Kininogen deficiency modulates chronic intestinal inflammation in genetically susceptible rats

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Received 23 October 2001; accepted in final form 4 February 2002

Isordia-Salas, Irma, Robin A. Pixley, Fengling Li, Irma Sainz, R. Balfour Sartor, Albert Adam, and Robert W. Colman. Kininogen deficiency modulates chronic intestinal inflammation in genetically susceptible rats. Am J Physiol Gastrointest Liver Physiol 283: G180–G186, 2002.—Genetically susceptible Lewis rats injected in the intestinal wall with peptidoglycan-polysaccharide (PG-APS) polymers develop chronic granulomatous enterocolitis concomitant with activation of the kallikrein-kinin system. To elucidate the role of high-molecular-weight kininogen (HK) in chronic enterocolitis, we back crossed Brown-Norway rats having a HK deficiency with Lewis rats for five generations. Two new strains were produced, wild-type F5 (F5WT) and HK deficient (F5HKd), each with a ~97% Lewis genome. The HK values of F5WT and F5HKd rat plasma were 0.62 ± 0.20 and 0.08 ± 0.03 U/ml, respectively. In PG-APS-injected rats, chronic inflammation was measured by using gross gut score, histological inflammation, liver granuloma, and white blood cell count. The mean gross gut scores were significantly lower in the F5HKd than in the F5WT rats. Plasma T-kininogen was significantly less in F5HKd. These results indicate the importance of the kallikrein-kinin system in this model of chronic enterocolitis and systemic inflammation.

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INFLAMMATORY BOWEL DISEASES (IBDs), including Crohn’s disease and ulcerative colitis, are disorders characterized by local and systemic chronic inflammation with unpredictable relapses and remissions (10). These disorders are immunologically mediated and have a genetic component (10, 20). In animal models, the aggressiveness and chronicity of the inflammatory process is dependent on the genetic background of the host (9).

Previous studies from our laboratory have used a rat model of acute and chronic granulomatous enterocolitis that permits detailed examination of the mechanisms of the genetically determined intestinal and systemic inflammation (22). Bacterial cell wall polymers, peptidoglycan-polysaccharide from group A streptococci (PG-APS), were injected intramurally to induce intestinal inflammation (17, 24). Acute inflammation at the injected area was observed in all rat strains included, but the spontaneous reactivation of chronic granulomatous inflammation was restricted to genetically susceptible Lewis and Sprague-Dawley rats and did not occur in Buffalo and Fisher rats (17, 22, 34). Female Lewis rats, the highest responders, develop acute intestinal inflammation that peaks 1–2 days after PG-APS injection and gradually decreases over the next 10 days. The enterocolitis spontaneously reactivates beginning on day 14, accompanied by peripheral erosive arthritis, granulomatous hepatitis, normochromic anemia, and leukocytosis (22). The histological findings of transmural inflammation, fibrosis, and granulomas show that the pattern of macrophage and Th1-derived cytokines resemble Crohn’s disease (14, 30). The inflammatory response initiated by PG-APS is similar to that present in human IBDs in that it is mediated by leukocyte activation with liberation of cytokines, eicosanoids, oxygen radicals, nitric oxide, and activation of complement, coagulation, and fibrinolytic cascades (21, 24, 28).

The plasma kallikrein-kinin system (KKS), or contact system, is comprised of factor XII (FXII), prekallikrein (PK), and factor XI (FXI), which are the zymogens of proteases, and high-molecular-weight kininogen (HK), which serves as the cofactor of contact activation and is the genesis of bradykinin release (7, 26). Bradykinin is a potent inflammatory mediator that exerts its biological effects by activating constitutive B2 and inducible B1 receptors on endothelial cells, smooth muscle cells, epithelial cells, and fibroblasts (2). This potent inflammatory peptide enhances vasodilation, increases vascular permeability, and influences intestinal motility and electrolyte secretion (2, 11), all of which are features of Crohn’s disease.
Negatively charged biological products, such as en-
odotoxin (18), or activated blood or endothelial cells are able to activate zymogen FXII, the initial component of
the contact system, to FXIa. FXIa converts PK to
kallikrein and causes activation of FXI to FXIa. In
addition, FXIa is an agonist for neutrophils (33) and
monocytes (31) and is capable of initiating the classic
complement cascade (12). Kallikrein in the presence of
HK produces neutrophil chemotaxis (18), aggregation
(16), and oxygen consumption (23) and induces elas-
tase release (35). Kallikrein stimulates the fibrinolytic
system by converting prourokinase to urokinase (15)
and activates the alternative complement pathway (8).

