Myeloid-derived growth factor regulates neutrophil motility in interstitial tissue damage

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Review Timeline:

| Event                          | Date       |
|-------------------------------|------------|
| Submission Date               | 2021-03-09 |
| Editorial Decision            | 2021-04-06 |
| Revision Received             | 2021-04-25 |
| Editorial Decision            | 2021-04-26 |
| Revision Received             | 2021-05-05 |

Monitoring Editor: Ian Macara

Scientific Editor: Andrea Marat

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1083/jcb.202103054
April 6, 2021

Re: JCB manuscript #202103054

Dr. Anna Huttenlocher
University of Wisconsin Madison
Dept of Pediatrics & Medical Microbiol & Immunol University of Wisconsin 4225 Microbial Sciences Building
1550 Linden Drive
Madison, WI 53706

Dear Anna,

We have now received three external reviews of your interesting manuscript "Myeloid-derived growth factor regulates neutrophil motility in interstitial tissue damage", and I am pleased to report that all of them are very positive, with only minor issues that will need to be addressed in order to make the study suitable for publication by JCB. Most of the reviewer comments can be addressed through modifications to the text and/or figures. For instance, reviewer #1 questions the correctness of some statements about known MYDGF function in mice. This reviewer and #3 also ask if there are effects of MDGF on inflammatory cell types other than neutrophils (such as macrophages). Reviewer #2 raises a point about controls for the morpholino studies. Overall, we consider these issues relatively minor and revisions should not need a second external review.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:
Text limits: Character count for a Report is < 20,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Reports may have up to 5 main text figures. To avoid delays in production, figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, https://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

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Supplemental information: There are strict limits on the allowable amount of supplemental data. Reports may have up to 3 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.
As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing measures that limit spread of COVID-19 also pose challenges to scientific researchers. Lab closures or occupancy limits especially are preventing scientists from conducting experiments to further their research. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors to decide on an appropriate time frame for resubmission. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers’ comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you’ve had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Ian Macara, Ph.D.
Editor

Andrea L. Marat, Ph.D.
Senior Scientific Editor

Journal of Cell Biology

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Reviewer #1 (Comments to the Authors (Required)):

Houseright and colleagues have studied the roles of MYDGF in sterile zebrafish injury. The manuscript is well written. I have the following comments.

1) The official (NCBI, UniProt) symbol (gene, protein) of MYDGF is MYDGF (or mydgf, depending on species). The gene/protein should not be dubbed "MyDGF" as done here. This needs to be changed throughout.

2) It is claimed in the introduction that "neutrophils distinguish between self-damage and microbial cues". It is not clear what this really means and what the differences in neutrophil behavior in these two situations may be.

3) What is meant by "self"-damage? In the manuscript, fish are injured by transection and burning.

4) It is stated on page 3, lines 61-63 that MYDGF’s "biologic function remains unclear". This is not quite correct. Korf-Klingebiel et al. have shown how inflammatory cell-derived MYDGF enhances angiogenesis (via ERK/Stat3) and protects cardiomyocytes (via PI3K/Akt) and how it thereby improves myocardial infarct repair in mice.

5) It is stated on page 4, line 66 that "the role of MyDGF in regulating inflammation remains
unknown”. This is not quite correct. Korf-Klingebiel et al. have investigated at serial time points after myocardial infarction in mice how genetic deletion of MYDGF affects inflammatory cell accumulation in the infarcted heart (neutrophils, monocytes, macrophages, T cells, and B cells).

6) On page 21, lines 452-455, the authors speculate that "It is possible that cardiac tissue repair is improved in MyDGF-deficient mice because of the inhibitory effects of MyDGF on neutrophils. Indeed, there are transient increases in neutrophils at the infarcts in MyDGF-deficient mice (Korf-Klingebiel et al., 2015)". This wild speculation needs to be deleted. In the cited publication, MYDGF deficient mice have only slightly elevated neutrophil counts in the infarcted myocardium on day 3, not on days 1 and 7. This may very well be a chance finding (inflammatory cell counts were obtained under 15 different conditions) and there are no data in the literature, suggesting that such a (small) difference would be associated with differences (let alone improvements) in cardiac tissue repair.

7) I did not quite understand why the authors expressed and purified recombinant MYDGF in BL21(DE3) cells (pages 7-8) and also prepared crude MYDGF-transfected HEK293 cell supernatants (pages 9-10), which can be expected to contain hundreds of proteins besides MYDGF?

