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

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ARTICLE INFO

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

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

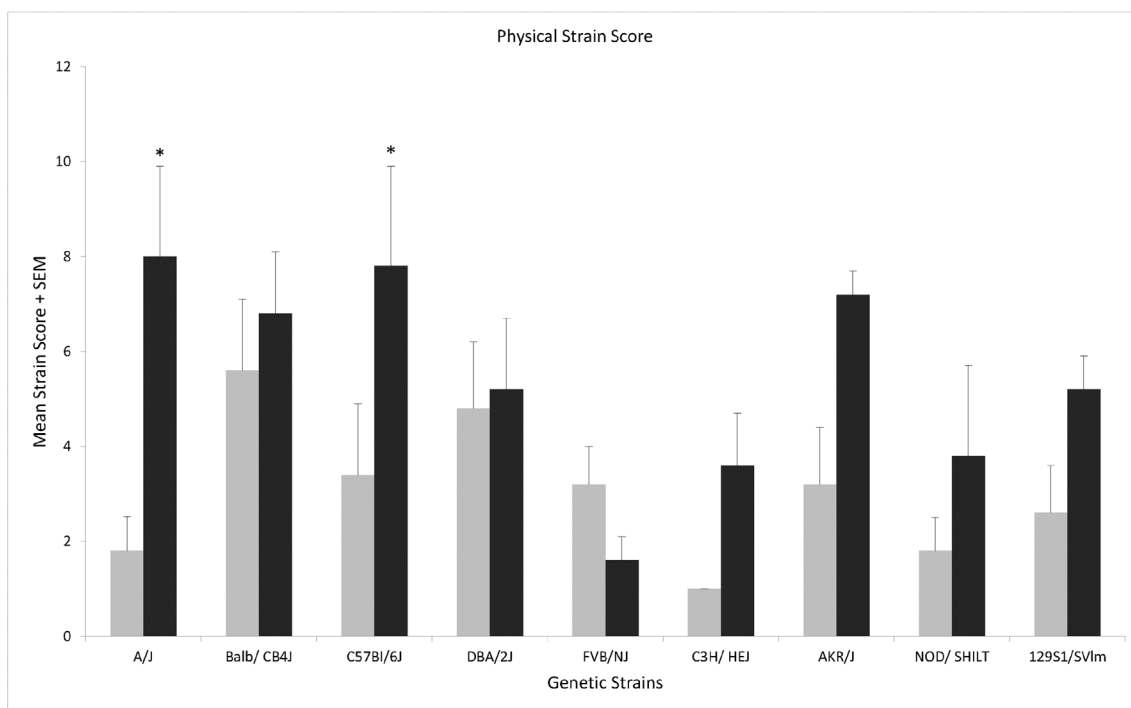Albumin concentration was measured in bronchoalveolar lavage

