Elevated numbers of infiltrating eosinophils accelerate the progression of Duchenne muscular dystrophy pathology in *mdx* mice

Marine Theret, Lucas Rempel, Joshua Hashimoto, Morten Ritso, Lin Wei Tung, Fang Fang Li, Melina Messing, Michael Hughes, Kelly McNagny and Fabio Rossi
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MS TITLE: Elevated numbers of infiltrating eosinophils accelerate the progression of Duchenne Muscular Dystrophy pathology in mdx mice

AUTHORS: Marine Theret, Lucas Rempel, Fang Fang Li, Joshua Hashimoto, Morten Ritso, Melina Messing, Michael Hughes, Kelly McNagny, and Fabio MV Rossi

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

This study investigates the effect that high levels of eosinophils (using an IL5 Tg mouse) have on muscle development and repair and regeneration in WT mice, and in mice which are a model of DMD (mdx).

Eosinophils are known to be important for muscle regeneration after acute injury. Studies have described high infiltrating eosinophil numbers in mdx mice and in DMD patients.
Previous studies show that decreasing the numbers of eosinophils in mdx mice did not improve the histopathology of the muscle and increasing them also had no reported effect. These studies focussed on young mice (3-5 weeks). The current study has focussed on the effects of eosinophilia in older mice. The authors report on the chronic effects of eosinophilia and their infiltration on muscle regeneration and repair and show that eosinophilia in a mdx DMD mouse model results in increased defects in the muscle and early death.

Overall I think that this paper shows interesting data indicating an important role that eosinophils play in muscle repair and degeneration in a mouse model of DMD. How the eosinophils cause this is not rigorously investigated – the section where the proposed mediator cells- FAPs and MPs and changes to macrophage populations- is rather inconclusive and possible expected effects on these cells that are not observed are not discussed (eg no enhanced stimulation of FAPS and MPs) beyond an imbalance in eosinophil numbers causing a problem . On the back of this study the authors reasonably propose that assessment of human eosinophils in human DMD is warranted. Some data from DMD patients (eg blood counts, although I appreciate this is not trivial data to get hold of) backing up the mouse work could strengthen the author's MS.
Eosinophils are relatively understudied inflammatory cells, and I think this report makes interesting observations for the DMD area of study; the IL5:mdx mouse has a significant and interesting phenotype, although experiments investigating the mechanistic side to how the eosinophils are acting are quite preliminary.

Comments for the author

Figure 1. Data shows that damaged muscle tissue with eosinophilia (IL5Tg), compared to without (WT), shows affected muscle regeneration after acute injury . This was quantified by counting cell nuclei in a cross section of the muscle -these are clearly visible on the figure- and by measuring the cross sectional area of the myofibres. On fig 1A it would be useful if the dimensions of a myofiber was indicated on 2 differing sections to illustrate what was measured. Supplementary data shows that the eosinophilia is observed in both the blood stream and the muscle tissue. It would be interesting to see eosinophil distribution in the muscle tissue which may be possible with PAS staining on similar sections.
The effects of increased eosinophil number on the proliferation of cells important to muscle regeneration were also investigated. These cells, FAP and subsequently MPs, are known to be stimulated by eosinophils, so their proliferation was studied in wounded IL5 and WT mice. Some transient increase in Edu+ MPs was observed, but overall their proliferation decreased, perhaps unexpectedly in the presence of high numbers of eosinophils. I was unsure if MPs being stimulated and them proliferating are the same thing? This could be clarified. No effects of eosinophilia were seen on FAP proliferation (again it could be clarified if this is a good measure of stimulation), despite the changes on their downstream effectors the MPs .
The effects of eosinophilia on macrophages was also studied. Eosinophils secreting TGFb and IL-4 can drive macrophages from the pro-inflammatory to pro-regenerative phenotype, however again perhaps surprisingly, high levels of eosinophils in the IL5 mice did not result in changes in the inflammatory/ regenerative balance of macrophages. However the authors do report an overall decrease in macrophage numbers. The authors conclude that muscle regeneration is affected by eosinophila. There is evidence for this statement in figure 1 A . The evidence for links between eosinophilia and muscle regeneration - effects on FAP and MP stimulation and the eosinophil mediated skewing of macrophages towards a regenerative phenotype – is less convincing and rather preliminary.
Fig 2 A double Tg mdx:IL5 mouse was studied to see the effects of hyper-eosinophilia on this model of chronic disease. Mdx mice with eosinophilia died earlier than mdx alone, double Tg mice had less

