Circulating von Willebrand factor in inflammatory bowel disease

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Abstract

Raised circulating von Willebrand factor is a recognised marker of vascular injury. To evaluate the role of vascular injury in the pathogenesis of inflammatory bowel disease, serum von Willebrand factor in Crohn's disease, ulcerative colitis, confirmed bacterial diarrhoea, and healthy subjects was measured. von Willebrand factor values were raised in 9/14 patients (p = 0.007) with active Crohn's disease, 15/28 (p = 0.004) with inactive Crohn's disease, 16/23 (p = 0.0003) with active ulcerative colitis, 9/27 (p = 0.04) with inactive ulcerative colitis, and 15/17 (p = 0.0001) patients with bacterial diarrhoea. Serum von Willebrand factor was unrelated to disease activity in Crohn's disease but was significantly raised in active (p = 0.02) compared with inactive ulcerative colitis. In contrast to controls, the detection of von Willebrand factor from inflammatory bowel disease sera and that from fractured endothelial cells was significantly inhibited by the reducing agent, dithiothreitol, suggesting the presence of an additional dithiothreitol sensitive form of the molecule derived from injured endothelial cells in inflammatory bowel disease. That serum von Willebrand factor is raised in quiescent as well as active Crohn's disease is compatible with the proposal that vascular injury is a fundamental abnormality in this disorder. The raised von Willebrand factor values in active inflammatory bowel disease and bacterial diarrhoea could be caused by either vascular injury, occurring secondary to bowel inflammation, or to an acute phase response resulting from endothelial cell stimulation by mediators released during the inflammatory process. Raised circulating von Willebrand factor could contribute to the increased risk of thrombosis associated with active inflammatory bowel disease.

Inflammatory bowel disease is characterised by focal or diffuse inflammation of the alimentary tract. Although its aetiology is unknown, intestinal vascular injury has been proposed as a major pathogenic factor. Ultrastructural abnormalities of the intestinal vasculature have been reported in Crohn's disease and ulcerative colitis, and affected patients 'show various coagulation abnormalities and an increased risk of thrombosis'. Increased circulating von Willebrand factor (factor VIII related antigen) has been proposed as a marker of vascular injury. von Willebrand factor is a high molecular weight multimeric glycoprotein, synthesised and released by vascular endothelial cells and megakaryocytes, that plays an important role in platelet-endothelial cell interactions and stabilising the factor VIII coagulation protein. Circulating von Willebrand factor is raised in experimental models of vascular injury and in disorders characterised by overt vascular inflammation.

In this study, by examining serum von Willebrand factor in active and quiescent inflammatory bowel disease, in patients with confirmed bacterial diarrhoea, and in healthy volunteers as controls, we have investigated the role of vascular injury in the pathogenesis of inflammatory bowel disease.

Methods

PATIENTS WITH INFLAMMATORY BOWEL DISEASE (Tables I and II)

In Crohn's disease (n = 42; 14 with active and 28 inactive disease) and ulcerative colitis (n = 50; 23 with active and 27 with inactive disease) were diagnosed by conventional clinical, radiological, endoscopic, and histological criteria.

Disease activity in Crohn's disease was determined by Harvey's clinical index, with an additional 1 point for the following laboratory variables: haemoglobin < 12 g/100 ml (men) and < 11 g/100 ml (women); erythrocyte sedimentation rate > 20 mm/hour; C reactive protein > 0.8

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Clinical details of patients with Crohn's disease (CD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>14</td>
</tr>
<tr>
<td>Site of disease: Small bowel</td>
<td>6</td>
</tr>
<tr>
<td>Ileocolonic</td>
<td>1</td>
</tr>
<tr>
<td>Colonic</td>
<td>7</td>
</tr>
<tr>
<td>Treatment: * Oral steroids</td>
<td>8</td>
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<tr>
<td>Sulphasalazine</td>
<td>5</td>
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<tr>
<td>Topical steroids</td>
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<tr>
<td>Metronidazole</td>
<td>3</td>
</tr>
<tr>
<td>None</td>
<td>5</td>
</tr>
</tbody>
</table>

*Some patients were on more than one treatment.
Figure 1: von Willebrand factor concentrations in inflammatory bowel disease and bacterial diarrhoea. von Willebrand factor was assayed in sera by ELISA. Results are expressed as percentage values, calculated from a standard dilution curve constructed from pooled healthy human sera. Horizontal bars represent median values and the upper limit of the normal range is indicated by the broken horizontal line. (*) = p < 0.05 v control, ** = p < 0.05 v inactive disease.)

