

Original article

T-cell activation in occupational asthma and rhinitis

Background: Allergic asthma and rhinitis are described as associated with a Th2 activation. However, recent works indicate that a Th1 activation can also be associated with these diseases, concomitantly to a defect in regulatory T (Treg) cell activation. Occupational asthma (OA) and occupational rhinitis (OR) are peculiar cases of these diseases in which the T-cell activation profile is largely unknown.

Objective: To characterize T-cell activation induced after a specific inhalation test (SIT) in OA and OR.

Material and methods: A total of 21 subjects with OA, 10 subjects with OR, 10 exposed nonallergic (ENA) subjects, and 14 healthy volunteers were included. The SIT with the incriminated substance was performed in patients and ENA subjects. Blood and induced sputum were obtained before and after SIT. T cells were analysed for CD69, CD25, IL-13, and IFN- γ expression by flow cytometry. IL-4 and IFN- γ were assayed by enzyme-linked immunosorbent assay (ELISA) in cell culture supernatants. Treg cells were identified as CD4⁺CD25^{high}CD45RO⁺CD69⁻ T cells in peripheral blood.

Results: Baseline IFN- γ production was decreased in OA and OR compared with controls. The SIT induced an increase in both Th1 and Th2 cells in blood and sputum from OA. In this group, the proportion of peripheral Treg cells decreased after SIT. Similar results were found in the CD8⁺ population. ELISA assays were concordant with flow cytometry. In OR, an attenuated activation profile was found, with an increase in the proportion of IL-13-producing T cells after SIT. By contrast, in ENA subjects, SIT induced Th2 activation, with an increase in Treg cells and a decrease in Th1 cells.

Conclusions: Our results demonstrate a gradient of T-cell activation from a tolerating profile in ENA subjects to an inflammatory profile in OA, with an intermediate stage in OR.

E. Mameessier¹, F. Milhe^{1,2}, C. Guillot³, J. Birnbaum^{1,2}, P. Dupuy⁴, A.-M. Lorec⁵, D. Vervloet^{1,2}, A. Magnan^{1,2}

¹Université de la Méditerranée; ²Fédération des Maladies Respiratoires; ³Institut Jean Roche, Faculté de Médecine, Université de la Méditerranée; ⁴Centre d'Investigations Cliniques, INSERM, AP-HM, Hôpital Ste Marguerite; ⁵Laboratoire Central, Hôpital Ste-Marguerite, Marseille, France

Key words: asthma; occupational diseases; rhinitis; sputum; T cells.

Prof. Antoine Magnan
UPRES 3287, Pathologie Respiratoire Liée à l'Environnement
Faculté de Médecine
27, Bd Jean Moulin
13285 Marseille
France

Accepted for publication 3 November 2006

Occupational factors have been implicated in 9–15% of adult asthma (1), making occupational asthma (OA) the main cause of occupational respiratory diseases. As a result of the large diversity of causative agents, the pathophysiology of OA is still controversial. It is admitted that immunological and nonimmunological reactions can be involved, and among the former, IgE-dependent and IgE-independent immune responses. IgE-dependent OA is mainly because of high molecular weight (> 5000 Da) molecules, such as vegetable proteins, and thought to be closely related to asthma unrelated to work. Low molecular weight agents such as isocyanates, aldehydes and other chemicals induce a form of asthma in which the implication of IgE is doubtful (2). However, in both cases of immunological OA, the airway inflammation is similar, with the presence of eosinophils, lymphocytes and mast cells (1). Previous studies in mice have demonstrated a Th2 cell activation, with an increase in IL-5 and IL-4 production in experimental OA (3, 4). Other mice studies have found a Th1 activation associated with the Th2

activation previously described, especially in the case of low molecular weight allergen exposure, with IFN- γ production (5, 6), notably by the CD8⁺ $\gamma\delta$ T cells (7). This view is reminiscent of what is currently known in non-occupational asthma, in which a Th2 activation predominates, accompanied by a Th1 activation (8). In addition, in non-occupational asthma, some evidences recently indicated that Th2 and Th1 cell activation could be concomitantly associated with a decrease in the regulatory T (Treg) cell frequency and activation (9). However, to date, no data about Treg cells in OA, in response to a specific inhalation test (SIT), have been published.

Occupational asthma is frequently preceded by occupational rhinitis (OR), notably in cases related to high molecular weight agents (1). Many studies clearly established that allergic rhinitis is Th2-mediated (10). In addition, a Th1 activation was also observed in allergic rhinitis during natural allergen exposure (11), and a deficiency in the Treg cell activation was clearly demonstrated in this disease (12). However, no report concerns specifically the immunopathology of OR.

The aim of this study was thus to characterize the human T-cell activation induced by occupational allergens, in OA and OR subjects. We took advantage, for this purpose, of SIT performed to diagnose an OA or rhinitis.

Materials and methods

Study population

Patients referred to our institution for clinical suspicion of OA and/or rhinitis were consecutively included from January 2003 to July 2005. Occupational asthma was diagnosed on a history of chronic cough and wheezing dyspnea exacerbated at work and improved during out-of-work periods, in patients with a positive methacholine test at least after SIT, and a positive SIT. Occupational rhinitis was diagnosed on chronic rhinorrhea and/or nasal obstruction, with sneezing and nasal itching, increased at work and improved out of work, with triggering of nasal symptoms during SIT. In addition, these patients had to display a negative SIT on lung function and a negative methacholine test. For high molecular weight allergens, skin prick tests were performed with commercial extracts (Stallergenes, Antony, France) (cereal flours and latex), or the native vegetable (cauliflower). For low molecular weight substances, SIT was the only diagnosis method used.

