmol/L IL-3 or GM-CSF. IL-4, HBSS, or heat-inactivated IL-4 or HBSS were included from the onset of culture (final concentration 600 pmol/L). Each point represents the mean \pm SD of six replicates. (■) 24°C; (▨) 100°C. (B) Spontaneous ^{51}Cr release after 18 hours in culture. ^{51}Cr -labeled monocytes were incubated for 18 hours in the presence or absence of 600 pmol/L IL-4, together with CSF or control medium. Before washing nonadherent cells from the wells, samples of supernatant were collected and counted. Results are expressed as total supernatant cpm (■), and compared at each point with total adherent cpm (▨). Each point is the mean \pm SD of six replicates in a representative experiment. Total cpm added to each well were 14,384.

inhibited approximately 80% of ^{125}I -IL-3 binding. These results confirm in 1-day-old cells the IL-3/GM-CSF cross-inhibition that we have previously reported in fresh monocytes.⁸ In contrast, IL-4 failed to inhibit ^{125}I -IL-3 binding, and inhibited only 15% of ^{125}I -GM-CSF binding. Overall binding in IL-4-pretreated cells was not significantly different from that in medium-treated cells ($P > .34$) and the IL-3/GM-CSF cross-inhibition was still evident. IL-4 did not alter ^{125}I -IL-3 or ^{125}I -GM-CSF binding (Table 3).

Table 2. LFA Surface Expression on Unstimulated and IL-4-Stimulated Monocytes

MoAb (antigen)	Stimulus	Experiment		Fluorescence Shift (mean \pm SD)
		1	2	
60.1 (MAC-1)	No CSF	92*	101	
	IL-4	122 (+30)†	121 (+20)	+25 \pm 5
MO-1 (MAC-1)	No CSF	63	65	
	IL-4	84 (+21)	85 (+20)	+21 \pm 1
TS1/22 (LFA-1)	No CSF	112	105	
	IL-4	121 (+9)	107 (+2)	+6 \pm 4
150/95 (p 150/95)	No CSF	41	41	
	IL-4	50 (+9)	52 (+11)	+10 \pm 1
60.3 (β chain)	No CSF	142	135	
	IL-4	157 (+15)	150 (+15)	+15 \pm 0
TS1/18 (β chain)	No CSF	128	124	
	IL-4	144 (+16)	135 (+11)	+14 \pm 3

Monocytes were incubated in suspension for 21 hours with or without 600 pmol/L IL-4, and stained according to the protocol in Materials and Methods. The specificities of the MoAb are: 60.1, MO-1: α chain MAC-1 (CD11b); TS1/22: α chain LFA-1 (CD11a); 150/95: α chain p150/95 (CD11c); 60.3, TS1/18: β chain LFA family (CD18). Parallel adhesion experiments on the same donor cells after overnight suspension culture yielded the following percent adhesion at 30 minutes. Exp. 1: no. CSF, 48 ± 3.3 ; IL-4, 21.4 ± 1.6 ; Exp. 2: no. CSF, 12.5 ± 0.5 ; IL-4, 7.1 ± 0.5 .

*Mean fluorescence, after subtraction of background fluorescence, measured on a logarithmic scale (arbitrary units).

†Shift in mean fluorescence relative to no CSF group. A mean fluorescence shift of 25.6 corresponds to a doubling of antigen density.

DISCUSSION

The data presented here show that IL-4 is a negative regulator of monocyte adhesion, and provide support for a possible anti-inflammatory role for this cytokine *in vivo*. Inhibition of adhesion was seen at concentrations of IL-4 as low as 20 pmol/L, while maximal inhibition required concen-

trations of 60 to 600 pmol/L, depending on the strength of the pro-adhesive stimulus. Both basal and CSF-stimulated adhesion were inhibited, and decreased adhesiveness was seen to endothelium and to plastic. The effects of IL-4 were also time-dependent; inhibition of adhesion was seen only after several hours in culture.

In a series of control experiments, no evidence was found for a direct toxic effect of the IL-4 preparation on monocytes. In particular, cell viability as measured by spontaneous release of ^{51}Cr from labeled monocytes and by Trypan-blue exclusion was not altered during the culture period, and the activity of the IL-4 preparation was destroyed by boiling.

Two lines of investigation were pursued in an attempt to understand the mechanism of IL-4 in these experiments. The dependence of CSF-stimulated adhesion on CD18⁵ and the partial dependence of basal monocyte adhesion on the same molecule^{5,9,12} suggested that IL-4 might exert an antiadhesive effect by downmodulation of this antigen. In fact, IL-4 induced the opposite change, with small increases in CD18, MAC-1, and, to a lesser extent, p150/95 expression at 21 hours. Similar results were obtained for two separate experiments, and parallel adhesion assays showed IL-4-induced inhibition of adhesion of approximately 50% (Table 2). These results are in keeping with a previous report that showed increased expression of MAC-1 and p150/95 but not LFA-1 on human monocytes after 3 days culture with IL-4.¹³ The dissociation of cell adhesiveness and the absolute level of expression of cell adhesion molecules may be explained in either of two ways. IL-4 may inhibit the expression of other, unknown adhesion structures responsible for the LFA-independent component of monocyte adhesion. Alternatively, IL-4 may influence the membrane distribution or functional status of LFA members, thereby altering cell adhesion. Support for the latter hypothesis comes from our own observations on the relative failure of IL-3 and GM-CSF to upregulate LFA-1 and