To evaluate the role of HK in intestinal and systemic
inflammation, we created a kininogen-deficient Lewis rat
by back crossing the Brown-Norway (Katholie strain)
HK-deficient rat (13) to normal Lewis rats (Table 1).

Preliminary studies indicated that Brown-Norway rats
did not develop chronic granulomatous inflammation
after PG-APS injection. After five generations, we produced
two new rat strains, one with <10% HK and a 97% Lewis
genetic background and a wild-type rat with normal
levels of HK and 97% Lewis background. We compared
the ability of intramural PG-APS to induce chronic gastro-
intestinal, joint, and systemic inflammation in rats
with normal vs. deficient plasma levels of HK.

METHODS

Production of HK-deficient Lewis rat. The Brown-Norway
Katholie rat strain has an Ala-to-Ser (amino acid 145, sequence without signal peptide) substitution in rat HK,
which leads to a secretion defect of the molecule in hepato-
cytes, resulting in plasma levels of HK ranging from 5–10%
of normal (13). Since the Brown-Norway-Katholie strain
was resistant to PG-APS-induced enterocolitis (data not
shown), we produced an HK-deficient rat strain susceptible
to chronic inflammation by back crossing Lewis rats for 5
generations with an offspring of a Brown-Norway-Katho-
liek × Lewis cross (Table 1). At the end of 5 back crosses to
Lewis rats, the Brown-Norway gene pool had been diluted
1/25 (or 3%). Therefore, the Lewis gene pool represented 97%
of the total gene pool in these rats. Two new strains were
produced: F5HKd (deficient in HK) and F5WT (wild-type, not
deficient in HK), each with 97% Lewis genetic material.

PG-APS polymers. Purified, sterile peptidoglycan-polysac-
charide fragments from the cell walls of group A, type 3,
strain D58 streptococci (Streptococcus pyogenes) were pre-
pared as described previously (22).

Experimental protocol and treatment. Two groups of fe-
male specific-pathogen crossed rats of ~155 g were used
(F5HKd, n = 9; F5WT, n = 5). The intestines of each animal
were exposed by laparotomy with sterile technique, and PG-
APS was injected (15 mg/g body wt), as previously described
(22), intramurally into five sites of the terminal ileum and
cecum. Animals were euthanized 21 days after surgery.

Quantification of intestinal inflammation. Intestinal in-
flammation was quantified by gross, biochemical, and immu-
nological and histological methods validated for this model
(17, 22). At necropsy, performed by a blinded observer, the
gross gut score was calculated by using the sum of 0–4 scores
of four independent parameters, including intestinal wall
thickening, adhesion, mesenteric contraction, and serosal
nodules (granulomas). The maximum score was 16. Compo-
nents of a blinded histological inflammatory score were based
on the number of infiltrating mononuclear cells (macro-
phages and lymphocytes; i.e., chronic inflammation), neutro-
phils, and edema (acute inflammation). The presence of crypt
abscesses and granulomas was noted. The maximum score
was 24 (acute 0–4 and chronic 0–4 in 2 sections of the
cecum).

Systemic inflammation. Rats were weighed before surgery
and at necropsy. At necropsy, liver and spleen weights were
recorded. Hepatic granulomas were quantified with a score of
0–4. Joint diameters were measured with calipers (22). Car-
diac blood was collected for hematological measurements
(white blood cell count, hematocrit, and hemoglobin) and
assays of inflammatory proteins at euthanasia.

Assays of contact activation in vitro. PK function levels
were performed by a microtiter, amidolytic assay by using a
chromogenic substrate, S-2302 (Pro-Phe-Arg-p-nitroanilide;
Chromogenix, Moindal, Sweden) as described in our labora-
tory (6). HK coagulant activity was evaluated by our modifi-
cation of an activated partial thromboplastin time test assay
(4, 27) using total kininogen-deficient plasma purchased from
George King (Overland Park, KS) (19). In addition, FXI and
FXII coagulant activity was performed by a similar method
using deficient plasma obtained from George King.

T-kininogen determination. Plasma T-kininogen was mea-
sured by sandwich enzyme-linked immunoabsorbent assay,
as described previously (1).

