Clin Exp Immunol 1997; **107**:601–607

Human blood dendritic cells: binding to vascular endothelium and expression of adhesion molecules

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(Accepted for publication 31 October 1996)

SUMMARY

To investigate the binding properties of dendritic cells (DC) to vascular endothelium, a comparative analysis was undertaken of DC, monocytes and lymphocytes isolated from the blood of 25 healthy subjects using monolayers of human umbilical vein endothelial cells as the adherence substrate. More blood DC (mean 24% adherence) were adherent to endothelial monolayers than monocytes (mean 18%; $P < 0{\cdot}001$) and lymphocytes (mean 12%; $P < 0{\cdot}001$). When the monolayers were pretreated with tumour necrosis factor-alpha (TNF-α) all leucocyte populations exhibited an increased attachment, but there was still greater binding of DC (mean 37% adherence) in comparison with monocytes (mean 23%; $P < 0{\cdot}001$) and lymphocytes (mean 18%; $P < 0{\cdot}001$). Flow cytometric analysis revealed that in relation to monocytes and lymphocytes the DC had a higher surface expression of the adhesion molecules CD11a ($P < 0{\cdot}05$), CD11c ($P < 0{\cdot}005$) and CD54 ($P < 0{\cdot}005$) but a lower prevalence of cells bearing CD49d (mean 38%; $P < 0{\cdot}05$) and the homing receptor CD62L (mean 14%; $P < 0{\cdot}001$). CD1a was present on 22% of DC and virtually absent from the surface of monocytes and lymphocytes. The intensity of expression of the β_1-integrins, CD49c, CD49d and CD49e was greater on DC than lymphocytes and monocytes ($P < 0{\cdot}05$). Antibody blocking studies demonstrated that DC binding to untreated and TNF-α-treated endothelium was dependent upon the expression of CD11a, CD18 and CD49d, and the simultaneous application of anti-CD18 and anti-CD49d antibodies produced an approximate 70% inhibition of adhesion ($P < 0{\cdot}001$). Thus, the expression of both β_1- and β_2-integrins contributes to the adhesive interaction between DC and endothelium.

Keywords dendritic cells adherence adhesion molecules endothelium

INTRODUCTION

Dendritic cells (DC) are highly potent at presenting antigen, stimulating resting and memory T lymphocytes and clustering T cells either spontaneously or by antigen dependence [1]. They originate from stem cells in bone marrow [2] and constitute ≈0·5–4% of blood mononuclear leucocytes [3]. Progenitors of tissue DC are also present in human cord blood and peripheral blood [4,5] and their numbers are expanded following stimulation with the cytokines tumour necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [5–7].

Blood DC have a high expression of class II antigens and are more efficient than monocytes and B lymphocytes at presenting antigen to T lymphocytes [8,9]. It is generally thought that tissue DC do not divide [10,11], but evidence of mitosis has been described for Langerhans cells [12,13]. Although the maintenance of DC numbers in normal tissue may be accounted for in part by proliferation of resident cells, it is most likely that an expansion in DC numbers at sites of continued antigenic challenge, e.g. tumours [14,15] and chronic inflammatory lesions [16], depends upon recruitment of mature or progenitor forms from the circulation. Therefore, the extravasation of blood DC across blood vessel walls may be a key event in activating and maintaining T cell reactivity.

The migration of blood mononuclear leucocytes into tissue is mediated by the expression of surface adhesion-promoting molecules such as the β_2-integrin family (CD11/CD18), which promote binding to endothelial cells, and by the β_1-integrins, which enhance adhesion to endothelium and to the extracellular matrix proteins [17]. When endothelium is activated by TNF-α its adhesiveness for lymphocytes and monocytes is enhanced [17]. This effect is due in part to the increased surface expression of CD54 (intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, which is a ligand for CD11a/CD18) and the induced expression of other ligands such as vascular cell adhesion molecule-1 (VCAM-1),

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which is recognized by the β_1-integrin CD49d (VLA-4) [18]. Adhesion molecules have been identified on the surface of human DC, but their expression in relation to function has been generally interpreted in terms of inducing lymphocyte clustering and proliferation [8] rather than promoting their interaction with components of blood vessel walls.

