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Human polymorphonuclear leucocytes stimulated by tumour necrosis factor-alpha show increased adherence to extracellular matrix proteins which is mediated via the CD11b/18 complex

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SUMMARY

The present study demonstrates that tumour necrosis factor (TNF) and FMLP, but not IL-1 or IL-8, enhanced the adherence of polymorphonuclear neutrophil (PMN) to fibronectin, an extracellular matrix protein. The adherence induced by FMLP was very rapid, within 5 min while the induction of adherence by TNF was much slower, reaching maximum at 60 min. TNF also enhanced an adhesion of PMN to other extracellular matrix proteins, such as laminin, collagen IV and gelatin II, but not to human serum albumin. Anti-CD18 MoAb completely inhibited the binding of TNF-stimulated PMN to fibronectin and partially inhibited the binding to laminin. Further investigation showed that adhesion of TNF-stimulated PMN to fibronectin and laminin was inhibited by anti-CD11b MoAb and to a lesser extent by CD11a MoAb. In contrast to TNF-stimulated PMN the binding of unstimulated PMN to fibronectin and laminin was only inhibited by anti-CD11a MoAb. Anti-CD11c had no effect on PMN adherence. These results suggest that unstimulated PMN adhere to extracellular proteins through the CD11a/18, while TNF-stimulated PMN adhere through the CD11b/18. These results suggest that TNF secreted at the site of inflammation may enhance the interaction of PMN with the extravascular environment through the CD11b/18 complex.

Keywords polymorphonuclear leucocytes adhesion tumour necrosis factor integrins

INTRODUCTION

Circulating and bone marrow neutrophils provide the front line of defence against many infectious agents [1,2]. During an inflammatory response these cells can be activated and rapidly mobilized to the inflammatory site [3]. Recent studies have shown that cellular adhesion proteins play an important role in many of the events involved in the PMN accumulation at inflammatory lesion, such as intravascular margination [4], adherence to endothelial cell (EC) [5], chemotactic migration [6,7], extravascular movement [8] and phagocytosis [3,9]. PMN, monocytes and lymphocytes from patients with leucocyte adhesion deficiency display an abnormal pattern of adherence-dependent functions, e.g. failure to accumulate granulocytes at the inflammatory lesion. This defect was found to be due to a deficiency of a family of functionally and structurally related surface glycoproteins, the CD11/18 conplex [3]. This family comprises the lymphocyte functional antigen, LFA-1/CD11a, Mac-1/CD11b and gp150,95/CD11c [10]. Furthermore it has also been shown that MoAb against the CD11b/18 antigen can inhibit adhesion of PMN to vascular endothelium [11,12], as well as to plastic/glass [13] and also interferes with chemotaxis [9] and intracellular killing [11,13].

In vivo studies have demonstrated that injection of inflammatory cytokines such as IL-1 and TNF [14–16] and chemotactic agents such as IL-8, FMLP and C5a cause PMN to accumulate at the injection site [17,18]. Both IL-1 and TNF have been demonstrated to induce the expression of adhesion proteins such as ICAM and ELAM on endothelial and fibroblast cells [19]. Many studies on the interaction of PMN with EC have demonstrated the importance of the CD11b/18 complex in the adhesive interaction of these two cell types [20–23]. However, for the PMN to penetrate into the tissues subsequent to migration between the endothelial cells it must also interact with exracellular matrix proteins present in the basement membrane and interstitial spaces [24,24]. At present there is little available information on adherence of PMN to connective tissue proteins in extracellular matrices including fibronectin, collagen and laminin. The present study was carried out to investigate the effect of inflammatory mediators on PMN adhesion to the extracellular proteins and to identify the responsible adhesion proteins.

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MATERIALS AND METHODS

Reagents

FMLP, fibronectin, laminin, collagen type IV and gelatin type II were all purchased from Sigma Chemical Co. (St Louis, MO). Human recombinant IL-1α, TNF-α and IL-8 were gifts of Dainippon Pharmaceutical Co. (Osaka, Japan).

