Role of interleukin 8 on leucocyte-endothelial cell adhesion in intestinal inflammation

H Arndt, M A Bolanowski, D N Granger

Abstract

Background—An important action of interleukin 8 (IL8) is stimulation of granulocytes. The object of this study was to assess the contribution of IL8 to the leucocyte-endothelial cell interactions associated with intestinal inflammation in the rat.

Methods—Two indomethacin injections (48 and 24 hours prior to the experiments) induced a longlasting ileitis in rats. The number of adherent and emigrated leucocytes, leucocyte rolling velocity, and shear rate were monitored in normal and inflamed mesenteric postcapillary venules. Some animals received a monoclonal antibody (MAb) against IL8 or CD11b/CD18 at 24 and 12 hours prior to the experiment.

Results—Indomethacin elicited a seven-fold increase in leucocyte adherence and a 5.4-fold increase in leucocyte emigration, while leucocyte rolling velocity was reduced by nearly 80%. The indomethacin induced increases in leucocyte adherence and emigration were significantly reduced (by 57% and 67%, respectively) while leucocyte rolling velocity was increased (to 63% of control) by the IL8-specific MAb. The level of inhibition seen with the IL8 MAb was similar to that associated with administration of a MAb directed against the leucocyte adhesion molecule CD11b/CD18.

Conclusions—IL8 contributes to the leucocyte-endothelial cell interactions elicited in mesenteric venules by indomethacin.

(Gut 1996; 38: 911–915)

Keywords: indomethacin, microcirculation, inflammatory bowel disease.

Cytokines have been invoked as modulators of immunological and inflammatory reactions associated with inflammatory bowel disease (IBD).¹ The tissue concentrations of several cytokines are increased in IBD. An increased synthesis of interleukin 1 (IL1), IL2 receptor, and IL6 has been shown in the inflamed intestinal mucosa of patients with Crohn's disease and ulcerative colitis.² IL2 and interferon values are increased in mucosal biopsy specimens from patients with Crohn's disease but not in those with ulcerative colitis,^{3,4} whereas IL10 mRNA is increased in ulcerative colitis but not in Crohn's disease.⁵

Interleukin 8 (IL8) is another cytokine that accumulates in inflamed lesions of patients with activated ulcerative colitis.⁶ Recent findings show that IL8 tissue values and the expression

of IL8 gene in biopsy samples from patients with IBD is related to the macroscopic and histological grade of active inflammation.^{7,8} After appropriate stimulation, IL8 is synthesised mainly by mononuclear phagocytes, however, fibroblasts, epithelial cells, and endothelial cells are all known to produce this powerful cytokine.^{9–11} In intestinal epithelial cell lines, IL8 synthesis can be stimulated by the inflammatory cytokines IL1 and tumour necrosis factor (TNF).¹² The biological activity of IL8 is very similar to chemotactic peptides like C5a and f-Met-Leu-Phe.¹³ Consequently, a major action of IL8 is activation of neutrophils,¹⁴ inducing directional migration, increasing the production of reactive oxygen metabolites, and increasing the expression and level of activation of adhesion molecules.^{15,16} There are also reports that suggest that the IL8 secreted by activated endothelial cells inhibits the adhesion of neutrophils to activated endothelial monolayers.^{17,18}

Although the published in vitro data suggest that IL8 may exert both pro-inflammatory and anti-inflammatory effects, the limited number of studies that consider IL8 function in vivo suggest that its pro-inflammatory actions dominate. In a rabbit model of lung reperfusion injury a monoclonal antibody against IL8 prevented neutrophil infiltration and tissue injury.¹⁹ Mulligan and coworkers have recently shown that a murine monoclonal antibody (DM/C7) to human IL8 largely abolishes the inflammatory cell infiltrate and vascular dysfunction observed in a rat model of IgG immune complex induced lung injury.²⁰ It was assumed (but not determined) that the IL8 MAb exerted its beneficial effects by limiting the recruitment of adherent and emigrated neutrophils in the pulmonary microvasculature. With the same monoclonal antibody neutrophil influx in a dermal immune complex induced inflammation in mice could be abolished.²¹ The objects of this study were to determine: (a) whether IL8 contributes to the inflammatory cell infiltrate and mucosal lesion formation observed in a rat model of intestinal inflammation, and (b) whether IL8 mediates the leucocyte-endothelial cell adhesive interactions in postcapillary venules that occurs in the same experimental model.