overall mass and some muscle mass in particular was decreased, small n numbers explained some lack of significance to the data. In older double Tgs measurable effects on myofibres were observed although interestingly these changes at younger stages were different to those seen in the WT v IL5 mouse on acute wounding.
Changes in older mice include reduction in myofiber size (although n.s.) and shape (more dramatic) and the authors speculate that this may reflect a known DMD phenotype. I was however unsure what the white asterisks were showing in Fig 2 G and H as there seem to be similar patterns elsewhere - this could be clarified to the reader as this being a reflection of the phenotype in humans is of course very interesting. Needless to say the data would be improved with higher n numbers.
PSR staining shows increased fibrosis in eosinophilic mice in various muscles including the diaphragm which they suggest is the likely cause of early death.
Fig 3 shows the effects of eosinophilia on the mass and myofibres of young double Tg mice (the myofibers of young double Tg mice are ok at 7 months) that have been repeatedly wounded to induce fibrotic scarring and model the chronic damage of DMD.
Muscle tissue mass loss in mdx mice as result of damage was exacerbated by eosinophilia (IL5, mdx mice). This reflected a defect in myofiber CSA, and was associated with increased collagen staining and scarring.

Typo on line 54, 'DMD' missing - I think.
Typo on line 147 'indicate/s'

Reviewer 2

Advance summary and potential significance to field

In the present study, Theret et al. use in vivo mouse models to rigorously address the role of eosinophils in muscular dystrophy and regeneration following an acute injury. The study is significant as it aims to address an area in the field that is quite perplexing. The role of eosinophils in muscle is complex, with distinct roles in acute muscle damage versus chronic muscle disease. Further, the role of eosinophils seems to vary depending of the stage of disease with growing evidence suggesting that eosinophils promote muscle fibrosis in dystrophic mice. However, a direct examination of the effect of increasing eosinophilia on muscle fibrosis has not been performed. The present study provides clear in vivo evidence through genetic models that increased eosinophils in dystrophic muscle exacerbates the development of fibrosis. The authors further show that increased eosinophils following acute injury impairs muscle regeneration. Although well-conducted, the study would be benefit from a larger n for some experiments. However, it is understandable that the increased mortality of dystrophic mice with increased eosinophilia places a significant barrier in achieving larger group size. The authors address this limitation through the use of younger mice undergoing microdamage. Collectively, the findings are convincing and well-interpreted. These findings should have a significant impact in the field by addressing the role of eosinophil in later stages of muscular dystrophy. Although I am overall enthused with this study, there are a few minor concerns/comments that require some editing of the manuscript before this study can be considered for publication. Please see below.

Comments for the author

1. There appears to be a disagreement between the present studies and those reported by Heredia et al. Do the muscle eosinophils in IL-5 Tg mice not express IL-4? If so, why is there a lack of an effect on the FAPs in the present study? A response can be incorporated into the discussion.
2. The sentence seems incomplete. "of" what?
More recently, the lack of Dystrophin in satellite cells (muscle stem cells) 54 has been shown to impair their behavior and contributes to the pathogenesis of
(Dumont et al., 55 2015).
3. I believe there is a typo in the sentence, below. Did the authors mean to cite figure 1D?
"Although we noticed an increase in activation one day post injury (Figure S1D) MP proliferation was decreased at 3 and 5 days post injury (Figure 1E), leading to an overall decrease in their total number (Figure 1E)."

