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Review COMMONS

Protein tyrosine phosphatase 1B is involved in efficient type I interferon secretion upon viral infection

Elisa Reimer, Markus Stempel, Baca Chan, Hanna Bley and Melanie M. Brinkmann
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Editor: Michael Way

Review timeline
Submission to Review Commons: 14 January 2020
Submission to Journal of Cell Science: 13 March 2020
Accepted: 19 March 2020

Reviewer 1

Evidence, reproducibility and clarity

The authors show that PTP1B is required for the secretion of Type I IFNs following activation of their expression, and that this activity did not require phosphatase activity. The data are convincing and the manuscript is written clearly. Some major issues still need attention, and the authors could easily provide additional evidence bearing on the mechanism of this effect.

The authors do not seem to fully understand the complexity of expression of Type I interferons, leading them to do some experiments with IFN beta and some with IFN alpha, with no apparent reason for the choice. However, the expression of the IFN alpha genes is driven by the prior synthesis of IFN beta in a process that requires the IFN receptor. Thus, if there is a problem in the secretion of IFN beta, there will of course be no synthesis of the IFN alphas. The authors need to do all of their experiments with IFN beta only and, if they wish repeat some of the work with IFN alpha, with appropriate discussion.

Also, in the introduction, it is stated that the JAKs associate with IFNAR in response to Type I IFNs, which is not true. They are associated constitutively without the need for IFN-signaling.

I also think that the authors could pay attention to the processing of IFN beta. It would be straightforward for them to find out whether the signal peptide is removed normally, or whether this step is inhibited in the absence of PTP1B.

Significance

The work in this manuscript represents a significant and important advance in our knowledge of how the secretion of IFN beta is regulated. It would be much more compelling with more mechanistic insight.

REVIEWERS CROSS COMMENTING:
Both reviewers focus on the desirability of providing additional information concerning the effects of PTB1B on cytokine secretion. Reviewer 1 agrees with the major comment of reviewer 3 and thinks that the additional work suggested by both should be completed in a revised version of the paper.

Reviewer 2

Evidence, reproducibility and clarity

Summary:

The authors demonstrate a role of PTB1B in promoting secretion of type I IFNs during infection or in response to TLR or cGAS/STING activation. The main focus is on primary macrophages. This effect was dependent on cellular localization of PTB1B to the ER, but was independent of PTB1B phosphatase activity. This suggests that PTP1B may mediate interactions that promote efficient traffic of these cytokines out of the host cell. Although the precise mechanism of this regulation remains unknown, the work here presents interesting new data that shed new light on PTP1B and type I IFN secretion. Overall, the conclusions are convincing and relatively preliminary or speculative claims are appropriately restricted to the discussion.

Major comments:

The premise put forth here is that type I IFNs are uniquely or at least somewhat uniquely affected by the PTP1B deficiency. However, the evidence supporting this as being a relatively unique phenomenon rests on the data showing secreted TNFa is similar in wt and mutant cells. Given that release of TNFa from the cells requires its cleavage, it would be important to perform total cell TNFa staining similar to that done for IFNb.

Another more conventionally-secreted cytokine should also be evaluated. For example IL-6.

Minor comments:

Presentation of data and methods presented is overall clear.
Statistical analysis is mostly fine, but for multipanel graphs, recommend using one-way ANOVA rather than t-test.

Significance

Describes a previously unknown function of PTB1B in regards its involvement in the regulation of type I interferon secretion. The authors cite existing studies with PTB1B that only investigate its phosphatase activity, and understanding how this protein can modulate the secretion of type I interferons independently of this activity is novel. Audiences include those that are interested in mechanisms of PTB1B's role during infection, and it's relation to type I interferon responses.

