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# Microglial PD-1 modulates neuroinflammation and Alzheimer's disease pathology by astrocytic PD-L1

Markus Kummer, Christina Ising, Christiane Kummer, Heela Sarlus, Angelika Griep, Ana Vieira-Saecker, Stephanie Schwartz, Annett Halle, Matthias Brückner, Kristian Händler, Joachim Schultze, Marc Beyer, Eicke Latz, and Michael Heneka
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## Review Timeline:


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|---|---|
| Submission Date: | 6th May 21 |
| Editorial Decision: | 2nd Jun 21 |
| Revision Received: | 27th Sep 21 |
| Accepted: | 25th Oct 21 |

*Editor: Karin Dumstrei*


## 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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Hi Michael,

Thanks for submitting your manuscript to The EMBO Journal. Your study has now been seen by two referees and their comments are provided below.

As you can see from the comments, both referees find the study interesting and suitable for publication in The EMBO Journal. The referees raise a few comments that should be fairly straightforward to sort out. Let me know if we need to discuss the points further - Happy to do so!

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Please also take a look at the attached Document for helpful tips on how to format the revision

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 31st Aug 2021.

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Referee #1:

In this paper, Kummer et al first observed the expression of PD-L1 in plaque-associated astrocytes from both AD patients and APP/PS1 mice, showing that astrocytic PD-L1 might be the major source of elevated CSF PD-L1 observed in AD patients. They next characterized the PMA-induced cleavage and γ-secretase-mediated cleavage of PD-L1 in different cell lines (HEK, Hela, C6 astrocytoma) and in vitro cultured mouse astrocytes. Then, Kummer et al focused on the function of microglial PD-1 in AD pathology and demonstrated that PD-1 KO APP/PS1 mice developed more Aβ plaques (quantified by IF staining and ELISA) and had impaired cognitive behaviors (Morris water maze) compared to APP/PS1 mice. This might be caused by defects of Aβ phagocytosis by PD-1-deficient microglia, as the authors showed that microglia with higher phagocytic ability expressed more PD-1, while PD-1 KO microglia expressed lower level of scavenger receptor CD36. Moreover, when comparing to WT microglia, the transcriptomic profiles PD-1 KO microglia were enriched in complement-, inflammasome- and pro-inflammatory cytokine-related gene sets, which is consistent with the immune suppressive function of PD-1.

The function of PD-1-PD-L1 axis in AD has been a long-standing debate in the past few years. Though some studies showed that AD mice treated with anti-PD1 antibodies had increased recruitment of monocyte-derived macrophages and exhibited less severe AD pathology, these results have been controversial. Here, Kummer et al provide new important insights on how PD-1 affect AD pathogenesis proposing that PD-1 is important for sustaining the phagocytic function of microglia. This paper is outstanding, convincing, well-performed and will contribute to the debate with new exciting and informative data. I have a few comments that the authors may consider addressing.

Major points

1. In figure 3, the authors show images that are a bit diffuse/odd together with FACS staining on cultured microglia stimulated with LPS o Abeta. It would be helpful to provide a better image and indicate the % of PD1+ microglia and how cells were gated (were dead cells excluded). The authors may consider to perform a FACS staining on microglia acutely isolated from APP/PS1 e APP/PS1 x PD1 -/-, if mice are available.
2. The data on γ-secretase cleavage of PD-L1 seems secondary to the main topic of the study, as the secretion of soluble sPD-L1 is mainly dependent on the PMA-induced S2 cleavage, not γ-secretase cleavage. Also, it has been reported that ADAM10

and ADAM17 can cleave PD-L1 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185206/), so the authors may consider reducing the focus on PMA-induced S2 cleavage in cell lines.
3. In Fig. 5B, the authors compared the effect of WT and PD-L1-KO astrocyte conditioned medium and found that astrocyte-derived sPD-L1 is important for microglia-mediated Aβ uptake. Overall, this is a nicely designed experiment and the results are convincing. However, it remains possible that there are variables between WT and PD-L1 KO astrocyte conditioned medium other than the level of sPD-L1, and these unknown variables could also contribute to the difference in microglia phagocytosis. The authors should cautiously discuss this possibility or, if possible, repeat the experiment including an anti-PD-L1 antibody to see if they abolish microglial Aβ uptake.
4. Since a previous paper has shown that PD-1 blockade can help recruit monocyte-derived macrophages, I wonder if the authors have observed a difference in CCR2 expression level in their RNA-seq of WT and PD-1 KO microglia. Alternatively, they could quantify the abundance of CCR2+ cells in ex vivo microglia by flow cytometry to see if there's a difference between APP/PS1 and PD-1 KO APP/PS1 mice.