PMN separation

Human PMN were isolated from peripheral blood by Ficoll-Paque centrifugation, followed by dextran sedimentation (3% w/v in PBS) for 20 min at room temperature [26,27]. The PMN rich supernatant was then collected and residual red blood cells removed by hypotonic lysis. The cells were washed in PBS and resuspended at 1×10^7/ml in RPMI with 1% fetal calf serum.

Adherence assay

Adhesion assays were performed in triplicate in 96-well microtitre plates [28]. 50 μl of 5 μg/ml solutions of fibronectin, laminin, collagen and gelatin were placed in individual wells and incubated overnight at 4°C. All the wells were then blocked for 2 h with 200 μl of HBSS containing 10 mg/ml HSA, washed three times with HBSS containing 0·1% Tween-20 and finally once in HBSS alone. After 5–120 min stimulation of the PMN, 5×10^5 cells were added to each well and the plates were then incubated for 10 min at 37°C. The wells were filled with HBSS, sealed using acetate tape and inverted for centrifugation at 150 *g* for 5 min. After discarding the non-adherent cells, the remaining cells were solubilized with 100 μl of PBS containing 1% Triton X100 and then assayed for myeloperoxidase (MPO) using *o*-phenylenediamine dihydrochloride [29]. 1% Triton was also added to control wells containing 5×10^5 cells to measure the total MPO added to the wells. The percentage adherence was calculated as: (amount of MPO in test/total amount of MPO added) × 100.

Adhesion inhibition assays

MoAb TS1/18 (anti-CD18), TS1/22 (anti-CD11a) and LM2 (anti-CD11b) were a gift from Dr R. Rothlein at Boehringer Ingelheim Pharmaceuticals Inc. SHCL3 (anti-CD11c) and Mo-p9 (anti-CD14) were obtained from Becton Dickinson. An MoAb against the common β chain of the β1 integrin or VLA antigen family, 4B4 (anti-CD29), was obtained from Coulter Corporation. The PMN were then mixed with 10 μg/ml of MoAb and incubated at room temperature for 10 min. 5×10^5 PMN were then added to each well and the adherence assay carried out as described above.

FACS

To measure the relative density of cell surface antigens, viable suspensions of stimulated and control PMN were incubated with 20 μl of test or control antibodies (anti-CD14) for 15 min at 4°C. The samples were then washed in PBS with 10% human AB serum and stained with fluorescein-labelled secondary Ab (FITC goat $F(ab')_2$ anti-mouse IgG, Tago Inc.). The cells were then fixed in 1% paraformaldehyde and analysed on an Epics Profile Analyzer. The results were calculated as the percentage of PMN expressing the test antigen and the relative fluorescence intensity of expression.

Statistical analysis

The data were expressed as mean ± s.d. Significance was determined using Student's *t*-test.

RESULTS

PMN adherence to extracellular proteins

As shown in Fig. 1, TNF and FMLP stimulated an increase in PMN adhesion to the fibronectin coated plates but not IL-1 or IL-8. This increase was observed very rapidly for FMLP within 5 min and slowly for TNF with maximal adherence at 60 min. The increased PMN adhesion was dose dependent for both these stimuli, with TNF maximum at 5 ng/ml and FMLP maximum at 10^{-8} M (Table 1). However no concentration of FMLP tested showed increased adhesion at 60 min. IL-1 and IL-8 were found to have no effect on PMN adherence at any time point or concentration tested (Table 1). Both TNF and FMLP were also found to increase adhesion of PMN to laminin, collagen and gelatin, but not to human serum albumin, suggesting that the increased adherence was specific for extracellular proteins (Table 2).

Inhibition of PMN binding to extracellular proteins by MoAbs against CD18/CD11b and CD29

Several studies have shown that the rapid increase in adhesion of PMN to extracellular matrices induced by FMLP is mediated through the CD18 pathway [20,21,25], so we investigated the ligand which mediated adhesion of PMN induced by TNF. MoAbs against CD18, but not CD14, were found to inhibit the increased PMN adherence stimulated by TNF to fibronectin and laminin (Fig. 2). In the case of fibronectin, addition of anti-CD18 MoAb resulted in the total inhibition of PMN adherence to the wells, i.e. it inhibited both baseline adherence and the enhanced adherence caused by TNF. Similar findings were also observed for collagen and gelatin (data not shown). In the case of laminin, 20% of added PMN were able to adhere even after treatment with anti-CD18 MoAb. To test if this adherence to laminin was due to the β1 integrin family which includes the laminin receptor, PMN stimulated with TNF were incubated

