nature portfolio

Peer Review File

Progenitor T cells drive chronic pulmonary type 2 inflammation

Corresponding Author: Dr Patrick Brennan

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

Version 0:

Decision Letter:

20th Aug 2025

Dear Dr Brennan,

Thank you for providing a point-by-point response to reviewers comments on your manuscript "Progenitor T cells drive chronic pulmonary type 2 inflammation". We would be willing to consider a revised version that addresses these comments, as you outlined. Please bear in mind that we will be reluctant to approach referees in the absence of major revisions.

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.

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* 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.

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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.

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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.

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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
Nature Immunology

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have fully addressed my previous comments. I appreciate the additional data and explanation they have provided and support publication of the manuscript.

Reviewer #2

(Remarks to the Author)
In this revised manuscript investigating the biology of progenitor Th2 cells in chronic allergic inflammation, Kratchmarov et al. have performed numerous new experiments that have significantly strengthened the manuscript. The authors have rigorously characterized a population of TCF1+ Th2 cells that arise during chronic allergic lung inflammation, which exhibit self-renewal and the ability to differentiate into effector Th2 cells that maintain the type 2 response. The authors new experiment using CD4-CreERT2 x Tcf7 fl/fl mice supports the importance of TCF1+ Th2 cells in vivo during chronic allergic lung inflammation. Their comparison of LN and NLT TCF1+ Th2 cells sheds new light on the unique features of NLT TCF1+ Th2 cells, which the authors complement with an IL-7 blockade experiment. In addition, the new scRNA-seq and spatial transcriptomics datasets provide a detailed characterization of the distinct features of acute and chronic allergic lung inflammation. These datasets will be a helpful resource for future investigations. I congratulate the authors on this rigorous and important experimental work. This manuscript will be of broad interest to the immunology community. The text is well written and I don't see any concerning issues with the methodology or statistical analyses. Below I outline some very minor comments, but I do not feel additional experimental work or significant revisions are necessary.

Minor comments:
1) On line 172-173, the authors write "Taken together, these data confirm that the GATA3+TCF1+ST2+ Th2 population has progenitor potential, coupling self-renewal with effector generation." Do the authors mean the GATA3+TCF1+ST2- Th2 population?

2) In lines 291-292, the authors write "These surface marker expression patterns suggest that the TCF1+ST2- compartment represents a state distinct from resident memory." I understand what the authors mean by this sentence, but after showing that TCF1+ST2-CD4+ T cells within the lungs are similar in number after FTY720 treatment (suggesting residency) and do not absolutely require ongoing antigen stimulation (a shared feature with memory T cells), this statement may be confusing

to the reader. Perhaps the authors can rewrite this sentence to more clearly explain the unique features of TCF1+ST2- cells?

3) There seem to be typos on lines 318 and 625. Also there is a typo in Figure 8F (right panel) in which the y-axis should presumably read “ILC2”.

4) In Figure 8d and the methods, the authors state that they are using an anti-IL-7R antibody, but in Figure 8e-f, the authors label the group as anti-IL-7. Although I appreciate the anti-IL-7R is not depleting and is blocking IL-7 signaling, the authors should be consistent with the antibody being used throughout the figure.

Reviewer #3

(Remarks to the Author)
I am quite positive of this resubmission by Kratchmarov and co-authors. The work has been dramatically improved and provides more conclusive evidence now than before that progenitor Th2 cells are in fact important for sustaining the effector Th2 population and that these have physiological relevance. The data on the progenitor niche and depletion experiments are also more conclusive. So I appreciate the effort.

However, I believe that the authors would do well to reproduce the data presented in Fig. 3c with conditional and temporal deletion of Tcf7. It appears that this was only conducted once and this is not sufficient from my perspective. It is the most important experiment in the study in my eyes and needs to be shown with more replicates. The effective deletion of Tcf7+ cells needs to be more clearly shown also, even in a temporal manner (how quickly is deletion detected) and this entire analysis needs to be broadened. Again, for me, this is an important part of the paper, even if tcf7 deletion if only partial, the authors should clearly show this.

Similarly, the IL7R blockade experiments presented in Figure 8 need to be reproduced as it is not apparent that this has been performed.

If these can be reproduced reliably, the study would be more conclusive.

Decision Letter:

20th Mar 2026

Dear Dr Brennan,

Thank you for providing your response to reviewers concerns on your article "Progenitor T cells drive chronic pulmonary type 2 inflammation". We think your proposed changes should satisfy reviewers concerns and we remain interested in the possibility of publishing your study in Nature Immunology, following the addition of the data as per your response.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file 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.

When revising your manuscript:

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* 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.

* Please include a revised version of any required reporting checklist. It will be available to referees to aid in their evaluation of the manuscript goes back for peer review. They are available here:

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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.

Please note, 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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We hope to receive your revised manuscript within two weeks. 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.

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

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.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,

Stephanie Houston, PhD
Senior Editor
Nature Immunology

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)
Thanks to the authors for pointing out the difficulties with the CD4 Cre TCF7 mice. This is a shame. Thanks also for conducting more experiments with IL7R blockade, which make the study more rigorous.
I think that the study goes a long way to establishing that TCF1+ progenitors support allergic lung inflammation and sustain long term Th2 cell responses. I still don't think that the results presented prove this beyond any doubt, but on the balance of all results, the study is convincing, novel and will be of interest to the field.

Decision Letter:

Our ref: NI-A41143B

19th May 2026

Dear Dr. Brennan,

Thank you for submitting your revised manuscript "Progenitor T cells drive chronic pulmonary type 2 inflammation" (NI-A41143B). It has now been seen by the one of the original referees and their comments are below. The reviewer 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

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

Sincerely,

Stephanie Houston, PhD
Senior Editor
Nature Immunology

Referee #1 (Remarks to the Author):

A. Kratchmarov and colleagues report that a population of TCF1+ CD4 T cells may act as a progenitor population for more differentiated Th2 cells. They show that TCF1+ cells become more and more prominent over time in chronic models of airway allergen instillation, but not in acute allergen or infection models (with H polygyrus bakeri) (Figs. 1 and 2). These TCF1+ cells are likely dependent on iBALT formation since depletion of B cells with an anti-CD20 antibody is able to significantly reduce this population (Ext data 3). In Fig. 3, Kratchmarov and colleagues define a transcriptional signature for progenitor Th2 cells in the chronic phase of allergen instillation which both shares and has unique features from progenitors in an LCMV dataset. Finally, the authors show that adoptive transfer of progenitors using the marker Ly108 as a marker for progenitors, leads to robust allergic inflammation in TCRb KO mice – at a greater extent than what is achieved after the adoptive transfer of differentiated Th2 cells. Transfer of this Ly108+ population into immune-proficient congenic mice also leads to their differentiation into ST2+ effector cells.

B. The study provides some support to the notion that a progenitor pool exists and has the potential to differentiate into effector Th2 cells. This is in line with the authors own previous study on human Th2 cells in various allergic diseases, and in that sense, this study does not appear to greatly shift our understanding of the function of this progenitor population.

In our initial human studies, we defined a tissue memory Th2 population which we named the Th2 multipotent progenitor. We further demonstrated that this population had the capacity to differentiate into Th2 effectors, TFH, and Treg while self-renewing, suggesting a role for these cells in human disease. However, this work was largely reliant on *ex vivo* human tissues, and our capacity to follow progenitor cells *in vivo* over time and dissect mechanisms involved in their maintenance was inherently limited.

We appreciate the frank assessment of the potential impact of our initial submission, which was helpful in planning additional experiments in this revised manuscript. Respectfully, we contend that we have learned a tremendous amount about the Th2 tissue progenitor in this study - with substantial new experimental data and ability to present our work in a long format, we hope that the reviewer will agree.