Minor comments
1. In Figure 2A, the authors detected PD-L1-CTF in DAPT-treated cells. However, the γ-secretase cleavage generates a shorter form of PD-L1-CTF, and should be detectable by Myc antibodies in the non-DAPT-treated cells (the third and fourth lanes). Could the authors explain why they didn't see those lower MW bands? Maybe they get rapidly degraded?
2. For Figure S8: it's hard to tell if there's a difference in Iba1 expression just by looking at microscopy images. It would be better if the authors could quantify the Iba1 coverage or Iba1+ microglia number.
3. The paper contains various errors and missing points, and needs further proof-reading. For example, the IF staining in Fig. 3B is CD11b according to the figure legend, but it is labeled as Iba1 in the figure; also, there is a figure legend for Fig. S3B, but Fig. S3 only has one panel; the authors may include the experimental procedure for Fig. 1F and Fig. 2 (concentration of PMA, IFNγ, TNFα, etc.); it's not mentioned in figure legend, methods, or anywhere in the main text; in the figure legend of 2F, the authors say that "lysate and CM were immunoblotted using a Myc antibody", but I don't think they can detect sPD-L1 with Myc antibody, so I assume that they are using AF1019 for CM? Please make sure that the methods and figure legends are accurate and detailed enough.

Referee #2:

In this manuscript, Kummer at al. proposed that astrocyte-secreted PD-L1 stimulated microglia via PD-1 signaling. Such astrocytic-microglial interaction enhanced CD36 expression and microglia capacity for uptaking Aß42, resulting reduced amyloid deposition and alleviation the cognitive deficits observed in AD disease.

The authors start by evaluating the PD-L1 expression in AD patients, observing increased PD-L1 levels in the cerebrospinal fluid of AD patients They verified this phenomenon in mice, and observed increased expression of PD-L1 by astrocytes surrounding the amyloid plaques. The PD-L1 expression apparently is an early phenomenon, as it is observed early on (4 months) when few amyloid plaques are detected. They performed a series of experiments in various cell lines, aiming to explain the shedding off of PD-L1 from astrocytes, concluding that PD-L1 is released by juxtamembrane cleavage by a PMA-inducible protease, with a further processing of the remaining fragment by a -secretase complex.

They then showed that PD-1 deficiency in APP/PS mice leads microglial changes, characterized by a decrease in CD36 expression and enhanced expression of inflammatory and coagulation markers. Such changes correlated with increase amyloid deposition in cortical and hippocampal areas and impaired spatial memory in APP/PS PD-1-/- mice.

Comments:

1. The authors suggest that PD-L1 is expressed on astrocytes and PD1 is expressed on microglia during AD disease. How about the opposite pattern? Could Aß42 induce PD-L1 expression on microglia and PD1 expression on astrocytes? Or at least a subset of them?

2. In AD patients, it seemed that PD-L1 was increased in CSF, but they proposed the beneficial function of PD-L1 in AD disease. To explain this contradiction, they suspect that "an unresolved, chronic inflammation that causes microglial exhaustion and disfunction is ongoing and that at very late diseases stages this immune checkpoint is not functional anymore (Dong & Chen, 2006)", furthermore they mentioned that "it has been reported that prolonged states of negative immune regulation results in PD-1 mediated immune exhaustion that goes along with reduced cell motility (Oxford, 2013)".

If PD-1 signaling could enhance clearance of amyloid but also induce immune exhaustion and associated dysfunction, it is probably important to strengthen PD1 signaling and release exhaustion by blocking other exhaustion-related factors (not PD1) for treatment of AD disease, they should more clearly discuss this aspect.

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It is mentioned that "but also at early time points at 4 months of age when very few amyloid plaques are present (Fig. 1 E, S1) implying that upregulation of PD-L1 is an early phenomenon.", Please clarify which data represented 4 months of APP/PS1 mouse in supplementary figure 1 legend.