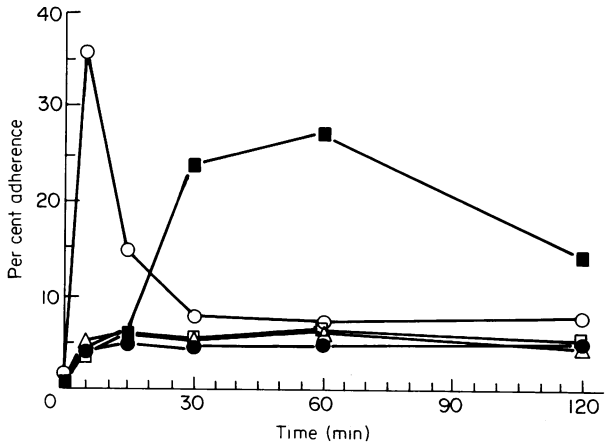

Fig. 1. Effect of a variety of mediators on adherence of PMN to fibronectin with time. (□——□) Control, (●——●) 10 ng/ml IL-1, (△——△) 10 ng/ml IL-8, (○——○) 10^{-8} M FMLP, (■——■) 5 ng/ml TNF. Results are the means of six experiments with the s.d. < 5%.

Table 1. Effect of different concentration of inflammatory mediators on adherence of PMN to fibronectin

Stimulant	Concentration	% Adherence 5 min	% Adherence 60 min
None		4·8±0·9	6·2±3·1
IL-1	1 ng/ml	4·8±0·9	4·3±1·4
	5	5·6±1·4	7·2±1·9
	10	7·1±2·2	5·6±1·1
IL-8	1 ng/ml	6·6±0·8	7·1±1·3
	5	4·9±1·7	6·2±2·1
	10	5·4±0·8	5·2±1·4
FMLP	10^{-9} M	12·2±1·6	7·7±1·5
	10^{-8} M	29·3±2·4	4·3±0·7
	10^{-7} M	10·6±3·1	6·6±1·9
TNF	1 ng/ml	6·9±2·3	12·3±2·9
	5	5·7±1·8	29·8±4·6
	10	7·3±2·7	27·4±5·1

PMN were stimulated for 5 or 60 min at 37°C. Results are expressed as mean±s.d. of six experiments.

Table 2. Effect of TNF and FMLP on adherence of PMN to a variety of extracellular matrix proteins

Substrate	% Adherence TNF	% Adherence FMLP
Laminin	62·2±5·6	45·8±1·4
Collagen	40·3±8·0	61·5±6·2
Gelatin	41·5±5·2	32·9±4·4
Plastic+HSA	2·2±1·9	3·1±0·7

PMN were incubated for 60 min with 5 ng/ml TNF or for 5 min with 10^{-8} M FMLP. Results are expressed as the mean±s.d. of six experiments.

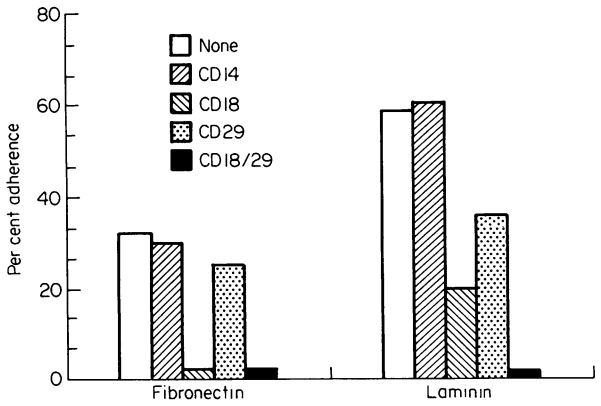

Fig. 2. Effect of preincubation with anti-CD18, anti-CD29 and anti-CD14 on TNF-stimulated PMN adherence to fibronectin or laminin. Results are the means of eight experiments with the s.d. <5%.

with anti-CD29 (antibody against the common β1 chain) and then tested for their ability to adhere to fibronectin or laminin. PMN adherence to fibronectin was unaffected by the anti-CD29, however binding to laminin was reduced by 24% of total PMN added (Fig. 2). Incubation of PMN with both anti-CD18 and anti-CD29 resulted in complete inhibition of PMN adherence to laminin.

