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Letter to the Editor

Clinical significance of invariant natural killer T cells and IL-5 in acute eosinophilic pneumonia

Dear Editor,

Acute eosinophilic pneumonia (AEP) is an allergic lung inflammation associated with acute fever, severe hypoxemia, diffuse pulmonary infiltration, and eosinophilia in BAL fluid. Although it is not known whether the pathogenesis of AEP differs from that of other eosinophilic lung diseases, such as chronic eosinophilic pneumonia (CEP), patients with AEP tend to experience more severe and more rapidly progressing symptoms, including fever, shortness of breath, and chest pain, as compared with patients with CEP. Preceding exposure to tobacco smoke or other inhaled substances has been suggested to contribute to the development of AEP but not CEP.[1]

Th2 lymphocytes and Th2 cytokines, such as IL-4, IL-5, and IL-13, have well-characterized roles in allergic inflammatory responses. However, the mechanisms by which inflammatory cells and cytokines promote eosinophilic inflammation, especially in AEP, are still obscure. A previous study suggested that natural killer (NK) cells and NK T (NKT) cells may also regulate eosinophilic pneumonias.[2] Although that study did not differentiate between patients with AEP and CEP, the data were interesting because they showed that a subset of invariant NKT (iNKT) cells, which use a limited number of T-cell receptor (TCR) Vα and Vβ chains, can affect the Th cell polarization early in an immune response and induce a Th2-biased response.[3] Here, we aimed to determine whether iNKT cells could contribute to the pathogenesis of AEP.

Between June 2000 and May 2020, we recruited 14 patients with AEP, 40 patients with CEP, and 14 healthy volunteers to participate in this study (Supplementary Table 1). This study was approved by the Research Ethics Committee of the University of Tsukuba Hospital (approval code H24-140). All subjects provided informed consent in accordance with institutional guidelines and the Declaration of Helsinki. The 14 patients with AEP included 4 men and 10 women aged 15–31 years, of whom 11 were current smokers and 3 were never-smokers. AEP was diagnosed on the basis of typical clinical presentation, i.e., acute fever with shortness of breath of less than 2 weeks' duration, diffuse pulmonary infiltrates on chest radiographs, and the presence of eosinophils as more than 25% of total leukocytes in the BAL fluid. The 40 CEP patients included 23 men and 17 women aged 31–83 years, of whom 10 were current smokers, 11 were ex-smokers, and 19 were never-smokers. CEP was diagnosed on the basis of chronic pneumonia with symptoms of slight fever and shortness of breath persisting over several weeks; bilateral pulmonary infiltrates, with peripheral dominant distribution; and alveolar eosinophilia as for AEP. The 14 healthy subjects included 11 men and 3 women aged 16–38 years, of whom 4 were current smokers and 10 were never-smokers. Methodological details can be found in the Supplementary Methods.

The eosinophil numbers in BAL fluid were significantly higher in both AEP and CEP patients as compared with healthy subjects, but there was no significant difference between the two eosinophilic pneumonia groups (Supplementary Table 1). The percentage and number of iNKT cells (defined as TCRVα24$^+$Vβ11$^+$) in BAL fluid were markedly higher for AEP patients compared with CEP patients or healthy controls (Fig. 1A, B), whereas total Th cells (CD3$^+$CD4$^+$) did not differ among these groups. Regarding the effect of smoking on the alveolar infiltration of iNKT cells, we found that the numbers of iNKT cells in BAL fluid were not affected by smoking status (current smoker vs. non- or ex-smoker) in any groups: AEP (134.2 ± 36.1 vs. 74.9 ± 58.0; $p = 0.44$), CEP (3.8 ± 1.3 vs. 7.7 ± 1.5; $p = 0.18$), and healthy control (1.5 ± 0.7 vs. 1.8 ± 0.4; $p = 0.69$). The level of IL-5, but not of IL-4 or IL-13, in BAL fluid was significantly higher in AEP patients compared with CEP patients or healthy subjects (Fig. 2A). Furthermore, quantitative reverse-transcription PCR analysis demonstrated that IL-5 mRNA levels were significantly higher in iNKT cells purified from the BAL fluid of AEP patients compared with CEP patients or healthy subjects, whereas the IL-5 mRNA levels in Th cells were comparable (Fig. 2B).

