

Correspondence

Natural killer T cells in bronchial biopsies from human allergen challenge model of allergic asthma

To the Editor:

Matangkasombut et al¹ have readdressed the debate about the role of invariant natural killer T (iNKT) cells in the lungs of patients with asthma.² Although they have addressed the issue of whether iNKT cells are present in bronchoalveolar lavage fluid, they have not looked in lung tissue itself or focused on the important issue of whether the number and percentage of iNKT cells change over time during asthma exacerbation and increased airway hyperresponsiveness (AHR). A key difference from mouse model studies showing a major role of iNKT cells is that those studies depend on synchronous disease induced by challenge. We have measured AHR by spirometry and methacholine PC₂₀ and taken bronchial biopsies at baseline, 24 hours, and 7 days after allergen challenge in patients with mild/moderate allergic asthma.³ This approach approximates the active allergen challenge used in mouse models. Our study has the advantage that it is internally controlled because we can compare allergen-challenged lung biopsies with control tissue and tissue from the same donors at baseline. Five patients with a fall in FEV₁ of >10% from baseline 24 hours after allergen challenge and 3 [F1-4/C] healthy controls were studied (Fig 1, A). We used phycoerythrin-conjugated CD1d/α-galactosylceramide (αGalCer) tetramers to stain bronchial biopsy cryosections and count CD3⁺ αGalCer-loaded CD1d tetramer⁺ cells (Fig 1, B). No sections contained any cells that were CD3⁺tetramer⁺, making it highly unlikely that this staining method detects nonspecific tetramer uptake, for example by monocytes.

At baseline, the mean number of iNKT cells/mm² of lung biopsy was 33 (range, 13-40) in patients with allergic asthma compared with none in the healthy control samples ($P < .003$; Fig 1, C). Thus, 9.8% (range, 5-20) of CD3⁺ cells were iNKT cells compared with controls, in whom no iNKT cells were identified ($P < .04$). Allergen challenges were performed. The mean fall in FEV₁ from baseline was -17.5% (± 1.6 SEM) at 24 hours ($P < 3.5 \times 10^{-6}$), and -6.6% (± 0.8 SEM) at 7 days ($P < 2.5 \times 10^{-5}$). AHR was increased at both 24 hours and 7 days after challenge compared with baseline (Fig 1, D and E). Importantly, we found that 24 hours after allergen challenge, the mean percent of iNKT cells increased to 15% (range, 7-29) at the same time as the fall in FEV₁ and increased AHR (9.8% ± 2.7 SEM) at baseline compared with 15% (± 4.1 SEM) at 1 day. The mean number of iNKT cells/mm² of lung biopsy increased from 33 (range, 13-40) to 59 (range, 8-107; 32.6 ± 5.1 SEM) at baseline compared with 59.0 (± 17.8 SEM) at 24 hours.

These findings support the hypothesis that although they constitute a minority of the CD4⁺ infiltrate, iNKT cells are present and may have a key role in allergic asthma; whether in an inflammatory or regulatory capacity remains to be determined. The absence of iNKT cells in healthy control lung biopsies, the presence of iNKT cells in patients with allergic asthma at baseline, and the increase in the number and percentage of iNKT cells with increased AHR at 24 hours are important and in line with murine studies in which AHR is abrogated in the absence of iNKT cells.⁴⁻⁶ Our study is limited by small numbers, but our results along with those of Matangkasombut et al¹ strongly suggest that further, larger studies are needed to examine how the number and function of iNKT cells contribute to AHR in allergic asthma.

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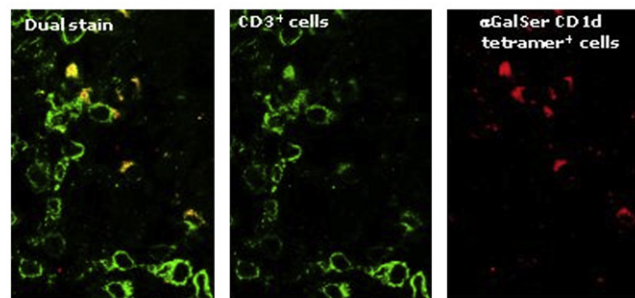
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A