Methods

Animal preparation

Male Sprague-Dawley rats (160–240 g) anaesthetised with 110 mg/kg Inactin (Na-5-ethyl-1 (1'-methyl-propyl) 2-thio-barbiturate, Byk Gülden, Konstanz, Germany) were

Department of Physiology and Biophysics, Louisiana State University – Medical Center, USA
D N Granger

Department of Internal Medicine I, University of Regensburg, Germany
H Arndt

Molecular and Cellular Biology, The Monsanto Company, St Louis, Missouri, USA
A Bolanowski

Correspondence to: D N Granger, PhD, Department of Physiology and Biophysics, Louisiana State University – Medical Center, 1501 Kings Highway, Shreveport, Louisiana 71130-3932, USA.

Accepted for publication 29 December 1995

catheterised (carotid artery) for continuous measurement and recording of systemic blood pressure and heart rate (Statham P23A Transducer, Oxnard, CA; Grass Recorder, Grass Instruments, Quincy, MA), tracheotomised for facilitating breathing during the experiment, and surgically prepared for microscopic observation of mesenteric venules.

Intravital microscopy

Animals were placed in a supine position on an adjustable Plexiglas microscope stage and the exteriorised segment of the mid-ileum was draped over an optically clear viewing pedestal permitting transillumination of a 2 cm² section, as described previously.^{22, 23} The exposed bowel wall was draped with saline soaked gauze, the mesentery was covered with Saran wrap (Dow Chemicals, Indiana) to minimise condensation on the objective lens, and continuously superfused with warm bicarbonate buffered salt solution (BBS, pH 7.4) bubbled with 5% CO₂ – 95% N₂. Animal core temperature was thermostated to 37°C.

Single unbranched mesenteric venules of 25–35 µm diameter and roughly 150 µm in length were transilluminated with a 12V–100W light source and observed through an intravital video microscope (Leitz Ortholux II, Germany) with a ×40 objective lens (Zeiss UD 40/0.65, Germany) and a ×10 eyepiece. A video camera (Hitachi WK-C150, Japan) mounted on the microscope projected the image onto a colour monitor (Sony PVM-2030, Japan). The images were recorded using a video cassette recorder (Panasonic NV8950, Japan) for playback analysis. Venular diameter (D_v) was measured on or off line using a video image shearing monitor (IPM, LaMesa, CA). Red blood cell centreline velocity (V_{rbc}) was measured on line with an optical Doppler velocimeter (Microcirculation Research Institute, Texas A and M University). Mean red blood cell velocity was calculated assuming $V_{mean} = \text{centreline velocity}/1.6$.²⁴ Venular shear rate ($\dot{\gamma}$) was calculated based on the Newtonian definition: $\dot{\gamma} = 8(V_{mean}/D_v)$.²⁵

The number of adherent and emigrated leucocytes were determined during playback of videotaped images. A leucocyte was defined as adherent to venular endothelium if it was stationary for at least 30 seconds. Leucocyte adherence was expressed as the number per 100 µm length of the venule. Leucocyte emigration was expressed as the number of white blood cells per microscopic field (1.7×10^{-2} mm²). Rolling leucocytes were defined as white blood cells moving at a slower velocity than erythrocytes in the same vessel. The leucocyte rolling velocity (V_{wbc}) was determined from the time a leucocyte required to move along 100 µm of the microvessel. A mean of 10 estimates of transit time was used to calculate V_{wbc} .²³