To investigate further the contribution of the CD18 complex to binding by TNF-activated PMN, the effect of MoAb against CD11a, CD11b and CD11c was also tested. PMN adherence to both fibronectin and laminin was inhibited after preincubation with MoAb against CD11b (Table 3). MoAb against CD11a also inhibited binding of the PMN although the decrease was only half as great as that observed for CD11b. Preincubation of PMN with anti-CD11c MoAb did not inhibit PMN binding to these extracellular proteins (Table 3).

In contrast to TNF-stimulated PMN, MoAb against CD11b were unable to block the binding of *unstimulated* PMN adherence to fibronectin and laminin (Table 4). Adherence of unstimulated PMN was more effectively inhibited by CD11a: in the case of fibronectin by 88±3·1 and for laminin by 56±0·7.

FACS analysis

The percentage of PMN expressing the antigens tested was approximately 99·9% and this was not affected by treatment

Table 3. Inhibition of adherence of TNF stimulated PMN adherence by MoAb against CD11a, 11b, 11c

Substrate	Pretreatment	% Inhibition
Fibronectin	None	—
	CD11a (TS1/22)	36·8±2·7*
	CD11b (LM2)	79·8±3·3*
	CD11c (SHCL3)	—
Laminin	None	—
	CD11a (TS1/22)	16·4±1·7*
	CD11b (LM2)	66·7±3·5*
	CD11c (SHCL3)	—

PMN were stimulated for 60 min with 5 ng/ml TNF and then preincubated with 10 μg/ml MoAb for 10 min at room temperature before adherence.

* Significant difference between test and control, $P>0·05$.

Table 4. Inhibition of adherence of unstimulated PMN to fibronectin and laminin by MoAb against CD11a and CD11b

Substrate	Pretreatment	% Inhibition
Fibronectin	None	—
	CD11a (TS1/22)	88·4±3·1*
	CD11b (LM2)	0·8±0·3
Laminin	None	—
	CD11a (TS1/22)	56·5±0·7*
	CD11b (LM2)	1·7±3·5

PMN were preincubated with 10 μg/ml MoAb for 10 min at room temperature before adherence.

* Significant difference between control and test, $P>0·05$.

Table 5. FACS analysis of PMN expression of the CD18/11 complex

	Relative mean fluorescence		
Stimulant	CD11a	CD11b	CD11c
None	157±25	221±30	117±31
TNF 5 ng/ml	150±44	418±41	102±26
FMLP 10^{-7} M	179±31	370±29	137±17

PMN were incubated for 60 min with TNF or FMLP. Results are expressed as mean±s.d. of six experiments.

with TNF or FMLP. A small but consistent increase in the fluorescence intensity of CD11b was observed for PMN stimulated with TNF and FMLP (Table 5). PMN incubated with TNF or FMLP showed no change in their surface expression of CD11a and CD11b.

DISCUSSION

After PMN have migrated through the endothelium, the next structures they encounter are the subendothelial basement membrane and interstitial space. The basement membrane and extravascular site are rich in matrix proteins such as laminin, fibronectin and collagen. The interaction of PMN with these matrix proteins has been shown to be very important both for PMN accumulation and their functional state at inflammatory lesions [30,31]. The present study demonstrates that stimulation of PMN by TNF results in a slow but prolonged increase in adherence to fibronectin, laminin, collagen and gelatin. An increase in PMN adhesion to extracellular proteins was also stimulated by FMLP, however in contrast to TNF this was a very rapid and transient process, suggesting that the different stimuli may perform different functions. FMLP is also known to induce a very rapid and transient adherence of PMN to EC [17,20,21]. Therefore it may be that FMLP is important for the rapid transient adherence of PMN to endothelial cells and for migration to the inflammatory site, while TNF stimulates prolonged adherence of PMN to extracellular matrix proteins to hold them at the inflammatory site.