A total of 14 healthy volunteers and 10 exposed nonallergic (ENA) workers to OA-inducing substances were included as controls. Healthy volunteers and ENA subjects were nonsmokers, and displayed normal pulmonary function with negative methacholine test.

All subjects gave a written informed consent, and the protocol was approved by the ethical committee Comité de Protection des Personnes Marseille 2.

Methacholine test

After baseline spirometry and plethysmography with measurement of airway resistance (R_{aw}) and functional residual capacity according to the method of Dubois et al. (13), patients inhaled successive doses of methacholine (40, 60 μ g, and 4 times 100 μ g), using an automatic dosimeter (ME-FAR; Electromedicali, Brescia, Italy). The R_{aw} and forced expiratory volume in 1 s (FEV1) were measured after each inhalation. The test was stopped when R_{aw} increased by three times or when FEV1 fell by 20%. Two puffs of albuterol (100 μ g) were given thereafter to reverse the bronchial obstruction. The test was considered positive if R_{aw} doubled and FEV1 dropped by more than 20% for a dose below 200 μ g of methacholine (14).

Specific inhalation tests

Anti-asthmatic medications were withheld according to their duration of action. The SITs were carried out in 5 m³ room equipped with an exhaust ventilation system and a small electric fan to ensure air mixing (15). The SIT was performed only in subjects with baseline FEV1 > 70% of predicted value.

Patients were progressively exposed to the respective suspected occupational agent in a dose as close as possible as the occupational usage, for a total duration of 60 min. Maximal expiratory volumes were measured before, every 5 min of exposure for the first 15 min of the procedure, and every 10 min till the end of the challenge. Patients were then kept in hospital and FEV1 was measured each hour till 6 h after the challenge to monitor any late-phase reaction.

The test was considered as positive if FEV1 dropped below 80% of the baseline value. In case of asthmatic reaction during SIT, the procedure was stopped. In case of a decrease of the FEV1 below 80% of baseline value, the patient was given 200–1000 μ g of albuterol to reverse the bronchial obstruction.

Nasal symptoms were monitored throughout the procedure and the presence of sneezing, rhinorrhea, nasal obstruction and pruritus were checked before each spirometry. Specific inhalation test was considered as positive for OR if significant nasal symptoms were triggered.

Induced sputum

Sputum was induced with hypertonic saline (4.5%) aerosol. Immediately, cellular plugs were separated from saliva and incubated with 0.1% dithiothreitol. Cells were filtered through 40- μ m cell strainer, washed, and resuspended in RPMI[®] (Gibco, Cergy-Pontoise, France) at a concentration of 10⁶ cells/ml. Total nucleated cell counts and viability were assessed after trypan blue exclusion. Samples were validated by a viability > 50% and a salivary cell contamination < 20%, according to ERS guidelines (15). Cells were then cytopspined on silane-coated slides (Sigma-Aldrich, Lyon, France) and stained with May-Grunwald-Giemsa coloration (Sigma-Aldrich). Results were expressed as percentages of leucocytes.

Assessment of T-cell activation

Whole blood cells were cultured at 37°C, 5% CO₂ in complete medium [RPMI 1640 10% heat-inactivated fetal calf serum, 100 U/ml penicillin G, 100 μ g/ml streptomycin, 2 mM/glutamine, pyruvate and 2-mercaptoethanol (Gibco)]. IFN- γ and IL-13 were assessed by flow cytometry after 6 h of stimulation by PMA (50 ng/ml; Sigma-Aldrich) and ionomycin (2 μ g/ml, Sigma-Aldrich) plus monensin (2 μ M) (Sigma-Aldrich).

Surface antibodies coupled to fluorochromes [anti-CD3-Cy5PE, anti-CD8-Cy5PE, anti-CD8-PE, anti-CD25-PE, anti-CD45RO-FITC, anti-CD69-APC (Immunotech, Marseilles, France), anti-CD3-FITC, anti-CD4-Cy5PE, anti-CD4-APC (Dako, Trappes, France)] were added at the recommended concentration in 50 μ l of whole blood. Cells were fixed in formaldehyde 4% for 10 min, and permeabilized with saponine 0.1%. Intracellular antibodies [anti-IFN- γ -FITC (Pharmingen, Le pont de Claix, France) and anti-IL13-PE (R&D system, Lille, France)] were added at the recommended concentrations for 30 min, cells were suspended in formol 0.1% before flow cytometric analysis. For each surface or intracellular staining, positive cells were identified by comparison to the fluorescence of cells stained with control IgG isotypes (Immunotech).

Fluorescence was detected with a 15 mW argon ion laser on a three-color FACScalibur[®] flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Standard acquisition and analysis were obtained through Cellquest Software (Becton Dickinson).

CD4⁺CD25^{high} T cells were considered as Treg cells based on the CD4⁺CD25^{high}CD45RO⁺C69⁻ phenotype. This subset was only studied in the peripheral blood samples because of too few cells recovered with induced sputum.

Enzyme-linked immunosorbent assay

IFN- γ and IL-4 were assessed by enzyme-linked immunosorbent assay (ELISA) in cell culture supernatants in wells cultured without monensin. Enzyme-linked immunosorbent assay was carried out according to manufacturer instructions. IFN- γ and IL-4 DuoSet[®] were obtained from R&D Systems. IFN- γ and IL-4 assay sensitivity was of 15 pg/ml.

