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Murine genotype impacts pancreatitis severity and systemic inflammation: An experimental study

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ABSTRACT

Background: Little is known regarding the impact of host response in acute pancreatitis. Here, we induce murine necrotizing pancreatitis in 9 different mouse strains.

Materials and methods: We examined 9 different mouse strains: Balb/CB4J, C3H/HEJ, NOD/SHILT, A/J, AKR/J, C57BI/6J, DBA/2J, FVB/NJ, 129S1/SvlmJ. 10 animals per strain were randomly allotted to two groups. Sterile necrotizing pancreatitis was induced by injection of taurocholate into the common bile duct. Control animals were injected with saline. Every 6 h, clinical parameters were examined and scored. After 24 h, animals were sacrificed to examine and compare serum enzymes, histology, bronchoalveolar lavage fluid, and serum IL-6.

Results: Histologically, taurocholate treated animals scored significantly higher than control animals. Concordantly, serum lipase and amylase were significantly elevated in pancreatitis animals in all strains. NOD/SHILT and AKR/J mice had the highest enzyme activity. 24 h after induction, there were no signs of increased pulmonary vascular leak in taurocholate animals. Remarkably, interleukin 6 was not increased at all in C57BL/6J, C3H/HeJ, and 129S1/SvlmJ mice compared to all other strains.

Conclusion: The genetic strain has an impact on pancreatitis severity and systemic inflammatory response in a murine taurocholate induction model. Analogous differences in humans may partially account for the disparity in post-ERCP pancreatitis.

1. Introduction

Acute biliary and post-ERCP pancreatitis ranges from a mild self-limiting disease to a severe and highly lethal illness involving Systemic Inflammatory Response Syndrome (SIRS) and often leading to pulmonary, cardiovascular and renal insufficiency [1]. To date, this disparity is poorly understood. Gathering data on the impact of genotype on disease severity in humans is difficult. This study is designed to collect data on this question in a feasible way using a mouse model.

Taurocholate induction models of acute necrotizing pancreatitis aim at mimicking pancreatitis arising from obstruction of the distal bile duct and are established procedures [2]. While the induction stimulus has become standardized and reproducible, little is known about host impact on pancreatitis severity and on SIRS. Several studies have tried to shed light on molecular variants that may influence pancreatitis severity [3–5]. To date, there is very little conclusive evidence though.

We hypothesized that the genotype of particular mouse strains impacts the severity of acute necrotizing pancreatitis. In this study, we determine histological and clinical parameters known to correlate with pancreatitis severity as well as IL-6 and bronchoalveolar parameters known to correlate with SIRS in 9 genetically different mouse strains to examine the relationship between genotype and disease severity.

2. Material and methods

2.1. Animals

All animal procedures were conducted according to the Federation of European Laboratory Animals Science Associations guidelines and approved by the local animal welfare committee (approval code: G-08-79). The experiments were performed in compliance with the ARRIVE criteria [6]. To avoid influence of the female hormonal cycle during the course of the experiments, we examined male mice. We used 9 genetic strains: Balb/CB4J, C3H/HEJ, NOD/SHILT, A/J, AKR/J, C57BI/6J, DBA/2J, FVB/NJ, 129S1/SvlmJ. Animals were ordered from Jackson Laboratory, Bar Harbor, Maine, USA. They had an average weight of 23.91 ± 1.98 g and an average age of 12–16 weeks and were housed under standard conditions with a 12 h dark/light cycle and standard pellet diet and water ad libitum. All animals were housed under SPF-conditions. Only 5 animals per group were ordered at a given time and

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housed together. Postoperatively, animals were housed separately until euthanized. 10 animals of each strain were evenly and randomly allotted to each treatment arm and operated in order of randomization. There was no additional blinding. Group sizes were based on the assumed difference in outcome parameters. Total mortality was 5% in the necrotizing pancreatitis group. These animals were replaced. Anesthesia was administered using Forene (Abbot GmbH & Co KG, Wiesbaden, Germany) and 0.15 mg/kg buprenorphine (Temgesic - Essex Pharma GmbH, München, Germany).

2.2. Treatment procedures

During the experiment 0.15 mg/kg buprenorphine was administered every 8 h as 0.5 ml injections. Studies were aborted if two of the following criteria were fulfilled: trunk or extremity paralysis, breathing noises, self-mutilation, or repeated utterance of pain upon handling.

After shaving the abdomen and skin disinfection, a midline laparotomy was performed and the proximal common bile duct was temporarily clamped by using a microvessel clip as previously described [2]. Administering 2 ml/kg 4% taurocholate induced sterile necrosis. Control animals received an injection of 2 ml/kg 0.9% sodium chloride into the common bile duct. After the infusion into the common bile duct, the needle was withdrawn, and the puncture site was closed using 8/0 Prolene (Ethicon Deutschland, Norderstedt, Deutschland). The microvessel clip was removed and physiological bile flow was restored. Finally, the abdomen was closed. 24 h after induction of pancreatitis, animals were sacrificed by cardiac puncture under general anesthesia with Forene. After re-laparotomy, organs were harvested and a bronchoalveolar lavage was performed.