Fig. 1. Physical strain score of controls (grey) compared to animals with taurocholate induced pancreatitis (black): In most groups, taurocholate treated animals scored higher. This difference was statistically significant in A/J and C57B/6J mice. In AKR/J mice, which displayed highly increased local and systemic parameters, the difference did not quite reach statistical significance.
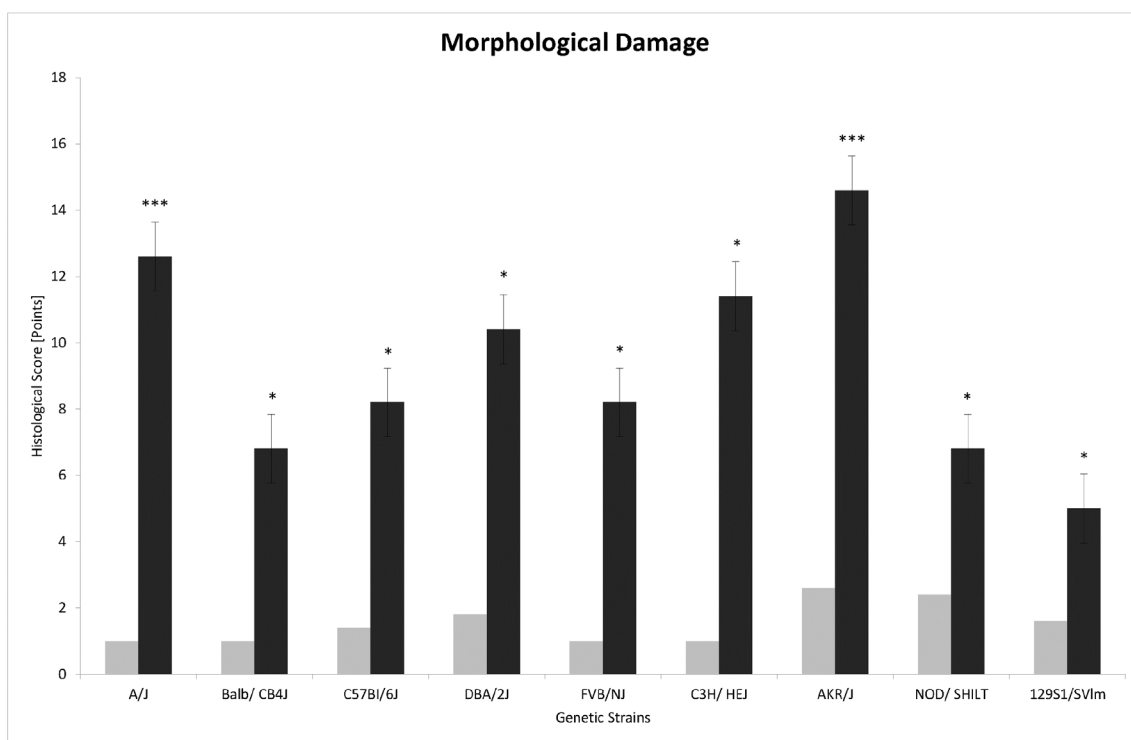