Our novel mouse model greatly extends our understanding of the Th2 progenitor state and provides clear contrast with the acute type 2 lung inflammation models commonly employed in the field. These studies move our initial finding from association toward causation, which is not a small feat. We developed a novel model of chronic type 2 inflammation using poly-allergen sensitization, enabling us for the first time to define and interrogate the development of type 2 progenitor cells. Moreover, we demonstrate that tissue progenitors are necessary and sufficient for the maintenance of type 2 inflammation. We directly compare acute to chronic type 2 inflammation and also contrast tissue Th2 cells with those in draining lymph nodes, identifying factors associated with chronic tissue inflammation. We identify lineage relationships among Th2 populations using scTCRseq and adoptive transfer mnethods. We compare chronic type 2 inflammation to chronic infection to give our findings broader context. We analyzed the whole lung microenvironment using scRNAseq and spatial transcriptomics, including stromal and parenchymal cells in the chronic state, identifying and functionally validating a role for B cells and IL-7 in the maintenance of Th2 progenitors. Together, we believe these findings will be impactful to Th2 biology and T cell biology in general.

C. The data and methodology used in this study are quite appropriate, but they still don't allow us to really gauge the importance of this TCF1+ population in repopulating the Th2 cell pool over time. It would be ideal if these progenitor cells could be specifically depleted in chronic models of allergen instillation and the impact of this depletion thereafter tested. A genetic model would be ideal - whether this can be achieved by temporal deletion of the Tcf1 gene is unclear. The authors went to some lengths to identify a transcriptional signature for progenitor cells, but have not validated these genes, nor shown what deletion of such genes may do to the progenitor population. Again, timed deletion of x gene may be able to impair this TCF1+ population in real time, but this is not shown.

We appreciate the reviewer's suggestion that a mechanistic understanding of Th2 progenitor cell function would be enhanced by a conditional genetic ablation approach. However, we had concerns about employing

widely used germline knockout strains, e.g. *Tcf7*$^{-/-}$ mice where naïve T cell-poeisis (*Nature* 1995. 374:70–74) and Th2 differentiation (*Nature Immunology* 2009. 10:992-9) are both profoundly perturbed, as it would not be possible to distinguish a role for *Tcf7* in development, from a role in acute differentiation, from a role in chronic inflammation. Given the role of *Tcf7* in acute Th2 differentiation, we were also reluctant to employ CD4-Cre, Lck-Cre, or Ox40-Cre mice. To address this important question, we have now generated inducible CD4 CreER^{T2} Tcf7fl/fl mice, allowing for temporal ablation. This approach led to partial *Tcf7* deletion, and as a result, we observed a decrease in total Th2 cells as well as tissue eosinophilia, supporting a role for TCF1 in the maintenance of chronic type 2 inflammation (new Figure 2c). Based on discussions with collaborators and our own experiences with other loci, partial deletion seems to be an inherent limitation of the CD4-CreER^{T2} driver, which is unfortunately the only widely available inducible T cell deleter that does not involve TCR transgenic approaches. Despite incomplete ablation, we still believe that this is important and meaningful new data.

To complement the *Tcf7* inducible deletion data, we also utilized an antibody blockade approach to target Th2 progenitors, motivated by scRNAseq analysis showing elevated IL-7R expression on tissue Th2 progenitors, and inspired by Reviewer 3 (Figure 8). We present functional data that local blockade of IL-7R through intranasal antibody administration is sufficient to deplete Th2 progenitor cells and decrease tissue inflammation (Figure 8). Through scRNAseq analysis, we also identified potential sources of local IL-7 production. Taken together, our genetic ablation of *Tcf7* and antibody-mediated depletion of Th2 progenitors support the requirement of the Th2 progenitor in the maintenance of chronic type 2 inflammation.

The authors have used anti-CD20 to impair TLS formation, but it would be ideal if the established TLS could be disrupted using a chemical approach – from this, the impact on subsequent TCF1+ cells and allergic immunity would need to be analysed.

We concur that the distinction between (1) disruption of already established TLS versus (2) impairing the *de novo* establishment of TLS is essential and carries implications for translational work in human diseases. As such, our approach was to treat with B cell depleting monoclonal antibody treatment at the chronic stage of inflammation when TLS is already established. Our findings therefore are in line with the reviewer's suggested approach. To clarify our experimental strategy for the revision, we now include clear sensitization and antibody administration timeline diagrams with the new Figure 6h.

D. Statistics appear ok.

E and F. I have outlined what I see as the key issue in section C. Being able to deplete TCF1+ cells in real time and analysing the impact of this would be important. As it is, it is hard to say whether these cells actually play a role in repopulating the Th2 cell pool.

Please see above response to the issue of TCF1+ cell depletion, which addresses this comment.

Several other issues should also be addressed. Firstly, the expression of TCF1 is poor, as detected by antibody staining. In Fig. 1D, the authors have used a gate for ST2 and TCF1 which may or may not be correct – it is hard to tell. The authors may be underestimating the TCF1+ gate in the acute phase simply because of background levels, since the quadrant appears to essentially cut through the TCF1+ cells in the acute FACS plot. It is evident that in cells from chronic mice, TCF1 staining is actually also brighter on ST2+ cells. This makes it difficult to have much confidence in the TCF1 stain. A TCF1 reporter mouse would help to solve this problem.

We share the reviewer's concern regarding the technical difficulties with intranuclear transcription factor staining and agree that separation of TCF1-hi and TCF1-low cells is not trivial. To address this important issue, we present two new, complimentary sets of data (new Figure 2), the combination of which provides us with confidence in the TCF1-expressing populations at distinct phases of inflammation.

1) We performed intranuclear staining with a different mouse monoclonal antibody obtained from BD Biosciences (Clone S33-966), which showed superior separation of TCF1-hi/low states (new Figure 2a).
2) We obtained *Tcf7*^{Gfp} reporter mice and performed experiments with both acute and chronic allergen challenge, followed by sorting of Tcf7-Gfp^{hi} and Tcf7-Gfp^{low} cells, followed by intranuclear staining for GATA3/Foxp3 to distinguish subsets of Th2 cells. Quantification of GATA3+Tcf7Gfp^{hi} (Th2 progenitor) and GATA3+ST2+Tcf7Gfp^{low} (Th2 effector) populations confirmed the expected temporal distributions from antibody staining for each subset.

Secondly, the comparison to H poly is strange. The authors use day 42 as a chronic timepoint, but this infection is not actually chronic. Hp is being eliminated at this time point and likely by 8 weeks post-infection, the worm is largely cleared. Thus, I don't really understand that importance of this comparison and it remains quite unclear to me whether there is truly a difference in the prevalence of TCF1+ cells in chronic allergen instillation experiments versus more acute models.

While it is true that *H. bakeri* is eventually cleared by immunocompetent mice, in our hands it can take up to ~90 days for full clearance, and infection is ongoing at day 40-50 when we interrogated the model. We now present fecal egg count data to support this assertion and respectfully submit that this model constitutes the best available option we have for comparing our "pathogenic" chronic type 2 inflammation to "physiologically appropriate" chronic type 2 inflammation. Notably, we do not expect that type 2 lung inflammation is the only setting in which progenitor Th2 cells accumulate (our human data suggests that Th2 progenitors are a common feature of pathogenic type 2 inflammation). Nevertheless, with the *H. bakeri* comparison, we demonstrate the concept that Th2 progenitor differentiation is not a default feature of type 2 immunity but rather that a context-dependent process, which we believe is a fair claim supported by our data.

In transfer experiments, the authors have used 10,000-20,000 cells and it is not clear to me why they would use different cell numbers when trying to compare the pathological effects of adoptive cell transfer.

We have clarified the methods section to specify that equal numbers of each subset were used for a given experiment but that there were different yields from different donor mice, as is common in adoptive transfer of T cells. That is to say, when 10,000 progenitor cells were transferred, this was compared to transfer of 10,000 effector cells from the same donor mouse. Results did not differ depending on the cell number transferred.

There are also questions that spring up here including, Do differentiated Th2 cells induce more robust inflammation early on? Do these cells have inherent difference in ability to home back to the lung that is independent of their progenitor/effector status? Would intranasal transfer yield the same effects?

