

Interferon-gamma and IL-10 may protect from allergic polysensitization in children: preliminary evidence

I. Prigione^{1*}, F. Morandi^{1*}, M. A. Tosca^{2*}, M. Silvestri², V. Pistoia¹, G. Ciprandi³ & G. A. Rossi²

¹Laboratory of Oncology; ²Department of Pulmonary Diseases, IRCCS G. Gaslini Institute; ³Semeiotica e Metodologia Medica I, DIMI, University of Genoa-Azienda Ospedaliera Universitaria San Martino, Genoa, Italy

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Correspondence

G. Ciprandi, MD
Semeiotica e Metodologia Medica I
DIMI Viale Benedetto XV 6
16132 Genoa Italy.
Tel.: +39 10 3533 8120
Fax: +39 10 3538 7573
E-mail: gio.cip@libero.it

*Equally contributed as first authors.

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Abstract

Background: A functional defect of T regulatory cells (Treg) has been proposed as pathogenic mechanism of allergic reaction. Polysensitization is a common feature of allergic patients.

Aim of the study: It was to investigate the possible role of Treg-Th1 cytokines, in the development of new sensitizations in childhood.

Methods: Forty monosensitized (MS) children with allergic rhinitis were evaluated and followed-up for 2 years. New sensitizations were investigated. IL-10 and IFN- γ were evaluated in *in vitro* experiments.

Results: Children remaining MS showed significant higher production of both IL-10 and IFN- γ .

Conclusion: This preliminary study provided evidence that IL-10 and IFN- γ production could be defective in allergic children prone to develop polysensitization.

The pathogenic mechanisms involved in allergic sensitization are complex and still partially understood. Current concepts recognize that the Th2-polarized immune response and the relative allergen-specific defect of Th1 cells characterize allergic sensitization (1, 2). It has been observed that the immune response to allergens in health and disease states is the result of a fine balance between allergen-specific T regulatory cells (Tregs) and allergen-specific Th2 cell (3–5).

Tregs include a broad spectrum of CD4⁺ T cell subpopulations such as Th3 cells, Tr1 cells, CD4⁺ CD25⁺ cells, and Natural Killer T (NKT) cells and are able to release IL10 and Transforming Growth Factor (TGF)- β (5, 6). These are key cytokines for switching allergen-specific immunoglobulin production respectively toward G and A isotypes. In addition, Tr1 cells produce IFN- γ , also, the characteristic 'Th1' cytokine. Indeed, defective IFN- γ production at birth is considered a predisposing factor for developing allergic

disorders (1) and an allergen-specific functional defect of Tregs, promoting Th2 polarization and IgE synthesis, has been detected in adult allergic patients (7, 8).

Some studies have demonstrated that sensitization is often an 'ongoing' phenomenon, frequently starting in childhood with sensitization to a single food protein or perennial allergen, and evolving in a significant proportion of individuals toward 'polysensitization', i.e. the sensitization to different allergens. Indeed, many monosensitized (MS) children become polysensitized (PS) in a relative short time (9–11).

A study was therefore designed to investigate the possible role of Treg-Th1 cytokines, in the development of new sensitizations in childhood.

Materials and methods

Forty children with moderate-severe persistent allergic rhinitis and/or rhinitis plus intermittent asthma, MS to

house dust mite (HDM), visited at Gaslini Institute, were followed up for 2 years : (1) to evaluate the development of new sensitizations and (2) to compare, in mono and PS children, IFN- γ and IL-10 production by peripheral blood mononuclear cells (PBMC) and allergen-specific T cell lines, at beginning and at the end of the study period.

Children were progressively recruited after visit at Allergy Center of Gaslini Institute, they were included in the study if fulfilled a series of criteria (see after). The diagnosis of allergic rhinitis was performed according to validated criteria (12).

The Institutional Ethical Committee of the G. Gaslini Institute approved the protocol. Signed informed parental consent was obtained.

The inclusion criteria were: clinical diagnosis (rhinitis, asthma, or both), type and severity grade assessment, age below 10 years; history of perennial allergic symptoms for at least 3 years; skin prick test and detection of serum IgE (allergen-specific IgE serum levels >3.5 kU/l) positive only for HDM, as markers of monosensitization; no chronic use of inhaled or systemic corticosteroids or prior sublingual immunotherapy. Children were evaluated at entry, performing a skin prick test and taking a blood sample both for IgE assessment and evaluation of cytokine production (cells were separated and frozen). After 2 years, children were re-evaluated, performing skin prick test. Randomly, four MS and four PS children were evaluated for cytokine production.

