Increased expression of cell adhesion molecule P-selectin in active inflammatory bowel disease

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Abstract

The pathogenic changes of inflammatory bowel disease (IBD) depend on migration of circulating leucocytes into intestinal tissues. Although leucocyte rolling and tenuous adhesion are probably regulated by inducible selectins on vascular endothelia, little is known about the expression of these molecules in Crohn's disease and ulcerative colitis. Using immunohistochemistry on surgically resected specimens, this study investigated endothelial P-selectin (CD62, granular membrane protein-140) in frozen sections of histologically uninvolved tissues adjacent to inflammation (Crohn's disease=10; ulcerative colitis=10), from highly inflamed areas (Crohn's disease=20; ulcerative colitis=13), and from normal bowel (n=20). By light microscopy, two forms of P-selectin immunoreactivity were detected that apparently corresponded ultrastructurally to stored and released distributions. Compared with the normal gut, there was a 3.7-fold increase of P-selectin immunoreactivity on veins ($p<0.0001$), venules ($p<0.0001$), and capillaries ($p<0.05$) in the highly inflamed gut, without differences between Crohn's disease and ulcerative colitis. In the uninvolved gut, P-selectin expression was similar to that seen in normal controls, except for a focal increase of P-selectin in the vicinity of small lymphocyte aggregates. The dramatic upregulation of P-selectin in the inflamed tissue and its potential role in leucocyte trafficking support the concept of P-selectin blocking therapy for the control of active IBD.

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proposed, entailing margination from the centreline of blood flow towards the vascular wall, rolling, tethering to the endothelia, stable adhesion, and finally, transendothelial migration.¹ Each of these steps involves specific families of adhesion molecules, which are expressed on endothelial cells and on circulating cells as their counterparts and ligands.^{2,3}

The selectin family of adhesion molecules, which comprises E-selectin, P-selectin, and L-selectin, predominantly mediates the first steps of cellular adhesion^{4,5} and several studies have shown upregulation of E-selectin on activated endothelial cells in a variety of tissues⁶⁻⁸ including the gut in patients with IBD.^{9,10} Little investigation has been made, however, of P-selectin in normal and diseased gut, although its DNA was cloned and sequenced in 1989.¹¹

P-selectin (also known as PADGEM, CD62, LECAM-3, or granular membrane protein-140) is stored in endothelial cells and platelets and is released after activation by mediators of inflammation,¹² allowing these cells to bind to their receptor/ligands, the carbohydrate structure of sialyl-Lewis X, present on neutrophils and monocytes.¹³⁻¹⁵ Furthermore, P-selectin binds to CD4+ lymphocytes,¹⁶ subpopulations of memory cells, and natural killer cells.¹⁷ Expression of P-selectin is upregulated by histamine, thrombin, tumour necrosis factor α ,^{12,18,19} and by oxygen radicals,²⁰ some of which have been shown to be present in excess in IBD.²¹⁻²³ P-selectin is expressed also on endothelial cells infected by viruses,²⁴ the presence of which has recently been reported in IBD.²⁵

In IBD, we have shown an increased percentage of P-selectin positive platelets in the peripheral blood of patients with Crohn's disease and ulcerative colitis.²⁶ In inflamed intestinal tissue, there is a single report of P-selectin in both Crohn's disease and ulcerative colitis²⁷ which, however, was confined to advanced lesions and provided only limited information about the topography and grade of P-selectin immunoreactivity.

In this study, we hypothesised that P-selectin is upregulated in the development of the inflammatory lesion and in active IBD. Upregulated P-selectin could induce the adhesion of circulating inflammatory cells and thus contribute to the genesis of the intestinal cellular infiltrate.

