Interleukin 27 in bronchoalveolar lavage fluid in patients with non–small cell lung cancer

To the Editor With great interest, we read the article by Kopiński et al[1] on increased levels of interleukin 27 (IL-27) in patients with early clinical stages of non–small cell lung cancer. We would like to ask several questions. First, the authors described that the middle lobe of the right lung (or alternatively the lingual of the left lung) was lavaged. On the other hand, they described that bronchoalveolar lavage (BAL) was performed as part of a routine diagnostic workup of a peripheral solitary pulmonary lesion. We would like to know whether patients with lung cancer had the primary lesion in the middle lobe or the lingual of the left lung, or perhaps a different site than the middle lobe was lavaged? Second, idiopathic pulmonary fibrosis (IPF) is a disease with a possible association with smoking,[2] and IL-27 levels in this study were measured only in nonsmoking patients with IPF.[1] We would like to know whether this was appropriate as data representative of patients with IPF. Third, what is the clinical significance of high levels of IL-27 in BAL fluid? Did high IL-27 levels have pathological meanings, not through the bloodstream but via the respiratory tract? Do the high levels of IL-27 have the same pathological significance in lung cancer and nonmalignant respiratory diseases? Fourth, it is interesting that the range of high and low IL-27 levels in BAL fluid was broad in patients with lung cancer. What is the authors' opinion on this? Is there a possibility that angiogenesis was pathologically extensive in lung cancer patients with high IL-27 levels in BAL fluid?

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Authors' reply We thank Dr. Satoh et al for their interest in our manuscript and for their insightful comments. Below we respond to all questions listed in their letter.

First, we completely agree that the lavaged lobe(s) should have been identified in the Methods section. Indeed, in all subjects with suspected interstitial lung disease, including controls, the right middle lobe/lingula was lavaged. However, in patients with lung cancer, bronchoalveolar lavage (BAL) was always performed in the direct vicinity of the tumor. Consequently, there were 4 patients lavaged in the right upper lobe; 3, in the right middle lobe; 2, in the right lower lobe; 5, in the left upper lobe, and 2, in the lingual. We are very sorry for this oversight.

Second, as the authors correctly emphasized, our study group included only nonsmoking patients with idiopathic pulmonary fibrosis (IPF). This was clearly mentioned in the Patients and methods and Results sections. Therefore, our results and conclusions refer to this group only

and are in no way representative of smokers with IPF. IPF is indeed more common in smokers. Yet, paradoxically bronchoalveolar lavage (BAL) fluid is difficult to obtain in this subgroup, as many smokers with IPF have contraindications to BAL. An overlap between the deleterious impact of smoking (as reflected by reduced forced expiratory volume in 1 second [FEV_1]) and other respiratory pathologies (eg, hypoxia due to IPF) precludes BAL in a number of patients with IPF. Low or unsatisfactory lavage representativeness (eg, BAL fluid recovery <30% of instilled volume, epithelial cell contamination >5%, etc) for the lower airways should also be considered.[1-3] Finally, we assessed interleukin (IL)-27 concentrations in BAL fluid, but from 2 smokers with IPF (15.7 and 0 pg/ml in BAL supernatant) only. This number is too low to allow any conclusions or comparisons.

Third, our opinion on the role of IL-27 in local immunity in non–small cell lung cancer (NSCLC) and ILD, was presented in the manuscript, though briefly due to word count limits. As indicated in the literature, IL-27 plays an important role in maintaining respiratory system homeostasis. Due to its versatile effect, particularly on T-lymphocyte subsets, macrophages, and airway epithelial cells, it regulates numerous pathological processes within the respiratory tract. It has been shown that IL-27 expression significantly affects, not necessarily in a positive way, the clinical course of tuberculosis, atopic bronchial asthma, chronic obstructive pulmonary disease, malignancies, and other inflammatory reactions in the lungs.

