nature portfolio

Peer Review File

Plasmacytoid dendritic cells are dispensable or detrimental in murine systemic or respiratory viral infections

Corresponding Author: Dr Elena Tomasello

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
An outstanding study that reveals important data on pDC function in vivo during the course of infection. The role of pDC IFN responses are shown to be detrimental to survival after infection with flu strains. Sars-CoV 2 infection is less affected, with only males showing a TLR7-dependent reliance on IFN for survival, with detrimental affects of pDC. MCMV infections seem agnostic to the presence or absence of pDC.

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Is it possible that these non-pDC cell types may also be affected in the pDC-less mice? Loss of these cells could potentially contribute to detrimental effects of 'pDC'. It is worth noting that Pacsin1 appears to be an ISG in various cell types (see Interferome for e.g.)

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A novel approach is taken to generate mice with loss of a gene specifically in pDCs – shield BM chimeras in pDC-less mice reconstituted with BM from knockout mice allowing for the first time to interrogate the specific function of selected genes in pDCs in vivo. Using this approach the authors show that lack of TLR7 or MyD88 or IRF7 specifically in pDCs reduced immunopathology and rescued the mice infected with Influenza A virus.
Finally, the authors investigate the role of pDCs in SARS-CoV-2 infection of hACE2-expressing mice. In this model, where IFN I and TLR7 play an important protective role, pDC-less female mice showed no difference in survival while pDC-less male mice are more protected. The authors conclude that other cells (not pDCs) producing IFN I in a TLR7-dependent manner are required for protection. By showing that pDCs produce type I IFN later in the infection they suggest that the early protective IFN I production is pDC-independent, while the delayed IFN I production by pDCs may contribute to pathology in SARS-CoV-2 pneumonia.
The manuscript is very well written and follows a logical order that is easy to follow. The experiments are thoroughly described in the text and in the legends including precise information on statistical testing and number of experiments and mice. The data is robust and support the interpretations and conclusions. The results are interpreted in the context of the relevant literature which is correctly cited.
This study is very relevant because it shows convincing data that pDCs are not required for antiviral protection in mice in several models of acute viral infection, and provides evidence for a pathology enhancing effect of pDCs, which leads to reduced survival thereby challenging the notion that pDCs are essential and beneficial for innate antiviral defense (in mice). The role of pDCs in MCMV and Influenza A virus infection has been investigated previously using other means of reducing the number of pDCs and the results presented here are consistent with the findings described previously. This study takes an additional step and addresses the mechanism of the pathology-enhancing effect of pDCs in pathogenic Influenza A virus infection and shows its dependence on TLR7 expression in pDCs and on IFNAR signaling. The investigation of pDC function in the SARS-CoV-2 mouse model is novel and suggests a pathology-enhancing role of pDCs also in this respiratory viral infection model suggesting that pDCs recruited to the SARS-CoV-2 infected lung may contribute to the delayed and prolonged type I IFN production that has been shown to promote inflammation and pathology in human patients with severe COVID-19. Although the results cannot be directly extrapolated to humans, these finding are potentially highly significant in the context of therapeutic approaches promoting or inhibiting pDC responses in acute respiratory viral infection warranting further investigation in non-human primate pre-clinical and in clinical studies.
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Decision Letter:

21st Jun 2024

Dear Dr Tomasello,

Your Article, "Plasmacytoid dendritic cells are dispensable or detrimental in murine systemic or respiratory viral infections" has now been seen by 2 referees. You will see from their comments copied below that while they find your work of considerable potential interest, they have raised quite substantial concerns that must be addressed. In light of these

comments, we cannot accept the manuscript for publication, but would be very interested in considering a revised version that addresses these serious concerns.

We hope you will find the referees' comments useful as you decide how to proceed. If you wish to submit a substantially revised manuscript, please bear in mind that we will be reluctant to approach the referees again in the absence of major revisions.

Reviewers comments regarding the role of non-hematopoietic cells during infection in pDC-less mice must be addressed before we will reconsider the study.

If you choose to revise your manuscript taking into account all reviewer and editor comments, please highlight all changes in the manuscript text file [OPTIONAL: in Microsoft Word format].

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

If revising your manuscript:

* Include a “Response to referees” document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions at http://www.nature.com/ni/authors/index.html. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

The Reporting Summary can be found here:
https://www.nature.com/documents/nr-reporting-summary.pdf

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-portfolio/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Extended Data figures and tables are online-only (appearing in the online PDF and full-text HTML version of the paper), peer-reviewed display items that provide essential background to the Article but are not included in the printed version of the paper due to space constraints or being of interest only to a few specialists. A maximum of ten Extended Data display items (figures and tables) is typically permitted. When re-submitting your manuscript, please ensure that any supplementary figures and tables that are more critical to the manuscript’s conclusions are converted to Extended data to increase these data’s visibility.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Immunology or published elsewhere.