Reviewer Expertise: innate immunity, type I interferon/cytokine biology, viral/bacterial infections

REVIEWERS CROSS COMMENTING:

Agree with reviewer #1. Also with this reviewer's suggestion to focus

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First revision

Author response to reviewers' comments

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

The authors show that PTP1B is required for the secretion of Type I IFNs following activation of their expression, and that this activity did not require phosphatase activity. The data are convincing and the manuscript is written clearly. Some major issues still need attention, and the authors could easily provide additional evidence bearing on the mechanism of this effect.

The authors do not seem to fully understand the complexity of expression of Type I interferons, leading them to do some experiments with IFN beta and some with IFN alpha, with no apparent reason for the choice. However, the expression of the IFN alpha genes is driven by the prior synthesis of IFN beta in a process that requires the IFN receptor. Thus, if there is a problem in the secretion of IFN beta, there will of course be no synthesis of the IFN alphas. The authors need to do all of their experiments with IFN beta only and, if they wish repeat some of the work with IFN alpha, with appropriate discussion.

We thank the reviewer for his/her appreciation of our work and this comment. While we agree that IFNß is a prerequisite for IFNα production, we want to emphasize the following:
(1) We originally planned to perform assays dissecting intracellular and extracellular IFNß protein levels, however, the levels at time points of interest were below the detection limit of available ELISA kits, thus we had to move to an IFNα ELISA which could detect the levels present at those early time point tested.
(2) Since we performed both IFNß and IFNα ELISAs for almost all our assays (Figures 2, 5, 6, S4) and always observed a similar phenotype, we would conclude that secretion of type I IFNs, i.e. IFNα and IFNß, is regulated by the same mechanism.
(3) If a defect in IFNß secretion in PTP1B$^{-/-}$ cells would affect the production of IFNα, this would lead to a general reduction of protein synthesis of IFNα in PTP1B$^{-/-}$ cells. However, as shown in Figure 4D, the total production of IFNα upon ISD stimulation and MCMV infection is similar in WT and PTP1B$^{-/-}$ cells, indicating that the induction and protein expression of IFNα is not affected. Since IFNß levels are not zero in PTP1B$^{-/-}$ cells, we are not surprised by this result – the levels of IFNß (albeit reduced in PTP1B$^{-/-}$) suffice to induce IFNα production via the IFNAR. This observation actually strengthens our conclusion that secretion of type I IFNs is positively regulated by PTP1B.

Also, in the introduction, it is stated that the JAKs associate with IFNAR in reponse to Type I IFNs, which is not true. They are associated constitutively without the need for IFN-signaling.

Thank you - we will adjust this in the manuscript.

I also think that the authors could pay attention to the processing of IFN beta. It would be straightforward fore them to find out whether the signal peptide is removed normally, or whether this step is inhibited in the absence of PTP1B.

In order to test if the signal peptide is removed in PTP1B$^{-/-}$ cells, we would have to perform an immunoblot and detect IFNß protein. For this, we would need to prevent secretion to allow IFNß to accumulate in the cell - otherwise it would be secreted and detection levels would be too low to be visualized by immunoblotting. To prevent secretion, brefeldin A treatment would need to be performed. We have tried to use Brefeldin A treatment, however, while upon treatment with Brefeldin A no type I IFN can be detected in the supernatants, as expected, we were also not able to detect intracellular IFN levels by ELISA, indicating that the disruption of ER and Golgi also impacts the production of type I IFNs (*data not shown*).

Moreover, we do not think that PTP1B affects removal of the signal peptide of IFNß. The signal peptide is cleaved within the ER lumen during translation of proteins. PTP1B is anchored to the ER membrane facing the cytosol, thus, there is no direct connection of PTP1B with the ER lumen and an impact on ER signal peptide cleavage is unlikely.

Reviewer #1 (Significance (Required)):

The work in this manuscript represents a significant and important advance in our knowledge of how the secretion of IFN beta is regulated. It would be much more compelling with more mechanistic insight.