Do the authors have the image of whole brain and quantitative ELISA results to compare the amount of 4-months and 9-months one?

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Author mentioned that "Animal; For the immunohistochemistry, biochemistry and in vivo-phagocytosis assay only female mice were used, whereas for the behavioral analysis mixed gender groups were used."
Female APP/PS1 mice have more amyloid plaque number and amount compared with same aged male mice. And the amyloid depositions started to appear earlier in female compared with male (Jun W et al (2003)).

Could the author observe increased amyloid plaques in male PD-1 deficient APP/PS1 mice compared with male APP/PS1 mice? Was there any correlation between the amount of amyloid and cognitive deficits both in male and female PD-1 deficient APP/PS1 mice?

Please mention if possible the gender of AD patients and control.

**Point-by-point response to Referees**
Referee comments in black, author responses in blue

Referee #1:

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Major points

**1.** In figure 3, the authors show images that are a bit diffuse/odd together with FACS staining on cultured microglia stimulated with LPS o Abeta. It would be helpful to provide a better image and indicate the % of PD1+ microglia and how cells were gated (were dead cells excluded).
Ad 1.1: Our imaging data (Fig. 3 A, B) show expression of PD-1 in microglia surrounding plaques in humans and mice. As microglia surround the plaque, the cells have close contact to Aβ (Fig. 3 B, second image) and those plaque-associated microglia show increased PD-1 mRNA levels (Fig. 3 C). These results prompted us to investigate if inflammatory stimuli such as Aβ are able to directly induce PD-1 expression on microglia. This, we then show in our flow cytometry analysis of microglia in culture, where treatment with either LPS or Aβ were sufficient to increase PD-1 levels on the cell membrane. For the flow cytometry analysis of cultured cells, no live/dead stain was applied. We exchanged the data set on PD-1 expression on microglia shown in Fig. 3 D to provide a better representation of our results and modified the appearance of the plot. Cells were pre-gated for CD11b-positivitiy, this information together with the % of PD-1-positive cells (29.6% in the control sample, 47.5% and 43.3% in LPS- and Aβ -treated samples, respectively) is included in the figure now.

The authors may consider to perform a FACS staining on microglia acutely isolated from APP/PS1 e APP/PS1 x PD1 -/-, if mice are available.
Ad 1.2: In Figure 5, we already show the quantification of FACS data from microglia acutely isolated from APP/PS1 and APP/PS1 PD-1$^{-/-}$ mice (Fig 5 D-G). Mice received a methoxy-XO4 injection prior to microglia isolation. As indicated by their positivity for the dye methoxy-XO4, we found less amyloid-positive microglia in APP/PS1 PD-1$^{-/-}$ mice (Fig. 5 D) together with less CD36 expression (Fig. 5 E) in those cells as compared to methoxy-XO4-positive microglia from APP/PS1 mice. Levels of CD11b and CD45 remained unchanged between the genotypes (Fig 5 F, G).
To complement the in vitro result on PD-1 increases upon Aβ treatment, we provided data on microglia from APP/PS1 and control mice (Fig. 3). Here, we show colocalization of PD-1 and Iba1 by immunohistochemistry (Fig 3 B) and increased RNA levels of PD-1 especially in plaque-associated microglia (Fig. 3 C).
In our flow cytometry analysis from mouse samples, a 7-AAD staining was performed to exclude dead cells from the analysis. This information was added to the methods section.

**2.** The data on γ-secretase cleavage of PD-L1 seems secondary to the main topic of the study, as the secretion of soluble sPD-L1 is mainly dependent on the PMA-induced S2 cleavage, not γ-secretase

cleavage. Also, it has been reported that ADAM10 and ADAM17 can cleave PD-L1 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185206/), so the authors may consider reducing the focus on PMA-induced S2 cleavage in cell lines.

Ad 2: PD-L1 can reportedly be found in the CSF (Saftig and Reiss, Semin Cell Dev Biol 2008) and we found increased levels of PD-L1 in the CSF of AD patients (Fig. 1 A). According to our hypothesis, the (PMA-inducible) S2 cleavage is most likely responsible for the detection of PD-L1 in the CSF. Therefore, we consider the data set on potential S2 cleavage mechanisms for PD-L1 as an important part of our study, providing a mechanistic explanation on how a transmembrane protein ends up in the CSF.

