Restoration of Type 17 immune signaling is not sufficient for protection during influenza-associated pulmonary aspergillosis

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Running title: Type 17 immunity in influenza-associated pulmonary aspergillosis

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Abstract:

Influenza-associated pulmonary aspergillosis (IAPA) is a severe complication of influenza infection that occurs in critically ill patients and results in higher mortality compared to influenza infection alone. Interleukin-17 (IL-17) and the Type 17 immune signaling pathway cytokine family are recognized for their pivotal role in fostering protective immunity against various pathogens. In this study, we investigate the role of IL-17 and Type 17 immune signaling components during IAPA. Wild-type mice were challenged with influenza A H1N1 (Flu) and then exposed to *Aspergillus fumigatus* ATCC42202 resting conidia on day 6 post-influenza infection, followed by the quantification of cytokines and chemokines at 48 hours post-fungal infection. Gene and protein expression levels revealed that IL-17 and Type 17 immune cytokines and antimicrobial peptides are downregulated during IAPA compared to mice singularly infected solely with *A. fumigatus*. Restoration of Type 17 immunity was not sufficient to provide protection against the increased fungal burden observed during IAPA. These findings contrast those observed during post-influenza bacterial super-infection, in which restoration of Type 17 immune signaling protects against exacerbation seen during super-infection. Our study highlights the need for future studies to understand the immune mechanisms that increase susceptibility to fungal infection.

Keywords: Interleukin-17; IAPA; antimicrobial peptides; *Aspergillus fumigatus*

Importance

IAPA significantly elevates the risk of mortality in patients with severe influenza. Type-17 immunity is critical to host defense during fungal infections and, therefore, vital to understand its role during IAPA. The observations in this study reveal that Type 17 immunity is impaired during IAPA, potentially increasing susceptibility to secondary infection with *Aspergillus fumigatus*. However, restoration of IL-17 signaling alone is not sufficient to

reduce fungal burden in our murine IAPA model. These observations differ from those observed in post-influenza bacterial super-infections, suggesting that the mechanisms underlying viral-fungal super-infection are different than those that underly viral-bacterial super-infection. By elucidating the complex interactions between the host immune system, influenza, and *A. fumigatus*, these findings are vital for developing strategies to enhance immune responses and improve survival rates during IAPA.

OBSERVATION

Influenza-associated pulmonary aspergillosis (IAPA) is a severe complication of influenza infection that occurs in critically ill patients and results in higher mortality compared to influenza infection alone (1). The pathology of IAPA manifests through invasive growth of *Aspergillus fumigatus* within the lungs during influenza infection (2). The interleukin-17 (IL-17) cytokine family is a pivotal component in mediating protective immunity against various pathogens (3); however, the specific involvement of IL-17 and its related cytokines in the context of IAPA has not been studied. IL-17 exerts its immunomodulatory effects by promoting the synthesis of pro-inflammatory molecules, including cytokines, chemokines, and antimicrobial peptides, which facilitate the recruitment of neutrophils and other immune effectors to sites of infection, thereby augmenting the host defense against invading pathogens (4). Our current study investigates the role of IL-17 and IL-17-related cytokines in IAPA, employing a murine model to delineate their impact on disease pathogenesis and immune responses. Our observation sheds light on the complex host immune system that occurs during IAPA, aiming to uncover novel therapeutic avenues and enhance patient outcomes in managing IAPA.

RESULTS

Type 17 cytokines are inhibited during IAPA

We have previously published a murine model of IAPA that demonstrates increased morbidity in mice co-infected with influenza and *A. fumigatus* (5). Type 17 immunity plays a critical role in host defense against *A. fumigatus* and other fungal pathogens (6-10). IL-17 and other Type 17 immune cytokines also play a critical role in the development of bacterial super-infection during influenza (11-12). Therefore, we hypothesized that the Type 17 immune response would play an essential role during IAPA. To model this hypothesis, C57BL/6J male mice were challenged with a sublethal dose of influenza A PR/8/34 H1N1 (100 PFU) for 6 days, followed by 2.5×10^7 *A. fumigatus* (ATCC strain 42202) conidia, and after 48 hours, fungal burden was assessed. Mice super-infected with influenza and *A. fumigatus* had decreased expression of IL-17, IL-22, IL-23, and IL-1β compared to those infected with *A. fumigatus* alone (**Fig 1A**). This downregulation could potentially contribute to increased morbidity, mortality, and fungal burden compared to singular infection with *A. fumigatus* alone. In addition to the gene expression changes, protein expression analysis also showed a decreased production of IL-17, IL-22, IL-23, and IL-1β proteins in super-infected mice (**Fig 1B)**.

