IL-13 signature in severe adult asthmatics with airway neutrophilia: a new endotype to treat!

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Editorial (invited)

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IL-13 signature in severe adult asthmatics with airway neutrophilia: a new endotype to treat!

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To the editor,

With great interest we read the article by Azim and co-workers [1] published in this issue of Allergy. To understand the inflammatory component of the peripheral airways of severe asthmatics uncontrolled by high dose inhaled corticosteroids (ICS) the authors examined the bronchoalveolar lavage (BAL) in two well-characterised asthma populations. In 78 severe asthma patients constituting the experimental cohort, an increase in IL-13 BAL levels associated with increased neutrophils without eosinophils, were reported. In the validation cohort (n=18), the authors confirmed this "non-eosinophilic" phenotype and further showed the presence of pathogenic bacteria, *Moraxella catarrhalis* , *Haemophilus sp* and *Streptococcus sp* . The significance of the study is three-fold. First, the identification of a severe T2 asthma population with an IL-13 signature and airway neutrophils that may be misclassified due to absence of the "classic" biomarker – eosinophils. Second, it highlights potential alternate cellular sources of IL-13 besides the T2 cells. Third, the association of high BAL IL-13 with microbial dysbiosis in a low-eosinophilic airway inflammation setting that indicates a novel pathogenetic role of IL-13, further contributing to asthma severity.

Almost two decades back Ward et al reported a high variability in the inflammatory BAL profile [2]. Therefore, the confirmation of a neutrophilic airway cellularity in a follow-up validation cohort was essential, and adds to the robustness of the study design. However, the presence of neutrophils in the BAL may simply be an innate immune response to the detected pathogenic bacteria or the ongoing effect of the corticosteroid therapy, rather than a direct IL-13 effect. Both confounders could have been easily addressed if the airway inflammation was investigated longitudinally after a course of antibiotics. In addition, the absence of eosinophils in the BAL could simply be a numerical anomaly where the increased number of neutrophils masks the eosinophil count. Again, this could have been checked by examining the cellularity in BAL post-infection (i.e. after a course of antibiotics) or by using a ratio between eosinophils and neutrophils instead of absolute values.

Close inspection of the reported data reveals that an underlying eosinophilic/T2 component in the high tertile IL-13 severe asthma group cannot be ruled out altogether. First, the distribution on the scatter plot (Figure 2 in Azim *et al* [1]) shows half of the patients having >2% BAL eosinophils. In the replication cohort, the BAL eosinophil% were higher in the high IL-13 group vs low (1.30±4.53 vs. 0.50±1.65) but multiple comparison tests have not been reported to ratify a statistical difference. IL-5 levels were significantly higher in the high IL-13 compared to the low tertile group (0.61 ± 1.53 vs 0.32 ±0.35 pg/mL, p=0.001). IL-13 correlated with both BAL neutrophil% (r=0.580, p<0.001) and BAL eosinophil%, (r=0.271, p=0.017), even though the latter was a weaker association. Using routine sputum cell differential, ˜10% of severe asthmatics consistently have a mixed granulocytic sputum, i.e. neutrophils >65% together with borderline eosinophils in the range of 2-3%. These patients usually have ongoing airway infection that masks their eosinophilia, as their eosinophilia re-emerges after the infection is resolved [3]. A mixed granulocytic phenotype based on sputum cytology has been reported both by the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) and Unbiased BIOmarkers in PREDiction of respiratory disease outcome (U-BIOPRED) cohorts. The patient population evaluated is similar to other observational studies where 7-10% of asthmatics have mixed phenotype [3], lowest lung function [4], and similar dysbiosis by 16s sequencing [5]. Taken together, the high tertile IL-13 "neutrophilic" group based on the BAL analysis may be representative of the mixed granulocytic phenotype.

The debate continues on whether neutrophils contribute to the pathology of severe asthma in the high tertile IL-13 group or whether they are only present as a consequence of infection or the use of corticosteroids. It must be pointed out that, targeting different drivers of neutrophilic inflammation (e.g. IL-17, IL-23, CXCR2 [reviewed extensively in [6]]) had discouraging results in the clinical trials, although patient selection was not optimal.

We next raise the question on whether increased IL-13 in the airway is contributory or collateral to the observed infections. The IL-4/IL-13 pathway has been implicated in dampening the neutrophil response to an infection by impairing IL-8 induced migration and neutrophil extracellular trap formation [7]. LPS (infection mimic) induces airway hyperresponsiveness and corticosteroid resistance via IL-13 dependent signalling of pulmonary macrophages [8]. Therefore, increased IL-13 due to an underlying T2 pathology may impair

host defence. Of interest, increased sputum IL-13 levels predicts the presence of airway autoantibodies in severe asthmatics with a mixed granulocytic phenotype. Moreover, autoantibody-induced macrophage dysfunction in the mixed phenotype can contribute to recurrent airway infections [9]. That said, inherent immunodeficiencies or neutrophil dysfunction were not ruled out in this study as other underlying reasons for the observed airway infection in the replication cohort.

