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To the
Science Editor
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Prof. Xue-Jiao Wang

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Resubmission of the manuscript CLS-D-18-00107

Dear Editor, dear Prof. Xue-Jiao Wang,

we followed all your suggestions and resubmit a revision of our manuscript. Please find enclosed our modified manuscript as well as a point to point reply to the comments of the reviewers.

In summary, we like to thank the editor and the reviewers for the quick review and their helpful work. We hope that the revised manuscript is now acceptable for publication.

Yours sincerely,

Dietmar Zechner, Ph.D.
(Corresponding author)

Reply to reviewer 1 (Ketan Ramesh Vagholkar)

We like to thank the reviewer for the quick review and the very constructive suggestions. In response to your comments we improved the title, abstract, core tip, method section and discussion. Please find our point to point response below:

1. Your comment:
   Title needs to be a little catchy. In fact the title itself gives away the conclusion thereby precluding the interest in reading the article.
   Our reply:
   We decided to change the title to: The impact of hyperglycemia on autoimmune pancreatitis and regulatory T-cells. We hope this title defines the contents of the manuscript without giving away the conclusion.

2. Your comment:
   Statement made in lines 47 to 49 of the abstract needs reframing as it is very misleading. What does the author wish to convey is a cause for some confusion.
   Our reply:
   We decided to change the text (now line 68 to 89 of abstract): Severe hyperglycemia did, however, not lead to an aggravation, but rather to a slight attenuation of autoimmune pancreatitis. In the pancreas both the histological score of the pancreas as well as the number of CD3+ lymphocytes (P<0,053) were decreased by hyperglycemia.

3. Your comment:
   The keywords seem to be satisfactory.
   Our reply:
   Since the key words are satisfactory, we did not change them.

4. Your comment:
   The statement made in the core tip that is line 68-72 should be rephrased.
   Our reply:
   We decided to change the text (now line 114-119) to: This preclinical study demonstrates that hyperglycemia does not lead to an aggravation but rather an attenuation of autoimmune pancreatitis. Thus, this result might have the clinical implication that a tight adjustment of blood glucose concentration in patients with autoimmune pancreatitis is not needed, because it might not have a beneficial effect on the progression of this disease.

5. Your comment:
   The clinical implications need to be stated in brief to develop interest in the mind of the reader.
   Our reply:
   We decided to state the clinical implications at the end of the core tip: Thus, this result might have the clinical implication that a tight adjustment of blood glucose concentration in patients with autoimmune pancreatitis is not needed, because it might not have a beneficial effect on the progression of this disease.

6. Your comment:
   Background of the article is well formulated and gives a fair idea of the whole issue being addressed.

Our reply:
Since the background is well formulated, we did not change it.

7. Your comment:
   Methods section is well planned. However a clear correlation to the patterns of assessment would have added to the quality of this section.
   Our reply:
   We changed the description of the histological evaluation slightly to the following text: The histology of the pancreas was investigated by light microscopy (Olympus Corporation, Tokyo, Japan). The severity of autoimmune pancreatitis was determined by scoring the degree of inflammatory cell infiltration and destruction of the parenchym as described by Kanno et al (0=none inflammation, healthy organ; 1=mild inflammation, mononuclear cells present in the interstitium but no destruction of parenchym; 2=moderate inflammation, focal destruction of parenchym with mononuclear cell infiltration; 3=moderate and diffuse or severe but focal inflammation, diffuse destruction of parenchym with residues of intact parenchyma; 4=severe and diffuse inflammation, extended mononuclear cell infiltrates with destruction of acini and replacement by adipose tissue)[20,21].

8. Your comment:
   The discussion component is too sketchy. Elaborate details of the cytomorphological-pathological correlation should have been elaborated.
   Our reply:
   We elaborated on the importance of the histological observations (line 322-339) by including the following text in the discussion: Another limitation of this study is the use of a histological score, which was defined by the ordinal numbers 0 to 4. Comparing the median of the histological score allows, therefore, only a restricted presentation of complex changes in the histology of the pancreas[20]. We observed that the median of this score was reduced from 2,25 in normoglycemic to 1,50 in hyperglycemic mice (Fig. 2B). However, this simple comparison underestimates the observed changes. The same data can also be presented as percentage of mice with a histological score of ≥3. Under normoglycemic conditions 42% of mice (8 from 19 mice) received a histological score of ≥3. Under hyperglycemic conditions only 6% (1 from 17 mice) received a score of ≥3. These high scores were assigned when moderate diffuse or severe focal inflammation plus a diffuse destruction of the acini (score 3) or severe and diffuse inflammation plus extended destruction of acini (score 4) was observed[20,21]. This impressive difference in the percentage of mice with a high histological score, therefore, suggests that hyperglycemia reduces the damage to the parenchym, which might indirectly reduce local inflammation. Alternatively, this observation could also suggest that hyperglycemia reduces inflammation and thus minimizes the damage to the parenchym. Due to the following observations, we prefer the second option.

