Adhesion molecules in inflammatory bowel disease

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Abstract

The ability of leucocytes to adhere to endothelium is essential for leucocyte migration into inflammatory sites. Some of these adhesion molecules are released from the cell surface and can be detected in serum. The soluble adhesion molecules intercellular adhesion molecule 1 (ICAM-1), E selectin, and vascular cell adhesion molecule 1 (VCAM-1) were studied in the serum of patients with Crohn's disease, ulcerative colitis, and healthy controls. A second blood sample was taken from patients with active disease after one month of treatment and a third two months after remission was achieved. Tissue expression of the same adhesion molecules was studied by immunohistology. Circulating VCAM-1 concentrations were significantly higher in patients with active ulcerative colitis ($n=11$, median=165 U/ml) compared with patients with inactive ulcerative colitis ($n=10$, median=117 U/ml, $p<0.005$), active Crohn's disease ($n=12$, median=124 U/ml, $p<0.02$), and controls ($n=90$, median=50 U/ml, $p<0.0001$). Within each disease group there were no significant differences in E selectin or ICAM-1 concentrations between the active and inactive states, however, patients with active Crohn's disease had significantly higher ICAM-1 concentrations ($n=12$, median=273 ng/ml) than controls ($n=28$, median=168, $p<0.003$). VCAM-1 concentrations fell significantly from pre-treatment values to remission in active ulcerative colitis ($p<0.01$). In Crohn's disease there was a significant fall in ICAM-1 both during treatment ($p<0.01$) and two months after remission ($p<0.02$). Vascular expression of ICAM-1 occurred more often and was more intense in inflamed tissue sections from patients with ulcerative colitis and Crohn's disease than from controls. Vascular labelling with antibody to E selectin also occurred more often in patients with active inflammatory bowel disease. In conclusion, increased circulating concentrations of selected adhesion molecules are associated with inflammatory bowel disease. There is also evidence of local upregulation, particularly of ICAM-1. Differential expression of adhesion molecules in tissue may play a part in the initiation of leucocyte migration and local inflammation; the function of circulating adhesion molecules is unknown, but may

play a physiological part in blocking adhesion.

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Inflammatory bowel disease is characterised by the infiltration of inflammatory cells derived from the circulation including monocytes, lymphocytes, and neutrophils. Several factors contribute to the local recruitment of inflammatory cells. These include the release of cytokines in the microenvironment and the interaction between adhesion molecules expressed on circulating inflammatory cells and those on their local target cells. The potential importance of these molecules in inflammatory conditions of the gastrointestinal tract is suggested by a recent paper¹ in which an inhibitor of expression of β_2 integrins involved in leucocyte adhesion (NPC 15669) attenuated acetic acid induced colitis in rats.

Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are cytokine inducible glycoproteins belonging to the immunoglobulin supergene family.² ICAM-1 is constitutively expressed on a limited number of cell types including endothelial cells (weakly)³ but is induced on a wide variety of cells by inflammatory cytokines such as interleukin 1 (IL1), tumour necrosis factor (TNF), and interferon γ (IFN γ).^{4,5} Susceptible tissues include haemopoietic cells, epithelial cells, and endothelial cells. ICAM-1 is a ligand for the leucocyte integrins leukocyte function associated molecule-1 (LFA-1) and Mac-1³ and participates in leucocyte adhesion to activated endothelial cells, T cell/antigen presenting cell, T cell/T cell, and T cell/B cell interactions.

VCAM-1 is less widely distributed than ICAM-1 and is expressed by germinal centre dendritic cells, interdigitating dendritic cells, Kupffer cells, synovial lining cells, and renal proximal tubule cells.⁶⁻⁹ Endothelial cells also express VCAM-1 after activation by cytokines such as TNF α , IL 1 β , and IFN γ . It is primarily involved in lymphocyte and monocyte-endothelial cell interactions and binds to an integrin of the VLA4 class expressed on all leucocytes except neutrophils. T cell activation leads to kinase induced conformational changes of VLA-4, increasing the avidity of the binding with VCAM.¹⁰

E selectin is transiently expressed on endothelial cells only, after induction by IL1, TNF or lipopolysaccharide.¹¹ IFN γ seems to

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stabilise E selectin surface expression without prolonging the period of synthesis.¹² It primarily mediates the neutrophil-endothelial cell interactions that modulate recruitment of neutrophils to sites of inflammation,¹³ but more recent studies have shown that it also mediates the adhesion of a subpopulation of resting CD4⁺ memory cells to activated endothelium.¹⁴