Group & ID	Age (y)	Sex	FEV ₁ % Predicted	PC ₂₀ Methacholine (mg/mL)	Allergen-specific IgE (IU/mL) C, HDM, G	Allergen Dose (BU)	Maximum FEV ₁ % Decrease EAR	LAR
Control 1	29	M	113.6	>16	All negative	NA	NA	
Control 2	28	M	99.2	>16	All negative	NA	NA	
Control 3	26	F	100.0	>16	All negative	NA	NA	
Asthma 1	23	F	95.7	6.16	36.4 (HDM)	400	-30	-42
Asthma 2	46	M	118.2	8.00	11.6 (G)	162	-27	-25
Asthma 3	21	F	88.7	1.74	20.5 (HDM)	1325	-41	-39
Asthma 4	19	F	90.3	1.40	0.93 (C)	5325	-56	-16
Asthma 5	22	M	95.1	3.20	54.80 (C)	390	-23	-23

Definition of abbreviations: BU = biologic units; C = cat dander; EAR = early asthmatic response; F = female; G = mixed grass pollen; HDM = house dust mite; M = male. Patients with a diagnosis of allergic asthma with either a 15% increase in FEV₁ after β_2 -agonist or methacholine PC₂₀ of 8 mg/mL or less, who developed a LAR to allergen challenge, had a baseline FEV₁ > 70% predicted and had positive skin prick tests (SPT) to 1 or more of the allergens HDM, C or G (ALK, Horsholm, Denmark) were studied. Patients were controlled with rescue β_2 -agonists only at the time of the study and those sensitive to pollens were studied out of season. All patients and controls were nonsmokers. A fall in FEV₁ > 15% of reference value was defined as an EAR. An LAR was defined as a fall in FEV₁ > 15% of reference value between 3 and 7 hours after allergen challenge. Royal Brompton & Harefield Hospital Ethics Committee approved the study, and volunteers gave written, informed consent.

B



C

Group & ID	number of cells/mm ² of lung biopsy tissue							
	Baseline			24 hours after allergen challenge			7 days after allergen challenge	
	CD3 ⁺ only	Tet ⁺ only	CD3 ⁺ /Tet ⁺	CD3 ⁺ only	Tet ⁺ only	CD3 ⁺ /Tet ⁺ Number(%CD3 ⁺) cells	CD3 ⁺ only	Tet ⁺ only
Control 1	160	0	0					
Control 2	324	0	0					
Control 3	56	0	0					
Asthma 1	200	0	40 (20)	317	0	91 (29)	ND	ND
Asthma 2	336	0	32 (10)	254	0	46 (18)	160	0
Asthma 3	250	0	13 (5)	120	0	8 (7)	238	0
Asthma 4	429	0	38 (9)	763	0	107 (14)	359	0
Asthma 5	804	0	40 (5)	578	0	43 (7)	586	0

After the initial screening visit, patients with asthma underwent spirometry, methacholine PC₂₀, and fiberoptic bronchoscopy with bronchial biopsies at baseline, 24 hours and 7 days after allergen challenge. Methacholine challenge was carried out according to American Thoracic Society guidelines. Allergen challenges were performed with either mixed grass, or *Dermatophagoides pteronyssinus* (Allergopharma, Reinbek, Germany), or cat dander (Leti, Barcelona, Spain). Extracts were chosen based on clinical history, SPT, and allergen-specific IgE. An incremental dosing schedule was used. Bronchial biopsies were dual stained using phycoerythrin-conjugated CD1dαGalCer tetramers and anti-CD3 and analysed by 2 independent investigators blind to the clinical data at the time of the analysis.

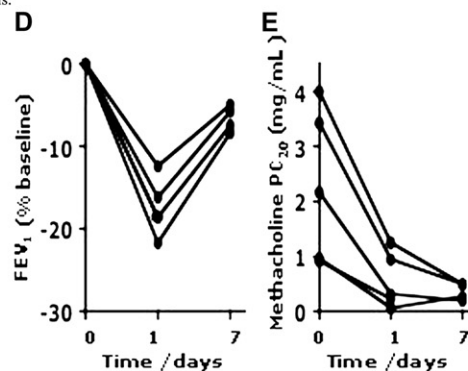


FIG 1. iNKT cells in allergen-challenged patients with asthma. **A**, Volunteer data and airway responses to allergen challenge. **B**, Representative laser confocal images of bronchial biopsy cryosections from patients with allergic asthma 24 hours after allergen challenge. Sections were dual-stained with anti-CD3 (green) and α -galactosylceramide-loaded CD1d-phycoerythrin tetramers (red), colocalization of both showing iNKT cells (orange/yellow). **C**, The number of iNKT cells/mm² of lung biopsy tissue taken from controls and patients with allergic asthma at baseline, 24 hours and 7 days after allergen challenge. **D**, Airway responses to allergen challenge. FEV₁ (**D**) and methacholine PC₂₀ (**E**) measurements 24 hours and 7 days after allergen challenge. SPT, Skin prick test.