Experimental protocol

Twenty two rats were divided into four groups. Three groups received two subcutaneous injections of indomethacin 48 and 24 hours

before the experiment (10 mg/ml 5% NaHCO₃, 7.5 mg/kg each).²⁶ One group was treated additionally with a MAb against IL8 (DM/C7, 3 mg/kg intravenously)²⁰ while another group received a MAb against the leucocyte adhesion glycoprotein CD11b/CD18 (MAb 17, 1.5 mg/kg intravenously)²⁷ under ether anaesthesia at 24 hours and 12 hours prior to the experiment. DM/C7 was provided by The Monsanto Company (St Louis, Missouri), MAb 17 by Repligen Corporation (Cambridge, Massachusetts). The indomethacin induced changes were not affected by isotype matched non-binding control antibodies (IgG1_K for IL8, IgG2_K for CD11b/CD18, PharMingen, San Diego) and none of the antibodies or its solvent (saline) had an intrinsic effect on the microcirculatory parameters in animals not treated with indomethacin (data not shown). The control group received two subcutaneous injections of the indomethacin vehicle, 5% NaHCO₃.

After all parameters measured on line (arterial pressure, V_{rbc} , D_v) were in a steady state, images from the mesenteric preparations – approximately 10 venules per animal – were videotaped for five minutes.

Tissue analysis

After intravital microscopy the animals were killed with an overdose of pentobarbital and the intestines were excised and opened longitudinally. Findings were ranked using the following criteria: 0 – no change in serosa or mucosa, 1 – hyperaemic lesions or petechial bleeding, or both, 2 – single mucosal erosion or ulceration, 3 – multiple erosions or ulcerations without any lesions in the serosa and mesentery, or single mucosal erosion or ulcer with hyperaemic, adhesive or haemorrhagic lesions in the serosa, 4 – multiple erosions or ulcerations (less than 10 cm of total length of the intestine involved) with hyperaemic, adhesive or haemorrhagic lesions in the serosa, 5 – multiple erosions or ulcerations (more than 10 cm of total length of the intestine involved) with hyperaemic, adhesive or haemorrhagic lesions in the serosa.²⁶

Myeloperoxidase activity

Intestinal tissue samples were rapidly excised, rinsed with ice cold saline, blotted dry, and frozen at –70°C until thawing for determination of myeloperoxidase activity using methods previously described.²⁸

Statistics

All data were analysed using standard statistical analysis – that is, analysis of variance with the Scheffe's (posthoc) test. All values are expressed as means (SEM), statistical significance was set at $p < 0.05$.

Results

Figure 1 illustrates the effects of the MAbs against IL8 and CD11b/CD18 on the indomethacin induced increase in leucocyte adherence in rat mesenteric venules compared

Figure 1 Effect of monoclonal antibodies to IL8 and CD11b on indomethacin induced leucocyte adherence in rat mesenteric venules. Shown are mean (SEM) values. * $p < 0.05$ relative to control, † $p < 0.05$ relative to indomethacin.

Figure 2 Effect of monoclonal antibodies to IL8 and CD11b on indomethacin induced leucocyte emigration in rat mesenteric venules. Shown are mean (SEM) values. * $p < 0.05$ relative to control, † $p < 0.05$ relative to indomethacin.

with untreated rats. Two subcutaneous injections of indomethacin elicited a nearly sevenfold increase in adherence (24.0 (1.6) *v* 3.6 (0.5) leucocytes per 100 µm venule in control), which was reduced by 57% by the anti-IL8 MAb (10.3 (0.7) and by 60% by the anti-CD11b MAb (9.6 (1.0)). The 5.4-fold increase in leucocyte emigration (Fig 2) caused by indomethacin injections (14.5 (1.0) *v* 2.7 (0.6) leucocytes per field in control) was significantly blunted by both monoclonal antibodies (anti-IL8 4.8 (0.5), anti-CD11b 5.9 (0.7)).

