

DENDRITIC CELLS INDUCED TH1/TH2 CYTOKINE BALANCE IN RESPONSE TO ALLERGEN CHALLENGE

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REZUMAT

Objective: Evaluarea comparativă a efectului lui Derp1, componenta alergenica majoră a acarianului prezent în praful de casa, Dermatophagoides pteronyssinus (Derp), asupra producției de citokine de tip Th2 de către celulele dendritice diferențiate din monocite provenite de la pacienții alergici la praful de casa și de la subiecții sanatoși. **Material și metodă:** Din sângele venos periferic al pacienților incluși în studiu am izolat monocite, din care am diferențiat celule dendritice utilizând GM-CSF și IL-4. Celulele dendritice izolate au fost stimulate cu Derp1 sau LPS, utilizând ca control al maturării acestora. Supernatantul de cultură a fost analizat în vederea prezentei IL-10 și IL-12. Celulele dendritice stimulate au fost co-cultivate împreună cu limfocite T CD4+ naive autologe, iar în supernatantul de cultură s-a analizat prezenta IL-4 și IFN- γ . **Rezultate:** Derp1 induce o expresie crescută a CD86 și CD83 pe suprafața celulelor dendritice provenite de la pacienții alergici și se asociază cu o producție crescută a acestora de IL-10 (58.81 ± 14.48 pg/ml), un promotor al răspunsului de tip Th2. Limfocitele T CD4+ naive autolog stimulate de către celulele dendritice pulsate cu Derp1 produc preferențial IL-4 (15.53 ± 8.38 pg/ml) comparativ cu IFN- γ (8.47 ± 2.54 pg/ml). În contrast cu pacienții alergici, limfocitele T provenite de la pacienții sanatoși prezintă o secreție semnificativ crescută de IFN- γ (32.71 ± 10.73 pg/ml) ($p < 0.001$). **Concluzii:** Expresia markerilor de suprafață a celulelor dendritice este diferită în funcție de tipul de Derp1 – CD86 și CD83 la pacienții alergici, respectiv CD80 la subiecții sanatoși. Limfocitele T naiv stimulate de către celulele dendritice pulsate cu Derp1 produc cantități mai mari de IL-4 comparativ cu cele de la pacienții sanatoși. Modularea răspunsului imunitar definește balanța între alergii și toleranță și sunt strâns dependente de statutul imun individual. **Cuvinte cheie:** alergii, celule dendritice, citokine, limfocite T CD4+, răspuns Th1/Th2

ABSTRACT

Objective: To evaluate the effect of Derp1, the major allergen of the house dust mite Dermatophagoides pteronyssinus (Derp), on Th2 cytokine production in coculture of naïve T cells and monocyte-derived DCs from Derp allergic patients and healthy donors. **Material and methods:** CD14+ monocytes were isolated from venous peripheral blood and differentiated to DCs using GM-CSF and IL-4. Generated DCs were pulsed with Derp1 or LPS, as a control of DC maturation. DCs supernatants were assayed for IL-10 and IL-12 production. Pulsed DCs were cocultured with autologous naïve CD4+ T cells and the supernatant was assayed for IL-4 and IFN- γ . **Results:** Derp1 up-regulates CD86 and CD83 expression on DCs from allergic patients and is associated with a higher production of IL-10 (58.81 ± 14.48 pg/ml), a promoter of Th2 response. Naïve T cells from allergic patients stimulated by autologous Derp1 pulsed DCs preferentially produced IL-4 (15.53 ± 8.38 pg/ml) rather than IFN- γ (8.47 ± 2.54 pg/ml). In contrast, T cells from healthy donors secreted significantly higher levels of IFN- γ (32.71 ± 10.73 pg/ml) ($p < 0.001$). **Conclusions:** Derp1 up-regulates different surface markers expression on DCs – CD86 and CD83 in allergic patients, and CD80 in healthy donors. Autologous naïve T cells from allergic patients in coculture with Derp1-pulsed DCs induced higher IL-4 production than in healthy donors. The modulation of immune response and the establishment of the balance between allergy and tolerance against an allergen are close dependent on the immune status of the individual. **Key Words:** allergy, dendritic cells, cytokines, CD4+ T cells, Th1/Th2 response