The purpose of the present study was to compare the adherent properties of human blood DC with those of lymphocytes and monocytes using endothelial monlayers as the adherence substrate. In addition, experiments were undertaken to examine the expression of known adhesion molecules on the surface of DC and to assess their contribution in binding to endothelial cells.

MATERIALS AND METHODS

Isolation of blood dendritic cells, monocytes and lymphocytes

Peripheral blood (70 ml) was donated by healthy volunteers, defibrinated by shaking with glass beads, and mononuclear cells were separated by density gradient centrifugation over Ficoll–Paque (Pharmacia, Uppsala, Sweden). The mononuclear cells from the interface were washed and cultured overnight at a concentration of 5×10^6 ml in 25 cm^3 tissue culture flasks (Falcon Co, Cockeysville, MD) in complete medium (RPMI 1640 Dutch modification, with 100 U penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (FCS)). Non-adherent cells were centrifuged over metrizamide (13·7% w/v) (Nyegaard, Oslo, Norway) at 600 ***g*** for 10 min. Low-density cells, enriched for DC, were collected from the interface and washed twice in medium. Adherent cells recovered from overnight cultures were used as a source of monocytes (purity >73%), whilst enriched lymphocytes (purity >94%) were recovered from the pellet of the metrizamide gradient.

Contaminating Fc receptor-positive monocytes were removed by incubation on human IgG-coated plates for 1 h at room temperature. The DC were further enriched by treatment with antibodies directed against CD3 (T cells), CD14 (monocytes), CD19 (B cells) and CD56 (natural killer (NK) cells), and panning for 1 h on flasks (Applied Immune Systems, Santa Clara, CA) coated with anti-mouse IgG. Cells prepared in this way (purity >76%) contained <2% of cells bearing markers for the contaminating cell types [19] and were highly HLA class II-positive. Viability of enriched cells was >99% as judged by trypan blue exclusion and the median cell yield was 2×10^6 cells. Ultrastructural analysis revealed that they possessed the characteristic morphology of DC, in that there were few cytoplasmic organelles, several cytoplasmic projections that ranged from a blunted to a veiled appearance [20], and the nucleus was irregularly shaped or oval. Only our enriched DC preparations were able to stimulate an allogeneic mixed leucocyte reaction (MLR), and these DC were more potent than monocytes in presenting recall antigens, purified protein derivative (PPD) influenza virus and tetanus toxoid, to lymphocytes [21].

Isolation and culture of endothelial cells

Endothelial cells were obtained from the veins of human umbilical cords by our routine method [22]. Briefly, endothelial cells were removed with 1 mg/ml collagenase solution (Class II, in RPMI; Sigma, St Louis, MO), and resuspended in Dulbecco's modified essential medium (DMEM) supplemented with 20% FCS, 2 mM glutamine, 200 U/ml penicillin, 100 U/ml streptomycin and gentamicin (GIBCO, Paisley, UK). Endothelial cells were grown in gelatin (1% w/v)-coated flasks (Costar, Cambridge, MA) in a 10% CO_2-humidified atmosphere at 37°C. When confluent, cells were detached with trypsin–EDTA (Sigma, Poole, UK), seeded onto gelatin-coated 96-well plates (Costar) and again grown to confluence. Identification of endothelial cells was confirmed by immunofluorescent staining with an anti-human Factor VIII-related antigen antiserum (Nordic Labs, Tilburg, The Netherlands) and by their characteristic morphology using light microscopy.

Adherence assay

Enriched preparations of DC, monocytes and lymphocytes were each radio-labelled with $Na_2\,{}^{51}CrO_4$ (Amersham International plc, Aylesbury, UK) according to standard procedure [22] for 1 h at 37°C and resuspended in DMEM with 10% autologous serum to a concentration of 1×10^6/ml. Before the adherence assay some endothelial monolayers were treated with 1, 10 or 100 U/ml recombinant TNF-α (supplied by Dr A. Meager, NIBSC, UK) for 5 h. All wells were washed twice with RPMI without serum before the introduction of 100 μl labelled leucocytes (1×10^5 cells/well). After incubation at 37°C for 1 h the non-adherent leucocytes were aspirated and the endothelial monolayers washed gently five times with DMEM, without serum, at 37°C to remove loosely attached adherent cells. In each microtitre well the endothelium with adherent leucocytes was osmotically disrupted by treatment with 0·2 ml of 0·1 M NaOH. The lysate was aspirated, collected and counted in an auto-gamma scintillation counter. All tests were performed in quadruplicate in randomly allotted wells. Leucocyte adhesion was expressed in terms of the percentage of leucocytes originally dispensed onto the endothelial monolayers. It was calculated as follows:

