nature portfolio

Peer Review File

Evidence for Interleukin-17C governing interleukin-17A pathogenicity and orchestrating asthma endotype switching in bronchiectasis

Corresponding Author: Professor Jin-Fu Xu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This well-written paper, authored by an experienced group, appears to introduce a novel connection between IL-17C and the modulation of IL-17A, leading to the shift from eosinophilic to neutrophilic asthma driven by Pseudomonas aeruginosa. While IL-17A's role in inflammation is recognized, IL-17C's contribution to this process in bronchiectasis with asthma may indeed represent a new and underexplored aspect, giving this research its novelty. Overall, a well structured paper, which is clearly presented. The findings are of potential importance and interest to the pulmonary scientific community and helps clinicians to understand the pathophysiology of neutrophilic driven asthma. S

However, there are a few key points that the authors should address before publication:
1. Tables 1/2:
a. It is important to clarify whether HRCT data are available for the listed patients.
b. Include FEV1 values to enhance the characterization of pulmonary function in the patient population.
c. How did you sample for microbiology results? Induced sputum?
2. FVC and FEV1/FVC Findings:
a. Clarify why patients with bronchiectasis and asthma (BA) show lower FVC but have a similar FEV1/FVC ratio when compared to asthmatic individuals. This raises questions about specific lung mechanics in BA patients that require further exploration.
3. Pseudomonas Aeruginosa and Th2 Endotype:
a. The concept of Pseudomonas aeruginosa driving a Th2 endotype is intriguing, but several points need addressing:
i. The majority of the supporting evidence stems from murine models, which calls for stronger human-based validation. Performing assays with human cells, such as ILC3s, would provide more relevant translational insight.
ii. It would be beneficial to include data on Pseudomonas aeruginosa colonization in the studied patient subsets and analyze correlations with Th2-associated markers (e.g., TSLP, CCL17). This would strengthen the manuscript's human relevance and address the weakness in linking P. aeruginosa to Th2 responses in humans.
iii. Consider referencing previous studies that examine the relationship between P. aeruginosa and Th2 responses in humans, such as this work: https://pubmed.ncbi.nlm.nih.gov/16387607/
and, this work:
https://pubmed.ncbi.nlm.nih.gov/38888974/

4. Discussion:
a. The current discussion is somewhat basic and lacks a strong translational perspective. The manuscript should address whether patients colonized by Pseudomonas aeruginosa should be treated differently based on their Th2 responses.
b. Lessons from cystic fibrosis (CF) management could offer valuable insights into how Pseudomonas aeruginosa infection might be treated in these patients. This could be an important avenue for further research and discussion.

Reviewer #2

(Remarks to the Author)
This study by Zhang et al. addresses current challenges in the treatment of patients with coexisting bronchiectasis and asthma. The authors sought to explore the asthma endotype in patients with non-cystic fibrosis bronchiectasis with asthma (BA) to identify new therapeutic targets. Using mouse models, the authors report a pathogenic interaction between IL-17C and ILC3s that potentiates IL-17A responses and switching to a neutrophilic asthma endotype. The study is interesting and warrants investigation, however, there are some major limitations.

- The authors' rationale for inducing P. aeruginosa infection followed by allergic sensitisation to Ova in their murine model lacks convincing justification. While individuals of all ages can have bronchiectasis, it is most common in the elderly. The EMBARC study explored this overlap between asthma and bronchiectasis and identified that asthma could be reported as an etiology of bronchiectasis. This is supported by the British thoracic society guideline for non-cystic fibrosis bronchiectasis recommendation for considering asthma as the cause of bronchiectasis in the absence of other etiological explanations. Others show that asthma severity is an independent risk factor for bronchiectasis, that there is a high percentage of bronchiectasis in patients with severe asthma, and that age of asthma onset and exacerbations are independently associated with the occurrence of bronchiectasis.

- Does P. aeruginosa infection alone adequately model the diverse immunopathological repertoire and pathophysiology of patients with bronchiectasis? This must be established to convince readers that the reported observations are not simply readouts of host:pathogen interactions e.g. the effects of an active infectious challenge on shaping allergic sensitisation and the immunological profile of the recall response.

- The potential influence of smoking history on the reported data is not discussed further than reported rates of "Smoking, %" in Table 1.

- The authors identify the paucity of data on asthma endotypes in the context of bronchiectasis-asthma overlap as a barrier to treatment decision-making, but do not present data on sputum inflammometry, which is a global benchmark for characterization and management guidance. This limits the translational applicability of the findings from the mouse experiments.

- The authors report a positive correlation between peripheral (serum) IL-17A and IL-17C in patients with bronchiectasis with or without asthma. It would be more informative to perform these analyses in patients with bronchiectasis alone, asthma alone, and with both bronchiectasis and asthma. The specific relevance of these findings in the context of ILC3 responses should then be demonstrated in patients with both bronchiectasis and asthma to support the contention that "IL-17C could serve as a potential therapeutic target for individuals of BA".

Reviewer #3

(Remarks to the Author)
MANUSCRIPT NCOMMS-24-60979
GENERAL COMMENTS.
This manuscript presents a clinical-translational study that addresses how bacterial colonization in patients with bronchiectasis and asthma alters immune signaling via the archetype Type 3 signaling pathway involving interleukin (IL)-17 cytokines, especially IL-17A and IL-17C. The data sets presented are extensive, consisting of data from humans, from cell and animal models. Taken together, the scientific basis for the manuscript and the presented conclusions is solid. Nevertheless, the results presented are important for the (improved) understanding of archetype immune signaling in patients with chronic airway disease and bacterial colonization, with future implications beyond asthma.
The scientific criticism relates mainly to the present focus on ILC 3 cells, a focus that leaves the expert reader with several unanswered questions regarding the role of Th17 and Tc17 cells. The mechanistic rationale for the critical role of IL-17A per se also deserves to be stated in a more precise manner, given its importance for this field of research (see also Specific comments below). Additional and important criticism relates to how the presentation of the underlying study is made, from a linguistic point-of-view. Key entities need to be handled in a more consistent manner than is now the case. Moreover, as the main text now stands, it is in need of editing by an expert in English. Once it is optimized in these respects, the manuscript is likely to become important for future research on chronic inflammatory airway disorders.
SPECIFIC COMMENTS.
A) Title: The title emerges as too definitive. Maybe "Evidence for IL-17C governing IL-17A pathogenicity..." would be better given that the focus is set mainly on ILC3 cells and not on additional sources of IL-17A? Another reason for this is that important evidence originates from experimental models and not humans in vivo.
B) Summary: The authors should make clear what evidence originates from humans and what evidence originates from experimental models. Apart from this, The scientific contents are fine but linguistic editing is needed.
C) Introduction:
1. Para 1 and 2: The text would benefit from the addition of information on Type 1 and Type 3-signaling and how these pathways relate to neutrophilic and eosinophilic inflammation. Here, key entities must be introduced and used in a more accurate and consistent manner than is now the case.
2. The entire study behind this manuscript study is based upon the role of IL-17A in mediating neutrophil accumulation in the lungs. Given this, a brief presentation of the basic evidence is appropriate, published in the original studies from Linden et al and from Kolls et al (summarized in Linden & Kolls, Immunity 2004; and in Linden & Dahlen, ERJ 2014). The original studies are also relevant for comments in the the results and the discussion (see below).
D) Results:

1. Table 2: Data sets on FEV1 and on ACT for healthy controls iare missing.
2. Text line 108: All FENO values are within the normal range, so the comment on a trend here doe s not seem meaningful.
3. Text line 165: These references are not adequate. See point C.2 for appropriate references.
4. Text line 281: The data set on MMP-9 emerges as important, not least given that this proteinase originates in part from neutrophils. The latter can be emphasized in the text here (as well as in the discussion and in the principal overview in Fig 8).
5. Apart from the above, the figures and tables seem adequate.
E) Discussion:
1. A general comment is that the text does not really make clear why the emphasis is set on ILC3 cells and not on other sourcces for IL-17A (such as Th17 or Tc17 cells).
2. Another general comment is that the discussion need to address the (potential) involvement of Type 1 signaling (via ILC 1 cells or via Th1 or Tc1 cells).
3. Text line 383: Figure 8 need to include MMP-9 given the current data and its relevance in neutrophilic inflammation in general (see also above).
4. Text line 413: Although this reviewer share the forwarded conclusions re. the current status of clinical evidence on inhibiting IL-17A signaling in asthma, the text here must be revised to make it easy to understand.
5. Text line 423: The presentation of published evidence on IL-17C i snow somewhat confusing and all publications on this may not be relevant for the current setting. This text can preferably be restricted to the most relevant publications.
6. Text line 460: The limitations of this study include the points forwarded as points E.1 and E.2 above.
7. Text line 469: The conclusion section should include comments on MP-9 as well (see above).
F) Methods:
1. Text line 610: The authors need to present the specific provider for each utilized assay, to make it possible for interested readers to reproduce data from this potentially important study.
2. Text line 644: The rationale for always using "two-sided significance" (regardless of pre-existing data and hypotheses) seem unclear and a rationale should be explained.
3. Except for the above, the methods seem adequately described.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
My questions and comments have been satisfactorily addressed with the support of new data. At this time, I have no further questions and would like to thank the authors for their detailed responses.

Reviewer #2

(Remarks to the Author)
The authors' response and rationale for inducing P. aeruginosa infection followed by allergic sensitisation to Ova in their murine model remains insufficiently justified. There has also been no attempt to examine the effects of the sequence of exposures experimentally where the authors could have investigated whether P. aeruginosa infection after the establishment of Ova-induced experimental asthma results in similar pathogenic interactions between IL-17C and ILC3s that potentiate IL-17A responses and a switch to the neutrophilic asthma endotype.

The additional information presented in Table 1 of the revised manuscript is informative, however, the potential influence of smoking history on the reported outcomes remains undiscussed.

There remains insufficient data to claim the study of asthma endotypes in bronchiectasis-asthma overlap. The absence of sputum inflammometry remains a critical limitation of the current study. This is inadequately justified by the authors' and their reference to previous work showing that neutrophilic inflammation is the primary phenotype in asthma-bronchiectasis overlap, and the unscientific statement that those data are suggestive for the current study's exploration of asthma endotypes in this overlap.

New data of correlations between serum IL-17A AND IL-17C in patients with bronchiectasis alone, asthma alone, and with both bronchiectasis and asthma are informative. However, these data show that positive correlations between IL-17A and IL-17C are also present in patients with asthma or bronchiectasis alone, yielding the same output across all groups in this comparison and weakening the authors' contention that pathogenic IL-17C uniquely governs IL-17A pathogenicity and asthma endotype switching in bronchiectasis. Therefore, sputum inflammometry is required to provide some insight into the mechanistic disease endotype present in the patients with asthma alone. This also highlights the unsuitability of PBS/OVA mice to model the 'asthma alone' cohort in this study as these animals did not exhibit allergic induction of IL-17.