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INTRODUCTION

Allergic diseases are inflammatory disorders characterized especially by accumulation of eosinophils, T cells and mast cell. The inflammation is controlled by T-helper type 2 cells (Th2) secreting cytokines such as interleukin IL-4, IL-5, and IL-13, which orchestrate key features of allergy. The role of individual chemokines and chemokine receptors in the pathogenesis of allergic inflammation is often uncertain. T cells are

unable to respond to antigen independently of antigen-presenting cells (APCs), particularly dendritic cells (DCs), whose involvement in Th2 immunity is highly relevant to allergic sensitization.¹

The physiologic role of DCs is to perform a continuous survey of the antigen-exposed sites of the body, and to orientate the response to a certain antigen towards an immunogenic or a tolerogenic pathway. Any inaccuracy in their function can lead either to a misbalanced immune defense response or to allergy, even the development of autoimmunity.^{1,2} DCs play an important role in the pathogenesis of allergic disorders through their ability to interact with T cells, which lead to initiation and amplification of Th 2 immune responses. Due to their location, immediately above the basement membrane of the airways epithelium, DCs have direct contact with incoming antigens.² Mucosal DCs are immature DCs, with high endocytic and antigen processing activity and a weak capability to activate T-cells. After antigen uptake, they lose their capacity to take up further antigens, they become mature DCs, and carry processed antigen from nonlymphoid tissues to the draining lymph nodes, where they become able to stimulate naive T cells. The result will be the proliferation of antigen-specific T cells, which differentiate into cytokine-producing effectors T cells, able to recirculate to the target tissues and to induce the immune response polarization toward a Th1 or a Th2.^{3,4}

Differentiation into Th1 or Th2 response depends on many factors like : the nature and the dose of the antigen, the route of exposure, the genetic background of the individual, the type of costimulatory molecules expressed on the surface of the DCs, the polarizing cytokines microenvironment during antigen presentation.

Depending on their origin and their capacity to induce preferentially, the differentiation of naive T cells into Th1 and Th2 effectors cells, there were described two subclasses of DCs - DC1 and DC2. Myeloid DCs (M-DC), derived from the bone marrow, are able to induce Th1 responses, whereas plasmacytoid DCs (P-DC), with lymphoid origin, induce Th2 responses.⁵ Allergic asthmatic patients seem to have more plasmacytoid DCs in peripheral blood compared with healthy individuals and this could be related with the Th2-dominant phenotype in asthma.⁶

It was shown also that the type of costimulatory molecules expressed on the DCs surface is essential for determining Th differentiation. The role of CD86 molecule seems to be more important than CD80 in allergic diseases development, based on its ability to induce the Th2 response. In contrast, CD80 is

preferentially associated with Th1 type responses.⁷ It was observed that B cells from patients with atopic dermatitis express CD86, and this expression is correlated with the total serum IgE level.⁸

The orientation toward Th1 or Th2 profile is also dependent on the prevalence of IL-10 or IL-12 secreted by DCs, as polarizing cytokines during the DCs - T cells interaction. It is postulated that IL-10 inhibits type 1 response, thus promoting a Th2 response, while IL-12 favours Th1 responses, by enhancing IFN- γ production by T cells.⁹ In contrast, it was demonstrated that low levels of IL-12 promote a type 2 response.¹⁰ Based on these data, it is possible to obtain a different Th response dependent on IL-12 production, which can vary genetically and with age.

The aim of the study was to evaluate the comparative effect of Der p1, one of the major allergens of the house dust mite *Dermatophagoides pteronyssinus* (Der p), and LPS - the major component of the outer membrane of Gram-negative bacteria, on monocyte-derived DCs obtained from patients sensitive to Der p and from healthy donors.