Nature Immunology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as ‘corresponding author’ on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on ‘Modify my Springer Nature account’. For more information please visit please visit www.springernature.com/orcid.

Please do not hesitate to contact me if you have any questions or would like to discuss the required revisions further.

Thank you for the opportunity to review your work.

Sincerely,

Stephanie Houston, PhD
Senior Editor
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Reviewers' Comments:

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I do not have further comments.

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All the questions and comments of the reviewers were adressed adequately. Additional experiments and data analysis were

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Decision Letter:

Our ref: NI-A37978A

2nd Jul 2025

Dear Dr. Tomasello,

Thank you for submitting your revised manuscript "Plasmacytoid dendritic cells are dispensable or detrimental in murine systemic or respiratory viral infections" (NI-A37978A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Immunology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

We will now perform detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

If you had not uploaded a Word file for the current version of the manuscript, we will need one before beginning the editing process; please email that to immunology@us.nature.com at your earliest convenience.

Thank you again for your interest in Nature Immunology Please do not hesitate to contact me if you have any questions.

Sincerely,

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Senior Editor
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NI-A37978 Point-by-point response to referees

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The co-expression of *Pacsin1* and *Siglech* is shared by brain microglia, endothelial cells and various neurons. See for eg: Broad Institute's SC Portal. It would seem sensory neurons can also express both. (Pain. 2023 Oct; 164(10): 2196–2215. Published online 2023 Jun 6. doi: 10.1097/j.pain.0000000000002934)- and associated RNAseq data at https://livedataoxford.shinyapps.io/drg-directory/.

Is it possible that these non-pDC cell types may also be affected in the pDC-less mice? Loss of these cells could potentially contribute to detrimental effects of 'pDC'. It is worth noting that Pacsin1 appears to be an ISG in various cell types (see Interferome for e.g.)

We analysed different public single cell RNA-Seq databases that were obtained after sorting and analysis of mouse brain cells. We used two databases available from the Broad Institute (**Fig.1a-b for referees**), as well as one database available from the Linnarsson lab, at the Karolinska Institute, Sweden (**Fig.1c for referees**). Altogether, these data show that, in the brain, *Pacsin1* is highly expressed in neuronal cells, while *Siglech* expression is restricted to microglial cells. Co-expression of these two genes was found exclusively in a rare fraction of microglial cells (**Fig.1c for referees**). To test whether microglial cells can be an off-target of our intersectional genetic-based strategy, we used our pDC-Tom mice, which are fluorescent pDC-specific reporter mutant mice deriving from the breeding of Siglech-iCre mice with Pacsin1-LSL-tdT mice. In pDC-Tom mice, we previously reported that the Tomato fluorescent protein was exclusively detected in "bona fide" pDCs (Valente et al. Nat Immunol 2023). Consistently, we did not detect any Tomato expression in sections of brains isolated from IAV-infected pDC-Tom mice, as compared to the background signal detected in the brain of IAV-infected control mice (**Fig.1d for referees**). Moreover, Tomato expression was not detectable by flow cytometry in CD45+ CD11b+ CX3CR1+ Siglech+ microglial cells isolated from the brain of pDC-Tom mice, when compared to their counterparts isolated from the brain of control mice (**Fig.1e for referees**). Finally, we detected significant alteration neither in the frequency nor in the activation state of microglial cells, neurons or astrocytes in the brain of uninfected versus IAV-infected pDC-less mice as compared to

control animals (**Supplementary Fig.1**), as detailed below. Altogether, our findings do not show any effect on cells other than pDC.

Other comments in order of occurrence:

1. Line 205-206: Irf7 ...expression was upregulated with similar kinetics...but poorly in organs from infected IFNAR1-/- mice:

No-the data of Fig 2d (spleen) show Irf7 gene upregulation/kinetics is similar in pDC-less and IFNAR-/- mice. The regulation of Isg15 is also similar across all genotypes in spleen and lungs.

According to both reviewer suggestion, we have modified the text, as follows.

When compared to uninfected mice, ~~*Irf7*, *Mx2*, *Isg15* and *Ifit2*~~ the expression of the ISGs *Mx2*, *Ifit2*, *Irf7* and *Isg15* was upregulated with similar kinetics in the organs of MCMV-infected control and pDC-less, mice (**Figure 2d** and **Extended data Figure 2d**), although we observed a high variability for each genotype and organ, with a trend to pDC-dependent expression for *Irf7* in the spleen and for *Mx2* and *Isg15* in the liver. In contrast, the expression of these ISGs was downregulated in most of the organs isolated from infected *Ifnar1*^{KO} mice.

We have also modified the related figures 2d and Extended 2d.

2. Lines 208-09: Splenic pDcs were also found to be the main source of IFN-III during MCMV infection....consistent with..

Yes- but low levels of transcript in liver not pDC-dependent. Overall transcript levels only here for IFN-III and should be noted.