Reviewer #3

(Remarks to the Author)
MAJOR COMMENTS
The authors have addressed most of the criticism in an adequate manner, and the manuscript i snow substantially improved.

There is only one issu ethat remain to be clarified (see below).

SPECIFIC COMMENTS
D. Results. Table 2: Data sets on FEV1 and ACT for healthy controls are missing. The authors need to clarify whether they have access to the requested data or not. If the data exists, the authors need to include the data sets in the table and address it in the results.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The authors have satisfactorily addressed my questions and comments with the support of new data, disclosures of the study's limitations, and textual revision.

Reviewer #3

(Remarks to the Author)
GENERAL COMMENTS:
In this manuscript, the authors present a range of interesting and potentially important findings on key mechanistic aspects of the involvement of IL-17C in the immunopathology of the combined clinical phenotype of allergic asthma and bronchiectasis with bacterial colonization. In essence, all the needed data sets were in place already in the first version of the manuscript. However, although the authors have improved some of the reasoning, writing and grammar in their most recent revision, there is still need for improved scientific clarity. There is also some concern about the novel data set added (see point C below).
SPECIFIC COMMENTS:
Response and revisions relating to comments from
A) Reviewer 1: Satisfactory.
B) Reviewer 2: The authors do not responde to the criotical comments from this reviewer in a satisfactory manner. Reviewer 2 makes an important point regarding the seemingly artificial order of establishing the principal pathogenic events in the mouse model. This "order of events" is yet to be justified with clear and precise reasoning and writing, by the authors. Intuitively, it does seem less feasible that bronchiectasis including colonization with P aeruginosa would precede allergic asthma in real life. It seems more feasible that allergic asthma comes first and bronchiectasis later, as a feasible consequence of impaired immunity and chronic inflammation.
In their most recent revision of the manuscript, the authors fail to write about the above related matters in a clear and concise manner. For example, the statement on line 296 could just as well have been written in the opposite manner and be just as true ("Bronchiectasis is commonly reported in asthma patients"). This illustrates a more universal problem with the way the authors seem to reason and write – they fail to argue in a clear and structured manner why the chose the "experimental approach" that they did.
Given the above, it does become problematic that authors now have chosen to introduce a new and limited data set that fails to prove a matching impact on the specific immunopathology, using a revised model where the order of establishing principial pathogenic events is seemingly more relevant (ie. allergic asthma precedes bronchiectasis including colonization with P aeruginosa). It is hard to understand why the authors chose to include so few observations (small "n") in their new data set, since this makes it hard to prove positive outcomes of immunopathological interest, when the alterations in cytokines and/or cells are small or variable. The small size of the novel material also makes it close to impossible to draw negative conclusions (due to risk for Type II error). Thus, by doing so, the authors weaken their own standpoint.
However, and importantly, given the current limitations of the scientific understanding of the clinical phenotype with both disorders (named "BA" in the manuscript), addressing the above is mainly a question of improving the way the authors present and discuss their evidence, although doubling the observations in their novel data set would clearly help in the current situation.
C) Reviewer 3: The response to this reviewer is acceptable but not fully satisfactory. The lack of basic clinical characterization of lung functon(spirometry) and symptoms (ACT) in the group of healthy subjects gives a poor impression in terms of clinical characterization, although it is understood that it is too late now to do anything about it now. Evidently, the authors cannot guarantee that their controls are truly healthy, although it seems feasible that this is the case. This reviewer thinks that this is acceptable, given that the problem is addressed as a "limitation" in the discussion.

Point-by-point responses

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author)

This well-written paper, authored by an experienced group, appears to introduce a novel connection between IL-17C and the modulation of IL-17A, leading to the shift from eosinophilic to neutrophilic asthma driven by Pseudomonas aeruginosa. While IL-17A's role in inflammation is recognized, IL-17C's contribution to this process in bronchiectasis with asthma may indeed represent a new and underexplored aspect, giving this research its novelty. Overall, a well structured paper, which is clearly presented. The findings are of potential importance and interest to the pulmonary scientific community and helps clinicians to understand the pathophysiology of neutrophilic driven asthma.

However, there are a few key points that the authors should address before publication:

1. Table 1/2:

a. It is important to clarify whether HRCT data are available for the listed patients.

Reply:

Thank you for your valuable comments. A HRCT scan is required to make the diagnosis of bronchiectasis, so it is important for the listed patients in the present study applying a HRCT scan to distinguish bronchiectasis with asthma from asthma alone. Of all 92 patients, most patients including bronchiectasis patients (30/31), asthmatics (29/30), patients of bronchiectasis with asthma (29/31) received the chest HRCT scan and HRCT data can be retrieved from Picture archiving and Communication System (PACS) in Shanghai Pulmonary Hospital, whereas remaining 4 patients underwent the chest HRCT scan in other hospitals within the last 1 month.

b. Include FEV1 values to enhance the characterization of pulmonary function in the patient population.

Reply:

Thank you for your constructive remark and the data on FEV1, FEV1% pred, FVC values have been supplemented in Table 2 of the revised manuscript as shown below. It is shown that in terms of FEV1 and FVC values, there were a remarkable decline in patients of bronchiectasis with asthma compared to asthmatics, whereas no significance

was detected in terms of FEV1% pred among all the patients.

Table 2. Clinical characteristics of all subjects

Variables	Healthy controls (n = 20)	Bronchiectasis (n = 31)	Asthma (n = 30)	Bronchiectasis with asthma (n = 31)	*P* value
Lung function	NA				
FEV1		1.34 (0.94-2.01)	1.6 (1.21-2.33)	1.24 (0.80-1.64) $^{\Delta}$	0.0321
FEV1% pred		59.09 (23.25)	66.19 (20.69)	54.16 (20.86)	0.1062
FVC		2.1 (1.53-2.66)	2.4 (1.98-2.99)	1.75 (1.22-2.15) $^{\Delta\Delta\Delta}$	0.0007
FVC% pred		71.09 (20.94)	79.2 (14.36)	62.92 (17.52) $^{\Delta\Delta}$	0.0028
FEV1/FVC, %		66.28 (13.69)	66.98 (13.01)	68.53 (15.5)	0.8487
FeNO	NA	10 (6.75-15.25) $^{\Delta}$	25 (12-112)	17 (9-27)	0.0143
ACT score	NA		17 (14-20)	16 (14-17)	0.0367
Peripheral blood eosinophils, %	1.6 (0.73-2.3)	1.7 (0.9-2.7) $^{\Delta\Delta\Delta\Delta}$	7.1 (3.3-9.9) ****	1.9 (1.3-2.8) $^{\Delta\Delta\Delta}$	<0.0001
Peripheral blood eosinophils $\times 10^9 \cdot L^{-1}$	0.085 (0.053-0.15)	0.10 (0.06-0.14) $^{\Delta\Delta\Delta\Delta}$	0.39 (0.20-0.66) ****	0.11 (0.07-0.17) $^{\Delta\Delta\Delta}$	<0.0001
Serum IgE	NA	$^{\Delta\Delta}$		$^{\Delta\Delta\Delta\Delta}$	<0.0001
<100		21 (70.0%)	7 (23.3%)	28 (90.3%)	
100-200		4 (13.3%)	8 (26.7%)	1 (3.2%)	
>200		5 (16.7%)	15 (50.0%)	2 (6.5%)	

Note: Quantitative data are summarized as mean (SD) for normally distribute variables or median (IQR) for non-normally distribute variables and qualitative data are presented as n (percentage).

P value: overall comparison among existing groups.

$^{****}P < 0.0001$ compared with healthy control group.

$^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$, $^{\Delta\Delta\Delta\Delta}P < 0.0001$ compared with asthma group.

Abbreviations: FEV1, Forced Expiratory Volume in 1 s; FVC, Forced Vital Capacity; FeNO: Fractional exhaled nitric oxide; ACT: Asthma Control Test; Ig: immunoglobulin; NA, not available.

c. How did you sample for microbiology results? Induced sputum?

Reply:

In the present study, induced sputum was not involved, so spontaneously expectorated sputum samples were lacking in all healthy subjects and the small minority of asthmatic patients (1/30). And microbiology results were available according to microbiological cultures of sputum or bronchoalveolar lavage fluid (BALF). Notably, among asthmatic patients, *P. aeruginosa* has been confirmed negative by microbiological culture in only sputum samples (12/30), only BALF (3/30) or both (12/30), whereas the remaining 3 asthmatics were absent for microbiological results due to the lacking of BALF or sputum samples and the delay in sputum sample delivery. Additionally, among all the patients with the isolation of *P. aeruginosa* including bronchiectasis patients (16/31), patients of bronchiectasis with asthma (11/31), only sputum cultures from bronchiectasis patients (6/16), patients of bronchiectasis with asthma (3/11) were positive for *P. aeruginosa*, while only BALF cultures from bronchiectasis patients (5/16), patients of bronchiectasis with asthma (4/11) and both sputum and BALF cultures from bronchiectasis patients (5/16), patients of bronchiectasis with asthma (4/11) were positive.

2. FVC and FEV1/FVC Findings:
a. Clarify why patients with bronchiectasis and asthma (BA) show lower FVC but have a similar FEV1/FVC ratio when compared to asthmatic individuals. This raises questions about specific lung mechanics in BA patients that require further exploration.

Reply:

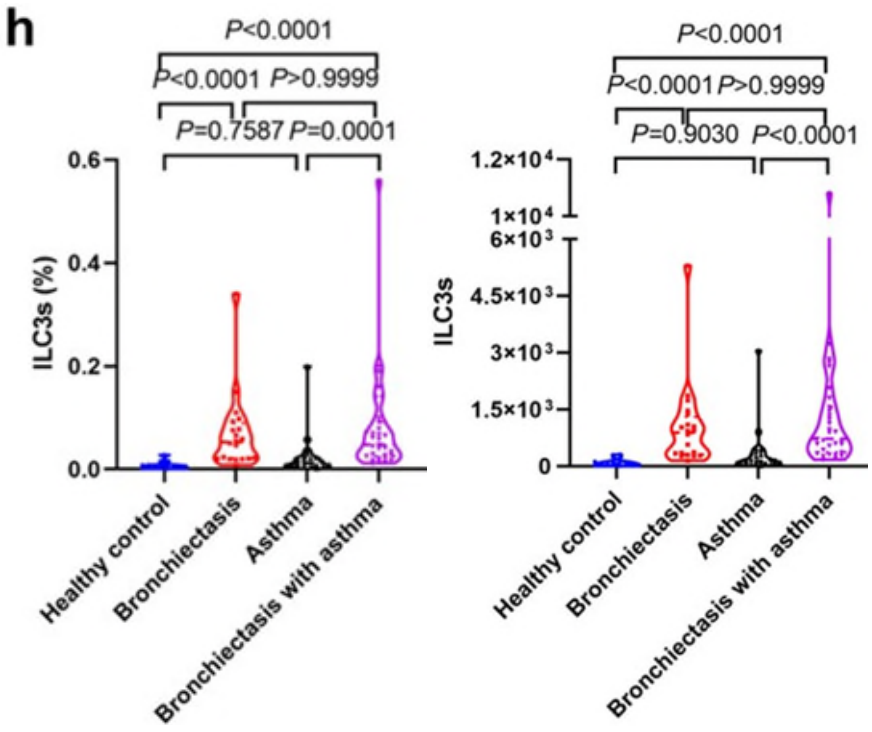
Given that FEV1 and FVC values were both lower in patients of bronchiectasis with asthma in comparison with asthmatics (Table 2), FEV1/FVC value, as the ratio of FEV1 to FVC, might be comparable among all the patients. Notably, there is limited

knowledge regarding the comparison for clinical characteristics especially the lung function between asthmatics and patients of bronchiectasis with asthma. Collectively, non-significant FEV1/FVC value raises questions about specific lung mechanics in patients with bronchiectasis with asthma and requires further exploration.