The Table presents the data for leucocyte rolling velocity, venular wall shear rate, macroscopic mucosal ulcerations, and myeloperoxidase activity. The reduction of leucocyte rolling velocity by 76% by indomethacin treatment was partly prevented by anti-IL8 and anti-CD11b MAb (63% and 67% reduction *v* control, respectively). The venular wall shear rate was not affected by indomethacin, indomethacin/anti-IL8, or indomethacin/anti-CD11b treatment and therefore not the cause for changed rolling and emigration.

Effects of indomethacin (INDO) and the monoclonal antibodies against IL8 and CD11b/CD18 on leucocyte rolling velocity (*V_{wbc}*), venular wall shear rate (*SR*), mucosal ulceration, and myeloperoxidase activity (*MPO*) as a parameter of granulocyte tissue infiltration

	<i>V_{wbc}</i> (mm/s)	<i>SR</i> (s ⁻¹)	Mucosal ulceration	<i>MPO</i> (U/g ww)
Control (n=6)	73.2 (6.4)	432 (28)	0	46.0 (14.0)
INDO (n=5)	17.0 (1.3)*	420 (38)	4.0 (0.5)*	89.1 (13.0)*
INDO+anti-IL8 MAb (n=6)	27.2 (4.5)*†	441 (35)	3.6 (0.6)*	35.9 (18.9)†
INDO+anti-CD11b MAb (n=5)	24.8 (2.1)*†	443 (29)	3.4 (0.5)*	45.9 (4.7)†

Shown are mean (SEM) values. *Denotes $p < 0.05$ relative to control, †shows $p < 0.05$ relative to INDO (n=number of animals per group).

The indomethacin induced increase of leucocyte adhesion in mesenteric venules was accompanied by macroscopic mucosal ulcerations of the small bowel (ulcer index 4.0 (0.5) *v* 0 in control) and an increase of myeloperoxidase (89.11 (13.0) U/g wet weight *v* 46.0 (14.0) in control), an index of granulocyte infiltration into the inflamed tissue. While the macroscopic extent of ulceration was not significantly reduced by treatment with either MAb, both the IL8 and CD11b MAb did blunt the indomethacin induced in Myeloperoxidase activity (Table).

Discussion

The accumulation of leucocytes in inflamed tissue is preceded by leucocyte adhesion to vascular endothelium. This rate limiting step in the inflammatory response is modulated by a variety of adhesion glycoproteins expressed on the surface of leucocytes and endothelial cells. Several cytokines affect the expression or state of activation of these cell adhesion molecules. For granulocyte-endothelial cell interactions, the synthesis of E- and P-selectin has been shown to be inducible by IL1 and TNF.²⁹⁻³¹ Granulocyte-macrophage colony stimulating factor and TNF α induce neutrophils and monocytes to shed L-selectin and simultaneously mobilise intracellular pools of the CD11b/CD18 to the leucocyte surface,^{32 33} thereby promoting attachment of neutrophils to endothelium.

The role of IL8 in modulating leucocyte-endothelial interactions is presently controversial. The IL8 produced by cytokine activated endothelial cells and by neutrophils in itself^{18 34} has been shown to stimulate the binding activity of CD11b/CD18 on the surface of neutrophils.¹⁵ Smith and coworkers have also shown that there is an IL8 dependent mechanism of neutrophil transmigration through cytokine activated endothelium, which is CD11b/CD18 dependent.³⁵ This pro-inflammatory action of IL8 was recently shown in a rat model of IgG immune complex induced lung inflammation and vascular injury.²⁰ Mulligan and coworkers showed a considerable reduction in the tissue influx of neutrophils and the vascular injury induced by IgG immune complexes in rats receiving the anti-human IL8 MAb DM/C7. On the other hand, there are reports that invoke an anti-inflammatory role of IL8. It has been shown that IL8 inhibits the adhesion of neutrophils to activated endothelial cells in vitro^{17 18} and reduces neutrophil accumulation in acutely inflamed tissues.³⁶