Peripheral blood mononuclear cells were cultured in 96-well flat-bottom plates (100 000 cells/w) in medium alone or in the presence of 10 μ g/ml of *Dermatophagoides farinae* (Df; Stallergenes, Milan, Italy) or *Candida albicans* (Ca, 2×10^6 autoclaved bodies/ml) for 6 days, or in the presence of 1 μ g/ml phytohemagglutinin (PHA; Sigma, St Louis, MO, USA) for 48 h. Cell-free culture supernatants were collected on day 3 (for allergen stimulation) or day 2 (for PHA stimulation) and stored at -80°C until use. The quantitative determination of IL-10 and IFN- γ were then performed using a commercial ELISA kit (Immunotools, Friesoythe, Germany) according to the manufacturer's instructions, the lowest threshold of detection being 2 pg/ml for IFN- γ and 1.2 pg/ml for IL-10. In addition, allergen-specific T-cell lines were generated from PBMC (1×10^6 /ml) stimulated with Df (10 μ g/ml). On day 7, activated T cells were purified using PERCOLL gradient and expanded with rIL-2. Flow cytometric analysis for intracellular IL-10 and IFN- γ production was performed on T-cell lines, as previously described (13). Anti-IFN- γ fluorescein isothiocyanate (FITC) mAb (Caltag, Burlingame, CA, USA) and anti-IL-10

purified extract mAb (BD Biosciences, San Jose, CA, USA) were used. Cells were analyzed on a Fluorescence Activated Cell Sorting (FACS) caliber cytometer, using the CELL QUEST software (BD Biosciences). The area of positivity was determined using isotype-matched control mAbs (BD Biosciences).

Statistical analyses were performed with Mann-Whitney test, using PRISM software (GraphPad, Milan, Italy).

Results

Forty children were evaluated (24 boys and 16 girls, mean age 7.2 years), 20 had allergic rhinitis, five asthma, and 15 both.

Out of the 40 children enrolled, one dropped out for familiar problems. At the follow-up visit, nine children became PS developing new sensitizations toward *Parietaria*, *Olea*, grasses and animal danders. Randomly, four MS children and four PS children were studied for immunologic evaluation.

At baseline, significantly higher levels of IFN- γ were detected in the PBMC supernatants in four MS children when compared with four PS children in all experimental conditions, i.e. upon stimulation with Df, Ca or PHA ($P = 0.02$, $P = 0.0019$ and $P = 0.0002$, respectively) (Fig. 1A,B,C). IL-10 was under the level of detectability in supernatants of PBMC from all children analyzed at baseline, either after allergen- or Ca- or PHA-driven activation (not shown).

Evaluation by flow cytometry of cytokine production by allergen-specific T-cell lines derived from four

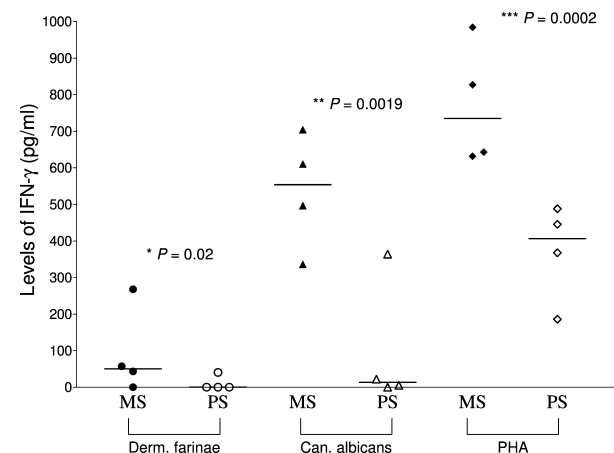


Figure 1 IFN- γ production of peripheral blood mononuclear cells from four children remaining monosensitized (MS) and four children who subsequently developed new allergic sensitization (polysensitized) after *Dermatophagoides farinae*- (A) or *Candida albicans*- (B) or phytohemagglutinin-driven (C) stimulation.