Statistical analysis. Statistical analysis was performed
using the unpaired Student’s t-test. P values < 0.05 were
considered significant.

RESULTS

Comparisons of contact system plasma proteins in rat
strains. One milliliter of Lewis rat plasma is defined as
containing 1.00 U/ml of rat HK. The parent Lewis
strain had a mean plasma HK concentration of 1.00 ±
0.14 U/ml, whereas the Brown-Norway strain had a
HK concentration of 0.09 ± 0.01 U/ml. The new strains
produced had 0.63 ± 0.20 U/ml (F5WT) and 0.08 ± 0.03
U/ml (F5HKd) HK (Fig. 1). Thus we produced two new
strains of rats composed of 97% Lewis genetic back-
ground with and without a severe deficiency of HK.

Other plasma contact factors were examined in these
strains (Fig. 2). The FXI levels in the noninflamed
parental Lewis strain and Brown-Norway strains
showed no significant differences (1.00 ± 0.14 and
1.18 ± 0.01 U/ml, respectively). The FXI level was
decreased in F5WT (0.70 ± 0.05 U/ml) but did not

Table 1. Animal back crossing

<table>
<thead>
<tr>
<th>Breeders</th>
<th>% Lewis Genome</th>
<th>Generation</th>
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<tbody>
<tr>
<td>HH × hh</td>
<td>50%</td>
<td>F1</td>
</tr>
<tr>
<td>F1HH × HH</td>
<td>75%</td>
<td>F2</td>
</tr>
<tr>
<td>F2HH × HH</td>
<td>87%</td>
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<td>94%</td>
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</tr>
<tr>
<td>F4HH × HH</td>
<td>97%</td>
<td>F5</td>
</tr>
<tr>
<td>F5HH × F5Hh</td>
<td>97%</td>
<td></td>
</tr>
</tbody>
</table>

Select by HK phenotype

<table>
<thead>
<tr>
<th>HH</th>
<th>97%</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5HKd (HK &lt; 10%)</td>
<td></td>
</tr>
<tr>
<td>F5WT (HK &gt; 90%)</td>
<td></td>
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</tbody>
</table>

HH = high-molecular-weight kininogen (HK)-dominant = Lewis rat; hh = HK-recessive = Brown-Norway-Katholiek rat.
differ significantly from the Lewis parental strain. The F5HKd contained a FXI value significantly lower than that observed in the F5WT. PK levels in the parent Lewis and Brown-Norway rats were not significantly different (1.00 ± 0.05 and 0.73 ± 0.05 U/ml, respectively). F5WT PK levels (0.98 ± 0.04 U/ml) were similar to those in the Lewis rat. The F5HKd (0.52 ± 0.04 U/ml) had a significantly lower PK concentration than the F5WT strain. The F5WT and F5HKd strains contained the same 97% genetic background; therefore, lower FXI and PK levels in the F5HKd rats are presumably a consequence of the lower functional HK levels in the plasma with which FXI and PK associate. This decreased level of PK is also observed in HK-deficient human plasma (4). FXII was not significantly different in all four strains. The similarity of the measured FXII levels in all of the groups indicates that the sample preparation methods did not affect the other protein values measured in our samples.

Effect of HK deficiency on experimental inflammation. In previous studies from our laboratory (22), we used a Lewis rat model of chronic granulomatous enterocolitis induced by intramural PG-APS injection into the cecum and ileum. Intramural injection of PG-APS in the two new rat strains, F5WT and F5HKd, induced chronic inflammation in both strains compared with the human serum albumin-injected controls (Fig. 3). However, there were significant differences in the values of many of the measured pathological parameters between the two new rat strains with normal vs. deficient plasma HK levels. These differences reflect decreased inflammation in the intestine and liver after PG-APS injection in the rats with low baseline HK levels. The gross gut score was significantly decreased (P = 0.024) in the F5HKd strain (7.8 ± 1.5) compared with the F5WT strain (13.0 ± 0.1). The total intestinal histological score compared with the F5WT rats (19.2 ± 1.5) was significantly decreased in the F5HKd rats (13.5 ± 1.8). Likewise, the liver granuloma score was significantly decreased in F5HKd rats (1.7 ± 0.3) compared with the F5WT (3.6 ± 0.3). The white blood cell count was significantly elevated (P = 0.028) in the F5WT strain (37.1 ± 5.3 × 10^3 cell/μl) compared with the F5HKd (24.1 ± 3.0 × 10^3 cell/μl). No significant differences were found between F5WT and F5HKd rats injected with PG-APS in liver weight, spleen weight, hemocrit, hemoglobin, or joint diameter (not shown).