Statistical analysis

Results were expressed as mean $\pm$ standard error. Data were normally distributed as indicated by graphic representation of the data and negative Kolmogorov-Smirnov test. Variations among controls, ENA subjects, OR, and OA patients were performed using ANOVA. If the ANOVA indicated significant variation of the mean, comparisons were carried out using parametric unpaired *t*-test between controls and the three other groups. Results obtained after specific challenge were compared with baseline by paired *t*-tests. A *P*-value below 0.05 was considered as statistically significant. Analyses were carried out using the Statview[®] software (SAS, Cary, NC, USA).

Results

Subjects

A total of 44 subjects (mean age 43 ± 6 years) were investigated for suspected OA or OR. After SIT, 21 were considered as OA, 10 as OR and 13, in whom SIT was negative, were excluded (Table 1). A total of 14 OA were exposed to low molecular weight allergens: 3 painters (isocyanates), 3 hospital workers (formaldehydes), 4 hairdressers (persulfates), 1 dental prosthesis manufacturer (diacrylate), 1 photographer (diacrylate), 1 ink industry worker (diacrylate), and 1 chemical industry worker (epoxy resin). Among the seven workers exposed to high molecular weight agents, three hospital workers were allergic to latex, two bakers were allergic to cereal flours, one vegetable merchant was allergic to cauliflower and a joiner was considered as allergic to wood dusts. Among OR, six patients were exposed to low molecular weight agents: formaldehydes in a hospital worker, persulfates in three hairdressers, aliphatic amines in a chemistry industry worker and diacrylates in a photographic processing worker. High molecular weight agents were incriminated in four patients: Cereal flour in two bakers, wood dust in a joiner and latex in a health care worker. Allergy to high molecular weight allergens was documented by positive skin prick tests at the exception of wood dust allergy. Ten ENA patients (mean age 32 ± 3 years) were included, among which two nurses were exposed to latex, a laboratory technician, a cleaner, a hospital worker, and a building worker were exposed to formaldehydes, a painter and a coachbuilder were exposed to isocyanates, and a chemical industry worker was exposed to epoxy (Table 1). All ENA subjects displayed negative methacholine test and SIT. Fourteen healthy subjects (mean age 31 ± 5 years) with negative methacholine test were included as controls. Specific inhalation test was performed in patients and ENA subjects only.

Induced sputum cell counts

Valid induced sputum was obtained in 18 OA, 4 OR, and 7 ENA subjects. As the number of valid samples from OR was less than five, results were not analysed in this group. Eosinophils were increased in OA compared with con-

Table 1. Patient characteristics

Gender	Suspected allergen	FEV1
Asthma		
F	Isocyanate	88
F	Latex	91
M	Wood	82
M	Isocyanate	118
F	Isocyanate	85
F	Persulfates	104
M	Flour	110
M	Diacrylates	120
M	Diacrylates	78
F	Formaldehydes	83
M	Diacrylates	76
F	Latex	69
F	Latex	71
F	Formaldehydes	101
M	Epoxy	92
F	Flour	97
F	Formaldehydes	89
M	Persulfates	76
M	Cabbage	88
M	Persulfates	79
F	Persulfates	101
Rhinitis		
F	Formaldehydes	101
M	Flour	125
F	Latex	105
F	Persulfates	95
M	Wood	96
F	Amine	90
F	Persulfates	100
M	Diacrylates	121
M	Flour	103
M	Persulfates	103
Exposed nonallergic subjects		
M	Latex	98
M	Formaldehydes	110
M	Formaldehydes	92
F	Amine	88
F	Formaldehydes	79
F	Latex	120
M	Isocyanate	105
M	Formaldehydes	76
M	Isocyanates	88
F	Epoxy	119

F, female; M, male; FEV1 given in percentage of predicted value.

trols at baseline, and significantly increased further after SIT ($P < 0.05$) (Fig. 1). In ENA subjects, sputum cell counts did not differ from controls and did not vary after SIT.

T-cell activation

The CD69 expression was not different in blood T cells in patients compared with controls and did not vary upon SIT (Table 2).

The baseline proportions of IL-13-producing T cells were similar between groups. After SIT, the proportions

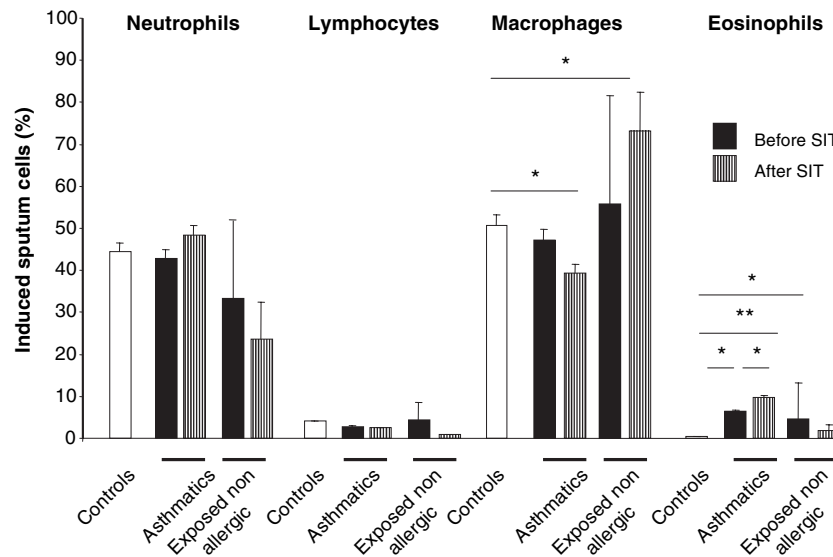


Figure 1. Induced sputum differential cells counts. Induced sputum cells were recovered in occupational asthma and exposed non-allergic individuals, before specific inhalation test (SIT) data are compared to control data by an unpaired *t*-test (*p* values indicated in the 'before SIT' columns); after SIT data are compared to before challenge data by a paired *t*-test (*p* values indicated in the 'after SIT' columns). Differential cell counts were obtained after cytospin and May-Grunwald-Giemsa staining. **P* < 0.05, ***P* < 0.005 ****P* < 0.0005. SIT, specific inhalation test. Results expressed as mean ± standard error.

