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## Polyfunctionality of bona fide resident lung CD69 + natural killer cells

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with the correlation of total IL-18 and macrophage activation syndrome (MAS)-prone conditions, they found elevated free IL-18 specifically in patients with Still's disease, and also observed a higher IL-18/IL-18BP affinity than previously described. Further investigation is warranted to better understand discrepancies between calculated and measured free IL-18, and appropriate caution should be taken in the interpretation of calculated free IL-18 until the aforementioned assumptions have been addressed.

This report provides the first clinical clues that the association between free IL-18 and MAS is no mere epiphenomenon. Specifically, the magnitude and chronicity of elevated IL-18 in NLR4-related MAS (and in most systemic juvenile idiopathic arthritis<sup>5</sup>) might suggest a "priming" effect for exuberant MAS-like responses. However, this patient's rapid improvement also implicates a role for IL-18 in propagating inflammation. In addition, the patient's enterocolitis was associated with substantially more free IL-18 in stool than in serum, suggesting less buffering by IL-18BP at mucosal surfaces. Overall, the important work initiated nearly 2 decades ago in describing the endogenous regulation of IL-18 continues to undergo scientific, and now clinical, refinement as we better understand the homeostatic and pathogenic functions of this cytokine.

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## Polyfunctionality of *bona fide* resident lung CD69<sup>+</sup> natural killer cells



To the Editor:

We read with great interest the recent article from Marquardt et al<sup>1</sup> dedicated to the detailed characterization of human lung natural killer (NK) cells. On the basis of the expression of CD69, they nicely distinguished *bona fide* resident CD69<sup>+</sup> from circulating CD69<sup>-</sup> CD56<sup>dim</sup> NK cells in a large cohort of patients undergoing pulmonary lobectomy. They have shown that NK cells in the lung are highly differentiated and predominantly circulating ones, which contrasts with lung CD3<sup>+</sup> T cells.

Although we do appreciate their comprehensive work, we would like to express 2 comments and queries.

In accordance with some of our previous works, the authors reported that NK cells were hypofunctional:

- In patients with lung carcinoma, we analyzed the phenotype and functions of lung NK cells and observed that the intratumoral NK cells were significantly hypofunctional and more commonly CD69 positive as compared with other NK cell populations.<sup>2</sup> In light with Marquardt et al,<sup>1</sup> we could then suggest that most of these intratumoral NK cells are resident.
- In patients with antisynthetase syndrome (aSS)—a rare autoimmune connective tissue disease characterized with interstitial lung disease (ILD) in the foreground<sup>3</sup>—peripheral blood NK cells are highly differentiated and hypofunctional as well, irrespective of the IL-15 serum titers.<sup>4,5</sup> Immunohistological studies in the lungs showed a massive infiltration of NK cells.<sup>4</sup> In addition, we recently observed in a 39-year-old woman with active aSS that the proportion of CD69<sup>+</sup> CD3<sup>-</sup> CD56<sup>+</sup> NK cells was higher in bronchoalveolar lavage as compared with blood (37% vs 16%, respectively). In respect with Marquardt et al,<sup>1</sup> this would suggest that NK cell infiltration in aSS-related-ILD mainly consists of resident NK cells.

We thus regret that the authors studied lung NK cell functions as a whole, regardless of their status of circulating or resident lung NK cells. We would be very interesting in knowing whether the authors showed any differences in polyfunction assays, according to the CD69 expression of the lung NK cells. Furthermore, hypofunctionality of lung NK cells was determined after *in vitro* stimulation with nonspecific stimuli, including K562 target cells and phorbol 12-myristate 13-acetate and ionomycin or IFN- $\alpha$ . We think it would be of interest to evaluate the proliferation and polyfunctional capacities of both CD69<sup>-</sup> and CD69<sup>+</sup> lung NK cells, following IL-15 stimulation. Indeed, IL-15 is locally produced within the lung<sup>1,6</sup> and involved in NK cell regulation (differentiation and functionality).<sup>7</sup>

We think that such complementary results might expand our knowledge about the distinct role of circulating and resident lung NK cells. Furthermore, these comprehensive data might help in understanding the involvement of NK cells in different diseases commonly affecting the lung, including cancer and ILD, and thus could lead to the development of new lung-targeted therapeutic strategies.<sup>7</sup>

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## Reply



To the Editor:

We thank Hervier et al<sup>1</sup> for providing comments on our article<sup>2</sup> characterizing natural killer (NK) cells in the human lung. Based on analysis of CD69 expression, our results suggest that most CD56<sup>bright</sup> NK cells are tissue-resident, whereas most CD56<sup>dim</sup> NK cells in the lung circulate.<sup>2</sup> Our results also indicate that the total lung NK cell population is largely hypofunctional. Hervier et al<sup>1</sup> regret the fact that we studied NK cell functionality only as a whole, that is, regardless of the cells' status as tissue-resident (CD69-expressing) or circulating.<sup>1</sup> Furthermore, they express that it also would have been of interest to study NK cell function following stimulation with IL-15, which is locally produced in lung. We believe that these are important questions worth considering in more detail.

In our study, we showed that both CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells in lung tissue are hypofunctional compared with the corresponding subsets in peripheral blood. Sorted CD56<sup>bright</sup> lung NK cells produced less TNF and GM-CSF after stimulation with PMA/ionomycin. Similarly, sorted CD56<sup>dim</sup> NK cells responded less well in response to stimulation with K562 cells and rituximab-coated 721.221 cells, as measured with assays for both degranulation and production of TNF. Overall, our results showed that lung CD56<sup>dim</sup> and CD56<sup>bright</sup> NK cells are hyporesponsive to several distinct stimuli and by multiple functional

readouts.<sup>2</sup> Given that most CD56<sup>bright</sup> NK cells express CD69, our results indicate that the lung-resident NK cells are also hyporesponsive. In preliminary experiments, lung NK cells responded to IL-15 by upregulation of Ki67, a marker of proliferation, and increased expression of granzyme B (unpublished data, Marquardt et al, 2017). However, more extensive comparative studies are required to draw more firm conclusions whether there are distinct effects of IL-15 on tissue-resident NK cells.

Several recent studies of tissue-resident lymphocytes, including NK cells, have revealed that there is considerable phenotypic and functional heterogeneity among tissue-resident lymphocyte subsets.<sup>3</sup> In addition to CD69, tissue-resident lymphocytes can be characterized by the expression of adhesion molecules, for example, CD49a and CD103. These molecules facilitate retention in specific parts of tissues. For example, CD103 binds to E-cadherin, which is predominantly expressed on epithelial cells and, as such, identifies intraepithelial tissue-resident lymphocytes.<sup>4,5</sup> Although the expression of CD69 on NK cells in tissues provides a good indication of tissue-residency, CD69 is also upregulated upon NK cell activation. Therefore, analysis of CD69 in combination with other markers of tissue-residency, for example, CD103 and CD49a, would be desirable to further dissect the characteristics of *bona-fide* human lung-resident NK cells.

The recent findings by Hervier et al of increased frequencies of NKp46<sup>+</sup> cells in lungs of patients with antisynthetase syndrome,<sup>6</sup> as well as reports on increased CD69 expression on intratumoral NK cells,<sup>7</sup> further highlight the need for more detailed studies of human lung-resident NK cells, in health and various settings of human disease. Indeed, a better understanding of these cells could lead to improved insights into disease pathogenesis, as well as promoting the identification of new drug targets or diagnostic tools in lung-associated conditions.

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