The reviewer has brought up an interesting issue of the early effector function and homing capacity of Th2 subsets. To address this question, we performed adoptive transfer experiments of Th2 progenitor and effector cells with subsequent readout at day 5 post-transfer. Interestingly, we saw no statistically significant difference in eosinophilia induced at this earlier timepoint. This suggests that early post-transfer, effector cells are active, home to the lung, and induce robust eosinophilia prior to cell death. This data also suggests that progenitor cells can rapidly differentiate and contribute to type 2 inflammation. The idea of an intranasal transfer is intriguing but given the profound mucous plugging that occurs in these models of pulmonary type 2 inflammation, and the lack of an understood role for the migration of T cells from the alveolar to the interstitial space, we do not believe it would be feasible to deliver reliable numbers of donor cells in a reproducible and physiologically relevant fashion.

The use of Ly108 versus ST2 also leads me to wonder how confident one can be about the antigen-specific status of this population. Adoptive transfer experiments with tetramer-specific cells, or with a TCR transgenic mouse would give more confidence here perhaps.

We studied polyclonal T cell responses to multiple antigens to better reflect human disease and also tracked antigen specificity using tetramers recognizing the 2W1S peptide. We now present data to clearly demonstrate that antigen-specific cells are present in these progenitor/effector compartments (new Figure 4f,g). Much of this data was condensed to summary plots in the initial submission and thus may not have provided the correct context. We could sort and transfer only a single tetramer positive cell population, but we are not sure that would strengthen our claims, and such an experiment would likely necessitate pooling of multiple donor mice in a non-physiologic way.

Ly108 as a marker is also not fully validated in Ext data 10. Are all of these Ly108+ST2- cells truly TCF1+ or are some of these TCF1-?

We selected Ly108 as a surrogate marker of TCF1 expression based on prior high-profile publications and internal validation, but we agree with the reviewer's concern regarding the purity of this population and the need for rigorous validation of the TCF1-expression status of any putative subset. To address this issue, we present Tcf7^{Gfp} reporter mouse experiments with concomitant LY108 staining (new Extended Data Figure 2b) and also perform definitive adoptive transfer experiments using *Tcf7*^{Gfp} donor cells (new Extended Dat Figure 2e,f), which provide complimentary readouts to address this issue.

H. The paper is generally well written. It is quite tricky I believe to understand the computational analysis that has been performed, which is also why validations for certain genes should be performed on this TCF1+ population.

We thank the reviewer for this comment. Transcriptomics is a rapidly evolving area, with new analytical tools made available on a regular basis, and we have applied multiple cutting-edge analysis techniques to answer specific questions. For example, co-varying neighborhood analysis (Reshef *et al.*, Nat Biotech, 2022) used in Figure 6 is not yet frequently applied in Immunology but was a powerful method to answer the questions posed by our data. To address this important comment and thus make our work more clear to a wide audience, we have added additional explanations of the informatic analyses in the revised, long-format manuscript, including lines 753-761, 774-776, and 784-791.

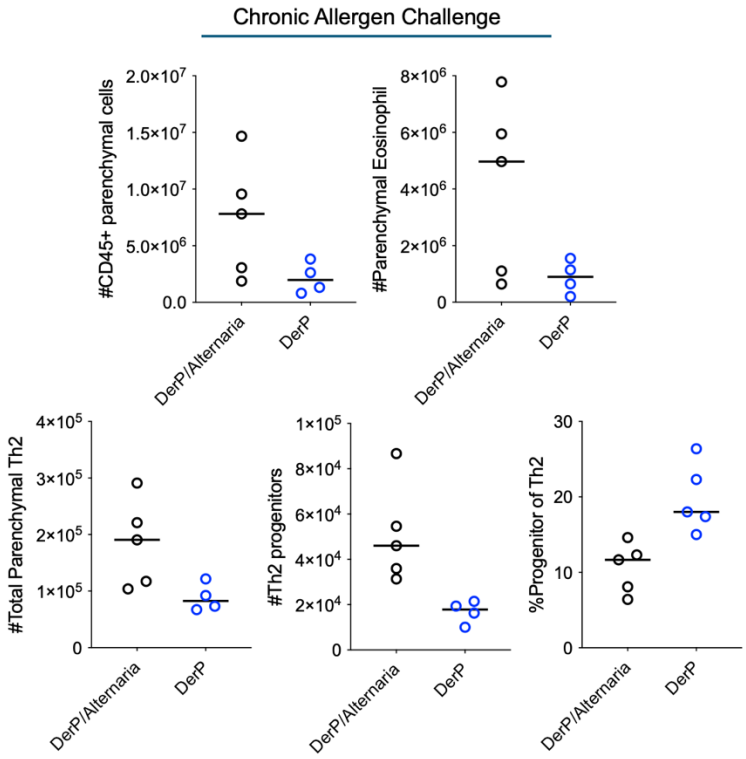
Referee #2 (Remarks to the Author):

This manuscript presents an in-depth dissection of chronic Th2 inflammation in the lung, highlighting the emergence of a TCF1+ progenitor CD4 T cells that maintain Th2 effector responses during allergic inflammation. The authors identify a transcriptionally distinct, tissue-resident subset of Th2 cells that retains stem-like features and gives rise to effector populations, even after antigen is removed. The study uses scRNAseq/TCR sequencing and comparative bioinformatic analysis with chronic infection to characterize stem like T cells outside of the CD8 exhaustion paradigm. The study raises new questions about TLS and B cells shape long term Th2 memory. There are a few points which could be addressed to clarify the findings.

• For readers outside the allergy field, could the authors describe the combined Derp2 + Alternaria approach and whether or not differences would be expected to the standard HDM model?

In this work, we sought to model and mechanistically dissect the pathophysiology of human allergic disease wherein patients are often polysensitized to multiple environmental allergens and encounter distinct stimuli in overlapping fashion, e.g. cockroach and dust mite in an apartment complex. HDM and Alternaria may indeed activate distinct innate signaling and alarmin pathways via different proteases and TLR ligands (e.g. *Science Immunology 2024, 9, eabq4341*). To address this question, we performed single-agent HDM sensitization through the chronic timepoint (please see inset figure). We found increased inflammation in the polysensitized mice, as indexed by total intraparenchymal CD45+ immune infiltrate and total lung eosinophil counts. This correlated with increased Th2 cell infiltration, and interestingly, increased total Th2 progenitor cell counts, despite a relatively lower frequency of this subset as a proportion of total Th2s. We conclude that the globally

increased inflammation in the polysensitization model results in increased Th2 infiltration/proliferation without disrupting the overall progenitor/effector hierarchy. We appreciate the question and provided this data here to address the question, but prefer to limit the manuscript to use of the combinatorial sensitization method as it allows us to withdraw/add signals from distinct stimuli. It could certainly be interesting for future studies to titrate single-agent Alternaria and single agent HDM to doses that elicit comparable eosinophilia and then dissect T cell phenotypes and microenvironment signals in an unbiased fashion. Notably, there is a wide range of reported doses for single-agent HDM sensitization and our conclusions with this benchmark experiment are limited to the dose of HDM that we have used for polysensitization.



• In their initial characterization of Th2 cells at the chronic phase the authors conclude that they have “minimal features of exhaustion” (line 91). Without assessing CTLA4, TIGIT, Tim3, Tox etc this seems a bit of a premature statement.

We concur with the reviewer that the phrase “minimal features of exhaustion” was unclear, and we should have avoided such ambiguous language. However, analysis of surface markers associated with activation and/or exhaustion is certainly valuable. To further characterize the expression of surface markers associated with activation and exhaustion, we performed flow cytometry analysis of Th2 cells with additional markers to clarify the phenotype. While Tigit expression did indeed increase with time, there are reports of TIGIT promoting type 2 inflammation/Th2 responses (J Immunol. 2016 May 1;196(9):3570-80). CTLA4 was most highly expressed by Tregs by several orders of magnitude, though also increased with chronic sensitization on Th2. PD1, the expression of which was equivalent between acute and chronic timepoints, is at once an exhaustion marker, a canonical TFH marker, and a marker of recent activation. TIM3 was not appreciably expressed at the acute or chronic timepoints. We would therefore advocate for caution in ascribing exhaustion or lack of exhaustion based on these markers and concur with the reviewer. We have therefore amended the text to state that Th2 cells have features of chronic activation, amidst evidence of significant ongoing pulmonary eosinophilia, fibrosis, and remodeling. We believe the functional data, e.g. adoptive transfers and disease readouts, provide a better assessment of these cells’ functional capacity.