Some authors suggested a significant negative effect of IL-27 on immune response against *Mycobacterium tuberculosis*.[4-6] In WSX-1–deficient subjects with impaired IL-27–dependent signaling, mycobacteria are more susceptible to phagolysosomal fusion in macrophages and are eliminated relatively fast.[7,8] Holscher et al[9] proved that mycobacteria elimination from infected macrophages in WSX-1–deficient subjects depends on increased secretion of proinflammatory cytokines—tumor necrosis factor (TNF) and IL-12p40 subunits—with subsequent Th-dependent interferon-γ (IFN-γ) production. In fact, they demonstrated the bidirectional activity of IL-27 in active tuberculosis: the WSX-1/IL-27 axis limits the immune reaction in tuberculosis and facilitates bacilli survival, and on the other hand, IL-27 reduces TNF and IL-12 release by activated macrophages and prevents extensive inflammatory response.[4,9] Another possible mechanism, namely, secretion of IL-10 in $CD4^+$ T cells, has been shown by Moreira-Teixeira et al.[10] Moreover, in tuberculosis-infected macrophages, IL-27 inhibits lysosomal acidification and phagolysosomal function (affecting numerous proteins: CD63, V-ATPase, and cathepsin D). As a result, IL-27 might support prolonged mycobacteria survival in macrophage-immature lysosomes.[5,11,12]

Yet, changes in local IL-27 concentrations significantly affect the spectrum of its biological effects: Zhang et al[13] showed that high IL-27 levels stimulate T cells, significantly promoting efficient mycobacteria elimination. Likewise, Ye et al[14] suggested that pleura-derived IL-27 affects mesothelial cells inducing their proliferation and stimulating local wound healing processes.

A number of in vivo studies have provided data on the IL-27/WSX-1 axis involvement in bronchial asthma. WSX-1–deficient mice with experimentally induced asthma demonstrated airway hyperresponsiveness, increased lung eosinophil migration, and overproduction of immunoglobulin, as compared with control animals with normal IL-27 receptor signalling. Th2-related cytokine secretion in WSX-1–deficient mice was also increased. It was suggested that IL-27 exerts an inhibitory effect on Th2 cells and therefore might protect against asthma.[15] On the other hand, IL-27 is implicated in airway hyperresponsiveness in patients with severe asthma. IL-27 synergizes with IFN-γ, affecting directly lung macrophages in a MyD88-dependent mechanism.[16] Additionally, it was demonstrated that respiratory syncytial virus-induced asthma exacerbation could be suppressed by neutralizing IL-27 and IFN-γ.[17]

In chronic obstructive pulmonary disease (COPD), Cao et al[18] observed the elevated IL-27 levels in the airways and implicated a number of cellular subsets as its source. Also, a negative correlation between IL-27 concentrations in sputum and FEV_1 seems to confirm its impact on COPD severity. According to Qiu et al,[19] high IL-27 expression in COPD is associated with both Th1 and Th17 cell activity, while its neutralization improves the clinical course of disease. IL-27 was also shown to induce the CXCL10 expression on lung fibroblasts and epithelial cells and therefore promote immune cell migration into the sites of inflammation, lymphoid follicle formation, and steroid resistance.[18,20,21] In secondary bacterial pneumonia and acute respiratory distress syndrome (ARDS), IL-27 levels negatively correlate with clinical outcome.[22] In ARDS, the ability of IL-27 to induce and intensify the inflammatory response seems particularly deteriorating,[23] while its deficit favors severe lung inflammation in experimental animals.[24] It has also been suggested that high IL-27 levels suppress IL-17 and increase susceptibility to bacterial pneumonia, as exemplified by a significantly increased risk of secondary respiratory infections (*Staphylococcus aureus, Streptococcus pneumoniae)* in the course of influenza.[25,26] In conclusion, we strongly believe that the biological effect of IL-27 is very much context dependent.

Finally, Dr Satoh et al very precisely indicated the wide range of IL-27 concentrations in BAL fluid from patients with NSCLC. This observation should not be surprising as, per definition, NSCLC is a rather heterogeneous group of lung cancers. Moreover, cytological, immunological, and biochemical variables of BAL fluid generally show non–Gaussian distribution. Relative neutrophil and eosinophil counts in BAL fluid are characterized by Poisson distribution, unlike in

peripheral blood.[27-30] This is in line with our data demonstrating a wide range of IL-27 concentrations in BAL fluid, some below the lower limit of detection.

A surprising observation that we were unable to adequately explain and therefore did not include it in the original manuscript is that alveolar lymphocytes positive for intracellular IL-27 were found in BAL fluid from all study participants, while no IL-27$^+$ lymphocytes in peripheral blood were ever observed. We believe that this finding provides yet another argument for the concept that BAL lymphocytes are indeed a local source of IL-27.

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