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Enhanced frequency of immunoregulatory invariant natural killer T cells in the airways of children with asthma

To the Editor:

Asthma is a disease characterized by eosinophil-rich airway inflammation, bronchospasm, and airway hyper-reactivity, which has recently increased dramatically in prevalence in the industrialized world.¹ In the past few decades, it has become clear that both pathogenesis and severity of asthma are mediated through T_H2 lymphocytes.² Recently, we and others have demonstrated that a new population of regulatory T cell, the Cluster of Differentiation 1d (CD1d)-restricted invariant natural killer T (iNKT) cell, plays a major role in the severity of experimental allergic asthma by enhancing harmful T_H2 immune responses.^{3,4} iNKT cells constitute a distinctive T-lymphocyte population that recognizes lipids presented by the nonpolymorphic MHC class I-like molecule, CD1d.^{5,6} They coexpress a highly restricted T-cell receptor (TCR) repertoire, composed of a single invariant V α 24J α 18 chain in humans, preferentially paired with a limited TCR V β chain repertoire.^{5,6} Another major feature of this T-cell population is its capacity to generate promptly a large range of immunomodulatory cytokines such as IL-4, IL-13, and IFN- γ .^{5,6} The influence of iNKT cells in the severity of experimental allergic asthma is presently well documented.^{3,4} However, it is still unclear whether iNKT cells influence the pathology of the human disease. One of the problems encountered in human studies comes from the difficulty to study iNKT cells locally at the site of the immune reaction rather than in peripheral blood, which is frequently the only material available. Yet, in atopic asthma, it is of foremost importance that the regulatory functions of these cells should be analyzed in the airways, where the symptoms do actually occur. Knowing that several key events that contribute to the development of allergic asthma take place in the early childhood,⁷ we analyzed here the frequency of iNKT cells in the airways of children suffering from T_H2 cell-mediated atopic asthma.

We examined 15 children with severe asthma, 12 children with airway infections (bronchiectasis), and 10

children with no obvious endobronchial inflammation or atopy (these controls are subjects with suspicion of foreign body in the airways). The children were recruited from the Necker Enfants Malades Hospital in Paris, France. Subject characteristics are outlined as follows: mean age of 4, 7, and 4 years (ranging from 6 months to 12 years) respectively, for asthma, infection, and control groups made up of female and male subjects. Severe asthma was treated according to the Global Initiative for Asthma/American Thoracic Society criteria, and all patients underwent computed tomography scan and bronchoscopy as part of their clinical work-up. The local ethical committee approved the study, and the parents gave written informed consent.

Bronchoscopy was performed under general anesthesia as described previously.⁷ Bronchoalveolar lavage fluid (BALF) was filtered on 100- μ m cell strainers to exclude debris. One aliquot was used for microbiologic evaluation. The number of total cells in the BALF was $100 \times 10^3 \pm 50 \times 10^3$, $980 \times 10^3 \pm 157 \times 10^3$, and $227 \times 10^3 \pm 41 \times 10^3$ per mL from asthma, infection, and control groups, respectively. To identify iNKT lymphocytes among the cells from BALF, we incubated them with α -galactosylceramide (α -GalCer) loaded CD1d/ β 2m tetramer-allophycocyanin (which specifically binds to the invariant V α 24J α 18 TCR of iNKT cells) or control (CD1d-unloaded or mock tetramers), as described.³ After washing, cells were further incubated with appropriate dilutions of anti-CD4-phycoerythrin, anti-TCR $\alpha\beta$ fluorescein isothiocyanate, and anti-CD8-phycoerythrin (from Immunotools, Germany). The cells were washed and analyzed on a FACScalibur (Becton Dickinson, Le Pont de Claix, France) using CellQuest software (Becton Dickinson). The percentage of the different T-cell subsets in BALF was evaluated in the lymphocyte gate according to forward-scatter/side-scatter characteristics. This lymphocyte gate represents $32\% \pm 3\%$, $22\% \pm 2\%$, and $29\% \pm 3\%$ of total BALF cells from asthma, infection, and control groups, respectively. Data are given as mean values $\pm$ SEM. The Mann-Whitney *U* test was used to establish statistical significance between patient groups.

Analysis of T-cell populations in BALF revealed a similar incidence of TCR $\alpha\beta$ ⁺ cells among children with asthma ($38\% \pm 9.9\%$) and controls ($43.2\% \pm 13.7\%$), whereas these cells were decreased in children with infections ($18.8\% \pm 3.6\%$). These findings suggest that the inflammation occurring in both pathologies could be mediated through distinct mechanisms resulting in the different recruitment of T cells to the airways.