MicroELISA plates (96 well) were coated with 100 ml of rabbit anti-human von Willebrand factor diluted 1/1000 in 0-05 mol/l carbonate buffer (pH 9-6) for one hour at room temperature. The plates were then washed in phosphate buffered saline (pH 7-4) containing 0-05% (v/v) Tween 20 (PBS-0-05% Tween), shaken dry, and 100 μl of a diluted standard or sample serum diluted 1/40 in PBS-0-1% Tween added to duplicate wells, and incubated at room temperature for one hour. PBS-0-1% Tween (100 μl) was also added to six microwells as blanks and a standard curve was determined for each plate by diluting (1/10-1/320) pooled healthy sera (the pooled serum standard had been previously confirmed to correspond to an NIBSC standard plasma for determination of von Willebrand factor. The plates were then washed three times by immersion into PBS-0-05% Tween, shaken dry, and 100 μl of rabbit anti-human von Willebrand factor peroxidase conjugate diluted 1/1000 in PBS-0-1% Tween were added to each well and incubated a further hour at room temperature. Finally, plates were washed three times by immersion in PBS-0-05% Tween and then once in citrate phosphate buffer (pH 5-0) before addition of 100 μl of 0-56 mg/ml 1-2 orthophenylenediamine dihydrochloride, dissolved in citrate phosphate buffer (pH 5-2), supplemented with hydrogen peroxide as substrate. Microwell plates were continuously agitated during colour development and absorbance values were measured at 492 nm on an automated ELISA reader (Dynatech MR 580). The average readings from the blanks were subtracted from all others and these were then read off the standard curve and multiplied by the dilution factor to give values of von Willebrand factor as a percentage of that in the pooled standard serum.

Dithiothreitol-modified ELISA
Serum samples or supernatants from cultured human umbilical vein endothelial cells (HUVE), subjected to four freeze-thaw cycles and then centrifuged at 3000 g, were incubated for 10 minutes with dithiothreitol (DTT) (0-6-2-4 mmol/l in PBS-0-1% Tween) at 37° C as described by Rose et al before determination of von Willebrand factor by ELISA.

Statistical analysis
Results within the normal range were defined as those values below the 95th centile value of the control group. Statistical significance of differences in group median values were determined by two tailed Mann-Whitney U test. Correlations were sought using Spearman’s rank correlation test. Statistical differences in the serial von Willebrand factor studies were determined by Wilcoxon’s (two tailed) signed rank test.

Results

Serum von Willebrand factor
Median serum von Willebrand factor concentrations were raised in all inflammatory bowel...
Disease groups compared with normal healthy controls (Fig 1). Sixty four per cent (p=0.007 for group median v control) of patients with active Crohn’s disease and 54% (p=0.004) with inactive disease had von Willebrand factor concentrations above normal (≥ healthy control 95th centile value, that is 132%). In the ulcerative colitis group, 70% (p=0.0003) of patients with active disease and 33% (p=0.04) with inactive disease had abnormally raised von Willebrand factor concentrations. Patients with inactive ulcerative colitis expressed significantly lower serum von Willebrand factor values than those with active disease (p=0.02). When von Willebrand factor concentrations in the Crohn’s disease and ulcerative colitis groups were examined as a function of individual clinical and laboratory parameters of disease activity, no significant correlations were found. In particular, there was no relation between serum von Willebrand factor concentrations and C reactive protein concentrations in the clinically inactive Crohn’s disease subgroup. von Willebrand factor values were also unrelated to the site of disease in Crohn’s disease (Table IV), extent of disease in ulcerative colitis (Table III), or drug treatments administered in either group.

High concentrations of serum von Willebrand factor were also observed in 88% of patients with confirmed bacterial diarrhoea (p=0.0001).

Serial studies in inflammatory bowel disease patients showed no statistically significant differences in circulating von Willebrand factor when a change in disease activity occurred (Fig 2). However, a fall in circulating von Willebrand factor accompanied the transition from active to inactive disease in 5 of 9 (p=NS) patients with ulcerative colitis. von Willebrand factor values also fell (p=0.0003) in all patients recovering from bacterial diarrhoea. No such trends were observed in patients with Crohn’s disease, where a change from active to inactive disease was accompanied by a rise in serum von Willebrand factor in 7/9 patients.