We have modified the text as follows:

Il28b expression was reduced in a pDC-dependent manner in the spleen, but not in the liver of MCMV-infected mice (**Extended Data Figure 2d**), consistent with enhanced expression of *Il28a* and *Il28b* genes in IFN-I-producing splenic pDCs[36].

3. Line 293- 'whole' pDCs- replace with 'total'

Thank you for catching this mistake. We have made the required correction.

4. Line 307: ''unique source of IFN-Is'

- It is worth differentiating here between IFN-a and IFN-b, where IFN-b production seems almost completely independent of pDC.

We have modified the text as follows:

During IAV infection, pDCs are a main source of the IFN-α, but not of the IFN-β, detectable in the lung ~~pDCs are a major, but not the unique source of IFN-Is in the lungs during IAV infection~~ (**Fig. 4** and **Extended Fig. 4**).

5. Line 330: "All IRF7ko and pDC-less pDC SBMC survived....

No: SBMS-IRF7KO shows death of one mouse (Fig 5e)

We have modified the text as follows:

Most of ~~All~~ *Irf7*^{KO} and pDC-less pDC SBMC survived, whereas 60% of CTR pDC SBMC died (**Fig. 5e** and **Extended Fig.5c**).

6. Line 332-334: Hence selective inactivation...in pDCs..confirmed the detrimental contribution of IFNs produced by lung pDCs.

I felt this statement was 'overstated'. The SBMC with either targeted MyD88, TLR7 or IRF7 all showed protection, though it wasnt complete, though pDC were complete KOs as shown in Fig 5B. Thus although pDC are definite major contributors, other mechanisms also seem to be at play –

We have modified the text as follows:

Hence, selective inactivation of TLR7/Myd88/IRF7 signaling in pDCs restored resistance to IAV infection in most of the pDC SBMC and ameliorated their clinical score, thus supporting the detrimental contribution of the IFNs produced by lung pDCs during IAV-induced lung immunopathology.

7. Line 379: 'most cytokines..significantly lower…- This was not the case for TNF, CXCL10, IL6, CCL5. Thus there is an unusual selective depoendence on pDC for select cytokines, some of which are associated with respiratory disease.

We have modified the text as follows:

BAL isolated from both strains of mice at days 2, 3 and 6 after infection did not differ in their content for proinflammatory cytokines, including TNF, CXCL10, CCL2 ~~and~~, IFN-γ, IL-6 and CCL5, and anti-inflammatory cytokines, such as IL-10 (**Fig. 6i** and **Extended Data Fig. 8d**). However, at day 8 after infection, the amounts of CXCL10, CCL2, IFN-γ and IL-10 ~~most cytokines~~ detectable in the BAL were significantly lower in the absence of pDCs (**Fig. 6i** and **Extended Data Fig. 8d**). In contrast, ~~while~~ TNF, IL-6 and CCL5 levels were not affected, suggesting pDC-independent sources. Hence, during late phases of IAV infection, enhanced

resistance of pDC-less mice correlated with reduced bronchioalveolar amounts of some of the cytokines known to promote ARSD.

In summary- addressing the comments 1-7 above would improve balance and robustness of the work. Given the importance of this model as a potential pDC-specific readout, I suggest to address the potential role/s of stromal and neuronal cells in the brain and potentially other organs.
For e.g. What does histology of the brain look like in pDC wt vs PDC-less mice upon viral infection?

We have performed an extensive histological analysis of the brains isolated from C57BL6 vs pDC-less mice, uninfected or at day 4 or 8 after IAV infection (**Supplementary Fig.1**). We have quantified the frequency of NeuN+ cells, corresponding to mature neurons, cFos+ cells, corresponding to activated neurons, Iba1+ cells, corresponding to microglial cells, and GFAP+ cells, corresponding to astrocytes. As the intensity of the staining was heterogenous within the brain, the quantification of positive cells was performed in the same selected brain areas in both strains of mice. Our data show no significant difference between control and pDC-less mice for any of the four cell types analysed, both in uninfected and infected conditions. Moreover, we did not detect any significant difference of expression of key neuronal genes, such as *S100a9*, *Ngfp*, *Plp* and *Dcx*, when analysed by RT-qPCR on RNA isolated from the brains of uninfected vs IAV-infected control and pDC-less mice (**Supplementary Fig.1**). Altogether, our results do not show any stromal or neuronal alteration in pDC-less mice, supporting the specificity of pDC targeting in our mutant mice.

Would it be possible to perform BM chimeras of this model- counter intuitive on the one hand, but may reveal non-hematopoietic intrinsic effects of loss of Pacsin1/Siglech positive cells.