Although IL8 has been implicated in the pathobiology of intestinal inflammation, no attempts have been made to directly assess the role of this cytokine in an experimental model of gut inflammation. We have previously shown that the indomethacin model of intestinal inflammation in the rat developed by Yamada and coworkers²⁶ is characterised by an intense recruitment of adherent and emigrated leucocytes in mesenteric venules, granulocyte accumulation in mucosal tissue,

and the development of mucosal ulcers.³⁷ The results of this study clearly suggest a role for IL8 in the recruitment of leucocytes elicited by indomethacin. The antihuman IL8 MAb DM/C7 profoundly attenuated the accumulation of adherent and emigrated leucocytes in postcapillary rat venules and the increased mucosal myeloperoxidase activity normally seen after indomethacin treatment. These findings are consistent with the beneficial effects of the same IL8 MAb (DM/C7) in a rat model of IgG immune complex induced lung inflammation and in a dermal immune complex induced inflammation in mice.^{20, 21}

Inasmuch as the anti-IL8 MAb was as effective as a MAb against CD11b/CD18 in reducing the recruitment of adherent and emigrated leucocytes in the same model of chronic intestinal inflammation, it seems likely that IL8 promotes the leucocyte-endothelial cell interactions by either increasing the surface expression or activation of CD11b/CD18 on leucocytes. This β_2 -integrin is the main ligand for high affinity binding of leucocytes to and transmigration through vascular endothelium.³⁸ However, we cannot exclude the possibility that IL8 may promote leucocyte-endothelial cell adhesion, at least partly by increasing the surface expression of endothelial cell adhesion molecules. There is evidence that IL8 can bind to the surface of endothelial cells, where it can act as an activator and chemoattractant of neutrophils.³⁹

In IgG immune complex induced lung inflammation in rats, IL8 MAb treatment effectively attenuated both the recruitment of inflammatory cells and the increased microvascular permeability to albumin.²⁰ However, in this study we observed that while the IL8 MAb reduced the accumulation of granulocytes normally observed in indomethacin induced intestinal inflammation, it did not blunt the accompanying mucosal ulceration response. The finding that the reduced mucosal accumulation of granulocytes was not accompanied by protection against indomethacin induced intestinal mucosal ulcerations is consistent with the results of Yamada *et al.*,²⁶ indicating that in this model of inflammation granulocyte infiltration into the mucosa is a consequence rather than a cause of the intestinal lesions. In this regard, it should be noted that treatment with DM/C7 offers no protection in a neutrophil independent model of IgA immune complex induced model of lung vascular injury.²⁰ Another explanation for the ineffectiveness of the anti-IL8 MAb could be the involvement of macrophages at the examined stage (50–54 hours after the first indomethacin administration) of the cascade of intestinal inflammation.⁴⁰ While the IL8 MAb did not prevent or reduce indomethacin induced mucosal ulceration, it may have afforded some protection against the microvascular injury that is likely to accompany the ulceration response.

1 Sartor RB. Cytokines in intestinal inflammation: pathophysiological and clinical considerations. *Gastroenterology* 1994; 106: 533–9.

