Genetic kininogen deficiency contributes to aortic aneurysm formation but not to atherosclerosis

Elena Kaschina, Monika Stoll, Manuela Sommerfeld, U. Muscha Steckelings, Reinhold Kreutz, and Thomas Unger. Genetic kininogen deficiency contributes to aortic aneurysm formation but not to atherosclerosis. Physiol Genomics 19: 41–49, 2004. First published July 6, 2004; doi:10.1152/physiolgenomics.00035.2004.—Brown Norway (BN) and BN Katholiek (BN/Ka) rat strains are both susceptible to develop lesions in the internal elastic lamina (IEL) of the aorta. BN/Ka rats are characterized by a single point mutation in the kininogen gene leading to deficiency in high- and low-molecular-weight kininogen. Recently, a suggestive quantitative trait locus for lesions in the IEL of the abdominal aorta was identified in an F2 intercross between Dahl salt-sensitive (SS) and BN rats, implicating kininogen as a positional candidate gene. Therefore, BN and BN/Ka rat strains represent ideal model organisms with which to study the contribution of kininogen to the genetic predisposition to IEL lesion formation and to characterize the early events underlying vascular remodeling. Here we present data demonstrating that genetic kininogen deficiency promotes the development of atherosclerosis upon 12-wk treatment with an atherogenic diet. Aneurysm formation was associated with an enhanced elastolysis, increased expression of MMP-2 and MMP-3, downregulation of TIMP-4, and with FasL- and caspase-3-mediated apoptosis. Kininogen-deficient animals also featured changes in plasma cytokines compatible with apoptotic vascular damage, i.e., upregulation of IFN-γ and downregulation of GM-CSF and IL-1β. Finally, in response to atherogenic diet, kininogen-deficient animals developed an increase in HDL/total cholesterol index, pronounced fatty liver and heart degeneration, and lipid depositions in aortic media without atherosclerotic plaque formation. These findings suggest that genetic kininogen deficiency renders vascular tissue prone to aneurysmatic but not to atherosclerotic lesions.

elastic lamina; Brown Norway rat; apoptosis; cytokine

KININOGENS ARE MULTIFUNCTIONAL proteins involved in the generation of kinins, modulation of platelet aggregation, angiogenesis, and acute-phase response (3). They belong to the extracellular inhibitors of cysteine proteases (cathepsins) (21, 34). Several reports have demonstrated the presence of a kallikrein-kinin system in the vascular wall (40). However, little is known about the role of kininogen deficiency in vascular disease or remodeling. The present study aimed to clarify whether genetic kininogen deficiency may contribute to the development of aortic lesions, aneurysms, or atherosclerosis, by comparing kininogen-deficient Brown Norway Katholiek (BN/Ka) rats with nondeficient Brown Norway (BN) rats after feeding them a high-fat (atherogenic) diet.

A trait of vascular fragility, characterized by the formation of abrupt defects within the elastic lamina of the abdominal aorta, has been identified in BN rats (5, 14, 24). Consequently, these rats are susceptible to the development of abdominal aortic lesions. Recently, two major quantitative trait loci (QTLs) for internal elastic lamina (IEL) lesions were identified on rat chromosomes 5 and 10 (12). An additional susceptibility locus for IEL lesions located on rat chromosome 11 was identified in an independent rat cross between Dahl salt-sensitive (SS) and BN (31), harboring the gene for kininogen. The BN/Ka rat strain features a deficiency in plasma kininogen. Hence, a point mutation (G to A transition) at nucleotide 487, resulting in an amino acid exchange from alanine to threonine, is responsible for the defective secretion of high-molecular-weight kininogen (HK) and low-molecular-weight kininogen (LK) by the liver of BN/Ka (23). Thus plasma HK is 38-fold higher and LK is 16.5-fold higher in normal BN compared with mutant BN/Ka animals (19). Therefore, the BN/Ka rat provides an ideal model organism with which to study the role of kininogen in the genetic predisposition to IEL lesion formation and its contribution to the development of aortic lesions and/or aneurysm formation. Since defects in the IEL have been implicated in early atherogenic events by allowing migration of medial muscular cells into the intima (7), we investigated the link between the susceptibility to IEL lesions and the pathogenesis of atherosclerosis, using BN and BN/Ka fed a high-cholesterol diet. We also studied the role of kininogen in the metabolic response to atherogenic diet and the pathophysiological mechanisms underlying aortic damage, including plasma lipid and cytokine profiling and apoptosis.

