

LETTER TO THE EDITOR

**PERIPHERAL TH-17 CELLS IN CHILDREN WITH ALLERGIC RHINITIS:
PRELIMINARY REPORT**G. CIPRANDI, A.M. CASTELLAZZI¹, D. FENOGLIO, F. BATTAGLIA and G. MARSEGLIA¹

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Th17 is a subset of T helper lymphocytes and exerts pro-inflammatory activities. Recently, it has been reported that serum IL-17 levels are high in the most severe patients with birch allergy studied both outside and during the pollen season. This study aims to compare the frequency of peripheral IL-17-producing T cells in children with allergic rhinitis and in healthy controls. Ten children with allergic rhinitis and 5 healthy non-allergic subjects were evaluated. Th17 were evaluated by intracellular staining in *ex-vivo* T cell compartment. *Ex-vivo* PBMC evaluation showed that allergic patients had higher frequencies of IL-17 producing T cells, both concerning CD4+ and CD8+ cells. In particular, there is a subset co-expressing IL-17 and IFN- γ both for CD4+ and CD8+ cells. In conclusion, this preliminary study suggests a possible role of Th-17 cells in the response to allergens in children.

Allergic rhinitis (AR) is characterized by a nasal mucosal inflammation dependent on a Th2 polarization. Indeed, it is well known that peripheral blood mononuclear cells (PBMC) of AR patients display a predominant IL-4 production by Th2 cells as compared to IFN- γ expression by Th1 cells. Another T lymphocyte subset, namely Th17 cells, has been recently discovered and it may be involved in the pathogenesis of several immune-mediated disorders (1). Th17 cells are characterized by the production of various cytokines, including IL-17, IL-6, TNF- α , and IL-22 (1). IL-17 is involved in tissue pathology in autoimmune disorders (1). Furthermore, IL-17 modulation is involved in neutrophil and eosinophil infiltration in experimental murine asthma models (2-4). IL-17 induces recruitment and increased survival for airway macrophages (5). These facts

initially suggested a regulatory role for IL-17 (6), even though the exact role of Th17 cells in nasal allergic inflammation is still debated. In this regard, a first study showed that patients with viral rhinitis had elevated concentrations of IL-17 in nasal secretions, whereas allergic patients had normal levels (7). However, it has been further shown that some patients with allergic rhinitis, due to birch allergy and evaluated outside the pollen season, may have detectable serum IL-17 levels which are related to some inflammatory parameters (8). More recently, it was shown that serum IL-17 levels were significantly related with clinical symptoms, drug use, and peripheral eosinophils in patients with pollen-induced AR evaluated during the pollen season (9). Thus, serum IL-17 might be considered a biomarker of severe allergy in AR patients (8-9). In

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addition, two recent studies reported an association between Th17 responses and symptom/medication score during SLIT, both in adults and children (10-11).

The present study was therefore aimed at investigating *in vitro* Th-17 allergen-specific repertoire in AR children during the pollen exposure.

MATERIALS AND METHODS

The study included 10 children (6 males and 4 females, mean age 10.1 years) with AR, and 5 healthy non-allergic subjects (2 males and 3 females, mean age 9.5 years).

The patients were included in the study on the basis of a clinical history of allergic rhinitis, positive skin prick test, and presence of nasal symptoms according to validated criteria (12). All subjects were healthy, except for AR, without any active disorder, including rhinosinusitis, and asthma was excluded in all patients.

The skin prick test and blood sampling were performed in all patients. The skin prick test was performed according to the guidelines of the European Academy of Allergy and Clinical Immunology (13), using a common panel as previously reported (8-9).

The study was approved by the local Ethics Committee and performed with the written informed consent of the children's parents.

Peripheral blood mononuclear cells (PBMC), obtained from Ficoll-Hypaque gradient of heparinized venous blood, were resuspended in RPMI 1640 (Invitrogen) containing 10% autologous plasma and used for *ex-vivo* CD4⁺ T lymphocyte evaluation of cytokine profile. Briefly, 2×10^6 PBMC were dispensed in Falcon tubes and activated with phorbol myristate acetate (PMA, 25 ng/ml, Sigma) and ionomycin (1 μ g/ml, Sigma) at 37°C for 5 h. In the last 3 h the Brefeldine-A (BFA, 10 μ g/ml, Sigma), was added to avoid cytokine secretion. After the incubation, the cells were washed with PBS and treated with PBS-0.2 μ M EDTA to eliminate cell aggregation. For the surface staining APC-Cy7-conjugated monoclonal antibodies, (MoAb) anti-human CD3 and Percp-Cy5-conjugated anti-human CD8 were used (Becton Dickinson, BD). The samples were fixed and permeabilized (Cytofix-cytoperm solution, BD), then FITC-conjugated anti-human IFN- γ , PE-conjugated anti-human IL-4 (BD), and AlexaFluor® conjugated anti-human IL-17A (eBioscience) were added for intracellular cytokine staining. Finally, samples were acquired and analyzed by FACS Diva software in a FACSCanto flow cytometer. The results are reported as percentage of cells positive for the tested cytokines.

Descriptive statistics were first performed and

quantitative parameters were reported as mean, standard deviation (SD), standard error of mean (SEM). The non-parametric Wilcoxon test was used to compare samples. The package "S-Plus" (MathSoft Corp.) was used for all analyses.

