

TH1 – TH2 Response and the Atopy Risk in Patients with Reproduction Failure

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Abbreviations:

ELISA, enzyme-linked immunosorbent assay; IVF, in vitro fertilization; LIF, lymphocyte inhibitory factor; M-CSF, macrophage colony-stimulating factor; PI, primary infertility; RPL, repeated pregnancy losses; SPT, skin prick test; TH, T-helper (lymphocyte)

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Introduction

More than 15% of the European population suffers from infertility. The etiology of female reproductive failure may be very complex owing to a neuro-endocrine-immune association. The immune causes of repeated pregnancy losses and/or repeated in vitro fertilization failure have been intensively investigated.^{1–8} Dysfunction of the immune system in various diseases is frequently associated with atopy.⁹ There is not enough evidence if this association exists also between atopy and pregnancy failure or primary infertility. Cytokines are critical immunoregulatory

Problem

Enhanced TH2 activity is characteristic for atopic diseases and is observed also in physiological pregnancy. The immune causes of repeated pregnancy losses and/or repeated in vitro fertilization failure may be associated with TH2 hypoactivity. The association with frequency of atopic diseases is unclear.

Method of Study

Intracellular production of IL-4 and IFN- γ by peripheral CD4+ T lymphocytes was studied, as well as serum levels of total and allergen specific IgE. Simultaneously skin prick tests with inhalant allergens were performed, and clinical features of atopy were registered by means of a questionnaire.

Results

Lower intracellular production of IL-4 by peripheral CD4+ T cells and lower frequency of elevated total and allergen specific IgE were found in women with reproduction failure compared to controls, as well as lower frequency of some symptoms possibly associated with atopy.

Conclusion

Our study showed the presence of TH2 hypoactivity in women with reproduction failure, which may be associated with lower occurrence of atopic diseases.

molecules, responsible for determining the nature of immune response. It has been proposed that the lower index of the TH1/TH2 immune response is supportive for physiological pregnancy,^{10,11} while increased TH1 immune response is associated with recurrent miscarriage.^{12,13} As cytokine production is partly under genetic control, it is possible that women suffering from a high incidence of abortions are genetically predisposed to mount a type of immune response inappropriate for pregnancy maintenance.^{14–19}

Saito¹¹ compared the distribution of TH1, TH2 and TH0 cell ratios in human peripheral and endometrial

T-cells. Piccinni²⁰ demonstrated the role of hormone-controlled level of the T-cell cytokines in the maintenance of pregnancy. She also showed²¹ that the simple activity of progesterone influencing TH1 type cytokines, which promote allograft rejection, may compromise pregnancy, whereas the TH2 type cytokines, by inhibiting TH1 response, promote allograft tolerance and therefore may improve fetal survival. However, TH1 cytokines are not always detrimental for pregnancy development. TH1 cytokines, depending on their time of expression, stage of gestation and relative concentrations, could have a positive role in a successful pregnancy. This proposition is partially in contrast with the published facts that female infertile patients have mainly an elevation of TH1 cytokine production.² Is the increase of the TH2 cytokines in the reproduction organs really a good immunological prognostic marker for physiological pregnancy? And what about the relation of atopic disorders and pregnancy? Atopy is a complex disease with a genetic predisposition to specific IgE production and allergic inflammation being caused by the infiltration of eosinophils, which depends on TH2 lymphocytes, producing mainly IL-3, IL-4 and IL-5 cytokines.^{9,22} We studied IL-4 and IFN- γ intracellular production by peripheral CD4+ TH cells, total and specific IgE levels and positivity of skin prick tests in infertile patients with or without atopic symptoms, in comparison with the healthy control group.

Patients and methods

A total of 42 patients (P) aged 26–41 years (mean age 31 years) were divided into two groups: (1) RPL - 13 patients with repeated pregnancy losses (3 or more), in seven of them antiphospholipid syndrome was proved. (2) PI - 29 patients with repeated (three or more) in vitro fertilization failures, never pregnant, assigned as primarily infertile women. Control group (C) was created by 21 healthy fertile women aged 23–40 years (mean age 25), with two and more children.

Methods

Detection of intracellular IL-4 and IFN- γ production
This was performed according to Prussin.²³

Cell isolation: Peripheral blood mononuclear cells were isolated by means of centrifugation on a den-

sity gradient using Ficoll® (Sigma, Steinheim, Germany).