Decreased IL-17-producing γδ T cells during IAPA

Flow cytometry analysis was conducted to quantify IL-17-producing T cells in our model. We measured $IL17^+$ $CD4^+$ T and γδ T cells in our model due to their well-established role as primary producers of IL-17. We observed a notable decrease in the total number of IL-17 producing γδ T cells in super-infected mice compared to singular *A. fumigatus* infection (**Fig 1C**). Comparatively, we observed no difference in the abundance of $IL17^+$ $CD4^+$ T cells between the different groups (**Fig 1C**). When interpreting these results, it is important to

106 consider the timing of mouse harvesting post- *A. fumigatus* infection. Specifically, the
107 analysis was conducted at a 48-hour time point post-infection. At this early stage, the immune
108 response is still evolving, with distinct subsets of immune cells playing varying roles. In
109 particular, it is noted that there were more innate γδ T cells present at this time point, with T
110 cells potentially infiltrating at later stages of infection.
111
112 **Reduced expression of IL-17/IL-22 associated antimicrobial peptides in IAPA**
113 As IL-17 and IL-22 are distinct lineages of Type 17 cells (13), we studied their effector
114 function by examining gene expression levels of IL-17- and IL-22- associated antimicrobial
115 peptides in our model. The expression levels of *Reg3β*, *Reg3γ, and Lcn2* were significantly
116 reduced in super-infected mice compared to those infected solely with *A. fumigatus* (**Fig 1D)**.
117 This reduction of IL-17- and IL-22- associated antimicrobial peptides may play a role in
118 fungal clearance during IAPA.
119
120 **Restoration of Type 17 immunity does not enhance fungal clearance during IAPA**
121 With the observation that Type 17 cytokines and antimicrobial peptides were significantly
122 downregulated during IAPA compared to *A. fumigatus* alone, we hypothesized that their
123 restoration could provide protection during IAPA. To test this hypothesis, we overexpressed
124 IL-17 proposing that it would rescue Type-17 immunity and enhance fungal clearance.
125 Overexpression of IL-17 significantly upregulated the levels of IL-17a mRNA (**Fig 2B**) and
126 downstream inflammatory mediators TNFα and CXCL1 during IAPA (**Fig 2C**) (14-15);
127 however, fungal burden remained unchanged **(Fig 2A)**. Interestingly, the total number of
128 inflammatory cells in the bronchoalveolar lavage fluid and the total numbers of neutrophils
129 and macrophages measured by cytospin differential also remained unchanged with
130 upregulation of Type 17 immunity (**Fig S1**). Additionally, we administered murine

recombinant IL-17 protein during IAPA and fungal burden was unchanged (**Fig S2**). Next, we restored components of the Type 17 immune signaling pathway that are both upstream and downstream of IL-17 to determine effects on fungal clearance. Since both IL-1β and IL-23 are known to induce IL-17 production from γδ T cell (16), and we observed decreased production during IAPA (**Fig 1A-B**), we administered IL-1β and IL-23+IL-1β together for synergistic effects during IAPA. There was a significant increase of IL-17 mRNA in the mice that received exogenous IL-1β and IL-23/IL-1β (**Fig 2E**); however, fungal burden remained unchanged (**Fig 2D**). Additionally, IL-1β and IL-23+IL-1β did not alter IL-22 mRNA expression (**Fig 2E**). Super-infected mice were administered either with Reg3β or Reg3γ to explore potential therapeutic interventions. Although both these peptide levels have been reduced in super-infected mice, neither changed fungal clearance during IAPA (**Fig 2F**). Additionally, we observed no difference in the total cell count of immune cells in bronchoalveolar lavage fluid (**Fig S3)**. Collectively, these results indicate that restoration of IL-17 signaling is not sufficient to restore fungal clearance during IAPA. These findings suggest the involvement of mechanisms beyond Type 17 inhibition causing decreased fungal clearance during IAPA and highlight the complexity of IAPA pathogenesis.