Radiation-induced lung injury (RILI) is a current complication associated with radiotherapy or experimental whole-body exposure to X-rays (Roy S. et al, Semin Radiat Oncol 2021 PMID: 33610273). RILI significantly increases host susceptibility to the infection by respiratory viruses, such as IAV. At early time points after irradiation increased susceptibility to respiratory infections is mainly due to impaired immune control. In contrast, several weeks after irradiation, enhanced susceptibility to IAV infection correlates with enhanced damages to lung epithelial integrity (Manning C et al, Int J Radiat Oncol Biol Phys 2013 PMID: 23195776). This is one of the main reasons why we developed and used shield BM chimeras, in which only the hind legs are irradiated, while the rest of the body, including the lungs, are shield-protected. Using CTR pDC SBMC, we clearly demonstrated that reconstitution with IFN-producing pDC is sufficient per se to enhance mouse susceptibility to IAV infection. Moreover, we showed the specific deleterious role of pDCs by using also BDCA2-hDTR mice, in which only pDCs are transiently deleted, without affecting any other hematopoietic or non-hematopoietic cells (Swiecki et al Immunity 2010).
However, in an effort to comply with the referee request, we generated whole body irradiated (WBI) chimeras using C57BL/6 (B6) and pDC-less mice as recipient or donor BM (**Fig.2a for referees**). As irradiation enhances mice morbidity and lung lesions associated with IAV infection(Manning C et al, Int J Radiat Oncol Biol Phys 2013 PMID: 23195776), we first calibrated the LD50 dose to administrate to WBI chimeras, by using exclusively BM B6 > B6 chimeras. We infected them with three different doses of IAV, 8 weeks after reconstitution (**Fig.2a for referees**). At the 145 pfu dose that we currently used in our experiments, all chimeras died; 80% succumbed at 120 pfu, whereas only 25% died when infected with 100 pfu (**Fig.2b for referees**). We thus decided to infect all the four types of chimeras with 110 pfu. Using these experimental settings, all types of chimeras displayed similar survival (**Fig.2c for referees**). Our interpretation is that, in these experimental conditions, irradiation-induced damages on epithelial lung cells overcome the benefit of pDC loss that we observed in both pDC-less and in BDCA2-hDTR mice. Therefore, WBI chimeras do not appear as a reliable model for studying the deleterious role of pDC during IAV infection.

Reviewer #2:

Remarks to the Author:
The authors introduce a new mouse model that lacks specifically plasmacytoid dendritic cells - pDC-less mice - generated by an intersectional genetic approach by crossing Siglech-iCre with Pacsin-LSL-DTA mice. Using this novel tool the authors revisit the role of pDCs in mouse models of murine cytomegalovirus (MCMV) infection and in a highly pathogenic Influenza virus infection model (H3N2 A/Scotland/20/74 IAV) where other methods of pDC depletion have produced inconclusive results which may be confounded by depletion of other cell types or unintended side effects. They show that while pDCs are the major source of type I IFN in MCMV infection they are not required for antiviral protection in this infection. In a specific Influenza A virus model that causes severe pneumonia in C57BL/6 mice, the authors show that pDC-less mice have less pathology (e.g. lung lesions and fluid leakage) and better survival. This outcome is expected given the contribution of pDCs to IFN I production (via TLR7) and the deleterious role of IFN I in this specific model.
A novel approach is taken to generate mice with loss of a gene specifically in pDCs – shield BM chimeras in pDC-less mice reconstituted with BM from knockout mice allowing for the first time to interrogate the specific function of selected genes in pDCs in vivo. Using this approach the authors show that lack of TLR7 or MyD88 or IRF7 specifically in pDCs reduced immunopathology and rescued the mice infected with Influenza A virus.
Finally, the authors investigate the role of pDCs in SARS-CoV-2 infection of hACE2-expressing mice. In this model, where IFN I and TLR7 play an important protective role, pDC-less female mice showed no difference in survival while pDC-less male mice are more protected. The authors conclude that other cells (not pDCs) producing IFN I in a TLR7-dependent manner are required for protection. By showing that pDCs produce type I IFN later in the infection they suggest that the early protective IFN I production is pDC-independent, while the delayed IFN I production by pDCs may contribute to pathology in SARS-CoV-2 pneumonia.
The manuscript is very well written and follows a logical order that is easy to follow. The experiments are thoroughly described in the text and in the legends including precise information on statistical testing and number of experiments and mice. The data is robust and support the interpretations and conclusions. The results are interpreted in the context of the relevant literature which is correctly cited.
This study is very relevant because it shows convincing data that pDCs are not required for antiviral protection in mice in several models of acute viral infection, and provides evidence for a pathology enhancing effect of pDCs, which leads to reduced survival thereby challenging the notion that pDCs are essential and beneficial for innate antiviral defense (in mice). The role of pDCs in MCMV and Influenza A virus infection has been investigated previously using other means of reducing the number of pDCs and the results presented here are consistent with the findings described previously. This study takes an additional step and addresses the mechanism of the pathology-enhancing effect of pDCs in pathogenic Influenza A virus infection and shows its dependence on TLR7 expression in pDCs and on IFNAR signaling. The investigation of pDC function in the SARS-CoV-2 mouse model is novel and suggests a pathology-enhancing role of pDCs also in this respiratory viral infection model suggesting that pDCs recruited to the SARS-CoV-2 infected lung may contribute to the delayed and prolonged type I IFN production that has been shown to promote inflammation and pathology in human patients with severe COVID-19. Although the results cannot be directly extrapolated to humans, these finding are potentially highly significant in the context of therapeutic approaches promoting or inhibiting pDC responses in acute respiratory viral infection warranting further investigation in non-human primate pre-clinical and in clinical studies.
We thank the reviewers for highlighting the careful description, robustness, novelty and relevance of our data, their potential impact for human health and the care we took in writing the manuscript in a logical and didactic manner easy to follow.