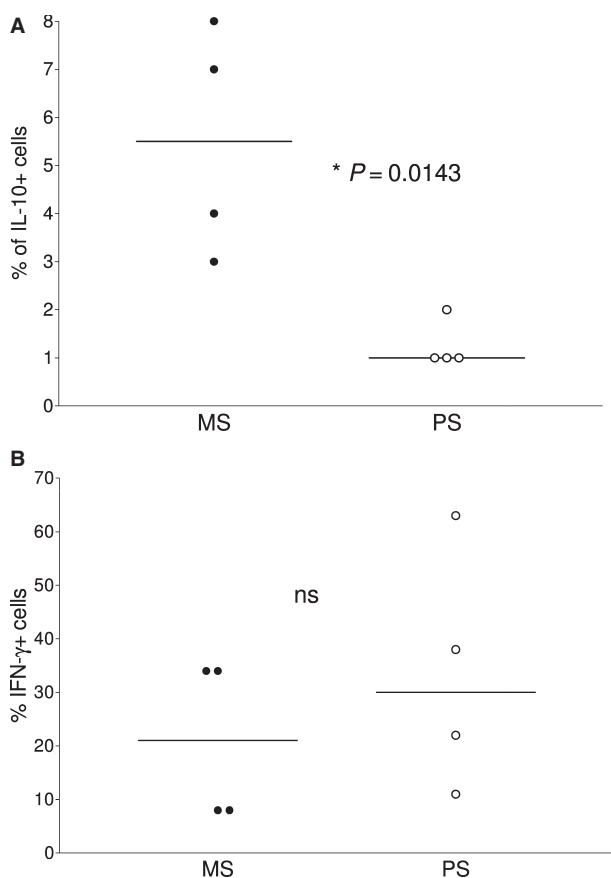


Figure 2 IL-10 (A) and IFN- γ (B) expression in *Dermatophagoides farinae*-specific T cell lines from four children remaining monosensitized (MS) and four children who subsequently developed new allergic sensitization (polysensitized). Analysis was performed by intracellular staining and flow cytometry.

MS and four PS patients demonstrated a significantly higher percentage of IL10-producing cells in MS children ($6.9 \pm 2.19\%$) than in PS children ($3.85 \pm 0.97\%$; $P = 0.0143$) (Fig. 2A). In contrast, no significant difference was found in the proportion of IFN- γ producing cells in T-cell lines generated from MS and PS (Fig. 2B).

At the follow-up visit, evaluation of cytokine production in allergen-specific T-cell lines derived from four MS and four PS patients showed again higher percentage of IL-10⁺ cells in MS children ($11 \pm 1.5\%$) than in PS children ($2.5 \pm 0.26\%$; $P < 0.0001$) and no differences in the proportion of IFN- γ ⁺ cells between MS and PS children (not shown).

Discussion

This preliminary study provides some interesting findings:

- a relevant percentage of MS children became PS in a short period (2 years), an observation that confirms previous studies and underlines this characteristic of sensitization in childhood;
 - children who remained MS had higher levels of IFN- γ in response to *Df*, *Ca* and PHA when compared with children who developed additional allergic sensitizations;
 - higher percentage of IL-10 producing cells was detected in allergen-specific T-cell lines derived from MS patients, when compared with those derived from patients who subsequently developed new sensitizations: this difference was still present 2 years later.
- These data may support the hypothesis that the development of new sensitizations may be favored by low production of some cytokines and especially of IFN- γ and IL-10 possibly related to Treg cells functional defects, a stigma of adult allergic patients (3, 5). However, this Tregs defect may be transitory, detectable at different levels and with different characteristics, when evaluating the cellular response to mitogens, recall antigens, such as *Candida*, or the relevant allergens. Tregs defects may also be reversible, either spontaneously or in response to specific therapeutic interventions, such as allergen-specific immunotherapy that may restore a physiologic Tregs function, with an increased IFN- γ and IL-10 production (7, 14).

This preliminary study provided the first evidence that children developing polysensitization might be characterized by a functional defect of IFN- γ and IL-10. However, a relevant limitation of this study is the very restricted number of evaluated children, therefore further studies should be conducted to confirm this hypothesis enrolling more numerous cohorts of MS children.

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