Cell stimulation and analysis: These cells were examined by means of the Cytodetect Kit® (IQ Products, Groningen, The Netherlands). After isolation and resuspension in RPMI 1640 medium, lymphocytes in concentration of 1×10^6 cells/mL were incubated 5 hr with stimulating reagents phorbol-myristate-acetate, monensin and ionomycin. After fixation and washing, cells were resuspended in HBSS. Fluorescein labeled monoclonal antibodies anti-CD45, anti-CD14, anti-CD3 or anti-CD4 were added to the cell suspension. After incubation, the cell membrane was permeabilized and fluorescein labeled antibody anti-IFN- γ or anti-IL-4 was added for staining of the intracellular cytokines.

Analysis of CD4+/IFN- γ + or CD4+/IL-4+ T cells was performed by means of a two-color FACSCAN® cytometer, Cellquest® software (Becton Dickinson, San Jose, CA, USA). Percentages of CD4+/IL-4+ or CD4+/IFN- γ + T-cells were calculated. See Fig. 1.

Measurement of serum total and specific IgE

Immediately after blood collection, serum was separated and stored at -20°C until testing. Total IgE was measured with a latex nephelometry analyzer (Dade-Behring, Marburg, Germany). Levels above 100 IU/mL were considered as elevated. Specific IgE, to a mixture of common inhalant allergens was detected by means of a fluoroenzymimmunoassay – Phadia-top®, UniCAP® (Phadia, Uppsala, Sweden). Levels above 0.7 IU/mL were considered to be positive.

Skin prick tests with inhalant allergens

This was performed using Alyostal prick® (Stallergenes, Antony, France). The following allergens were tested: House dust mites (*Dermatophagoides pteronyssinus* and *farinae*), mixture of birch tree pollens, mixture of grass pollens, dog, cat, and molds (*Alternaria*, *Aspergillus*, *Cladosporium*). The Wheal reaction of a minimum diameter of 3 mm was considered as positive reaction.

Questionnaire concerning the presence of possible atopy symptoms

All the women answered a questionnaire concerning the presence of possible atopy symptoms in their personal history (23 questions concerning