$$\%\ \text{adherence} = \frac{\begin{array}{c}\text{mean ct/min of endothelial monolayer}\\ \text{with adherent leucocytes} - \text{ct/min background}\end{array}}{\begin{array}{c}\text{mean ct/min of 100 } \mu\text{l radiolabelled}\\ \text{suspension} - \text{ct/min background}\end{array}} \times 100$$

Neither treatment of the endothelial cells with TNF-α, incubation with DC, monocytes or lymphocytes, nor washing five times to remove loosely adherent leucocytes resulted in a disruption of the monolayers as judged by visual inspection. In agreement with our earlier observations, the only vascular adhesion molecule readily identified on untreated endothelial cells was ICAM-1 [23]. However, incubation of the monolayers with TNF-α resulted in an up-regulation of ICAM-1 and induction of VCAM-1 and E-selectin [23].

Pretreatment of DC with antibodies against CD11a, CD18 and CD49d

Dendritic cells, at a concentration of 2×10^6/ml in culture media, were incubated for 45 min at room temperature with either anti-CD11a, anti-CD18, anti-CD49d MoAbs (equivalent to 25 μg/10^6 cells) or irrelevant (control) antibodies whose concentration and subclass were the same as those of the other antibodies at room temperature. The anti-CD11a (R.7.01) and anti-CD18 (R.15.7/H4) antibodies were supplied by R. Rothlein (Boehringer Ingelheim, Ridgefield, USA), the anti-CD49d antibody (L25) by L. C. Fritz (Athena Neurosciences, San Francisco, CA) and control antibodies (anti-retinal S antigen) by M. Stanford (UMDS).

Analysis of the expression of adhesion molecules

Direct labelling of leucocytes. Lymphocytes and monocytes (10 μl at a concentration of 6×10^6–10×10^6 cells/ml) were

Table 1. Antibodies used for flow cytometry

CD*	Conjugate†	Clone	Specificity	Source‡
1a	F	WM35	Thymocytes, Langerhans cells	Cy
3	P	Leu 4	Mature T cells; receptor associated	B
11a	F	MHM24	Leucocytes; LFA-1; β_2-integrin α-chain	D
11b	F	44	Leucocytes; CR3; β_2-integrin α-chain	S
11c	F	KB90	Leucocytes; p150/90; β_2-integrin, α-chain	D
14	P	LeuM3	Monocytes; LPS–LPS-binding protein receptor	B
15	F	LeuM1	Granulocytes and monocytes; Lewis X	B
16	P	Leulle	PMN, cytotoxic T cells and NK cells; IgG receptor FcRIII	B
18	F	IOT18	Leucocytes; β_2-integrin β-chain	I
19	P	Leu12	B cells	B
29	F	IOT29	Leucocytes; β_2-integrin β-chain of VLAs	I
44	F	IOL44	Hermes antigen; hyaluronate receptor	I
49b	U	IOP49b	Leucocytes; α_2-integrin α-chain of VLA-2	I
49c	U	IOP49c	Leucocytes; α_3-integrin α-chain of VLA-3	I
49d	U	IOP49d	Leucocytes; α_4-integrin α-chain of VLA-4	I
49e	U	IOP49e	Leucocytes; α_5-integrin α-chain of VLA-5	I
49f	U	IOP49f	Leucocytes; α_6-integrin α-chain of VLA-6	I
54	U	ICAM	Intercellular adhesion molecule-1 (ICAM-1)	I
56	P	Leu 19	T cells and NK cells	B
62L	F	Leu 8	Leucocytes; L-selectin	B
HLA-DR	Bi	L243	Monocytes, B-cells, dendritic cells	Co

*Cluster of differentiation.

†Bi, Biotin; F, FITC; P, phycoerythrin; U, unconjugated.