Increased expression of adhesion molecules has been described in a variety of inflammatory disorders such as dermatoses,¹⁵ asthma,¹⁶ and arthritis.¹⁷ Soluble variants of some adhesion molecules such as circulating E selectin, ICAM-1, and VCAM-1^{18–19} have been found in normal serum and at increased concentrations in several disorders including malignant diseases,^{20–21} HIV infection,²² psoriasis,²³ and uveitis.²⁴

In addition, Leeuwenberg *et al.*,²⁵ found that the amount of soluble adhesion molecules released from human umbilical vein endothelial cells stimulated with TNF, IL 1, or lipopolysaccharide, correlated directly with cell surface expression. At the present time, the significance and mechanism of release of these molecules is unknown.

The aims of this study were twofold: firstly, to investigate concentrations of circulating forms of the adhesion molecules E selectin, VCAM-1, and ICAM-1 in inflammatory bowel disease, and their correlation with clinical and immunological parameters of disease activity and von Willebrand factor. Von Willebrand factor is important in the adhesion of platelets to endothelial cells²⁶ and its release is thought to reflect vascular injury.²⁷ The second aim was to study the local expression of these three adhesion molecules in patients with ulcerative colitis, Crohn's disease, and controls.

Methods

Patients

Blood samples were collected from 43 patients with inflammatory bowel disease defined according to standard clinical, histological, and radiological methods. Twenty two patients had Crohn's disease (age range 19–68) (12 active: defined as Crohn's disease activity index (CDAI) >150²⁸), and 21 had ulcerative colitis (age range 18–80) (11 active: defined as mild (two patients), moderate (nine patients), or severe according to Truelove and Witt's criteria²⁹). A second blood sample was taken from patients with active disease after one month of treatment and a third, two months after remission was achieved. Samples were allowed to clot, the serum removed within 30 minutes of venepuncture, and stored at –70°C until analysis.

Active disease – 13 patients were treated with 30 mg prednisolone daily, three patients who relapsed while receiving corticosteroids were given azathioprine, another patient opted for treatment with a polymeric diet (Triosorbon, E Merck, Alton, Hampshire, UK), the other three had distal ulcerative colitis and received

either 5-ASA or corticosteroid enemas, but one of these failed to respond and required oral prednisolone. Two patients with Crohn's disease and one with ulcerative colitis who relapsed while taking prednisolone were referred for surgery. Seven patients with active ulcerative colitis were taking aminosalicylates (maintenance dose) and these were continued in the same dose throughout the study.

Inactive disease – of the patients with inactive Crohn's disease, two were taking prednisolone (5 mg and 10 mg) and azathioprine, two azathioprine alone, two prednisolone alone (2.5 and 10 mg), and four were receiving no treatment. Three patients included in the inactive ulcerative colitis group were taking prednisolone (5, 7.5, and 10 mg) and aminosalicylates, three were taking aminosalicylates alone, and four patients were not taking any drugs.

Samples were also obtained from 90 healthy laboratory and clinical personnel and blood donors (age range 18–60) and assayed for E selectin and VCAM-1. In the case of ICAM-1, a subgroup of 27 of the control samples (age range 24–54) were assayed.

Endoscopic biopsy specimens or surgical sections of small or large bowel, or both, were taken from eight patients with Crohn's disease and nine patients with ulcerative colitis. These included biopsy specimens from six patients with active ulcerative colitis and two patients with active Crohn's disease who also had serum samples taken. Endoscopic biopsy specimens from eight patients undergoing colonoscopy for investigation of abdominal pain (in whom no abnormality was subsequently found) or sections from surgical specimens of patients with colonic carcinoma (obtained well away from the tumour area) and diverticular disease were used as normal controls.

Assay of soluble adhesion molecules

Concentrations of circulating ICAM-1 were measured with a commercial enzyme linked immunosorbent assay (ELISA) kit (British Biotechnology Products, Oxford, UK). Concentrations of circulating E selectin and VCAM-1 were measured using dual monoclonal antibody two site ELISAs as previously described.^{18–20–30} The assays were standardised using a recombinant soluble form of E selectin or VCAM-1 lacking their transmembrane and cytoplasmic domains and given an arbitrary unitage against which all samples were measured. Inter and intra-assay coefficient of variation for all three ELISAs were <10% and <6% respectively over a range of values.

Von Willebrand factor assay

Assay of von Willebrand factor antigen was performed by ELISA using rabbit antihuman von Willebrand factor polyclonal antibodies (Dako Ltd, UK) according to the method described by Short *et al.*³¹ Intra-assay and interassay coefficients of variation were 3.1% and 3.6% respectively.