Table 2. Blood T-cell phenotype and cytokine production

	Exposed nonallergic subjects (<i>n</i> = 10)			Rhinitis (<i>n</i> = 10)		Asthmatics (<i>n</i> = 21)	
	Controls (<i>n</i> = 15)	Before SIT	After SIT	Before SIT	After SIT	Before SIT	After SIT
Flow cytometry (% of positive cells)							
CD3 ⁺ CD69 ⁺	9.86 (±2.6)	11.63 (±1.09)	11.80 (±2.1)	10.17 (±1.5)	11 (±2.68)	9.24 (±3.9)	10.5 (±3.12)
CD3 ⁺ IL13 ⁺	1.01 (±0.2)	0.63 (±0.05)	1.98 (±0.91)*	0.87 (±0.15)	1.52 (±0.36)*	1.05 (±0.18)	2.34 (±0.5)**
CD8 ⁺ IL13 ⁺	0.6 (±0.11)	0.85 (±0.37)	0.48 (±0.12)	0.8 (±0.11)	1.08 (±0.22)*	1.6 (±0.45)	2.79 (±0.77)***
CD3 ⁺ IFN-γ ⁺	18.32 (±2.23)	19.25 (±3.61)	13.79 (±2.68)*	9.89 (±1.2)***	11.46 (±1.4)	12.77 (±1.45)*	15.78 (±1.55)*
CD8 ⁺ IFN-γ ⁺	27.1 (±2.86)	26.00 (±5.96)	19.96 (±2.71)	13.9 (±1.14)***	13.5 (±2.2)	19.9 (±1.98)*	24.06 (±2.6)*
CD4 ⁺ CD25 ⁺	7.87 (±1)	8.71 (±0.95)	11.72 (±1.58)*	7.5 (±1)	6.32 (±0.5)	7.24 (±1)	5.4 (±0.7)*
ELISA (pg/ml)							
IFN-γ	39366 (±5194)	30290 (±6856)	14977 (±1996)*	24198 (±7813)	22866 (±6972)	40164 (±4572)	47129 (±5953)***
IL-4	79 (±8.5)	108 (±27.44)	138 (±22.5)	86 (±19)	142 (±24)*	110 (±22.4)	130 (±24.3)*

Results are expressed as mean ± standard error.

**P* < 0.05.

***P* < 0.0005. Before specific inhalation test (SIT), data are compared with control data by an unpaired *t*-test (*P*-values indicated in the 'before SIT' columns); After SIT data are compared with before challenge data by a paired *t*-test (*P*-values indicated in the 'after SIT' columns).

****P* < 0.005.

of CD3⁺ and CD8⁺ IL-13⁺ cells increased in both OA and OR (Fig. 2A). Concordant results were found by ELISA with regard to IL-4, which significantly increased after SIT in both groups (*P* < 0.05) (Table 2). In OA, SIT induced an increase in CD3⁺ but not in CD8⁺ IL13⁺ cells, and a nonsignificant IL-4 increase.

At baseline, IFN-γ⁺ cells were decreased in both groups of patients compared with controls, both in the whole T-cell population and in the CD8⁺ subset

(Fig. 2B). Specific inhalation test increased the percentage of IFN-γ-producing T cells in OA but not in OR. Again this result was found at the CD3⁺ and the CD8⁺ level (Table 2). This result was confirmed by ELISA in culture supernatants, with an increase in IFN-γ levels after SIT compared with baseline in OA (*P* < 0.005) (Table 2). By contrast, in ENA subjects, SIT decreased the percentage of CD3⁺IFN-γ⁺ cells (Fig. 2B). This decrease was significant at the level of the whole T-cell population