2.3. Physical strain score

All animals were closely observed and physical strain was scored with a score developed at the Julius-Maximilian University of Würzburg for determining the humane end point of experiments with laboratory animals. Bodyweight alterations, particularly weight loss, general condition, spontaneous behaviour, and clinical findings were recorded and scored. Strain was classified in 4°: 0 (0 points, no strain), 1 (little strain, 1 to 9), 2 (middle strain, 10 to 19), 3 (severe strain, euthanasia).

2.4. Histology

Tissue samples of the pancreas were formalin fixed and embedded in paraffin. 4 μm sections were stained with hematoxylin and eosin (H & E). Histological examination was conducted by two independent observers for 3 independent, randomly numbered and blinded sections of each animal. Severity of pancreatitis was diagnosed by scoring edema, leukocyte infiltration, parenchymal necrosis, fatty tissue necrosis and hemorrhages following the scoring system of Spormann et al. [7,8]. With this score, edema and leukocyte infiltrate are graded on a scale from 0 to 3, while acinar cell necrosis, fatty tissue necrosis and hemorrhage are graded from 0 to 7.

2.5. Serum lipase and amylase

Serum lipase and amylase were determined by routine clinical chemistry methods.

2.6. Enzyme-linked immunosorbent assays

Albumin concentration in BAL fluid was determined in duplicate by enzyme-linked immunosorbent assay (ELISA) (Albumin - Bethyl Laboratories, Montgomery, USA). Myeloperoxidase (MPO) concentration in BAL fluid was determined by MPO ELISA (Hycult Biotechnology, Uden, Netherlands) with undiluted samples. The assays were performed in duplicate according to the supplier's instructions. Serum IL-6 concentration was determined using an ELISA in duplicate form (IL-6 - BioLegend, San Diego, USA).

2.7. Statistics

The data were analyzed using SPSS Software (Version 16 for Mac OS, LEAD Technologies, Chicago, USA) and are displayed as mean ± SEM. All figures depict mean values with standard error of mean. To assess statistical significance we used Tukey's Test. Significance is assumed for $p < 0.05$.

3. Results

3.1. Clinical observations

All animals were weighed at onset and after 24 h, and the difference in bodyweight in percent was calculated for each group. There were no statistically significant differences between the groups. In the pancreatitis groups, weight loss ranged from 3.01 ± 1.1% in 129S1/SvlmJ animals to 8.03 ± 0.8% in AKJ animals. In control animals, weight loss ranged from to 3.6 ± 0.44% in C3H/HEJ animals to 5.8 ± 1.16% in DBA/2J animals. Clinical strain scores differed significantly between taurocholate animals and controls in A/J and C57BI/6J animals ($p < 0.05$). In the case of C57BI/6J mice, these clinical observations correlated with other parameters of disease severity. AKR/J mice, which showed a high increase of other disease parameters, had high strain scores, but did not quite reach statistical significance (Fig. 1).

3.2. Histology

Histological sections were examined and scored according to Spormann (Fig. 2) [2,8]. Ten random sections from the pancreatic head, body and tail were examined by two independent examiners at 20 times magnification. Edema, inflammatory infiltrate, parenchymal necrosis, fatty tissue necrosis and hemorrhage were scored by both examiners, and the mean score was used. High scores correlate with pancreatitis severity.

In both groups, edema of the parenchyma was observed. As described previously, local necrosis was exclusively observed in the taurocholate groups [2]. All strains showed significant score differences between controls and taurocholate animals ($p < 0.05$). In both groups, AKR/J mice reached the highest scores (14.6 ± 2.29 and 2.6 ± 0.83 respectively). Both AKR/J and A/J taurocholate animals had significantly higher scores than 129S1/SvlmJ mice, and the first scored significantly higher than NOD/ShiLtJ and Balb/cJ mice ($p < 0.05$).

3.3. Serum lipase and amylase

Serum lipase was significantly increased after 24 h in all strains in taurocholate animals compared to controls (highest $p = 0.001$, Fig. 3A). The highest serum enzyme activity was seen in Balb/cJ mice (4840 ± 3755 U/l), the least activity in DBA/2J mice (436 ± 171 U/l). These strains differed significantly ($p = 0.0001$). Furthermore, AKR/J mice showed significantly higher serum activity than C57BL/6J, DBA/2J, and FVB/NJ animals ($p < 0.05$).

Changes in serum amylase are illustrated in Fig. 3B and followed a similar pattern. AKR/J mice showed the highest serum enzyme activity in the taurocholate group (74,608 ± 23,200 U/l). This was significantly higher than in DBA/2J mice (9540 ± 1496 U/l; $p = 0.0001$). Similar to serum lipase, all amylase activity was significantly higher in all taurocholate animals compared to their respective controls ($p < 0.05$), except in FVB/NJ mice ($p = 0.079$).

3.4. Bronchoalveolar lavage

Albumin concentration was measured in bronchoalveolar lavage