4. The use of red asterisks versus red p-values in the figure legend for various panels versus directly over the bar in a graph is confusing and not defined in the figure legend text.
5. Figure 3B is not cited in the results section, although the results are discussed.
6. Considering the following study https://pubmed.ncbi.nlm.nih.gov/24803842/ in relationship the statement below.
"While muscle eosinophilia has previously been associated with myositis, it does not seem to be always associated with poor outcomes (Selva-O'Callaghan et al., 2014) and, while muscle eosinophilia has been investigated in autosomal-recessive limb girdle muscular dystrophies (LGMD2A/2C), to our knowledge, it has not been investigated in the DMD."

First revision

Author response to reviewers' comments

We would like to thank the reviewers for their time and the feedbacks. As requested, we have added 2 to 3 n for the experiments in Figure 1, which has changed the interpretation of the data. We have also added additional n to the *mdx*:IL-5Tg experiments but these were limited to 2 control *mdx* and 1 *mdx*:IL-5Tg for logistical reasons. We noted that the latest died at 6 months of age. Nevertheless, we decided to add this mouse and its two control to the data. We have assigned the dead mice with a Δ in the Figure 3 and changed the legend to 6/7 month instead of 7 months only.
We hope the new version answer the comments and is suitable for publication. Changes can be found in red in the manuscript. We also have made significant changes in the formatting of the manuscript by merging the results and discussion sections in order to follow the guidelines provided by Development.

Reviewer 1
Advance Summary and Potential Significance to Field:
This study investigates the effect that high levels of eosinophils (using an IL5 Tg mouse) have on muscle development and repair and regeneration in WT mice, and in mice which are a model of DMD (mdx).
Eosinophils are known to be important for muscle regeneration after acute injury. Studies have described high infiltrating eosinophil numbers in mdx mice and in DMD patients.
Previous studies show that decreasing the numbers of eosinophils in mdx mice did not improve the histopathology of the muscle, and increasing them also had no reported effect. These studies focussed on young mice (3-5 weeks). The current study has focussed on the effects of eosinophilia in older mice. The authors report on the chronic effects of eosinophilia and their infiltration on muscle regeneration and repair and show that eosinophilia in a mdx DMD mouse model results in increased defects in the muscle and early death.

1. Overall I think that this paper shows interesting data indicating an important role that eosinophils play in muscle repair and degeneration in a mouse model of DMD. How the eosinophils cause this is not rigorously investigated – the section where the proposed mediator cells- FAPs and MPs and changes to macrophage populations- is rather inconclusive and possible expected effects on these cells that are not observed are not discussed (eg no enhanced stimulation of FAPS and MPs) beyond an imbalance in eosinophil numbers causing a problem.
This is an excellent point and we tried to address with two new experiments (Figure 2 and Figure S2).
First, we performed RNAseq on eosinophils from WT and IL5-Tg muscles. We screened for known markers of Th1 and Th2 inflammation, as well as various cytokines known to be secreted by eosinophils. Of interest, IL5-Tg eosinophils present a mixed phenotype as they express both Th1 and Th2 markers as it has been recently described in (Dolitzky et al., 2021). IL5-Tg eosinophils express less *Il4*, and more enzymes such as *Epx* and *Prg2* (Figure 2) which have been described as toxic for myofibers (Cai et al., 2000; Schröder et al., 2013; Wehling-Henricks, 2004; Wehling-Henricks et al., 2008). This suggested that the eosinophils found in the IL5-Tg are qualitatively different and possibly more toxic.

To characterize such differences functionally, we co-cultured FAPs from IL5-Tg mice withWT single myofibers. We analysed satellite cell fate at 48h (activation/proliferation) and 72h (proliferation/differentiation). We did not see any major changes induced by FAPs coming from IL5-Tg mice compared to WT FAPs. This fits with previous findings suggesting that eosinophils activate FAPs through IL-4 secretion. As IL5-Tg eosinophils do not produce more IL-4, IL5-Tg FAPs are unable to stimulate more MP proliferation (Figure S2).
Altogether, this suggests that eosinophils from IL5-Tg mice may directly interfere with myogenesis and myofiber growth, possibly through increased expression of toxic products.