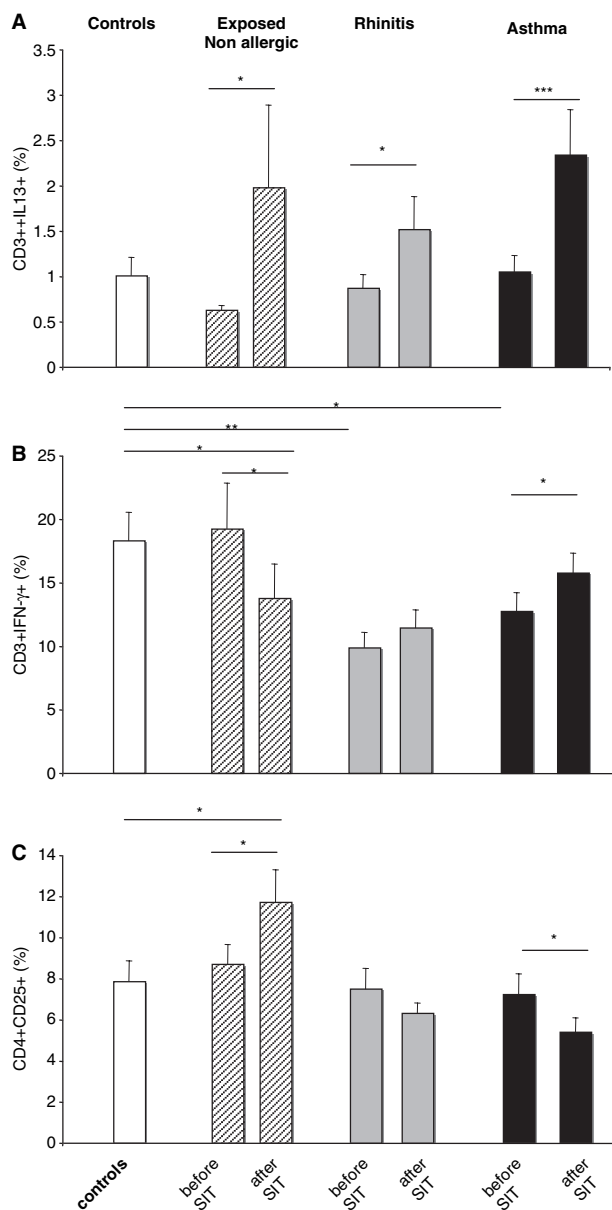


Figure 2. Blood T-cell activation in occupational asthma (OA) and rhinitis, and in exposed nonallergics (ENA). (A) Percentages of blood IL-13-producing T cells before and after specific inhalation test (SIT) in OA and rhinitis, in ENA subjects and in controls. (B) Percentages of blood IFN- γ -producing T cells before and after SIT in OA and rhinitis, in ENA subjects and in controls. (C) Percentages of blood CD4⁺CD25⁺CD45RO⁺CD69⁻ T cells before and after SIT in OA and rhinitis, in ENA subjects and in controls. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$. Results expressed as % $\pm$ SEM.

(CD3⁺) only. In cell culture supernatants, results were concordant with a decrease by 50% of IFN- γ levels (Table 2).

No difference was found among groups before challenge, regarding the proportion of the CD4⁺CD25⁺CD45RO⁺CD69⁻ Treg cell population. After SIT, OA

patients displayed a significant decrease in the regulatory T-cells population ($P < 0.05$) (Fig. 2C). By contrast, in ENA subjects, a significant increase in Treg cell percentages occurred after SIT.

Specific inhalation test increased induced sputum CD69⁺ ($P < 0.005$), IFN- γ ⁺ ($P < 0.05$) and IL-13⁺ ($P < 0.05$) T-cell proportions in OA patients (Fig. 3).

Comparisons according to exposure to low or high molecular weight agents

Results obtained in OA and OR were then analysed according to the type of putative causal agent. In all cases, results were concordant in both groups, although not necessarily significant, because of small effectives. Results are showed for CD3⁺IL13⁺, CD3⁺IFN- γ ⁺, and Treg cells in Fig. 4.

Discussion

Allergic asthma is characterized by Th2 activation, mainly attributed to CD4⁺ T cells, which, by producing IL-4 and IL-5, respectively, induce IgE production and bronchial eosinophilia, two main features of asthma. This concept, named the Th2 paradigm in allergy, is to date considered as a dogma in asthma pathophysiology (16).

Despite an intrinsic defect in Th1 cells concordant with this Th2 paradigm, we found that in OA patients, SIT induced a mixed Th2/Th1 response. In addition, CD8⁺ cells were the main producers of IFN- γ .

In OA, the T-cell activation was previously studied in a murine model of isocyanate-induced asthma, a major form of OA because of a low molecular weight allergen. In this model, the implication of T cells was clearly demonstrated. Indeed, T cells from sensitized animals were sufficient to transfer the disease to naïve mice (17). Conversely in CD4⁻ but also in CD8⁻ mice, isocyanate-induced airways hyper-responsiveness was impaired, together with airways eosinophilia and specific IgE production. A Th2 activation was demonstrated, with an increase in IL-4 and IL-5 production, but also a Th1 activation mainly characterized by IFN- γ increase (6). In humans, hyper-responsive to isocyanates, bronchial biopsy studies have long been established an *in situ* activation of eosinophils, CD4⁺ and CD8⁺ T lymphocytes (18). Therefore, animal and human data are concordant with our findings of a mixed Th1/Th2 activation with implication of the CD8⁺ T cells in OA.

This profile could be specific of the low molecular weight agent used in these studies, namely isocyanates: OA is due to a large variety of allergens, among which high molecular weight allergens are frequently distinguished from low molecular weight allergens with regard to the induced pathology. Notably, the former are thought to induce a form of asthma similar to nonoccupational allergic asthma, in which atopy is a risk factor,

