

INTERFERON-GAMMA MAY BE AN EARLY MARKER OF RESPONSE TO SUBLINGUAL IMMUNOTHERAPY

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Allergic rhinitis (AR) is characterized by Th2 polarized immune response. Allergen-specific subcutaneous immunotherapy may restore a physiologic Th1 profile. However, there are few studies investigating the immunological effects of sublingual immunotherapy (SLIT). The aim of this study is to investigate whether a pre-seasonal SLIT course could affect IFN- γ production. Forty-four AR patients with pollen allergy assumed pre-seasonal SLIT for 3 months. IFN- γ -specific producing cells were assessed by cytokine ELISPOT before and 3 months after the beginning of SLIT. Visual analogue scale (VAS) for symptoms and medication score was also evaluated. The frequency of IFN- γ -specific producing cells significantly increased after SLIT ($p < 0.01$), and this increase was significantly associated with improvement of both symptoms ($p < 0.001$) and medication use ($p < 0.01$). In conclusion, these results may be considered clinically relevant as SLIT treatment may induce a quick IFN- γ response that is related to clinical improvement.

Allergic rhinitis (AR) prevalence is progressively increasing worldwide (1). AR is characterized by mucosal inflammation. This allergic inflammation displays a predominant interleukin (IL)-4 production by Th2 cells over interferon (IFN)- γ expression by Th1: the so-called Th2 polarization (2).

AR causal therapy is based on allergen-specific immunotherapy (SIT) (3). SIT reduces both the allergic symptoms and the need for medications (4). These effects depend on the development of the tolerance toward the causal allergen (5). A recent WHO position paper has provided evidence that SIT is effective in patients with allergic rhinitis and mild

forms of asthma (6).

As the classical subcutaneous administration of allergen extracts (SCIT) may involve the risk of serious adverse reactions, the alternative route of sublingual immunotherapy (SLIT) has been developed (7). The clinical SLIT efficacy has been documented in several studies (6-8) and two meta-analyses on patients with AR and asthma (9-10). However, the SLIT mechanism of action is still obscure (11). On the other hand, it has been demonstrated that SCIT is capable of inducing a shift from Th2-polarization to a prevalent Th1-polarization (12-14). In contrast, only two studies

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have demonstrated that SLIT is able to increase allergen-driven IFN- γ production (15-16), whereas another two studies were conflicting (17-18).

Therefore, this study aims to investigate whether a pre-seasonal SLIT course could affect IFN- γ production in a group of patients suffering from AR due to pollen allergy.

MATERIALS AND METHODS

Patients

Forty-four patients with AR due to pollen sensitisation were included in the study which was conducted with the approval of the local Ethics Committee and carried out with the written informed consent of all participants. AR was diagnosed according to validated criteria (8). The mean age of the analyzed AR patients (25 females and 19 males) was 38.4 years (range 14-74).

The patients' blood samples were collected before SLIT, and a 3 month pre-seasonal course.

Sublingual Immunotherapy

Patients received a pollen allergen extract (Anallergo, Florence, Italy) of *Betula alba* (19 cases, the accumulate dose was 30 mcg), *Parietaria judaica* (18 cases, the accumulate dose was 12 mcg), and a mix of 7 *Graminaceae* (7 cases, the accumulate dose was 12 mcg) for at least 3 months before the pollen season (such as pre-seasonal immunotherapy). The dose in the maintenance phase was 1,000 U.RAST/ml (internal reference): five drops 3 times per week.

Clinical assessment

Allergy symptoms and the use of medication were scored at the end of the pollen season. The form included a visual analogue scale (VAS) of nasal symptoms (19). During the pollen season patients could assume lyophilised ebastine tablets (10 mg, 1 or 2) on demand as rescue medication. The medication scoring was: not at all (0), occasionally/seldom (1), occasionally/often (2), almost daily (3), continuously with the lowest dose (4), continuously with the maximal dose (5).