The aim of the study therefore was to investigate qualitatively and quantitatively the expression of immunoreactive P-selectin on endothelial cells in both uninvolved and highly inflamed areas of Crohn's disease and ulcerative colitis, using light microscopy of operative

The migration of leucocytes into tissues is the central event in inflammation and in an immune response. In inflammatory bowel disease (IBD), there is a dense intestinal infiltrate of inflammatory and activated immune cells with a differential distribution pattern for Crohn's disease and ulcerative colitis. For the development of the local intestinal cellular infiltrate, circulating cells must stick to the intestinal vascular endothelium and transmigrate into the tissue, where the immunoinflammatory reaction is created.

Recently, a multistep cascade of adhesion of circulating cells to endothelial cells has been

TABLE I Clinical and histological features of 63 patients with inflammatory bowel disease and 21 control patients

	Crohn's disease ileum	Controls ileum	Ulcerative colitis colon	Controls colon
Patients				
Number (male:female)	35 (24:11)	7 (5:2)	28 (16:12)	14 (9:4)
Mean age (range) (years)	34 (18-70)	60 (38-81)	39 (28-53)	53 (25-77)
Preoperative therapy				
Glucocorticosteroids	28	0	23	0
Sulphasalazine	4	0	22	0
Azathioprine	3	0	10	0
Operations				
Resection of ileocolonic anastomosis	2	0	0	0
Ileal resection	7	0	0	0
Ileocaecal resection	26	0	0	0
Right sided colectomy	0	7	0	3
Left sided or subtotal colectomy	0	0	3	7
Total colectomy	0	0	25	4
Tissues*				
Histological grade of inflammation				
None (grade 0)	12	7	18	14
Mild inflammation (grade 1)	3	0	8	0
Intermediate (grade 2)	11	0	6	0
High (grade 3)	29	0	16	0

*=Total numbers of tissue samples studied from study A and Study B.

specimens. We also examined the intracellular expression of P-selectin in IBD by electron microscopy.

Methods

PATIENTS AND TISSUES

Surgically resected specimens from a total of 35 patients with Crohn's disease and 28 patients with ulcerative colitis were obtained within 30 minutes of removal. For the first part of the study, tissue samples were collected from either macroscopically uninvolved areas at a distance of 2-4 cm from the inflamed area or from the centres of inflammation (study A; one sample per case). In addition, to study the expression cell adhesion of molecules at different distance from the main lesion within the same patient, up to six samples per specimen were collected from uninvolved, intermediate, and severely affected areas from each of a further five Crohn's disease patients and five ulcerative colitis patients (study B; several samples per case). Indications for surgery were chronic stenosis or disease refractory to treatment, or both, in Crohn's disease and longstanding pancolitis or left sided colitis in ulcerative colitis. Non-inflamed control tissues were taken from hemicolectomy specimens resected for cancer, at least 5 cm from the malignancy (n=17), and from total colectomy specimens resected for familial adenomatosis coli (n=4) (see Table I for further details). In all cases, diagnosis was confirmed by histopathological examination of the resected specimen.

TABLE II Antibody characteristics

Antibodies to	Dilution	Type	Source
P-selectin (CD62)	1:1000	m/c	A Mazurov*
PECAM-1 (CD31)	1:8000	m/c	A Mazurov*
von Willebrand factor	1:200 000	m/c	Serotec, UK
CD45RO (UCHL1)	1:8000	m/c	Dako, Denmark
Interleukin 2 receptor (CD25)	1:100	m/c	Dako, Denmark
Pan-T cell (CD3)	1:800	p/c	Dako, Denmark
Macrophages (CD68)	1:200	m/c	Dako, Denmark

m/c=Mouse monoclonal; p/c=rabbit polyclonal; *antibodies were kindly donated by AM, Institute of Experimental Cardiology, Cardiology Research Centre, Moscow, Russia.

HISTOLOGICAL ASSESSMENT

Tissues were fixed by immersion in Zamboni's solution (saturated picric acid; 0.1 M phosphate buffer; 2% w/v formalin pH 7.2) and rinsed in 15% (w/v) sucrose in 0.1 M phosphate buffered saline with 0.01% (w/v) sodium azide. Cryostat blocks were prepared and sectioned at 6 µm thickness. One section from each sample was stained with haematoxylin and eosin for histological determination of inflammation according to a previously published method,²⁸ grading from '0' (non-inflamed) to '3' (highly inflamed). Only tissues without histological signs of inflammation (grade '0') were included as 'uninvolved' (Table I).