2. On the back of this study the authors reasonably propose that assessment of human eosinophils in human DMD is warranted. Some data from DMD patients (e.g. blood counts, although I appreciate this is not trivial data to get hold of) backing up the mouse work could strengthen the author's MS.
We understand the reviewer's point. Indeed, obtaining blood samples from DMD patients and age matched controls would be challenging but not impossible. However, currently, the vast majority of DMD patients are undergoing treatment that affects eosinophil countsin the blood (corticosteroids/prednisone). This is a significant confounding factor that would interfere with the interpretation of the data.
We did not have access to human DMD patient donor muscle tissue samples that we couldstain for Siglec-8 or Major Basic Protein. However, this quantification has been recently published by Dr. Armando Villata (Kastenschmidt et al., 2021), showing that DMD musclesdisplay higher eosinophil infiltration than control muscles. We have cited this work in theintroduction.

3. Eosinophils are relatively understudied inflammatory cells, and I think this report makes interesting observations for the DMD area of study; the IL5:mdx mouse has a significant and interesting phenotype, although experiments investigating the mechanistic side to how the eosinophils are acting are quite preliminary.
We hope that the new experiments performed in Figure 2 and S2 can answer the reviewer's comment.

Reviewer 1 Comments for the Author:
4. Figure 1. Data shows that damaged muscle tissue with eosinophilia (IL5Tg), compared to without (WT), shows affected muscle regeneration after acute injury. This was quantified by counting cell nuclei in a cross section of the muscle -these are clearly visible on the figure- and by measuring the cross sectional area of the myofibres. On fig 1A it would be useful if the dimensions of a myofiber was indicated on 2 differing sections to illustrate what was measured.
This has now been added to the figure.

5. Supplementary data shows that the eosinophilia is observed in both the blood stream and the muscle tissue. It would be interesting to see eosinophil distribution in the muscle tissue which may be possible with PAS staining on similar sections.
We have performed SiglecF/Laminin staining at 7, 14, and 28 days post injury and added them in figure S1D. To note, we have also done PAS staining but we were not convinced the staining was specific to eosinophils in these settings. Attached are images of the PASstaining for future reference (Figure R1).