PMN adherence to extracellular protein was not increased in response to other inflammatory cytokines such as IL-1 or IL-8. The failure of IL-1 to increase PMN adherence may be as a result of lack of specific receptors for IL-1 on PMN, but this would seem unlikely as PMN have been shown to contain approximately 700 high affinity receptors for IL-1 [32]. Alternatively it could be that some intracellular signalling mechanisms for IL-1 are absent, as IL-1, unlike TNF, does not induce PMN chemotactic activity, but does appear to activate some functions in PMN such as an enhanced respiratory burst [32]. Therefore the ability of IL-1 to induce PMN accumulation at inflammatory sites could be indirect, through its ability to stimulate expression of adhesion molecules on endothelial cells or release of chemotactic cytokines such as IL-8 from endothelial or fibroblast cells [19,33]. IL-8 was also shown to have no effect on PMN adherence to extracellular proteins. This is similar to the findings of Gimbrone *et al.* [34] who found that IL-8 inhibited the adherence of PMN to stimulated HEC. However, in contrast, Carveth *et al.* [25] showed that IL-8 was able to stimulate increased PMN adherence to subendothelial matrixes which contained laminin. The reason for the differences is not clear although it may simply be due to concentration differences, as Carveth *et al.* [25] used 8 μg/ml while in the present study only 10 ng/ml of IL-8 was used which has been shown to be the maximal concentration for chemotaxis [33].

PMN binding to the extracellular proteins was inhibited by MoAb to CD18 complex. The involvement of the CD11/18 complex in the binding of PMN to extracellular proteins has been suggested by several investigators [35,36]. Our study shows that the adherence of TNF-stimulated PMN to fibronectin and laminin appears to be closely linked to the CD11/18 complex as binding was completely abolished for fibronectin and dramatically reduced for laminin by MoAb against the CD11a for unstimulated PMN and by anti-CD11a and CD11b MoAb for TNF-stimulated PMN. It is possible that the effect of the anti-CD18 MoAb was not direct but due to inhibition of IgG Fc receptor closely associated with the CD11/18 complex as shown in monocytes by Brown *et al.* [37]. However incubation of PMN with anti-CD18 Ab had no effect on the phagocytosis of IgG coated erythrocytes, suggesting that the CD18 Ab were not simply interfering with FcR molecules closely associated with the CD11/18 antigens. Alternatively it is possible that the inhibition of adherence by the anti-CD18 was acting by negative signalling by interfering with CD11/18-independent pathways as shown by van Noesel *et al.* [38] in T lymphocytes. However, we do not think this is likely as the MoAb used in this study, anti-CD18, TS1/18, was the one anti-CD18 MoAb which was not found to have an inhibitory effect on T lymphocyte proliferation. Therefore we think that it is most likely that the inhibition by CD11/18 MoAb observed in the present study is direct.

In contrast to fibronectin, binding of TNF-stimulated PMN to laminin was only partially inhibited by anti-CD18 MoAb or anti-CD11a together with anti-CD11b MoAb. This finding is similar to that of Bohnsack *et al.* [39] who found that adherence of PMN stimulated by PMA to laminin was partially inhibited by MoAb against CD18. Therefore it would appear that PMN can bind laminin by, at least, two different receptor–ligand interactions. The CD18 independent mechanism is likely to be attributable to a member of the β1 integrin family (CD29) which are known to be present on myeloid cells and bind to a wide variety of matrix proteins such as laminin, collagen and fibronectin [40,41]. Early evidence had suggested that PMN did not express β1 integrins [42], however more recent studies have been able to detect β1 integrins of the laminin receptor and fibronectin receptor in stimulated PMN [43,44]. The reason for this discrepancy is not clear although it has been suggested that oxidative products from the PMN may interfere with the MoAb binding to its antigen [43]. Further evidence for the involvement of the β1 integrin family in PMN adherence laminin was shown in the present study by the ability of an anti-CD29 MoAb to partially inhibit the binding of TNF stimulated PMN binding to laminin.