3. Pseudomonas Aeruginosa and Th2 Endotype:
a. The concept of Pseudomonas aeruginosa driving a Th2 endotype is intriguing, but several points need addressing:
i. The majority of the supporting evidence stems from murine models, which calls for stronger human-based validation. Performing assays with human cells, such as ILC3s, would provide more relevant translational insight.

Reply:

Thanks to the efforts from the journal editor for instantly sharing the review report that they have received with us in early November, we were able to plan relevant experiments as soon as possible and conscientiously completed all scheduled scientific experiments as suggested above. In the revised manuscript, we supplemented the ratio of ILC3s among lymphocytes and total numbers in peripheral blood mononuclear cells (PMBCs) among healthy subjects, asthmatics, patients with bronchiectasis with or without asthma by flow cytometry analysis, yielding extra data on Supplementary Fig. 2h. Our data implied that patients of BA presented higher ratio and cell counts of ILC3s compared to asthmatic patients or healthy subjects. The finding corresponded to more abundant ILC3s in mice exposed to PA/OVA, laid the basis for investigating IL-17C modulating ILC3 response in asthma endotype switching within the context of bronchiectasis with asthma and provided more relevant translational insight.



Supplementary Figure 2

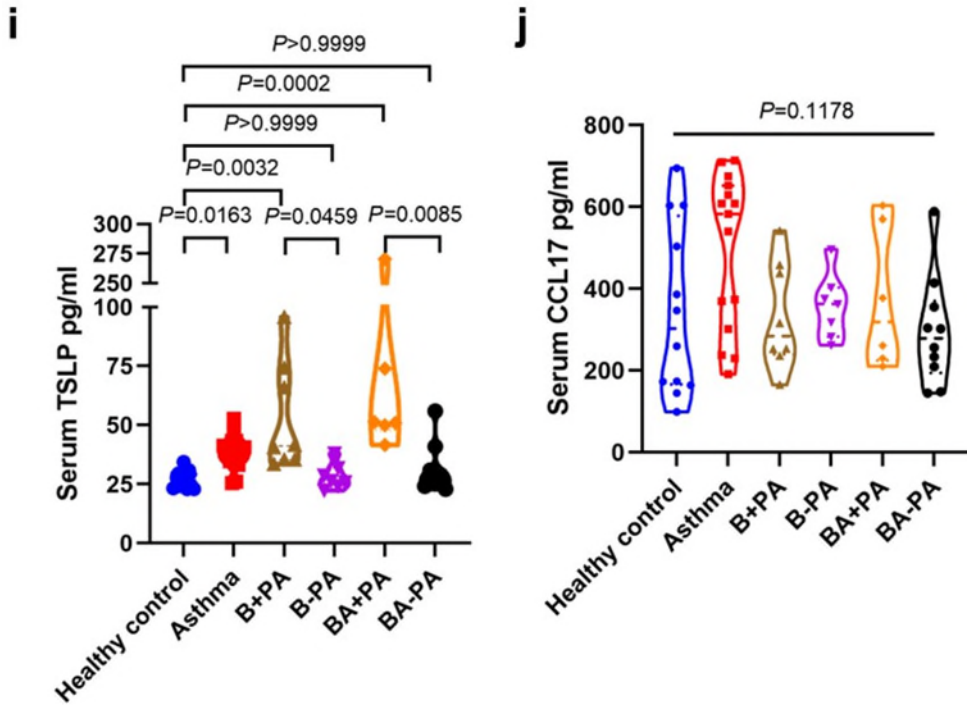
ii. It would be beneficial to include data on Pseudomonas aeruginosa colonization in the studied patient subsets and analyze correlations with Th2-associated markers (e.g., TSLP, CCL17). This would strengthen the manuscript's human relevance and address the weakness in linking P. aeruginosa to Th2 responses in humans.

iii. Consider referencing previous studies that examine the relationship between P. aeruginosa and Th2 responses in humans, such as this work: https://pubmed.ncbi.nlm.nih.gov/16387607/ and, this work: https://pubmed.ncbi.nlm.nih.gov/38888974/.

Reply:

Thank you for the above suggestion. According to previous studies from Hartl et al. and Rehman et al. (summarized in Hartl et al, J Allergy Clin Immunol 2006; and in Rehman et al, JCI Insight 2024) listed in the References section of the revised manuscript (Ref. 37, 38), we examined the serum levels of Th2-associated markers, TSLP and CCL-17 among healthy subjects, asthmatics, *P. aeruginosa*-infected, noninfected patients with bronchiectasis with or without asthma by ELISA analysis and added extra data on Supplementary Figure 2 of the revised manuscript to establish the link of *P. aeruginosa*

infection to Th2 responses in human. Our data showed raised TSLP in *P. aeruginosa*-infected patients with bronchiectasis with or without asthma, whereas no significance in CCL-17 levels was observed (Supplementary Fig. 2i, j).



Supplementary Figure 2

4. Discussion:
a. The current discussion is somewhat basic and lacks a strong translational perspective. The manuscript should address whether patients colonized by Pseudomonas aeruginosa should be treated differently based on their Th2 responses.
b. Lessons from cystic fibrosis (CF) management could offer valuable insights into how Pseudomonas aeruginosa infection might be treated in these patients. This could be an important avenue for further research and discussion.

Reply:

Thank you for your valuable comments. Following the suggestion, we expanded the Discussion section in Para 3 as follows: *Our findings linked P. aeruginosa infection to Th2 responses. P. aeruginosa infection is correlated with significantly higher levels of Th2 cells and CCL-17 in part of cystic fibrosis patients (summarized in Hartl et al, J*

Allergy Clin Immunol 2006; and in Rehman et al, JCI Insight 2024). Th2-endotype of bronchiectasis characterized by raised blood eosinophils or FeNO has been increasingly recognized (summarized in Martins et al, Pulmonology 2023). The evidence of elevated TSLP in P. aeruginosa-infected patients with bronchiectasis with or without asthma might facilitate us to provide precision medicine approach based on Th2 responses for these patients. Lessons from cystic fibrosis management could offer valuable insights into the treatment for P. aeruginosa infection in these patients. This could be an important avenue for further research.

Reviewer #2 (Remarks to the Author)

This study by Zhang et al. addresses current challenges in the treatment of patients with coexisting bronchiectasis and asthma. The authors sought to explore the asthma endotype in patients with non-cystic fibrosis bronchiectasis with asthma (BA) to identify new therapeutic targets. Using mouse models, the authors report a pathogenic interaction between IL-17C and ILC3s that potentiates IL-17A responses and switching to a neutrophilic asthma endotype. The study is interesting and warrants investigation, however, there are some major limitations.

- The authors' rationale for inducing P. aeruginosa infection followed by allergic sensitisation to Ova in their murine model lacks convincing justification. While individuals of all ages can have bronchiectasis, it is most common in the elderly. The EMBARC study explored this overlap between asthma and bronchiectasis and identified that asthma could be reported as an etiology of bronchiectasis. This is supported by the British thoracic society guideline for non-cystic fibrosis bronchiectasis recommendation for considering asthma as the cause of bronchiectasis in the absence of other etiological explanations. Others show that asthma severity is an independent risk factor for bronchiectasis, that there is a high percentage of bronchiectasis in patients with severe asthma, and that age of asthma onset and exacerbations are independently associated with the occurrence of bronchiectasis.

Reply:

Thank you for your valuable comments. As shown above, previous studies and guideline have indicated that asthma is a potential cause of bronchiectasis and the severity can be recognized as an independent risk factor for bronchiectasis. Notably, in

the present study, to identify the difference in clinical characteristics of patients with coexisting bronchiectasis and asthma from patients with bronchiectasis alone or asthma alone, we recruited patients with primary diagnosis of bronchiectasis and coexisting asthma. Therefore, we established a mouse model of ovalbumin-induced asthma within the context of *P. aeruginosa* chronic lower respiratory tract infection to investigate underlying mechanisms involving in the endotype of bronchiectasis and asthma overlap.

- Does P. aeruginosa infection alone adequately model the diverse immunopathological repertoire and pathophysiology of patients with bronchiectasis? This must be established to convince readers that the reported observations are not simply readouts of host:pathogen interactions e.g. the effects of an active infectious challenge on shaping allergic sensitisation and the immunological profile of the recall response.

Reply:

Globally, the lack of mature animal models in analogy with bronchiectasis has become a barrier to exploring the pathogenesis of bronchiectasis and potential effective managements. Bronchiectasis is a complex and heterogeneous condition manifesting unknown etiology and highly variable clinical presentation, so there are no animal models representing bronchiectasis. During the design phase, we found that in numerous researches such as previous studies from Martin et al., Ward et al., Collin et al. and Frija et al. (summarized in Martin et al, Eur Respir J 2011; Ward et al, Eur Respir J 2011; Collin et al, EBioMedicine 2020; Frija-Masson et al, Eur Respir J 2017), *P. aeruginosa* chronic lower respiratory tract infection has been widely employed to explore the underlying mechanisms involved in immunopathological repertoire and pathophysiology of bronchiectasis. Consequently, by referring to previous work from Facchini et al., Rossi et al. and Codagnone et al., (summarized in Facchini et al, J Vis

Exp 2014; Rossi et al, J Cyst Fibros 2023; Codagnone et al, Mucosal Immunol 2018), we established *P. aeruginosa* chronic lower respiratory tract infection through intratracheal (i.t.) instillation with *P. aeruginosa*-laden beads. Undoubtedly, the model that we established has yet to be optimized, which is the ongoing work of our team. Importantly, we explained the purpose of the model we used in the last paragraph of the Introduction section in the revised manuscript.

- The potential influence of smoking history on the reported data is not discussed further than reported rates of "Smoking, %" in Table 1.

Reply:

Thank you for your constructive remark. And in the present study, smoking status was defined as never smokers, ex-smokers and current smokers as depicted in the table below and in Table 1 of the revised manuscript.