MATERIAL AND METHODS

Study groups

The study included two groups of donors: house dust mite allergic patients (n = 8) and healthy donors (n = 7) with age between 22 and 60 years (39.46 ± 13.65 years). Allergic patients suffered for allergic rhinitis \pm allergic asthma defined according to ARIA criteria and GINA criteria, respectively^{11,12}. House dust mite allergic patients were characterized by highly positive skin prick test for Der p (wheal bigger than histamine response \pm pseudopodes), total IgE serum level between 199.7 and 806.2 UI/ml (502.4 ± 186.87 UI/ml) and specific IgE anti-Der p in class 4 or above, while in healthy donors skin prick test was negative, and total IgE serum level ranged between 18.3 and 145.9 UI/ml (66.98 ± 53.49 UI/ml). (Table 1) Participants in this study had not used antihistaminics and corticosteroids for at least 4 weeks before study entry. Any other medication was discontinued 12 h prior to blood sampling. The healthy donors were non-allergic and non-asthmatic, with normal lung function and no history of respiratory diseases.

Differentiation of DCs

Peripheral venous blood was collected from house dust mite allergic patients (n = 8) and healthy donors (n = 7). After platelet-rich plasma depletion, blood cells were diluted in RPMI 1640 (Promocell) and layered over a Ficoll gradient. After centrifugation (4000 rpm, 15 minutes), peripheral blood mononuclear

cells (PBMCs) were harvested, washed 3x with RPMI 1640, centrifugated (1500 rpm, 5 min) and suspended in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 1% L-glutamine, 1% Pen/Strep. Purified PBMCs (5×10^6 /ml) were incubated into 6-well flat-bottomed culture plates at 37°C, 5% CO₂ for 90 minutes for aderation. Adherent cells were cultured for 7 days into 6-well culture plates in RPMI supplemented with 10% FCS, 1% L-glutamine and 1% Pen/Strep and in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) (1000 U/ml) and IL-4 (400 U/ml) (R&D Systems).

Table 1. Characteristics of the study participants.

| | Allergic patients (n=8) | Healthy donors (n=7) |
|---------------------|-------------------------|----------------------|
| Age (years) | 39.37±15.07 | 38.42±13.43 |
| Sex (M/F) | 6/2 | 4/3 |
| TotalserumIgE(U/ml) | 502.40±186.87 | 66.98±53.49 |

Data are mean ± Std. Dev.

Stimulation of immature DCs with Der p 1 and LPS

Immature DCs were pulsed for 24 hours with 10ng/mL Der p 1 (Genway Biotech.) or with 1µg/mL LPS (Sigma), as a control of DC maturation.

DC surface marker analysis (phenotypic analysis)

Cells were collected, washed 2x in standard RPMI, centrifuged (1600 rpm, 4 min) and re-suspended in PBS adjusting the concentration at 1×10^5 cell/ml. They were incubated for 15 minutes with different monoclonal antibodies (mAbs): anti-CD14, anti-HLA-DR, anti-CD83, anti-CD80, anti-CD86, anti-CD1a (BD Biosciences). After washing, the cells were analyzed using a FACS Sort (Software CellQuestPro).

T cells proliferation assay

The negative cells from blood samples were collected, washed, marked with CFSE according to manufactured protocol and added into the wells containing allogenic DCs pulsed with Der p 1 or LPS (ratio 1:10) at 37°C and 5%CO₂ in RPMI supplemented with 10% FCS, 1% L-glutamine and 1% Pen/Strep for seven days.

Cytokine profile secreted by DCs assay

Supernatants of DCs pulsed or not with Der p 1 or LPS were harvested after 24 hours and assayed for the presence of IL-10, and IL-12 by specific enzyme-linked immunosorbent assay (ELISA) using Eli-pairs (R&D Systems). The sensitivity of cytokine detection was 0.5 pg/ml.

Purification of autologous naive CD4+ T cells

Naïve CD4+ T cells were isolated by depletion of non-T helper cells and memory CD4+ T cells from PBMCs, using an indirect magnetic labelling system with a cocktail of biotin-conjugated monoclonal antibodies (Miltenyi Biotec).