The following were also measured for each patient: haemoglobin, white cell and platelet

Figure 1: Serum concentrations of soluble forms of the adhesion molecules: (A) E selectin, (B) VCAM-1, and (C) ICAM-1 in patients with active and inactive inflammatory bowel disease and in controls. The horizontal lines represent median values.

count (automated Coulter Counter, Coulter Electronics, Luton, Bedfordshire, UK); plasma viscosity (Coulter Viscometer, Counter Electronics); erythrocyte sedimentation rate (ESR) (Westergren method); serum C reactive protein (Behring Nephelometer, Hoechst UK, Hounslow, UK) and serum albumin (sequential multiple auto-analyser with computer; Technicon, Basingstoke, Hampshire, UK).

Immunohistochemistry

Paraffin wax embedded sections were dewaxed in xylene and rehydrated. Endogenous peroxidase activity was blocked by incubating sections in 0.3% hydrogen peroxide in methanol for 10 minutes at room temperature followed by incubating with 0.1% trypsin at 37°C for 20 minutes. After incubating in normal mouse serum (1 in 5), goat polyclonal antibodies to E selectin, VCAM-1, or ICAM-1 (from Dr A Gearing) diluted 1 in 200 or control (normal goat serum) were then applied and left overnight at room temperature. After washing, biotinylated monoclonal anti-goat antibody (B3148, Sigma Chemicals), diluted 1 in 250 was applied and sections incubated for 30 minutes at room temperature. After a further washing step, colour was developed using an avidin-biotin-peroxidase complex (Dako

ABC-HRP complex kit) according to the manufacturer's instructions followed by washing and incubation in DAB (diaminobenzidine).

Histological assessment of biopsy specimens and surgical sections

All sections were assessed by a single observer (blinded to the antibody applied to the section), for the degree of positivity in all vessels and other tissue types by the antibodies.

Statistics

Methods appropriate to non-parametric data were used throughout: the Mann-Whitney U test to assess differences between the different patient groups, Wilcoxon matched pairs signed rank sum test to determine differences in the same patient group after treatment, and Spearman's rank coefficient to assess correlation. The analyses were performed by computer using the statistical analysis program CSS.

Results

Circulating adhesion molecules (Fig 1A–C)

VCAM-1 concentrations were significantly higher in patients with active ulcerative colitis (median=165 U/ml) compared with patients with inactive ulcerative colitis (median=117, $p<0.005$), active Crohn's disease (median=124, $p<0.02$), and controls ($n=90$, median=50, $p<0.0001$). VCAM-1 concentrations were also significantly greater in patients with both active and inactive Crohn's disease than controls ($p=0.0001$). There were no significant differences in E selectin or ICAM-1 concentrations between patients with active and inactive disease, although patients with active Crohn's disease had significantly higher ICAM-1 concentrations (median=273 ng/ml) than controls ($n=28$, median=168, $p<0.003$). VCAM-1 concentrations fell significantly from pretreatment values to remission in active ulcerative colitis (pretreatment median=165, post-treatment median=138, $p<0.01$). In Crohn's disease concentrations of soluble adhesion molecules were available after treatment in 11 of 12 patients with active disease. There was a significant fall in ICAM-1 both during treatment ($p<0.01$) and two months after remission (pretreatment median=262, post-treatment=205, $p<0.02$) (Fig 2).

There was no significant difference in sICAM-1 concentrations between the patients with inactive disease who were taking corticosteroids compared with those who were not. The two highest values of sICAM-1 (500 and 344) occurred in patients with inactive Crohn's disease who were taking 10 and 5 mg prednisolone respectively.

In patients with ulcerative colitis, sVCAM-1 correlated with von Willebrand factor ($r=0.67$, $p<0.003$), platelet count ($r=0.43$, $p=0.05$), ESR ($r=0.62$, $p<0.01$), and inversely with haemoglobin ($r=-0.65$, $p<0.004$) and albumin ($r=-0.65$, $p<0.006$). sICAM-1

Figure 2: Serum concentrations of soluble forms of: (A) VCAM-1 and (B): ICAM-1 before and after treatment of active ulcerative colitis and Crohn's disease.

concentrations correlated with von Willebrand factor ($r=0.49$, $p<0.03$), C reactive protein ($r=0.57$, $p<0.01$), and inversely with albumin ($r=-0.60$, $p<0.01$). In active Crohn's disease, ICAM-1 concentrations were inversely correlated with haemoglobin ($r=-0.73$, $p<0.03$).