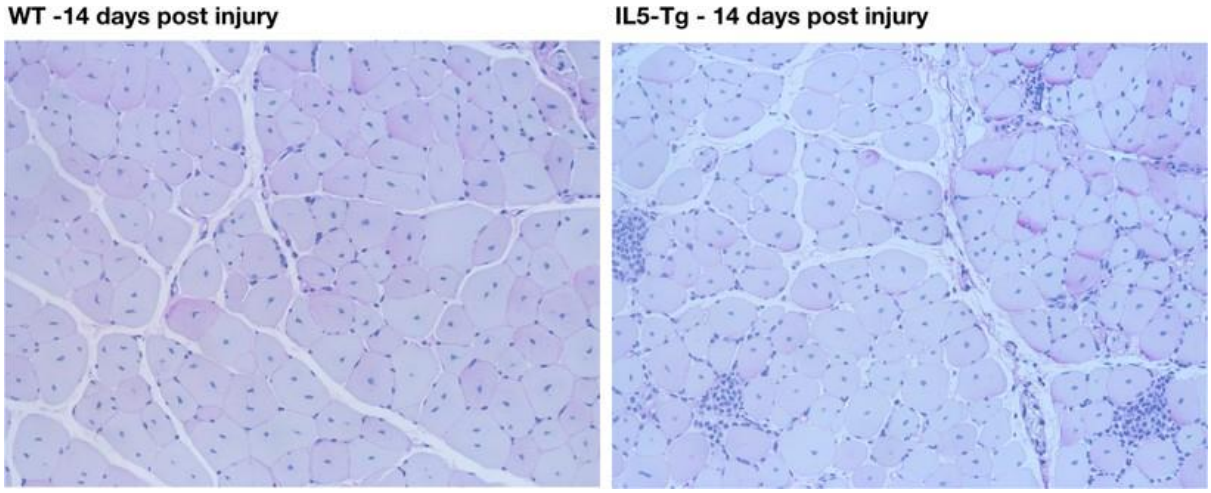

Figure R1: PAS staining on paraffin-embedded section of WT and IL5-Tg muscles 14 days after notexin damage.

6. The effects of increased eosinophil number on the proliferation of cells important to muscle regeneration were also investigated. These cells, FAP and subsequently MPs, are known to be stimulated by eosinophils, so their proliferation was studied in wounded IL5 and WT mice. Some transient increase in Edu+ MPs was observed, but overall their proliferation decreased, perhaps unexpectedly in the presence of high numbers of eosinophils.
I was unsure if MPs being stimulated and them proliferating are the same thing? This could be clarified. No effects of eosinophilia were seen on FAP proliferation (again it could be clarified if this is a good measure of stimulation), despite the changes on their downstream effectors, the MPs.
We refer to the response to comment #1, showing that while there is a difference in the expression of potentially myotoxic perforins in IL5-Tg eosinophils, the expression of *Il4*, through which eosinophils have been proposed to act on FAPs and indirectly on MPs, is downregulated in IL5-Tg eosinophils. We have also made changes in the manuscript to clarify the difference between stimulation and proliferation.

7. The effects of eosinophilia on macrophages was also studied. Eosinophils secreting TGFb andIL-4 can drive macrophages from the pro-inflammatory to pro-regenerative phenotype, however again perhaps surprisingly, high levels of eosinophils in the IL5 mice did not result in changes in the inflammatory/ regenerative balance of macrophages. However the authors do report an overall decrease in macrophage numbers. The authors conclude that muscle regeneration is affected by eosinophila. There is evidence for this statement in figure 1 A . The evidence for links between eosinophilia and muscle regeneration - effects on FAP and MP stimulation and the eosinophil mediated skewing of macrophages towards a regenerative phenotype – is less convincing and rather preliminary.
Regarding eosinophil and FAP/MP fate, please see the responses to comments #1 and #6.
The relatively small effect of eosinophilia on macrophage skewing is a novel and somewhat surprising finding. Eosinophils have been shown to be required for the maintenance of pro-regenerative macrophages in fat depots (Wu et al., 2011). In contrast, here we show that it does not have an effect in the context of acute skeletal muscle injury. The data from the RNAseq experiments confirm that IL5-Tg eosinophils are not classic Th2 effectors as they also express high levels of various Th1 markers (Dolitzky et al., 2021). We have measured the expression of Ly-6C on blood monocytes as well as tissue resident macrophages in skeletal muscle and fat at steady state and while we show a drastic change of the ratio in favour of Ly-6C- monocytes in the blood, we found the opposite was true in both muscle and adipose tissue (Figure 2). This suggests a coordinated systemic response to hyper-activated eosinophils. They are also consistent with a more modern view of the Th1/Th2 paradigm, away from the simplistic dogma that Th1 environments are associated with damage and Th2 pro-regenerative, and towards a view in which excessive polarization in either direction is deleterious, and the two types of environment need to be appropriately balanced for correct tissue homeostasis and repair.