Table 1. Baseline information of healthy controls, bronchiectasis patients, asthmatic patients and patients of bronchiectasis with asthma

	HC	Bronchiectasis	Asthma	Bronchiectasis with asthma
Subjects	20	31	30	31
Age (y)	40.5 (36.25-46.75)	57 (54-68)	59.5 (50.75-66)	61 (53-69)
Sex				
Male	8 (40%)	12 (38.7%)	11 (36.7%)	5 (16.1%)
Female	12 (60%)	19 (61.3%)	19 (63.3%)	26 (83.9%)
Smoking status				
Never smokers	16 (80%)	26 (83.9%)	24 (80%)	29 (93.5%)
Ex-smokers	0 (0%)	5 (16.1%)	2 (6.7%)	0 (0%)
Current smokers	4 (20%)	0 (0%)	4 (13.3%)	2 (6.5%)
Duration of symptoms (y)	NA	12 (5-30)	4 (2-10.25)	30 (5-40)
PA isolation, %	NA	51.6	0	35.5

- The authors identify the paucity of data on asthma endotypes in the context of bronchiectasis-asthma overlap as a barrier to treatment decision-making, but do not present data on sputum inflammometry, which is a global benchmark for characterization and management guidance. This limits the translational applicability of the findings from the mouse experiments.

Reply:

As shown above, the lack of sputum cytology does indeed limit the translational applicability of the findings from the mouse experiments, which has been stated in the limitations in the Discussion section of the manuscript. Neutrophils in sputum can be assessed, however, the patients listed in the study are situated across the country, and it is pretty difficult to recruit them again for collecting sputum samples and further sputum cytology. Notably, previous study from Kupczyk et al. (summarized in Kupczyk et al, Allergy 2014) suggested that biomarkers such as sputum inflammometry are often unstable over time and it is insufficient to determine asthma phenotype according to sputum cytology. Additionally, existing reports from O'Byrne et al. and Chung (summarized in O'Byrne et al, Lancet Respir Med 2016; Chung, Lancet Respir Med 2016) have identified neutrophilic asthma by blood inflammometry among patient with severe asthma. Interestingly, previous work from Ma et al. (summarized in Ma et al, Arch Bronconeumol 2024) showed that neutrophilic inflammation is the primary phenotype in patients with the overlap between asthma and bronchiectasis, which may be suggestive for our study exploring the asthma endotype within the context of bronchiectasis-asthma overlap. And if sputum samples are available in the future, we would conduct sputum cytology in these patients.

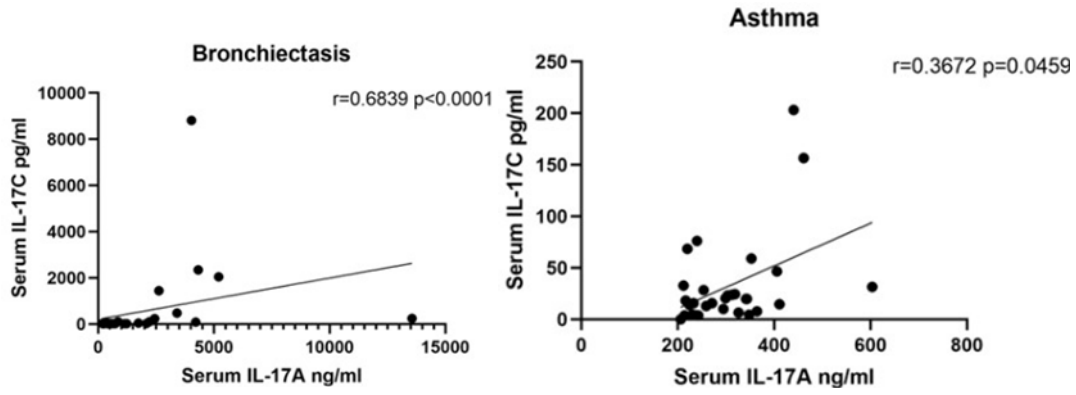
- The authors report a positive correlation between peripheral (serum) IL-17A and IL-17C in patients with bronchiectasis with or without asthma. It would be more

informative to perform these analyses in patients with bronchiectasis alone, asthma alone, and with both bronchiectasis and asthma. The specific relevance of these findings in the context of ILC3 responses should then be demonstrated in patients with both bronchiectasis and asthma to support the contention that "IL-17C could serve as a potential therapeutic target for individuals of BA".

Reply:

Thank you for your valuable comments and we redrew the figure 5j with the supplement of Figure 5k as suggested above. As shown in the figure below and Fig. 5j, k, we performed the correlation analysis between IL-17A and IL-17C in patients with bronchiectasis alone, asthma alone, and with both bronchiectasis and asthma, indicating a positive correlation between serum IL-17C levels and IL-17A levels among these patients (Fig. 5j). Importantly, to investigate the specific relevance of these findings in the context of ILC3 responses, given ILC3s as the source of IL-17A and augmented numbers of ILC3 in peripheral blood of patients with bronchiectasis with asthma (Supplementary Fig. 2h in the revised manuscript), we identified the correlation between ILC3s ratios or counts and IL-17C levels in peripheral blood of patients with bronchiectasis with asthma and also found a positive correlation between these indicators (Fig. 5k), which contributed to support the role of IL-17C as a potential therapeutic target in patient with bronchiectasis with asthma.

j



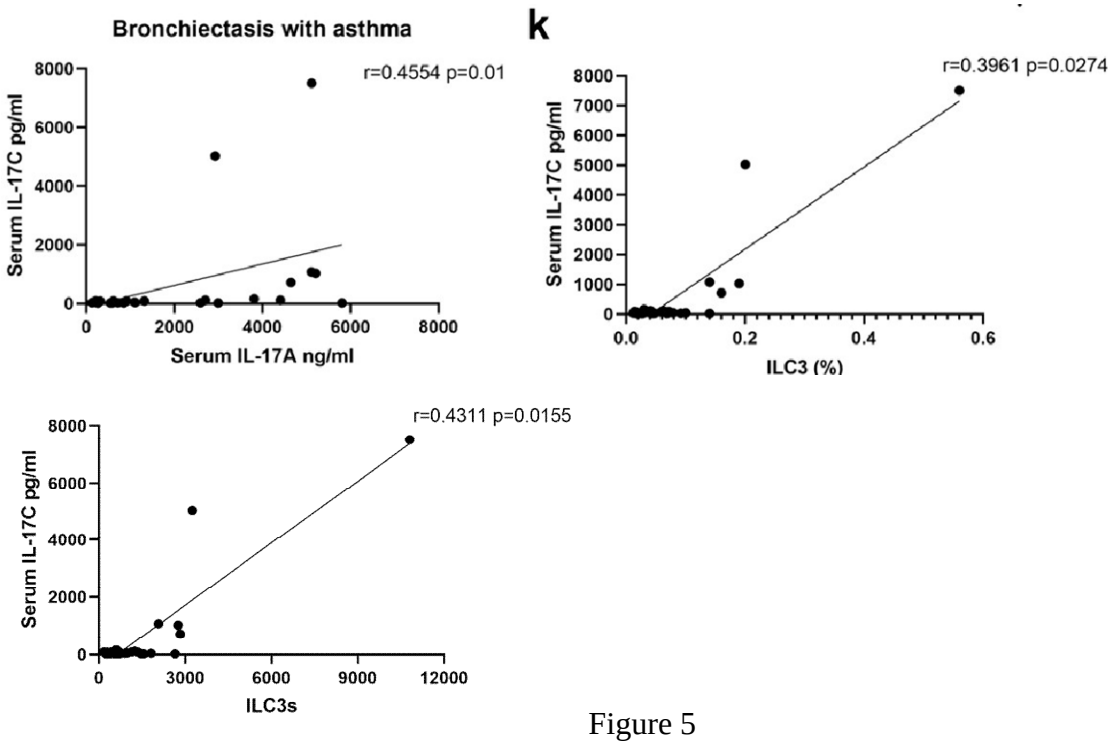


Figure 5

Reviewer #3 (Remarks to the Author):
MANUSCRIPT NCOMMS-24-60979
GENERAL COMMENTS.
This manuscript presents a clinical-translational study that addresses how bacterial colonization in patients with bronchiectasis and asthma alters immune signaling via the archetype Type 3 signaling pathway involving interleukin (IL)-17 cytokines, especially IL-17A and IL-17C. The data sets presented are extensive, consisting of data from humans, from cell and animal models. Taken together, the scientific basis for the manuscript and the presented conclusions is solid. Nevertheless, the results presented are important for the (improved) understanding of archetype immune signaling in patients with chronic airway disease and bacterial colonization, with future implications beyond asthma.
The scientific criticism relates mainly to the present focus on ILC3 cells, a focus that leaves the expert reader with several unanswered questions regarding the role of Th17 and Tc17 cells. The mechanistic rationale for the critical role of IL-17A per se also deserves to be stated in a more precise manner, given its importance for this field of research (see also Specific comments below). Additional and important criticism relates to how the presentation of the underlying study is made, from a linguistic point-of-view. Key entities need to be handled in a more consistent manner than is now the case. Moreover, as the main text now stands, it is in need of editing by an expert in English. Once it is optimized in these respects, the manuscript is likely to become important for future research on chronic inflammatory airway disorders.
SPECIFIC COMMENTS.

A) Title: The title emerges as too definitive. Maybe "Evidence for IL-17C governing IL-17A pathogenicity..." would be better given that the focus is set mainly on ILC3 cells and not on additional sources of IL-17A? Another reason for this is that important evidence originates from experimental models and not humans in vivo.

Reply:

Thank you for your valuable suggestion and we modified the title of the revised manuscript as follows: *Evidence for IL-17C governing IL-17A pathogenicity and orchestrating asthma endotype switching in bronchiectasis.*

B) Summary: The authors should make clear what evidence originates from humans and what evidence originates from experimental models. Apart from this, The scientific contents are fine but linguistic editing is needed.

Reply:

Thank you for your valuable comments. As instructed above, inadequacies have been modified in the Summary section of the revised manuscript.

C) Introduction:
1. Para 1 and 2: The text would benefit from the addition of information on Type 1 and Type 3-signaling and how these pathways relate to neutrophilic and eosinophilic inflammation. Here, key entities must be introduced and used in a more accurate and consistent manner than is now the case.

Reply:

Thank you for your constructive comments. The information of type 1 and type 3-signaling and the association with neutrophilic and eosinophilic inflammation have been supplemented in Para 1 of the revised manuscript.

Key entities such as type-2 high (T2), type-2 low (non-T2) in Para 1 and *nontypeable Haemophilus influenzae* (NTHi) in Para 2 have been revised in the manuscript. And key entities such as bronchiectasis with asthma (BA) in Para 2 were introduced with reference to previous studies from Ma et al. and Polverino et al. (summarized in Ma et

al, Arch Bronconeumol 2024; Polverino et al, J Allergy Clin Immunol 2024).