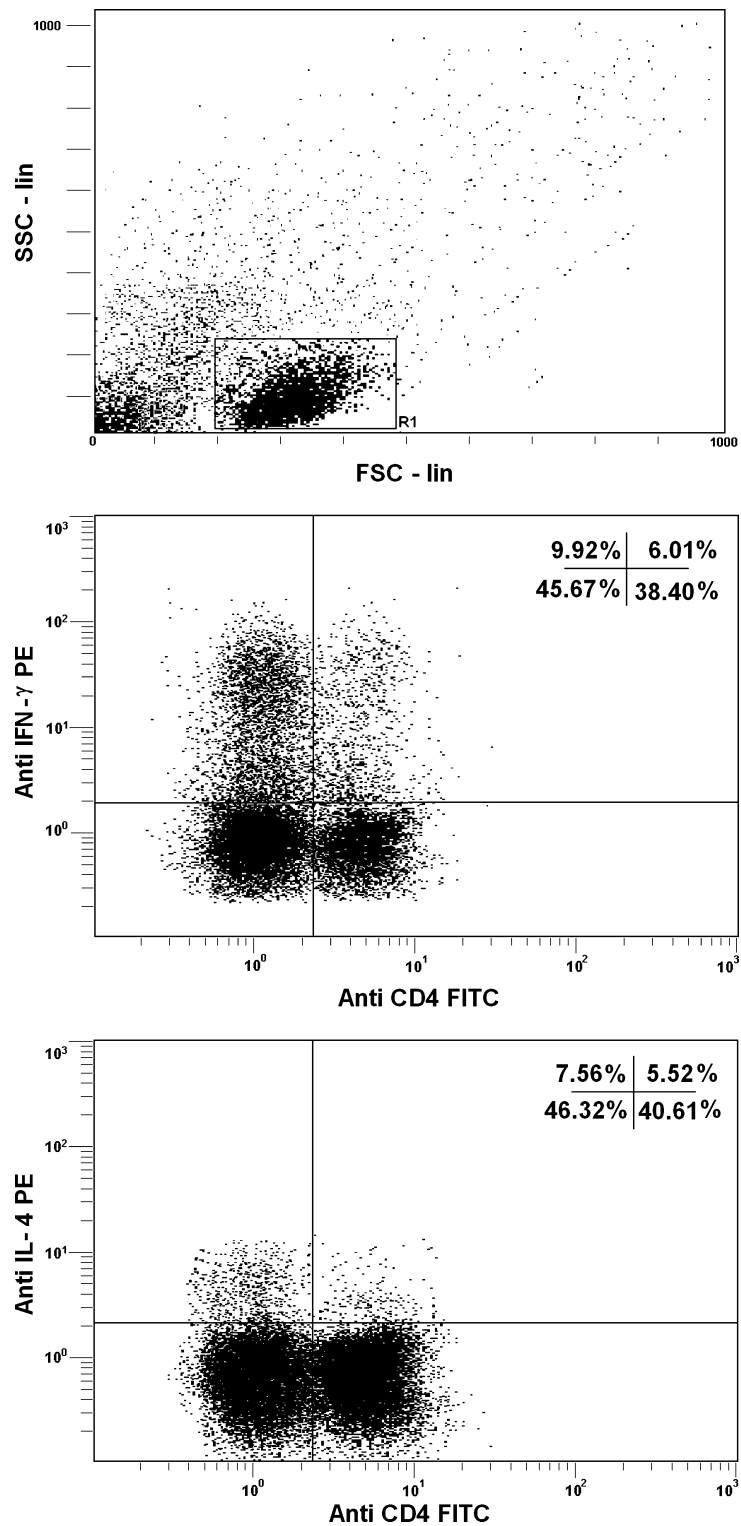


Fig. 1 Dot – Plots of IFN- γ and IL-4 Production by TH cells.

occurrence of seasonal or perennial rhinitis, conjunctivitis, sneezing, itching, bronchial asthma symptoms as cough, wheezing or dyspnea, and skin allergy symptoms like urticaria, atopic dermatitis or Quincke edema, allergic reaction after insect stings or food). Only unambiguous answers (yes or no) were assessed.

Statistical Analysis

Comparisons of IL-4 and IFN- γ production by CD4+ T cells were performed by means of Kruskal–Wallis test and comparison of IL-4/IFN- γ index by means of Wilcoxon test. Frequencies of the elevated total IgE, frequencies of the positivity of specific IgE and questionnaire results were analyzed by means of the Fisher's test. Skin prick tests positivities were analyzed by means of chi-square test.

Results

IL-4 and IFN- γ Production

IL-4 production

Intracellular production of IL-4 was detected on the average in 13.3% (S.D. 12.7, median 9.9) of CD4+ T lymphocytes in group P, in 5.0% (S.D. 5.7, median 1.6) in subgroup RPL and in 15.5% (S.D. 13.5, median 11.4) in subgroup PI, in comparison to 31.1% (S.D. 20.1, median 35.2) in group C. The differences of P versus C and RPL versus C were statistically significant ($P = 0.021$, $P = 0.021$ respectively). See Fig. 2.

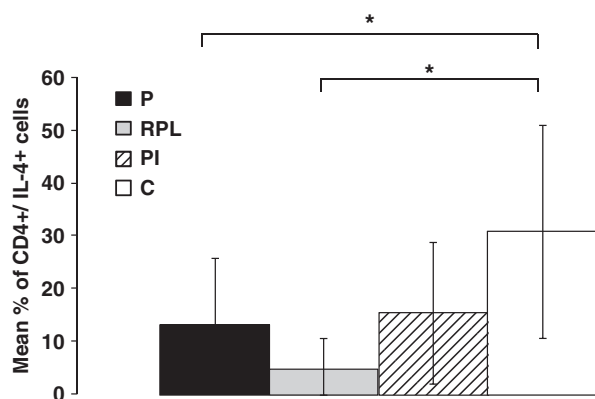


Fig. 2 Intracellular Production of IL-4.

IFN- γ production

No significant differences in groups P, RPL, PI, and C concerning intracellular production of IFN- γ were detected. Intracellular production of IFN- γ was detected on the average in 10.8% (S.D. 5.9, median 9.6) of CD4+ T lymphocytes in group P, in 11.4% (S.D. 6.3, median 10.1) in subgroup RPL, in 10.4% (S.D. 5.8, median 9.1) in subgroup PI and in 8.6% (S.D. 3.2, median 8.0) in group C. See Fig. 3.