DISCUSSION

Influenza and *A. fumigatus* have individually been studied due to their clinical significance; however, the synergy and complexities that arise when these pathogens co-exist within the same host remain poorly understood. Aspergillus species can also cause super-infection during SARS-CoV-2 (17,18). The Type 17 cytokine family is recognized for its pivotal role in fostering protective immunity against a spectrum of pathogens (19). Previous research has substantiated the involvement of IL-17 in the context of viral-bacterial superinfections (11). The IL-17 pathway has also promoted *Aspergillus* clearance within pulmonary tissues (6-8).

This study aims to delineate the specific roles of IL-17 and Type 17 immune-associated cytokines and chemokines in IAPA.

Our results demonstrate that preceding infection impairs Type17 immunity during IAPA; however, restoration of IL-17 signaling does not alter the host's susceptibility to secondary fungal infection. Both gene expression and protein quantification of key Type 17 immune cytokines are decreased during IAPA. IL-17, a pro-inflammatory cytokine, plays an essential role in fungal infections, particularly in the lungs, by recruiting neutrophils and other immune cells to the site of infection and by inducing the production of antimicrobial peptides (20). IL-17 synergistically collaborates with IL-22 for a robust immune response against fungal infection (21). Our results also demonstrate the inhibition of IL-17- and IL-22- associated antimicrobial peptides during IAPA. Despite the observed reduction in Type 17 immunity during IAPA, restoration of various components of the IL-17 signaling pathway, both upstream and downstream of IL-17, did not lead to improved fungal clearance. In contrast to prior studies that showed restoration of Type 17 immune pathway components rescued bacterial clearance during post-influenza bacterial super-infection (11,12,22), the current study indicates that restoration of IL-17 signaling alone is not sufficient to reduce fungal burden in a murine IAPA model. IL-17 may play a protective role but not a restorative role during IAPA. Notably, the immune response is dynamic during infection and augmentation of Type 17 signaling at other time points during IAPA may produce different results. Interestingly, downregulation of IL-1β in our mouse model is consistent with the findings in human patients with IAPA versus influenza alone (23). This consistency between our findings in mice and human patients strengthens the validity of our results and suggests that the observed decrease in cytokines is a robust effect that is relevant across species. Importantly, these studies show that immune regulation during post-influenza fungal super-infection and post-influenza bacterial super-infection are not driven by the same mechanisms. It

underscores the need for additional studies to understand the immune mechanisms that increase susceptibility to fungal infection during influenza and how delineation of the specific cell types and immune pathways that are necessary for fungal host defense during viral infection may lead to future therapeutics.

ETHICS APPROVAL

The University of Pittsburgh Institutional Animal Care and Use Committee approved the study under Protocol No. 21063690.

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DATA AVAILABILITY

All the associated data is provided with this manuscript or in supplementary files.

DECLARATION OF INTEREST

The authors have no conflict of interest with any organization or entity with a financial interest in the subject matters or materials discussed in this manuscript.

SUPPLEMENTAL MATERIAL

Supplemental Materials and Methods and Figures S1-S3.

REFERENCES

1. Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, Lagrou K, Verweij PE, Van de Veerdonk FL, Gommers D, Spronk P, Bergmans DCJJ, Hoedemaekers A, Andrinopoulou ER, van den Berg CHSB, Juffermans NP, Hodiamont CJ, Vonk AG, Depuydt P, Boelens J, Wauters J, Dutch-Belgian Mycosis study group. 2018. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: A retrospective cohort study. Lancet Respir Med 6(10): 782–792. https://doi.org/10.1016/S2213-2600(18)30274-1.

2. van de Veerdonk FL, Kolwijck E, Lestrade PPA, Hodiamont CJ, Rijnders BJA, van Paassen J, Haas P-J, Oliveira Dos Santos C, Kampinga GA, Bergmans DCJJ, van Dijk K, de Haan AFJ, van Dissel J, van der Hoeven HG, Verweij PE, Dutch Mycoses Study Group. 2017. Influenza-associated aspergillosis in critically ill patients. Am J Respir Crit Care Med https://doi.org/10.1164/rccm.201612-2540LE.