Von Willebrand factor was significantly higher in patients with active (median=1.32 IU/ml, range: 0.82–1.85) rather than inactive ulcerative colitis (median=0.83, range: 0.47–2.54, $p<0.03$), but there were no significant differences between patients with active and inactive Crohn's disease (medians=0.99, range 0.55–2.76 and 1.04, range 0.41–1.92 respectively). In patients with ulcerative colitis, von Willebrand factor correlated with platelet count ($r=0.52$, $p<0.02$), ESR ($r=0.79$, $p<0.0008$), C reactive protein ($r=0.49$,

$p<0.03$), and inversely with haemoglobin ($r=-0.47$, $p<0.04$) and albumin ($r=-0.63$, $p<0.007$). In patients with Crohn's disease there was an inverse correlation between von Willebrand factor and albumin in patients with active disease only ($r=-0.686$, $p=0.02$).

E selectin concentrations did not correlate with any parameters.

Local expression of adhesion molecules

The Table summarises the results of endothelial labelling.

Controls ($n=8$) – blood vessels were all negative for E selectin apart from one case, which had minimal labelling. Seven cases were positive VCAM-1 and five for ICAM-1. Figure 3A shows a control section labelled with ICAM-1 antibody (regarded as being negative). The occasional pigmented macrophage seen in this section represents pseudomelanosis coli.

Ulcerative colitis (nine involved and one uninvolved) – in four of the inflamed specimens there was endothelial positivity for E selectin. In one of these cases a section was also available taken proximal to the inflamed area, which was negative. Nine of 10 sections contained vessels positive for VCAM-1, although staining did not seem to be any more extensive than seen in controls. The most striking finding in this group was that all patients had vessels that were positive for ICAM-1, most of them strongly so. Figure 3B illustrates a section showing appreciable endothelial positivity for ICAM-1. More patients with ulcerative colitis tended to express E selectin than controls.

Crohn's disease – there were nine involved cases (six colonic, three ileum) and three uninvolved (one caecum, two ileum). Of the involved cases, four contained vessels positive for E selectin (one case of patchy involvement was positive only in the inflamed areas), all cases were positive for VCAM-1, six moderately so. All cases had positive vascular labelling for ICAM-1, which was intense in three sections. The three cases of uninvolved bowel were moderately positive for both VCAM-1 and ICAM-1, but not E selectin.

Other tissue components – occasional inflammatory cells in the lamina propria were positive for ICAM-1 expression with a slight increase in the number of positive cells in ulcerative colitis and Crohn's disease compared with controls.

Endothelial labelling results

	Number of cases with vascular endothelium positive for adhesion molecule		
	Controls	Ulcerative colitis (involved)	Crohn's disease (involved)
E selectin	1 of 8	4 of 9	4 of 9
VCAM-1	7 of 8	8 of 9	9 of 9
ICAM-1	5 of 8	9 of 9 (6 intense)	9 of 9 (3 intense)

Local expression of the adhesion molecules E selectin, VCAM-1, and ICAM-1 in sections taken from inflamed areas of small or large bowel in patients with Crohn's disease and ulcerative colitis and in 'controls'. Control tissue was taken from patients undergoing resection for carcinoma or diverticular disease, well away from the affected area or from patients who had normal colonoscopic and histological findings.

Figure 3: Sections taken from (A): control patient (negative for vascular adhesion molecule expression) and (B): patient with active ulcerative colitis showing intense labelling with ICAM-1 antibody. The occasional pigmented macrophage seen in the section taken from the control patient represents *pseudomelanosis coli*.

No cells in the lamina propria (other than endothelial cells) showed specific labelling for E selectin or VCAM-1. There was epithelial connective tissue labelling in a few cases. In these cases there was diffuse epithelial labelling present in an identical pattern with all three antibodies, which was considered to be non-specific. In one case of active Crohn's disease, there was a distinct pattern of focal epithelial labelling with the ICAM antibody only, which may therefore be specific.

There was no obvious relation between the tissue concentrations of adhesion molecules and their circulating concentrations in the eight patients who had both measured.

Discussion

This study has shown that the concentration of selected circulating adhesion molecules and the expression of endothelial cell surface adhesion molecules are increased in inflammatory bowel disease. There was a dissociation between circulating and local expression. VCAM-1 concentrations were significantly

increased in the circulation of patients with active ulcerative colitis without prominent local upregulation compared with controls. This may reflect differences in turnover rates. There was also no obvious relation between the tissue concentrations of adhesion molecules and their circulating concentrations in the eight patients who had both measured.