The following major points would need to be addressed in my opinion:

1. The pathogenic Influenza virus infection model with the H3N2 A/Scotland/20/74 virus strain was used here to investigate the role of pDCs in that model. Previously published data cited by the authors showed that pathology and survival in this model depends in IFNAR signaling. This has also been shown in the similar X31 model and in that model depletion of pDCs using anti-BST2 antibodies also reduced the pathology. While it is interesting that pDC-less are protected against the immunopathology elicited by these Influenza A viruses, where IFN-alpha/-beta contributes to the inflammation and severity of the pneumonia, it is also an expected result. However, in other Influenza virus infection models where type I IFN is more important for virus control, do pDCs contribute to the IFN-dependent protection?

We agree with the reviewer that our conclusions regarding IAV infection have been drawn based on the exclusive use of the Scotland H3N2 strain. We have specified this in all the sections of the paper. We chose this strain, as it induces lung immunopathology in mice on C57BL/6 background (Le Goffic et al. Plos Pathog 2006), but with reduced severity as compared to the currently used PR8 (H1N1). A recent publication has proposed that, in mice infected with PR8, IFN-III would be the first line of host protection and that both IFN-III and IFN-I would be required at lethal dose (Galani et al. Immunity 2017). We thus investigated the impact of IFN-III loss in mice infected with the Scotland IAV strain. Our novel data, included in the revised version of our paper, show that 90% of the mice deficient for Ifnl2/3 succumb to the infection, whereas more than 85% of the mice deficient for the response to type I and type II interferons survive. Thus, IFN-III contribute to host defense in our IAV infection model, although these cytokines are produced by pDCs. Our bulk RNA-Seq data show that IFN-III levels are not significantly reduced in the lungs of infected pDC-less mice, thus showing that other cells than pDCs contribute to of the production of protective IFN-III. A protective role of IFN-I was proposed in mice bearing a functional allele of *Mx1*, a major restriction factor against IAV replication (Kaminski et al. J Gen Virol 2012). However, even in an Mx1-competent context, viral control upon infection by different IAV strains appears to be affected more strongly by unresponsiveness to IFN-III than to IFN-I, especially when the virus is administered in conditions allowing selective infection of the upper respiratory tract (Klinkhammer et al, eLife, 2018). We generated B6.A2G-Mx1-*Ifnar1*^{KO} and B6.A2G-Mx1-pDC-less mice and infected them with high doses of the Scotland IAV strain, in comparison to B6.A2G-Mx1 animals. As shown in the revised version of the manuscript, all three Mx1-competent mouse strains survived to the infection (Ext Fig.3c), although at day 2 after infection viral titers tended to be higher in B6.A2G-Mx1-*Ifnar1*^{KO} mice (Ext Fig.3d). Depending on the mouse genetic background and on the IAV strain, IFN-III can contribute to viral control, eventually in cooperation with IFN-I. Therefore, future studies going beyond the scope of our current manuscript will be needed to disentangle the complexity of the roles of the different type of IFN in the balance between immune protection and immunopathology during IAV infections.

2. The mechanism of the detrimental function of pDCs in SARS-CoV-2 infection in male mice is very interesting (data in Figure 7 and Ext Fig 8). Here the mechanism is not fully worked out. It would be interesting to know if the IFN I response itself (IFN-alpha subtypes and IFN-beta) is reduced in pDC-less mice and in TLR7ko mice. The kinetic of pDC numbers in the lung would be important to understand their contribution to host response and pathology.