Table 3. Binding of ^{125}I -CSF to Monocytes After Preincubation With or Without IL-4

Preincubation	Competitor	Radioligand	
		^{125}I -IL-3	^{125}I -GM-CSF
Medium	Nil	100*	100
	IL-4	91 \pm 9† ($P = .47$)‡	85 \pm 1 ($P = .001$)
	IL-3	—	32 \pm 1 ($P < .0001$)
	GM-CSF	17 \pm 4 ($P < .0001$)	—
IL-4 (600 pmol/L)	Nil	123 \pm 17	115 \pm 13
	IL-4	115 \pm 34 ($P = .97$)	115 \pm 8 ($P = .99$)
	IL-3	—	27 \pm 11 ($P = .014$)
	GM-CSF	15 \pm 4 ($P = .008$)	—

Monocytes were incubated for 18 hours in suspension with or without 600 pmol/L IL-4. Cells were harvested by centrifugation and binding of ^{125}I -CSF was measured as described in Materials and Methods. Both radioligands were used at concentrations of 100 pmol/L. Competitors were included in the binding mixture at 100-fold excess molar concentration.

*Data for each radioligand are expressed as a percentage of the specific binding seen in medium-preincubated cells.

†Mean \pm SEM for three experiments with two replicates per point per experiment.

‡Significance, compared with nil competitor, by Student's *t*-test. IL-4-pretreated cells did not display significant differences in ^{125}I -CSF binding compared with control-pretreated cells ($P > .34$).

p150/95 expression in monocytes, despite stimulating large increases in LFA-1- and p150/95-dependent adhesion.⁵ Other groups have reported more direct evidence for functional change in myeloid cell adhesion molecules.¹⁴⁻¹⁶

We also investigated the possibility that IL-4 might inhibit CSF-induced monocyte adhesion by altering the expression of, or by interacting with, the membrane receptors for IL-3 or GM-CSF. We have previously shown that IL-3 and GM-CSF show cross-inhibition of binding to human monocytes,⁸ and this interaction is again demonstrated in Table 3. In contrast, significant inhibition by IL-4 was only seen for the binding of ¹²⁵I-GM-CSF and was of small magnitude (Table 3). It would seem unlikely that inhibition of CSF-binding of such a magnitude would be sufficient to explain IL-4-induced inhibition of adhesion in GM-CSF-treated cells. Similarly, preincubation of monocytes overnight with IL-4 did not result in significant modulation of CSF receptor expression (Table 3). While our own data fail to demonstrate a clear mechanism for IL-4-induced inhibition of adhesion on these cells, it is apparent that such inhibition is independent of changes in surface expression of LFA molecules or of CSF receptors.

The effects of IL-4 on adhesion may provide an explanation for certain other inhibitory effects of IL-4, including those of inhibition of IL-1 and TNF- α production.¹⁷⁻¹⁹ Adhesion has been shown to be an important signalling step as by itself it induces expression of a number of monokines, including IL-1 β , TNF- α , M-CSF, IL-6, and the oncogene product *c-fos* in human monocytes.^{20,21} IL-4-induced inhibi-

tion of monocyte adhesion to the culture vessel may therefore provide a mechanism for the inhibition of production of IL-1 and TNF- α . While unproven, this hypothesis has the attraction of providing for a common mechanism for the anti-inflammatory effects of IL-4. The converse hypothesis, namely that IL-1 and TNF- α promote monocyte adhesion, and that inhibition of their production might therefore cause cell detachment, might also be proposed. However, experiments in this laboratory with exogenous TNF- α have shown the absence of pro-adhesive effects of this cytokine on human monocytes.⁴

Recent reports have indicated that IL-4 stimulates endothelial cells, leading to enhanced adhesion of lymphocytes but not neutrophils.^{22,23} However, no reports could be found of inhibitory effects of IL-4 on cell adhesion. Indeed, there is a paucity of information on cytokines which downregulate the adhesion process^{24,25} and none on inhibitors of monocyte adhesion. The demonstration of such inhibitory activity for IL-4 suggests that this factor may play a central role in the modulation of inflammatory and vascular diseases *in vivo*.

ACKNOWLEDGMENT

We thank Dr S.C. Clark for generous gifts of IL-3 and GM-CSF, the Adelaide Red Cross Transfusion Service for the supply of blood packs, the staff of the Labour Ward, Queen Victoria Hospital for collecting the umbilical cords, Julie Halsall for excellent technical assistance, and Helen Mayberry and Barbara Meakins for excellent secretarial assistance.