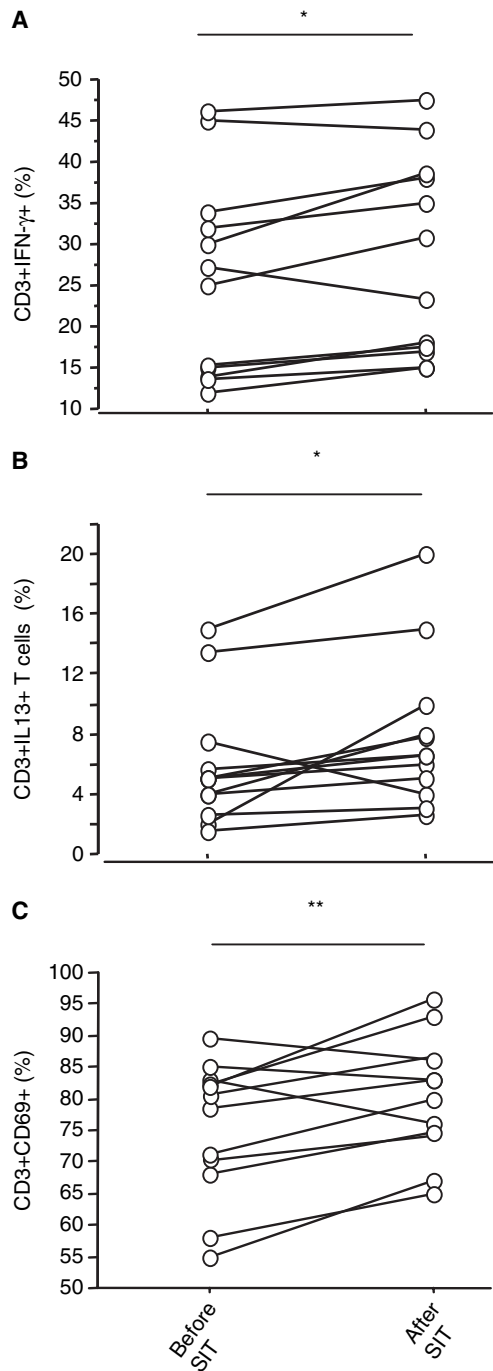


Figure 3. Induced sputum T-cell activation in occupational asthma (OA). (A) Percentages of induced sputum IFN- γ -producing T cells before and after SIT in OA. (B) Percentages of induced sputum IL-13-producing T cells before and after specific inhalation test (SIT) in OA. (C) Percentages of induced sputum CD3⁺CD69⁺ T cells before and after SIT in OA. * $P < 0.05$, ** $P < 0.005$. Results expressed as % $\pm$ SEM.

specific IgEs are present in serum, and skin prick tests are relevant to the diagnosis. In OA related to low molecular weight agents, atopy is not a risk factor, specific IgEs are

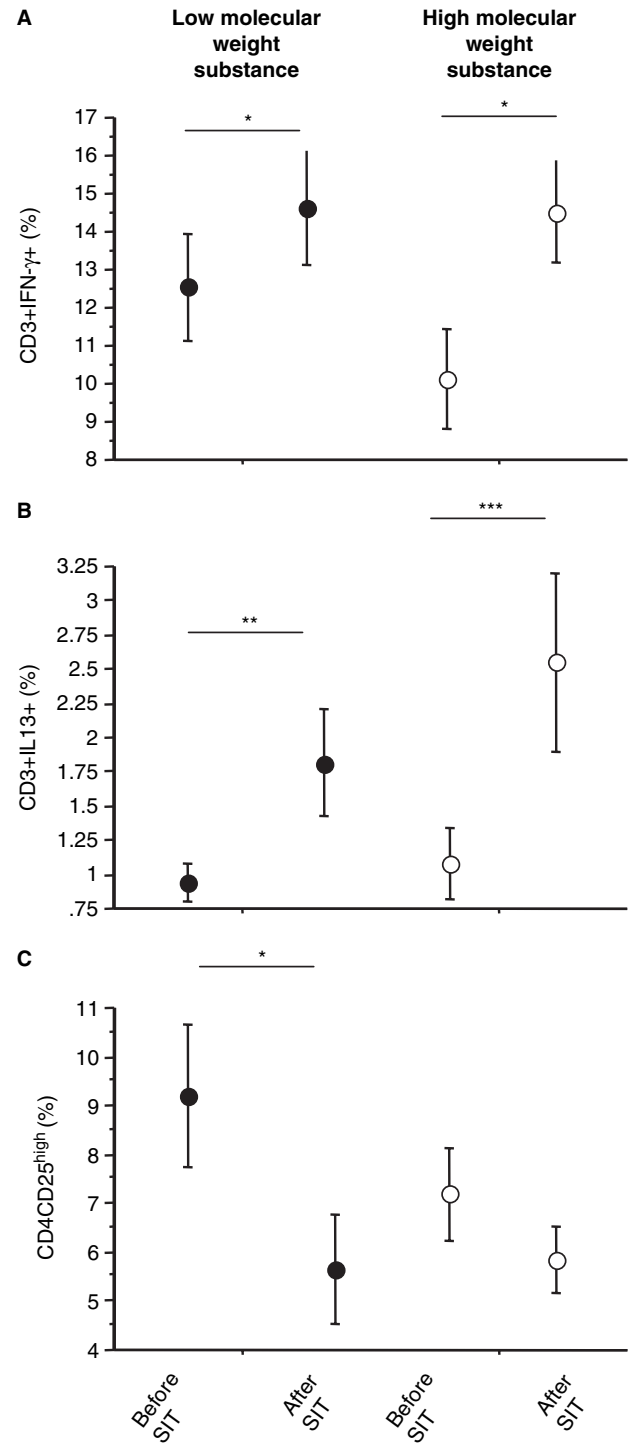


Figure 4. T-cell activation according to type of exposure. (A) Percentages of blood IFN- γ -producing T cells before and after SIT in OA or rhinitis. (B) Percentages of blood IL13-producing T cells before and after specific inhalation test (SIT) in occupational asthma (OA) or rhinitis. (C) Percentages of blood Treg cells before and after SIT in OA or rhinitis. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$. Results expressed as % $\pm$ SEM.

frequently not found, and thus skin prick tests are poorly contributive to the diagnosis (1). However the T-cell activation profile between both forms of substances involved was similar, which suggest that although IgE are not necessarily involved in both cases, some mechanisms are shared.