‡B, Becton Dickinson; Co, Coulter; Cy, Cymbus Bioscience (Southampton, UK); D, Dako (High Wycombe, UK); I, Immunotech; S, Serotec (Kindlington, UK); Fifth Human Leucocyte Antigen Workshop (Dr J. Kawahara).

labelled with the vital nuclear fluorescent dye LDS-751 (Exciton Inc., Dayton, OH) and mixed with 4 μl anti-adhesion molecule antibodies conjugated with FITC or PE [24]. They were kept for 10 min on ice, adjusted to 1 ml with 10 mM HEPES-buffered Hanks' balanced salt solution pH 7·3, containing 0·5% bovine serum albumin (HBSS–BSA) (Sigma) and examined by flow cytometry.

Dendritic cells (10 μl at a concentration of 1×10^6/ml) were mixed with 4 μl FITC-conjugated antibodies and with 10 μl of a mixture of anti-CD3-PE, CD14-PE, CD16-PE, CD19-PE, CD56-PE and biotinylated HLA-DR (see Table 1). They were kept for 10 min on ice before adding streptavidin-conjugated quantum red (QR-SA) in HBSS for a further 10 min.

Indirect labelling of leucocytes. Lymphocytes and monocytes labelled with LDS-751 were mixed with 4 μl of unlabelled antibodies for 10 min on ice and then incubated with 10 μl of a 2:5 dilution of FITC-labelled $(Fab)_2$ fragments of rabbit anti-mouse immunoglobulin for 10 min before being adjusted to 1 ml with HBSS–BSA for flow cytometry [24]. Dendritic cells were treated similarly, except that before flow cytometry they were incubated with 10 μl of the PE-HLA-DR cocktail (described above), followed by incubation for a further 10 min with 10 μl of a 1:5 dilution of QR-SA.

Flow cytometry. Samples were examined in a FACScan flow cytometer (Becton Dickinson, San Jose, CA) equipped with Consort 32 Lysys II (version 1.02) software. Leucocytes were identified by their location in two-dimensional plots of side light scatter (SSC) *versus* staining intensity using the nuclear dye LDS 751 (monitored in the FL3 channel) [24]. Dendritic cells were identified by their poor reactivity to PE-conjugated antibodies (Table 1) against CD3, CD14, CD16, CD19 and CD56 (monitored in the FL2 channel) and strong staining with HLA-DR linked to QR monitored in the FL3 channel. In both instances the binding of FITC-labelled antibodies was monitored in the FL1 channel. All samples were stained with anti-adhesion molecule antibodies (Table 1) and with the appropriate negative isotype controls. Results were expressed as the percentage of cells which stained positively and the level of adhesion molecule expression as the mean fluorescence intensity.

Statistical analysis

All results are expressed as the mean ± s.e.m. except for the antibody blocking studies (Table 3), which are presented as the mean ± s.d. For the comparative study of the adherence properties of DC, monocytes and lymphocytes the data were approximately normally distributed and Student's *t*-test was used to determine the significance of differences between these mononuclear populations. Differences in distribution and expression of adhesion molecules were analysed by the Mann–Whitney *U*-test, whilst the blocking effect of antibody to adhesion molecules on DC adherence (Table 2) was evaluated by the Wilcoxon signed rank test.

RESULTS

Adherence of DC to endothelial monolayers

The study assessed the adherence properties of DC, monocytes and lymphocytes isolated from the blood of 25 healthy subjects. In each of these experiments DC, monocytes and lymphocytes were added to untreated and TNF-treated endothelial cells propagated from one umbilical cord vein. At an early stage of the investigation it was evident that the three leucocyte populations occasionally differed

Table 2. Distribution and intensity of expression of adhesion molecules on dendritic cells (DC), lymphocytes and monocytes

a. Prevalence of mononuclear cells expressing adhesion molecules

β_1-integrins	Dendritic cells (mean %)	Lymphocytes (mean %)	*P	Monocytes (mean %)	*P
CD49b	30 ± 8	6 ± 1	<0·02	67 ± 8	<0·005
CD49c	17 ± 5	7 ± 2	NS	32 ± 9	NS
CD49d	38 ± 10	85 ± 3	<0·005	93 ± 2	<0·001
CD49e	42 ± 11	45 ± 5	NS	94 ± 3	<0·001
CD49f	45 ± 11	47 ± 8	NS	78 ± 6	<0·05
CD29	73 ± 5	60 ± 4	NS	98 ± 1	<0·005
Others					
CD15	35 ± 8	13 ± 2	NS	86 ± 3	<0·05
CD44	77 ± 9	94 ± 5	NS	99 ± 1	<0·05
CD54	83 ± 5	13 ± 6	<0·001	85 ± 4	NS
CD62L	14 ± 6	73 ± 3	<0·001	86 ± 2	<0·001
CD1A	22 ± 6	1 ± 1	<0·02	2 ± 1	<0·001

b. Intensity of expression of adhesion molecules (MFI)

