Factor V Leiden and the etiology of inflammatory bowel disease

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Summary
Inflammatory bowel disease (IBD) refers to two chronic diseases that cause inflammation of the intestines: ulcerative colitis and Crohn's disease. Patients suffering from IBD have a threefold increased risk of venous thrombosis compared with matched controls. Importantly, thromboembolic disease is a significant cause of morbidity and mortality in patients with IBD. However, despite several supporting observations it is still elusive whether activation of the blood coagulation cascade is involved in the etiology and pathogenesis of IBD. To confirm or refute the hypothesis that activated blood coagulation aggravates the development of IBD, we subjected wildtype and homozygous FV Leiden mice to a model of DSS-induced colitis. Experimental colitis led to a reduction in body weight, shortening of the colon and increased colon weight. In addition, DSS treatment led to ulcerations, edema formation, crypt loss, fibrosis and the influx of inflammatory cells into the colon. However, the FV Leiden genotype had no significant effect on any of the DSS-induced symptoms of colitis. We therefore conclude that the FV Leiden allele has no effect in murine colitis and we thus question the importance of activated blood coagulation in the etiology or pathogenesis of IBD.

Keywords
Animal models, inflammatory mediators, protein C/S pathway

Introduction
Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis, are life-long and recurrent disorders of the gastrointestinal tract with unknown etiology. These idiopathic diseases are characterized by the development of intestinal inflammation resulting from the transmural infiltration of neutrophils, lymphocytes, monocytes or macrophages, and plasma cells, accompanied by the overproduction of oxygen free radicals, ultimately leading to mucosal disruption and ulceration (1).

Patients suffering from IBD have a three-fold increased risk of venous thrombosis compared with age, sex and geographically matched controls (2) and this complication is a significant cause of morbidity and mortality (3). The quoted frequency of venous thrombosis in IBD patients is reported to be between 1 and 8%, whereas in postmortem studies rates reach 40% (4). The risk may be increased with active disease, although more than a third of patients have inactive disease at the time of thrombosis (3). Hence, it is apparent that IBD predisposes to venous thrombosis. A more controversial issue is whether exacerbated coagulation is involved in the etiology of IBD.

A vascular component to the pathogenesis of IBD was already proposed in 1934 (5). Later, in 1989, a series of changes comprising vascular injury, focal arteritis, fibrin deposition, arterial occlusion and micro-infarction was proposed as pathogenic sequence in Crohn's disease (6). As the early vascular changes appeared to precede mucosal changes, it was suggested that they were more likely to cause than result from the pathologic features of Crohn's disease.

Additional suggestive findings in support of an important role of ongoing blood coagulation in the etiology of IBD are observations that the risk of IBD is lower than expected in patients with inherited bleeding disorders (7). The observed number of cases of IBD in hemophilia A, hemophilia B and/or Von Willebrand's disease was approximately a third of the expected number on the basis of population studies. In addition, several observational studies using heparin treatment suggest a beneficial role of this inhibitor of blood coagulation in the pathogenesis of IBD (8–11). A small clinical randomized trial including 100


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mild to moderate active ulcerative colitis patients could however not confirm this beneficial effect of heparin (12). Whether the lack of efficacy in this trial is due to the small patient panel, the inclusion criteria of mainly mildly affected patients, the short duration of low-molecular-weight heparin (LMWH) treatment or the fact that blood coagulation has no role in the etiology of IBD remains to be established.

In the current study, we sought to test the hypothesis that activated blood coagulation exacerbates IBD by subjecting mice with a genetic predisposition to thrombosis (factor V [FV] Leiden; FVL) to a state of the art model of experimental IBD.

Materials and methods

Animals
FVL mice carrying a R504Q single amino acid mutation have been described previously (13, 14). R504Q mice (on a mixed 129Sv-C57BL/6J background) were backcrossed to C57BL/6J mice for four generations (N4), and N4 R504Q heterozygous mice were intercrossed to produce homozygous and wildtype offspring. All mice were bred and maintained at the animal facility at the Academic Medical Center according to institutional guidelines, with free access to food and water. Animal procedures were carried out in compliance with the Institutional Standards for Humane Care and Use of Laboratory Animals. All mice were housed in the same temperature-controlled room with alternating 12-hour light/dark cycles. Mice at an age of 8–10 weeks were used in the colitis model as described below.

Induction of IBD
In dextran sulfate-induced colitis, mice were fed 1.5% (w/v) DSS (mol wt 40 kDa; TdB Consultancy, Uppsala, Sweden) in their drinking water for seven days. As solvent controls, mice received drinking water without DSS. The mice were killed on day 7.

Assessment of inflammation
Body weights were recorded daily. Experimental colitis is characterized by shortening of the colon due to its thickening due to inflammation, edema, and muscular hypertrophy. Therefore, the total length of the colon (cm) was measured as a parameter for disease severity. Due to the infiltration of inflammatory cells into the intestinal wall, experimental colitis is characterized by an increase in colon weight. The wet weight of the colon (g/cm) was recorded and used as an index of disease-related intestinal wall thickening. Subsequently, the colons were longitudinally divided in two parts, one of which was used for histological examination.