9. Your comment:
   A brief implication on the clinical outcome should have been given which would have enabled the reader to consider new paradigms for future research.
   Our reply:
   We describe the clinical implication of our manuscript at the end of the discussion section with the following text: Although our study has the limitation to be only a preclinical study on mice, it also suggests that an aggressive adjustment of blood glucose concentration might not be necessary as a prerequisite for the treatment of AIP. For final clarification of this issue, a clinical study evaluating if a tight adjustment of

blood glucose in addition to steroid therapy is beneficial or harmful to patients with autoimmune pancreatitis might need to be pursued.

10. Your comment:
    The illustrations, tables and statistical evaluation is quite good. The overall flow of the presentation is quite good. The referencing is adequate with relevant ones being used. All the necessary rules of conformity in basic research have been satisfactorily complied with.
    Our reply:
    Since these points are quite good, we did not change it.

11. Your comment:
    The important fact highlighted by this research is the immunological basis for the effects of hyperglycemia on disease progression. The startling fact was improvement in the severity of the disease process which is a novel finding. The article provides a thought provoking insight into the effect of hyperglycemia on this not so well understood disease. The conclusions satisfactorily explains the results of the study. The quality of the manuscript is satisfactory. However a few statement are misleading or confusing. The main issue with the study is the way it can be extrapolated with the human disease. The study provides a new avenue for research that is to study the mechanism of improvement of the disease process by hyperglycemia.
    Our reply:
    Thank you for your reflections, we agree with your comments.

Reply to Reviewer 2 (anonym)

We like to thank the reviewer for the quick review and the very critical but also very constructive suggestions. In response to your comments we improved the introduction and the discussion sections. Please find our point to point response below:

1. Your comment:
   I acknowledge the authors' track record in exploring the basic pathophysiology of acute pancreatitis, However, the discussion is flawed as to extrapolation to clinical practice. It is virtually dogma that both acute and chronic hyperglycemia are deleterious to every organ system including the immune system. The migration and bacteriocidal capacity is hindered by acute hyperglycemia. Diabetics are clearly more prone to infection.
   Our reply:
   We agree with the reviewer. Indeed hyperglycemia has a deleterious effect on many organs and also on the immune system. This might exactly be the cause why diabetes might be harmful during infections, but beneficial during an autoimmune disease. We discuss this issue using the following text: A beneficial effect of hyperglycemia on autoimmune pancreatitis seems to be counterintuitive, since hyperglycemia has been demonstrated to have a deleterious effect on many organs. However, hyperglycemia can also have an inhibitory effect on the immune system. One could speculate that this impact on the immune system may have a beneficial effect on the progression of some autoimmune diseases.

2. Your comment:
   The discussion is not fluent in some segments and requires more polishing. I would keep the title and other sections but redo the discussion.
   Our reply:
   Due to reviewer 1, we had to change the titel to: The impact of hyperglycemia on autoimmune pancreatitis and regulatory T-cells. We hope this title defines the contents

of the manuscript without giving away the conclusion. In addition, we extended and corrected many parts of the discussion. We hope the discussion is now easier to understand.

3. Your comment:
   Noting esoteric references to support not treating hyperglycemia runs against the grain of clinical practice. What I would conclude is that aggressive therapy of hyperglycemia may not be warranted and hyperglycemia may have a surreptitious effect on the immune correlates of AIP within the pancreas.
   Our reply:
   We agree with the reviewer that it might be awkward to suggest not to treat hyperglycemia, that our study has its limitations and that the cited literature might run against the grain of clinical practice. However, we believe in our data and could also find some studies that support the idea not to treat hyperglycemia during autoimmune pancreatitis. We changed some of the references and have modified the last paragraph of the discussion. The following text is included: For example, in one third of all cases, DM even worsened after insulin therapy[15]. Moreover, it was reported that treatment of diabetes with insulin lead to hypoglycemic attacks in 10 from 50 AIP patients[13]. In addition, several clinical studies could demonstrate that DM improved in many cases automatically after steroid therapy[15,30]. These clinical studies argue against a tight control of blood glucose in AIP patients. Although our study has the limitation to be only a preclinical study on mice, it also suggests that an aggressive adjustment of blood glucose concentration might not be necessary as a prerequisite for the treatment of AIP. For final clarification of this issue, a clinical study evaluating if a tight adjustment of blood glucose in addition to steroid therapy is beneficial or harmful to patients with autoimmune pancreatitis might need to be pursued.