IMMUNOCYTOCHEMISTRY

Tissue sections were immunostained using a range of antibodies (see Table II) by an indirect immunoperoxidase method²⁹; sections serial to those used for staining P-selectin, were stained with monoclonal antibody against platelet endothelial cell adhesion molecule-1 (PECAM-1) for the identification of microvessels.^{30 31} Tissues with P-selectin showing a punctate staining pattern were also immunostained for von Willebrand factor on serial sections. Infiltrating mononuclear cells were further characterised by immunostaining for CD68 (macrophages), CD3 (T cells), and CD25 (interleukin 2 receptor) and CD45RO (memory cells) as markers of T cell activation. Sections were counterstained with neutral fast red and mounted with glycerol gelatin.

ELECTRON MICROSCOPY

Pre-embedding transmission electron microscopic immunohistochemistry was performed on selected cases of both normal and Crohn's disease gut to elucidate the cause of the differential staining pattern of P-selectin seen by light microscopy on particular endothelia. Serial sections of 40 µm thickness were cut and stained as mentioned above, free floating, in a 12 well tissue culture plate. After staining, the sections were fixed in 1% (w/v) osmium tetroxide for two hours at 4°C, washed in phosphate buffered saline, dehydrated in graded ethanols, and flat embedded in epoxy resin on a slide covered with an acetate sheet.³² After removal of the sheet, the flat embedded sections were observed under a light microscope. To analyse the same vessels at both light and electron microscopic level, areas of interest, for example vessels showing a punctate staining pattern, were cut out and re-embedded in plastic capsules, modified by trimming off the cone shaped tips of standard EM capsules. Ultrathin sections of silver-gold interference colour were stained with 4% (w/v) uranyl acetate in methanol followed by Reynolds's lead citrate for one to two minutes and observed in a Zeiss 10 CR electron microscope.

EVALUATION

Five visual fields within the mucosa and submucosa were chosen randomly from each

TABLE III Differential expression of P-selectin on vascular endothelia in normal gut and in inflammatory bowel disease

<i>P-selectin score</i>	<i>Normal ileum (n=7)</i>				<i>Uninvolved Crohn's disease (n=10)</i>						<i>Highly inflamed Crohn's disease (n=20)</i>				
	<i>A</i>	<i>Aa</i>	<i>V</i>	<i>Vv</i>	<i>Cap</i>	<i>A</i>	<i>Aa</i>	<i>V</i>	<i>Vv</i>	<i>Cap</i>	<i>A</i>	<i>Aa</i>	<i>V</i>	<i>Vv</i>	<i>Cap</i>
4	0	0	0	0	0	0	0	0	0	0	0	0	4	13	1
3	0	0	0	1	0	0	0	0	0	0	0	2	9	5	4
2	0	0	0	1	0	0	0	0	0	0	0	4	4	2	5
1	0	1	0	3	2	0	0	1	2	2	3	3	0	0	3
0	7	6	7	2	5	10	10	9	5	6	17	11	3	0	7

<i>P-selectin score</i>	<i>Normal colon(n=14)</i>				<i>Uninvolved ulcerative colitis (n=10)</i>						<i>Highly inflamed ulcerative colitis (n=13)</i>				
	<i>A</i>	<i>Aa</i>	<i>V</i>	<i>Vv</i>	<i>Cap</i>	<i>A</i>	<i>Aa</i>	<i>V</i>	<i>Vv</i>	<i>Cap</i>	<i>A</i>	<i>Aa</i>	<i>V</i>	<i>Vv</i>	<i>Cap</i>
4	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
3	0	0	0	3	1	0	0	0	0	0	0	0	10	3	0
2	0	3	2	2	2	0	0	0	3	2	0	0	3	1	8
1	0	2	2	4	2	0	0	2	3	2	0	8	0	0	3
0	14	9	10	5	9	10	10	8	4	6	13	5	0	0	2