8) The authors have nicely demonstrated in loss-of-function and gain-of-function experiments, that MYDGF controls neutrophil recruitment and egress from sterile wounds in zebrafish. Are there effects on other inflammatory cell types? Considering that MYDGF promotes angiogenesis in mouse infarcts (a sterile wound), could MYDGF have an effect on angiogenesis in zebrafish wounds?

9) One of the coauthors (V.B.) has published data proposing MYDGF as an ER-resident protein. In this publication, MYDGF is presented as a growth factor (in agreement with other reports in the literature). As MYDGF is expressed in neutrophils themselves (Suppl. Fig. 1A), neutrophil behavior may be influenced in zebrafish from within (ER) or without (autocrine/paracrine from monocytes). Could this be experimentally addressed? At least some more discussion is needed.

10) Labeling in the data panels needs to be improved, color codes (dark rose, light rose, dark grey, light grey) and group allocations need to be better explained, preferably in the figure panels themselves. Are summary data presented with SD or SEM?

Reviewer #2 (Comments to the Authors (Required)):

Housерight and colleagues have submitted a manuscript demonstrating a role for MyDGF in the resolution of neutrophils during an inflammatory response. The gene was identified in a screen for genes that respond specifically to sterile injuries and the followup demonstrates that in the absence of MyDGF, neutrophils remain at the wound site for a longer period of time and as a consequence, inhibit the normal wound healing response. This work would be of general interest to the readers of JCB. I only have two minor comments:

1) The generally accepted control for morpholino studies is to use two different morpholinos with non-overlapping sequences to show the same phenotype (mRNA rescue is unlikely to work at the ages tested in this study). There are two MyDGF morpholinos listed, but it is unclear if both were used. Data for both should be included. Although the amt-1 morpholino has been published, I would still request a similar control be performed with another amt-1 morpholino. Alternatively both
experiments could be repeated with an orthogonal knockdown approach such as crispant targeting for each gene.

2) The authors show convincingly that MyDGF KO in zebrafish harms tissue regeneration, yet in the discussion the authors state "It is possible that cardiac tissue repair is improved in MyDGF-deficient mice because of the inhibitory effects of MyDGF on neutrophils." How do the authors explain these apparently contradictory observations?

Reviewer #3 (Comments to the Authors (Required)):

This is a nice, thorough study, in zebrafish, of the function of myeloid-derived growth factor (MyDGF) in regulation of neutrophil recruitment/retension/resolution to/from a site of inflammation. The authors identify MyDGF to be upregulated by inflammatory cells following mechanical or thermal lesioning of the larval zebrafish tail, but not post bug injection into the otic vesicle, suggesting this is a wound but not infection activated response. They go on to generate a CRISPR KO of MyDGF which has no morphological defects and is not needed for normal embryogenesis or developmental dispersal of immune cells (although the latter not explicitly demonstrated).

The authors then perform wounding studies on mydgf-/- larvae to test what the impact on inflammation might be. Wounding the otic vesicle of -/- fish results in increased neutrophil recruitment by comparison to control larvae (their sentence on line 361 might be less prone to ambiguity if it finished "control larvae", rather than just "control"). But if recombinant MyDGF is injected into the ear then neutrophil numbers are reduced in control larvae and even rescued back to low levels in the -/- fish. If, instead, bugs are injected into the ear then there is no apparent difference in recruitment of neutrophils to -/- versus wt ears, indicating that MyDGF limits neutrophil recruitment to damage, functioning like a brake, but not to infection.

The authors go on to make tail transection wounds in MyDGF -/- larvae and see retention of neutrophils through their altered migratory behaviour after having arrived at the wound. They then perform a dendra photoconversion study which confirms that there is more recruitment to the wound (whether mechanical or thermal) and less resolution away from the wound. Next they attempt to dissect the mechanism for MyDGF action and specifically to test whether it might be regulated by HIF signalling, which they achieve through use of a HIF1 disrupting morpholino targeting arnt-1; this treatment rescued wound recruited neutrophil numbers back to WT levels in MyDGF -/- larvae, and together with the observation of increased phd3 mRNA levels is quite suggestive that HIF1-a might be mediating these effects.