REFERENCES

- Joris I, Zand T, Nunnari JJ, Krolikowski FJ, Majno G: Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. *Am J Pathol* 113:341, 1983
- Endemann G, Pronczuk A, Friedman G, Lindsey S, Alderson L, Hayes KC: Monocyte adherence to endothelial cells *in vitro* is increased by VLDL. *Am J Pathol* 126:1, 1987
- Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA: Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J Clin Invest* 76:2003, 1985
- Gamble JR, Elliott MJ, Jaipargas E, Lopez AF, Vadas MA: Regulation of human monocyte adherence by granulocyte-macrophage colony-stimulating factor. *Proc Natl Acad Sci USA* 86:7169, 1989
- Elliott MJ, Vadas MA, Cleland LG, Gamble JR, Lopez AF: IL-3 and GM-CSF stimulate two distinct phases of adhesion in human monocytes. *J Immunol* 145:167, 1990
- Sanchez-Madrid F, Nagy J, Robbins E, Simon P, Springer TA: A human leukocyte differentiation antigen family with distinct alpha subunits and a common beta subunit: The lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1) and the p150/95 molecule. *J Exp Med* 158:1785, 1983
- Wall RT, Harker LA, Quadracci LJ, Striker GE: Factors influencing endothelial cell proliferation *in vitro*. *J Cell Physiol* 96:203, 1978
- Elliott MJ, Vadas MA, Eglinton JM, Park LS, Bik-To L, Cleland G, Clark SC, Lopez AF: Recombinant human interleukin 3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) show common biological effects and binding characteristics on human monocytes. *Blood* 74:2049, 1989
- Keizer GD, Te Velde AA, Schwarting R, Figdor CG, De Vries JE: Role of p150, 95 in adhesion, migration, chemotaxis and phagocytosis of human monocytes. *Eur J Immunol* 17:1317, 1987
- Mentzer SJ, Crimmins MAV, Burakoff SJ, Faller DU: Alpha and beta subunits of the LFA-1 membrane molecule are involved in human monocyte-endothelial adhesion. *J Cell Physiol* 130:410, 1987
- Arnaut MA, Lanier LL, Faller DU: Relative contribution of the leukocyte molecules Mol, LFA-1, and p150, 95 (LeuM5) in adhesion of granulocytes and monocytes to vascular endothelium in tissue- and stimulus-specific. *J Cell Physiol* 137:305, 1988
- Carlos TMA, Dobrina A, Harlan JM: Multiple receptors on human monocytes are involved in adhesion to cultured human endothelial cells. *Proc Am Soc Hematol* 143a:483, 1989 (abstr)
- Te Velde A, Klomp JPG, Yard BA, De Vries JE, Figdor CG: Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. *J Immunol* 140:1548, 1988
- Buyon JP, Abraham SB, Phillips MR, Slade SG, Ross GD, Weissmann G, Winchester RJ: Dissociation between increased surface expression of Gp 165/95 and homotypic neutrophil aggregation. *J Immunol* 140:3156, 1988
- Altieri DC, Bader R, Mannucci PM, Edgington TS: Oligospecificity of the cellular adhesion receptor MAC-1 encompasses an inducible recognition specificity for fibrinogen. *J Cell Biol* 107:1893, 1988
- Altieri DC, Edgington TS: A monoclonal antibody reacting with distinct adhesion molecules defines a transition in the functional state of the receptor CD11b/CD18 (MAC-1). *J Immunol* 141:2656, 1988

17. Hurme M, Palkama T, Sihuola M: Interleukin-4 inhibits interleukin-1 synthesis by a post-transcriptional mechanism. *Biochem Biophys Res Commun* 157:861, 1988
18. Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA: Potential anti-inflammatory effects of interleukin-4: Suppression of human monocyte tumor necrosis factor α , interleukin-1 and prostaglandin E_2 . *Proc Natl Acad Sci USA* 86:3803, 1989
19. Essner R, Rhoades K, McBride WH, Morton DL, Economou JS: IL-4 down regulates IL-1 and TNF gene expression in human monocytes. *J Immunol* 142:3857, 1989
20. Haskill S, Johnson C, Eierman D, Becker S, Warren K: Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. *J Immunol* 140:1690, 1988
21. Navarro S, Debili N, Bernaudin J-F, Vainchenker W, Doly J: Regulation of the expression of IL-6 in human monocytes. *J Immunol* 142:4339, 1989
22. Thornhill MH, Kyan-Aung U, Haskard DO: IL-4 increases human endothelial cell adhesiveness for T cells but not for neutrophils. *J Immunol* 144:3060, 1990
23. Masinovsky B, Urdal D, Gallatin WM: IL-4 acts synergistically with IL-1 β to promote lymphocyte adhesion to microvascular endothelium by induction of vascular cell adhesion molecule-1. *J Immunol* 145:2886, 1990
24. Gimbrone MA Jr, Obin MS, Brock AF, Luis EA, Hass PE, Hébert CA, Yip YK, Leung DW, Lowe DG, Kohr WJ, Darbonne WC, Bechtol KB, Bake JB: Endothelial interleukin 8: A novel inhibitor of leukocyte-endothelial interactions. *Science* 246:1601, 1989
25. Gamble JR, Vadas MA: Endothelial adhesiveness for blood neutrophils is inhibited by transforming growth factor β . *Science* 242:97, 1988