Plasma T-kininogen, an acute-phase protein unique to rats, was measured antigenically (1) as another indicator of the inflammatory response in these rats (Fig. 4). The levels were significantly increased in both PG-APS-treated F5 groups compared with their untreated values (P < 0.001). When PG-APS-injected rats were compared, the F5HKd group had a significantly lower T-kininogen level compared with the F5WT rats (P = 0.023).

Examination of microscopic sections of the cecum showed that the degree of cellular infiltration and inflammatory changes were increased in F5WT rats injected with PG-APS (Fig. 5B). The HK-deficient group (F5HKd) showed a minimal to moderate thickening of the mucosa, with moderate mononuclear cell infiltration in the lamina propria and submucosa and...
There was a relative absence of neutrophils, crypt abscesses, and necrosis. In contrast, the F5WT group showed marked thickening of the submucosa, with crypt abscesses in the mucosa, necrotic granulomas in the submucosa, and severe chronic inflammation and fibrosis (Fig. 5B).

KKS. As observed previously in the Lewis rat (22), injection of PG-APS caused a marked decrease in the F5WT rat plasma HK concentrations from initial normalized levels (1.0 ± 0.2 to 0.2 ± 0.1 U/ml). This observation indicates an extreme in vivo activation of the contact system as a consequence of intestinal PG-APS injection (P = 0.04; Fig. 6). No change occurred from the low HK values in F5HKd rats with PG-APS injection. Significant differences (P < 0.05) occurred in either plasma FXI or PK between the treated F5WT and F5HKd rats, but, when corrected by normalization for the pretreatment values, there were no differences in the percent change. No significant differences were found in the FXII levels in any of these groups. We have determined that a 5% presence of HK in vitro is able to support sustained PK activation compared with normal HK values (25). This observation may explain the lack of significant differences in the values of FXI and PK between these two rat strains on PG-APS treatment.

![Fig. 3. Comparison of the gross gut score, total histological score, liver granuloma score, and white blood cell count (WBC) were blindly determined in the F5 rats 21 days after peptidoglycan-polysaccharide (PG-APS) injection. The short bar in the first column of each graph represents mean normal value of non-PG-APS-treated parental Lewis rats, which were albumin injected as reported in a previous study (35). Values are means ± SE. Gross gut score, P = 0.024 vs. F5WT. Intestinal histological score, P < 0.05. Liver granuloma score, P = 0.02 vs. F5WT. *P < 0.05; **P < 0.02.](image)

![Fig. 4. Plasma T-kininogen antigenic levels of the F5 strains. Pretreatment and PG-APS-injected comparisons are shown. Values are means ± SD. A significant difference (*P = 0.023) was observed between levels in the F5WT (solid bars) and F5HKd (open bars) PG-APS-injected rats.](image)

![Fig. 5. A: chronic colitis in the HK-deficient (F5HKd) rat 21 days after PG-APS. Note the moderate degree of mononuclear cell infiltration in the lamina propria and submucosa (SM), with fibrosis, but relative absence of neutrophils, crypt abscesses (CA), and necrosis. M, mucosa. B: chronic colitis in the F5WT rat 21 days after treatment. Note crypt abscess, marked thickening of the submucosa, and necrotic granulomas (NG).](image)
DISCUSSION

Inflammation is accompanied by activation of the KKS and resulting alterations of its component plasma proteins. The KKS is one of the participants in the pathogenesis of inflammatory reactions involved in cellular injury, including coagulation, fibrinolysis, kinin formation, complement activation, cytokine secretion, and release of proteases. Protein components of the KKS bind to and interact with leukocytes, platelets, and endothelial cells. The formation of plasma kallikrein has at least two effects: neutrophil stimulation and bradykinin release. KKS activation has been demonstrated in various experimental and human disease states caused by bacterial infection and/or immunological injury (4), including ulcerative colitis and Crohn’s disease. Direct involvement of the KKS in the pathogenesis of the rat acute and chronic enterocolitis (27, 28), acute arthritis (3), and experimental human sepsis (5) has been documented by previous studies from our laboratory.