Previous studies in inflammatory bowel disease have shown that vascular endothelium and lamina propria mononuclear phagocytes express ICAM-1.³²⁻³⁴ Our results are in agreement with this, but we did not see such extensive lamina propria mononuclear cell staining. VCAM-1 is constitutively expressed in lymphoid aggregates in normal colonic mucosa but is not significantly enhanced in inflammation, conversely, E selectin was not detected in normal mucosa but was present on endothelial surfaces in association with active inflammation.³⁴⁻³⁶ Several epithelial cell types including human colonic adenocarcinoma cell lines and colonic cancer cells *in vivo* express ICAM-1.³⁷⁻³⁸ In a few of our cases there was epithelial labelling that seemed to be non-specific, in that it was diffusely present with all three antibodies. In one case, however, the epithelial labelling did seem to be specific in that there was a distinct pattern of reactivity only seen with the ICAM antibody and not with other antibodies of the same species.

The differential local expression of adhesion molecules that we found may reflect differences in local cytokine concentrations. The expression of E selectin, VCAM-1, and ICAM-1 on endothelial cells is induced by IL1 and TNF³⁹⁻⁴⁰; both of which are produced in increased amounts in the inflammatory bowel disease.⁴¹⁻⁴³ The mechanism by which soluble adhesion molecules are released remains unclear. The differential serum concentrations that we saw may be caused by differences in the rate of shedding of the molecule from vascular endothelial cells induced by differences in cytokine profile or concentration.

The concentration of soluble ICAM-1 in patients with active Crohn's disease and VCAM-1 in patients with ulcerative colitis fell after corticosteroid treatment. Corticosteroids are known to affect ICAM-1 expression.⁴⁴ Although, as stated previously, in the inactive disease group there was no significant difference between patients taking corticosteroids and those who were not. However, patients were receiving lower doses of corticosteroids than the active group. The reduction may result from down regulation of the release or expression of adhesion molecules, or both. By down regulating the expression of these molecules on endothelium, their interaction with immune cells will be reduced and inflammatory activity diminished. This mechanism of action may be of importance in inducing remission in inflammatory bowel disease.

Von Willebrand is increased as part of the acute phase response in humans⁴⁵ and is increased in inflammatory bowel disease.⁴⁶ Stevens *et al*⁴⁶ found, as we did, that serum von Willebrand activity was unrelated to disease activity in Crohn's disease but was

significantly raised in active compared with inactive ulcerative colitis. They suggested that the increase is compatible with the proposal that vascular injury is an early event in the pathogenesis of Crohn's disease.⁴⁷ If circulating concentrations of adhesion molecules are a reflection of vascular inflammation, the increased concentrations of sICAM-1 in a few patients with inactive Crohn's disease would be compatible with continuing vascular inflammation in patients with inactive Crohn's disease.

It is possible that the positive correlation between sICAM-1 and the acute phase protein C reactive protein, and the negative correlation with the negative acute phase protein albumin reflects the activity of circulating cytokines. As previously discussed, cytokines such as IL1 and TNF α , are important in upregulating expression of ICAM-1. These cytokines, together with IL6, can differentially regulate production of acute phase proteins.⁴⁸

The reason why two of the healthy controls had VCAM concentrations of >1000 U/ml is unclear but probably does not result from assay interference as normal values of ICAM-1 and E selectin were detected in these samples using assays of a similar design. Similar concentrations of VCAM-1 were detected in repeat samples two months later.

The physiological importance of these soluble molecules is unclear; they may merely represent a spill over from the cell bound molecule, alternatively, they may regulate cell adhesion by competition as indeed, both sICAM-1 and sVCAM-1 can support leucocyte adhesion when immobilised to plastic.⁴⁹ By binding to receptors on leucocytes, soluble adhesion molecules may prevent adherence of inflammatory cells to vascular endothelium and hence diminish inflammation. On the other hand, they may trigger a response in a ligand bearing cell.

In conclusion, raised circulating concentrations of selected adhesion molecules are associated with inflammatory bowel disease and are related to disease activity in ulcerative colitis. There is also evidence of local upregulation, particularly of ICAM-1. Differential expression of adhesion molecules in tissue may play a part in the initiation of leucocyte migration and local inflammation; it is possible that circulating forms play a protective part in inflammation by competing in cell to cell adhesion. Further studies are required to determine their role in the inflammatory process.

Some of these data have previously been published in abstract form (*Gut* 1993; 34 (suppl 1): S12).

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