Index of the number of cells producing cytokines

This IL-4/IFN- γ was on the average 1.7 (S.D. 1.9, median 1.1) in group P, 0.4 (S.D. 0.5, median 0.2) in subgroup RPL and 2.0 (S.D. 2.1, median 1.2) in subgroup PI in comparison to 5.0 (S.D. 3.4, median 5.2) in healthy controls C. Significant differences were found between groups P and C as well as RPL and C (more pronounced) ($P = 0.003$, $P = 0.009$ respectively). See Fig. 4.

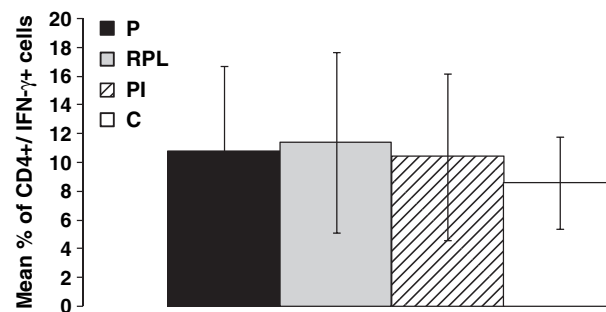


Fig. 3 Intracellular Production of IFN- γ .

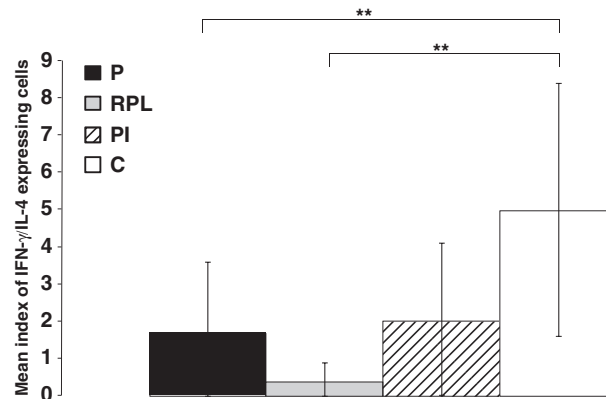
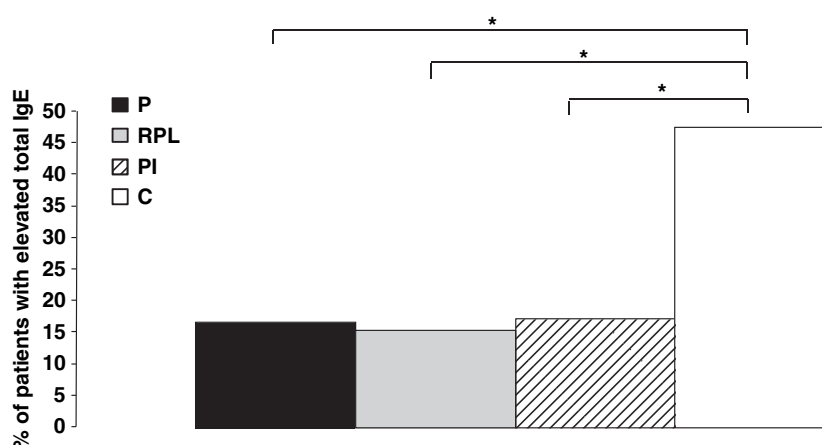


Fig. 4 Proportion of CD4+ IL-4+ & CD4+ IFN- γ + cells.

Fig. 5 Frequency of total IgE higher than 100 IU/mL.



Total and Specific Serum IgE

Total IgE levels

The levels of total serum IgE were found as follow: Group P mean 72 IU/mL (S.D. 133, median 29.0), subgroup RPL mean 51 IU/mL (S.D. 43, median 27.0), subgroup PI mean 79 IU/mL (S.D. 154, median 31.0), and the control group C mean 120 IU/mL (S.D. 115, median 88.0). No statistical differences among groups were found. When comparing frequency of elevated levels of total serum IgE, significantly lower frequency was found in the group P (16.7%) compared to controls C (47.6%) ($P = 0.033$). Similarly lower frequencies were found in subgroups RPL (15.4%) ($P = 0.041$) and PI (17.2%) ($P = 0.038$) compared to controls. See Fig. 5.