Fig. 2. Histomorphological scoring of the pancreas according to Spormann: The pancreas was examined microscopically, and edema, inflammatory infiltrate, parenchymal necrosis, fatty tissue necrosis, and hemorrhage were scored. Scores of controls (grey) were compared to animals with taurocholate induced pancreatitis (black). This figure illustrates that taurocholate induction is indeed a reliable trigger of necrotizing pancreatitis. All strains showed significant score differences between controls and the taurocholate animals (*). Tissue necrosis was exclusively observed in pancreatitis groups. A/J and AKR/J mice scored significantly higher not only compared to their respective control groups, but also compared to 129S1/SvlmJ, NOD/ShiLtJ and Balb/cJ mice (***).
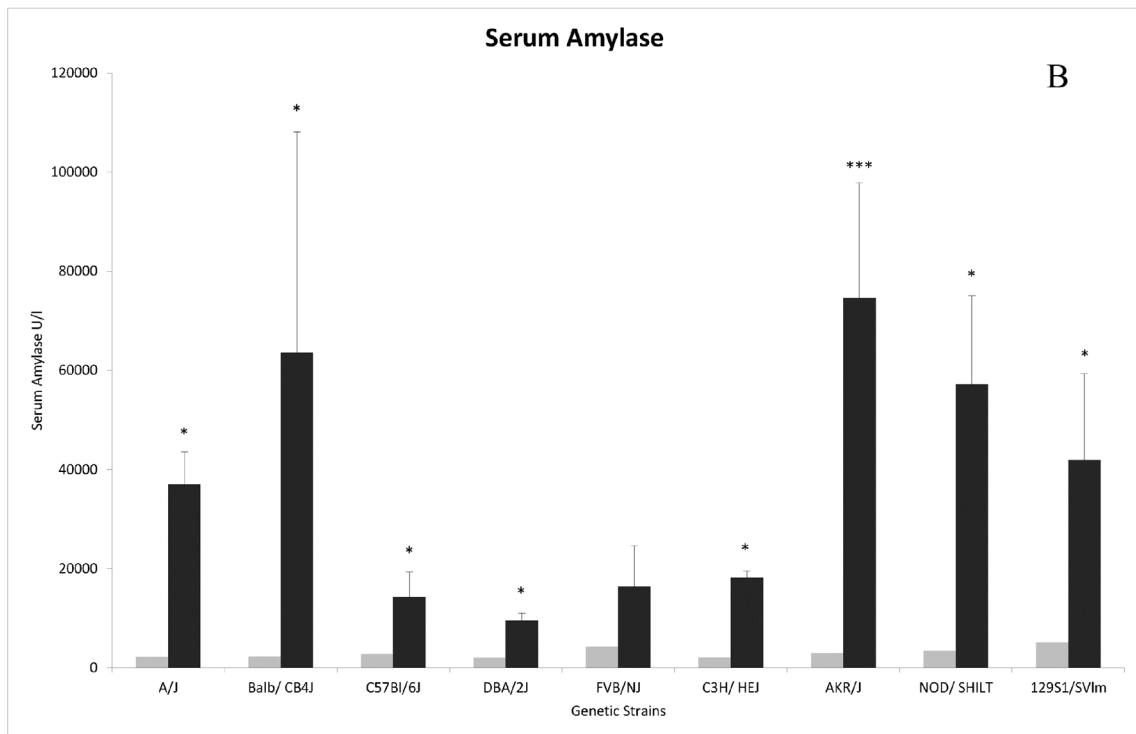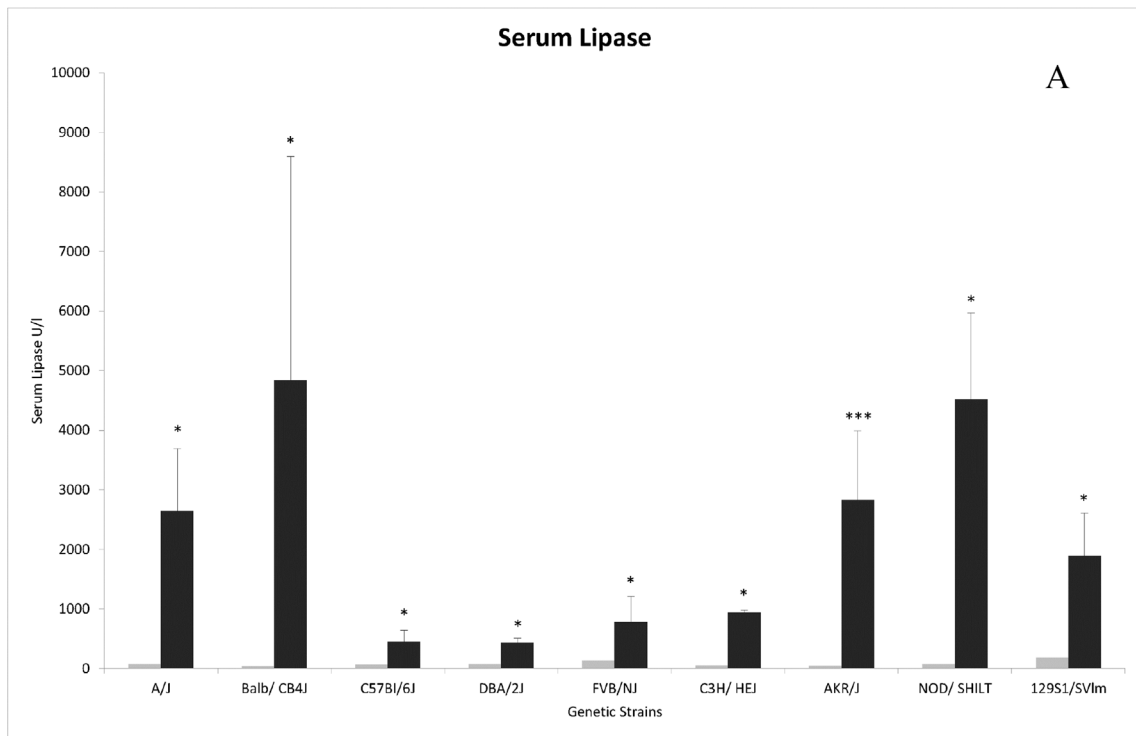
Fig. 3. A: Serum lipase in controls (grey) compared to animals with taurocholate induced pancreatitis (black): In all groups, serum lipase activity was significantly increased after 24 h in taurocholate animals compared to controls (*). AKR/J mice showed significantly higher serum activity