In addition, more and more publications indicate that in common non-OA, a Th1 activation accompanies the Th2 cytokine production (8). Indeed, whereas in some models of asthma the induction of Th1 cells was protective (19), in other cases, the presence of IFN- γ -producing cells exacerbated the asthmatic phenotype (20). In addition, in humans, a production of IFN- γ was found, correlated to asthma severity (21), symptoms (22), and bronchial hyper-responsiveness (23). It is noteworthy that IFN- γ -producing T cells in asthma were frequently CD8⁺ cells (23). This is in keeping with results found in ENA subjects. Indeed, whereas in these patients the SIT-induced Th2 activation was not found at the CD8⁺ level. In addition, a decrease of IFN- γ -producing T cells occurred, suggesting a crucial role for IFN- γ -producing T cells, notably CD8⁺, in the inception of asthma in exposed subjects.

This raises the question of the role of CD8⁺ cells in asthma. These cells are considered as a heterogeneous population, including effector cytotoxic T cells and cytokine-producing T cells but also NK cells, NKT cells, and $\gamma\delta$ T cells. Furthermore, the bronchial infiltration by the CD8⁺ T cells was associated with the decline of FEV1 in asthma (24) and asthma mortality (25).

Therefore, the involvement of IFN- γ ⁺ and of CD8⁺ cells in asthma could be a trait of increased severity. In this view, it is not surprising to find this trait in OA patients, as this form of asthma is considered as more severe than non-OA (1). No relationship was found between asthma severity or specific or nonspecific bronchial hyper-responsiveness and CD8⁺ or IFN- γ ⁺ T cells in this study, probably because of the limited number of patients explored.

It was also recently pointed out that a deficiency in the Treg cell population could preclude to the mixed Th2/Th1 activation seen in asthma (26). Indeed, Treg cells regroup small populations of T cells, which constitutively produce immunosuppressive cytokines such as IL-10 and/or TGF- β . These cells are thought to be responsible for the immune homeostasis, preventing the organism from any unwarranted inflammation, notably against self-antigens and allergens (27). In this view, a deficiency in

Treg cells could be responsible for the increase of auto-immune and allergic diseases observed in the last decades (28). A Treg cell deficiency was previously showed in asthma (8) and allergic rhinitis (12). The decrease of the Treg cells observed in OA patients after SIT, but overall the increase of Treg cells induced in ENA subjects are concordant with this hypothesis. The variation in the proportion of the Treg population was not previously investigated in OA after inhalation challenge. Hoffman and coll (29) assessed the Treg cells in blood after ingestion challenge in patients with OA, because of cereal flour exposure. In these conditions, they observed a decrease of CD4⁺CD25⁺ cells after ingestion but only when compared with placebo and not when comparing baseline to post-challenge data. A similar profile of mixed Th1/Th2 activation and a decrease of Treg cells were recently demonstrated in exacerbations of asthma, a condition that can be compared with allergen challenge (30).

T-cell activation has not been previously investigated in OR. These patients are rare; OR is frequently neglected and therefore unexplored. Herein, we demonstrate a T-cell activation profile reminiscent of what is described in non-occupational rhinitis. Indeed, as asthma, rhinitis is largely associated to a Th2 activation at baseline, enhanced under allergen stimulation (7). We found a deficient IFN- γ production in T cells at baseline, which is in keeping with an intrinsic Th2 skewing and a SIT-induced Th2 activation. This profile can be considered as different from the one seen in asthma, as Th1 activation and Treg cell decrease were not found in OR. However, it is more likely that the OR T-cell activation profile is intermediate, with an elevation of IFN- γ production and a decrease of Treg cells that do not reach significance in our study. Indeed, the mean cytokine and T-cell variation was concordant with OA results in a smaller extent.

In conclusion, our results demonstrate a gradient of T-cell activation from a tolerating profile in ENA subjects (decrease of Th1, increase of Th2 and Treg cells after SIT) to an inflammatory profile in OA (increase of both Th1 and Th2, decrease of Treg cells after SIT), with an intermediate stage in OR.

Acknowledgment

This work was supported by a grant from Comité National de Lutte contre Les Maladies Respiratoires, AP-HM.

