CD301 mediates fusion in IL-4-driven multinucleated giant cell formation

Patricia J. Brooks, Yongqiang Wang, Marco A. Magalhaes, Michael Glogauer and Christopher A. McCulloch
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Original submission

First decision letter

MS ID#: JOCES/2020/248864

MS TITLE: The C-type lectin CLEC10 Mediates Fusion in IL-4-Driven Multinucleated Giant Cell Formation

AUTHORS: Patricia J Brooks, Yongqiang Wang, Marco Magalhaes, Michael Glogauer, and Christopher A McCulloch
ARTICLE TYPE: Research Article

First of all, I am very sorry for the long delay in getting the reviews back on your manuscript. As you will see both reviewers have suggested improvements to the manuscript that should not be too arduous and involve substantial new experimentation.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. 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 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. Please attend to all of the reviewers' comments. 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

In this manuscript, Brooks et. al. investigated the molecules that determine the formation of multinucleated giant cells (MGCs). The authors revealed that CD301b/Mgl2, a C-type lectin receptor, was strongly upregulated in primary murine macrophages and mouse RAW264.7 macrophages undergoing IL-4-induced MGC formation. Function-inhibiting CD301 antibodies or deletion of the mouse CD301b significantly inhibited IL-4-induced ligand binding and reduced MGC formation. In addition, CD301-positive MGCs were identified in biopsy specimens of human inflammatory lesions and human homolog of CD301b mediated IL-4-induced human MGC formation. The authors conclude that in monocytes, IL-4 increases CD301b expression, which mediates intercellular adhesion and fusion required for the formation of MGCs.
This is an interesting study identifying a novel component in IL-4-induced MGC formation Â– Mgl2 or CD301b in mouse and human, respectively. Experiments are well designed and results are clearly presented.

Comments for the author

1. In Fig. 1E, the authors stated that CD301 showed increased abundance at plasma membrane with the increased number of nuclei per cell. However, the images show evenly distributed CD301 in the cytoplasm. In addition, some multinucleated cells in Fig. 3A appear to be CD301 negative. Co-staining with a cell membrane marker and showing the single Z layer of confocal image would help presenting the data more clearly. Western blotting for CD301 in the cell membrane vs. cytosolic fractions could also be used to validate this hypothesis.

2. The authors showed that the transcription of Mgl1 and Mgl2 is upregulated in RAW264.7 cells by IL-4 treatment although “the other groups of cells” show no change of expression of these two genes. The authors should provide more details of “the other groups of cells”. What are these cells and what cytokines were used to treat these cells?

3. The numbers of quantified cells in Fig. 2E are too small (<2 cells per group). Or is there a labeling error along the Y axis?

4. The authors stated that introducing exogenous Mgl2 was able to bring the fusion rate of Mgl1&Mgl2 dKO cells without IL-4 treatment to the level of WT cells under IL-4 treatment, indicating that Mgl2 is the major mediator of IL-4-induced MGC formation. However, the images in Fig. 4B shows dramatically less fusion of DKO rescue cells without IL-4, compared to the DKO rescue cells with IL-4, although the qualification shows similar level of fusion between the two groups. More representative images should be shown.

5. It is difficult to see fused cells in all the fluorescent images. It would be very helpful if all multinucleated cells need are outlined on these images.

6. CLEC10 is used in the title and only two other places. CD301 should be included in the title.

7. Statistical significance (P-values) are missing in Fig. 1A.

8. Representative images need to be provided for Fig. 5D.

Reviewer 2

Advance summary and potential significance to field

The authors aim was to uncover mechanisms of multinucleated giant cell formation in response to IL-4 treatment of monocytes. Using murine monocytes treated with cytokines combined with isotope-labeling, they identified that mgl2 (CD301 in human and also known as CLEC-10) was substantially increased in monocytes treated with IL-4. Blocking of Mgl2 or deletion of Mgl1 and Mgl2 substantially reduced the formation of MGCs. Interestingly, RANKL treatment of monocytes was unaffected by the loss of mgl2 suggesting that multinucleation induced by RANKL is mgl2-independent. Overall this is a very interesting study and provides important insight into the mechanisms driving MGC formation.