• The inclusion of the helminth model and analysis of nasal tissues after allergic sensitization are interesting. However, to better understand how the tissue vs inducing agent contribute to Th2 differentiation it would be better to compare apples to apples, for example, Derp1 sensitization intranasally (focusing on lung) versus oral gavage (focusing on LP).

We agree with the reviewer’s concern that the properties of the allergen may play a role equal to the microenvironment for modulating Th2 cell fate. We would also note that it is difficult to make direct comparisons between tissues in different models, and our claims with a worm model are limited to providing an example of tissue type 2 inflammation that is not associated with expanded Th2 progenitors. However, we found the reviewer’s question very interesting, and to address this issue, we have now analyzed a scRNAseq dataset that characterizes T cells in the GI tract following oral HDM/DerP sensitization in a model of eosinophilic esophagitis (new Extended Data Figure 4g). We found minimal *Tcf7* and *Gata3* co-expression and no distinct progenitor-like cluster, particularly when accounting for cells that were present in the homeostatic (saline sensitization) condition.

• After antigen withdrawal in Figure 2 there are decreased numbers of ST2+ cells while TCF1+ cells are less affected. Do TCF1 cells have differences in proliferation (before/after antigen withdrawal)? And do they require ongoing inflammation (after antigen withdrawal) for their maintenance?

To address this question, we now show frequencies of Ki67+ cells under these conditions, which showed a decrease in proliferation following antigen withdrawal (new Figure 4f). Regarding the question of whether ongoing inflammation is required, this is a fascinating issue that relates to the core difference between a "stem/progenitor" cell and a "memory" cell. We know from the "resting" condition (Extended Data Figure 5) where inflammation is resolving that GATA3+TCF1+ST2- cells persist, but we cannot say whether these cells are the same as their progenitor ancestors or if they transition into a more conventional TRM-like state since we did not thoroughly compare these stages. Understanding the connections between T memory states in chronic inflammation, resolving inflammation, and resting memory is certainly an interesting topic for future study and we thank the reviewer for this question.

• As FTY720 treatment can be less efficient during chronic inflammation, would the authors please provide data to show that FTY720 efficiently removes T cells from the circulation (as stated inline 156). In experiment 2e, the MFI of TCF1 appears to be a bit lower after FTY720 treatment (flow plot). This could reflect a decrease in T cells recruited from the dLN (despite the fact that the cell numbers are not changed). Could the authors show that proliferation of TCF1+ cells with and without FTY720 treatment is unchanged?

To address this question, we now show depletion of intravascular-labeled, i.e. circulating, lymphocytes after FTY720 treatment in Extended Data Figure 4e.

We had not considered that there could be reduced recruitment from the lymph node in the setting of FTY720 treatment which is then compensated for by increased local proliferation to yield comparable numbers and thank the reviewer for this suggestion. Upon further analysis of our prior experiments, we found that TCF1 MFI was unchanged in the progenitor compartment with or without FTY720 treatment (new Extended Data Figure 4f). These data, coupled with the lack of a change in Ki67 expression (Extended Data Figure 4f), suggests that this is ultimately a less likely scenario. Nevertheless, the idea is intriguing and the idea of a finite tissue niche and competition for space between *de novo* recruited cells and already tissue resident cells is an essential question in TLS biology.

• In extended data figure 3 the authors show that B cell depletion strongly reduces TLS size in the lung. The number of TCF1+ cells also decreases but does not seem to be a strongly impacted. Might there be other factors that help sustain TCF1+ cells after B cell depletion? Are other features of TLS maintained after depletion; would blocking CXCL13 or CCL19/21 or LTBR-Fc to act directly on stromal cells have an identifical effect?

We agree with the reviewer that it would be very interesting to address the stromal, myeloid, and endothelial cell constituents of TLS, as well as the interactions between these populations. However, given the partial phenotype we saw with B cell depletion, and the partial phenotype with LTBR-Fc reported by Marion Pepper and colleagues in the initially co-submitted manuscript to *Nature* (which the reviewer may not have known about but has since been made available on BioRxiv: https://www.biorxiv.org/content/10.1101/2025.10.03.680350v1), we suspect there may be multiple overlapping and redundant mechanisms at play. Additionally, CXCR5 (receptor for CXCL13) is not appreciably expressed by our Th2 subsets, so we did not pursue CXCL13 blockade. While an interesting suggestion, we were unable to find commercially available neutralizing monoclonal antibodies for CCL19 or CCL21.

Given the importance of this line of inquiry, we performed new scRNAseq of whole lung populations as well as VisiumHD spatial transcriptomics at the acute and chronic timepoints to analyze cell-cell interactions using bioinformatic approaches (new Figure 6 and 7). These analyses identified many cell-cell and molecular signaling interactions that could be relevant to the chronic inflammatory microenvironment. Among these

factors, we provide further bioinformatic dissection of possible T-B cell interactions (new Figure 6, 7 and Extended Data Figure 11) that could explain the phenotype observed with B cell depletion. We also identified a possible role of IL-7 though our informatic analyses and demonstrate a functional role for IL-7 in the maintenance of the Th2 progenitor population (new Figure 8).

• Regarding the pseudobulk analysis (line 239, extended data Fig 5), this is not the optimal approach. Doing DE between clusters which you've defined based on the gene transcription is using the same data twice - once to create the clusters, and once to compare. It would be better to follow the approach introduced in Extended Data 6 and line 299.

The reviewer has brought up an interesting technical point that we agree merits close consideration. The clusters were defined based on unbiased k-means clustering using principal components, i.e. we did not select markers for cluster creation. The data in the DotPlot in Extended Data Figure 5a merely shows a curated highlight of transcripts to orient readers to significant cluster markers from the “FindAllMarkers” Seurat function output. Some of the highlighted genes on the volcano plot in the pseudobulk analysis (Extended Data Figure 6) do indeed contribute strongly to some of the principal components, but in fact every single highly variable transcript (e.g. the 2,000 most variable transcripts by default Seurat settings) has a “loading” on every single principal component, and thus technically every one of the 2,000 most variable genes contributes to the clustering, so we are not sure there is any way to avoid “double counting” the data. Even the Extended Data Figure 7 approach that the reviewer has suggested will suffer from “double counting”. Nevertheless, we did attempt the approach suggested by the reviewer as a complementary analysis, but it did not yield significant differentially expressed genes because there are too few “Chronic” cells in the Acute Effector cluster and vice versa. We believe that the original analyses are informative since the clusters compared are nearly exclusive to the acute vs. chronic state.

The comparisons in Extended Date Figure 6 are meant to highly broad differences between the effector cell clusters, similar to the “FindAllMarkers” function which is used in Seurat to define key markers of all clusters. Pseudobulk differential gene expression is preferable to the FindAllMarkers function for this purpose, as the latter suffers from (1) p-value inflation (from considering each cell a biological replicate), (2) few tunable parameters, and (3) uses the entire remainder of the dataset as the comparison group, which can obscure more subtle differences.

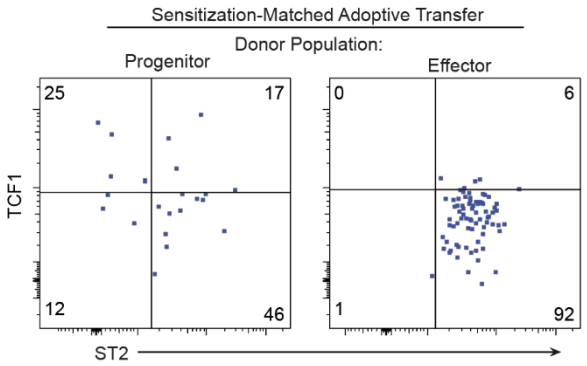
As they are not central to our major claims, we are open to removing these volcano plots from the manuscript in consultation with the Referees and Editor, and could instead present a heatmap of the results from the FindAllMarkers function output, as is common in the field. However, we feel this would be less meaningful for understanding differences between Effector clusters at different timepoints than the current approach.