We found a similar percentage of CD4⁺ cells among gated BALF TCR $\alpha\beta$ ⁺ lymphocytes, whether they were recovered from patients of the asthma ($35\% \pm 3\%$), the infected ($39\% \pm 4\%$), or the control group ($34\% \pm 3.5\%$). CD8⁺ cells were the predominant T-cell population in the BALF of patients with asthma ($59\% \pm 5\%$) as well as in the other 2 groups ($52\% \pm 5\%$ and $63\% \pm 4\%$ for infected and control group, respectively).

This is the first evidence for the presence of iNKT cells in BALF of children with asthma (Fig 1) using specific detection by the CD1d/ α -GalCer tetramers. Their

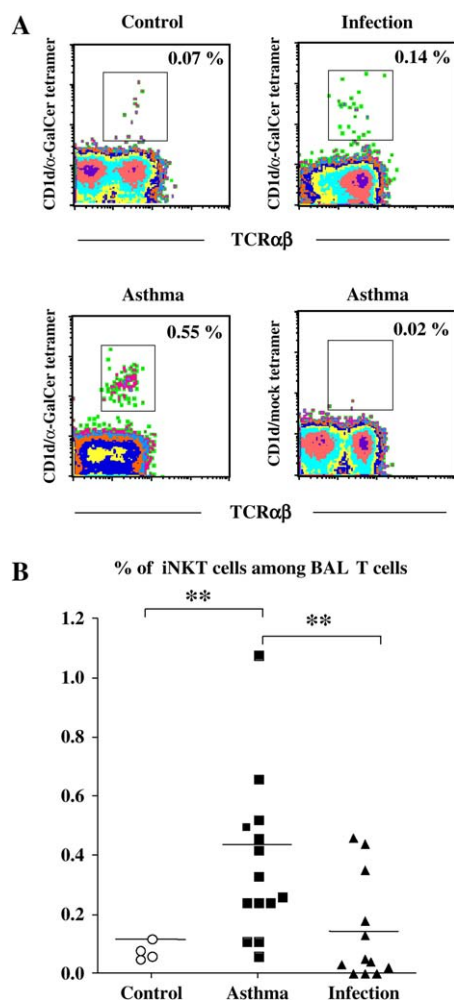


FIG 1. A, Representative fluorescence-activated cell sorting plots compare the frequency of CD1d/α-GalCer tetramer⁺ cells among gated TCRαβ⁺ lymphocytes from BALF of children with asthma, infections, or nonatopic controls. **B**, Percentages of CD1d/α-GalCer tetramer⁺ iNKT cells among gated TCRαβ⁺ BALF T cells from controls or patients with asthma or infections. The mean frequencies are represented by a horizontal bar. ***P* < .01.

frequency among T cells was significantly higher in BALF of patients with asthma ($0.435\% \pm 0.09\%$, mean $\pm$ SEM) than in nonatopic controls ($0.116\% \pm 0.03\%$, mean $\pm$ SEM; *P* < .01). This is why we addressed the question whether the enhanced frequency of iNKT in BALF of patients with asthma is a typical feature of this pathology or occurs in association with other diseases resulting in airway inflammation. The observation that iNKT cells are more frequent in BALF of patients with asthma than of infected patients ($0.142\% \pm 0.05\%$; Fig 1) argues in the favor of this specificity. Moreover, it has been reported that iNKT cells are absent in the BALF of patients with sarcoidosis, another type of lung inflammation.⁸

The limited percentage of airway iNKT cells is not surprising considering the huge diversity of TCR repertoire among BALF T cells.⁹ However, their immunoregulatory properties and their well established implication in several experimental diseases, including asthma, suggest

that their detection in the airways might provide a new means of diagnosis.

In conclusion, we demonstrate for the first time that iNKT cells are increased in BALF of children with asthma, supporting their role in the pathogenesis of the disease. We have already reported that iNKT cells enhance the severity of experimental asthma.^{3,4} Knowing that iNKT cells are decreased in the peripheral blood of atopic patients,¹⁰ it is plausible that they migrate from the periphery to inflammatory sites in the lung where, through their capacity to produce IL-4 and IL-13, they could aggravate the severity of asthma. Further studies are required to verify this hypothesis and establish their mechanism of action in human asthma.

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