METHODS

Animals and Study Design

Male Wistar rats (Wistar), BN (Winkelmann), and BN/Ka (Charité, Berlin, Germany) were kept under conventional conditions in our animal facility. Rats of each strain were divided into two groups \( n = 20 \) each): standard chow diet (CD) and atherogenic diet (AD). At 10 wk of age, the animals of the AD group were fed a high-fat/cholesterol diet containing 15% dairy fat, 2% cholesterol and 0.5% cholic acid (Altromin, Munich, Germany) for a period of 12 wk. Rats were killed at 22 wk of age, and blood was taken immediately. Plasma lipoproteins, cytokines, and protein expression were analyzed together with histological examinations. Heart, liver, and kidney weight and the
organ/body mass ratio were calculated. All experiments were performed in accordance with the German law on animal protection as revised in 1993.

Genotyping

Genetic characterization of Wistar, BN, and BN/Ka and verification of the homogeneity of each strain was performed using 80 polymorphic SSLP markers at an average spacing of 25 cM across the genome. DNA was extracted according standard procedures from tail-tip biopsies. All genetic markers were based on amplification by PCR of polymorphic microsatellites. Oligonucleotide primer pairs for genetic markers were obtained from public databases (http://www.rgd.mcw.edu). PCR was performed on 50 ng of genomic DNA in a final volume of 10 µl, which contained 100 mMol of each primer, 200 µmol/l dNTPs, 1.5 mMol/l MgCl2, 50 mMol/l KCl, 10 mMol/l Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, and 0.25 U of Taq DNA polymerase (Promega). The forward primer was labeled with [γ-32P]ATP by T4 polynucleotide kinase. PCR products were processed and subsequently analyzed by autoradiography after polyacrylamide gel electrophoresis.

Sequencing of HK cDNAs from BN, BN/Ka, and Wistar Rats

Total RNA was isolated from livers using the TRIzol method. First-strand cDNA synthesis was done from 2 µg total RNA in a total volume of 20 µl using the first-strand cDNA synthesis kit from MBI Fermentas according to manufacturer’s recommendations. The primers HK-F ACGAGTACCACTGTCTGGG and HK-R TGTTTGCA were used to amplify a 200-bp fragment spanning the bases 430 to 629 of the rat HK-kinogen cDNA (GenBank accession no. L29428.1) in a 50-µl reaction containing 200 nM of each primer, 250 µM dNTPs, 1.5 mM MgCl2, 2 U BioTherm DNA polymerase (GeneCraft, Münster, Germany), and reaction buffer supplied by the manufacturer. PCR reactions were carried out on a PTC-200 Peltier Thermal Controller (MJ Research) with the following touchdown protocol: initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 30 s, a starting annealing temperature of 65°C (reduced by 0.5°C/cycle) for 30 s, and extension at 72°C for 40 s, 10 additional cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and amplification at 72°C for 40 s, and a final extension step of 72°C for 3 min. The PCR fragments were purified using the QiaQuick PCR purification system (Qiagen) and then sequenced in both directions with the primers HK-F and HK-R on an ABI Prism 377 DNA sequencer according to manufacturer’s recommendations. By using this method, we could detect the G to A point mutation at position 504, which corresponds to base 487 of the coding sequence of rat HK and results in an Ala163 to Thr amino acid exchange in the polypeptide.

Plasma Cytokine Profiling and Lipid Measurements

Serum samples were collected after overnight fasting. Plasma interleukin (IL), IL-1α, IL-1β, IL-2, IL-4, IL-6, and IL-10, granulocyte/macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) were analyzed using Bio-Plex Protein Array System (Bio-Rad). Monocytic chemoattractant protein 1 (MCP-1) levels were assessed by a commercially available ELISA kit (Biosource International). Plasma total cholesterol, high-density lipoprotein (HDL), and triglyceride levels were measured by colorimetric enzymatic assays (Cypress Diagnostics).

Histological analysis

Aortic tissue was removed from the ascending aorta to the iliac bifurcation, fixed with 10% formaldehyde overnight, and examined under a binocular microscope for aneurysm formation. Quantification of aneurysms was based on the percent incidence. The aortas were opened longitudinally, stained with Sudan III, and examined for lesions under the stereomicroscope.