• Could the authors show the gating and sort strategy for adoptive transfer experiments shown in Figure 4 and validate the use of Ly108 in this model?

We have included these details in the new Extended Data Figure 2 and moreover performed analogous validation experiments using *Tcf7*^{Gfp} reporter mice as suggested by Reviewer 1. We thus have confidence in the lineage relationships established by the new Figure 3.

Is it possible that survival, homing and differentiation potential of transferred cells is affected by their transfer into naïve (as opposed to sensitized/inflamed) mice?

This is an important consideration, and we appreciate the suggestion which we have now addressed by performing adoptive transfer experiments with congenic recipient mice that have been sensitized in parallel to the donor mice with Alternaria/DerP, analogous to “infection-matched” adoptive transfers used in the LCMV literature (please see inset figure). Interestingly, while we observed identical differentiation patterns for the Th2 subsets, we recovered many fewer cells after their transfer into inflamed tissues, which suggests that the progenitor cell niche might be finite and not necessarily accessible at all times after sensitization. In fact, the number of cells recovered in the lung was often close to the limit of detection, e.g. 100 cells/lung digest, which presented technical difficulties in accurately obtaining cell counts. This observation opens up a fascinating series of additional experiments that we feel would form an excellent follow up study of “niche accessibility”.



I’m also intrigued by the intermediate population that expresses both TCF1 and ST2. In CD8 T cells activated during chronic LCMV, IL-33 can support Tcf1/Tscm cells, in part by counterbalancing type 1 IFN signals (Marx et al Immunity 2023). Could the authors comment on whether a similar mechanism could apply to this intermediate TCF1+ST2+ subset? Given the integration of sc data already presented, it would be informative to further connect this newly discovered CD4 progenitor T cell biology to the more widely characterized CD8 stem-like T cell paradigm.

We agree with the reviewer’s comments and share their interest in the intermediate population, which our data demonstrates has some proliferative and differentiation capacity (Figure 3b) and also expands with time (Figure 2b). We suspect that this population is an intermediate state, possibly with distinct supporting factors. Given the expression of ST2, it is certainly possible that IL-33 provides important signals to the intermediate state. We have included a bioinformatic comparison of the Th2 progenitor to CD4+ cells during infections (Figure 5) and have now, in an expanded discussion section, cited and discussed the IL-33/CD8 stemness paper with an emphasis on divergent signals in distinct inflammatory states.

While several of the Figures highlight TFH cells/features there is very little discussion about TFH cells in the written text. Are TFH cells distinct from progenitor/TFH or progenitor/TFH-like cells (both wordings are used). In line 162, the authors justify B cell depletion on the basis of interaction with B cells and CD8 T cells in iBALT but do not mention TFH-like cells which are known to be present. In line 259 Izumo1r is referred to as a Treg marker but not a TFH marker. Are there differences in FR4 (encoded by Izumo1r) or Bcl6 or IL-21 expression in progenitor/intermediate populations by flow cytometry? It would be helpful to provide a bit more explanation for how TFH or TFH-like cells connect to the TCF1+ progenitor population.

TFH cells are certainly important in models of allergic pulmonary sensitization, and we concur that establishing a framework for distinguishing these cells from progenitors is important. We have performed FR4, BCL6, and CXCR5 staining to complement the bioinformatic assessment of TFH markers from Extended Data Figure 6b. While we could detect TFH cells reliably in the draining lymph node, including GATA3+ TFH2/13 phenotype cells, these were rare in the lung tissue, with frequencies less than 5%. FR4 expression increased with time, though was clearly uncoupled from the PD1/CXCR5/BCL6 expression. While TFH cells are likely important players in type 2 inflammation, including in the lung at earlier timepoints post-sensitization, they seem to not be a dominant population with chronicity in our model. Regarding the wording, we have updated the main text and the “progenitor/TFH” designation is now used solely in Extended Data Figure 9, because the comparison group in that analysis, i.e. LCMV Clone13, are cells taken from spleen and thus contain TFH cells, which did not separate into different clusters in our bioinformatic integration.

Referee #3 (Remarks to the Author):

In this manuscript, Kratchmarov et al. investigate the biology of progenitor Th2 cells in chronic allergic inflammation. A notable feature of chronic allergic diseases is the absence of apparent T cell exhaustion despite long-term antigen exposure. Defining the immune mechanisms that maintain chronic allergic disease is of critical importance to the allergy field. The authors previously defined a population of TCF1+LEF1+ Th2 cells with progenitor features in human allergic diseases, which they termed Th2 multipotent progenitor cells. In the current manuscript, the authors use murine models of type 2 inflammation and define a population of TCF1-expressing Th2 cells with progenitor features that expand during chronic allergic inflammation and are sufficient to promote allergic inflammation upon adoptive transfer. Interesting, the authors show that progenitor Th2 cells exhibit a higher "stemness" score compared to progenitor T cells in chronic viral infection. Overall, the manuscript is well-written and the quality of the experimental work, including data presentation, is very well done. I congratulate the authors on this experimental work, which represents an important contribution to our understanding of Th2 cell biology during chronic allergic inflammation. However, there are limitations to the current manuscript that reduce my enthusiasm for the study. As the authors note, TCF1-expressing, progenitor CD4+ T cells have been characterized in various other contexts (PMID: 35637103; PMID: 37672564; PMID: 38168955; PMID: 39396521) and the authors have previously characterized a TCF1-expressing Th2 cell population in humans. While defining the TCF1-expressing, progenitor Th2 cell population in mice is important for investigating their development and function, the preceding studies reduce the novelty of the current manuscript. In addition, there are some limitations to the experimental work. Notably, the experiment testing the function of progenitor Th2 cells relies on adoptive transfer and does not definitively assess the contribution of progenitor and effector Th2 cells in vivo. In addition, there is a lack of mechanistic insight into the development of progenitor Th2 cells, beyond an undefined signal from B cells.

Major comments:

1) After characterizing progenitor Th2 cells, including the contexts giving rise to this Th2 cell state and characterizing their transcriptional signature, the authors assess the function of progenitor versus intermediate versus effector Th2 cells via an adoptive transfer approach. This approach is convincing in demonstrating that progenitor Th2 cells exhibit the ability to differentiate into effector Th2 cells. However, because of the limitations of tissue digestion and the adoptive transfer approach, the contribution of progenitor versus effector Th2 cells in vivo remains unclear. Notably, in the CD8+ T cell field, numerous studies demonstrated that tissue-resident memory T cells (Trm) poorly proliferate when stimulated ex vivo likely due to poor survival compared to circulating memory CD8+ T cells after isolation from tissues. Subsequent intravital imaging experiments showed that CD8+ Trm robustly proliferate in vivo (PMID: 29311694). Consequently, it's possible that progenitor Th2 cells orchestrate greater allergic inflammation than effector Th2 cells upon adoptive transfer, in part, due to the latter population being more vulnerable to death or poor proliferation upon being extracted from tissues. While the adoptive transfer approach is reasonable for assessing a lineage question (progenitor Th2 cells give rise to effectors and not vice versa), it still remains unclear to what extent progenitor Th2 cells and effector Th2 cells contribute to maintaining chronic allergic inflammation over time in vivo. Can the authors provide an alternative experimental approach to test the function of progenitor and effector Th2 cells in vivo? While Tcf7 is required for initial Th2 cell priming, could the authors use a tamoxifen-inducible CD4-Cre crossed to Tcf7 floxed mice to address the role of TCF1 in CD4 T cells in maintaining chronic allergic inflammation?

