

Letter to the Editor

Natural killer T cells in the lungs of patients with asthma*To the Editor:*

On the basis of studies in mice, an important role for invariant natural killer T (*i*NKT) cells in the pathogenesis of asthma has been proposed.¹⁻⁴ However, the role of *i*NKT cells in human asthma is controversial because there is disagreement on whether *i*NKT cells are present in the lungs of patients with asthma.^{5,6} To address this controversy, we examined bronchoalveolar lavage fluid (BALF) from asthmatic subjects with a broad range of severity and symptom control.

The study was approved by the Institutional Review Boards at Children's Hospital Boston and Brigham and Women's and

Connolly Hospitals. All 18 subjects provided written informed consent and underwent fiberoptic bronchoscopy. Five had severe asthma (based on the National Heart, Lung, and Blood Institute Severe Asthma Research Program criteria),⁷ 8 had well-controlled asthma (based on the National Heart, Lung, and Blood Institute Expert Panel Report 3 Guidelines), and 5 had no history of asthma (Table I). BALF samples were analyzed for the presence of *i*NKT cells by means of flow cytometry (8-Color BD FACSCanto, FlowJo Software; TreeStar, Inc, Ashland, Ore) by investigators who were blinded to clinical data at the time of the analysis. BALF cells were filtered through 70- μ m cell strainers to remove mucous and examined for viability with trypan blue (mean viability, 95%) before treatment with human IgG to block Fc receptors.

TABLE I. Subjects' demographic and clinical information

Group	Subject ID	Sex	Age (y)	FEV ₁ (%) [*]	Exacerbation [†]	Duration (y) [‡]	ICS dose [§]	Other medication	Allergies [¶]	Concomitant disease
Severe asthma	P1	Female	50	78	1	50	1000	Salmeterol, Montelukast	Cockroach	Hypertension
	P2	Male	31	56	2	25	1000	Salmeterol, Tiotropium, Montelukast	None	None
	P3	Male	50	41	2	50	1000	Salmeterol, Theophylline	Cockroach	Diabetes, Hypertension
	P4	Male	21	58	1	15	1000	Salmeterol	Cockroach, Cat, Mite	None
	P5	Male	49	73	1	40	1880	Salmeterol, Montelukast	Alternaria, Tree, Dog, Cat	Diabetes, Hypertension
	Mean		40	61	1.4	36	1176			
Well-controlled asthma	W1	Female	35	87	0	25	500	Salmeterol	None	None
	W2	Male	41	109	0	20	500	Salmeterol	None	None
	W3	Female	22	90	0	3	500	Salmeterol	Cat	None
	W4	Male	33	103	0	30	500	Salmeterol	Cockroach	None
	W5	Female	47	86	0	40	440	None	Cockroach, Rat, Mite	None
	W6	Female	56	83	1	3	800	Montelukast	None	None
	W7	Female	59	82	1	1.5	264	None	None	Hypertension, Ischemic heart disease
	W8	Female	47	99	0	2	0	None	ND	Peptic ulcer, Dilated cardiomyopathy
	Mean		43	92	0.25	16	438			
No asthma	H1	Male	43	ND	0	0	0	None	ND	Sarcoidosis
	H2	Male	39	ND	0	0	0	None	ND	Bronchiectasis
	H3	Male	49	99	0	0	0	None	ND	Peptic ulcer
	H4	Female	31	114	0	0	0	None	ND	Sarcoidosis
	H5	Female	50	89	0	0	0	None	None	None
	Mean		41	107	0	0	0			

Six other BALF samples were evaluated by using a different methodology. These samples could not be directly compared with the final 18 samples because the sensitivity for detecting natural killer T cells was not comparable. The trend of results with the 6 other samples was similar to what is reported for the 18 samples.

ND, Not done.

^{*}Percent predicted FEV₁ at the time of study entry.

[†]Number of acute asthma exacerbation requiring oral corticosteroid, an emergency department visit, or an urgent care visit in the past 12 months.

[‡]Duration that the subject has received a diagnosis of asthma.

[§]Inhaled corticosteroid dose (fluticasone or fluticasone equivalent in micrograms per day). Medications were not changed for at least 4 weeks before bronchoscopy.

^{||}Other medications used for asthma control (not including rescuer medication).

[¶]Positive response on allergen skin prick testing.