3. Kuwabara T, Ishikawa F, Kondo M, Kakiuchi T. 2017. The role of IL-17 and related cytokines in inflammatory autoimmune diseases. Mediators Inflamm 2017:3908061. https://doi.org/10.1155/2017/3908061

4. Korn T, Bettelli E, Oukka M, Kuchroo VK. 2009. IL-17 and Th17 Cells. Annu Rev Immunol 27:485–517. https://doi.org/10.1146/annurev.immunol.021908.132710

5. Tobin JM, Nickolich KL, Ramanan K, Pilewski MJ, Lamens KD, Alcorn JF, Robinson KM. 2020. Influenza suppresses neutrophil recruitment to the lung and exacerbates secondary invasive pulmonary aspergillosis. J Immunol 205:480–488. https://doi.org/10.4049/jimmunol.2000067

6. Werner JL, Gessner MA, Lilly LM, Nelson MP, Metz AE, Horn D, Dunaway CW, Deshane J, Chaplin DD, Weaver CT, Brown GD, Steele C. 2011. Neutrophils produce interleukin 17A (IL-17A) in a dectin-1- and IL-23-dependent manner during invasive fungal infection. Infect Immun 79:3966–3977. https://doi.org/10.1128/iai.05493-11

7. Taylor PR, Leal SM, Sun Y, Pearlman E. 2014. *Aspergillus* and *Fusarium* corneal infections are regulated by Th17 cells and IL-17-producing neutrophils. J Immunol 192:3319–3327. https://doi.org/10.4049/jimmunol.1302235

8. Gessner MA, Werner JL, Lilly LM, Nelson MP, Metz AE, Dunaway CW, Chan YR, Ouyang W, Brown GD, Weaver CT, Steele C. 2012. Dectin-1-dependent interleukin-22 contributes to early innate lung defense against *Aspergillus fumigatus*. Infect Immun 80:410–417. https://doi.org/10.1128/iai.05939-11

9. Sparber F, LeibundGut-Landmann S. 2019. Interleukin-17 in antifungal immunity. Pathogens 8. https://doi.org/10.3390%2Fpathogens8020054

10. Malacco NLS de O, Rachid MA, Gurgel IL da S, Moura TR, Sucupira PHF, de Sousa LP, de Souza D da G, Russo R de C, Teixeira MM, Soriani FM. 2018. Eosinophil-associated innate IL-17 response promotes *Aspergillus fumigatus* lung pathology. Front Cell Infect Microbiol 8:453. https://doi.org/10.3389/fcimb.2018.00453

11. Kudva A, Scheller EV, Robinson KM, Crowe CR, Choi SM, Slight SR, Khader SA, Dubin PJ, Enelow RI, Kolls JK, Alcorn JF. 2011. Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice. J Immunol 186:1666–1674. https://doi.org/10.4049/jimmunol.1002194

12. Robinson KM, Choi SM, McHugh KJ, Mandalapu S, Enelow RI, Kolls JK, Alcorn JF. 2013. Influenza A exacerbates *Staphylococcus aureus* pneumonia by attenuating IL-1β production in mice. J Immunol 191:5153–5159. https://doi.org/10.4049/jimmunol.1301237

13. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, Husain S, Kreindler JL, Dubin PJ, Pilewski JM, Myerburg MM, Mason CA, Iwakura Y, Kolls JK. 2008. IL-22 mediates mucosal host

defense against Gram-negative bacterial pneumonia. Nat Med 14:275–281. https://doi.org/10.1038/nm1710

14. Wang CQF, Akalu YT, Suarez-Farinas M, Gonzalez J, Mitsui H, Lowes MA, Orlow SJ, Manga P, Krueger JG. 2013. IL-17 and TNF synergistically modulate cytokine expression while suppressing melanogenesis: potential relevance to psoriasis. J Invest Dermatol 133:2741–2752. https://doi.org/10.1038%2Fjid.2013.237

15. Sharma AK, Mulloy DP, Le LT, Laubach VE. 2014. NADPH oxidase mediates synergistic effects of IL-17 and TNF-α on CXCL1 expression by epithelial cells after lung ischemia-reperfusion. Am J Physiol Lung Cell Mol Physiol 306:L69-79. https://doi.org/10.1152/ajplung.00205.2013

16. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KHG. 2009. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity 31:331–341. https://doi.org/10.1016/j.immuni.2009.08.001

17. Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, Zhu F, Zhu B, Cui L. 2020. Co-infection with respiratory pathogens among COVID-2019 cases. Virus Res 285:198005. https://doi.org/10.1016/j.virusres.2020.198005

18. van Arkel AL, Rijpstra TA, Belderbos HN, Van Wijngaarden P, Verweij PE, Bentvelsen RG. 2020. COVID-19–associated pulmonary aspergillosis. Am J Respir Crit Care Med 202(1):132-5. https://doi.org/10.1164/rccm.202004-1038LE