β_1-integrins	Dendritic cells (mean MFI)	Lymphocytes (mean MFI)	*P	Monocytes (mean MFI)	*P
CD49b	127 ± 30	24 ± 3	<0·02	44 ± 8	NS
CD49c	99 ± 20	24 ± 4	<0·005	41 ± 6	<0·05
CD49d	111 ± 18	42 ± 3	<0·01	60 ± 4	<0·05
CD49e	118 ± 20	26 ± 1	<0·001	103 ± 6	<0·05
CD49f	125 ± 23	29 ± 2	<0·01	85 ± 15	NS
CD29	94 ± 11	39 ± 3	<0·001	125 ± 12	NS
Others					
CD15	120 ± 16	145 ± 35	NS	288 ± 83	NS
CD44	305 ± 50	320 ± 16	NS	739 ± 22	<0·001
CD54	161 ± 30	22 ± 2	<0·002	35 ± 2	<0·005
CD62L	61 ± 14	79 ± 5	NS	107 ± 11	<0·05
CD1A	93 ± 19	35 ± 21	<0·05	27 ± 17	<0.05

Results are expressed as the mean values ± s.e.m. of lymphocytes, monocytes and DC isolated from 11 healthy subjects.

*P values relate to results from lymphocytes and monocytes that were compared with those of DC.

NS, Not significant.

from one another in the concentration of TNF-α needed to generate optimal binding to endothelial monolayers. Consequently, in evaluating the adherence properties of leucocytes to cytokine-treated endothelium, results were expressed as the maximum increase in adhesion of DC, monocytes and lymphocytes to endothelial cells pretreated with 1, 10 or 100 U/ml TNF-α relative to their binding to untreated endothelium. Figure 1 shows that a mean 24% of DC bound to untreated endothelial monolayers in comparison with 18% monocytes ($P < 0{\cdot}001$) and 12% lymphocytes ($P < 0{\cdot}001$). All mononuclear cell preparations demonstrated an increased adhesion to endothelial cells treated with TNF-α ($P < 0{\cdot}001$), although the percentage of DC (mean 37%) binding to TNF-α-stimulated monolayers was again greater than that of monocytes (mean 23%; $P < 0{\cdot}001$) and lymphocytes (mean 18%; $P < 0{\cdot}001$). These findings illustrate that within the mononuclear leucocyte population, blood DC have the highest adherence propensity for endothelial cells.

Expression of adhesion molecules

Flow cytometry demonstrated that several of the adhesion molecules investigated were highly expressed on the surface of DC (Fig. 2). The percentage of DC and monocytes expressing the β_2-integrins was similar and both populations had a greater number of CD11b- and CD11c-bearing cells in comparison with lymphocytes (Fig. 3a). Figure 3b shows that in comparison with monocytes and lymphocytes, DC have a higher level of expression of CD11a ($P < 0{\cdot}05$ and $P < 0{\cdot}02$, respectively) and CD11c ($P < 0{\cdot}001$ and $P < 0{\cdot}005$, respectively).

The distribution and expression of the other adhesion molecules investigated in this study are shown in Table 2. In comparison with monocytes, there were fewer DC expressing CD49b, CD49e, CD49f, and CD29 and, although most lymphocytes and monocytes expressed CD49d, only a small proportion of DC were positive. However, the intensity of expression of CD49b, CD49c, CD49d and CD49e was higher on DC than lymphocytes and

Table 3. Anti-adhesion molecule antibodies inhibit the binding of dendritic cells (DC) to endothelial monolayers