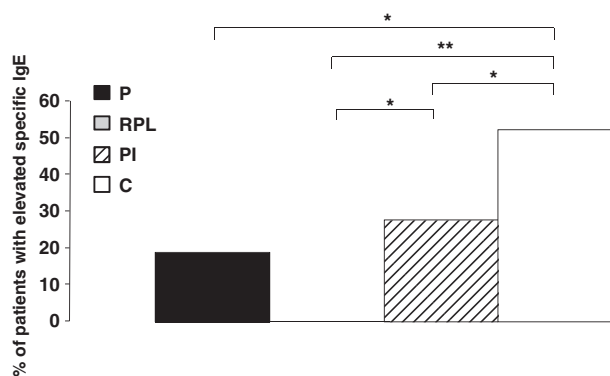


Fig. 6 Frequency of specific IgE (Phadiatop®) higher than 0.7 IU/mL.

Specific IgE levels

The mean levels of specific IgE (Phadiatop®) in group P was 1.1 IU/mL (S.D. 3.6, median 0.0), in RPL 0.3 IU/mL (S.D. 0.6, median 0.0) and in PI 1.5 IU/mL (S.D. 4.2, median 0.0). The tendency to higher levels in control group C (mean 8.7 IU/mL, S.D. 11.1, median 1.1) did not reach the statistical significance. The frequency of Phadiatop® positivity was significantly lower in group P (19.0%) compared to controls C (52.4%) ($P = 0.039$). Similarly lower frequencies of positivity were seen in both subgroups – RPL (0%) ($P = 0.008$) and PI (27.6%) ($P = 0.048$) compared to controls. More, significant difference was found also in comparison of subgroup RPL to subgroup PI ($P = 0.032$). See Fig. 6.

Skin Prick Tests (SPT)

Wheal reaction at least 3 mm in diameter was considered as a positive reaction. Patients who showed a positive reaction to at least one tested allergen were considered to be positive in SPT.

Skin prick tests were positive in 71% of patients in group P, in 69% of patients in subgroup RPL, in 72% of patients in subgroup PI and in 65% of women in group C. No significant differences were found between the groups. See Fig. 7.

Questionnaire

All participants were asked to fill in the questionnaire concerning atopic symptoms in their history. In the group P and in both subgroups RPL and PI, less

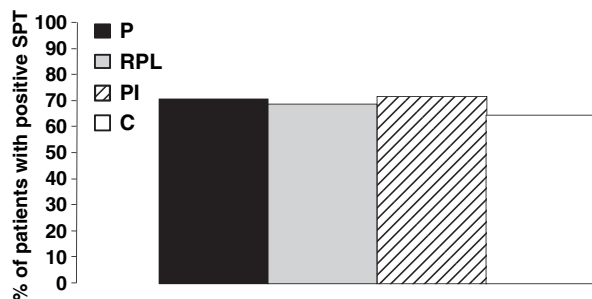


Fig. 7 Frequency of at least one Spt wheal ≥ 3 mm.

positive answers concerning chronic rhinitis and conjunctivitis were noted in comparison to the controls C. Positive answers for rhinitis were noted in 15% of the patients in group P ($P = 0.002$), 0% in subgroup RPL ($P = 0.002$), and in 21% in subgroup PI ($P = 0.037$) in comparison to 53% in group C (controls). Similar differences were found in symptoms of conjunctivitis—positive answers for conjunctivitis were noted in 17% of patients in group P ($P = 0.014$), 0% in subgroup RPL ($P = 0.001$), and in 24% in subgroup PI (NS) in comparison to 47% in group C. Difference between subgroups RPL and PI was also significant ($P = 0.012$). Positive answers for cough or asthma were noted in 10% of patients in group P, 8% in subgroup RPL, 10% in subgroup PI, and 26% in group C. Positive answers for dermatitis or edema were noted in 14% of patients in group P, 38% in subgroup RPL, 3% in subgroup PI, and 47% in group C. None of these differences was significant. See Fig. 8.