19. Huangfu L, Li R, Huang Y, Wang S. 2023. The IL-17 family in diseases: from bench to bedside. Signal Transduct Target Ther 8:402. https://doi.org/10.1038/s41392-023-01620-3

20. Puerta-Arias JD, Mejía SP, González Á. 2020. The role of the interleukin-17 axis and neutrophils in the pathogenesis of endemic and systemic mycoses. Front Cell Infect Microbiol 10:595301. https://doi.org/10.3389/fcimb.2020.595301

21. Sawada Y, Setoyama A, Sakuragi Y, Saito-Sasaki N, Yoshioka H, Nakamura M. 2021. The role of IL-17-producing cells in cutaneous fungal infections. Int J Mol Sci 22. https://doi.org/10.3390/ijms22115794

22. Robinson KM, McHugh KJ, Mandalapu S, Clay ME, Lee B, Scheller EV, Enelow RI, Chan YR, Kolls JK, Alcorn JF. 2014. Influenza A virus exacerbates *Staphylococcus aureus* pneumonia in mice by attenuating antimicrobial peptide production. J Infect Dis 209:865–875. https://doi.org/10.1093/infdis/jit527

23. Feys S, Gonçalves SM, Khan M, Choi S, Boeckx B, Chatelain D, Cunha C, Debaveye Y, Hermans G, Hertoghs M, Humblet-Baron S, Jacobs C, Lagrou K, Marcelis L, Maizel J, Meersseman P, Nyga R, Seldeslachts L, Starick MR, Thevissen K, Vandenbriele C, Vanderbeke L, Vande Velde G, Van Regenmortel N, Vanstapel A, Vanmassenhove S, Wilmer A, Van De Veerdonk FL, De Hertogh G, Mombaerts P, Lambrechts D, Carvalho A, Van Weyenbergh J, Wauters J. 2022. Lung epithelial and myeloid innate immunity in influenza-associated or COVID-19-associated pulmonary aspergillosis: an observational study. Lancet Respir Med 10:1147–1159. https://doi.org/10.1016/S2213-2600(22)00259-4

24. Robinson KM, Ramanan K, Clay ME, McHugh KJ, Pilewski MJ, Nickolich KL, Corey C, Shiva S, Wang J, Muzumdar R, Alcorn JF. 2018. The inflammasome potentiates influenza/*Staphylococcus aureus* superinfection in mice. JCI Insight 3. https://doi.org/10.1172/jci.insight.97470

25. Robinson KM, Ramanan K, Clay ME, McHugh KJ, Rich HE, Alcorn JF. 2018. Novel protective mechanism for interleukin-33 at the mucosal barrier during influenza-