The reviewer has raised an interesting question that alludes to broader experimental issues regarding whether a cell population is necessary, sufficient, or both for a given inflammatory process. While we feel that we have established both the lineage relationship of the Th2 compartment as well as the sufficiency of Th2 progenitors for initiating and sustaining inflammation, the question of "necessity" remains open, and we agree with the reviewer regarding its central importance. Please see our response to a similar critique from Reviewer #1 for discussion of this point, reproduced here for ease of reference:

To address the reviewer's question, we generated inducible CD4 CreER^{T2} Tcf7 fl/fl mice for temporal ablation. This approach led to partial *Tcf7* deletion, and as a result, we observed a decrease in total Th2 cells as well as tissue eosinophilia, supporting a role for TCF1 in the maintenance of chronic type 2 inflammation. Based on discussions with collaborators and our own experiences with other loci, partial deletion seems to be an inherent limitation of the CD4-CreER^{T2} driver, which is unfortunately the only widely available inducible T cell deleter that does not involve TCR transgenic approaches. To complement our data with the *Tcf7* inducible deletion, we also utilized an antibody blockade approach, motivated by scRNAseq analysis showing elevated

IL-7R expression on tissue Th2 progenitors, inspired by Reviewer 3 (Figure 8). We present functional data that local blockade of IL-7R through intranasal antibody administration is sufficient to deplete Th2 progenitor cells and decrease tissue inflammation (Figure 6). Through scRNAseq analysis, we also identified potential sources of local IL-7 production. Taken together, our genetic ablation of *Tcf7* and antibody-mediated depletion of Th2 progenitors support the requirement of the Th2 progenitor in the maintenance of chronic type 2 inflammation.

2) The authors propose a model in which progenitor Th2 cells expand in the context of chronic type 2 inflammation and play a critical role in replenishing effector Th2 cells, raising the question of which signals promote progenitor Th2 cell development and maintenance. The authors provide evidence that cognate antigen is dispensable for maintaining progenitor Th2 cells, at least on the order of weeks, and that depletion of B cells partially reduces progenitor Th2 cell numbers. However, there isn't any deeper insight into the signals promoting the development/expansion of progenitor Th2 cells. Can the authors provide a mechanism promoting progenitor Th2 cell development or maintenance? Is there a specific signal provided by B cells that promotes their expansion or maintenance?

We agree with the reviewer that identification of the signal(s) derived from TLS cells will be critical for defining Th2 progenitor cell function, but we would also assert that there are likely complex/redundant interactions involving multiple cell types, including stromal cells, macrophages, and endothelial cells. Indeed, as antigen is dispensable for progenitor cell maintenance for several weeks of time, it is unclear if B cells directly interact with T cells or rather if they are involved in organizing the global architecture of the TLS. To address this important question, we have performed scRNAseq of whole lung populations at the acute and chronic timepoints for subsequent cell-cell interaction analysis and additional hypothesis generation. These experiments support a role for B cells, and additionally suggested a role for IL-7 that we were able to test functionally. Understanding the complex temporal and spatial cellular interactions in tissue is a fascinating long-term goal, and we anticipate that this dataset will also form the foundation of several additional future studies for our team and the immunology community.

For additional details regarding new data added that addresses this question, please see our related response to a query from Reviewer #2, reproduced here for ease of reference:

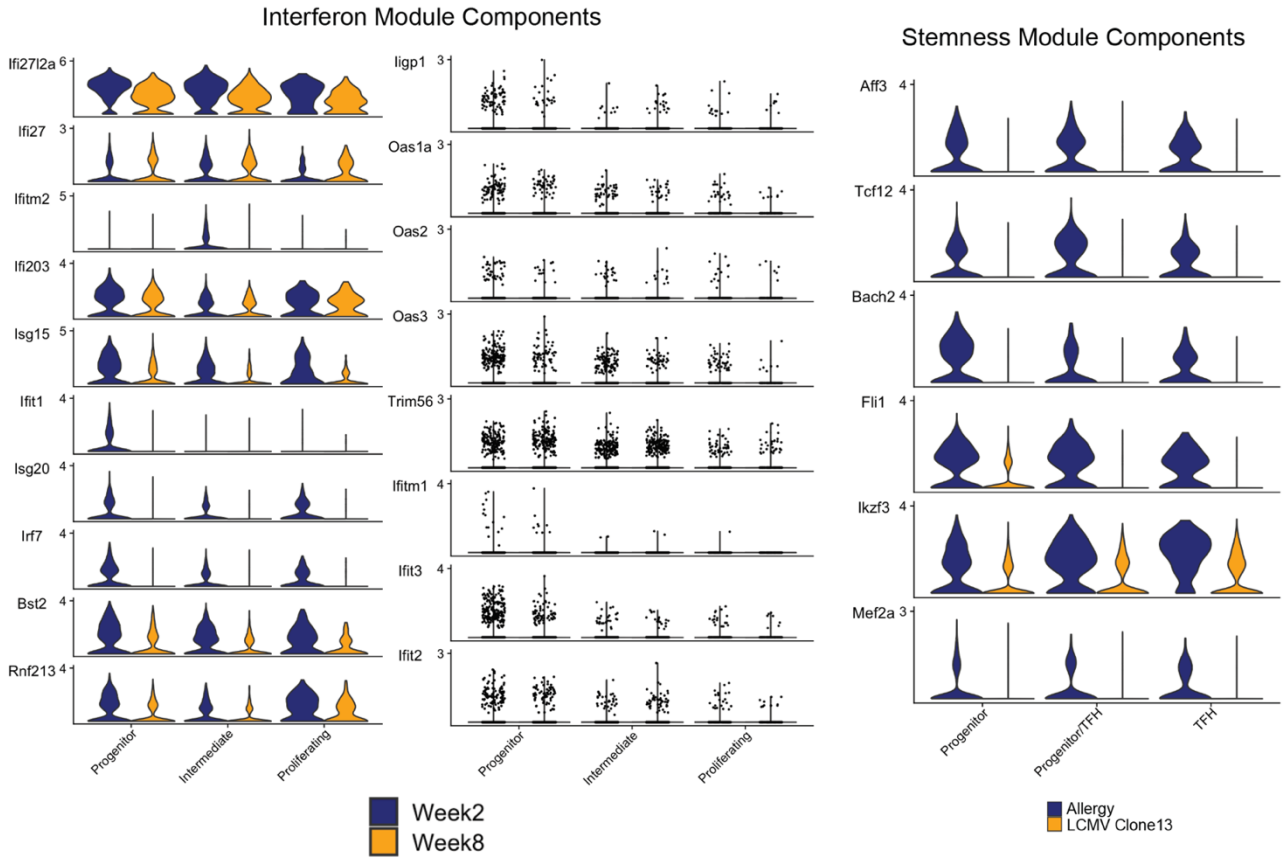
Given the importance of this line of inquiry, we performed scRNAseq of whole lung populations as well as VisiumHD spatial transcriptomics at the acute and chronic timepoints to analyze cell-cell interactions using bioinformatic approaches (new Figure 6. These analyses identified many cell-cell and molecular signaling interactions that could be relevant to the chronic inflammatory microenvironment. Among these factors, we provide further bioinformatic dissection of possible T-B cell interactions (new Extended Data Figures 11, 12, 13) that could explain the phenotype observed with B cell depletion. We also identified a possible role of IL-7 though our informatic analyses and demonstrate a functional role for IL-7 in the maintenance of the Th2 progenitor population (new Figure 7).

3) The authors suggest that some chronic type 2 responses require contributions from lymph node (LN) progenitor Th2 cells whereas others are maintained by non-lymphoid tissue (NLT) progenitor Th2 cells. Besides differences in location, can the authors define any other differences in LN and NLT progenitor Th2 cells?

We appreciate this question and lymph node progenitor cells are an ongoing area of interest for our group. To address the issue, we have performed scRNAseq analyses of T cells from the mediastinal lymph node (mLN) in our model. We identified mLN progenitor-like T cells, a portion of which had features of TFH cells and then subsequently compared these clusters to tissue progenitors (new Figure 8a). Promising tissue adaptation candidates including *Il7r, Icos,* and *Nr3c1* were identified, among others. Of particular interest, we were able to demonstrate a functional role for IL-7R signaling in Th2 progenitor maintenance through antibody blockade (Figure 8c). We greatly appreciate the reviewer's suggestion as the newly generated scRNAseq dataset provided the key inspiration for this experimentation.