than C57BL/6J, DBA/2J, and FVB/NJ animals (***). B: Serum amylase in controls (grey) compared to animals with taurocholate induced pancreatitis (black): As illustrated in this diagram, differences between taurocholate and control animals concerning serum amylase were analogous to serum lipase changes and statistically significant (*). AKR/J mice showed the highest serum enzyme activity in the taurocholate group (74,608 ± 23,200U/l) (***). In FBB-NJ mice, the difference did not reach statistical significance.
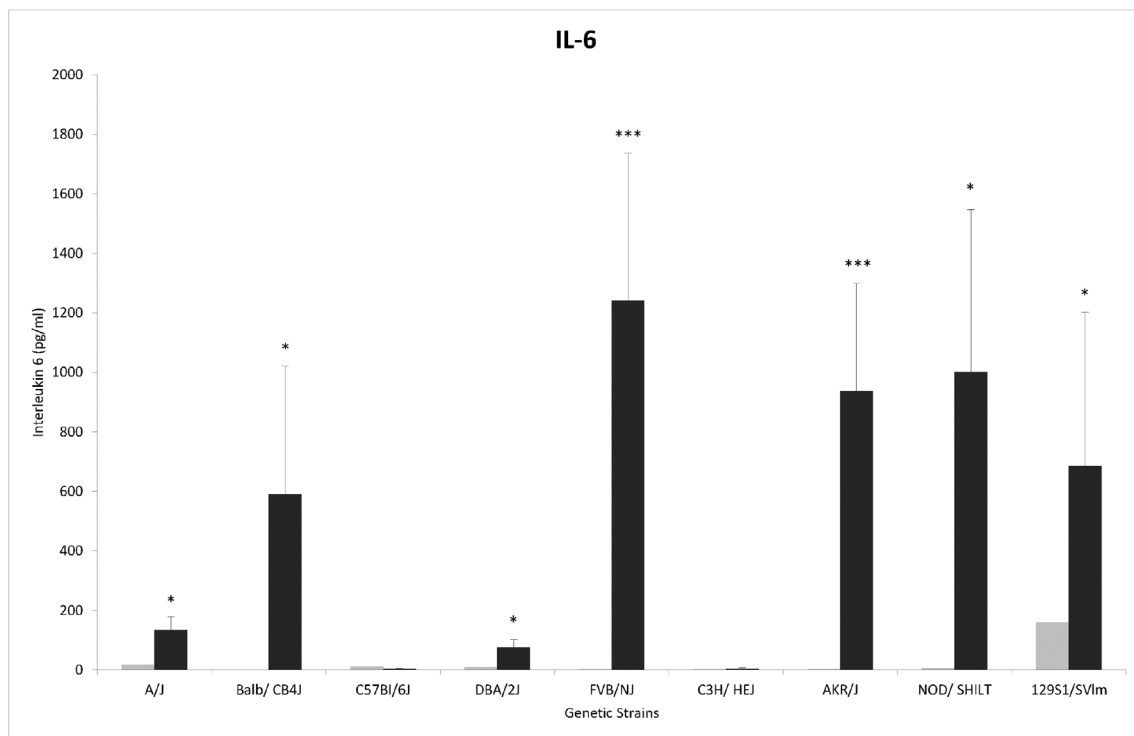
Fig. 4. Serum IL-6 of controls (grey) compared to animals with taurocholate induced pancreatitis (black): Surprisingly, in two groups we observed practically negligible IL-6 values. In all other groups, the differences between taurocholate animals and controls were significant (*). Nonetheless, the increase of this parameter varies dramatically. AKR/J and FVB/NJ animals showed significant higher values compared to C57BL/6J and C3H/HEJ animals (***). In FVB/NJ animals, this increase contrasts with serum enzyme levels, which were only moderately increased compared to other groups.