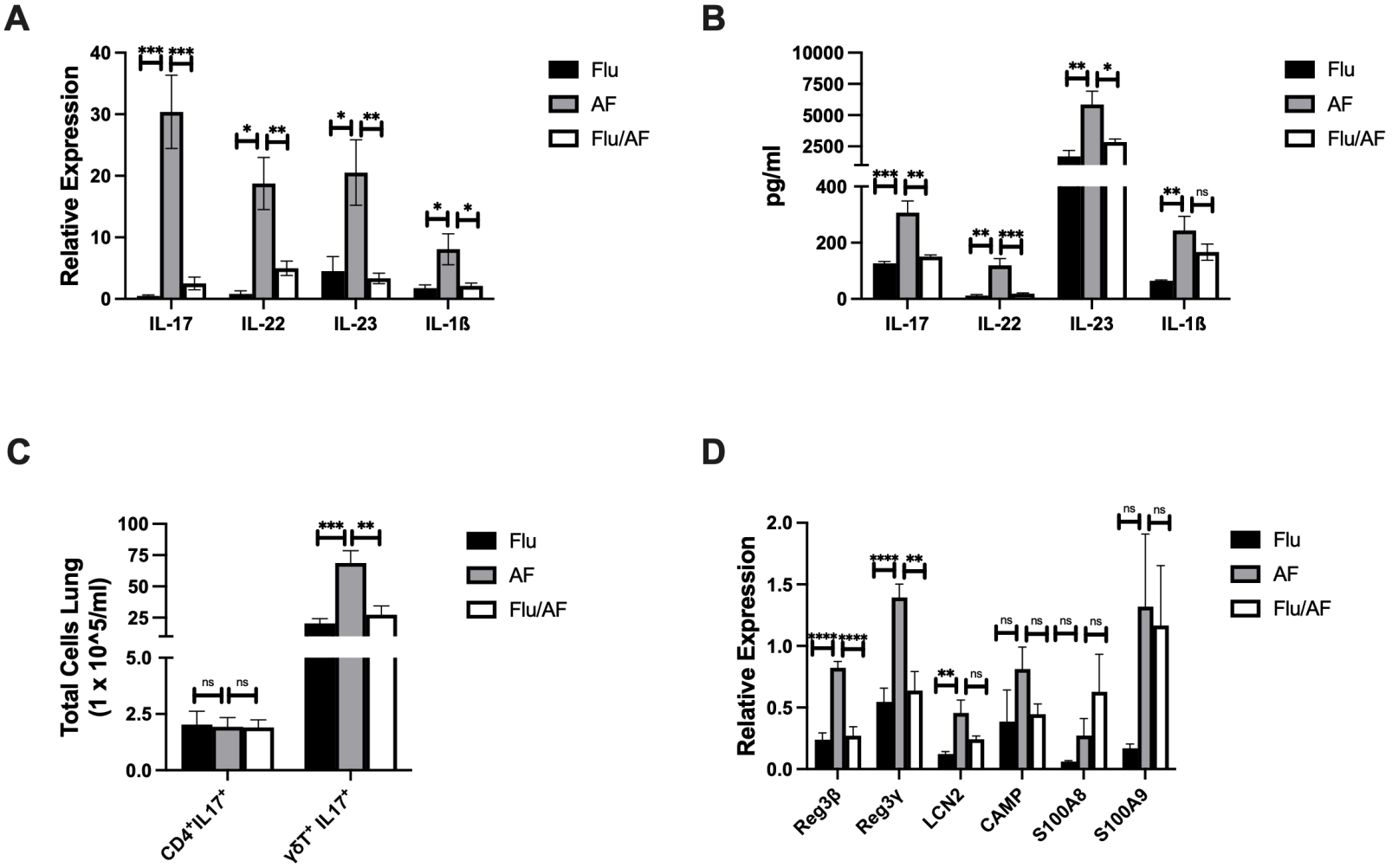
associated bacterial superinfection. Mucosal Immunol 11:199–208. https://doi.org/10.1038/mi.2017.32

FIGURE LEGENDS

Figure 1: **Type 17 immune pathway is downregulated during influenza-associated pulmonary aspergillosis (IAPA).** WT mice were infected with influenza A H1N1 PR/8/34 on day 0 and subsequently challenged with 2.5×10^7 *aspergillus fumigatus* (AF) at 6 dpii. Lung samples were collected at 48 h post-AF challenge. (**A**) IL-17 and related pro-inflammatory cytokine gene expression quantified by RT-qPCR, (**B**) Protein levels of IL-17-related cytokines quantified using Lincoplex assay or ELISA, (**C**) Flow cytometry analysis of IL-17-producing cells, $CD4^+$ $IL\text{-}17^+$ T cells and γδ T cells, (**D**) IL-17/IL-22-associated antimicrobial peptide gene expression quantified by RT-qPCR. Data were compiled from two independent experiments and are presented as means ± SEM, with statistical significance marked as $*p < 0.05$, $** p < 0.005$, and $*** p < 0.0005$ by unpaired student's T-test.

Figure 2: Restoration of Type 17 immune signaling is not sufficient to provide protection during IAPA. WT mice were infected with influenza A H1N1 PR/8/34 on day 0 and subsequently challenged with 2.5×10^7 *aspergillus fumigatus* (AF) at 6 dpii. Lung samples were collected at 48 h post-AF challenge. (**A-C**) Mice were administered IL-17 expressing adenovirus (IL-17) or control adenovirus during IAPA and (**A**) fungal burden measured by CFU, (**B**) IL-17a gene expression quantified by RT-qPCR, and (**C**) TNFα and CXCL1 cytokines measured by ELISA. (**D-E**) Mice were administered IL-1β or IL-1β+IL-23 murine recombinant protein during IAPA and **(D)** fungal burden measured by CFU, (**E**) IL-17a and IL-22 gene expression quantified by RT-qPCR. **(F)** Mice were administered Reg3β or Reg3γ murine recombinant proteins during IAPA and fungal burden measured by CFU. Data were compiled from two independent