2. The entire study behind this manuscript study is based upon the role of IL-17A in mediating neutrophil accumulation in the lungs. Given this, a brief presentation of the basic evidence is appropriate, published in the original studies from Linden et al and from Kolls et al (summarized in Linden & Kolls, Immunity 2004; and in Linden & Dahlen, ERJ 2014). The original studies are also relevant for comments in the the results and the discussion (see below).

Reply:

In the present study, as indicated above, it is imperative to stress the role of IL-17A. Given this, we referred to the original studies from Linden et al and from Kolls et al (summarized in Linden & Kolls, Immunity 2004; and in Linden & Dahlen, ERJ 2014) listed in the Reference section of the revised manuscript (Ref. 6, 7) and supplement a brief presentation of the evidence supporting the role of IL-17A in mediating neutrophil accumulation in Para 3 of the Introduction section, the Results section and the Discussion section.

D) Results:

1. Table 2: Data sets on FEV1 and on ACT for healthy controls are missing.

Reply:

In the present study, the assessment of FEV1 and ACT was for individuals manifesting clinical symptoms and these ones were willing to receive inspections such as lung function test and ACT. Notably, the healthy controls that we recruited lacked clinical symptoms, and therefore identifying FEV1 and ACT values of these subjects within normal range.

2. Text line 108: All FENO values are within the normal range, so the comment on a trend here does not seem meaningful.

Reply:

As suggested above, all the medians of FeNO values were within the normal range and

more importantly, no significance was detected for FeNO values in patients of BA in comparison with asthmatic patients. Accordingly, we have made correction in the revised manuscript.

3. Text line 165: These references are not adequate. See point C.2 for appropriate references.

Reply:

Thank you for your suggestion and we have supplemented the references in the revised manuscript as instructed in point C.2 (Ref. 6, 7).

4. Text line 281: The data set on MMP-9 emerges as important, not least given that this proteinase originates in part from neutrophils. The latter can be emphasized in the text here (as well as in the discussion and in the principal overview in Fig 8).

Reply:

Thank you for your constructive remark. As indicated above, we have supplemented neutrophil-derived MMP-9 in the Result section, Para 6 and Para 8 of the Discussion section and the principal overview of Figure 8.

5. Apart from the above, the figures and tables seem adequate.

Reply:

We have comprehensively revised the inadequacies in the manuscript according to the above comments.

E) Discussion:

1. A general comment is that the text does not really make clear why the emphasis is set on ILC3 cells and not on other sources for IL-17A (such as Th17 or Tc17 cells).

Reply:

Thank you for your valuable comment and we have made relevant modifications to Para 5, Para 6 and Para 7 of the Discussion section. Although the focus of present study is the modulation of ILC3 responses by IL-17C, the role of Th17 cells in asthma

endotype within the context of *P. aeruginosa* chronic infection has been investigated in the study. PA/OVA exposure increased the cell numbers of Th17 cells (Fig. 2c, d). Notably, the modulation of Th17 cells by IL-17C has been widely discussed in numerous researches such as previous study from Chang et al. (summarized in Chang et al, Immunity 2011), which coincides with that ablation of *Il17re* reduced the levels of Th17 cells in lung tissues from mice exposed to PA/OVA (Fig. 6n). Inspired by this study, when our preliminary results indicated that ablation of *Il17re* attenuated ILC3 response, we asked a question that whether IL-17C may regulate ILC3 responses, which promoted further exploration and consequently discovered that IL-17C directly bound to ILC3s by interacting with IL-17RE and subsequently potentiated IL-17A expression. However, as indicated above, given that IL-17C governed IL-17A pathogenicity, we didn't rule out the potential role of Tc17 cells which can secret IL-17A as the same as Th17 cells and ILC3s in experimental asthma model within the context of *P. aeruginosa* chronic lower respiratory tract infection. Such inadequacy has been stressed in the fifth limitation in the Discussion section of the revised manuscript, and the conclusion that IL-17C governs IL-17A pathogenicity by interacting with ILC3s should be interpreted cautiously due to this limitation.

2. Another general comment is that the discussion need to address the (potential) involvement of Type 1 signaling (via ILC 1 cells or via Th1 or Tc1 cells).

Reply:

Thank you for your suggestion and we made relevant modifications to Para 4 and Para 7 of the Discussion section. In the Results section of the manuscript, we investigated the potential involvement of ILC1, Th1 cells associated with type 1 signaling and our

data suggested that *P. aeruginosa* chronic lower respiratory tract infection failed to affect ILC1 and Th1 responses in mice exposed to PA/OVA (Supplementary Fig. 2e-g), which was stressed in Para 4 of the Discussion section. However, apart from ILC1s and Th1 cells, we didn't rule out the potential role of Tc1 cells in experimental asthma models within the context of *P. aeruginosa* chronic infection. Such inadequacy has been emphasized in the fifth limitation of the Discussion section in the revised manuscript.

3. Text line 383: Figure 8 need to include MMP-9 given the current data and its relevance in neutrophilic inflammation in general (see also above).

Reply:

Thank you for the above suggestion and we introduced neutrophil-derived MMP-9 to Figure 8 of the revised manuscript.

4. Text line 413: Although this reviewer share the forwarded conclusions re. the current status of clinical evidence on inhibiting IL-17A signaling in asthma, the text here must be revised to make it easy to understand.

Reply:

We have re-written the part in Para 4 of the Discussion section in the revised manuscript according to the above suggestion.

5. Text line 423: The presentation of published evidence on IL-17C is now somewhat confusing and all publications on this may not be relevant for the current setting. This text can preferably be restricted to the most relevant publications.

Reply:

Thank you for your constructive suggestion. To better convey the idea that the role of IL-17C in regulating tissue inflammation has been controversial, the removal, addition and a brief introduction of the most relevant publications have been made in Para 5 of the Discussion section in the revised manuscript.

6. Text line 460: The limitations of this study include the points forwarded as points E.1 and E.2 above.

Reply:

As indicated in E.1 and E.2, we have listed the potential involvement of type 1 and type 3 signaling as the fifth limitation in the revised manuscript.

7. Text line 469: The conclusion section should include comments on MMP-9 as well (see above).

Reply:

As indicated in D.4, we have introduced neutrophil-derived MMP-9 and its role in regulating IL-17C production to the conclusion section of the revised manuscript.

F) Methods:

1. Text line 610: The authors need to present the specific provider for each utilized assay, to make it possible for interested readers to reproduce data from this potentially important study.

Reply:

Thank you for your valuable remark. In the flow cytometry analysis section, due to numerous antibodies for use in the experiment, we briefly introduced the providers of these antibodies. Consequently, we have provided the specific providers of each utilized assay in Supplementary Table 2 and indicated it at the end of the paragraph.

2. Text line 644: The rationale for always using "two-sided significance" (regardless of pre-existing data and hypotheses) seem unclear and a rationale should be explained.

Reply:

As shown above, the rationale for always using “two-sided significance” weren’t be clearly explained in the manuscript and we have supplemented the rationale in the statistical analysis section of the revised version. Our study mainly concerns the correlation analysis, one-way ANOVA, two-way ANOVA in terms of statistical analysis. And the null hypotheses we establish are that there is no significant correlation between

two variables and all the medians or means are equal. Considering the paucity of pre-existing data on the endotype of bronchiectasis and asthma overlap, we are unable to determine the preset results of experiments (in which identifying a>b or a<b was absolutely correct) and therefore adopting two-sided test and recognizing two-sided significance level as 0.5. Moreover, more importantly, in the same situation, the power of one-sided test is higher, whereas two-sided test is comparatively conserve, which means that in case of one-sided test, false positive conclusions would be drawn, so two-sided test should be adopted in the present study to avoid false positives and gain reliable results.

3. Except for the above, the methods seem adequately described.

Reply:

We have made corrections accordingly in the Methods section of the revised manuscript in light of the above suggestions.

Point-by-point responses
REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):
My questions and comments have been satisfactorily addressed with the support of new data. At this time, I have no further questions and would like to thank the authors for their detailed responses.

Reply:

Thank you for your professional review work on our manuscript. Meanwhile, we were very pleased that our responses have addressed your concerns and met with approval.

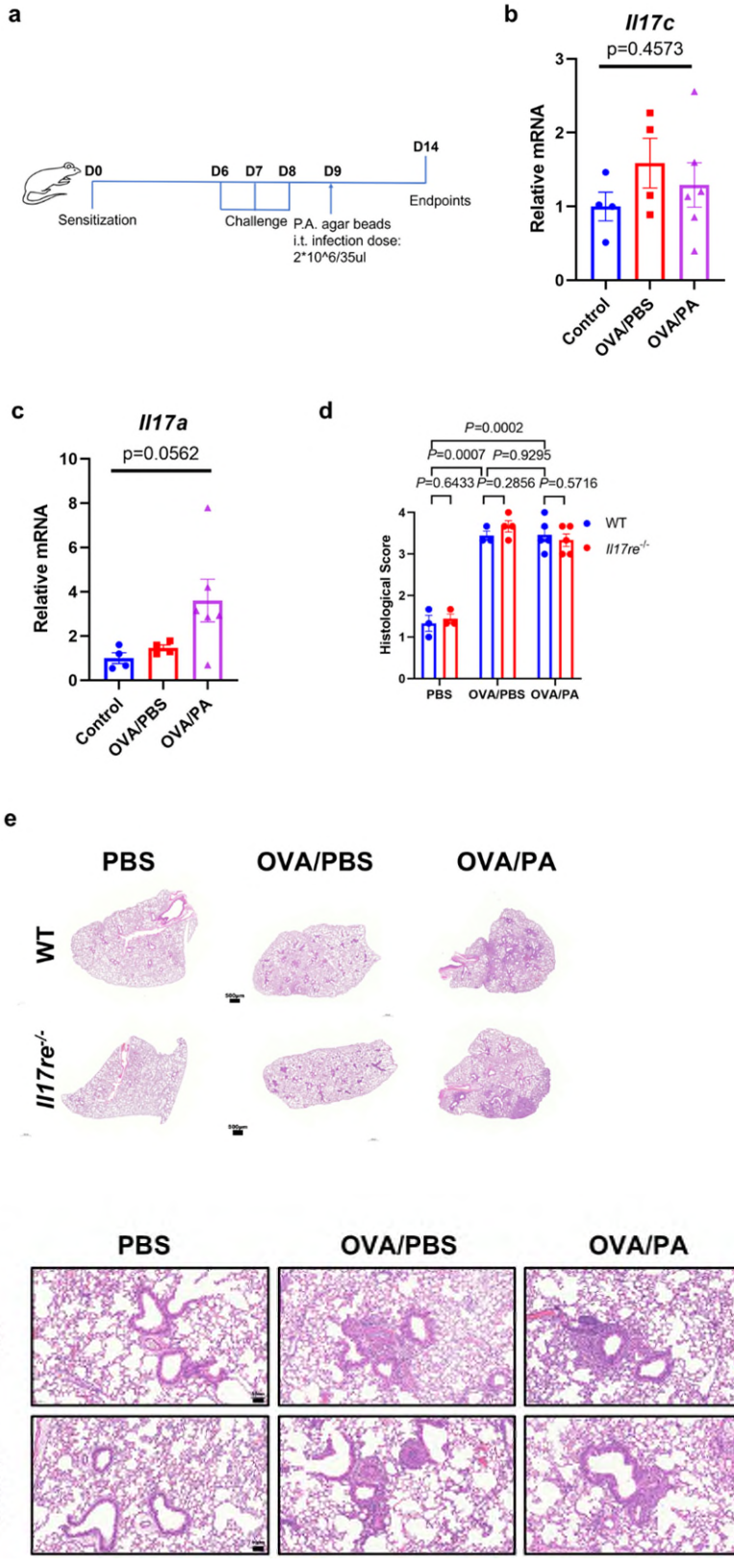
Reviewer #2 (Remarks to the Author)
The authors' response and rationale for inducing P. aeruginosa infection followed by allergic sensitisation to Ova in their murine model remains insufficiently justified. There has also been no attempt to examine the effects of the sequence of exposures experimentally where the authors could have investigated whether P. aeruginosa infection after the establishment of Ova-induced experimental asthma results in similar pathogenic interactions between IL-17C and ILC3s that potentiate IL-17A responses and a switch to the neutrophilic asthma endotype.