fluid to assess signs of early pulmonary damage and vascular leak. These parameters were shown to be transiently elevated in animals with necrotizing pancreatitis and significantly elevated in animals with infected necrotizing pancreatitis in our previous work. However, we neither recorded significant changes in taurocholate treated animals compared to their controls nor were any groups particularly sensitive. These results are concordant with above mentioned data that show a significant, early increase of parameters for pulmonary lesion that is no longer significant after 24 h [2]. Analogously, we determined myeloperoxidase (MPO) concentration in bronchoalveolar fluid to assess pulmonary inflammation. However, this parameter too was not significantly altered after 24 h.

3.5. Il-6

Il-6 is a known marker for systemic inflammatory response. In previous experiments, taurocholate induced pancreatitis was accompanied by marked increase of IL-6. Here, we made quite remarkable observations (Fig. 4). First of all, with the exception of C57BL/6J p (p = 0.655) and C3H/HeJ (p = 0.694) animals, which showed almost no expression of IL-6, all strains showed a statistically significant increase of IL-6 in taurocholate treated animals compared to their respective controls (p < 0.05). Among the taurocholate groups, FVB/NJ and AKR/J had the highest concentrations of IL-6 and differed significantly compared to the above mentioned animals (p < 0.05). In general, there appeared to be a correlation between serum enzyme levels and degree of systemic inflammation. In the case of FVB/NJ animals, this is not the case though. While serum enzyme activity was moderately elevated, IL-6 levels were clearly increased.

4. Discussion

Several animal models are used to study the mechanisms of acute pancreatitis. Recent data on an L-Arginine induction model suggest that

mouse strains may vary in susceptibility to a particular induction method [9]. We hypothesized that genetic mouse strains may differ in their response to induction of necrotizing pancreatitis. Here, we compare local and systemic disease parameters in 9 different mouse strains using a well-established murine model of taurocholate induced pancreatitis.

Clinical observations show that there is a wide range of susceptibility to pancreatitis triggers in humans as well. Most cases of acute pancreatitis are self-limiting and have a good prognosis. However, in 15–20% of cases, patients develop severe pancreatitis [10]. In necrotizing pancreatitis, acinar cell death triggers a pro-inflammatory cascade that produces systemic reactions including ARDS, SIRS, and ultimately multi-organ failure, leading to a grim mortality rate of up to 50% [11,12]. Little is known about which factors contribute to systemic involvement and how come some patients suffer from severe local pancreatitis, but display little systemic affection.

The main limitation of our study is the low number of animals per group. This study was intended to scan for clear-cut differences between different strains. Larger numbers would be necessary to examine the difference between individual strains more subtly. Animals were not operated on in a blinded fashion in this study, and it is necessary to bear in mind that this is a model of necrotizing pancreatitis. While these results certainly cannot be directly translated, we find that this work stimulates further animal research. On the one hand, further experiments using far fewer strains might elucidate the mechanisms behind results we report on. On the other hand, other models that trigger SIRS may be helpful in testing whether host response is a variable in other disease entities as well. We believe a replacement of animal research is not feasible at this time due to the complexity of the disease.