One striking finding was the marked elevation of iNKT cells in the lungs of AEP, but not CEP patients, despite comparable numbers of eosinophils. iNKT cells are a unique lymphoid subset distinct from either NK or T cells. In addition to expressing a very limited repertoire of TCR α and β chains, the cells recognize glycolipid antigens in a CD1d-restricted manner. Despite their low abundance compared with conventional T cells, iNKTs can rapidly secrete large amounts of cytokines, including IL-5, upon stimulation.[3] Thus, it is reasonable to consider that iNKT cells may play a central role in promoting eosinophilic pneumonia, as well as asthma.[4] The reason why iNKT cells are preferentially enriched in the lungs of patients with AEP remains unclear, but there are several possible explanations. First, iNKT cells can respond more rapidly than other cells during an immune response,[3] which is consistent with a role in an acute stage of eosinophilic inflammation. Second, despite our data showing that the number of iNKT cells was not affected by the patients' current smoking status, there remains a possibility that iNKT cells could be activated by oxidative damage resulting from smoke exposure, as indicated previously.[5] Finally, the pathogenic antigenic stimuli for immune cells, including iNKT cells, in

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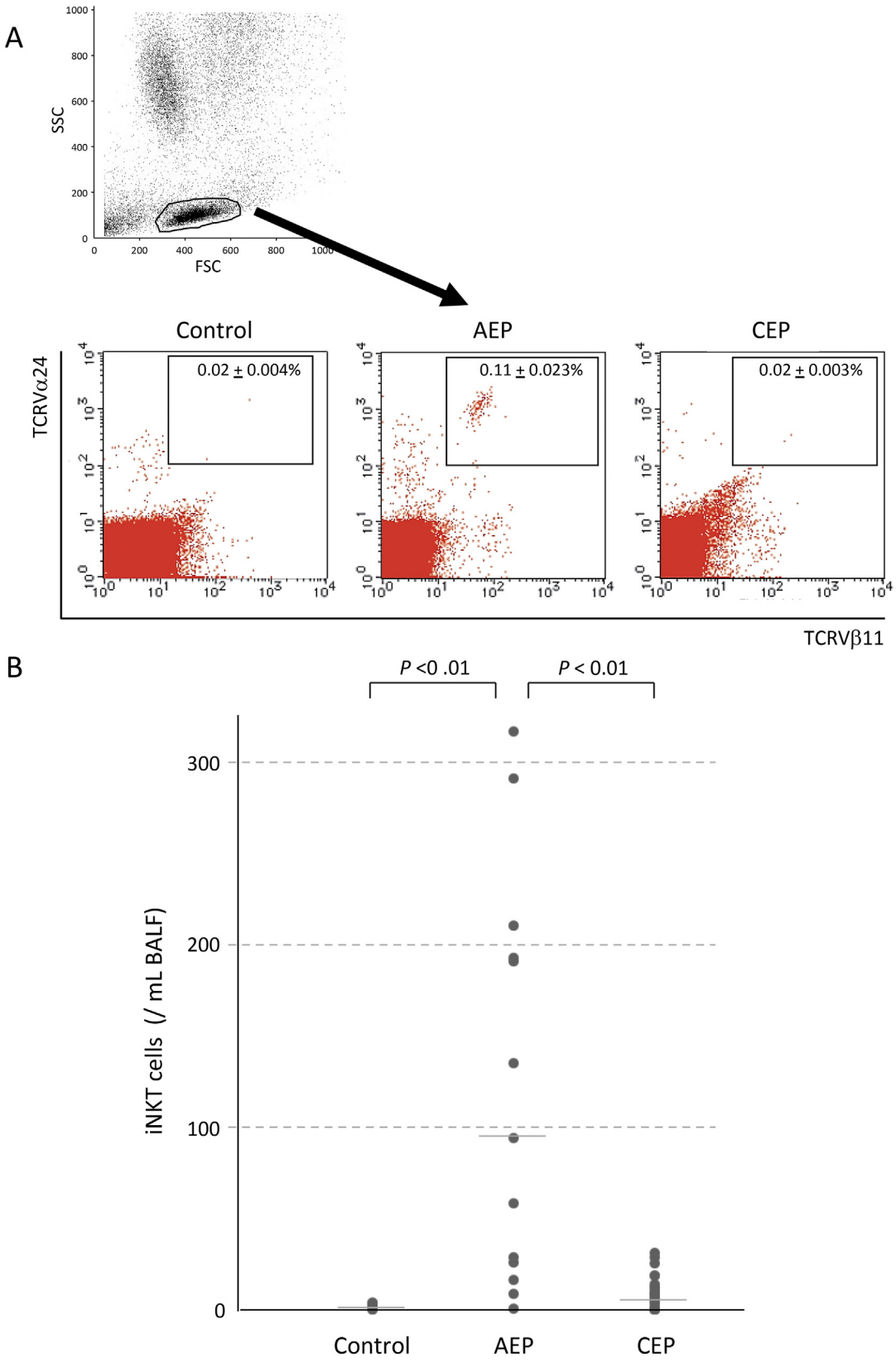