A=arteries; Aa=arterioles; V=veins; Vv=venules; Cap=capillaries.

section for semi-quantitative light microscopic analysis. Two independent observers who did not know the diagnosis of the tissues assessed the extent of endothelial expression of P-selectin separately for each type of vessel. They counted the number of PECAM-1 positive vessels and then counted the number of P-selectin positive vessels on a section serial to that stained for PECAM-1. The percentage of PECAM-1 positive vessels that coexpressed P-selectin was estimated and the results were expressed as scores according to criteria similar to those reported elsewhere,³³ meaning 0=no staining; 1=30% of all vessels of a specific vessel type stained; 2=31–60%; 3=61–90%; 4=more than 90% stained. Comparisons between groups were made using the Mann-Whitney two tailed test for unpaired samples. Correlations were tested using Spearman's rank correlation test.

Results

P-SELECTIN IN THE NORMAL GUT

In normal gut, there was sporadic endothelial expression of P-selectin in all layers of the intestinal wall without differences between ileum and colon. Vascular endothelium was more often P-selectin positive in the mucosa and submucosa than in the subserosa or muscularis propria. P-selectin immunoreactivity was mostly a feature of venules and, far less, capillaries (Table III). There was no staining

on arteries and only very little on arterioles and veins. P-selectin immunoreactivity in an individual vessel usually was either strong or could not be detected; only very few vessels showed weak staining. The controls were older than the disease group but there was no significant decrease in expression of endothelial P-selectin with age (control ileum rank correlation=−0.24 (p=0.56), control colon rank correlation=−0.10 (p=0.78)). Intravascular platelets, as identified by PECAM-1, strongly coexpressed P-selectin without an apparent difference between the groups.

P-SELECTIN IN THE UNINVOLVED GUT ADJACENT TO INFLAMMATION

In the uninvolved areas of Crohn's disease and ulcerative colitis, P-selectin showed distribution patterns and staining intensity similar to those seen in the normal gut. As in controls, immunoreactivity for P-selectin was restricted to venules, capillaries, and some veins but was not detected on arterial vessels (Table III). Statistical evaluation of semiquantitative analysis showed no significant differences between uninvolved areas of IBD and the normal gut (Fig 1 and Fig 2).

In some vessels (<5% of all P-selectin positive vessels), P-selectin immunoreactivity appeared as small black dots and patches (Fig 3) rather than as homogeneous bands, as was usually seen (Fig 4). The patchy distribution pattern was found on venules and, very rarely,

Figure 1: P-selectin immunoreactivity in venules in normal ileum (n=7) and in uninvolved (n=10) and highly inflamed (n=20) gut in Crohn's disease (CD).

Figure 2: P-selectin immunoreactivity in venules in normal colon (n=14) and in uninvolved (n=10) and highly inflamed (n=13) gut in ulcerative colitis (UC).

Figure 3: P-selectin on a submucosal venule in normal ileum. Note the punctate intracytoplasmatic staining pattern in the endothelium (arrow). Intraluminal platelets are positive (original magnification $\times 460$).

Figure 5: Von Willebrand factor on a submucosal venule in normal ileum on a section serial to that of Fig 3. The punctate intracytoplasmatic staining pattern is even more pronounced than that in Fig 3 (original magnification $\times 510$.)

on arterioles. Few small veins and venules expressed both types of P-selectin immunoreactivity within the same vessel. Staining for von Willebrand factor showed a similar and even more punctate staining pattern than that occasionally seen by staining for P-selectin (Fig 5) suggesting that the punctate appearance of P-selectin immunoreactivity could mean colocalisation of both molecules. On ultrastructural analysis of these areas it seemed that the punctate staining pattern corresponded to P-selectin stored in Weibel-Palade bodies (Fig 6). In contrast, the homogeneous longitudinal immunoreactive bands seemed to correspond to P-selectin, redistributed along the endothelial cell membrane (Fig 7). Surprisingly, both distribution patterns of P-selectin immunoreactivity could sometimes be detected within an individual endothelial cell (Fig 8).