- 2 Isaacs KL, Sartor RB, Haskill JS. Cytokine mRNA in inflammatory bowel disease mucosa detected by PCR amplification. *Gastroenterology* 1992; 103: 1587–95.
- 3 Mullin GE, Lazenby AJ, Harris ML, Bayless TM, James SP. Increased interleukin-2 messenger RNA in the intestinal mucosal lesions of Crohn's disease but not ulcerative colitis. *Gastroenterology* 1992; 102: 1620–7.
- 4 Breese E, Braegger CP, Corrigan CJ, Walker-Smith JA, MacDonald TT. Interleukin-2- and interferon- γ -secreting T cells in normal and diseased human intestinal mucosa. *Immunology* 1993; 78: 127–31.
- 5 Mullin GE, Vezza FR, Sampat A, Maycon Z, Kalz R, Eisenstein, *et al.* Abnormal IL-10 mRNA production in the intestinal mucosal lesions of inflammatory bowel disease. *Gastroenterology* 1993; 104: A751.
- 6 Mahida YR, Ceska M, Effenberger F, Kanlak L, Lindley I, Hawkey CJ. Enhanced synthesis of neutrophil-activating peptide-1/interleukin-8 in active ulcerative colitis. *Clin Sci* 1992; 82: 273–5.
- 7 Mitsuyama K, Toyonaga A, Sasaki E, Watanabe K, Tateishi H, Nishiyama T *et al.* IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. *Clin Exp Immunol* 1994; 96: 432–6.
- 8 Mazzucchielli L, Hauser C, Zgraggen K, Wagner H, Hess M, Laissue JA, *et al.* Expression of interleukin-8 gene in inflammatory bowel disease is related to the histological grade of active inflammation. *Am J Pathol* 1994; 144: 997–1007.
- 9 Peveri P, Waltz A, Dewald B, Baggiolini M. A novel neutrophil-activating factor produced by human mononuclear phagocytes. *J Exp Med* 1988; 167: 1547–59.
- 10 Strieter RM, Phan SH, Showell HJ, Remick DG, Lynch JP, Genord M, *et al.* Monokine-induced neutrophil chemotactic factor gene expression in human fibroblasts. *J Biol Chem* 1989; 264: 10621–6.
- 11 Schroeder JM, Christophers E. Secretion of a novel and homologous neutrophil-activating peptides by LPS-stimulated human endothelial cells. *J Immunol* 1989; 142: 244–51.
- 12 Eckmann L, Jung HC, Schürer-Maly C, Panja A, Morzycka-Wroblewska E, Kagnoff MF. Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. *Gastroenterology* 1993; 105: 1689–97.
- 13 Thelen M, Peveri P, Kernen P, Tschärner V, Walz A, Baggiolini M. Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. *FASEB J* 1988; 2: 2702–6.
- 14 Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin-8, a novel cytokine that activates neutrophils. *J Clin Invest* 1989; 84: 1045–9.
- 15 Detmers PA, Lo SK, Olson-Egbert E, Walz A, Baggiolini M, Cohn ZA. Neutrophil-activating protein-1/interleukin-8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. *J Exp Med* 1990; 171: 1155–62.
- 16 Baggiolini M, Imboden P, Detmers P. Neutrophil activation and the effects of interleukin-8/neutrophil-activating peptide-1 (IL-8/NAP-1). In: Baggiolini M, Sorg C, eds. Vol 4. *Cytokines* 1992; Basel: Karger, 1–17.
- 17 Wheeler ME, Lusinskas FW, Bevilacqua MP, Gimbrone Jr MA. Cultured human endothelial cells stimulated with cytokines or endotoxin produce an inhibitor of leukocyte adhesion. *J Clin Invest* 1988; 82: 1211–8.
- 18 Gimbrone Jr MA, Obin MS, Brock AF, Luis EA, Hass PE, Hébert CA, *et al.* Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. *Science* 1989; 246: 1601–3.
- 19 Sekido N, Mukaida N, Harada A, Nakanishi I, Watanabe Y, Matsushima K. Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8. *Nature* 1993; 365: 654–7.
- 20 Mulligan MS, Jones ML, Bolanowski MA, Baganoff MP, Deppeler CL, Meyer DM, *et al.* Inhibition of lung inflammatory reactions in rats by an anti-human IL-8 antibody. *J Immunol* 1993; 150: 5585–95.
- 21 Zhang Y, Ramos BF, Baganoff MP, Deppeler CL, Meyer DM, Widomski DL, *et al.* The role of IL-8 and mast cell-generated TNF α in neutrophil recruitment. *Inflammation* 1995; 19: 132.
- 22 Granger DN, Benoit JN, Suzuki M, Grisham MB. Leukocyte adherence to venular endothelium during ischemia/reperfusion. *Am J Physiol* 1989; 257: G683–8.
- 23 Arndt H, Smith CW, Granger DN. Leukocyte-endothelial cell adhesion in spontaneously hypertensive and normotensive rats. *Hypertension* 1993; 21: 667–73.
- 24 Davis MJ. Determination of volumetric flow in capillary tubes using an optical Doppler velocimeter. *Microvasc Res* 1987; 34: 223–30.
- 25 House SD, Lipowsky H. Leukocyte-endothelium adhesion: microhemodynamics in mesentery of the cat. *Microvasc Res* 1987; 34: 363–79.
- 26 Yamada T, Deitch E, Specian RD, Perry MA, Sartor RB, Grisham MB. Mechanisms of acute and chronic intestinal inflammation induced by indomethacin. *Inflammation* 1993; 17: 641–62.
- 27 Freyer DR, Morganroth ML, Todd RF. Surface Mo1 (CD11b/CD18) glycoprotein is upmodulated by neutrophils recruited to sites of inflammation in vivo. *Inflammation* 1989; 13: 495–505.
- 28 Grisham MB, Benoit JN, Granger DN. Assessment of leukocyte involvement during ischemia and reperfusion of intestine. *Methods Enzymol* 1990; 186: 729–42.
- 29 Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone Jr MA. Identification of an inducible