Comments for the author

Major Comments:
1. The data shown in Figure 1E and quantified in Figure 1F sre meant to demonstrate the membrane localization of Mgl proteins (anti-CD301 antibody), but the images do not reflect a membrane staining and in fact show more distribution in the cytoplasm. The authors should either show more representative images, perform z-stack analysis of treated cells, or perform a biotin labeling of treated cells to more effectively quantify the levels of Mgl1/2 (CD301) on the cell surface of IL-4 treated cells.

2. I would suggest that the authors show enlarged images of those shown in Figure 2C, since they quantify the data in Figure 2D. As shown, the images are too small to appreciate the fusion that has happened or not in the presence or absence of the blocking antibody. This is also true for Figure 4B and 4C.

3. In panel 2E it is unclear how the multinucleated cells were quantified based on the legend. What are the values? Is it 2 cells with 6-9 nuclei per XX number of cells counted? Field of view? This needs to be better explained.

4. In Figure 5A it is again very difficult to see what cells are being considered/quantified as being fused/multinucleate. I would suggest either showing representative enlarged images of the fused cells and place the images shown in Figure 5A in a supplemental figure.

Minor Comments:
1. Placing the labels (A, B, C, ...) in the figures below the data panels are quite distracting. It is much more commonplace to have these labels adjacent to the top of the panel.
3. Number of technical and experimental replicates should be mentioned in the figure legends where statistical methods are employed.
2. there are a few typos throughout the manuscript that need attention.

First revision

Author response to reviewers' comments

Responses to Previous Reviews

Reviewer 1 Comments for the Author:
1. *In Fig. 1E, the authors stated that CD301 showed increased abundance at the plasma membrane with the increased number of nuclei per cell. However, the images show evenly distributed CD301 in the cytoplasm. In addition, some multinucleated cells in Fig. 3A appear to be CD301 negative. Co-staining with a cell membrane marker and showing the single Z layer of confocal image would help presenting the data more clearly. Western blotting for CD301 in the cell membrane vs. cytosolic fractions could also be used to validate this hypothesis.*

We have followed up with this helpful idea and now include a new figure (Figure 2) with two different approaches for examining the spatial relationship between CD301 and plasma membrane labeling with concanavalin A; separately, we prepared plasma membrane and cytosolic fractions and immunoblotted these fractions for a membrane protein and for CD301. We provided detailed methods for these new data in the revised manuscript.

2. *The authors showed that the transcription of Mgl1 and Mgl2 is upregulated in RAW264.7 cells by IL-4 treatment although "the other groups of cells" show no change of expression of these two genes. The authors should provide more details of "the other groups of cells". What are these cells and what cytokines were used to treat these cells?*

In the revised manuscript we improved the wording of the text to indicate that the groups were treated with no cytokines, RANKL, IL-1¤, and TNF¤ and that these groups showed no change in expression of these genes.

3. *The numbers of quantified cells in Fig. 2E are too small (<2 cells per group). Or is there a labeling error along the Y axis?*

We revised the text in the Figure Legend and the Results and the y-axis title of the figure itself to indicate that this histogram shows the mean number of cells with the identified numbers of nuclei per high power microscopic field.

4. *The authors stated that introducing exogenous Mgl2 was able to bring the fusion rate of Mgl1&Mgl2 dKO cells without IL-4 treatment to the level of WT cells under IL-4 treatment, indicating that Mgl2 is the major mediator of IL-4- induced MGC formation. However, the images in Fig. 4B shows dramatically less fusion of DKO rescue cells without IL-4, compared to the DKO rescue cells with IL-4, although the qualification shows similar level of fusion between the two groups. More representative images should be shown.*

In the new Figure 5B we highlighted (by circling) those cells with at least 3 nuclei per cell to more clearly indicate the fused cells.

5. *It is difficult to see fused cells in all the fluorescent images. It would be very helpful if all multinucleated cells are outlined on these images.*

We addressed this suggestion by highlighting the multinucleated cells.