Whilst I really liked this study and I think its insights merit publication in JCB, I have several questions. What about macrophages; the authors need to tell us something about whether or not MyDGF impacts on macrophage wound recruitment? And on page 5 they tell us how similar zf MyDGF is to its human equivalent, so why not even a brief discussion of any insights from GWAS or other population health studies of associations with any clinical inflammatory conditions?

Lastly, and probably trivial, the authors generate a new antibody to zfish MyDGF for western studies; it would be nice know whether they tested whether their antibody is good for immunofluorescence imaging studies also?
Response to review

We thank the reviewers for their enthusiasm. Below we provide a point by point response to review.

Response to editor comments

Most of the reviewer comments can be addressed through modifications to the text and/or figures. For instance, reviewer #1 questions the correctness of some statements about known MYDGF function in mice.

We have revised the text based on the reviewer comments.

This reviewer and #3 also ask if there are effects of MDGF on inflammatory cell types other than neutrophils (such as macrophages).

We have included a new figure in the supplemental figures to address this concern. We did not observe a change in macrophage number or contacts with neutrophils. A new section is included in the results section to address this critique.

Reviewer #2 raises a point about controls for the morpholino studies.

We have included two separate morpholinos and found the same results that we observed with the mutant. This is generally a sufficient control. Rescue with overexpression of MYDGF was not effective because the overexpression of MYDGF RNA was toxic to the larvae.

Reviewer comments

Reviewer 1

1) The official (NCBI, UniProt) symbol (gene, protein) of MYDGF is MYDGF (or mydgf, depending on species). The gene/protein should not be dubbed "MyDGF" as done here. This needs to be changed throughout.

This has been corrected.

2) It is claimed in the introduction that "neutrophils distinguish between self-damage and microbial cues". It is not clear what this really means and what the differences in neutrophil behavior in these two situations may be.

The wording has been changed for clarity.

3) What is meant by "self"-damage? In the manuscript, fish are injured by transection and burning.
The manuscript has been edited for clarity.

4) It is stated on page 3, lines 61-63 that MYDGF's "biologic function remains unclear". This is not quite correct. Korf-Klingebiel et al. have shown how inflammatory cell-derived MYDGF enhances angiogenesis (via ERK/Stat3) and protects cardiomyocytes (via PI3K/Akt) and how it thereby improves myocardial infarct repair in mice.

The manuscript has been edited for clarity.

5) It is stated on page 4, line 66 that "the role of MyDGF in regulating inflammation remains unknown". This is not quite correct. Korf-Klingebiel et al. have investigated at serial time points after myocardial infarction in mice how genetic deletion of MYDGF affects inflammatory cell accumulation in the infarcted heart (neutrophils, monocytes, macrophages, T cells, and B cells).

We have edited this to say remains unclear.

6) On page 21, lines 452-455, the authors speculate that "It is possible that cardiac tissue repair is improved in MyDGF-deficient mice because of the inhibitory effects of MyDGF on neutrophils. Indeed, there are transient increases in neutrophils at the infarcts in MyDGF-deficient mice (Korf-Klingebiel et al., 2015)". This wild speculation needs to be deleted. In the cited publication, MYDGF deficient mice have only slightly elevated neutrophil counts in the infarcted myocardium on day 3, not on days 1 and 7. This may very well be a chance finding (inflammatory cell counts were obtained under 15 different conditions) and there are no data in the literature, suggesting that such a (small) difference would be associated with differences (let alone improvements) in cardiac tissue repair.

We have revised the manuscript as suggested by the reviewer.

7) I did not quite understand why the authors expressed and purified recombinant MYDGF in BL21(DE3) cells (pages 7-8) and also prepared crude MYDGF-transfected HEK293 cell supernatants (pages 9-10), which can be expected to contain hundreds of proteins besides MYDGF?

We used the recombinant MYDGF for generation of the antibody and the MYDGF transfected cells for the rescue experiment because of issues with protein folding.

8) The authors have nicely demonstrated in loss-of-function and gain-of-function experiments, that MYDGF controls neutrophil recruitment and egress from sterile wounds in zebrafish. Are there effects on other inflammatory cell types? Considering that MYDGF promotes angiogenesis in mouse infarcts (a sterile wound), could MYDGF have an effect on angiogenesis in zebrafish wounds?
We have now included a new supplemental figure 3 showing that there are no specific defects in macrophage recruitment.

