Reply: *Ex vivo* SOCS3 gene responsiveness to alarmins in eosinophils of mepolizumab-treated patients is as yet of unknown biological significance

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Reply to S. Couillard:

We thank S. Couillard for his interesting comments related to our publication on the effects of interleukin (IL)-5 deficiency on the transcriptome of residual eosinophils of *Il5*-deficient mice and mepolizumab-treated patients [1]. We indeed document stronger upregulation of the expression of the SOCS3 gene following stimulation with IL-33 *ex vivo* that is conserved between eosinophils from mepolizumab-treated patients and from *Il5*-deficient mice.

We do not question that this specific response of IL-5-deprived eosinophils might have biological implications since SOCS3 is an important gene in asthma. Indeed, Seki *et al*. [2] reported that SOCS3 gene expression in peripheral T cells correlates with asthma severity and that transgenic mice in which the *Socs3* gene is overexpressed specifically in T cells develop more robust T-helper type 2 (Th2) responses and associated airway allergy. Further, genetic association studies pinpointed the SOCS3 locus as a suspected driver of asthma risk [3]. We discussed these findings.

Nonetheless, we advise caution in interpreting and extrapolating our specific observation in eosinophils for several reasons. From a purely technical standpoint first, our study of the response to IL-33 was performed *ex vivo* on isolated eosinophils, as a way of modelling eosinophil activation. It remains to be determined whether a similar difference in SOCS3 gene induction in eosinophils would be observed in the airways of mepolizumab-treated asthma patients or *Il5*-deficient mice, in which the immune microenvironment is obviously more complex. In addition, caution is warranted when extrapolating gene expression data, as changes in gene expression are not always reflected at the protein, and hence functional level. It remains unknown whether SOCS3 protein levels would be sufficiently affected in eosinophils of IL-5-depleted hosts to the point of altering eosinophil behaviour.

Second, from a conceptual standpoint, S. Couillard proposes an interesting but speculative extrapolation. He proposes that increased SOCS3 gene responsiveness to IL-33 in IL-5-deprived eosinophils could make them more prone to recruitment or activation and contribute to asthma exacerbations in a subset of asthma patients treated with anti-IL-5 biologicals, as reported in the MEX study [4]. It has however to be noted that SOCS3 is mostly suspected to affect asthma risk through the T cell intrinsic impact it has on T cell polarisation into Th2 cells [2, 5], which are major drivers of the atopic T2 asthma endotype [6]. The role of SOCS3 in eosinophils themselves remains in comparison poorly studied and there has been no experimental evidence so far that SOCS3-overexpressing eosinophils would be more prone to activation, or "trigger happy" as S. Couillard puts it. It is yet a hypothesis worth testing, as we discussed.

We actually believe that two non-mutually exclusive models of eosinophil biology should be considered to better understand clinical observations such as those of the MEX study. A first model, in line with S. Couillard's hypothesis, would be that eosinophil responses may be intrinsically and systemically "imprinted" during eosinophil development by changes in the immunological context, such as a deficit in IL-5 signalling. We recently conceptualised this possibility that the immune environment in which eosinophil progenitors mature might generate different eosinophil "endotypes", involved in different

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It is too early to conclude on a potential biological significance of increased SOCS3 gene expression in blood eosinophils of mepolizumab-treated patients stimulated with interleukin-33 *ex vivo* https://bit.ly/3wuXMit

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asthma phenotypes [7]. Circulating "trigger happy" eosinophils could arise from such a process. Nonetheless, a second competing or complementary model would be that eosinophils see their function plastically "tailored" by the local lung tissue environment, which could increase eosinophil recruitment and shape the function of invading eosinophils from mere bystanders to direct actors of pathological manifestations [7]. Patients with severe eosinophilic asthma who develop eosinophilic exacerbations in the MEX study displayed, on average, more elevated exhaled nitric oxide fraction (F_{ENO}). It is also remarkable that patients treated by anti-IL5 biologicals generally also retain high F_{ENO} levels outside exacerbation periods, despite a sharp reduction in the magnitude of airway eosinophilic inflammation [8]. This suggests some patients harbour elevated type 2 immune activation, which could locally promote eosinophil recruitment and activation.

Altogether, our study and the comments of S. Couillard highlight the remaining uncertainties about the mechanisms of action of precision therapeutics for asthma, and the continued need for translational research to better understand the contribution of eosinophils, and IL-5, to pathological manifestations in eosinophil-associated diseases.

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References

1 Van Hulst G, Jorssen J, Jacobs N, *et al.* Anti-IL-5 mepolizumab minimally influences residual blood eosinophils in severe asthma. *Eur Respir J* 2021; 59: 2100935.
2 Seki Y, Inoue H, Nagata N, *et al.* SOCS-3 regulates onset and maintenance of TH2-mediated allergic responses. *Nat Med* 2003; 9: 1047–1054.
3 Hao K, Bossé Y, Nickle DC, *et al.* Lung eQTLs to help reveal the molecular underpinnings of asthma. *PLOS Genet* 2012; 8: e1003029.
4 McDowell PJ, Diver S, Yang F, *et al.* The inflammatory profile of exacerbations in patients with severe refractory eosinophilic asthma receiving mepolizumab (the MEX study): a prospective observational study. *Lancet Respir Med* 2021; 9: 1174–1184.
5 Rottenberg M, Carow B. SOCS3, a major regulator of infection and inflammation. *Front Immunol* 2014; 5: 58.
6 Hammad H, Lambrecht BN. The basic immunology of asthma. *Cell* 2021; 184: 1469–1485.
7 Van Hulst G, Bureau F, Desmet CJ. Eosinophils as drivers of severe eosinophilic asthma: endotypes or plasticity? *Int J Mol Sci* 2021; 22: 10150.
8 Schleich F, Graff S, Nekoee H, *et al.* Real-world experience with mepolizumab: does it deliver what it has promised? *Clin Exp Allergy* 2020; 50: 687–695.