6. *CLEC10 is used in the title and only two other places. CD301 should be included in the title.*

We corrected this point as suggested.

7. *Statistical significance (P-values) are missing in Fig. 1A.*

We added these values as suggested to the figure.

8. *Representative images need to be provided for Fig. 5D.*

We assembled a new figure (Figure 6) and in this figure, panel E now provides the representative images that were requested.

Reviewer 2 Comments for the Author:

Major Comments:

1. The data shown in Figure 1E and quantified in Figure 1F are meant to demonstrate the membrane localization of Mgl proteins (anti-CD301 antibody), but the images do not reflect a membrane staining and in fact show more distribution in the cytoplasm. The authors should either show more representative images, perform z-stack analysis of treated cells, or perform a biotin

labeling of treated cells to more effectively quantify the levels of Mgl1/2 (CD301) on the cell surface of IL-4 treated cells.

This suggestion is very much in line with the first reviewer's comment and we have addressed this point by the addition of the data shown in the new Figure 2.

2. I would suggest that the authors show enlarged images of those shown in Figure 2C, since they quantify the data in Figure 2D. As shown, the images are too small to appreciate the fusion that has happened or not in the presence or absence of the blocking antibody. This is also true for Figure 4B and 4C.

We have circled enlarged images of these cells to facilitate their identification.

3. In panel 2E it is unclear how the multinucleated cells were quantified based on the legend. What are the values? Is it 2 cells with 6-9 nuclei per XX number of cells counted? Field of view? This needs to be better explained.

This commentary is very similar to the first reviewer's comment and we have now corrected in this text and in the figure as described in our response to point #3 above.

4. In Figure 5A it is again very difficult to see what cells are being considered/quantified as being fused/multinucleate. I would suggest either showing representative enlarged images of the fused cells and place the images shown in Figure 5A in a supplemental figure.

We have addressed this useful commentary by drawing circles around the multinucleated cells as suggested above.

Minor Comments:

1. Placing the labels (A, B, C, ...) in the figures below the data panels are quite distracting. It is much more commonplace to have these labels adjacent to the top of the panel.

We moved all of the figure labeling to the top of the data panels as suggested.

2. Number of technical and experimental replicates should be mentioned in the figure legends where statistical methods are employed.

We have now indicated the sample sizes for all experiments in the Statistical analysis section of the revised manuscript.

3. There are a few typos throughout the manuscript that need attention.

We found 7 typographical errors in the manuscript which have now been corrected.

Second decision letter

MS ID#: JOCES/2020/248864

MS TITLE: The C-type lectin CLEC10 Mediates Fusion in IL-4-Driven Multinucleated Giant Cell Formation

AUTHORS: Patricia J Brooks, Yongqiang Wang, Marco Magalhaes, Michael Glogauer, and Christopher A McCulloch
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but reviewer 1 has raised some critical points that will require amendments to your manuscript. I don't feel that a new membrane marker aside from ConA needs to be pursued. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

We are aware that you may be experiencing disruption to the normal running of your lab that makes 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.

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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. Please attend to all of the reviewers' comments. 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

Comments:

In the revised manuscript, the authors made efforts to address most of my comments. In particular, they used ConA to label the plasma membrane. However as shown in Fig. 2A, ConA appears to primarily stain the reticular network in the cytosol instead of the plasma membrane. Most likely, ConA was internalized during the incubation process, and therefore cannot be used as a reliable plasma membrane marker here. The authors should consider using a plasma membrane-targeted fluorescent protein to mark the membrane.

Comments for the author

Other comments:

1. How many cells or fields in total were quantified per group for Fig. 3E? The numbers of cells showed in the Y axis are very small (<2 cells per group). It is necessary to count more cells.

2. The authors stated that GalNAc treatment promoted THP-1 cells fusion to a similar level of IL-4 treatment alone. However, Fig. 6D appears to show significantly less fusion in M-CSF+GalNAc cells compared to M-CSF+IL4 cells. The authors should address the inconsistency between the image and graph.

3. It would be very helpful to outline multinucleated cells by tracing the plasma membrane instead of using red circles, which are rather inaccurate.