According to the excellent suggestions of the reviewer, we have analysed more in depth the IFN-I production and response during SARS-CoV2 infection. First, we have added new data obtained after analysis of Bulk RNA-Seq performed on lungs isolated from K18:hACE mice at different time points after infection (Fig. 8 and Ext Fig.9). These data show that: i) IFN-I expression reaches its peak at day 2 p.i.; ii) *Ifna4* and *Ifnb1* expression persists until day 4 p.i.; iii) ISG expression persists for longer times, being still detectable at day 6 p.i (Fig. 8d, Ext Fig 9a-b). Second, we have compared the kinetics of pDC recruitment in the lungs, viral titers, IFN-I and ISG expression between control, pDC-less and *Tlr7*^{KO};K18:hACE mice at days 2 and 4 after infection (Fig. 8g, Ext Fig9 e-f). pDC increased starting from day 2 p.i., with no major impact of Tlr7 deficiency. Viral control was not altered in the absence of pDC or of Tlr7 signalling. Interestingly, infection-dependent induction of *Ifna1* and *Ifna5* expression was affected in both female pDC-less and *Tlr7*^{KO} mice, while *Ifna4* and *Ifnb1* were reduced exclusively in

female *Tlr7*^{KO} mice. IFN-I were barely detectable in infected males at day 2 p.i., with a late pDC-independent, but Tlr7-dependent expression of some IFN-I genes at day 4 p.i. These results are thus consistent with Tlr7-dependent, but pDC-independent, production of protective IFN-I. Despite similar viral control in infected animals across the different mouse strains examined, the expression of ISGs was reduced by the lack of pDCs or of Tlr7 signalling, especially in females. Finally, we have also integrated new data regarding pDC kinetics (Fig. 8e, Ext. Fig.9c-d).

3. Abstract:"…were more resistant to intranasal infection with influenza virus…" This suggests that pDC-less mice are less infected (lower viral titer), which is not the case. I would therefore suggest to formulate this differently, and say that pDC-less mice show less immunopathology and higher survival.
We thank the reviewer for suggesting this excellent correction. The abstract has been modified accordingly as follows:

pDC-less mice ……., and showed higher survival and less lung immunopathology ~~were more resistant~~ to intranasal infection with influenza virus and SARS-CoV2.

4. Introduction: It would be helpful to explain in the introduction the H3N2 A/Scotland/20/74 Influenza virus infection model and how the immunopathology develops in that model.

We thank the reviewer for this excellent suggestion. We have modified the introduction, as follows:

Moreover, pDC-less mice showed an enhanced resistance to respiratory infections with the influenza virus strain Scotland H3N2[33] or with SARS-CoV2 that induce a severe lung immunopathology associated to a local cytokine storm.

5. All Figures: It is unclear what the genitype of the control mice is. Do they express Cre or not? Are they from the same breeding, similar housing conditions? It wold be important to make that clear from the start.

We thank the reviewer for this important question. We are sorry for not having explained clearly enough the exact nature of the control animals used. As control animals, we used mostly C57BL/6 mice from a commercial provider. As C57BL/6 come from Janvier lab and not directly from our own mouse, we let them to adapt to our mouse house for at least two weeks before performing experiments. Moreover, for each set of data in Figures 1,2,3 and 6, we also used as additional control homozygous *Pacsin1*-DTA or *Siglech*-Cre mice, as these two mouse strains are bred together to generate double heterozygous pDC-less. We did no detect any difference between commercial C57BL/6 mice and *Pacsin1*-DTA or *Siglech*-Cre mice. This has now been explained in the M & M section of the revised version, as follows:

C57BL/6, coming from an external provider, as well as *Siglech*^{iCre} and *Pacsin1*$^{LSL-DTA}$ mice, bred in the same mouse house as pDC-less mice, gave similar readouts and were indistinctly used as control in experiments with MCMV or IAV.

6. Figure 2d: It could be mentioned that there is high variability, but a trend towards reduced ISG expression in pDC-less mice is observed, seen for example for Irf7 in spleen and liver, Mx2 in liver and Isg15 in liver.

We have modified the text according to the suggestions from reviewers, as follows.

When compared to uninfected mice, ~~*Irf7*, *Mx2*, *Isg15* and *Ifit2*~~ the expression of the ISGs *Mx2*, *Ifit2*, *Irf7* and *Isg15* was upregulated with similar kinetics in the organs of MCMV-infected control and pDC-less, mice (**Figure 2d** and **Extended data Figure 2d**), although we observed a high variability for each genotype and organ, with a trend to pDC-dependent expression for *Irf7* in the spleen and for *Mx2* and *Isg15* in the liver. In contrast, the expression of these ISGs was downregulated in most of the organs isolated from infected *Ifnar1*^{KO} mice.

7. Line 247: It would be helpful to insert a sentence that makes clear why the experiment with mice that have a functional Mx1 allele was performed. What was the purpose of the experiment?