Paraffin-embedded cross sections of thoracic and abdominal aorta (4 µm) were stained (hematoxylin-eosin, Weigert’s method for elastic fibers, Sirius red-picric acid stain for collagen) and analyzed by quantitative morphometry (AxioVision 3.1). Serial 10-μm-thick cryo-sections from the aortic arch and abdominal aorta were stained with Oil red for neutral lipids.

The aortas were examined for plaques and lesions, and the numbers of elastic lamellae in each of four quadrants were counted using light microscopy. The elastin and total collagen content was determined in histological sections in the masked parts of media 100 × 100 pixel using the Velocity Image system (Openlab 3, Improvision). Liver, heart, and kidney were also examined histologically (hematoxylin-eosin stain).

Immunohistochemistry. Immunohistochemistry was performed using the avidin-biotin complex method according to the manufacturer’s instructions (Vectorstain ABC, Vector Laboratories). Paraffin-embedded tissue sections were stained using Fas ligand (FasL), caspase-3 antibodies (Santa Cruz Biotechnology), and rat monocytes/macrophages ED1 antibody (Acris) followed by biotinylated anti-rabbit IgG (Vector Laboratories). Peroxidase activity was visualized by 3-amino-9-ethylcarbazole (AEC, Vector Laboratories).

Detection of apoptotic cells. For the identification of DNA fragmentation, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining of aortas, liver, and heart was performed using an in situ death detection kit for immunohistochemical detection and quantification of apoptosis at single cell level according to manufacturer’s protocol (Roche). After the reaction with DAB substrate, the stained specimens were analyzed by light microscopy.

Western Blot

Tissue samples were homogenized in lysis buffer (50 mM Tris-HCl, 500 mM EDTA, 150 mM NaCl, 0.1% Triton X-100) supplemented with 100 µg/ml PMSF and protease-inhibitor cocktail (Roche) followed by centrifugation. Protein concentrations were determined by the method of Bradford using BSA as a standard. Proteins (25 µg per line) were separated by 10% SDS-PAGE and transferred to the nitrocellulose membranes (Amersham). Membranes were blocked in 5% nonfat milk/TBBS, then incubated consecutively with primary antibody and horseradish peroxidase-conjugated secondary antibody (Dako). Immunoreactive proteins were detected using ECL reagents (Amersham). The following primary antibodies were used: tissue inhibitor of metalloproteinases-4 (TIMP-4), metalloproteinase-2 (MMP-2), MMP-3 (Chemicon), Fas-L, Bcl-2, and caspase-3 (Santa Cruz Biotechnology). Quantification of Western blots was performed on a computer using the NIH image analysis system (Scion, Frederick, MD). To demonstrate equal protein loading of the gel, membranes were reprobed with GAPDH antibody (Acris).

Statistical Analysis

Values are expressed as means ± SE. Comparisons among the different groups were performed by two-way ANOVA followed by an unpaired Student t-test to detect differences between two groups. P < 0.05 was considered to be statistically significant.

RESULTS

Genetic Characterization of BN, BN/Ka, and Wistar Rats

Each strain was proven to be genetically homogeneous and can be considered “inbred.” At the kininogen locus the presence of the point mutation (G to A) at nucleotide 487 of the coding sequence of rat HK, resulting in the amino acid exchange from alanine to threonine, which was first reported by
Oh-ishi et al. (23) in 1994, was confirmed in all BN/Ka animals used in our experiments.

**Kininogen Deficiency Promotes the Development of Aneurysm Formation in the Abdominal Aorta**

BN and BN/Ka rats, but not Wistar rats, developed a widening of the abdominal aortic wall (Fig. 1A). Under baseline conditions, aneurysms were detected in 32% (6/20) of the control BN and in 50% (10/20) of the BN/Ka rats. The atherogenic diet increased the incidence of aneurysm formation in both rat strains: to 55% (11/20) in BN and to 100% in BN/Ka (20/20). The aneurysms in BN/Ka featured a pronounced dilated form (Fig. 1C, panel 3a), whereas BN had often numerous additional vessels of the elastic type surrounding the aneurysms (Fig. 1C, panel 2a). This observation confirms a recent report about suppressed angiogenesis in kininogen-deficient rats (13) as well as our earlier findings on enhanced capillarization following bradykinin potentiation by ACE inhibitor treatment (9, 37).