DCs-naïve T cells cocultures

Generated DCs from allergic patients or from healthy donors previously pulsed or not with Der p 1 or LPS for 24 hours were cocultured with the autologous naive CD4+ T cells (ratio 1:10). After 24 or 48 hours of coculture, supernatants were harvested and assayed for IL-4 and IFN-γ production using a specific ELISA (R&D Systems). The sensitivity of detection of IL-4 and IFN-γ was 0.22 pg/ml and 8 pg/ml, respectively.

Statistical analysis

Statistical analysis of DC surface markers and cytokine production were performed using Student t test. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Effect of Der p 1 on the expression of DC surface markers

We analyzed the expression of DC markers CD1a, CD11c and HLA-DR and the costimulatory molecules CD80, CD86 and CD83. Immature DCs, obtained after 7 days of culture, had the same characteristics in both healthy donors and allergic patients: high expression of CD1a and CD11c, and low expression of HLA-DR. Mature DCs were characterized by a very intense expression of HLA-DR, CD80 and CD86 (HLA-DR⁺⁺⁺/CD80-CD86⁺⁺⁺), an intense expression of CD83 and CD1a (CD83⁺⁺/CD1a⁺⁺) and a low expression of CD14 (CD14⁻). (Fig. 1) However, CD83 expression was visible lower on Der p 1-pulsed DCs when compared with LPS-pulsed DCs.

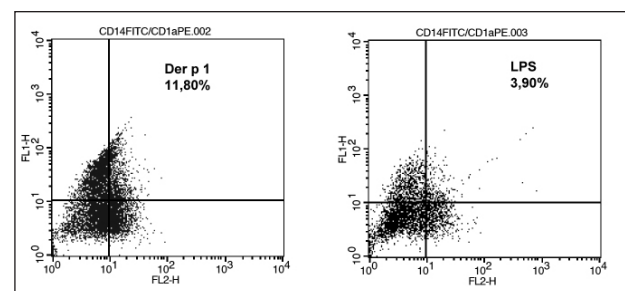


Figure 1. CD14/CD1a expression on the surface of DCs matured with Der p 1, LPS respectively.

Der p 1 exposure induced an up-regulates CD80 expression on DCs in healthy donors and CD86 and CD83 expression on DCs in house dust mite allergic patients. (Fig. 2)

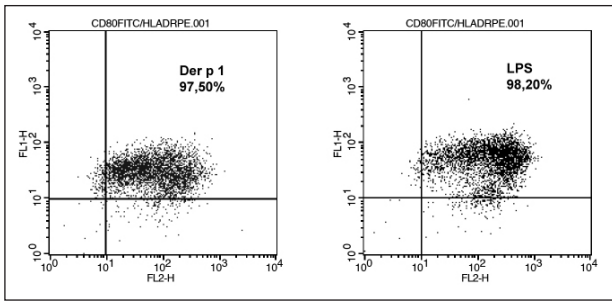


Figure 2. CD80/HLA-DR expression on the surface of DCs matured with Der p 1, LPS respectively.

In contrast, LPS enhances CD80, CD86 and CD83 expression in both groups. (Fig. 3)

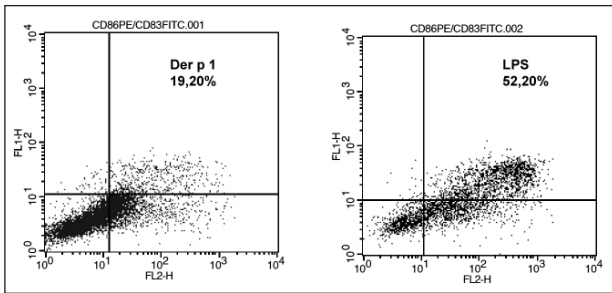


Figure 3. CD86/CD83 expression on the surface of DCs matured with Der p 1, LPS respectively.