	Percentage inhibition of adhesion							
	Anti-CD11a		Anti-CD18		Anti-CD49d		Anti-CD18 + anti-CD49d	
Expt	−TNF-α	+TNF-α	−TNF-α	+TNF-α	−TNF-α	+TNF-α	−TNF-α	+TNF-α
1	43	50	33	44	13	35		
2	20	22	27	59	11	26		
3	55	17	55	54	8	41		
4	36	48	44	53				
5			50	57	10	57	65	86
6			56	46	15	22	70	68
7			64	40	15	19	70	64
Mean	*38	*34	*47	*50	12	*33	*68	*73
± s.d.	±15	±17	±13	±7	±3	±14	±3	±12

DC from healthy subjects were pretreated with antibodies directed against CD11a, CD18, CD49d, or a combination of antibodies against CD18 and CD49d, before introduction into the adherence assay. Results are expressed as percent inhibition of adhesion to untreated (−TNF) and TNF-α-treated (+TNF) endothelial monolayers. Pretreatment of DC with an isotype-matched irrelevant antibody did not modify attachment to untreated or TNF-α-treated endothelium. Each experiment used DC from a different normal subject.

*$P < 0{\cdot}01$ compared with lymphocytes incubated with irrelevant antibody.

monocytes, and the intensity of CD49f and CD29 expression was greater on DC than lymphocytes, but not monocytes. CD1a is regarded as a marker of Langerhans cells, and this molecule was expressed by 22% of blood DC. Most DC (mean 83%) and monocytes (mean 85%) were positive for CD54 (ICAM-1), but the intensity of expression was greater on DC ($P < 0{\cdot}005$). The homing receptor CD62L (L-selectin) was present on most lymphocytes (mean 73%) and monocytes (mean 86%), in contrast to its expression by a few DC (mean 14%).

Adhesion molecules and the attachment of DC to endothelium

To assess the contribution of CD11a, CD18 and CD49d in promoting the binding of DC to endothelium, DC were incubated with antibodies to each of these adhesion molecules before being overlaid onto monolayers of untreated and TNF-α-treated endothelium. Table 3 shows that anti-CD11a and anti-CD18 antibodies inhibited the attachment of DC to resting and cytokine-activated endothelial cells by approx. 35% and 50%, respectively ($P < 0{\cdot}01$). Anti-CD49d impeded the interaction of DC with endothelial cells treated with TNF-α (mean 33% inhibition; $P < 0{\cdot}01$), but it had no significant effect upon the adhesion of DC to untreated endothelium. When DC were incubated with both anti-CD18 and anti-CD49d antibodies their attachment to untreated and TNF-α-treated monolayers was markedly inhibited (approx. 70% inhibition; $P < 0{\cdot}01$).

DISCUSSION

A prerequisite to the passage of leucocytes across blood vessel walls is binding to endothelial cells; the present study demonstrates that DC isolated from human blood are more adherent to untreated umbilical vein endothelial monolayers than either lymphocytes or monocytes, and that this adhesion is dependent upon CD18 and CD49d expression. Dendritic cells, like other mononuclear cell populations, exhibited an increased adhesion to TNF-α-stimulated endothelium, and this finding is in accord with the report that blood DC are more adherent to IL-1-treated endothelium than resting endothelium [25]. However, we noted that a greater number of DC bound to TNF-α-treated monolayers than monocytes and lymphocytes.