9) One of the coauthors (V.B.) has published data proposing MYDGF as an ER-resident protein. In this publication, MYDGF is presented as a growth factor (in agreement with other reports in the literature). As MYDGF is expressed in neutrophils themselves (Suppl. Fig. 1A), neutrophil behavior may be influenced in zebrafish from within (ER) or without (autocrine/paracrine from monocytes). Could this be experimentally addressed? At least some more discussion is needed.

Due to space limitations, this is beyond the scope of the current paper.

10) Labeling in the data panels needs to be improved, color codes (dark rose, light rose, dark grey, light grey) and group allocations need to be better explained, preferably in the figure panels themselves. Are summary data presented with SD or SEM?

Clarification in the figure legend is now included. We have reviewed the labeling concerns and do not agree with the reviewer.

Reviewer 2

1) The generally accepted control for morpholino studies is to use two different morpholinos with non-overlapping sequences to show the same phenotype (mRNA rescue is unlikely to work at the ages tested in this study). There are two MyDGF morpholinos listed, but it is unclear if both were used. Data for both should be included. Although the arnt-1 morpholino has been published, I would still request a similar control be performed with another arnt-1 morpholino. Alternatively both experiments could be repeated with an orthogonal knockdown approach such as crisptant targeting for each gene.

We included two distinct morpholinos (this has been clarified) and found similar phenotypes that recapitulated the mutant phenotype. This the accepted controls for MO. It is also standard to include just one arnt1 MO with an established MO (published in Blood).

2) The authors show convincingly that MyDGF KO in zebrafish harms tissue regeneration, yet in the discussion the authors state "It is possible that cardiac tissue repair is improved in MyDGF-deficient mice because of the inhibitory effects of MyDGF on neutrophils." How do the authors explain these apparently contradictory observations?

Thank you for picking up this error. The cardiac repair was impaired in the MYDGF-deficient mice. This has been corrected.
Reviewer 3

Whilst I really liked this study and I think its insights merit publication in JCB, I have several questions.

What about macrophages; the authors need to tell us something about whether or not MyDGF impacts on macrophage wound recruitment?

We have now included a new supplemental figure 3 to show the macrophage data.

And on page 5 they tell us how similar zf MyDGF is to its human equivalent, so why not even a brief discussion of any insights from GWAS or other population health studies of associations with any clinical inflammatory conditions?

This is an important point. Because of the limited space this is beyond the scope of the current paper.

Lastly, and probably trivial, the authors generate a new antibody to zf MyDGF for western studies; it would be nice know whether they tested whether their antibody is good for immunofluorescence imaging studies also?

We tried this but it did not work for immunofluorescence and have added a sentence about this in the methods section.
April 26, 2021

RE: JCB Manuscript #202103054R

Dr. Anna Huttenlocher
University of Wisconsin Madison
Dept of Pediatrics & Medical Microbiol & Immunol University of Wisconsin 4225 Microbial Sciences Building
1550 Linden Drive
Madison, WI 53706

Dear Anna,

Thank you for submitting your revised manuscript entitled "Myeloid-derived growth factor regulates neutrophil motility in interstitial tissue damage". We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

1) Text limits: Character count for Reports is < 20,000, not including spaces. Count includes title page, abstract, introduction, combined results and discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

2) Figures limits: Reports may have up to 5 main text figures.

3) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (either in the figure legend itself or in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, if you used parametric tests, please indicate if the data distribution was tested for normality (and if so, how). If not, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."
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7) Please be sure to provide the sequences for all of your primers/oligos and RNAi constructs in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers (where appropriate) for all of your antibodies. Please also indicate the acquisition and quantification methods for immunoblotting/western blots.

8) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
   a. Make and model of microscope
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   c. Temperature
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   f. Camera make and model
   g. Acquisition software
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9) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

10) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Reports may have up to 3 supplemental display items (figures and tables). Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section.

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12) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."

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14) A separate author contribution section following the Acknowledgments. All authors should be mentioned and designated by their full names. We encourage use of the CRediT nomenclature.

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB’s Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and video files: See our detailed guidelines for preparing your production-ready images, https://jcb.rupress.org/fig-vid-guidelines.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

**It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.**

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Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Ian Macara, Ph.D.
Editor

Andrea L. Marat, Ph.D.
Senior Scientific Editor

Journal of Cell Biology