T cells proliferation assay

Pulsed DCs have an intense capacity to induce the *in vitro* autologous T-cells proliferation. LPS induced a significant higher T cell proliferation (75.80%) than Der p 1 (57.08%) in both allergic patients and healthy donors. (Fig. 4) When compared T-cells proliferate response induced by Der p 1-pulsed DCs from allergic patients, it was higher than the one observed healthy donors. (Fig. 5)

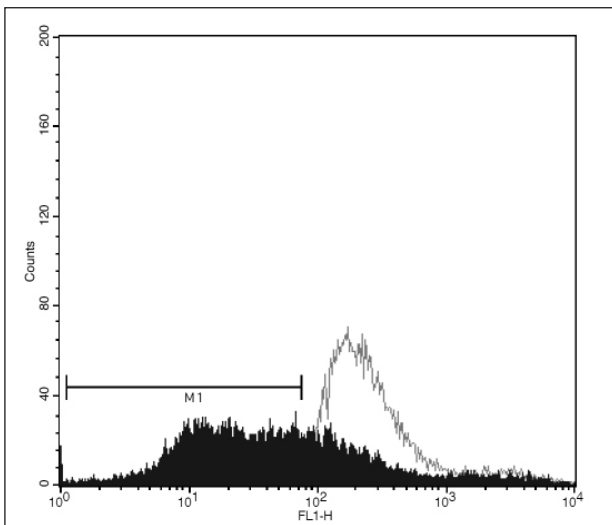


Figure 4. T-cells proliferative response induced by Der p 1-pulsed DCs, represented as filled histogram (57.08%) vs. controls (T cells + PHA), represented as empty histogram.

IL-10 and IL-12 production by pulsed DCs

IL-10 and IL-12 are known to play a very important role in the orientation of the immune modulation and because of that it was evaluated their production as a DCs response at antigen pulsation.

Der p 1 pulsed-DCs from house dust mite allergic patients produced higher levels of IL-10 (58.81 ± 14.48 pg/ml) in comparison with IL-10 production of the DCs from healthy donors (18.12 ± 16.24 pg/ml). (Table 2) In contrast with the high IL-10 production, Der p 1 pulsed-DCs produced low amounts of IL-12 (0.28 ± 0.09 pg/ml), especially in allergic patients.

Table 2. Cytokine productions after pulsation with Der p 1 or LPS.

| | IL-10 (pg/ml) | | IL-12 (pg/ml) | |
|-------------------|-------------------|---------------------|-----------------|------------------|
| | Der p 1 | LPS | Der p 1 | LPS |
| Allergic patients | 58.81 ± 14.48 | 40.53 ± 16.98 | 0.28 ± 0.09 | 9.32 ± 6.85 |
| Healthy donors | 18.12 ± 16.24 | 218.46 ± 116.43 | 1.03 ± 1.19 | 12.47 ± 6.13 |

Data are mean \pm Std. Dev.

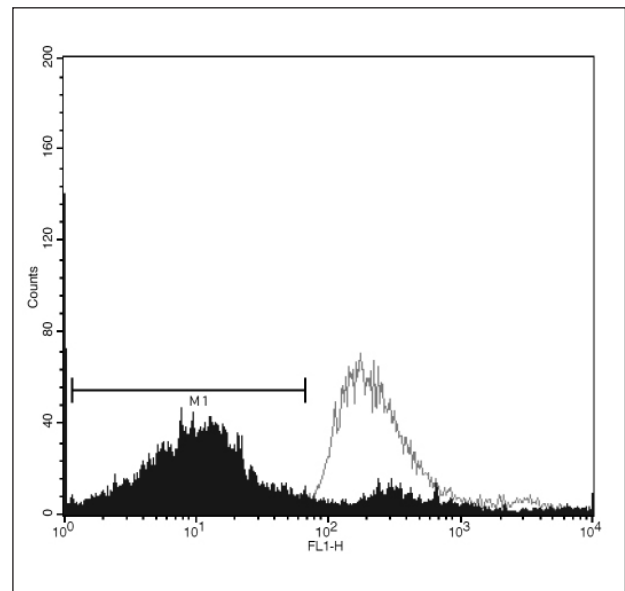


Figure 5. T-cells proliferative response induced by LPS-pulsed DCs, represented as filled histogram (75.80%) vs. controls (T cells + PHA), represented as empty histogram.