DC were isolated by means of their distinct physical properties

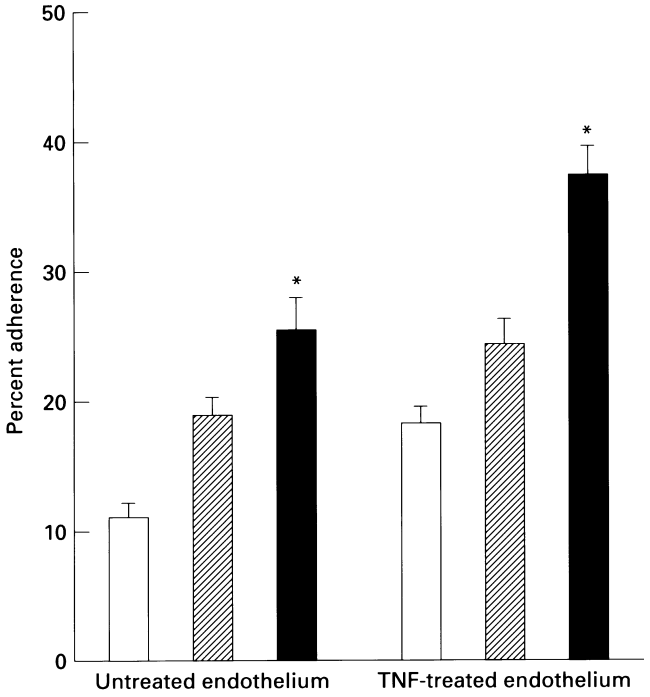

Fig. 1. Comparative adherence of dendritic cells (DC; ■), monocytes (▨) and lymphocytes (□) to endothelial monolayers. Results are the mean values from studying the attachment of DC, monocytes and lymphocytes from 25 healthy subjects. Vertical lines represent s.e.m. For each subject, populations of mononuclear cells were added to monolayers pretreated for 5 h with 1, 10 and 100 U/ml tumour necrosis factor-alpha (TNF-α) so as to obtain a value for the maximum percentage of cells binding to TNF-α-treated endothelium. *$P < 0{\cdot}001$ compared with binding of monocytes and lymphocytes to both untreated and TNF-α-treated endothelium.

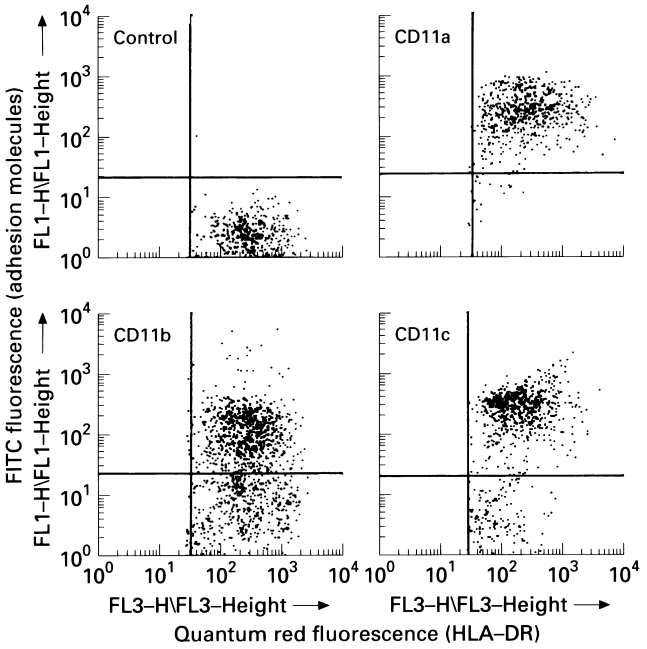

Fig. 2. Scatter plots of CD11/CD18 adhesion molecules on dendritic cells (DC). Results are from a typical experiment in which DC were identified on the basis of positive staining by anti-HLA-DR antibodies labelled with quantum red and negative staining by PE-labelled antibodies directed against CD3, CD14, CD16, CD19 and CD56. Cells were stained with FITC-labelled antibodies directed against CD11a, CD11b, CD11c or with isotype control antibody.

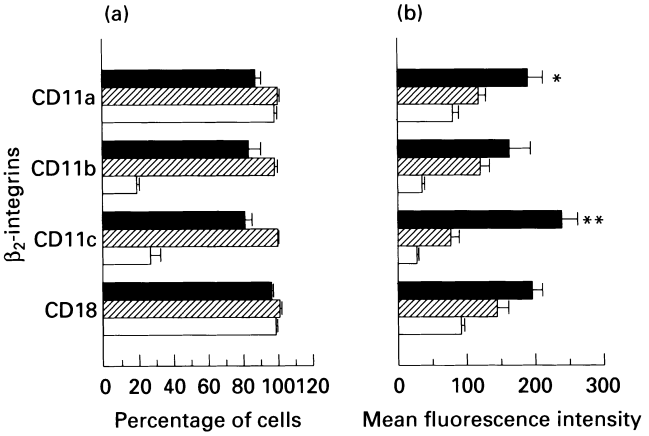

Fig. 3. Prevalence and surface expression of β_2-integrins on dendritic cells (■), monocytes (▨) and lymphocytes (□). Results are the mean values ± s.e.m. of leucocytes isolated from 12 healthy subjects. The percentage of cells expressing the integrins are shown in (a), and the intensity of expression (mean fluorescence intensity) shown in (b). $*P < 0{\cdot}05$; $**P < 0{\cdot}001$ compared with both monocytes and lymphocytes.