It is interesting to note that both TNF and FMLP stimulation of PMN was shown to produce a slight increase in the surface expression of the CD11b antigen at 60 min, although only TNF-stimulated PMN showed increased adhesion at this time; the FMLP induced adhesion had occurred much earlier at 5 min. This lack of correlation between increased CD11b

expression (shown by TNF and FMLP) and increased adhesion to extracellular proteins (only shown by TNF) suggests that increased adherence of PMN to extracellular matrix proteins may not simply be due to increased surface expression of integrins and that the CD11b/18 may undergo some alteration with time or stimulation. Schleiffenbaum *et al.* [45] found that increased adherence of PMN to EC observed with TNF, FMLP and platelet activating factor was not associated with an increase in CD11b expression but was inhibitable by an anti-CD11b MoAb. A candidate for the modification of CD11/18 and its activation is phosphorylation/dephosphorylation and recent studies have shown a correlation between increased phosphorylation and increased adherence of PMN in response to PMA and FMLP [46,47]. The phosphorylation induced in the CD11b/18 complex by FMLP was very rapid and transient which correlated with the increased adherence induced by the FMLP. These studies suggest that phosphorylation may be important for the activity of the CD11b/18. Therefore, while FMLP induces a very rapid and transient phosphorylation of CD11b/18 complex, TNF may induce a slower and more prolonged phosphorylation of the complex. Alternatively, TNF might modify the CD11b/18 antigen in some other way that allows it to interact more efficiently with its ligands. Further work will be needed to identify if increased phosphorylation or some other modification of the CD11b/18 complex can account for TNF- stimulated PMN adhesion in the present study.

The present study demonstrates that TNF stimulated a slow but sustained increased adherence of PMN to extracellular matrix proteins, in contrast to FMLP which induced a rapid and transient increase in adherence. This increased adherence stimulated by TNF appears to be due to the CD11b/18 (Mac-1) receptor. Thus it would appear that during an inflammatory response, the release of TNF by EC or fibroblasts would lead to an increased adhesion of PMN to fibronectin and laminin allowing the PMN at the lesion to interact efficiently with the extravascular environment and accumulate at sites of inflammation or infection.