Reply:

Previous work from Mao et al. and Chen et al. (summarized in Mao et al, Eur Respir J 2016; Chen et al, J Thorac Dis 2016) indicated that asthma can be reported as a comorbidity of bronchiectasis. Meanwhile, previous studies from Ma et al. and Coman et al. (Ma et al, Arch Bronconeumol 2024; Coman et al, Ann Allergy Asthma Immunol 2018) showed that bronchiectasis is one of comorbidities in severe asthma. Notably, bronchiectasis-asthma overlap and asthma-bronchiectasis overlap are both clinical issues. At this time, in the present study, we focused on the former and investigated the asthma endotype within the context of bronchiectasis-asthma overlap. Accordingly, we recruited the patients with primary bronchiectasis and coexisting asthma. Considering *P. aeruginosa* chronic lower respiratory tract infection has been widely employed to explore the underlying mechanisms involved in bronchiectasis, we established a model

of *P. aeruginosa* infection followed by Ova-induced asthma to investigate the underlying mechanisms involving in the asthma endotype within the context of bronchiectasis-asthma overlap.

Indeed, asthma-bronchiectasis overlap is a pivotal and interesting clinical issue. To explore potential role of IL-17C in the endotype of asthma-bronchiectasis overlap, we constructed an alternative model of Ova-induced experimental asthma followed by *P. aeruginosa* infection (Fig. a). As depicted below, the preliminary results indicated that *P. aeruginosa* infection didn't alter the levels of *Il17c*, *Il17a* mRNA and IL-17A as well as the cell counts of AM, neutrophils, ILC3. Airway inflammation, eosinophils, ILC2 together with the levels of IgE, IL-5, IL-13 and total cell numbers were elevated from OVA/PA-exposed mice compared with their control mice, whereas comparable between OVA/PA-exposed mice and OVA/PBS-exposed mice (Fig. b-o). Furthermore, ablation of *Il17re* didn't affect eosinophilic inflammation and ILC3 counts in mice exposed to OVA/PA or OVA (Fig. d-p). However, potential factors affecting the asthma endotype in an experimental asthma model followed by *P. aeruginosa* infection could be the time interval between the last challenge and i.t. infection with *P. aeruginosa* accompanied by duration of infection. Thus, the interesting scientific issue remains to be further investigated in the future work.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D0
D6
D7
D8
D9
D14
Sensitization
Challenge
P.A. agar beads
i.t. infection dose:
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Endpoints
b
Il17c
p=0.4573
Relative mRNA
Control
OVA/PBS
OVA/PA
c
Il17a
p=0.0562
Relative mRNA
Control
OVA/PBS
OVA/PA
d
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P=0.0007
P=0.9295
P=0.6433
P=0.2856
P=0.5716
Histological Score
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Il17re-/-
PBS
OVA/PBS
OVA/PA
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OVA/PBS
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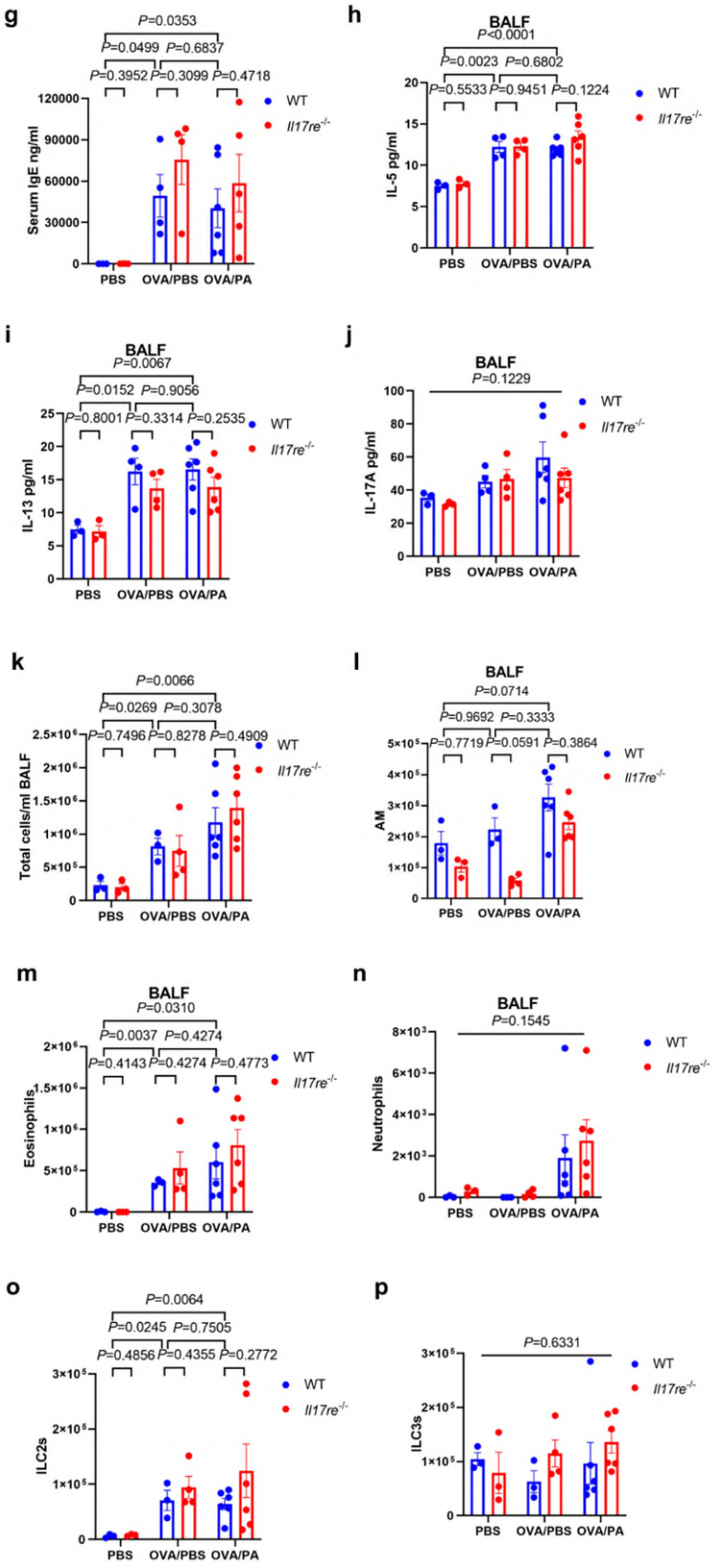
WT
Il17re-/-



Figure

a Schematic diagram of OVA treatment by i.p. delivery and airway challenge followed by i.t. instillation with P. aeruginosa-laden agar beads at Day 9 in a model and BALF and lung tissues were collected at Day 14. **b, c** mRNA levels of *Il17c* and *Il17a* in lungs from mice exposed to OVA/PBS

(n = 4), OVA/PA (n = 6) and their control (n = 4) were determined. **d-f** Semiquantified inflammation of lungs and representative images of H&E-stained lung sections from wild-type (WT; PBS, n = 3; OVA/PBS, n = 3; OVA/PA, n = 5) and *Il17re*$^{-/-}$ mice (PBS, n = 3; OVA/PBS, n = 4; OVA/PA, n = 5) in the model (**e** Scale bars, 500 μm; **f** Scale bars, 50 μm). **g-j** Protein levels of IgE, IL-5, IL-13, and IL-17A in serum or BALF from WT (PBS, n = 3; OVA/PBS, n = 4; OVA/PA, n = 6) and *Il17re*$^{-/-}$ mice (PBS, n = 3; OVA/PBS, n = 4; OVA/PA, n = 6) were assessed. **k-p** Quantification of total cells, AM, eosinophils and neutrophils in BALF together with total numbers of ILC2s, and ILC3s in lungs from WT (PBS, n = 3; OVA/PBS, n = 3; OVA/PA, n = 6) and *Il17re*$^{-/-}$ mice (PBS, n = 3; OVA/PBS, n = 4; OVA/PA, n = 6) as determined by flow analysis. Data are shown as mean ± SEM. Significance was calculated by one-way ANOVA (**b-d, g-p**).

The additional information presented in Table 1 of the revised manuscript is informative, however, the potential influence of smoking history on the reported outcomes remains undiscussed.

Reply:

In the present study, the aim is to investigate the asthma endotype in patients of bronchiectasis with asthma and airway inflammation is identified as an important indicator for evaluation.

In the revised version of the manuscript, we supplemented *P* value of smoking rates across the four groups in para 1 of the Result section, and further discussion concerning the potential influence of smoking history on the reported outcomes was inserted into para 2 of the Discussion section as follows: Notably, another potential factor that could influence asthma endotype might be smoking history being associated with airway inflammation. Nevertheless, the percentages of subjects with smoking history were comparable across the groups involved in the study (*P*=0.4304). With limited sample size, we couldn't perform subgroup analysis of reported outcomes based on smoking history among the patients of bronchiectasis with asthma.

There remains insufficient data to claim the study of asthma endotypes in bronchiectasis-asthma overlap. The absence of sputum inflammometry remains a critical limitation of the current study. This is inadequately justified by the authors' and their reference to previous work showing that neutrophilic inflammation is

the primary phenotype in asthma-bronchiectasis overlap, and the unscientific statement that those data are suggestive for the current study's exploration of asthma endotypes in this overlap.