LPS stimulation induced in both groups a significantly increased production of IL-10 ($p < 0.01$), and IL-12 ($p < 0.001$). (Fig. 6)

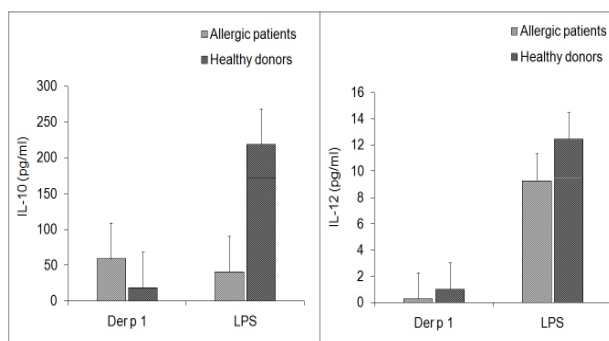


Figure 6. IL-10 and IL-12 production by DCs from house dust mite allergic patients and from healthy donors pulsed with Der p 1 (10 ng/ml) or LPS (1 µg/ml) for 24 h. The levels were evaluated by ELISA and results are expressed as the mean ± std. dev. $P < 0.05$.

Production of IL-4 and IFN-γ after autologous T cells stimulation

In allergic patients, autologous T cells in coculture with Der p 1-pulsed DCs produced higher levels of IL-4 (15.53 ± 8.38 pg/ml) when compared to healthy donors and low IFN-γ production (8.47 ± 2.54 pg/ml), indicating the DCs capacity to modulate the immune response towards the Th2 type in allergy. (Fig. 7) In contrast with allergic patients, T cells from healthy donors increase extremely significantly their production of IFN-γ ($p < 0.001$).

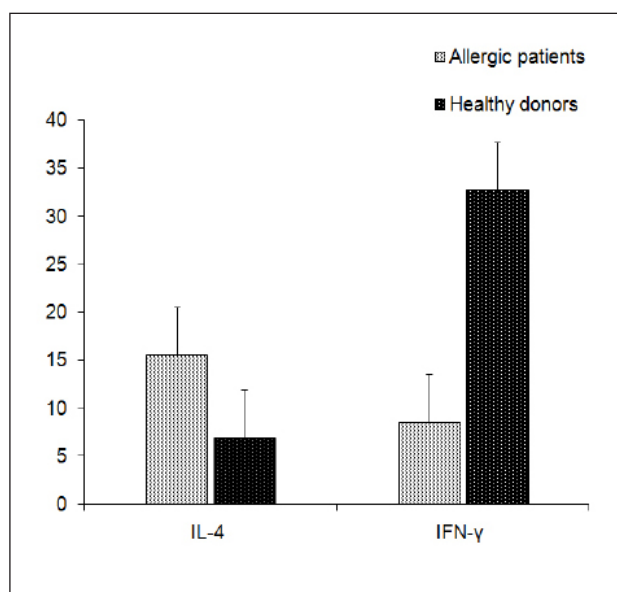


Figure 7. IL-4 and IFN-γ production by autologous T cells stimulated with Der p 1-pulsed DCs from allergic patients and healthy donors. The levels were evaluated by ELISA and results are expressed as the mean ± std. dev. $P < 0.05$

Supernatants of the healthy donors contained higher amounts of IFN-γ (32.71 ± 10.73 pg/ml) and lower levels of IL-4 (6.86 ± 5.42 pg/ml) corresponding to a Th1 immune response. (Table 3)

Table 3. Cytokine levels produced by autologous T cells

| | IL-4 (pg/ml) | IFN-γ (pg/ml) |
|-------------------|------------------|-------------------|
| Allergic patients | 15.53 ± 8.38 | 8.47 ± 2.54 |
| Healthy donors | 6.86 ± 5.42 | 32.71 ± 10.73 |

Data are mean ± Std. Dev.