P-SELECTIN IN THE INFLAMED GUT

In highly inflamed areas of Crohn's disease and ulcerative colitis (grade 3), P-selectin immunoreactivity was upregulated dramatically (Table III, Fig 9). In comparison with

normal ileum, inflamed lesions of Crohn's disease showed a significant increase in P-selectin immunoreactivity score on venules ($p=0.001$; Fig 1, veins ($p<0.0001$) and capillaries ($p=0.05$). P-selectin was slightly, but not significantly upregulated on arteries that were positive in nine of 20 cases (Table III). Corresponding changes were seen on veins and venules in ulcerative colitis (Fig 2), when compared with normal colon. There were no differences in P-selectin expression between Crohn's disease and ulcerative colitis. In the inflamed gut, only very few venules showed the punctate immunoreactivity for P-selectin found in normal controls and in uninvolved IBD gut, most vessels in inflamed sections showing homogenous expression along the entire endothelial lining.

P-SELECTIN IN RELATION TO DEGREE OF INFLAMMATION

The intraindividual relation of P-selectin to grade of inflammation was studied in a further 25 specimens taken from five patients with Crohn's disease (histograde '0', $n=2$; '1', $n=3$; '2', $n=11$; '3', $n=9$) and on 24 specimens taken from five patients with ulcerative colitis (histograde '0', $n=8$; '1', $n=8$; '2', $n=6$; '3', $n=2$) (study B). P-selectin immunoreactivity seemed to be increased and became more

Figure 4: P-selectin on a small mucosal (m) vein close to the submucosal (sm) border in uninvolved ileum from Crohn's disease. Note the homogeneous longitudinal immunoreactive band along the entire endothelium (original magnification $\times 430$).

Figure 6: Electron micrograph of an endothelial cell in normal ileum on a section serial to that of Fig 3. P-selectin immunoreactivity is present in a cytoplasmic granule (arrow) near the abluminal plasma membrane (original magnification $\times 68\,000$; lu=lumen).

Figure 7: Electron micrograph of an endothelial cell in uninvolved ileum from Crohn's disease on a section serial to that of Fig 5. P-selectin immunoreactivity is present along the luminal plasma membrane and in an invagination of the plasma membrane (insert). (Original magnification $\times 20\,000$; insert: $\times 63\,000$; er=erythrocyte.)

homogeneous across sections with the grade of inflammation. For venules (Table IV), the expression of P-selectin in a less inflamed tissue was always lower than or equal to its expression in a section displaying a higher grade of inflammation.

CELLULAR ENVIRONMENT OF P-SELECTIN POSITIVE VESSELS

Although evaluation of the total section areas showed clear cut differences between IBD tissues and normal controls, P-selectin immunoreactivity within an individual section was usually quite heterogeneous. P-selectin was expressed more frequently on venules, there was excessive cellular infiltrate in active disease, but also in vessels situated close to small cellular aggregates in the uninvolved gut. In some cases, those parts of an individual vessel that were close to surrounding cellular clusters were P-selectin positive, whereas other segments abutting uninvolved tissue were P-selectin negative (Fig 10). Aggregating mononuclear cells at the site of P-selectin positive vessels were mostly CD3 positive T cells and CD68 positive macrophages. Most of the T cells were CD45RO positive and some of

Figure 8: Electron micrograph of an endothelial cell in uninvolved colon from ulcerative colitis. Note that P-selectin immunoreactivity is present along the outer surface of luminal plasma membrane, in folds of abluminal plasma membrane, and in a cytoplasmic granule (arrow). (Original magnification $\times 11\,000$; nu=nucleolus; lu=lumen.)

Figure 9: P-selectin is upregulated on most of the submucosal blood vessels in a representative section of highly inflamed Crohn's disease. Intraluminal platelets are positive (original magnification $\times 280$).

them coexpressed the interleukin 2 receptor, according to their state of activation. P-selectin was induced at sites of mucosal ulceration and was upregulated throughout the gut wall in cases of transmural inflammation and in the vicinity of granulomas.