As the reviewer pointed out, PD-L1 can reportedly be cleaved by ADAM10 and ADAM17 (Orme et al., Oncoimmunology 2020). In line with this, we show that knockdown of ADAM17 inhibits secretion of soluble sPD-L1 (Appendix Fig. S5 B), but our data add BACE1 as another candidate sheddase (Appendix Fig. S5 A), Further, we provide evidence for the importance of the luminal, juxtamembrane region of PD-L1 in this cleavage process (Fig. S3 and former Fig. 2 F,G, now Appendix Fig. S4 B, C). But we agree that the subsequent cleavage by γ-secretase is secondary to the main topic of the study. Blocking the γ-secretase activity (Fig. 2 B, C) served mainly to show that first a luminal, juxtramembrane cleavage occurs, which led us to investigate this cleavage in more detail as presented in this part of our study.

However, at the request of the reviewer, we decided to reduce the overall focus on PD-L1 cleavage in cell lines and moved the data on the importance of the luminal, juxtamembrane region for S2 cleavage (former Fig. 2 F, G, H) to the Appendix (now Appendix Fig. S4 B, C, D).

3. In Fig. 5B, the authors compared the effect of WT and PD-L1-KO astrocyte conditioned medium and found that astrocyte-derived sPD-L1 is important for microglia-mediated Aβ uptake. Overall, this is a nicely designed experiment and the results are convincing. However, it remains possible that there are variables between WT and PD-L1 KO astrocyte conditioned medium other than the level of sPD-L1, and these unknown variables could also contribute to the difference in microglia phagocytosis. The authors should cautiously discuss this possibility or, if possible, repeat the experiment including an anti-PD-L1 antibody to see if they abolish microglial Aβ uptake.

Ad 3: The reviewer is of course right that there may be other variables in the astrocyte-conditioned medium and we have therefore modified the text accordingly. The explanatory sentence following Fig. 5 B now reads: “While we cannot rule out the involvement of other astrocyte-derived factors, this suggests that astrocytic PD-L1 is a modulating factor for microglial Aβ uptake.“

4. Since a previous paper has shown that PD-1 blockade can help recruit monocyte-derived macrophages, I wonder if the authors have observed a difference in CCR2 expression level in their RNA-seq of WT and PD-1 KO microglia. Alternatively, they could quantify the abundance of CCR2+ cells in ex vivo microglia by flow cytometry to see if there's a difference between APP/PS1 and PD-1 KO APP/PS1 mice.

Ad 4: As the reviewer pointed out, a previous paper showed that PD-1 blockade led to the recruitment of monocyte-derived macrophages in an AD mouse model and an alleviated phenotype (Baruch et al., Nat Med 2016). Blocking PD-L1 led to similar beneficial results and also a tauopathy mouse model benefited from inhibition of the PD-1/PD-L1 signaling axis and showed an enhanced recruitment of monocyte-derived macrophages to the brain (Rosenzweig et al., Nat Commun 2019). Recently, the same group has shown that recruitment of the monocyte-derived macrophages requires CCR2 (Ben-Yehuda et al., Mol Neurodegener 2021). However, no report shows expression of CCR2 on microglia. In another report on glioblastomas, the PD-1/PD-L1 axis was shown to be involved in CCR2-based recruitment of myeloid-derived suppressor cells (MDSCs) into the tumor. In this paper, the authors were able to show that MDSCs, but not microglia, express CCR2 (Flores-Toro et al., PNAS 2020). In line with this, CCR2 expression was not detected in our RNA-seq experiment with WT and PD-1-KO microglia.

Minor comments

5. In Figure 2A, the authors detected PD-L1-CTF in DAPT-treated cells. However, the γ-secretase cleavage generates a shorter form of PD-L1-CTF, and should be detectable by Myc antibodies in the non-DAPT-treated cells (the third and fourth lanes). Could the authors explain why they didn't see those lower MW bands? Maybe they get rapidly degraded?

Ad 5: We thank the reviewer for this careful evaluation of our mechanistic work. As the reviewer pointed out, cleavage of PD-L1-CTF by γ-secretase is generating a shorter, myc-tagged form of PD-

L1-CTF that should in theory be present in the non-DAPT-treated cells. However, detectable levels of the “normal” PD-L1-CTF are already very low and our failure to detect the smaller fragment could be due to either technical issues with the detection of such small protein fragments or could also be related to a rapid degradation of these fragments.