Histological analysis
Histological analysis was performed essentially as described before (15, 16). The longitudinally divided colons were rolled up, fixed in 4% formalin, and embedded in paraffin for routine histology. Two investigators who were blinded for treatment allocation of the mice scored the following parameters: 1) percentage of area involved, 2) number of follicle aggregates, 3) edema, 4) fibrosis, 5) erosion-ulceration, 6) crypt loss, and infiltration of 7) mono- and 8) polymorphonuclear cells. The percentage of area involved and the crypt loss were scored on a scale ranging of 0–3 as follows: 0, normal; 1, <10%; 2, 10–50%; 3, >50%. Erosions were defined as 0 if the epithelium was intact, 1 for the involvement of the lamina propria, 2 ulcerations involving the submucosa, and 3 when ulcerations were transmural. The severity of the other parameters was scored on a scale of 0–3 as follows: 0, absent; 1, weak; 2, moderate; 3, severe. The score ranges from 0 to a maximum of 24 points.

Statistical analysis
Values are given as mean and standard error of the mean (S.E.M.) per treatment group. Differences between groups were analyzed using the non-parametric Mann-Whitney U test. P<0.05 was considered significant.

Results

Body weight
As evident from Figure 1, DSS treatment significantly reduced body weight indicative of the development of colitis. After seven days, body weight was reduced by 8.4 ± 2.7 g in wildtype mice receiving DSS as compared to no significant loss of body weight (0.9 ± 1.0 g) in mice receiving solvent control. FVL mice receiving DSS treatment showed a similar reduction in body weight (9.7 ± 0.9 g) as the wildtype mice.

Colon length
As shown in Figure 2, colitis induction indeed reduced colon length form 8.1 ± 0.2 cm in solvent control treated mice to 6.4 ± 0.1 cm in wildtype mice treated with DSS. No significant difference of colitis-induced reduction in colon length was observed between wildtype and FVL mice (in which colon length was reduced to 6.5 ± 0.1 g).
Colon weight
As shown in Figure 3, DSS treatment significantly increased colon weight (31.8 ± 0.4 mg/cm and 40.3 ± 1.4 mg/cm for untreated vs. DSS-treated wildtype mice, respectively). Again, no significant difference between wildtype and FVL mice was observed (colon weight of 44.1 ± 1.1 mg/cm for DSS-treated FVL mice).

Histological score
The administration of DSS to the drinking water of wildtype mice clearly led to histological signs of experimental colitis. As shown in Figure 4, approximately 50% of the colon was affected by DSS treatment as evident from edema formation, crypt loss, fibrosis and the influx of inflammatory cells into the colon. Control mice showed no severe evidence of experimental colitis, and only showed some follicle aggregates and minor signs of edema formation. The total histological score or the individual histological scores were not significantly different between FVL and wildtype mice.

Discussion
The reduced number of cases of IBD in hemophilia and Von Willebrand's disease in combination with observational studies showing that heparin treatment was beneficial in IBD suggested that a thrombophilic state would be detrimental in the etiology or pathogenesis of IBD. Our data, however, do not support this hypothesis and question the role of activated blood coagulation in IBD.

In the current study, we determined whether the murine FVL allele influences experimental colitis by challenging wildtype and FVL mice with DSS. FVL, an arginine to glutamine missense mutation in the factor V (FV) gene at position 506 (17), is the most prominent risk factor for venous thromboembolism (18, 19). The amino acid substitution in the activated protein C (APC) cleavage site of FV leads to increased thrombin generation due to decreased APC-mediated inactivation of FV and due to decreased FV cofactor activity for FVIIa inactivation (20). In mice, the corresponding mutation in the FV gene mimics the human phenotype as evident from a marked increase in spontaneous tissue fibrin deposition (13). However, despite the severe thrombotic phenotype of the FVL mice, we did not observe any effect of the FVL genotype on body weight, colon length and/or weight and histological signs of colitis.

Genetic studies determining the prevalence of the FVL allele in IBD have mostly shown no difference in allele frequency between IBD patients and healthy controls (21). However, two studies did show that the FVL allele is more frequent in IBD patients than in healthy controls (22, 23). Although somewhat inconclusive, these genetic studies suggest that the FVL allele is of
minor importance in the etiology or pathogenesis of IBD. However, the lack of association between the FVL allele and IBD in these studies might be caused by the small number of patients in these studies and the confounding influence of the varying geographic distribution for the FVL mutation (21). Furthermore, it is important to realize that homozygous carriers of the FVL allele are rare and that all genetic studies are performed using heterozygotes which have a milder thrombotic phenotype.

Several issues should be kept in mind when interpreting our data. First, in the present study we experimentally induced colitis by the daily administration of DSS. Although this is a well-established model of IBD mimicking human disease (24), there are some obvious differences between the development of human disease and murine DSS-induced colitis. For instance, in human disease, IBD develops over a long period of time, whereas in the murine model colitis is induced within seven days. As a consequence, we cannot generally conclude that FVL does not influence the etiology or pathogenesis of IBD. For this reason, the effect of FVL might be investigated in other models of experimental colitis, such as genetic models in which colitis develops spontaneously. Second, we used an inbred mouse strain in which we induced colitis, while IBD patients form a very heterogeneous group both with regard to ethnic background, gender and pre-existing medical conditions.

In summary, we sought to confirm the aggravating role of activated blood coagulation in IBD by subjecting wildtype and homozygous FVL mice to a DSS colitis model. Our data, however, do not confirm any role of the FVL allele and question the general effect of blood coagulation in the etiology and pathogenesis of IBD.

References