Reply:

Thank you for comments. In our study, the asthma endotype in patients of bronchiectasis with asthma may tend to be type 2-low asthma characterized by lower eosinophil counts, higher levels of IL-17A, ILC3s and difficultly controlling asthma symptoms (Table 1, Supplementary Fig. 2h and Fig. 5h). Consistently, the data from animal model used for investigating the asthma endotype in bronchiectasis-asthma overlap indicated neutrophilic asthma with airway hyperreactivity, lower levels of IL-5, IL-13, IgE and abundant IL-17A (Fig. 1b-n). Indeed, sputum inflammometry is very important for identifying the asthma endotype among the patients of bronchiectasis-asthma overlap. And unavailable direct data on the asthma endotype using sputum inflammometry involving sample collection, storage, procession and cell isolation among the patients across the country have remained a limitation of the present study, which remain to be the focus of the future work. However, the findings of BALF cytology from mouse experiments revealed that *P. aeruginosa* chronic lower respiratory tract infection drove asthma endotype switching towards neutrophilic asthma, which can be an indirect indication for the asthma endotype of bronchiectasis-asthma overlap. Importantly, as mentioned above, we misquoted previous work based on the patients of asthma-bronchiectasis overlap for supporting the contention that neutrophilic inflammation could be the primary phenotype in bronchiectasis-asthma overlap.

New data of correlations between serum IL-17A AND IL-17C in patients with bronchiectasis alone, asthma alone, and with both bronchiectasis and asthma are informative. However, these data show that positive correlations between IL-17A

and IL-17C are also present in patients with asthma or bronchiectasis alone, yielding the same output across all groups in this comparison and weakening the authors' contention that pathogenic IL-17C uniquely governs IL-17A pathogenicity and asthma endotype switching in bronchiectasis. Therefore, sputum inflammometry is required to provide some insight into the mechanistic disease endotype present in the patients with asthma alone. This also highlights the unsuitability of PBS/OVA mice to model the 'asthma alone' cohort in this study as these animals did not exhibit allergic induction of IL-17.

Reply:

Thank you for comments. The positive correlations between IL-17A and IL-17C were universally applied for patients with asthma alone, bronchiectasis alone, and bronchiectasis-asthma overlap, however, precise modulation of IL-17C on IL-17A and asthma endotype switching was on the basis of the findings from mouse experiments in the present study. Notably, according to the findings from animal study, we didn't observe airway hyperreactivity reflecting asthma feature in mice exposed to *P. aeruginosa* infection alone with abundant IL-17C. Moreover, ablation of *Il17re* purely affected IL-17A-mediated inflammation and asthma endotype switching in PA/OVA-exposed mice rather than OVA-exposed mice, indicating the role of IL-17C in governing IL-17A pathogenicity and orchestrating asthma endotype driven by *P. aeruginosa* infection. Together, there remain insufficient human data and therefore revising the title as follows: *Evidence for IL-17C governing IL-17A pathogenicity and orchestrating asthma endotype switching in bronchiectasis.*

Reviewer #3 (Remarks to the Author):

MAJOR COMMENTS

The authors have addressed most of the criticism in an adequate manner, and the manuscript i snow substantially improved. There is only one issu ethat remain to be clarified (see below).

SPECIFIC COMMENTS

D. Results. Table 2: Data sets on FEV1 and ACT for healthy controls are missing. The authors need to clarify whether they have access to the requested data or not.

If the data exists, the authors need to include the data sets in the table and address it in the results.

Reply:

Thank you for comments. In terms of data sets on FEV1 and ACT for healthy controls, in our study, considering that the assessment of FEV1 and ACT was for individuals manifesting clinical symptoms, the healthy controls that we recruited lacked clinical symptoms, and therefore the requested data being inaccessible.

Point-by-point responses
REVIEWER COMMENTS

Reviewer #2 (Remarks to the Author):
The authors have satisfactorily addressed my questions and comments with the support of new data, disclosures of the study's limitations, and textual revision.

Reply:

Thank you for your insightful remarks on our manuscript. Meanwhile, we were very grateful that our work has addressed your concerns and met the expectations.

Reviewer #3 (Remarks to the Author)
GENERAL COMMENTS:
In this manuscript, the authors present a range of interesting and potentially important findings on key mechanistic aspects of the involvement of IL-17C in the immunopathology of the combined clinical phenotype of allergic asthma and bronchiectasis with bacterial colonization. In essence, all the needed data sets were in place already in the first version of the manuscript. However, although the authors have improved some of the reasoning, writing and grammar in their most recent revision, there is still need for improved scientific clarity. There is also some concern about the novel data set added (see point C below).
SPECIFIC COMMENTS:
Response and revisions relating to comments from
A) Reviewer 1: Satisfactory.
B) Reviewer 2: The authors do not respond to the critical comments from this reviewer in a satisfactory manner. Reviewer 2 makes an important point regarding the seemingly artificial order of establishing the principal pathogenic events in the mouse model. This "order of events" is yet to be justified with clear and precise reasoning and writing, by the authors. Intuitively, it does seem less feasible that bronchiectasis including colonization with P aeruginosa would precede allergic asthma in real life. It seems more feasible that allergic asthma comes first and bronchiectasis later, as a feasible consequence of impaired immunity and chronic inflammation.
In their most recent revision of the manuscript, the authors fail to write about the above related matters in a clear and concise manner. For example, the statement on line 296 could just as well have been written in the opposite manner and be just as true ("Bronchiectasis is commonly reported in asthma patients"). This illustrates a more universal problem with the way the authors seem to reason and write – they fail to argue in a clear and structured manner why they chose the "experimental approach" that they did.
Given the above, it does become problematic that authors now have chosen to introduce a new and limited data set that fails to prove a matching impact on the specific immunopathology, using a revised model where the order of establishing

principial pathogenic events is seemingly more relevant (ie. allergic asthma precedes bronchiectasis including colonization with P aeruginosa). It is hard to understand why the authors chose to include so few observations (small "n") in their new data set, since this makes it hard to prove positive outcomes of immunopathological interest, when the alterations in cytokines and/or cells are small or variable. The small size of the novel material also makes it close to impossible to draw negative conclusions (due to risk for Type II error). Thus, by doing so, the authors weaken their own standpoint.
However, and importantly, given the current limitations of the scientific understanding of the clinical phenotype with both disorders (named "BA" in the manuscript), addressing the above is mainly a question of improving the way the authors present and discuss their evidence, although doubling the observations in their novel data set would clearly help in the current situation.

Reply:

Thank you for your valuable comments. Previous work from Mao et al. and Chen et al. (summarized in Mao et al, Eur Respir J 2016; Chen et al, J Thorac Dis 2016) indicated that asthma can be reported as a comorbidity of bronchiectasis. Meanwhile, previous studies from Ma et al. and Coman et al. (Ma et al, Arch Bronconeumol 2024; Coman et al, Ann Allergy Asthma Immunol 2018) showed that bronchiectasis has been reported in 25-80% of patients with severe asthma.

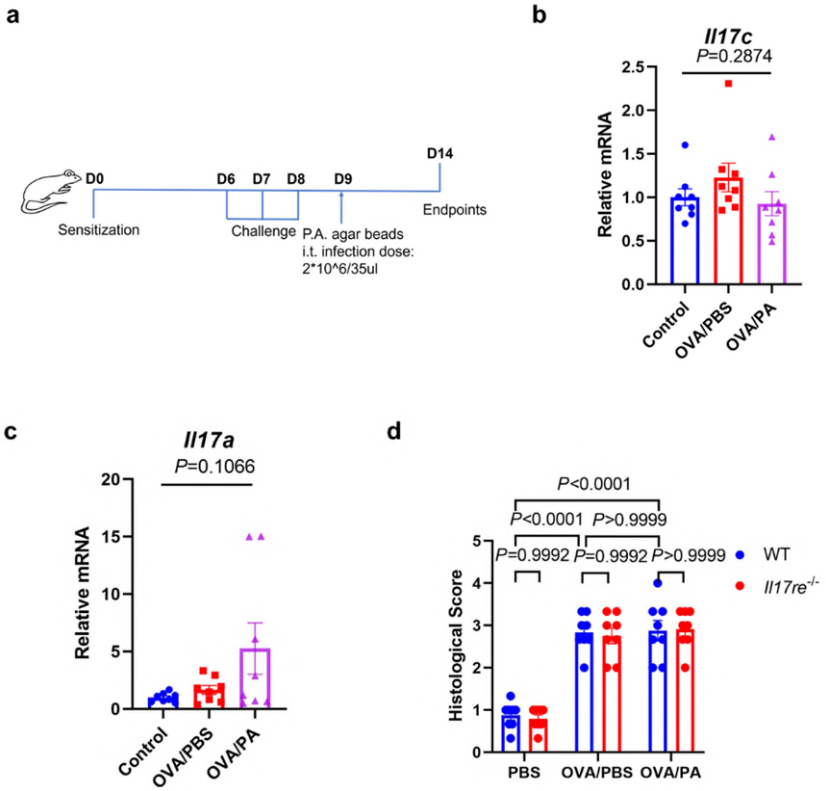
As suggested by the reviewer, we have added more details of the rationale behind experimental design on the order of events that *P. aeruginosa* chronic lower respiratory tract infection precedes allergic asthma in the introduction, results, discussion and methods section of the revised manuscript as follows: In clinical practice, bronchiectasis and asthma frequently coexist, however it is uncertain whether there is a cause-effect relationship between these two disorders. The overlap between bronchiectasis and asthma can be viewed as asthma-bronchiectasis overlap (ABO), and bronchiectasis-asthma overlap (BAO) with asthma accounting for 3-8% of bronchiectasis cases, which are both clinical issues. Despite the fact that asthma-

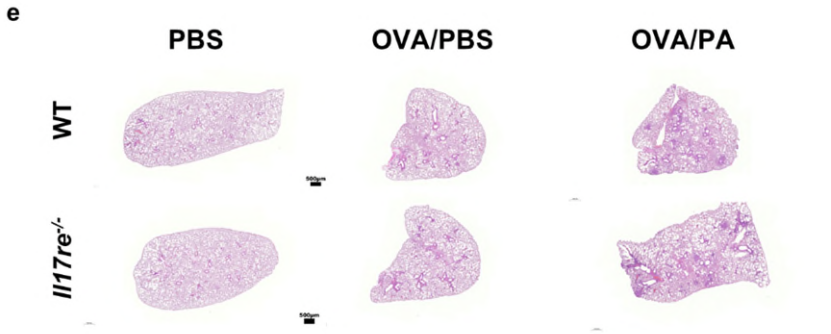
bronchiectasis overlap might be more prevalent globally, at this time, in the present study, we focused on the endotype of bronchiectasis-asthma overlap among the patients with primary diagnosis of bronchiectasis and coexisting asthma, and identifying the underlying mechanisms is therefore of key importance in precision medicine. Moreover, managing patients with bronchiectasis-asthma overlap poses challenges, particularly when it comes to the use of corticosteroids. Literature on bronchiectasis-asthma overlap is still scarce, and the endotype of bronchiectasis-asthma overlap remains to be fully defined, which has become a barrier to treatment decision-making. Furthermore, the lack of mature animal model mimicking bronchiectasis-asthma overlap has limited the availability of basic research data.