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REFERENCES

1 Spitznagel JK. Non-oxidative antimicrobial reactions of leukocytes. Contemp Top Immunobiol 1984; **14**:283–343.
2 Gabay JE. Microbicidal mechanisms of phagocytes. Curr Opin Immunol 1988; **1**:36–40.
3 Anderson DC, Springer TA. Leukocyte adhesion deficiency: An inherited defect in the Mac-1, LFA-1 and p150,95 glycoproteins. Ann Rev Med 1987; **38**:175–194.
4 Fehr J, Jacob HS. In vitro adherence and in vivo margination: two associated complement-dependent functions. Studies based on the acute neutropenia of filtration neutrophoresis. J Exp Med 1977; **146**:641–52.
5 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 1985; **76**:2003–11.
6 Zimmerman GA, Wisemasn GA, Hill HR. Human endothelial cells modulate granulocyte adherence and migration. J Immunol 1988; **134**:1866–74.
7 Furie MA, McHugh DD. Migration of neutrophils across endothelial monolayers is stimulated by treatment of the monolayers with interleukin-1 or tumour necrosis factor-α. J Immunol 1989; **143**:3309–17.
8 Brown AF. Neutrophil adhesion and locomotion on collagen substrata and collagen matrices. J Cell Sci 1982; **58**:455–67.
9 Anderson DC, Miller LJ, Schmalstieg FC, Rothlein R, Springer TA. Contribution of the Mac-1 glycoprotein family to adherence-dependent granulocyte functions: structure-function assessments employing sub-unit specific monoclonal antibodies. J Immunol 1986; **137**:15–27.
10 Sanchez-Madrid F, Nagy JA, Robbins E, Simon P, Springer TA. A human differentiation antigen family with a distinct α-subunit and a common β-subunit: the lymphocyte function-associated antigen, the C3bi complement receptor (OKM1/Mac-1) and the p150,95 molecule. J Exp Med 1983; **158**:1785–803.
11 Harlan JM, Killen PD, Senecal FM, Schwartz BR, Yee EK, Taylor RF, Beatty PG, Price TH, Ochs HD. The role of neutrophil membrane glycoprotein GP-150 in neutrophil adherence to endothelium. Blood 1985; **66**:167–78.
12 Pohlman TH, Stanness KA, Beatty PG, Ochs HD, Harlan JM. An endothelial surface factor(s) induced in vitro by lipopolysaccharide, interleukin-1 and tumour necrosis factor-α increases neutrophil adherence by a CDw18-dependent mechanism. J Immunol 1986; **136**:4548–53.
13 Beatty PG, Ledbetter JA, Martin PJ, Price TH, Hansen JA. Definition of a common leukocyte surface antigen (Lp 95–150) associated with diverse cell-mediated immune functions. J Immunol 1983; **131**:2913–20.
14 Movat HZ, Burrowes CE, Cybulsky MI, Dinarello CA. Acute inflammation and a Shwartzman-like reaction induced by interleukin-1 and tumour necrosis factor: synergistic action of the cytokines in the induction of inflammation and microvascular injury. Am J Pathol 1987; **129**:463–9.
15 Sayers TJ, Wiltrout TA, Bull CA, Denn III AC, Pilaro AM, Lokesh B. Effect of cytokines on polymorphonuclear neutrophil infiltration in the mouse: prostaglandin- and leukotriene-independent induction of infiltration by IL-1 and tumour necrosis factor. J Immunol 1988; **141**:1670–7.
16 Wankowicz Z, Megyeri P, Issekutz A. Synergy between tumour necrosis factor alpha and interleukin-1 in the induction of polymorphonuclear migration during inflammation. J Leukocyte Biol 1988; **43**:349–57.
17 Tonnesen M, Smedly L, Goins A, Henson P. The microvasculature in inflammation. Interaction between neutrophils and vascular endothelial cells. Agents Action 1982; **11** (suppl 1):125–8.
18 Baggolini M, Walz A, Krunkel SL. NAP/IL-8 a novel cytokine that activates neutrophils. J Clin Invest 1989; **84**:1045–57.
19 Dustin ML, Springer TA. Lymphocyte function-associated antigen LFA-1 interaction with intracellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. J Cell Biol 1988; **107**:321–31.
20 Smith G, Rothlein R, Hughes BJ, Mariscalco MM, Rudloff HE, Schmalstieg FC, Anderson DC. Recognition of an endothelial determinant for CD18-dependent human neutrophil adherence and transmigration. J Clin Invest 1988; **82**:1746–59.
21 Zimmerman GA, McIntyre TM. Neutrophil adherence to human endothelium in vitro occurs by CDw18 (Mol/Mac-1/LFA-1/GP150, 95) glycoprotein dependent and independent mechanisms. J Clin Invest 1988; **81**:531–7.
22 Luscinkas FW, Brock AF, Arnaout MA, Gimbrone Jr MA. Endothelial-leukocyte adhesion molecule dependent and leukocyte (CD11/18) dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine-activated human vascular endothelium. J Immunol 1989; **142**:2257–63.
23 Detmers PA, Lo SK, Olsen-Egbert E, Walz A, Baggiolini M, Cohn ZA. Neutrophil activating protein 1/IL-8 stimulates binding activity

of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. J Exp Med 1990; **171**:1155–62.

24 Hemler ME. Adhesive protein receptors on hematopoietic cells. Immunol Today 1988; **9**:109–13.

25 Carveth HJ, Bohnsack JF, McIntyre TM, Baggiolini M, Prescott SM, Zimmerman GA. Neutrophil activating factor (NAF) induces polymorphonuclear leukocyte adherence to endothelial cells and to subendothelial matrix proteins. Biochem Biophys Res Commun 1989; **162**:387–93.

26 Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Lab Cli Invest 1969; **21** (suppl 97):77–86.

27 Roos D, Loos JA. Changes in the carbohydrate metabolism of mitogenically-stimulated human peripheral lymphocytes. I Stimulation by phytohaemagluttin. Biochem Biophys Acta 1975; **222**:565–82.

28 Geng J-G, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, Bliss GA, Zimmermann GA, McEver RP. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature 1990; **343**:757–60.

29 Janusz MJ, Austen KF, Czop K. Lysosomal enzyme release from human monocytes by particulate activators is mediated by β-glucan inhibitable receptors. J Immunol 1987; **138**:3897–901.