Discussion

This is the first comprehensive report on the endothelial expression of P-selectin in Crohn's disease and ulcerative colitis. P-selectin was highly upregulated in inflamed areas of IBD, suggesting that P-selectin may participate in the recruitment of circulating inflammatory cells into the lesion.

Electron microscopic colocalisation studies using double labelling experiments with antibodies to both von Willebrand factor and P-selectin have shown that, before release, P-selectin is stored, together with von Willebrand factor, in endothelial intracellular organelles known as Weibel-Palade bodies.¹² After stimulation, intracytoplasmic Weibel-Palade bodies migrate to the cell membranes and fuse with the membrane to degranulate and allow P-selectin to redistribute along the endothelial cell surface.¹² In our tissues, although intense black P-selectin immunoreactivity prohibited the visualisation of tubular morphology characteristic for Weibel-Palade bodies at the electron microscopic level,³⁴ the typical shape and intracellular localisation of the P-selectin immunoreactive organelles left little doubt that they were Weibel-Palade bodies. This finding and the electron microscopic demonstration of

TABLE IV Expression of P-selectin on venules in tissues with different grades of inflammation taken from the same specimens

Patient	Crohn's disease	Patient	Ulcerative colitis
1	0*= $\dagger$ 0<1<3	1	0=0=0<2=2
2	1=1=2=2=2=3	2	0<1<2=3=3
3	2<2<3=3=3	3	1<1=1<1
4	2<2=2<2=3	4	0=0=0<0=1=1
5	2=2=3=3<3	5	1=2<2<2

*=Histological grade of inflammation; $\dagger$ =immunoreactivity of P-selectin is lower (<) or equal (=) in comparison with sections of tissue with the same or increasing grades of inflammation.

Figure 10: P-selectin on two small veins packed with blood cells in highly inflamed Crohn's disease. Only those parts of the vessels that are close to clusters of inflammatory cells (ic) are positive (original magnification $\times 280$).

P-selectin redistributed on the endothelial surface (Figs 7 and 8) suggest that, by means of immunocytochemistry and light microscopy, two different forms of P-selectin can be differentiated: (a) a stored form (seen as cytoplasmic, punctate immunoreactivity), (b) a released form (seen as longitudinal bands along the endothelial surface). The storage form was found predominantly in the normal and uninvolved IBD gut whereas the released form was mainly detected in highly inflamed tissues.

In IBD, perhaps surprisingly, no significant upregulation of P-selectin was shown in histologically non-inflamed tissues, adjacent to inflamed lesions. Given the susceptibility of any segment of the gastrointestinal tract to be affected by Crohn's disease and the continuous spread of ulcerative colitis, areas in the vicinity of inflammation will probably become inflamed in the later course of disease and thus may be regarded as 'early lesions'. We and others have shown that these areas, although lacking evidence of inflammation by cell infiltrates, are not entirely normal, displaying, for example, changed distribution of vasoactive intestinal polypeptide containing nerves³⁵ and increased mucus production.³⁶ Furthermore, increased expression of major histocompatibility complex antigens on nerve bundles³⁷ and endothelial cells³⁸ and upregulated lymphocyte function associated antigen-1 on mononuclear cells³¹ point to immunoactivation in the vicinity of IBD lesions.

Although the regulators of P-selectin expression are not yet fully understood, most of the known stimulants are produced by macrophages (for example, histamine, tumour necrosis factor α , oxygen radicals), granulocytes (leukotriene C₄, oxygen radicals) or T cells (tumour necrosis factor α), none of which occur

in high numbers in uninvolved areas of intestine. Unchanged expression of P-selectin in these tissues may thus result from a lack of appropriate stimulants. Another factor that may explain the unchanged expression of P-selectin in early lesions may be the time course of its release. P-selectin appears on the cell surface of endothelial cells five to 30 minutes after stimulation but, in cultures, disappears five to 10 minutes later in the absence of neutrophils.³⁹ Thus, it may not have been possible to detect increased expression of P-selectin if it happens less often in the early lesion than in advanced disease.