6. For Figure S8: it's hard to tell if there's a difference in Iba1 expression just by looking at microscopy images. It would be better if the authors could quantify the Iba1 coverage or Iba1+ microglia number.
Ad 6: As this analysis is not an essential part of our study, we decided to remove Fig. S8 to alleviate the reviewers concern. The already presented quantification of amyloid uptake as well as some microglia-activation markers on isolated microglia (Fig. 5 D-G) represents the more meaningful and in-depth analysis of microglia in this model.

7. The paper contains various errors and missing points, and needs further proof-reading. For example, the IF staining in Fig. 3B is CD11b according to the figure legend, but it is labeled as Iba1 in the figure; also, there is a figure legend for Fig. S3B, but Fig. S3 only has one panel; the authors may include the experimental procedure for Fig. 1F and Fig. 2 (concentration of PMA, IFNγ, TNFα, etc.); it's not mentioned in figure legend, methods, or anywhere in the main text; in the figure legend of 2F, the authors say that "lysate and CM were immunoblotted using a Myc antibody", but I don't think they can detect sPD-L1 with Myc antibody, so I assume that they are using AF1019 for CM? Please make sure that the methods and figure legends are accurate and detailed enough.
Ad 7: We apologize for the various errors that have been introduced while formatting our manuscript to meet the requirements of the EMBO journal. The text has been carefully checked for errors and more specific information in the figure legends and methods have been added wherever necessary. Among other things, the concentration of PMA (1 μM), IFN-γ (100 U/μl) and TNF-α (10 μg/ml) was added to Fig. 1 F and Fig. 2. The antibody used for detection of PD-L1 in former Fig. 2F, now Appendix Fig. S4 B, was corrected. We did not use the mouse-specific antibody AF1019 in this case, but the human-specific E1L3N antibody, as the experiment was performed with a human cell line (Hela cells).

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Comments:

1. The authors suggest that PD-L1 is expressed on astrocytes and PD1 is expressed on microglia during AD disease. How about the opposite pattern? Could Aß42 induce PD-L1 expression on microglia and PD1 expression on astrocytes? Or at least a subset of them?
Ad 1: In our immunohistochemical staining, we did not observe expression of PD-L1 on microglia nor do transcriptomic data in mice suggest that the receptor PD1 is expressed on astrocytes. Therefore, a signaling from astrocytes to microglia is more likely and represents the hypothesis we favor in our manuscript.

2. In AD patients, it seemed that PD-L1 was increased in CSF, but they proposed the beneficial function of PD-L1 in AD disease. To explain this contradiction, they suspect that "an unresolved,

chronic inflammation that causes microglial exhaustion and disfunction is ongoing and that at very late diseases stages this immune checkpoint is not functional anymore (Dong & Chen, 2006)", furthermore they mentioned that "it has been reported that prolonged states of negative immune regulation results in PD-1 mediated immune exhaustion that goes along with reduced cell motility (Oxford, 2013)".

If PD-1 signaling could enhance clearance of amyloid but also induce immune exhaustion and associated dysfunction, it is probably important to strengthen PD1 signaling and release exhaustion by blocking other exhaustion-related factors (not PD1) for treatment of AD disease, they should more clearly discuss this aspect.
Ad 2: We took the possibility of such a combinatorial treatment into account and added the following sentence to the discussion in our manuscript: “However, as PD-1 is important for Aβ uptake, another possible approach could be a combination of a treatment that on the one hand strengthens PD-1 signaling and on the other hand inhibits other exhaustion-related factors.”

Minor points:

3. It is mentioned that "but also at early time points at 4 months of age when very few amyloid plaques are present (Fig. 1 E, S1) implying that upregulation of PD-L1 is an early phenomenon.", Please clarify which data represented 4 months of APP/PS1 mouse in supplementary figure 1 legend.
Ad 3: Data of 4-month-old mice are shown in Fig. 1 E and Appendix Fig. S1 D, E, revealing astrocytic PD-L1 expression in APP/PS1 mice already at this young age. We have clarified this in the corresponding figure legends.