Frequency of IFN- γ -specific producing cells by cytokine ELISPOT

PBMC were isolated from a heparinized blood sample by Fycoll-paque (Lymphoflot, Biotest, Germany) density gradient centrifugation. The PBMC were washed with PBS and resuspended in complete medium (RPMI-1640 with 2mmol/l L-glutamine, Invitrogen) with 10% autologous plasma at 2×10^6 /ml. ELISPOT was performed in accordance with the manufacturer's

instructions. Briefly, polyvinylidene difluoride plates (Diacclone, France) were coated overnight with IFN- γ capture mAb. After saturation with RPMI-10% fetal calf serum (FCS, Invitrogen), 100 μ l of PBMC suspension was also added as. The cells were stimulated with phytoemagglutinine (PHA; 1 μ g/ml; Sigma Aldrich, Italy) as positive control, with allergen extracts used for SLIT for 20 hrs at 37°C and 5% CO₂ in humidified air. The allergen concentration of the whole extract of birch, parietaria, and grasses (kindly provided by Anallergo, Florence, Ital) in the final volume of culture medium were respectively 2.5, 1, and 1 mcg/ml, based on assessment in preliminary experiments. After incubation, the plates were washed with PBS-1% bovine serum albumine (BSA) and incubated with biotinylated detection antibody for 1 h and 30 min at room temperature (RT). After the washing, streptavidin-Alkaline phosphatase conjugate was added for 1 h at RT. Finally, BCIP/NBT was distributed in wells. Elispot assays were set in duplicate, and detection of spots representing cytokine-producing cells was performed by bioreader (BIO-SYS, Germany).

Statistical analysis

Comparisons between the number of spots before and after 3 months of SLIT were performed using a Wilcoxon test for paired data. Correlations were assessed using the Spearman rank correlation coefficient.

RESULTS

Assessment of IFN- γ -specific producing cells by cytokine ELISPOT

In Fig. 1 the median number of spots is reported for all the allergens before SLIT: the median number

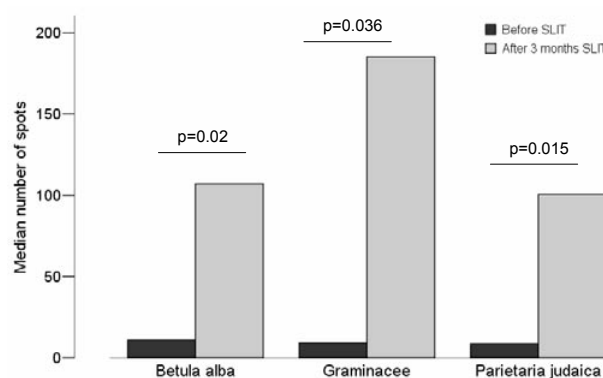


Fig. 1. Median number of spots for all the allergens before and after 3 months SPLIT.

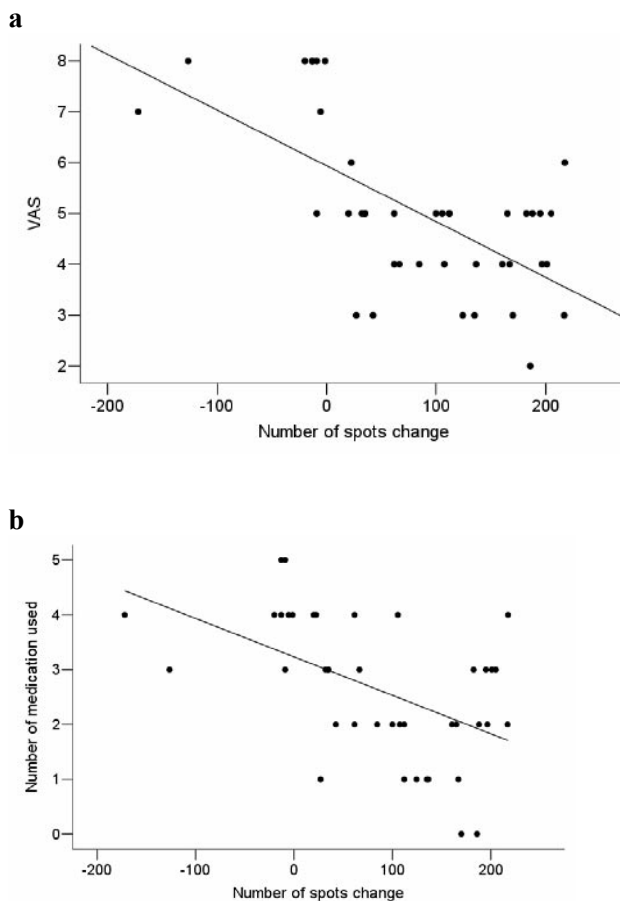


Fig. 2. Correlation between IFN- γ increase and VAS (a) and between IFN- γ increase and medication use (b).