4. Your comment:
   I would elaborate more on the detriments of the study including species difference and the unclear correlation of the parameters studies and the clinical course of AIP, I take exception with sentence linked to reference 5. Nowadays with EUS-FNA and laparoscopy, less patients with ultimate AIP need pancreas resection
   Our reply:
   In this latest version of the manuscript we elaborated more on the problematic aspects of this study and included a whole paragraph describing limitations (second paragraph of the discussion section). We also stress that this is only a preclinical study in mice and that a final clarification of the issue a clinical study might be necessary (last paragraph of discussion). The sentence linked to referenced 5 was changed to reflect the fact that fewer pancreas resections are needed nowadays (first paragraph of introduction). The following text was included in the manuscript: The differentiation to pancreatic neoplasia is difficult, which sometimes can lead to unnecessary pancreatectomy[5].

Reply to Reviewer 3 (anonym)

We like to thank the reviewer for his review and the very helpful suggestions. In response to your comments we corrected figure 1, all figure legends and the discussion section. Please find our point to point response below:

1. Your comment:

STZ has been widely used to induce diabetic model in rats and mice as well as other animals because STZ can relatively and selectively destroy cells of pancreatic islet. Therefore the hyperglycemia is prominent in this type-1 diabetic model. However, how authors in this study can exclude the effect of STZ on AIP? This issue may be needed to discussion.
Our reply:
We agree with the reviewer that we cannot completely exclude that STZ has an influence on AIP. We discuss this option in the discussion section using the following text: Our study has several limitations. For example, we cannot completely exclude that instead of high glucose concentration in the blood, the drug, we used for inducing hyperglycemia (STZ), influences AIP directly. However, in our opinion it is very unlikely, that the alkylating agent STZ, which has been demonstrated to be cytotoxic to cells, directly cures AIP.

2. Your comment:
In this study, authors only overserved effect of hyperglycemia on AIP for one time point. As indicated by authors, A temporary or sustained hyperglycemia can be observed in AIP patients, therefore is may also important to study how different time periods of hyperglycemia affect AIP.
Our reply:
We agree with the reviewer that it might be nice to study how different time periods of hyperglycemia affect AIP. We discuss this option in the discussion section using the following text: We also limited our study to evaluate the effect of sustained hyperglycemia on AIP, and can therefore not conclude, if a transient hyperglycemia also has beneficial effects on the progression of AIP.

3. Your comment:
Authors suggested that adjusting blood glucose concentration might not have a beneficial influence on the progression of autoimmune pancreatitis in diabetic patients. However, authors may also have some other good options and suggestions for treating AIP in diabetic patients.
Our reply: We do think that it is too early to publish suggestions for treating AIP in diabetic patients based on our preclinical study in mice. Instead we suggest that a clinical study evaluating if a tight adjustment of blood glucose in addition to steroid therapy is beneficial or harmful to patients with autoimmune pancreatitis should be pursued (last paragraph of discussion section).

4. Your comment:
About groups of animals In the legend of figure 1, authors indicated that 28-40 week old- MRL/Mp mice were i.p.-injected with streptozotocin (STZ) on day 1-5 (group: AIP+STZ), while one age-matched control cohort was i.p.-injected with the appropriate vehicle (group: AIP). However, in Figure 2 to figure 5, authors use STZ and Sham for two different groups. Please be identical **!**
Our reply:
We completely agree with the reviewer and apologize. We corrected Figure 1 and all figure legends and only use STZ or Sham to define the groups.

5. In each figure (Figure 1 to 5), authors repeat “Box plots indicate the median, the 25th and 75th percentiles in the form of a box, and the 10th and 90th percentiles as whiskers.” in each legend. It may not be necessary to repeat it in the legend of each figure.
Our reply:
We completely agree with the reviewer. We now define the percentiles only in figure legend 1.