4) One of the main conclusions of the paper is that chronic type 2 inflammation is maintained despite chronic antigen exposure due to progenitor Th2 cells possessing a greater “stemness” program than progenitor T cells in chronic type 1 responses. This is an interesting concept and the authors support this hypothesis by comparing the transcriptomic signatures of progenitor T cells responding to chronic type 2 inflammation versus LCMV clone 13. They generate a “stemness” module, showing that progenitor, progenitor/TFH, and TFH cells in chronic type 2 inflammation, compared to LCMV clone 13, exhibit a greater stemness module score. While this data is intriguing, could the authors provide additional data beyond the stemness module score to support the concept that progenitor T cells in chronic type 2 inflammation exhibit greater stem-like properties than T cells in a chronic type 1 response? My specific concern is that relatively small differences in expression of a large number of genes could yield an overall difference in the module score without being biologically meaningful. Could the authors provide normalized reads of transcripts involved in stem-like properties or provide some other biology to support the concept that progenitor T cells exhibit greater stem-like properties during chronic type 2 versus type 1 responses? Furthermore, as interferon signaling is known to limit T cell stemness, is the difference in stemness between progenitor T cells in chronic type 2 versus type 1 responses predominantly driven by interferon signaling (PMID: 28018990; PMID: 36809763; PMID: 27941247; PMID: 40062995)?

We share the reviewer’s concern regarding the biological relevance of small effect sizes and recognize that the AddModuleScore function developed by the Seurat team has the potential to identify differentially expressed genes with variable effect sizes. To investigate this further, we reviewed the code for the AddModuleScore function, which demonstrates that it calculates an average of Z score deviations for each gene in the module, and there is actually no additive effect from having more genes; in fact, including many transcripts with small deviations would deflate the overall ModuleScore. Second, in accordance with the reviewer’s suggestion, we visualized normalized reads for each transcript as a conventional ViolinPlot (presented below). For the interferon ModuleScore, we found that only several key transcripts, e.g. IRF7 a critical transcription factor induced by interferon, showed large differences between timepoints, whereas many others had minimal differences. This indicates that the inclusion of a whole set of interferon-stimulated genes actually deflated the ModuleScore. Nevertheless, we feel that showing the entire ModuleScore, as in the first submission, rather than a selection of transcripts that were significant, could allow for broad application in subsequent studies to other T cell subsets and also limits selection bias.



We agree with the reviewer that the bioinformatics analysis of interferon signaling opens up an interesting follow up series of experiments that we would eagerly take on in follow up studies.

Minor comments:

1) The authors use the term “sensitization” to describe allergen treatment over weeks. It seems reasonable to reserve sensitization for initial priming of the adaptive immune response. In the chronic allergen exposure models, perhaps the authors should use “sensitization” and “challenge” or simply “treatment” as I don’t think sensitization is the best term to use for chronic allergen exposures.
2) The authors use the phrase “collapse of adaptive immunity” in the abstract to refer to T cell exhaustion in the context of chronic infection and malignancy. While exhaustion certainly limits T cell function, even exhausted T cells still exhibit effector function, including cytokine production, so I don’t think “collapse” is the correct term here.

We appreciate this this comment. We have now refined our word choice for these two issues in the revision. As a single dose of intranasal Alternaria/DerP was sufficient for “sensitization” in our pilot experiments, we decided to use the word “challenge” in instances referring to chronic stimulation. Some instances of the word “sensitization” remain where it refers appropriately to acute sensitization.

3) The authors use Ki67 as a marker of proliferation, but it should be noted that Ki67 positivity simply shows that cells have the capacity to cycle. That is, Ki67 positive cells are not in G0. However, it does not reflect the number or cycles of proliferation. To determine this, injection with BrdU or equivalent agent would be needed.

To address this important point, we have performed *in vivo* EdU labeling experiments to further assess cell cycle state of Th2 cells at the acute and chronic timepoints. Indeed, as suggested by the reviewer, EdU positive cycling cells were found to be only a small percentage of the total Ki67+ pool, though the overall trends between timepoints were the same (Figure 1c).

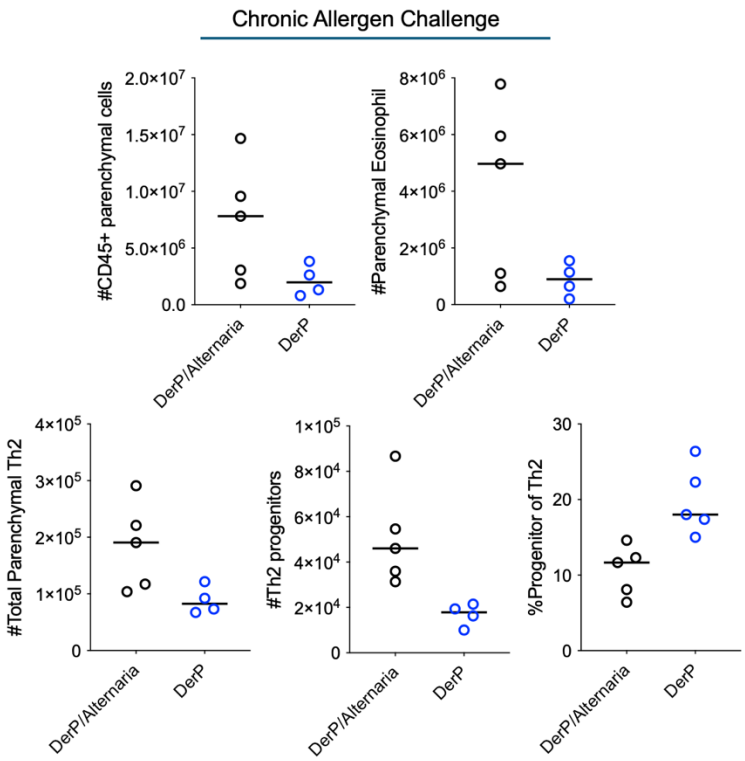
4) There seems to be a typo on line 266 with “Figure 4d” supposed to be “Figure 3d”

We corrected this typo and thank the reviewer for pointing this out.

5) Can the authors provide an explanation of why they used a mixed allergen extract model?

We thank the reviewer for this question and have now amended the text to explain the rationale for using the model in this work. This issue was also raised by Reviewer #2 and we have included our response below for reference:

In this work, we sought to model and mechanistically dissect the pathophysiology of human allergic disease wherein patients are often polysensitized to multiple environmental allergens and encounter distinct stimuli in overlapping fashion, e.g. cockroach and dust mite in an apartment complex. HDM and Alternaria may indeed activate distinct innate signaling and alarmin pathways via different proteases and TLR ligands (e.g. *Science Immunology 2024, 9, eabq4341*). To address this, we performed single-agent HDM sensitization through the chronic timepoint (please see inset figure). We found increased inflammation in the polysensitized mice, as indexed by total intraparenchymal CD45+ immune infiltrate and total lung eosinophil counts. This correlated with increased Th2 cell infiltration, and interestingly, increased total Th2 progenitor cell counts, despite a relatively lower frequency of this subset as a proportion of total Th2s. We conclude that the globally increased inflammation in the polysensitization model results in increased Th2 infiltration/proliferation without disrupting the overall progenitor/effector hierarchy. We prefer the combinatorial sensitization method as it allows us to withdraw/add signals from distinct stimuli. It could be interesting for future studies to titrate single-agent Alternaria and single agent HDM to doses that elicit comparable eosinophilia and then dissect T cell phenotypes and microenvironment signals in an unbiased fashion. Notably, there is a wide range of reported doses for single-agent HDM sensitization and our conclusions with this benchmark experiment are limited to the dose of HDM that we have used for polysensitization.