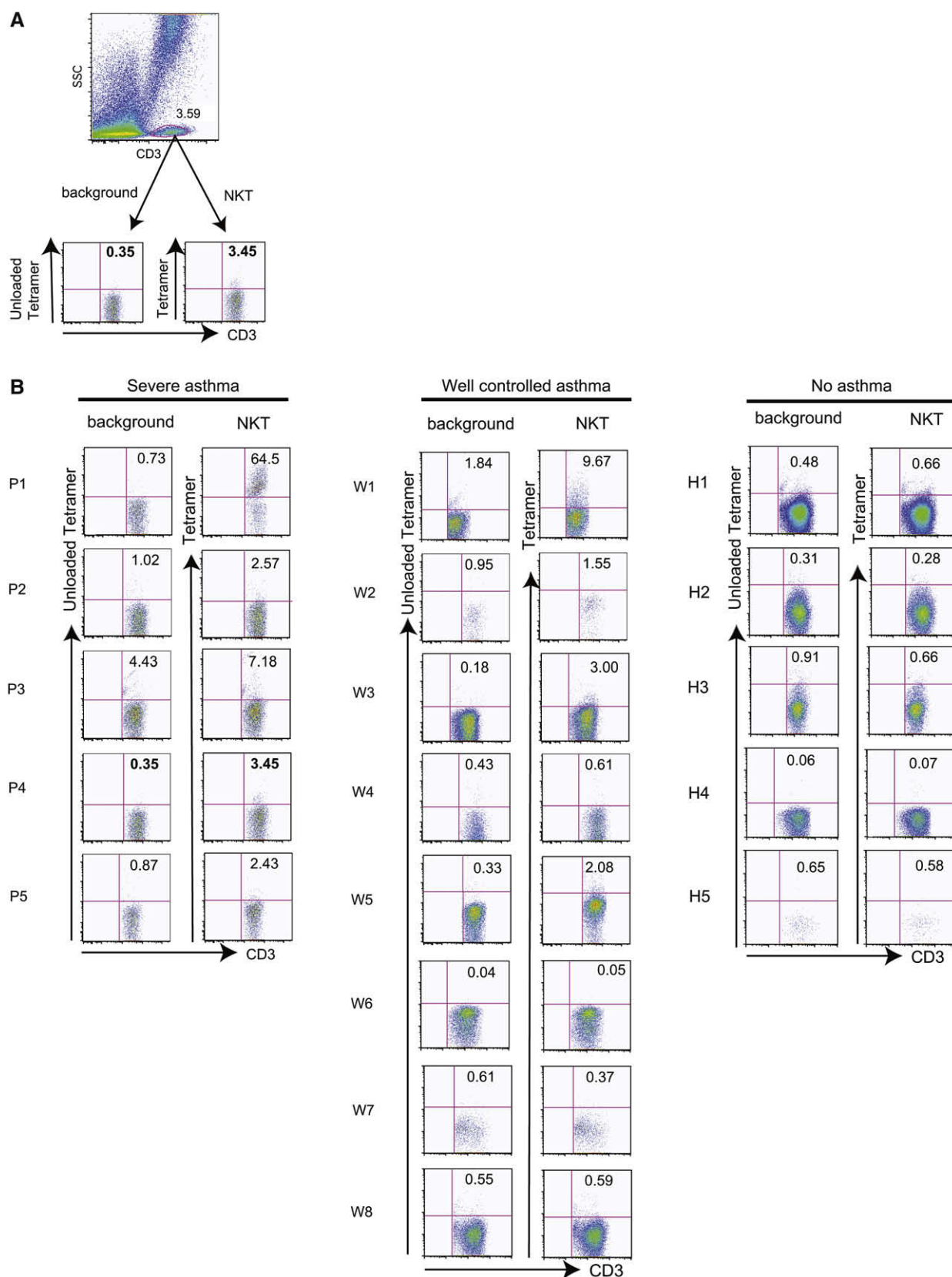


FIG 1. A, iNKT cells in BALF. We gated on CD3⁺ low side-scatter cells (T cells) and then analyzed for expression of PBS57-loaded CD1d tetramer (y-axis) versus CD3 (x-axis; iNKT, right panel of each pair) or for expression of unloaded CD1d tetramer (y-axis) versus CD3 (x-axis; background, left panel of each pair). **B**, iNKT cells and background staining for each subject categorized by asthma severity/symptom control.

The observation that *i*NKT cells are present in some patients with asthma suggests that *i*NKT cells might contribute to the pathogenesis of asthma. This question is of much greater importance but must be addressed by means of examination of *i*NKT cell function in asthma. Such functional studies are difficult to perform in human subjects for ethical and safety reasons, but studies in mice and nonhuman primates provide a very compelling argument regarding the importance of *i*NKT cells in the development of airway hyperreactivity (AHR). Thus in 3 distinct models of asthma in mice induced with allergen^{1,2} or virus⁴ or by means of exposure to ozone (a component of air pollution),³ AHR did not develop in the absence of *i*NKT cells. In each of these models, a different subset of *i*NKT cells was required for AHR. Moreover, functional studies of *i*NKT cells, performed by using direct and specific activation of *i*NKT cells in naive mice¹⁰ or in cynomolgus monkeys,¹¹ resulted in the development of AHR, suggesting that even the few *i*NKT cells present in the normal lung, when directly activated, can result in AHR. These functional studies of *i*NKT cells together suggest that *i*NKT cells might represent an important common element that links many different forms of asthma.

including allergic and nonallergic asthma. These results also suggest that the absolute number of *i*NKT cells present in the lungs might not be as important as their functional capacity when activated to contribute to the development of AHR.

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