We thank the reviewer for this excellent suggestion. The text has been modified as follows:

In most murine strains, including C57BL/6, Mx1, the main virulence restriction factor against IAV, is defective, due to a loss-of-function genetic mutation. Congenic C57BL/6 mice expressing at least one functional *Mx1* allele are resistant to IAV infection, even at high viral loads[42]. This resistance was proposed to be dependent on the response to IFN-I, downstream of the Tlr7-Myd88 signaling pathway[42]. Lung viral titers were increased in IAV-infected *Mx1*$^{+}$ mice depleted of BST2^{+} cells, leading to the hypothesis that pDCs are a source of protective IFN-I in *Mx1*$^{+}$ mice[42]. Thus, we used our pDC-less mice to determine whether their selective depletion reveals a beneficial role of pDCs in C57BL/6 mice expressing a functional Mx1.

8. Line 252: “pDC-less mice survived to the infection” – delete “to”

We thank the reviewer for catching this typographical mistake. It has been corrected.

9. Line 253: “displayed poor morbidity” – I would suggest to formulate this more directly and say that the mice survived and didn’t show any weight loss.

We thank the reviewer for this suggestion. The text has been modified accordingly as follows:

To this aim, we bred our *Pacsin1*$^{LSL\text{-}DTA}$ mice with B6.A2G-*Mx1*$^{+/+}$ animals, obtaining *Pacsin1*$^{LSL\text{-}DTA}$;*Mx1*$^{+/+}$ mice, which we then crossed with *Siglech*^{iCre} mice to generate *Mx1*$^{+}$ pDC-less mice. B6.A2G-*Mx1*$^{+/+}$ mice were also bred with *Ifnar1*^{KO} mice, generating *Mx1*$^{+}$;*Ifnar1*^{KO} animals. Surprisingly, B6.A2G-*Mx1*$^{+}$, *Mx1*$^{+}$ pDC-less and *Mx1*$^{+}$;*Ifnar1*^{KO} mice, all survived the infection with high viral loads of IAV ($10^{\wedge 6}$ pfu) and did not show any significant difference in weight loss ~~displayed poor morbidity~~ (**Extended Data Fig. 3c**), despite a trend to increased viral titers in *Mx1*$^{+}$;*Ifnar1*^{KO} mice at day 2 p.i. (**Extended Data Fig. 3d**).

10. Line 267 “lung pDCs increase” change to “lung pDCs increase in numbers”?

11. Line 287: “IFN-a were significantly“ change to “IFN- a levels were”?

12. Line 305: “responsible of” change to “responsible for”

We thank the reviewer for these suggestions. The text has been edited accordingly.

13. Figure 4a: Unclear which comparisons the p values belong to – this depiction is a bit confusing and should be clarified.

We thank the reviewer for pointing out this issue. This figure has been replaced by another one showing exclusively pDC. We hope that the data shown and their related statistics are now clearer.

14. Figure 5c, d, e and ext Fig. 5: The difference in survival is not reflected in a difference in weight loss (it can't really be seen very well because the size of the symbols is too large). To understand why the mice have better survival it would be important to know if the viral titers are different and what the clinical and histopathological signs were.

We thank the reviewer for pointing out this issue. We have modified the Ext Fig.5 by reducing the size of the symbols in the weight curves and by adding new data regarding SBMC clinical scores during IAV infection. We would like to underline that even plain pDC-less vs control mice do not significantly differ for their weight loss (Extended Fig.3). Although SBMC do not show main differences for their weight loss, they are quite different for their clinical scores. Indeed, control SBMC harbored more ruffled fur, were less active and drunk less water than pDC-less and *Irf7*^{KO} SBMC mice. Together with their non-significant trend towards higher weight loss or slower weight recovery, this led to significantly higher clinical scores for control SBMC as compared mutant SBMC.
Moreover, according with the reviewer request, we have added in Fig.5 new data showing that :

a. viral titers are comparable among the different SBMC ;
b. at the transcriptional level, the expression of *Ifna1*, *Ifna2* and *Ifna5* is reduced in both *Irf7*^{KO} and pDC-less SBMC, as compared to CTR SBMC. In contrast, *Ifna4* and *Ifnb1* are expressed at comparable levels in the three SBMC types;
c. at the protein level, IFN-α levels are reduced both in *Irf7*^{KO} and pDC-less SBMC, wheraes IFN-β levels are comparable in the three SBMC types.
   Data in b. and c. confirm the results obtained with plain pDC-less mice and shown in Figure 4 and Extended Figure 4.

15. Figure 6: Significant increases in eosinophils and neutrophils (but unaltered CD45+ cell numbers) are shown in pDC-less mice, despite them showing less lung inflammation. How could this be explained?
In pDC-less mice, we did observe a tendency towards higher numbers of CD45+ cells the lungs, although this was not statistically significant. As eosinophils and neutrophils are much less frequent in CD45+ cells than other immune cells whose numbers were not affected, such as B, T, NK cells and monocytes, their specific increase in numbers did not bear a significant impact on the total number of CD45+ cells.

16. Ext Figure 6: CD11b BUV395 axis labels: sometimes the "b" in CD11b is lacking.
We thank the reviewer for catching these mistakes. We have corrected them.