6) In Fig 1C, the authors show that the %IL-13-expressing CD4+ T cells is reduced between acute and chronic type 2 inflammation. From the representative figure, the gMFI of IL-13 also seems reduced during chronic type 2 inflammation, suggesting less IL-13 production on a per cell basis. Can the authors also present IL-13 gMFI from this experiment?

We appreciate this suggestion. To address this query, we quantified the IL-13 MFI from these experiments (new Figure 1d) which indeed demonstrates a modest but significant decrease, as predicted by the reviewer.

7) The authors use the term “chronic asthma” to describe their long-term intranasal allergen treatment model, but I think this term should be avoided when describing the murine model. Although intranasal allergen administration in mice induces allergic lung inflammation with features shared with human allergic asthma, the murine model does not completely mimic human asthma. For example, the murine model exhibits type 2 pneumonitis whereas inflammation is restricted to the airways in human allergic asthma. Also, mice don’t exhibit airway hyper-responsiveness with allergen treatment unless methacholine is administered whereas humans exhibit airway hyper-responsiveness with allergen exposure alone. Consequently, it may be more accurate to use the term “chronic type 2 inflammation” or something similar rather than the term “chronic asthma” for the murine model.

We thank the reviewer for this suggestion and concur that “chronic asthma” is not the most accurate term for the type 2 inflammation described in our model. We have refined the word choice for the revision for accuracy.

Point-by-point response to the most recent reviews:

Reviewer #1

(Remarks to the Author)
The authors have fully addressed my previous comments. I appreciate the additional data and explanation they have provided and support publication of the manuscript.

We thank the reviewer for their suggestions and appreciate their endorsement of our work.

Reviewer #2

(Remarks to the Author)
In this revised manuscript investigating the biology of progenitor Th2 cells in chronic allergic inflammation, Kratchmarov et al. have performed numerous new experiments that have significantly strengthened the manuscript. The authors have rigorously characterized a population of TCF1+ Th2 cells that arise during chronic allergic lung inflammation, which exhibit self-renewal and the ability to differentiate into effector Th2 cells that maintain the type 2 response. The authors new experiment using CD4-CreERT2 x Tcf7 fl/fl mice supports the importance of TCF1+ Th2 cells in vivo during chronic allergic lung inflammation. Their comparison of LN and NLT TCF1+ Th2 cells sheds new light on the unique features of NLT TCF1+ Th2 cells, which the authors complement with an IL-7 blockade experiment. In addition, the new scRNA-seq and spatial transcriptomics datasets provide a detailed characterization of the distinct features of acute and chronic allergic lung inflammation. These datasets will be a helpful resource for future investigations. I congratulate the authors on this rigorous and important experimental work. This manuscript will be of broad interest to the immunology community. The text is well written and I don't see any concerning issues with the methodology or statistical analyses. Below I outline some very minor comments, but I do not feel additional experimental work or significant revisions are necessary.

Minor comments:
1) On line 172-173, the authors write “Taken together, these data confirm that the GATA3+TCF1+ST2+ Th2 population has progenitor potential, coupling self-renewal with effector generation.” Do the authors mean the GATA3+TCF1+ST2- Th2 population?

We appreciate the reviewer pointing out this typo and have corrected the error.

2) In lines 291-292, the authors write “These surface marker expression patterns suggest that the TCF1+ST2- compartment represents a state distinct from resident memory.” I understand what the authors mean by this sentence, but after showing that TCF1+ST2-CD4+ T cells within the lungs are similar in number after FTY720 treatment (suggesting residency) and do not absolutely require ongoing antigen stimulation (a shared feature with memory T cells), this statement may be confusing to the reader. Perhaps the authors can rewrite this sentence to more clearly explain the unique features of TCF1+ST2- cells?

We have revised this section for clarity and accuracy and appreciate the reviewer’s perspective here. We feel that the new text, highlighted in the revised submission, focuses on the conclusions that can be directly drawn from the data and does not overstate parallels. The new text, lines 292-295, now reads:

"These surface marker expression patterns suggest that the TCF1+ST2- compartment has overlapping features of chronic activation, residency, and memory. Taken together, these findings demonstrate that specialized Th2 responses are associated with chronicity."

3) There seem to be typos on lines 318 and 625. Also there is a typo in Figure 8F (right panel) in which the y-axis should presumably read "ILC2".

We appreciate the reviewer pointing out these typos, which have been corrected.

4) In Figure 8d and the methods, the authors state that they are using an anti-IL-7R antibody, but in Figure 8e-f, the authors label the group as anti-IL-7. Although I appreciate the anti-IL-7R is not depleting and is blocking IL-7 signaling, the authors should be consistent with the antibody being used throughout the figure.

We have revised the labeling for consistency and appreciate the reviewer bringing this to our attention. All labels now indicate that the experimental intervention blocks IL-7R.

Reviewer #3

(Remarks to the Author)
I am quite positive of this resubmission by Kratchmarov and co-authors. The work has been dramatically improved and provides more conclusive evidence now than before that progenitor Th2 cells are in fact important for sustaining the effector Th2 population and that these have physiological relevance. The data on the progenitor niche and depletion experiments are also more conclusive. So I appreciate the effort.

However, I believe that the authors would do well to reproduce the data presented in Fig. 3c with conditional and temporal deletion of Tcf7. It appears that this was only conducted once and this is not sufficient from my perspective. It is the most important experiment in the study in my eyes and needs to be shown with more replicates. The effective deletion of Tcf7+ cells needs to be more clearly shown also, even in a temporal manner (how quickly is deletion detected) and this entire analysis needs to be broadened. Again, for me, this is an important part of the paper, even if tcf7 deletion if only partial, the authors should clearly show this.

The data on inducible *Tcf7* deletion presented in Figure 3 are a composite of multiple experiments, not a single replicate. These mice have remarkably inefficient breeding, and the modest sample size in Figure 3 reflect 3 experiments carried out over 6 months to ensure carefully matched littermate mice were used in parallel. For reasons that remain unclear, combining the Tcf7 fl/fl strain (which is already noted by The Jackson Laboratory to have 20% non-productive homozygous breeding pairs) with the CD4-CreER^{T2} driver further worsened the fertility of this strain and resulted in male CD4-CreER^{T2} Tcf7 fl/fl offspring that were completely sterile, as well as female CD4-CreER^{T2} Tcf7 fl/fl dames that generated small litters, i.e. 1-3 pups, with CD4-CreER^{T2} Tcf7fl/fl pups present in non-Mendelian ratios. We have updated the figure legend to indicate that the data presented shows independent, littermate-matched experiments. We believe that the data shown are clear, and we remain confident in the conclusions. However, we appreciate the reviewer's concerns, and to provide further supporting details of these experiments, we have now included additional data showing kinetic protein-level

TCF1 deletion at steady state that shows deletion occurs within 1 week of tamoxifen pulse, as well as deletion efficiency in the chronic phase (Extended Data Figure 2g). To further assess the downstream effects of *Tcf7* deletion, we also show new additional data on histologic assessment of tissue inflammation and BALT aggregates (Figure 3c). We respectfully assert that increasing biological replicates for the data already shown would not substantially alter the conclusions of the larger manuscript as the data are supported by orthogonal readouts, i.e. flow cytometry and distinct histological assessments.

The reviewer does indirectly raise a fascinating question – what is the broader role of TCF1 in supporting the progenitor state? This manuscript interrogates and defines the function and phenotype of progenitor cell state rather than the cell intrinsic function of TCF1, which recent work has suggested might be more of a “cog in the wheel” than a master regulator (*Immunity*, 2025, PMID: 41043414). Understanding the role of TCF1 and other transcription factors in Th2 memory states is certainly an area of interest for future work.

Similarly, the IL7R blockade experiments presented in Figure 8 need to be reproduced as it is not apparent that this has been performed.

We agree that experiments using blocking antibodies can show variability, and we hope to demonstrate our key findings in a clear and rigorous manner. We have therefore repeated the IL-7R blockade experiment, with the aggregated results presented in Figure 8e,f.

If these can be reproduced reliably, the study would be more conclusive.