An examination of aortic specimens stained with Sudan III did not reveal atherosclerotic lesions, neither in Wistar nor in BN and BN/Ka atherogenic diet groups. However, all BN and BN/Ka aortas contained numerous IEL lesions (Fig. 1B). In BN/Ka, particularly in the atherogenic diet group, the aortic lesions were localized in both abdominal and thoracic parts and were more profound when compared with BN. The atherogenic diet exaggerated the aortic damage but did not promote the development of atherosclerosis. The cross-sectional area of the lumens of the abdominal aorta of BN/Ka revealed a dilatation with pronounced remodeling in the adventitial space (Fig. 1C). All aneurysms were characterized by thinning of the aortic wall, fragmentation and rupture of elastic membranes across the media, and cystic medial degeneration. Interestingly, in the abdominal aortas of the animals exposed to the atherogenic diet, mainly in BN/Ka, pronounced lipid depositions between elastic lamellae were found, indicating fatty degeneration of aorta (Fig. 1D).

BN/Ka CD and AD rats had less elastic lamellae (CD, 6.9 ± 0.4 vs. 5.5 ± 0.3; AD, 6.0 ± 0.2 vs. 4.5 ± 0.3; P < 0.005) and, correspondingly, decreased elastin content compared with the BN group. Total collagen content was increased in BN/Ka, resulting in a decrease of the elastin/collagen ratio (CD, 0.5 ± 0.03 vs. 0.83 ± 0.05; P < 0.005; AD, 0.48 ± 0.03 vs. 0.85 ± 0.04; P < 0.005). These observations point to enhanced elastolysis in the aorta.

**Induction of the MMP Proteolytic Cascade: Upregulation of MMP-2 and MMP-3 and Downregulation of TIMP-4 in the Abdominal Aorta**

MMP-3 has been demonstrated to promote aneurysm formation in a mouse model (30). Comparing the protein content of MMP-3 in abdominal aortas of different rat strains, we found an increased expression in BN/Ka CD under basal conditions and in response to atherogenic diet (Fig. 2A). The MMP-2 protein revealed a band ~72 kDa and was upregulated only in BN/Ka AD (data not shown). The proteolytic activities of MMPs are precisely controlled by TIMPs. TIMP-4, one of the four members of TIMP family, interacts with or blocks the action of MMPs 1, 2, 3, and 9. In the rats with aneurysm formation, BN CD and BN/Ka CD, the protein expression of the unglycosylated TIMP-4 form in the aorta was strongly downregulated (Fig. 2B). In fact, TIMP-4 protein was detected only in Wistar CD, which had no aneurysms. Atherogenic diet stimulated the expression of TIMP-4 in BN, but not in BN/Ka, pointing once more to the differences between kininogen-deficient and nondeficient rats.

**Activation of Apoptosis in the Aorta**

Nuclear fragments exhibiting a positive TUNEL stain were localized in the media and adventitia in the BN/Ka AD, whereas in BN AD less apoptotic cells were found in the media and adventitia (Fig. 3, A–C). Western blot analysis of proteins also revealed an upregulation of the apoptotic markers, FasL and caspase-3, in the abdominal aorta of BN/Ka. Since MMPs, which were upregulated in aortic aneurysms in our experiments, can cleave the membrane-bound FasL to yield the active soluble form (16), it was interesting to study FasL expression. The atherogenic diet induced the expression of the active soluble form of FasL in all rat strains (see intensity of Western blot signal) (Fig. 2C). An upregulation of FasL was found in the abdominal aortas of BN/Ka AD compared with Wistar and BN. These data were confirmed by immunohistochemistry: in the aortic tissue from BN/Ka AD, FasL staining was widespread in the intima and media. In the BN AD, staining was concentrated in the lower layers of the media and in adventitia, whereas in the Wistar AD group no staining in the media and adventitia was found (Fig. 3, D–F).

Caspase-3 is known to be essential for Fas-mediated apoptosis (32). Caspase-3 was significantly upregulated in BN/Ka CD compared with BN and Wistar and extremely upregulated after the atherogenic diet (Fig. 2D). We did not find significant changes of the bcl-2 protein known to inhibit apoptosis (data not shown). This may be due to the fact that bcl-2 is regulated at an earlier time point of the apoptotic process.