In summary, elevated levels of IL-13 in the peripheral airways with increased neutrophils and low levels of eosinophils reveal an underlying T2 pathway for the mixed granulocytic asthma phenotype, together with alternate cellular sources of IL-13 besides eosinophils or the T2 cells, such as alveolar macrophages or mast cells (Figure 1). The study also adds a novel potential pathogenetic pathway (innate immune response impairment with microbial dysbiosis) to IL-13's "classic" contribution to asthma severity, including but not limited to, airway smooth muscle phenotype switch, mucus hypersecretion, eosinophil recruitment, and epithelial activation (Figure 1). Pending further validation through longitudinal studies, a therapeutic focus for this particular severe asthma endotype is therefore IL-13, a cytokine that is truly pleiotropic for its heterogeneity of cellular source and downstream functions. Targeting the IL-13/IL-4 pathway using an anti-IL-4Ra monoclonal antibody (dupilumab) was more successful than targeting IL-13 alone [10]. This might be relevant for the population in question as IL-4 levels (though lowly detected) were correlated with IL-13 levels.

We conclude by congratulating the authors [1] for their distinct observation of a severe asthma endotype with raised IL-13, neutrophilia and dysbiosis in the peripheral airways. This population may benefit from an IL-4/IL-13 targeted therapy, although the "classic" T2 selection biomarker – eosinophilia is not immediately noticeable. The work also reinforces the value of using airway secretions, instead of blood, to investigate asthma endotypes.

Author contributions : MM and AI wrote the manuscript.

Figure:

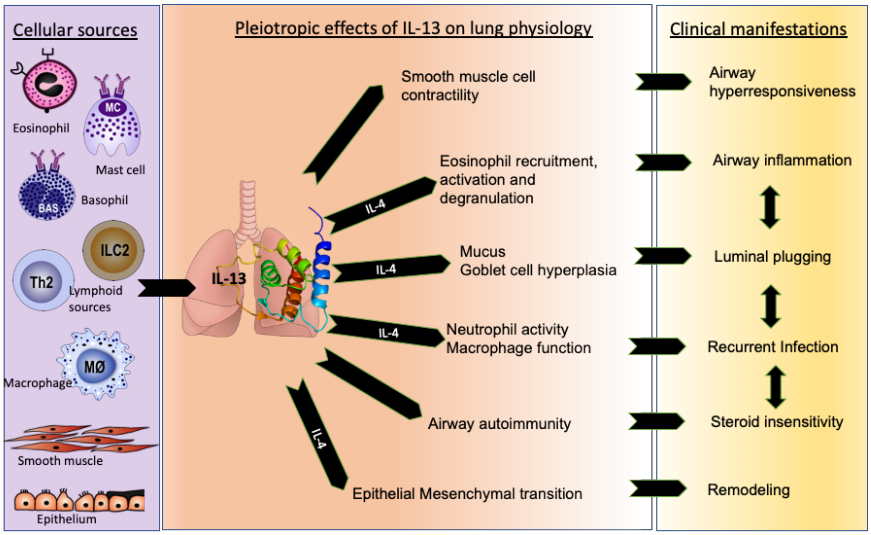

Figure legends:

Fig 1: Pleiotropic effects of IL-13 in severe asthma: The schematic highlights the diverse cellular source of IL-13, and its varied downstream effect on both immune and structural cells, reflected in heterogenous clinical manifestations.

Reference

1. Azim A, Green B, Lau L, Rupani H, Jayasekera N, Bruce K, Howarth P. Peripheral airways type 2 inflammation, neutrophilia and microbial dysbiosis in severe asthma. *Allergy* 2021, Jan 7(doi: 10.1111/all.14732.).

2. Ward C, Gardiner PV, Booth H, Walters EH. Intrasubject variability in airway inflammation sampled by bronchoalveolar lavage in stable asthmatics. *Eur Respir J* 1995: 8(11): 1866-1871.

3. Aleman F, Lim HF, Nair P. Eosinophilic Endotype of Asthma.*Immunology and Allergy Clinics of North America* 2016: 36(3): 559-568.

4. Chu DK, Al-Garawi A, Llop-Guevara A, Pillai RA, Radford K, Shen P, Walker TD, Goncharova S, Calhoun WJ, Nair P, Jordana M. Therapeutic potential of anti-IL-6 therapies for granulocytic airway inflammation in asthma. *Allergy, Asthma & Clinical Immunology* 2015: 11(1): 1-6.

5. Cox C, Kjarsgaard M, Surette M, Cox P, Nair P. A multidimensional approach to the management of severe asthma: Inflammometry, molecular microbiology and bronchial thermoplasty. *Canadian Respiratory Journal* 2015: 22(4): 221 - 224.

6. Sze E, Bhalla A, Nair P. Mechanisms and therapeutic strategies for non-T2 asthma. *Allergy* 2020: 75(2): 311-325.

7. Impellizzieri D, Ridder F, Raeber ME, Egholm C, Woytschak J, Kolios AGA, Legler DF, Boyman O. IL-4 receptor engagement in human neutrophils impairs their migration and extracellular trap formation. *J Allergy Clin Immunol* 2019: 144(1): 267-279.e264.

8. Hadjigol S, Netto KG, Maltby S, Tay HL, Nguyen TH, Hansbro NG, Eyers F, Hansbro PM, Yang M, Foster PS. Lipopolysaccharide induces steroid-resistant exacerbations in a mouse model of allergic airway disease collectively through IL-13 and pulmonary macrophage activation.*Clin Exp Allergy* 2020: 50(1): 82-94.

9. Mukherjee M, Nair P. Autoimmune Responses in Severe Asthma.*Allergy Asthma Immunol Res* 2018: 10.

10. Nair P, O'Byrne PM. The interleukin-13 paradox in asthma: effective biology, ineffective biologicals. *Eur Respir J* 2019: 53(2).