**Sensitivity of von Willebrand factor from inflammatory bowel disease sera to DTT**

Preincubation of sera with the reducing agent DTT (0.6–2.4 mmol/l) reduced, in a dose dependent manner, the amount of von Willebrand factor subsequently detected by ELISA (data not shown). Maximum inhibition by DTT, however, was related to the initial von Willebrand factor concentration: subjects with a raised concentration – that is, inflammatory bowel disease and bacterial diarrhoea patients – were more susceptible to inhibition than controls. A plot of the initial concentration against maximal inhibition by DTT (Fig 3) shows that a proportion of the von Willebrand factor measured by ELISA was completely resistant to the effects of DTT and that this proportion was greatest in subjects with normal serum von Willebrand factor concentrations. When von Willebrand factor released from fractured cells was tested for DTT sensitivity, the results were similar to those obtained from sera containing high concentrations (Fig 3).

### Discussion

Median serum von Willebrand factor concentrations were significantly raised in patients with Crohn’s disease, ulcerative colitis, and bacterial diarrhoea compared with healthy controls. Serum concentrations were significantly higher in patients with active ulcerative colitis than those with inactive disease (Fig 1). Furthermore, the transition from active to inactive disease in nine patients with ulcerative colitis was accompanied by a fall in von Willebrand factor in five of them (Fig 2). The high values of circulating von Willebrand factor in patients with active bacterial diarrhoea also fell towards normal on recovery. In contrast, no relation between serum von Willebrand factor concentrations and disease activity was found in Crohn’s disease patients (Figs 1 and 2).

Our results confirm and extend those of Gris et al who reported raised plasma von Willebrand factor concentrations in a small mixed group of patients with active inflammatory bowel disease.
Circulating von Willebrand factor in inflammatory bowel disease

Figure 3: Relation between the initial von Willebrand factor concentration and maximum inhibition by diethylthiourea in serum samples from healthy subjects (Ο), Crohn's disease (▲), ulcerative colitis (●), bacterial diarrhoea (△) and freeze thawed endothelial cell supernatant (▽). von Willebrand factor values were determined by ELISA after sample incubation for 10 minutes at 37°C with buffer or a maximal inhibitory concentration of diethylthiourea (DTT). Results were determined as previously described and maximal inhibition is given as \((\text{vWF} + \text{buffer} - \text{vWF} + \text{DTT}) / (\text{vWF} + \text{buffer})\).

Circulating von Willebrand factor (vWF) is increased in inflammatory bowel disease (IBD). The inverse relation between endothelial von Willebrand factor and bacterial diarrhoea suggests the presence of an acute phase reaction. The increased sensitivity of von Willebrand factor to DTT inhibition in subjects with raised serum concentrations, and the occurrence of a greater proportion resistant to DTT in controls, may indicate the presence of more than one form of von Willebrand factor in the circulation. The data suggest that at least a proportion of the von Willebrand factor in inflammatory bowel disease is derived from injured endothelial cells and that this proportion is highly sensitive to DTT. In our studies, von Willebrand factor released from fractured HUVE was more sensitive to inhibition by DTT than that released constitutively — that is, the von Willebrand factor circulating in normal healthy subjects — and probably represents the large multimeric forms stored in the Weibel-Palade bodies, which are released by specific or injurious stimuli in vitro. Further investigation into the molecular composition of von Willebrand factor by agarose gel electrophoresis is under way to clarify the presence of abnormal (large multimer) forms in the circulation of inflammatory bowel disease patients. While the increased incidence of venous thrombosis in inflammatory bowel disease could be partly related to endothelial cell damage, it has also been attributed to a hypercoagulation state. The importance of raised circulating von Willebrand factor in such a scenario is unknown. However, if the circulating von Willebrand factor in inflammatory bowel disease consists, as is suggested by our results, partly of the storage form of the molecule, which contains large protein multimers that are potent mediators of platelet adhesion/aggregate reactions, it may play a role in the pathogenesis of thrombosis as well as being a marker of vascular injury in inflammatory bowel disease. More work is needed to evaluate this hypothesis.

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Membrane attack complex (MAC). It is possible that activated complement components are partly responsible for the raised serum von Willebrand factor in inflammatory bowel disease since: (a) in ulcerative colitis, deposition of the complement MAC; reduced staining of endothelial von Willebrand factor, and the raised circulating von Willebrand factor concentrations reported here are each related to disease activity; (b) in Crohn's disease, increased secretion of C4 and C3 from perfused unaffected jejunum was, like serum von Willebrand factor, found in quiescent as well as active disease. 20