**Changes in Plasma Cytokine Profiles**

Since the degradation of the extracellular matrix is induced partly by cytokines, which, in turn, may promote apoptosis and tissue damage, we determined plasma cytokines (Fig. 4). IFN-γ was drastically upregulated in both BN and BN/Ka and was increased even more in BN/Ka upon fatty diet. Anti-apoptotic cytokines, such as GM-CSF and IL-1β, were significantly downregulated in BN/Ka. BN/Ka CD showed a decrease in IL-1β (3-fold) and GM-CSF (4-fold) compared with BN CD. IL-4 and IL-10 were also downregulated in BN/Ka, compared with BN. The expression of MCP-1 is essential for the early cellular response of atherogenesis. There were no differences in the MCP-1 plasma levels between Wistar, BN, and BN/Ka CD groups. After atherogenic diet, Wistar and BN/Ka groups developed twofold MCP-1 increases, whereas BN had a sixfold increase.

**Kininogen Deficiency Influences Lipid Metabolism**

Significant differences in the metabolic responses to dietary saturated fat and cholesterol were observed in different rat strains (Fig. 5). Despite the fact that all lipid indices in BN and BN/Ka CD groups were lower compared with Wistar CD, they showed a significant increase in cholesterol, as well as in HDL, following the atherogenic diet. These results are in agreement
KININOGEN DEFICIENCY CONTRIBUTES TO ANEURYSMS

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**Fig. 2.** Western blot analysis of metalloproteinase-3 (MMP-3, **A**), tissue inhibitor of metalloproteinase-4 (TIMP-4, **B**), Fas ligand (FasL, **C**), and caspase-3 (**D**) content in abdominal aortas of BN, BN/Ka, and Wistar rats on standard chow diet (CD, left) and atherogenic diet (AD, right). Representative images and densitometric data of proteins are means ± SE (n = 6). *P < 0.05 and **P < 0.005, BN/Ka vs. BN. *P < 0.05 and ##P < 0.005, BN/Ka vs. Wistar. §P < 0.05 BN vs. Wistar. The density of digitalized signals was quantified as percentage of the signal density of Wistar, which was set to 100%. **A**: an increased expression of MMP-3 protein was found in BN/Ka CD and AD rats. **B**: TIMP-4 protein was found as a 29-kDa glycosylated and a 23-kDa unglycosylated form. Unglycosylated TIMP-4 form was strongly downregulated in BN CD, BN/Ka CD, and BN/Ka AD. **C**: the 30-kDa soluble FasL protein was slightly upregulated in BN CD, BN/Ka CD, and BN/Ka AD. All AD groups revealed a strong double band of FasL protein. BN/Ka AD exhibited a significant upregulation of FasL compared with BN and Wistar. **D**: caspase-3 protein was strongly upregulated in both BN/Ka CD and AD.
with a recent study demonstrating a twofold increase of liver cholesterol in BN fed a high-cholesterol diet for 4 wk (4).

The response to the atherogenic diet was different in BN/Ka, compared with BN and Wistar. Plasma total cholesterol was lower in both BN and BN/Ka CD groups than in Wistar CD. After 12 wk on the atherogenic diet, plasma cholesterol in Wistar increased 1.6-fold. BN AD and BN/Ka AD showed a significantly enhanced cholesterol increase (3-fold and 5-fold, respectively) compared with the respective CD groups (Fig. 5A). Plasma HDL cholesterol levels were significantly lower in the BN and BN/Ka CD groups compared with Wistar (Fig. 5B). After the atherogenic diet, HDL cholesterol decreased in Wistar, but increased in BN and, particularly (10-fold), in BN/Ka. The HDL cholesterol/total cholesterol index was twofold lower compared with Wistar. After the atherogenic diet, HDL cholesterol/total cholesterol index increased 2.5-fold in BN/Ka, whereas in BN and Wistar it decreased (Fig. 5C).