REVIEWERS CROSS COMMENTING:

Both reviewers focus on the desirability of providing additional information concerning the effects of PTB1B on cytokine secretion. Reviewer 1 agrees with the major comment of reviewer 3 and thinks that the additional work suggested by both should be completed in a

revised version of the paper.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

Summary:

The authors demonstrate a role of PTB1B in promoting secretion of type I IFNs during infection or in response to TLR or cGAS/STING activation. The main focus is on primary macrophages. This effect was dependent on cellular localization of PTB1B to the ER, but was independent of PTB1B phosphatase activity. This suggests that PTP1B may mediate interactions that promote efficient traffic of these cytokines out of the host cell. Although the precise mechanism of this regulation remains unknown, the work here presents interesting new data that shed new light on PTP1B and type I IFN secretion. Overall, the conclusions are convincing and relatively preliminary or speculative claims are appropriately restricted to the discussion.

We thank the reviewer for these encouraging words. Yes, indeed, if the mechanism how type I IFNs are secreted could be revealed would be fantastic, because hardly any data exists that addresses this important biological question. However, what we deliver in this manuscript is also a new mechanism: we reveal a role of PTP1B in positive regulation of the type I IFN response and dissect the mechanism at which step it acts: trafficking/secretion of type I IFN. We hope that this is novel enough and agree that more work needs to be done to address the molecular details how IFNs are secreted from cells.

Major comments:

The premise put forth here is that type I IFNs are uniquely or at least somewhat uniquely affected by the PTP1B deficiency. However, the evidence supporting this as being a relatively unique phenomenon rests on the data showing secreted TNFa is similar in wt and mutant cells. Given that release of TNFa from the cells requires its cleavage, it would be important to perform total cell TNFa staining similar to that done for IFNb.

We do not understand the rationale of this suggestion. Since we do not see any difference between WT and PTP1B$^{-/-}$ cells regarding TNFα levels in the supernantant, we would not expect an effect on total intracellular TNFα levels.

Another more conventionally-secreted cytokine should also be evaluated. For example IL-6.

This is a valuable point and we now included data sets showing that IL6 levels in supernatants of PTP1B$^{-/-}$ cells, similar to TNFα, are not reduced. This fortifies our conclusions. These data are now included as new Figure S1A (total BM cells) and S1B (primary BMDM). Former Figures S1A+B have been renamed and are now Figure S1C and Figure S1D.

Minor comments:

Presentation of data and methods presented is overall clear.
Statistical analysis is mostly fine, but for multipanel graphs, recommend using one-way ANOVA rather than t-test.

Since we always compared our phenotypes to the WT control rather than between different sample groups, we chose the standard t-test as statistical analysis.

Reviewer #2 (Significance (Required)):

Describes a previously unknown function of PTB1B in regards its involvement in the regulation of type I interferon secretion. The authors cite existing studies with PTB1B that only investigate its phosphatase activity, and understanding how this protein can modulate the secretion of type I interferons independently of this activity is novel. Audiences include

those that are interested in mechanisms of PTB1B's role during infection, and it's relation to type I interferon responses.

Reviewer Expertise: innate immunity, type I interferon/cytokine biology, viral/bacterial infections

REVIEWERS CROSS COMMENTING:

Agree with reviewer #1. Also with this reviewer's suggestion to focus

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Decision letter

MS ID#: JOCES/2020/246421

MS TITLE: Protein tyrosine phosphatase 1B is involved in efficient type I IFN secretion upon viral infection

AUTHORS: Elisa Reimer, Markus Stempel, Baca Chan, Hanna Bley, and Melanie M Brinkmann
ARTICLE TYPE: Research Article

First of all, thank you for sending your manuscript to Journal of Cell Science through Review Commons. Second, I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

I would also like to draw your attention to our upcoming Special Issue on Host-Pathogen interactions. We think your paper would fit nicely in this issue, so please do consider it. You should note that if you would like your paper to appear in this issue, its publication will not be delayed. We employ a continuous publication model so will publish your article as soon as post-acceptance processing is completed, when it will be appear in PubMed, and it will then be collected into a special issue early next year. Please let us know if you would like to take up this option.