experiments and are presented as means ± SEM, with statistical significance marked as *$p < 0.05$, ** $p < 0.005$, and **** $p < 0.0001$ by unpaired student's T-test.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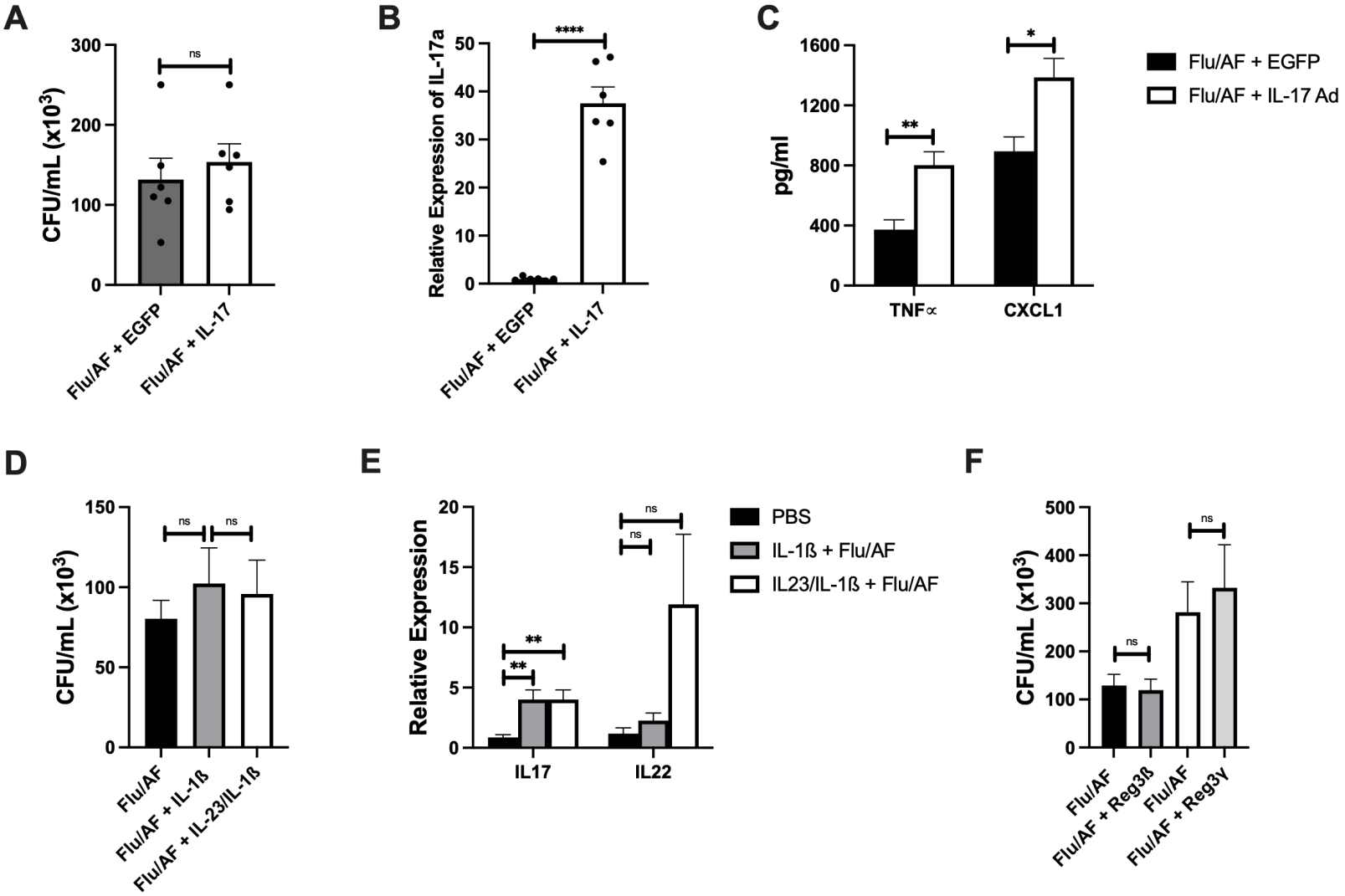
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IL23/IL-1ß + Flu/AF
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CFU/mL (x10^3)
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Flu/AF
Flu/AF + Reg3ß
Flu/AF
Flu/AF + Reg3γ