30 Bohnsack JF, Kleinman HK, Takahasi T, O'Shea JJ, Brown EJ. Connective tissue proteins and phagocytic cell function: laminin enhances complement mediated and Fc mediated phagocytosis by human cultured macrophages. J Exp Med 1985; **161**:912–23.

31 Nathan CF. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophage and lymphocytes. J Clin Invest 1987; **80**:1550–61.

32 Rhyne JA, Mizel SB, Taylor RG, Chedid M, McCall CE. Characterization of the human interleukin 1 receptor on human polymorphonuclear leukocytes. Clin Immunol Immunopath 1988; **48**:354–61.

33 Matsushima K, Oppenheim JJ. Interleukin 8 and MCAF: novel inflammatory cytokines induced by IL-1 and TNF. Cytokine 1990; **1**:2–13.

34 Gimbrone MA, Obin MS, Brock AF, Luis EA, Hass PE, Herbert CA, Yip YK, Leung DW, Lowe DG, Kohr WJ, Darbonne WC, Bechtol KB, Baker JB. Endothelial interleukin inhibitor of endothelial-leukocyte interactions. Science 1989; **246**:1601–3.

35 Wright SD, Weitz JI, Huang AJ, Levin SM, Silverstein SC, Loike JD. Complement receptor type three (CD11b/CD18) of human polymorphonuclear leukocytes recognizes fibrinogen. Proc Natl Acad Sci USA 1988; **85**:7734–8.

36 Anderson DC, Schmalsteig FC, Arnout MA, Kohl S, Tosi MF, Dana N, Hughes BJ, Brinkley BR, Dickey WD, Abramson JS, Springer TA, Boxer LA, Hollers JM, Smith CW. Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of high molecular weight surface glycoproteins (gp138), a common relationship to diminished cell adherence. J Clin Invest 1984; **74**:536–51.

37 Brown EJ, Bohnsack JF, Gresham HD. Mechanism of inhibition of immunoglobulin G-mediated phagocytosis by monoclonal antibodies that recognize the Mac-1 antigen. J Clin Invest 1988; **81**:365–75.

38 van Noesel C, Miedema F, Brouwer M, de Rie MA, Aarden LA, van Lier RAW. Regulatory properties of LFA-1 α and β chains in human lymphocyte activation. Nature 1988; **333**:850–2.

39 Bohnsack JF, Akiyama SK, Damsky CH, Knape WA, Zimmermann GA. Human neutrophil adherence to laminin *in vitro*. Evidence for a distinct neutrophil integrin receptor for laminin. J Exp Med 1990; **171**:1221–37.

40 Ruoslahti E, Pierschbacker MC. New Perspectives in cell adhesion, RDG and intergrins. Science 1987; **238**:491–6.

41 Springer TA. Adhesion receptors of the immune system. Nature 1990; **346**:425–34.

42 Pischel KD, Hemler ME, Huang C, Bluestein HG, Woods VL. Use of the mAb 12FL to characterize the differentiation antigen VLA-2. J Immunol 1987; **138**:226–33.

43 Greshan HD, Goodwin JL, Allen PM, Anderson DC, Brown EJ. A novel member of the integrin receptor family mediates Arg-Gly-Asp-stimulated neutrophil phagocytosis. J Cell Biol **108**:1935–43.

44 Singer II, Scott S, Kawka DW, Kazazis DM. Adhesomes: specific granules containing receptors for laminin, C3bi/fibrinogen, fibroectin and vitronectin in human polymorphonuclear leukocytes and monocytes. J Cell Biol 1989; **109**:3169–82.

45 Schleiffenbaum B, Moser R, Patarroyo M, Fehr J. The cell surface glycoprotein Mac-1 (CD11b/CD18) mediates neutrophil adhesion and modulates degranulation independently of its quantitative cell surface expression. J Immunol 1989; **142**:3537–45.

46 Merrill JT, Slade SG, Weissman G, Winchester R, Buyon JP. Two pathways of CD11b/18-mediated neutrophil aggregation with different involvement of protein kinase C-dependent phosphorylation. J Immunol 1990; **145**:2608–15.

47 Chatila TA, Geha RS, Arnout MA. Constitutive and stimulus-induced phosphorylation of CD11/18 leukocyte adhesion molecules. J Cell Biol 1990; **109**:3435.