In the highly inflamed gut, we found an increased expression of endothelial P-selectin in confirmation of preliminary findings by Nakamura *et al.*²⁷ Although our study was based on morphological findings, some functional implications can be drawn. Upregulation of P-selectin immunoreactivity was mostly a feature of (postcapillary) venules – that is, the important sites of leucocyte migration in inflammation.⁴⁰ As the mediation of cell adhesion is the only function of P-selectin established so far, increased P-selectin is probably involved in the recruitment of various circulating inflammatory cells into the IBD lesion; neutrophils, basophils, eosinophils, natural killer cells, and monocytes all have been shown to bind to endothelial P-selectin.^{13–15 17} Specifically, as T lymphocytes represent a major proportion of the cellular infiltrate in IBD and bind to P-selectin,¹⁶ P-selectin could mediate recirculation of these cells, which, after local intestinal antigen stimulation, proliferate elsewhere and migrate back to the intestinal lesion. Subsequent reciprocal interaction between endothelium and circulating cells – that is, induction of P-selectin on endothelial cells by stimulants released from inflammatory cells and recruitment of inflammatory cells into the lesion by endothelial P-selectin – could result in a self-perpetuating vicious circle leading to the development of the inflammatory infiltrate. Further indirect evidence for the participation of endothelial P-selectin in intestinal cell adhesion in IBD derives from its coexpression with intercellular adhesion molecule-1 (ICAM-1) found in our previous study³¹ and by others.^{27 41} ICAM-1 mediates steps of the adhesion cascade subsequent to those mediated by P-selectin.⁴ Given the high constitutive expression of endothelial ICAM-1 in the gut,³¹ however, upregulation of ICAM-1 in IBD is less dramatic in comparison with that seen for P-selectin. Thus, in active disease, continuous migration of circulating cells into the inflamed tissue is essentially mediated by P-selectin and may contribute to the maintenance and spread of inflammation.

Recent evidence exists for possible additional functions of P-selectin. Blocking the molecule's action showed that P-selectin mediates vascular permeability and haemorrhage after intravenous administration of cobra venom factors in rats⁴² and participates in tissue necrosis and oedema after transection and replantation of the rabbit ear.⁴³ P-selectin also contributes to the pulmonary microvascular dysfunction seen after intestinal

ischaemia/reperfusion.⁴⁴ Although some of these effects may be caused by P-selectin released from activated platelets,¹² the dramatic upregulation of endothelial P-selectin seen in active disease may contribute to various pathological events in IBD, either by local action, or, systemically, by shedding of the molecule.⁴⁵

Interrupting cellular recruitment into the lesion in IBD is an important therapeutic aim. Preliminary experimental data show that cellular adhesion can be blocked by monoclonal antibodies against adhesion molecules. For example, local systemic application of antibodies against lymphocyte function associated antigen-1 in animals significantly reduces the cellular infiltrate and tissue damage in cardiac⁴⁶ and intestinal⁴⁷ ischaemia reperfusion injury and in experimental rat colitis.⁴⁸ For P-selectin, application of a monoclonal antibody significantly decreases adherence of polymorphonuclear cells to stimulated endothelial cells and protects feline heart in myocardial ischaemia and reperfusion injury.⁴⁹ In rats, endotoxin induced neutropenia and polymorphonuclear cell accumulation in tissues can be blocked by treatment with antibodies to P-selectin.⁵⁰ Infusion of sialyl-Lewis X, a ligand for P-selectin, significantly reduces lung injury and diminishes the tissue accumulation of neutrophils in a P-selectin dependent model of rat lung injury.⁵¹ The dramatic increase of P-selectin in active IBD shown in this study supports the concept that blockade of P-selectin may have a therapeutic use in Crohn's disease and ulcerative colitis.

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