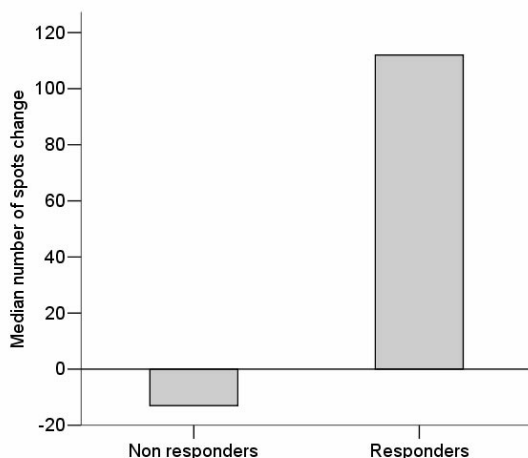


Fig. 3. Median number of spots change in non-responders and responders.

of spots is 10.5 (range 1-175) for *Betula alba*, 7.5 (range 3-13) for *Graminaceae* and 6 (range 2-130) for *Parietaria judaica* (overall median 7.5, range 1-175). After 3 months SLIT a significant spot increase is detectable both globally ($p < 0.001$) and singularly for each allergen (Fig. 1): the median number of spots after 3 months SLIT is 100 (range 1-220) for *Betula alba*, 177.5 (range 2-230) for *Graminaceae* and 100.5 (range 4-209) for *Parietaria judaica* (overall median 107.5, range 1-230). The median overall increase in the number of spots after SLIT was 100 (range -172, +217).

Relationship with clinical outcomes

A correlation between IFN- γ increase and VAS ($r = -0.56$, $p < 0.001$) and between IFN- γ increase and medication use ($r = -0.53$, $p < 0.001$) was evident during the pollen peak (Figs. 2, 3).

DISCUSSION

Allergic rhinitis is sustained by a Th2-polarized inflammation. IL-4 and IL-13 are typical Th2-dependent cytokines: in fact, they are capable of inducing, maintaining, and amplifying inflammatory allergic inflammation (20). On the other hand, Th1-related cytokines, such as IFN- γ and IL12, may suppress and counteract this Th2 polarization (21).

It has been widely reported that SIT is the unique causal treatment of AR as it is able to restore a physiologic Th1-response (22). Indeed, SCIT is associated with a decrease in IL-4 and IL-5 production by CD4⁺ Th2 cells, and induces a shift towards increased IFN- γ production by Th1 cells (12-14).

However, the SLIT mechanism of action on immune response is still obscure as few studies have been performed and are conflicting. A possible explanation of this discrepancy might depend on several methodological biases, including the age of patients, the study design, the dose of allergen extract, and the duration.

Therefore, this study was designed to investigate whether a single pre-seasonal SLIT course with pollen extract could affect the IFN- γ production.

The first finding is that within 3 months of SLIT initiation, IFN- γ producing cells significantly increased. In addition, this study shows that 80% of patients assuming SLIT had a clinical response

well-related to the increased IFN- γ production. This high percentage could depend on different factors: adequate screening of patients, their education and their motivations.

Moreover, a possible explanation of this successful immunological finding is based on the fact that the ELISPOT assay is the *ex-vivo* assay with the lowest limit of detection of IFN- γ producing T cells, allowing the reliable detection of as few as 10 specific T cells per 1 million PBMC (23-24).

In conclusion, the present study shows that SLIT is capable of inducing Th1-response directed to environmental antigens in allergic patients presenting with AR due to pollen sensitisation. In addition, SLIT induced systemic immunologic changes very rapidly which are well-related to the clinical outcomes. Therefore, these results may be considered clinically relevant as SLIT treatment induces quick Th1 response and clinical improvement. This Th1 response may also be useful to precociously identify responder patients, avoiding useless waiting. Therefore, IFN- γ evaluated by ELISPOT 3 months after SLIT beginning may be considered as an early marker of SLIT response. These preliminary findings need to be confirmed by further studies.

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