Reviewer 2

Advance summary and potential significance to field

The authors have satisfactorily addressed my concerns. I feel that the manuscript should now be acceptable for publication in the JCS.

Comments for the author

The authors have satisfactorily addressed my concerns. I feel that the manuscript should now be acceptable for publication in the JCS.

Second revision

Author response to reviewers' comments

November 5, 2020

Daniel Billadeau
Editor, Journal of Cell Science

Dear Dr. Billadeau,

Re: JOCES/2020/248864
TITLE: The C-type lectin CLEC10 Mediates Fusion in IL-4-Driven Multinucleated Giant Cell Formation
AUTHORS: Patricia J Brooks, Yongqiang Wang, Marco Magalhaes, Michael Glogauer, and Christopher A McCulloch

Thanks for arranging for the review of this paper and in particular for enabling the really helpful comments provided by the reviewers. Indeed, your organization and recruitment of the reviewers and the provision of their comments has allowed us to really improve the quality of the manuscript. We have followed your suggestions and not pursued the new membrane marker but we have made all of the other suggested modifications. These modifications are described below and are noted in the revised manuscript with yellow highlighting. For responding to the reviewer's comments, we respond below each point with our text.

Reviewer 1 Comments

In the revised manuscript, the authors made efforts to address most of my comments. In particular, they used ConA to label the plasma membrane. However, as shown in Fig. 2A, ConA appears to primarily stain the reticular network in the cytosol instead of the plasma membrane. Most likely, ConA was internalized during the incubation process, and therefore cannot be used as a reliable plasma membrane marker here. The authors should consider using a plasma membrane-targeted fluorescent protein to mark the membrane.

Response: As suggested by the Editor, we have not pursued this issue further.

Other comments

1. How many cells or fields in total were quantified per group for Fig. 3E? The numbers of cells showed in the Y axis are very small (<2 cells per group). It is necessary to count more cells.

Response: We have possibly written this section in a manner that was confusing and consequently we have re-written this. The data that are shown in this figure were obtained from at least 25, high power microscopic fields that contained at least 5 or more cells with >2 nuclei per cell. The y-axis label refers to the % of cells in each field that contained more than two nuclei and these data

were then grouped into two (3-5 nuclei per cell; 6-9 nuclei per cell). We have re- written the legend for Figure 3E as follows: “Quantification of number of cells with >2 nuclei per high power field. Data were obtained from analysis of at least 25 high power microscope fields. The data from the groups were divided into multinucleated cells with 3-5 nuclei/cell or cells with 6-9 nuclei/cell.”

2. The authors stated that GalNAc treatment promoted THP-1 cells fusion to a similar level of IL-4 treatment alone. However, Fig. 6D appears to show significantly less fusion in M-CSF+GalNAc cells compared to M-CSF+IL4 cells. The authors should address the inconsistency between the image and graph.

Response: We recognize and appreciate the reviewer’s point. We have now highlighted (with red dashed lines) the multinucleated cells for the various treatments in Figure 6D and the result of this clarification shows in the image, similar fusion in the M-CSF+GalNAc-treated cells compared with the M-CSF+IL4-treated cells, which is consistent with the histogram in Figure 6d.

3. It would be very helpful to outline multinucleated cells by tracing the plasma membrane instead of using red circles, which are rather inaccurate.

Response: We have outlined the plasma membrane of the cells in Figure 5C with red dashed lines as suggested to facilitate the locale of the multinucleated cells.

Reviewer 2 Comments for the Author
The authors have satisfactorily addressed my concerns. I feel that the manuscript should now be acceptable for publication in the JCS.

Response: We appreciate the reviewer’s support of the manuscript as was submitted.

Yours sincerely,
Chris McCulloch

Third decision letter

MS ID#: JOCES/2020/248864

MS TITLE: The C-type lectin CLEC10 Mediates Fusion in IL-4-Driven Multinucleated Giant Cell Formation

AUTHORS: Patricia J Brooks, Yongqiang Wang, Marco Magalhaes, Michael Glogauer, and Christopher A McCulloch
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.