Here the management of bronchiectasis-asthma overlap has become an important clinical issue to be addressed in our study. Considering that *P. aeruginosa* chronic infection model has been widely used in a repertoire of research on bronchiectasis, consistent with the patients with primary diagnosis of bronchiectasis and coexisting asthma, we report a model of *P. aeruginosa* chronic lower respiratory tract infection followed by ovalbumin-induced experimental asthma mimicking bronchiectasis-asthma overlap to investigate the underlying mechanisms involved in the endotype of bronchiectasis-asthma overlap.

Indeed, asthma-bronchiectasis overlap is also a pivotal and interesting clinical issue. To explore potential role of IL-17C in the endotype of asthma-bronchiectasis overlap, we constructed an alternative model of ovalbumin-induced experimental asthma followed by *P. aeruginosa* infection and conducted repeated experiments (Fig. a). As depicted

below, consistent with our previous animal experiment data, the preliminary results indicated that *P. aeruginosa* infection didn't alter the levels of *Il17c*, *Il17a* mRNA and IL-17A together with the cell counts of AM, neutrophils and ILC3. Airway inflammation, eosinophils, ILC2 together with the levels of IgE, IL-5, IL-13 and total cell numbers were elevated from OVA/PA-exposed mice compared with their control mice, whereas comparable between OVA/PA-exposed mice and OVA/PBS-exposed mice (Fig. b-o). Furthermore, ablation of *Il17re* didn't affect eosinophilic inflammation and ILC3 counts in mice exposed to OVA/PA or OVA (Fig. d-p). The interesting scientific issue remains to be further investigated and accordingly the relevant data are still unavailable in the revised manuscript.

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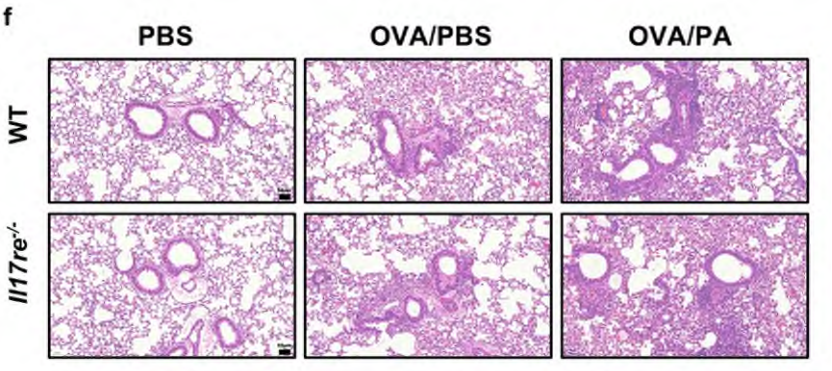
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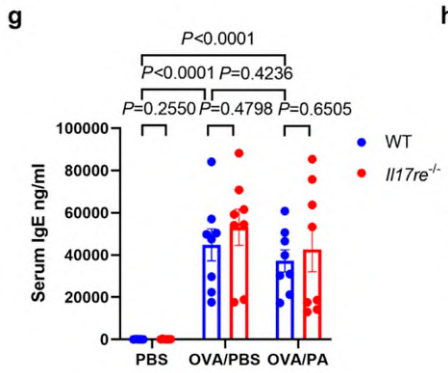
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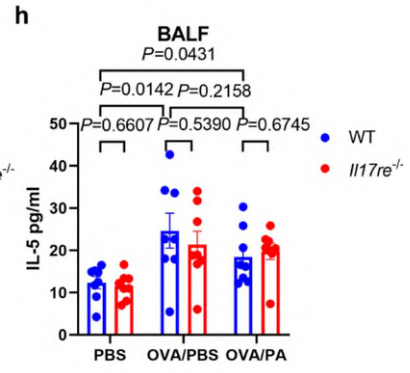
40000
20000
0
PBS
OVA/PBS
OVA/PA
WT
Il17re-/-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30
20
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0
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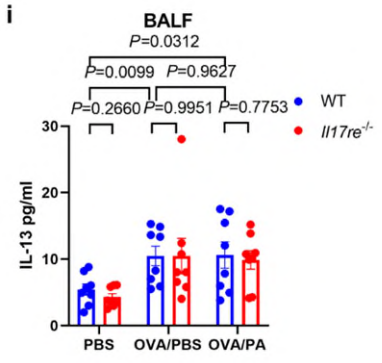
OVA/PBS
OVA/PA
WT
Il17re-/-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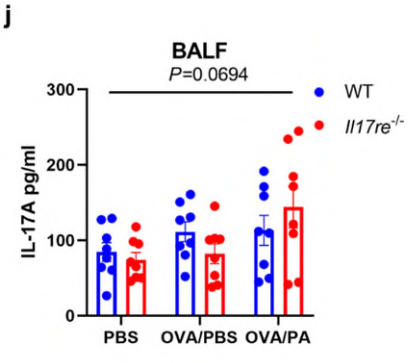
Il17re-/-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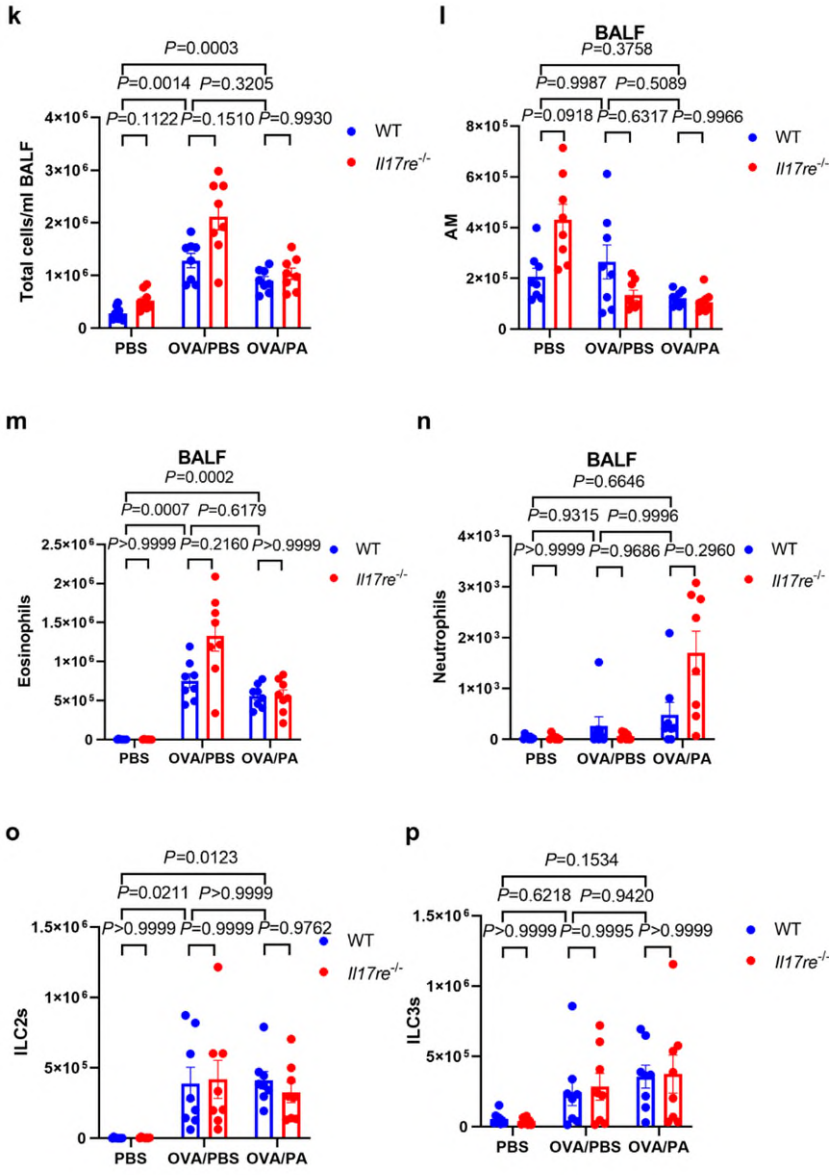
OVA/PBS
OVA/PA
WT
Il17re-/-



Figure

a Schematic diagram of OVA treatment by i.p. delivery and airway challenge followed by i.t. instillation with P. aeruginosa-laden agar beads at Day 9 in a model and BALF and lung tissues were collected at Day 14. **b, c** mRNA levels of *Il17c* and *Il17a* in lungs from mice exposed to OVA/PBS (n = 8), OVA/PA (n = 8) and their control (n = 8) were determined. **d-f** Semiquantified inflammation of lungs and representative images of H&E-stained lung sections from wild-type (WT; PBS, n = 8; OVA/PBS, n = 8; OVA/PA, n = 8) and *Il17re*$^{-/-}$ mice (PBS, n = 8; OVA/PBS, n = 8; OVA/PA, n = 8) in the model (**e** Scale bars, 500 μm; **f** Scale bars, 50 μm). **g-j** Protein levels of IgE, IL-5, IL-13, and IL-17A in serum or BALF from WT (PBS, n = 8; OVA/PBS, n = 8; OVA/PA, n = 8) and *Il17re*$^{-/-}$ mice (PBS, n = 8; OVA/PBS, n = 8; OVA/PA, n = 8) were assessed. **k-p** Quantification of total cells, AM, eosinophils and neutrophils in BALF together with total numbers of ILC2s, and ILC3s in lungs from WT (PBS, n = 8; OVA/PBS, n = 8; OVA/PA, n = 8) and *Il17re*$^{-/-}$ mice (PBS, n = 8; OVA/PBS, n = 8; OVA/PA, n = 8) as determined by flow analysis. Data are shown as mean ± SEM. Significance was calculated by one-way ANOVA (**b-d, g-p**).

C) Reviewer 3: The response to this reviewer is acceptable but not fully satisfactory. The lack of basic clinical characterization of lung functon(spirometry) and symptoms (ACT) in the group of healthy subjects gives a poor impression in terms of clinical characterization, although it is understood that it is too late now to do anything about it now. Evidently, the authors cannot guarantee that their controls are truly healthy, although it seems feasible that this is the case. This reviewer thinks that this is acceptable, given that the problem is addressed as a "limitation" in the discussion.

Reply:

We agree with the reviewer's comment and have addressed the missing values of spirometry and ACT score among the healthy controls as the second limitation in the revised manuscript.

Point-by-point responses
REVIEWER COMMENTS

Comments from reviewer #3 were mediated by in house editors and considered to be addressed.

Reply:

Thank you for your insightful remarks on our manuscript. Meanwhile, we were very grateful that our work has addressed your concerns and met the expectations with the substantial help from house editors.