4. Do the authors have the image of whole brain and quantitative ELISA results to compare the amount of 4-months and 9-months one?
Ad 4: We have not performed an analysis of amyloid plaque load or quantitative ELISA of 4-month-old mice. However, the strong increase in plaque pathology and cognitive deficits we see in PD-1 deficient APP/PS1 mice at 9 months of age together with the upregulated levels of PD-L1 already in 4-month-old mice (Fig. 1 E, Appendix Fig. S1 D, E) suggests an early involvement of the receptor and its ligand. As our data point towards a deficit in Aβ removal, we predict that PD-1 deficient APP/PS1 mice present with a stronger phenotype already early on. Investigating this in more detail would be important in a study looking at modulators of the PD-1/PD-L1 axis as a potential treatment target, but is out of the scope of our current study.

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Ad 5: As pointed out in our response above, we have not performed an analysis of amyloid plaque load or quantitative ELISA of 4-month-old mice. Following the exact disease progression is not the focus of our current study, in which we aimed to provide evidence for the involvement of microglial PD-1 and astrocytic PD-L1 in AD.

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Female APP/PS1 mice have more amyloid plaque number and amount compared with same aged male mice. And the amyloid depositions started to appear earlier in female compared with male (Jun W et al (2003)).

Could the author observe increased amyloid plaques in male PD-1 deficient APP/PS1 mice compared with male APP/PS1 mice? Was there any correlation between the amount of amyloid and cognitive deficits both in male and female PD-1 deficient APP/PS1 mice?
Ad 6: We have assessed amyloid plaques in female mice only to avoid high variabilities due to the sex differences observed in the APP/PS1 model and therefore cannot comment on the increase of amyloid plaques in male APP/PS1 / PD-1$^{-/-}$ mice. As behavioral analyses require high numbers of animals, we decided to include both sexes in these experiments, but used equal numbers of male and female mice in the APP/PS1 and APP/PS1 / PD-1$^{-/-}$ groups. The mice analyzed for plaque burden and behavior were separate groups of mice and we cannot provide any correlation data.

7. Please mention if possible the gender of AD patients and control.

Ad 7: The CSF samples used to measure PD-L1, Aβ 42 and pTau 181 in Fig. 1A included samples from 8 male and 2 female controls and 3 male and 7 female AD patients. This information was added to the methods sections, which also contains information on the average age (65.8 +/- SD 6.7 for control and 72.8 +/- SD 6.4 for AD) and average MMSE scores (29.8 +/- SD 0.42 for control and 20.4 +/-SD 2.75 for AD).

Dear Michael,

Thank you for submitting your revised manuscript. I have now had a chance to take a careful look at everything and all looks good.

I am therefore very pleased to accept your manuscript for publication in The EMBO Journal.

Congratulations on a nice study!

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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| Corresponding Author Name: Markus P. Kummer and Michael T. Heneka |
| Journal Submitted to: EMBO Journal |
| Manuscript Number: EMBOJ-2021-108662 |

**Reporting Checklist For Life Sciences Articles (Rev. June 2017)**

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

## A- Figures

### 1. Data

**The data shown in figures should satisfy the following conditions:**

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

### 2. Captions

**Each figure caption should contain the following information, for each panel where they are relevant:**

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

**In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.**

## B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

| | |
|---|---|
| 1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? | Except for the Abeta-FAM phagocytosis experiments, all cell culture experiment were repeated at least 3 times. For in vivo studies, the software G Power v3.1 was used to determine sample size. |
| 1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. | Software G Power v3.1 was used to determine sample size. |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | All animals were healthy and generated specifically for the experiments described. Animals or samples were excluded from analysis only in the instance of technical failure. |
| 3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe. | The animals were littermates, and inbred lines were used, where the individual mice were identical, therefore no specific randomization was needed. Mice were grouped according to genotype before they were randomly assigned to the experimental conduct (e.g. Morris Water Maze test). |
| For animal studies, include a statement about randomization even if no randomization was used. | The animals were littermates, and inbred lines were used, where the individual mice were identical, therefore no specific randomization was needed. Mice were grouped according to genotype before they were randomly assigned to the experimental conduct (e.g. Morris Water Maze test). |
| 4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe. | Researchers performing animal experiments and/or data analysis were blinded to the genotype of the mice. Cell culture experiments were performed blinded as one researcher performed the experiment and another researcher did the analysis wherever possible. |
| 4.b. For animal studies, include a statement about blinding even if no blinding was done | All researchers performing animal experiments and/or data analysis were blindedto the genotype of the mice. |
| 5. For every figure, are statistical tests justified as appropriate? | Appropriate statistical test were used, dependent on the experimental setup. |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | We have a lot of experience with the techniques and models used in this study and where therefore able to predetermine if our data meet the assumptions of the tests. |
| Is there an estimate of variation within each group of data? | Statistical analysis was performed using GraphPad Prism 5 and each analysis provided us with an estimate of variation. |

## USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-report

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jij.biochem.sun.ac.za
https://osp.od.nih.gov/biosafety-biosecurity-and-emerging-biotechnology/
http://www.selectagents.gov/

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| Is the variance similar between the groups that are being statistically compared? | Yes |

## C- Reagents

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| 6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right). | PD-1 was detected using PD-1 antibodies #4065 (ProSci, Poway, CA) and PDCD1 #PAB13253 (Abnova, Heidelberg, Germany). PD-L1 was detected using antibody #AF1019 (R&D, Wiesbaden, Germany) and PD-L1 (E1L3N) XP (#13684, Cell Signaling, Leiden, The Netherlands). Characterization of microglia was performed using antibodies #MCA711 against CD11b (AbSerotec, Düsseldorf, Germany) and anti-Iba1 (#019-19741, Wako, Neuss, Germany). Astrocytes were detected using GFAP-antibody #Z0334 (Dako, Hamburg, Germany). Aβ was detected using IC16 (Jager et al, 2009) against Aβ1-17, APP and APP-CTF using antibody 6E10 (#803010, Biolegend, San Diego, CA) and 140 (CT15, anti-APP C- terminal 20 aa; 1:2500 (Wahle et al, 2006)). EEA1 was detected using antibody #610456 (BD Bioscience, Heidelberg, Germany). Myc epitopes were detected using antibody 9E10 and tubulin using antibody E7 (both DSHB, Iowa City, IO). |
| 7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. | C6, Hela and HEK293 cells were all purchased from DSMZ, Braunschweig, Germany.) HEK293 cells stably transfected with human PS1 or PS1D246A mutant constructs were kindly provided by Dr. Jochen Walter, University of Bonn. Cells were not tested for mycoplasma contamination in our laboratory. |

* for all hyperlinks, please see the table at the top right of the document

## D- Animal Models

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| 8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals. | APP/PS1 heterozygous transgenic mice expressing mouse APP containing the human amyloid β domain as well as the Swedish mutation and the presenilin 1 Δexon 9-mutation, both under the control of the prion promoter (Jankowsky et al, 2001), and PD-L1 and PD-1 deficient mice were kindly provided by Dr. Heinz Wiendl (University of Münster). APP/PS1 mice were also bred with CX3CR1-EGFP mice (Jung et al, 2000). Mice were housed under standard conditions at 22 °C and a 12 h light-dark cycle with free access to food and water. |
| 9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments. | Animal care and handling complied with relevant ethical regulations and was performed according to the declaration of Helsinki and as approved by the local ethical committee (LANUV NRW). |
| 10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance. | We confirm compliance. |

## E- Human Subjects

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| 11. Identify the committee(s) approving the study protocol. | Use of the samples was approved by local ethical committees (Ethical committee of University of Bonn Medical Center). |
| 12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report. | Informed consent was obtained from all subjects. The experiments were in conformity with the principles of the declaration of Helsinki and the Department of Health and Human Services Belmont Report. |
| 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. | NA |
| 14. Report any restrictions on the availability (and/or on the use) of human data or samples. | NA |
| 15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. | NA |
| 16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list. | NA |
| 17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines. | NA |

## F- Data Accessibility

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| 18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'.<br><br>Data deposition in a public repository is mandatory for:<br>a. Protein, DNA and RNA sequences<br>b. Macromolecular structures<br>c. Crystallographic data for small molecules<br>d. Functional genomics data<br>e. Proteomics and molecular interactions | RNA sequencing data of wildtype and PD-1-/- microglia were deposited in the GEO functional genomics data repository (GSE77643). |
| 19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right). | NA |
| 20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right). | NA |
| 21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information. | NA |

## G- Dual use research of concern

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| 22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could. | NA |