DISCUSSION

It is known that the orientation of immune response depends on multiple factors, DCs being considered to play a key role in the initiation of primary immune response and in the amplification of secondary immune response. Balbo P and co (2001) confirmed that differential expression of DC surface markers influences Th1/Th2 balance.¹³ The most important DCs costimulatory molecules involved in Th differentiation are CD80 and CD86. Allergic status of the patient is characterized by a predominant expression of CD86, which has been demonstrated to play a role in up-regulation of B cells.¹⁴ This study revealed that Der p 1 up-regulates different costimulatory molecules on DCs membrane: CD80 expression in healthy donors and CD86 and CD83 expression in house dust mite allergic patients. The low expression of CD80 in healthy donors showed a close association between this costimulatory molecule and Th1 response modulation. Another study, performed by Xue-Qin C (2006) on patients with allergic asthma had the same results on CD86 expression. This was higher on DCs from allergic asthmatics than that on DCs from healthy donors, and naive T cells stimulated by DCs from these patients produced more IL-4.¹⁵

Expression of CD83, a maturation marker of PBMC-derived-DC, is visible lower on Der p 1-pulsed DCs comparatively with LPS-pulsed DCs. This demonstrates that LPS has a higher capacity to induce DC maturation when compared with the major allergen Der p 1. CD83 expression did not differ significantly between allergic patients and healthy donors.

Stimulation with LPS enhances CD80, CD86 and CD83 expression in both groups. The study of Langenkamp (2000) showed that LPS-DCs stimulation induced a high expression of CD80 with intense IL-12 production favouring a Th1 immune response, and then, after 24 h, observed an increase in CD86 expression with progressively losing of their capacity to produce this cytokine, leading to a Th2 response.¹⁶

The modulation of immune response into a Th1 or Th2 type depends on microenvironment cytokines present during the interaction between DCs and naive T cells, specially IL-10 and IL-12, both produced by

DCs. The main roles of IL-10 are the limitation of inflammatory response through down-regulation of Th1 proinflammatory cytokine expression (IFN- γ , IL-2, IL-6, IL-8, IL-12, and TNF- α) and the stimulation of B-lymphocytes proliferation and differentiation.¹⁷ Therefore, IL-10 is a Th2 response promoter. Our results showed an IL-10 intense production after Der p 1 pulsation in allergic patients, thus developing a Th2 response. In contrast, pulsation with LPS induced down-regulation of IL-10 production and increased amounts of IL-12 synthesis. Charbonnier et al. (2003) showed that M-DC and P-DC play a distinct role in orchestrating the initiation of the immune response and the determination of the Th polarization. Their study reported that M-DC from allergic patients pulsed with Der p 1 produced low levels of IL-10, whereas M-DC from healthy donors secreted significantly higher amounts of IL-10. Concerning P-DC, the incubation with Der p 1 induced low IL-10 production in both groups.¹⁸ Mosca (2000) demonstrated that IL-12 is a T cells stimulatory factor involved in Th1 differentiation of naive T cells. IL-12 plays an essential role in stimulation of IFN- γ and TNF- α production by T cells and reduces IFN- γ suppression IL-4 mediated.¹⁹

In response to Der p 1 stimulation, DCs from allergic patients induce on autologous T cells an increased IL-4 production comparison with IFN- γ production. In DCs from healthy donors the same type of stimulation up-regulate IFN- γ production, orientated the response toward a Th1 response. A study showed that the level of IFN- γ was low and did not significantly differ between the two groups¹⁵. Van der Pouw Kraan reported that in patients with allergic asthma IL-12 and IFN- γ production in whole blood cultures was lower significantly compared with healthy donors.²⁰

All these data lead to the conclusion that the modulation of immune response and the establishment of the balance between allergy and tolerance against an allergen are close dependent on immune status of the individual. Allergic response characterized through a high IL-10 production could be orchestrated by a DCs dysregulation. In the future, the new strategies for allergic diseases therapies should be focused on the essential role of DCs played in the establishment of the immune profile.

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