Discussion

Primary infertility and repeated pregnancy losses present a big problem in the world, including the

highly developed countries. At least some of these disorders may be immunologically determined. The genetic predisposition for cytokine production has been determined, and its influence on certain patients suffering from high incidence of abortions is proved to be directed by the genetic controlling system.^{4,14} Some authors^{12,16} believe that only TH1 type of the immune response is associated with recurrent miscarriages, in contrast to our results which also point to possible role of TH2 hypoactivity, because we observed the lower percentage of the peripheral CD4+/IL-4+ T cells in women with reproduction failure. Simultaneously, we did not observe any differences in the percentage of the peripheral CD4+/IFN- γ + T cells, IFN- γ being the most important marker of TH1 response. We also observed other TH2 hyperactivity markers to be associated with reproduction failure like elevated total and specific IgE, more frequent atopy symptoms and positive allergy skin prick tests. Our findings of elevated total and allergen specific IgE levels in patients slightly differ from the results of other studies.^{24–26} This difference may be due to the fact that we studied a group of patients with idiopathic reproduction failure without any further immunologic abnormalities, which was not the case in the above mentioned studies (e.g. women with anti-sperm antibodies were excluded from our study not being regarded as idiopathic reproduction failure).

It is known that in normal pregnancy immune response is more TH2-like, which protects the fetus from being rejected. Ekerfeld and others^{13,18,19,21,27} expect that significantly larger numbers of IL-4-secreting cells in the second and third trimesters of pregnancy can be even more induced by paternal leukocytes compared to unrelated leukocytes. The

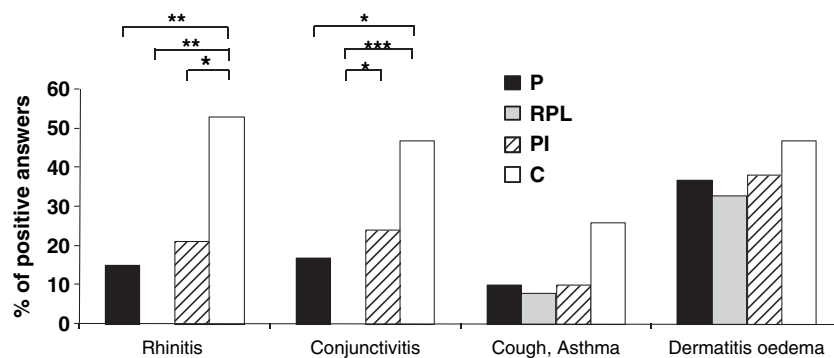


Fig. 8 Atopy Symptoms (rhinitis, conjunctivitis, cough & asthma, dermatitis and oedema) in personal history.

immune deviation toward TH2, which may protect the fetus from rejection, is an important homeostatic mechanism in normal pregnancies.

On the other hand, Piccinni et al.^{20,21} showed that TH1 cytokines, depending on their time of expression, stage of gestation and relative concentrations, could have a positive role in a successful pregnancy. But also other cytokines (LIF, M-CSF) produced by T cells seem to be important for the maintenance of the pregnancy. Hormones present in the microenvironment of the decidual T cells could be responsible, at least in part, for the cytokine profile of the T cells.¹¹ Immunomodulatory properties of progesterone play an important role as a potent inducer of TH2 type cytokines (e.g. IL-4 and IL-5), LIF and M-CSF production by T cells, whereas relaxin induces T cells to produce IFN- γ .²⁰ Pregnancy depends on many mechanisms induced by different types of cells. TH2 cells, specific and non-specific antibodies, idiotype, and anti-idiotype networks could make a good attribution to a prosperous pregnancy or pregnancies.^{3–7} Routine and relatively simple examination of sperm and zona pellucida antibodies, profile of antiphospholipid antibodies, and an examination of thrombophilic factors are also important and very useful indicators in human infertility.^{4–8}

Conclusions

Our study showed the presence of TH2 hypoactivity in women with reproduction failure, which is also connected with a lower load of atopy markers and symptoms. The percentage of the peripheral CD4+/IL-4+ T cells, total and specific IgE and atopy symptoms in the personal history were lower in women with reproduction failure than in healthy women. The differences were generally more pronounced in the subgroup of repeated pregnancy losses than in the subgroup of primary infertility.

We did not find any difference between the intracellular productions of TH1 cytokine IFN- γ by CD4+ peripheral T cells, however, the level of the TH2 cytokine IL-4 had a notable impact on the TH2/TH1 index IL-4/IFN- γ .

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Competing Interests

The authors declare that they have no conflict of interests.

Authors' Contributions

All authors have read and approved the final version of the manuscript.

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