RESULTS

Ex-vivo PBMC

Table I and Fig.1 report the mean ($\pm$ SD) of the percentages of CD3⁺, CD4⁺, and CD8⁺ peripheral T cell subsets by *ex-vivo* evaluation. The results of intracellular cytokine staining highlight a major frequency of IL-17 producing T cells in CD3⁺CD4⁺ T subset, in fact the mean of CD4⁺IL-17⁺ and CD4⁺IFN- γ IL-17⁺ was higher in allergic patients than in normal controls. Likewise, the mean of CD8⁺IL17⁺ and CD8⁺IFN- γ IL-17⁺ was significantly higher in allergic patients than in normal controls ($p < 0.01$ and $p < 0.001$, respectively).

DISCUSSION

The dichotomic functional Th1-Th2 paradigm had dominated the attention on CD4⁺ T cells over the past 20 years (14). Nowadays, Th1 and Th2 have had to make way for a third effector lineage, Th17, the discovery of which was sustained by identification of new cytokines: IL-17 and IL-22 (15). With the characterization of the Th17 lineage, a more unified model of CD4 orchestration of monocytic (Th1), mast cell/basophilic/eosinophilic (Th2), and neutrophilic (Th17) patterns of inflammation by distinct CD4⁺ T-cell subpopulations is now apparent. A unified understanding of the inductive-effector cytokines specific for each effector subset has emerged. Indeed, IFN- γ and IL-12 are characteristic Th1-dependent cytokines, IL-4, IL-5, and IL-13 are Th2 cytokines. The Th17-associated cytokines may be subdivided as inductive (IL-6, TGF- β , and IL-23) and effector (IL-17A, IL-17F, and IL-22).

Recently, a growing list of chronic inflammatory disorders has been linked to Th17. In this regard, the recent progress in characterizing the pro-inflammatory IL-17 cytokine family has added an additional layer of complexity on the regulation of allergic inflammation (16). In addition, IL-17A may play a role in the development of various allergic diseases that have classically been considered to be

Th2-mediated disorders (17). Moreover, IL-17A may be considered a new biomarker of disease progression and allergy severity as recently demonstrated in allergic rhinitis (8-9). However, to date there have only been studies that investigated IL-17 at serum level. The present study therefore aims to evaluate *in vitro* characterization of Th17 subsets at single cell level in children with allergic rhinitis.

Ex-vivo analysis of peripheral CD3+CD4+

T lymphocytes suggests that allergic children may have more elevated percentages of both CD4+IL-17+ and CD4+IFN- γ +IL-17+ cells than normal controls. This fact underlines the possible role of Th17 in allergic inflammation.

Moreover, this study provides the first evidence that another two subsets of T effector cells belong to the Th17 family: CD8+ IL-17+ and CD8+IFN- γ +IL-17+. The two subpopulations are significantly more

Table I. Percentages (mean $\pm$ SD) of positive *ex-vivo* PBMC for different markers in allergic patient and normal subjects.

<i>Ex-vivo</i> PBMC	Allergic children	Normal subjects
% CD3+	65.00 $\pm$ 8.72	68.80 $\pm$ 9.01
% CD3+CD4+	62.33 $\pm$ 2.89	66.40 $\pm$ 5.68
% CD4+IFN- γ +	9.47 $\pm$ 4.36	6.58 $\pm$ 4.42
% CD4+ILg4+	0.13 $\pm$ 0.12	0.14 $\pm$ 0.26
% CD4+IL-17+	0.63 $\pm$ 0.55	0.30 $\pm$ 0.32
% CD4+IFN- γ +IL-17+	0.23 $\pm$ 0.21	0.02 $\pm$ 0.05
% CD8+IFN- γ +	29.60 $\pm$ 14.74	14.84 $\pm$ 8.37
% CD8+IL-4+	0	0.12 $\pm$ 0.22
% CD8+IL-17+	0.93 $\pm$ 0.25 *	0.16 $\pm$ 0.31
% CD8+IFN- γ +IL-17+	0.50 $\pm$ 0.1 **	0.04 0.05

* $p=0.01$

** $p<0.001$

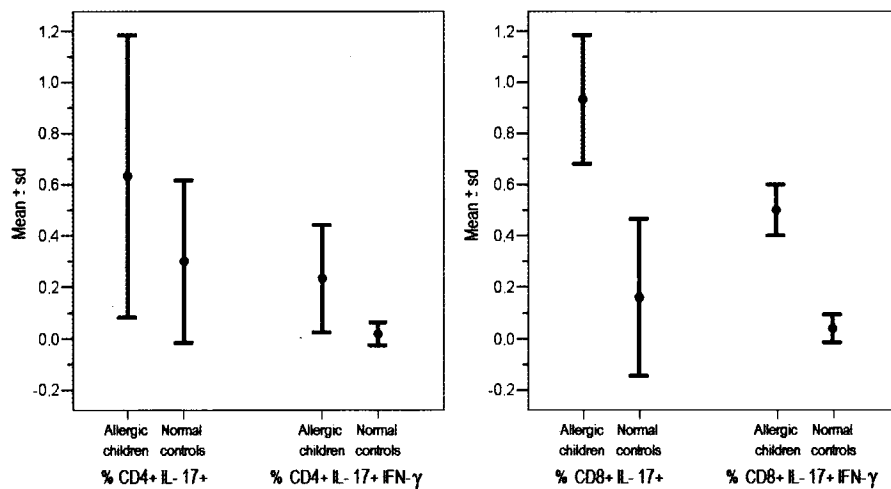


Fig. 1. Mean percentages of CD4+ and CD8+ cells positive for IL-17 and IFN- γ evaluated on *ex-vivo* PBMC of allergic children and normal subjects.

frequent in allergic children than in normal subjects. Obviously, further studies must be addressed to clarify this issue and their possible functional contribution to allergic inflammation.

In conclusion, this preliminary study provides the first evidence that Th-17 cells may be involved in the response to causal allergens in children.

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