(low density and transient adherence to plastic), and by applying negative selection by panning with antibodies directed against CD3, CD14, CD19 and CD56. With this procedure it was possible to obtain sufficient numbers of well characterized DC; many had the classical ultrastructure of large mononuclear cells, with oval or irregular shaped nuclei, few cytoplasmic organelles and protruding short veiled projections. They possessed a high expression of HLA-DR, HLA-DQ, initiated an MLR and were very potent at presenting recall antigens at low target-to-effector ratio; properties that are characteristic of DC [26].

Isolating neutrophils and mononuclear leucocytes from blood often results in cell activation [27], and a similar caveat is likely to apply to the enrichment of circulating DC. Thus, the phenotype of DC described in our study may be more representative of activated DC than resting cells. To address this question we are currently comparing the expression of adhesion molecules on isolated DC with those on DC in whole blood. The β_2-integrins CD11a and CD11c were highly expressed on DC, and antibody inhibition studies demonstrated that CD11a and CD18 expression was necessary for binding to untreated and TNF-treated endothelium, probably through recognition of ICAM-1 which is constitutively expressed on resting endothelium and up-regulated by the activity of TNF-α [17]. Contrary to our findings are reports stating that CD11b is poorly expressed on DC [8], and that anti-CD18 antibodies do not modify DC attachment to cultured endothelium [25]. Such discrepancies may arise from differences in experimental design, particularly in the method of DC separation. For example, our isolation procedure was completed within 24 h, whereas in the other studies it took 48 h, during which time further maturation of DC may have occurred. Activation of endothelial cells with TNF-α also induces the expression of VCAM-1, which is a ligand for CD49d [18].

We found that binding of DC to TNF-α-treated endothelial cells was CD49d-dependent. This adhesion molecule was present on fewer DC (38%) than other mononuclear cells, although its level of expression on DC was higher. The intimate association between DC adhesiveness and expression of CD18 and CD49d was further illustrated by experiments in which the combined treatment of DC with antibodies against both of these adhesion molecules produced the largest inhibition of adhesion to endothelial monolayers. CD62L (L-selectin) is the receptor which promotes the 'homing' of lymphocytes to secondary lymphoid tissue [28] by interacting with 'addressins' such as Gly-CAM-1 [29] on the luminal surface of high endothelial venules. In view of the low prevalence of blood DC bearing CD62L it seems that few DC will enter secondary lymphoid tissue by this recognition pathway.

Differences between the phenotype and function of DC from various tissues, and even within the same tissue, raise the question of whether the extravasation of DC from the blood is already 'programmed' or whether it is a random process in which the cells thereafter acquire the characteristics of tissue DC by the action of the local microenvironment. Distinct subsets of DC are believed to be present in the skin [30] and blood [20] of healthy subjects. Langerhans cells, in contrast to lymphoid DC, are phagocytic and express both CD11b and Fc receptors [31], and based upon differences in morphology there are three types of blood DC whose appearance resembles the Langerhans cells of skin or the veiled and interdigitating cells of lymphoid tissue [20]. Also, blood DC, unlike lymphoid DC, do not generate primary proliferative responses to contact sensitizers and are less efficient at inducing an allogeneic MLR [32]. However, under appropriate culture conditions blood DC are capable of maturing into potent immunostimulating cells [33]. The co-expression of CD1a with HLA-DR occurs only on Langerhans cells [34], and the current finding that 22% of blood DC identified in the present study were $CD1a^+$ adds support to the view some DC may be committed to leave the circulation at distinct anatomal locations.

On entering tissue, DC may down-regulate adhesion molecules

that governed their attachment to endothelium and increase the expression of those determinants that will enhance interaction with lymphocytes and tissue proteins. In support of this view is the low expression of β_2-integrins on DC in the lung [35] and on epidermal Langerhans cells [7,36]. Should the adherence properties of isolated blood DC reflect their *in vivo* properties then an enhanced adhesion to untreated and TNF-treated endothelium could underlie the maintenance of DC numbers in normal tissue, particularly where there is a high turnover of DC, such as thymus [38], spleen and lymph nodes, and in sites of inflammation [40] where inflammatory cytokines such as TNF-α are generated.

ACKNOWLEDGMENTS

This work was supported in part by the Trustees of Henry Smith's Charity.

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