Plasma triglycerides levels in BN and BN/Ka CD groups were twofold lower compared with Wistar. After the athero-
genic diet, there was no significant increase of triglycerides in Wistar, whereas in BN and in BN/Ka the increase was significant compared with BN and BN/Ka CD (Fig. 5D). The changes in plasma lipid profile in BN and BN/Ka were accompanied by fatty liver and heart degeneration and lipid deposits in the aortic media without pronounced atherosclerosis. Macroscopic analysis of the organs revealed an intra-abdominal and subcutaneous fat accumulation in the Wistar AD group. In contrast, BN and BN/Ka AD developed an accumulation of visceral fat: enlargement and fatty degeneration of the liver, heart, and kidneys. Body weight of Wistar gradually increased with time and, at the end of the study, was 9% higher in the AD group compared with the CD group. The body weight of the BN and BN/Ka increased at the beginning of the feeding period, but from weeks 9–12 on diet it gradually decreased. At the end of the experiment, body weight was 32% and 35% lower in BN and in BN/Ka, respectively, compared with CD rats. The liver/body weight ratio was 4.0-fold higher in BN and 4.2-fold higher in BN/Ka AD, whereas Wistar AD developed only a 1.3-fold liver/body weight ratio increase compared with control. The heart/body weight ratio was significantly increased only in the BN/Ka diet group (3.38 ± 0.09 vs. 2.7 ± 0.07, P < 0.0005) corresponding to pronounced fatty heart degeneration. Histologically, fatty liver and heart degeneration was extremely pronounced in BN/Ka AD.

DISCUSSION

In the present study, we provide evidence that genetically determined kininogen deficiency promotes the development of aneurysms formation. Comparing the incidence of aneurysms, the severity of aortic lesions, and the elastin and collagen content in BN/Ka and BN, we conclude that kininogen deficiency exerts a negative influence on this pathologic process. The atherogenic diet exaggerated the aortic damage but did not promote the development of atherosclerosis.

The dissolution of elastic lamina is known to be determined by serine proteases, neutrophil elastases, MMPs, and cysteine proteases (cathepsins) (10). Macrophage-mediated elastin degradation has been linked to the expression of cathepsins L, S, and K, whose elastinolytic potential exceeds that of all elastases (25). Kininogens are known to be extracellular inhibitors of the protease family. Since kininogens inhibit cysteine proteases, this inhibitor, the protease/antiprotease equilibrium may be modified in favor of enhanced proteolysis.

Induction of the MMP-2 and MMP-3 proteolytic cascade adds to the tissue destruction. Our data are consistent with recent experimental evidence that enhanced MMP activity contributes to a reduction of atherosclerotic plaque size but promotes aneurysm formation (30). MMPs are known to cleave the membrane-bound FasL to yield the active soluble form (16), thus initiating a program leading to apoptotic cell death. The exact mechanism responsible for vascular smooth muscle cell (VSMC) apoptosis in the aortas of kininogen-deficient rats remains to be elucidated, but current evidence indicates that an enhanced production of FasL and caspase-3 participates in this process. Caspases belong to the cysteine protease family. Since kininogens inhibit cysteine proteases, apoptosis in BN/Ka may occur more easily. We also observed pronounced apoptosis as well as upregulation of FasL in the
Fig. 4. The concentration of cytokines in plasma in different rat strains on CD and AD. Values are means ± SE. *P < 0.05, **P < 0.005, and ***P < 0.0005, AD vs. corresponding CD group. *P < 0.05, **P < 0.005, and ***P < 0.0005, BN vs. corresponding BN/Ka. *P < 0.05, **P < 0.005, and ***P < 0.0005, BN vs. corresponding Wistar. *P < 0.05, **P < 0.005, and ***P < 0.0005, BN/Ka vs. corresponding Wistar.

Fig. 5. Lipid profiling. A: plasma total cholesterol. B: HDL cholesterol. C: HDL cholesterol/total cholesterol index. D: plasma triglycerides. Values are means ± SE. *P < 0.05, **P < 0.005, and ***P < 0.0005, AD vs. corresponding CD group. *P < 0.05, **P < 0.005, and ***P < 0.0005, BN vs. corresponding BN/Ka. *P < 0.05, **P < 0.005, and ***P < 0.0005, BN vs. corresponding Wistar. *P < 0.05, **P < 0.005, and ***P < 0.0005, BN/Ka vs. corresponding Wistar.
other organs (liver and heart) of BN/Ka after atherogenic diet, supporting the hypothesis that kininogen deficiency promotes apoptosis. Given that aortic changes were more pronounced in the groups on atherogenic diet, it is tempting to speculate that the atherogenic diet induced oxidative stress and lipotoxicity, which further increased the tissue damage.