The extent and severity of local pancreatic damage following taurocholate induction of pancreatitis has been described in detail previously [2]. In our current trial, several observations are worth mentioning concerning the induction of acute pancreatitis. First, all strains developed necrotizing pancreatitis following taurocholate. In the

control groups, pancreatic damage was far less. This confirms that retrograde taurocholate injection is a very reliable pancreatitis model across strains. However, the degree of pancreatic damage among strains varied considerably. Especially AKR/J mice showed particularly high morphological scores; A/J and C3H/HeJ mice showed severe histological lesions as well. The respective control groups showed surprisingly few histological alterations. These results raise the question whether some strains are more susceptible to bile acidic trauma than others.

In analogy to our histological findings, significant differences in serum α-Amylase, which is indicative of pancreatitis severity in the experimental setting, were observed. AKR/J mice, which showed the most histological damage, had the highest amylase levels. In contrast, DBA/2J showed the lowest serum enzyme activity levels. Looking at lipase values, it is noteworthy that similar to the amylase values, C57BL/6J, DBA/2J, FVB/NJ, and C3H/HeJ mice showed considerably lower enzyme activity than other strains. AKR/J mice showed prominent values. In both cases, there was no strict correlation between morphological damage and serum enzyme activity.

Systemic complications determine the course of pancreatitis. Thus, we were interested in determining albumin and myeloperoxidase concentrations in bronchoalveolar lavage fluid to assess early pulmonary damage as well as neutrophil granulocyte invasion. However, similar to earlier observations that show a transient increase of these parameters during the first 16 h after induction, we did not see a significant difference among controls and taurocholate treated animals with respect to these parameters [2].

IL-6 is one of the mediators of systemic inflammatory response to a diverse set of lesions. In acute pancreatitis, high levels of IL-6 often precede severe cases and SIRS. As expected, already after 24 h, the taurocholate groups showed high levels of IL-6 compared to control groups. Thus, there was no systemic inflammatory response to manipulation and retrograde saline injection alone. C3H/HeJn and C57BL/6J animals showed almost negligible levels of IL-6. Not in all strains was there a correlation between histological damage, enzyme activity and IL-6 levels. FVB/NJ animals showed the most obvious discrepancy between IL-6 levels and other disease parameters. This implies that some strains are more susceptible to systemic affection than others. Comparing host response to other SIRS stimuli in these mouse strains might shed more light on this question.

Clinical observations in post-ERCP pancreatitis mirror the findings of our study well. In a large review, 3.5% of patients were seen to develop post ERCP acute pancreatitis with variable severity. 0.4% of patients suffered severe pancreatitis, and 0.11% of patients died [13]. In clinical studies, risk factors for the development of post-ERCP pancreatitis have been identified and stratified. However, much remains to be explored concerning the pathological mechanisms as well as the predisposition toward systemic affection [14].

5. Conclusion

This study is the first to demonstrate that data on acute pancreatitis may vary considerably not only dependent on the experimental model chosen, but also on the genetic strain used. Thus, our data explains differences in pancreatitis severity between experiments that utilize different mouse strains. Finally, this model is suitable to examine the mechanisms behind clinical differences in sensitivity to retrograde manipulation of the pancreas during ERCP.

Ethical approval

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).

Author contribution

Karoline Sander and Sabine Richter conducted the experiments and performed the stains. Uwe Wittel established the experimental models, formulated the driving hypothesis, and supervised the study. Gabriel Seifert participated in study design, performed data analysis with Uwe Wittel and drafted and completed the manuscript. All authors gave feedback to the manuscript and approved of its final form.

Conflicts of interest

Registration of research studies

No human participants.

Guarantor

Gabriel Seifert and Uwe Wittel are the guarantors of this study.

Consent

No human participants.

Availability of data and material

The raw data described in this manuscript is not deposited in a public repository.

Acknowledgements

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