We have described kallikrein-kallikrein activation (22) in a well-described Lewis rat inflammation model by using a bacterial cell wall polymer (PG-APS) injected intramurally to induce intestinal inflammation. We correlated changes in the KKS system with pathophysiological measurements of the inflammation (22). Both the acute and chronic changes in genetically susceptible Lewis rats are accompanied by evidence of activation of the KKS, yet this system is not activated during acute inflammation in Buffalo rats, which is equal in intensity to that of Lewis rats (22). Of considerable interest, Buffalo rats fail to develop the chronic, T cell-mediated phase of enterocolitis, nor do they develop systemic inflammation after PG-APS injection (17, 22). In vitro studies show more rapid degradation of HK and liberation of bradykinin in Lewis rat plasma vs. Buffalo plasma (22). These results agree with our present observations that our F5HKd rats deficient in HK have less active chronic inflammation than our F5WT rats with normal kininogen levels.

A specific boronic acid inhibitor of plasma kallikrein (P-8720) modulates the chronic local and systemic inflammation in Lewis rats injected with PG-APS, as demonstrated previously (28). The P-8720-treated group showed a highly significant decrease in all components of the gross and histological inflammatory scores and liver and spleen enlargement, leukocytosis, and arthritis associated with chronic intestinal inflammation. There was a significant decrease in plasma FXI and HK in the untreated group compared with the P-8720-treated group. This study strongly implicated kallikrein activation in the pathogenesis of chronic PG-APS-induced inflammation. Results using this pharmacological inhibitor are not definitive due to the possible confounding influence of hepatic toxicity and because inhibition of kallikrein activity will also prevent HK activation, since plasma kallikrein cleaves HK to release the biologically active peptide bradykinin. Therefore, it is impossible to determine whether the kallikrein inhibitor modulates inflammation by acting directly on kallikrein to prevent bradykinin formation.

Parental Lewis rat FXI was decreased in the F5HKd strain before injection of PG-APS. PK levels (an important participant in the KKS system) appeared normal in the F5WT strain and reduced in the F5HKd strain. The reduced level of PK in this deficient strain is similar to what is found for PK levels in human patients with HK deficiency (4). We have shown in these human individuals that this difference can be overcome by adding HK in vitro, indicating that this result is due to the participation of HK in the assay to detect PK activity (25).

Unfortunately, functional plasma PK activity is also low in the presence of HK deficiency, therefore limiting assessment of the comparative roles of HK or plasma kallikrein in intestinal and systemic inflammation. However, the consistent ability of a selective kallikrein inhibitor and the endogenous HK in the present deficiency study to modulate intestinal and systemic inflammation firmly implicates the KKS in general and the participation of PK and HK in the pathogenesis of chronic immune-mediated enterocolitis. F5HKd rats...
developed significantly less chronic intestinal inflammation, hepatic granulomas, and leukocytosis than F5WT rats following intestinal PG-APS injection, which is consistent with our previous observations with pharmacological blockade of kallikrein (28). However, HK deficiency did not protect against PG-APS-induced arthritis in Lewis rats, in contrast to prevention of acute and chronic phases of arthritis with P-8720 (28). The reasons for this discrepancy are unclear. One possibility is that kallikrein stimulates neutrophils distinct from HK cleavage and bradykinin liberation. The difference may reflect a nonspecific effect of the pharmacological inhibition or a requirement for more aggressive blockade of the KKS in arthritis than enterocolitis. However, a specific bradykinin receptor 2 inhibitor attenuated PG-APS-induced arthritis but not enterocolitis (32) and treatment with recombinant interleukin (IL)-1 receptor antagonist and IL-10 more easily suppressed arthritis than intestinal inflammation in this model (14, 17).

In summary, our results indicate that relative plasma HK deficiency in genetically susceptible Lewis rats results in decreased chronic enterocolitis, hepatic granulomas, and leukocytosis but no change in arthritis. Together with our previously reported inhibition of intestinal and systemic inflammation following selective pharmacological blockade of plasma kallikrein, we demonstrate an important role for the KKS in the pathogenesis of chronic granulomatous inflammation. These observations lay the foundation for selective inhibition of kallikrein and bradykinin in human IBD, particularly Crohn’s disease, which shares a number of pathological and immunological features with experimental PG-APS-induced granulomatous inflammation.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants RO1-DK-43735 and P30-DK-34987 as well as a research grant from the Crohn’s and Colitis Foundation of America.

REFERENCES


AJP-Gastrointest Liver Physiol • VOL 283 • JULY 2002 • www.aajpgi.org