Fig 2
8. A double Tg mdx:IL5 mouse was studied to see the effects of hyper-eosinophilia on this model of chronic disease. Mdx mice with eosinophilia died earlier than mdx alone, double Tg mice had less overall mass and some muscle mass in particular was decreased, small n numbers explainedsome lack of significance to the data.
In older double Tgs measurable effects on myofibres were observed although interestingly these changes at younger stages were different to those seen in the WT v IL5 mouse on acute wounding.
We have slightly increased the n of this experiment, however due to difficulties in breeding, we were able to only add one additional animal to the mdx:IL5-Tg group and two in the control group. Moreover, consistent with what we saw previously, this last mdx:IL5-Tg mouse was found dead at 6 months of age and harvested less than 24h post mortem. We have modified the graphs of Figure 3 (former Figure 2) and noted the post-mortem mice with a Δ symbol for transparency.
Lastly, It is believed that the response in younger mdx mice resembles acute damage, while older mdx mice display a more chronic inflammation / fibrotic phenotype. However, our results confirm that, not surprisingly, the phenotype in younger mdx and that in acutely damaged animals are not exactly the same.

9. Changes in older mice include reduction in myofiber size (although n.s.) and shape (more dramatic) and the authors speculate that this may reflect a known DMD phenotype. I was however unsure what the white asterisks were showing in Fig 2 G and H as there seem to be similar patterns elsewhere - this could be clarified to the reader as this being a reflection of the phenotype in humans is of course very interesting.
Needless to say the data would be improved with higher n numbers. PSR staining shows increased fibrosis in eosinophilic mice in various muscles including the diaphragm which they suggest is the likely cause of early death.

We have modified the star to an arrowhead symbol and we hope that it makes this clearer. As explained in comment #8, we have had issues with breeding, hence the low number of replicates. To partially compensate for this issue, we performed the microD experiments in Figure S4 (formerly Figure 3).

10. Fig 3 shows the effects of eosinophilia on the mass and myofibres of young double Tg mice (the myofibers of young double Tg mice are ok at 7 months) that have been repeatedly woundedto induce fibrotic scarring and model the chronic damage of DMD.
Muscle tissue mass loss in mdx mice as result of damage was exacerbated by eosinophilia (IL5, mdx mice). This reflected a defect in myofiber CSA, and was associated with increased collagenstaining and scarring.
Indeed, the microD experiment allowed us to confirm the results from the ageing experiment with higher n and more robust statistic tests. It is now clear that hyper- eosinophilia accelerates DMD histopathology, especially by inducing more fibrosis.

11. Typo on line 54, 'DMD' missing - I
think.Typo on line 147 'indicate/s'
Thank you for these corrections, we have made the changes.

Reviewer 2
Advance Summary and Potential Significance to Field:
12. In the present study, Theret et al. use in vivo mouse models to rigorously address the role of eosinophils in muscular dystrophy and regeneration following an acute injury. The study is significant as it aims to address an area in the field that is quite perplexing. The role of eosinophilsin muscle is complex, with distinct roles in acute msuscle damage versus chronic muscle disease. Further, the role of eosinophils seems to vary depending of the stage of disease with growing evidence suggesting that eosinophils promote muscle fibrosis in dystrophic mice.
However, a direct examination of the effect of increasing eosinophilia on muscle fibrosis has not been performed. The present study provides clear in vivo evidence through genetic models that increased eosinophils in dystrophic muscle exacerbates the development of fibrosis.
While we have not performed *in vitro* co-culture experiment with eosinophils and FAPs due to the extreme fragility of activated eosinophils, we think that Figure S4 (formerly Figure 3) is

sufficient to conclude that hyper-eosinophilia induces fibrosis. Furthermore, we have now generated RNAseq data showing IL5-Tg eosinophils express more *Tgfb1*, a well-known pro-fibrotic cytokine that has more recently been described to affect MP fusion (Girardi et al., 2021).

13. The authors further show that increased eosinophils following acute injury impairs muscle regeneration. Although well-conducted, the study would be benefit from a larger n for some experiments.
We have now added 2-3 n in the acute damage experiment and we tried our best to increasethe n for the *mdx*:IL5-Tg experiments, although we were only partially successful. Please see response to comment #8.