- endothelial-leukocyte adhesion molecule. *Proc Natl Acad Sci USA* 1987; **84**: 9238–42.
- 30 Weller A, Isenmann S, Vestweber D. Cloning of the mouse endothelial selectins. Expression of both E- and P-selectin is inducible by tumor necrosis factor. *J Biol Chem* 1992; **267**: 15176–83.
 - 31 Sanders WE, Wilson RW, Ballantyne CM, Beaudet AL. Molecular cloning and analysis of in vivo expression of murine P-selectin. *Blood* 1992; **80**: 796–800.
 - 32 Arnaout MA, Wang EA, Clak SC, Sieff CA. Human recombinant granulocyte-macrophage colony-stimulating factor increases cell-to-cell adhesion and surface expression of adhesion-promoting surface glycoproteins on mature granulocytes. *J Clin Invest* 1986; **78**: 597–601.
 - 33 Griffin JD, Spertini O, Ernst TJ, Belvin MP, Levine HB, Kanakura Y, *et al.* Granulocyte-macrophage colony-stimulating factor and other cytokines regulate surface expression of the leukocyte adhesion molecule-1 on human neutrophils, monocytes, and their precursors. *J Immunol* 1990; **145**: 576–84.
 - 34 Hébert CA, Luscinskas FW, Kiely J-M, Luis EA, Darbonne WC, Benett GL, *et al.* Endothelial and leukocyte forms of IL-8: conversion by thrombin and interactions with neutrophils. *J Immunol* 1990; **145**: 3033–40.
 - 35 Smith WB, Gamble JR, Clark-Lewis I, Vadas MA. Chemotactic desensitization of neutrophils demonstrates interleukin-8 (IL-8)-dependent and IL-8-independent mechanisms of transmigration through cytokine-activated endothelium. *Immunology* 1993; **78**: 491–7.
 - 36 Hechtman DH, Cybulski MI, Fuchs HJ, Baker JB, Gimbrone Jr MA. Inhibitor of polymorphonuclear leukocyte accumulation at sites of acute inflammation. *J Immunol* 1991; **147**: 883–92.
 - 37 Arndt H, Palitzsch K-D, Grisham MB, Granger DN. Metronidazole inhibits leukocyte-endothelial cell adhesion in rat mesenteric venules. *Gastroenterology* 1994; **106**: 1271–6.
 - 38 Furie MB, Tancino MCA, Smith CW. Monoclonal antibodies to leukocyte integrins CD11a/CD18 and CD11b/CD18 or intercellular adhesion molecule-1 inhibit chemoattractant-stimulated neutrophil transendothelial migration in vitro. *Blood* 1991; **78**: 2089–97.
 - 40 Nygard G, Anthony A, Piasecki C, Trevethick MA, Hudson M, Dhillon AP, *et al.* Acute indomethacin-induced jejunal injury in the rat: early morphological and biochemical changes. *Gastroenterology* 1994; **106**: 567–75.