References

1. Mapp CE, Boschetto P, Maestrelli P, Fabbri LM. Occupational asthma. *Am J Respir Crit Care Med* 2005;**172**:280–305.
2. Jeal H, Draper A, Harris J, Taylor AN, Cullinan P, Jones M. Modified th2 responses at high-dose exposures to allergen: using an occupational model. *Am J Respir Crit Care Med* 2006;**174**: 21–25.
3. Maestrelli P, Occari P, Turato G, Papiris SA, Di Stefano A, Mapp CE et al. Expression of interleukin (IL)-4 and IL-5 proteins in asthma induced by toluene diisocyanate (TDI). *Clin Exp Allergy* 1997;**27**:1292–1298.
4. Hardy CL, Kenins L, Drew AC, Rolland JM, O'Hehir RE. Characterization of a mouse model of allergy to a major occupational latex glove allergen Hev b 5. *Am J Respir Crit Care Med* 2003;**167**:1393–1399.
5. Maestrelli P, Del Prete GF, De Carli M, D'Elios MM, Saetta M, Di Stefano A et al. CD8 T-cell clones producing interleukin-5 and interferon-gamma in bronchial mucosa of patients with asthma induced by toluene diisocyanate. *Scand J Work Environ Health* 1994;**20**: 376–381.
6. Herrick CA, Xu L, Wisniewski AV, Das J, Redlich CA, Bottomly K. A novel mouse model of diisocyanate-induced asthma showing allergic-type inflammation in the lung after inhaled antigen challenge. *J Allergy Clin Immunol* 2002;**109**:873–878.
7. Wisniewski AV, Herrick CA, Liu Q, Chen L, Bottomly K, Redlich CA. Human gamma/delta T-cell proliferation and IFN-gamma production induced by hexamethylene diisocyanate. *J Allergy Clin Immunol* 2003;**112**:538–546.
8. El Biaze M, Boniface S, Koscher V, Mamessier E, Dupuy P, Milhe F et al. T cell activation, from atopy to asthma: more a paradox than a paradigm. *Allergy* 2003;**58**:844–853.
9. Karagiannidis C, Akdis M, Holopainen P, Wooley NJ, Hense G, Ruckert B et al. Glucocorticoids upregulate Foxp3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004;**114**:1425–1433.
10. Varga EM, Jacobson MR, Till SJ, Masuyama K, O'Brien F, Rak S et al. Cellular infiltration and cytokine mRNA expression in perennial allergic rhinitis. *Allergy* 1999;**4**:338–345.
11. Koscher V, Milhe F, El Biaze M, Vervloet D, Magnan A. Variation of T cell activation in allergic subjects during natural pollen exposure. *Allergy* 2006;**61**:35–42.
12. Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J et al. Relation of CD4+ CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004;**363**:608–615.
13. Dubois AB, Dautrebande L. Acute effects of breathing inert dust particles and of carbachol aerosol on the mechanical characteristics of the lungs in man; changes in response after inhaling sympathomimetic aerosols. *J Clin Invest* 1958;**12**:1746–1755.
14. Badier M, Guillot C, Delpierre S, Vanuxem P. Bronchial provocation tests. Comparison of 2 inhalation methods. *Presse Med* 1993;**22**:1169–1174.
15. Vandenplas O, Malo JL. Inhalation challenges with agents causing occupational asthma. *Eur Respir J* 1997;**10**: 2612–2629.
16. Kay AB. The role of T lymphocytes in asthma. *Chem Immunol Allergy* 2006;**91**:59–75.
17. Scheerens H, Buckley TL, Davidse EM, Garssen J, Nijkamp FP, Van Loveren H. Toluene diisocyanate-induced in vitro tracheal hyperreactivity in the mouse. *Am J Respir Crit Care Med* 1996;**154**: 858–865.
18. Bentley AM, Maestrelli P, Saetta M, Fabbri LM, Robinson DS, Bradley BL et al. Activated T-lymphocytes and eosinophils in the bronchial mucosa in isocyanate-induced asthma. *J Allergy Clin Immunol* 1992;**89**:821–829.
19. Wiley R, Palmer K, Gajewska B, Stampfli M, Alvarez D, Coyle A et al. Expression of the Th1 chemokine IFN- γ inducible protein 10 in the airway alters mucosal allergic sensitization in mice. *J Immunol* 2001;**166**:2750–2759.
20. Randolph DA, Stephens R, Carruthers CJ, Chaplin DD. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J Clin Invest* 1999;**104**:1021–1029.
21. Corrigan CJ, Kay AB. CD4 T-lymphocyte activation in acute severe asthma. *Am J Respir Dis* 1990;**141**:970–977.
22. Boniface S, Koscher V, Mamessier E, El Biaze M, Dupuy P, Lorec AM et al. Assessment of T lymphocyte cytokine production in induced sputum from asthmatics: a flow cytometry study. *Clin Exp Allergy* 2003;**33**:1238–1243.
23. Magnan A, Mély L, Camilla CA, Badier M, Bongrand P, Vervloet D. Assessment of the TH1/TH2 paradigm in whole blood in atopy and asthma: increased IFN-gamma-producing CD8+ T cells in asthma. *Am J Respir Crit Care Med* 2000;**161**:1790–1796.
24. O'Sullivan S, Cormican L, Faul JL, Ichinohe S, Johnston SL, Burke CM et al. Activated, cytotoxic CD8(+) T lymphocytes contribute to the pathology of asthma death. *Am J Respir Crit Care Med* 2001;**164**:560–564.
25. van Rensen EL, Sont JK, Evertse CE, Willems LN, Mauad T, Hiemstra PS et al. Bronchial CD8 cell infiltrate and lung function decline in asthma. *Am J Respir Crit Care Med* 2005;**172**:837–841.
26. Magnan A, Humbert H. Is deficient tolerance the true paradigm for atopic diseases? *Clin Exp Allergy* 2005;**35**: 1507–1510.
27. Romagnani S. Regulatory T cells: which role in the pathogenesis and treatment of allergic disorders? *Allergy* 2006;**61**:3–14.
28. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002;**347**: 911–920.
29. Hoffmann HJ, Skjold T, Raithel M, Adolf K, Hilberg O, Dahl R. Response of respiratory flour allergics in an ingested flour challenge may involve plasmacytoid dendritic cells, CD25+ and CD152+ T cells. *Int Arch Allergy Immunol* 2006;**140**:252–260.
30. Mamessier E, Nieves A, Badier M, Vervloet D, Magnan A. Induced sputum and blood T lymphocyte activation during exacerbations of severe asthmatics: the ISEA study. *Eur Respir J* 2006;**28**(Suppl. 1):S600(Abstract 3534).