17. Figure 7d: The FITC molecules in the airway should be the same amount in the healthy versus the leaky epithelium, but the FITC amount in the blood vessel should be higher when there is damage in the epithelium, so the green dots should be moved to the blood vessel to indicate this.

We thank the referee for his/her suggestions to ameliorate this scheme. We have modified the figure accordingly and hope that t will be clearer for the readers.

18. Figure 8f, line 453: YFP expression could be delayed compared to actual IFN-beta expression – therefore it is not possible to really say that "IFN production by pDCs was delayed with respect to ISG induction".

In our previous paper using *Ifnb1*^{EYFP} reporter mice (Abbas et al. 2020 Nat. Immunol.), we indeed showed that early IFN-producers pDC can express *Ifna* and *Ifnb1* transcripts and proteins, but not yet YFP, whereas, at the peak of their IFN-I production, pDC express both and then keep YFP expression for 12/24 hours after stopping IFN secretion. We have thus revised the text specifying this point. Moreover, we have added novel data to better document IFN expression in the presence or the absence of pDCs or Tlr7 signalling.

19. Figure 8d, e, f, Legend: It is unclear if male or female mice or both sexes were used. This should be written in the legend and described in the results if there were any differences between the sexes with regard to ISG expression

We thank the reviewer for pointing out this issue and apologize for it. In the revised version of the paper, we have specified the gender of the mice in each panel and added new experiments comparing female vs male control, pDC-less and *Tlr7*^{KO} K18-hACE mice.

20. Line 415: C57BL/6 mice are not resistant to SARS-CoV2 omicron variant – so this general statement is not correct.
We agree with the reviewer comment that SARS-CoV2 strains can vary in their binding affinity to mouse ACE and that the Omicron strain can efficiently bind to mACE. However, despite this feature, Omicron induces neither detectable infection nor pathology in C57BL/6 mice. We have modulated our text to better explain this, as follows, and we specified that we have used for our infection the δ Wuhan strain.
C57BL/6 mice usually develop neither detectable infection nor pathology upon exposure to most SARS-CoV2 strains, including because these viral strains display low affinity binding to mouse angiotensin-converting enzyme 2 (ACE2), the main ~~as~~ receptor for cell entry.

21. Line 436: "significantly worsened susceptibility", could be changed to "significantly increased susceptibility"
We thank the reviewer for this suggestion and have edited the text accordingly.

22. Line 507: I would be cautious to draw conclusions regarding pDC function in respiratory viral infection in humans. pDCs and TLR expression pattern show differences between mice and humans that may be relevant in response to viral infection.
We have removed the conclusions on humans from the corresponding sentence.
However, we would like to emphasize that the molecular make-up and functions of pDCs are strongly conserved between mice and humans, as strongly as that of B lymphocytes, and more strongly than that of other DC types or of NK and T lymphocytes. For example, likewise to mouse and human B cells, mouse and human pDCs co-cluster together in pangenomic transcriptomic analyses simultaneously analysing a variety of immune cell types, in contrast to other DC types and to NK or T lymphocytes that tend to cluster by species (Robbins et al. Genome Biology. 2008; Crozat et al. Immunol Rev. 2010). Contrary to the expression pattern of TLR9, the expression pattern of TLR7 is strongly conserved across cell types between mice and humans (Crozat et al. Immunol Rev. 2009). As we discussed later in the manuscript, one study has reported data supporting the proposal of a deleterious role of pDCs in severe human SARS-Cov2 infection (Laurent P … Barrat FJ. Sensing of SARS-CoV-2 by pDCs and their subsequent production of IFN-I contribute to macrophage-induced cytokine storm during COVID-19. Sci Immunol. 2022. doi: 10.1126/sciimmunol.add4906. PMID: 36083891). One study has reported data

supporting that pDC are redundant for SARS-CoV2 protection in humans (Momenilandi M … Béziat V. FLT3L governs the development of partially overlapping hematopoietic lineages in humans and mice. Cell. 2024. doi: 10.1016/j.cell.2024.04.009. PMID: 38701783). Hence, it is likely that pDC function could be redundant or detrimental in certain respiratory viral infections in humans, including in SARS-CoV2, as we discussed later in the manuscript.

23. Line 545-546: This sentence is hard to understand – would suggest to formulate differently for more clarity.

We apologize for difficulty to understand of this sentence. We have modified the text as follows:

Here, we showed that, during SARS-CoV2 infection of mice, pDC can be dispensable or deleterious, whereas Tlr7 and IFN-I responses are protective. pDC are increased in the lungs and contribute to local IFN-α production during infection. However, Tlr7 deficiency more severely and broadly decreases both IFN-α and IFN-β production in the lungs. Thus, our findings support a scenario where the IFN-I produced by pDC are not required for survival and can even be detrimental, whereas other Tlr7-dependent cellular sources of IFN-I are required for host resistance.