Cytokines produced by immune cells exert profound effects on vascular endothelial and smooth muscle cells (11). On the other hand, vascular cells can also produce large amounts of cytokines (6). We have shown that kininogen deficiency is associated with downregulation of plasma interleukins and GM-CSF and upregulation of IFN-γ. These changes may be a result of cytokine-vascular interactions, but at the same time they also may contribute to aneurysm formation. A suppressive cytokine, IFN-γ, known to activate the Fas/FasL system (39) and the MMP cascade (18) and to inhibit VSMC proliferation as well as synthesis of matrix proteins (1) was strongly upregulated in BN/Ka. These data are consistent with a report that IFN-γ predicts an increased rate of expansion in abdominal aortic aneurysms (15). On the other hand, the deficiency in the anti-apoptotic and necrosis-inhibiting cytokine GM-CSF may also contribute to aneurysm formation. GM-CSF is produced by macrophages, endothelial cells, and smooth muscle cells and is thought to act as a necrosis-inhibiting factor against arterial macrophages (36) and as an anti-apoptotic factor in neutrophils (27). The proinflammatory cytokine IL-1β can activate the transcription nuclear factor-kB (NF-kB), which may upregulate MnSOD, the cellular inhibitors of apoptosis 1 and 2, and members of the Bcl-2 family (20). Thus the decrease of GM-CSF and IL-1β and the increase of IFN-γ may simultaneously influence apoptosis and tissue damage. IL-10 was also downregulated in BN/Ka, compared with BN. IL-10 exerts anti-inflammatory effects on the vascular system and decreases superoxide anion production (33). The downregulation of IL-10 might promote oxidative stress, inducing tissue damage in BN/Ka.

Higher concentrations of MCP-1 are associated with hypercholesterolemia (8). Nevertheless, hypercholesterolemia in BN AD did not induce the infiltration of monocytes/macrophages and subsequent lesion formation in the vessel wall despite definite upregulation of plasma MCP-1. Thus, obviously additional factors are necessary for the activation of atherosclerosis.

Wistar rats are known to develop atherosclerosis after 5–6 mo of a high-fat diet, although a lipid infiltration of the liver appears after 6 wk starting the diet (38). BN and BN/Ka rats could not be fed an atherogenic diet for such a long period, since we observed a high mortality in both strains after exposure to the atherogenic diet already after 9–10 wk. BN/Ka differed in their metabolic response to an atherogenic diet from BN by exhibiting higher levels of cholesterol and HDL and a higher HDL/total cholesterol index. The inverse relationship between HDL levels and the occurrence of coronary heart disease has long been known. Recently, it has been suggested that HDL may play a major role in atherosclerotic lesion dynamics (28). High levels of HDL in BN AD and extreme high levels in BN/Ka AD, resulting in the increase of HDL/total cholesterol index, may certainly explain the absence of atherosclerotic lesion in these rat strains. Lower triglycerides levels in BN CD and AD and BN/Ka CD and AD groups compared with corresponding Wistar groups further add to the anti-atherogenic effect.

The fatty degeneration of organs was also more pronounced in the BN/Ka strain. Lipid depositions were even found in abdominal aortas between elastic lamellae. A similar metabolic response (high HDL levels) associated with fatty liver degeneration and, at the same time, absence of atherosclerosis has been described in heavy alcohol drinkers (2). The mechanisms protecting against atherosclerosis in this population are far from being understood. Nonalcoholic fatty liver disease also occurs in humans with lipoatrophy, who have a high incidence of insulin resistance and decreased leptin levels (26). It has been suggested that bradykinin, which is released from HK by plasma kallikrein, improves insulin sensitivity (35) and is involved in leptin-mediated glucose uptake in skeletal muscle (29) and in glycogen synthesis and lipogenesis (17). Thus the kallikrein-kinin system appears to have substantial impact on glucose and lipid metabolism. Further elucidation of the metabolic differences between BN and BN/Ka strains may shed some light on the genetic determinants of the relationship between atherosclerosis, obesity, and diabetes.

Conclusions

Our data show for the first time that a genetically determined kininogen deficiency promotes the formation of abdominal aorta aneurysms and is associated with enhanced elastolysis, FasL- and caspase-3-mediated apoptosis, the induction of the MMP-2 and MMP-3 proteolytic cascade, and downregulation of TIMP-4. Kininogen deficiency is also associated with changes in plasma cytokines, especially upregulation of IFN-γ and downregulation of GM-CSF and IL-1β. Kininogen deficiency plays a role in the changes in lipid metabolism following atherogenic diet, characterized by high levels of cholesterol, HDL, and an increased HDL/total cholesterol index, as well as pronounced fatty liver and heart degeneration together with lipid depositions in aortic media without atherosclerotic plaque formation.

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