14. However, it is understandable that the increased mortality of dystrophic mice with increased eosinophilia places a significant barrier in achieving larger group size. The authors address this limitation through the use of younger mice undergoing microdamage.
Collectively, the findings are convincing and well-interpreted. These findings should have a significant impact in the field by addressing the role of eosinophil in later stages of muscular dystrophy.
Although I am overall enthused with this study, there are a few minor concerns/comments that require some editing of the manuscript before this study can be considered for publication. Please see below.
Thank you for this positive comment. We hope the new data we provide addresses the concerns below.

Reviewer 2 Comments for the Author:
15. There appears to be a disagreement between the present studies and those reported by Heredia et al. Do the muscle eosinophils in IL-5 Tg mice not express IL-4? If so, why is there a lack of an effect on the FAPs in the present study? A response can be incorporated into the discussion.
This is an excellent point. Indeed, our results and the results from Heredia et al., 2013 seem discordant, especially as the new RNAseq from WT and IL5-Tg eosinophils confirms that IL5-Tg eosinophils indeed express *Il4*.
Yet, we do not think that our results entirely oppose the previous findings. Indeed, we believe it is likely that an appropriate number of eosinophils may well have a positive effecton skeletal muscle regeneration, as proposed by Heredia et al., but based on our data this is clearly not the case when too many of these cells are present in the tissue. Their abundance and their inflammatory profile, like for macrophages for example, need to be finely tuned to ensure a successful repair process. We refer to the answers provided to comment #1 and we have added a sentence in the discussion.

16. The sentence seems incomplete. “of” what?
More recently, the lack of Dystrophin in satellite cells (muscle stem cells) 54 has been shown to impair their behavior and contributes to the pathogenesis of (Dumont et al., 55 2015).
We have corrected the sentence and added “DMD” (line 54)

17. I believe there is a typo in the sentence, below. Did the authors mean to cite figure 1D?
“Although we noticed an increase in activation one day post injury (Figure S1D) MP proliferation was decreased at 3 and 5 days post injury (Figure 1E), leading to an overall decrease in their total number (Figure 1E).”
The reference to the figure 1D was indeed missing, and we have now addressed this.

18. The use of red asterisks versus red p-values in the figure legend for various panels versus directly over the bar in a graph is confusing and not defined in the figure legend text.
The red stars represent the p-values of the comparison of the IL5-Tg to the WT mice. Weused red # in the Figure S2 to compare WT or IL5-Tg to Controls, and in the Figure S4 to compare the micro-D to the non-damaged side. We hope this now makes more sense andwe have checked all the figure legends.

19. Figure 3B is not cited in the results section, although the results are discussed.
The reference to the figure 3B was indeed missing, we apologize for this oversight. This isnow Figure S4B

20. Considering the following study https://pubmed.ncbi.nlm.nih.gov/24803842/ in relationship thestatement below.
“While muscle eosinophilia has previously been associated with myositis, it does not seem to be always associated with poor outcomes (Selva-O’Callaghan et al., 2014) and, while muscle eosinophilia has been investigated in autosomal-recessive limb girdle muscular dystrophies (LGMD2A/2C), to our knowledge, it has not been investigated in the DMD.”
The reviewer is correct and we have added this reference at this location

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Second decision letter

MS ID#: DEVELOP/2021/200112

MS TITLE: Elevated numbers of infiltrating eosinophils accelerate the progression of Duchenne Muscular Dystrophy pathology in mdx mice

AUTHORS: Marine Theret, Lucas Rempel, Joshua Hashimoto, Morten Ritso, Lin Wei Tung, Fang Fang Li, Melina Messing, Michael Hughes, Kelly McNagny, and Fabio MV Rossi
ARTICLE TYPE: Research Report

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 2

Advance summary and potential significance to field

The revisions have improved the quality of the manuscript and its findings are likely to have an impact in the field by advancing our understanding of the role of eosinophils in chronic muscle degenerative disorders (e.g. DMD)

Comments for the author

My prior comments were adequately addressed.