Fig. 1. Representative flow cytometric profiles and percentages (**A**), and the number of TCRVα24^{+}Vβ11^{+} invariant natural killer T (iNKT) cells (**B**) among lymphocytes in BAL fluid from healthy subjects or patients with acute eosinophilic pneumonia (AEP) or chronic eosinophilic pneumonia (CEP) (n = 14 healthy subjects, n = 14 AEP patients, and n = 40 CEP patients). Results are shown as the mean ± SEM.

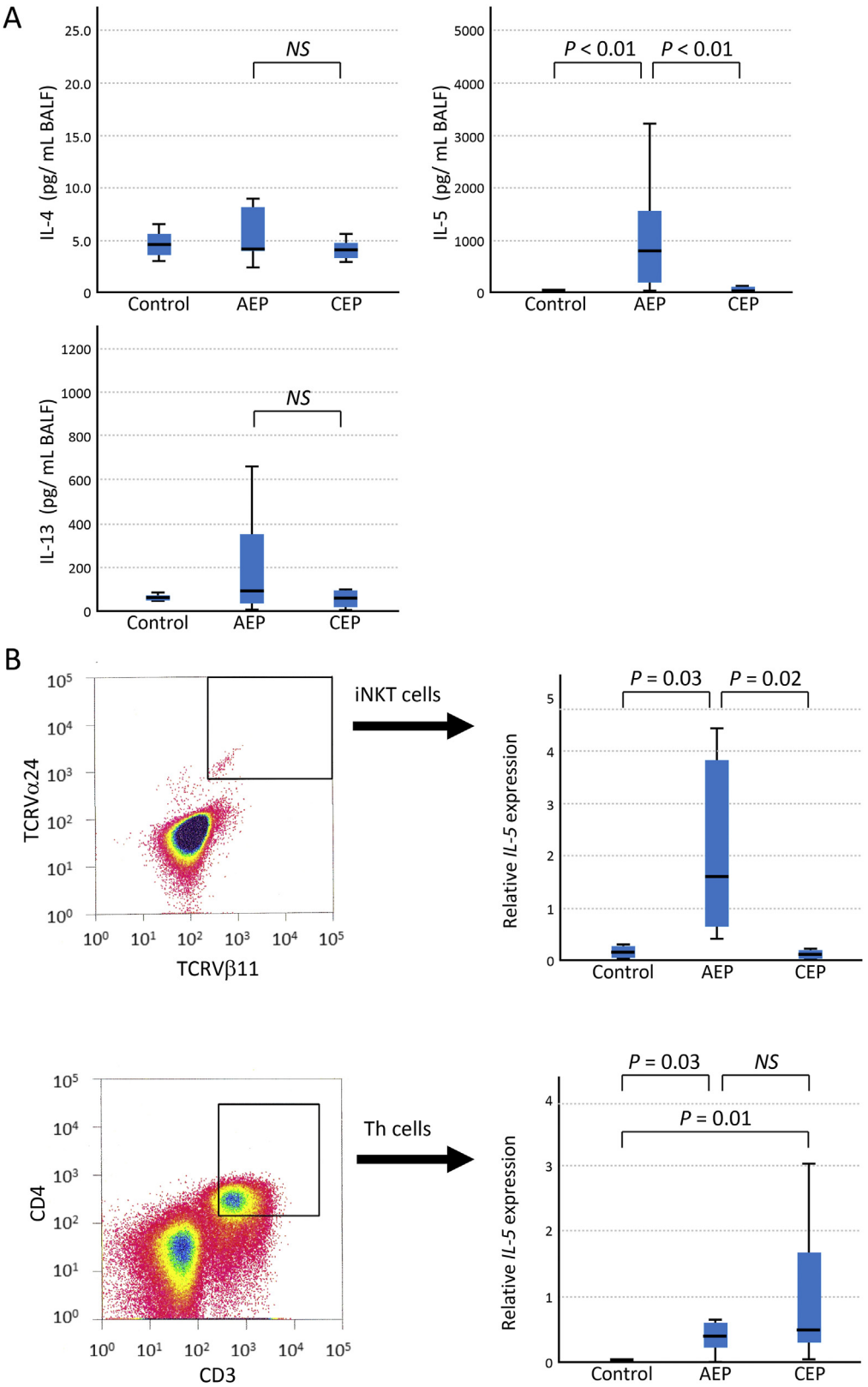

Fig. 2. (**A**) IL-4, IL-5, and IL-13 concentrations in BAL fluid from healthy subjects and patients with acute eosinophilic pneumonia (AEP) or chronic eosinophilic pneumonia (CEP) (n = 14 healthy subjects, n = 12 AEP patients, n = 17 CEP patients). (**B**) IL-5 mRNA levels in invariant natural killer T (iNKT) and T helper cells sorted from the BAL fluid of the three groups (n = 5 healthy subjects, n = 7 AEP patients, n = 6 CEP patients). Results are shown as the mean ± SEM. NS, not significant.

AEP may differ from those in CEP[6]; thus, they may each promote eosinophilic inflammation by altering different immune pathways.

Another interesting finding here was that IL-5, but not other Th2 cytokines, was significantly elevated in the BAL fluid from AEP patients but not CEP patients. IL-5 is a major Th2 cytokine, with roles in stimulating eosinophil proliferation, maturation, migration, and activation, as well as in facilitating allergic reactions in concert with other mediators. Our result confirms those of previous reports examining IL-5, IL-4, and IL-13 levels in BAL fluid from patients with AEP and CEP.[7,8] Moreover, IL-5 levels in BAL fluid have been reported to decrease in parallel with the clinical improvement of AEP patients.[9] The finding that IL-5 mRNA was elevated in iNKT cells, but not Th cells, points to iNKT cells as a potential source of IL-5 in AEP. One limitation of the present study is its relatively small sample size, especially for the ELISA and real-time PCR analyses, owing to the rarity of AEP and the retrospective nature of our study. To better understand the shared and distinct mechanisms of AEP and CEP, further investigations with more samples and analyses of other mediators, including cytokines (such as IL-25, IL-33, and TSLP), chemokines, lipid mediators, damage-associated molecular pattern molecules, and extracellular matrix proteins, all of which are linked to eosinophil accumulation and activation,[10] are required. Examining the different cell types, such as ILC2, that secrete IL-5 might also provide valuable information for unraveling the complex pathogenesis of AEP. Nevertheless, our findings support a continued focus on iNKT cells, as they may play a pathogenic role in AEP through novel mechanisms of action.

In conclusion, our results provide the first evidence that iNKT cells are a potential source of IL-5 and may play a crucial role in orchestrating eosinophilic inflammatory responses in AEP.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2020.09.013.

Conflict of interest

The authors have no conflict of interest to declare.

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