

Der p 1-pulsed myeloid and plasmacytoid dendritic cells from house dust mite-sensitized allergic patients dysregulate the T cell response

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Abstract: Although reports suggest that dendritic cells (DC) are involved in the allergic reaction characterized by a T helper cell type 2 (Th2) profile, the role of myeloid (M-DC) and plasmacytoid DC (P-DC), controlling the balance Th1/Th2, remains unknown. Here, we showed that in *Dermatophagoides pteronyssinus* (Dpt)-sensitized allergic patients and in healthy donors, M-DC displayed a higher capacity to capture Der p 1, a major allergen of Dpt, than did P-DC. However, Der p 1-pulsed M-DC from healthy subjects overexpressed CD80 and secreted interleukin (IL)-10, whereas M-DC from allergic patients did not. In contrast, with Der p 1-pulsed P-DC from both groups, no increase in human leukocyte antigen-DR, CD80, and CD86 and no IL-10 secretion were detected. When cocultured with allogeneic naive CD4⁺ T cells from healthy donors, Der p 1-pulsed M-DC from allergic patients favored a Th1 profile [interferon (IFN)- γ^{high} /IL-4^{low}] and Der p 1-pulsed P-DC, a Th2 profile (IFN- γ^{low} /IL-4^{high}). In healthy donors, no T cell polarization (IFN- γ^{low} /IL-4^{low}) was induced by Der p 1-pulsed M-DC or P-DC, but in response to Der p 1-pulsed M-DC, T cells secreted IL-10. The neutralization of IL-10 produced by Der p 1-pulsed M-DC from healthy donors led to an inhibition of IL-10 production by T cells and a polarization toward a type 1. Thus, IL-10 produced by M-DC might be an essential mediator controlling the balance between tolerance and allergic status. In addition, P-DC could contribute to the steady state in healthy donors or to the development of a Th2 response in allergic donors. *J. Leukoc. Biol.* 73: 91–99; 2003.

Key Words: allergy · steady state · Th1/Th2 response

INTRODUCTION

Dendritic cells (DC) represent a large network of immunosurveillance all over the organism. They reside in peripheral

tissues (i.e., respiratory tract, gut, and skin) and in secondary lymphoid organs (i.e., lymph nodes, tonsils, and spleen). In peripheral tissues, DC actively capture and process antigens and then migrate to draining lymph nodes, where they stimulate T cells [1–3]. DC are involved in T cell polarization toward a T helper cell type 1 (Th1) response, a phagocyte-dependent immune response leading to the clearance of virus and microorganisms, or a Th2 response, involved in the clearance of parasites or related to the allergic reaction. In peripheral blood (PB), two DC populations [myeloid DC (M-DC) and plasmacytoid DC (P-DC)], differing in their developmental pathways, were recently described [4, 5]: M-DC express the receptor for granulocyte macrophage-colony stimulating factor and other myeloid cell markers such as CD11c, CD13, and CD33, and P-DC, also present in bone marrow and in secondary lymphoid tissues, express the receptor for interleukin 3 (IL-3; CD123), lack myeloid cell markers, and give rise to plasmacytoid cells [6–8]. In vitro, PB-DC populations were first described to exhibit different functions: M-DC promote a Th1 immunity, whereas P-DC promote a Th2 immunity [4]. However, recent data suggest that the dichotomic T cell response is not exclusively governed by the DC type [9]. Monocyte-derived DC (MDDC), related to M-DC, promote a Th1 or a Th2 response according to the nature and/or the dose of the antigen [10–12], the DC T cell ratio [13], and their maturation state (active or exhausted) [14]. In addition, P-DC as the principal type 1 interferon (IFN)-producing cells, in response to viruses such as Herpes simplex virus (HSV) or influenza virus [15–17], could be considered as playing a key role in the antimicrobial immunity characterized by a type 1 T cell response. Moreover, in vivo, the DC microenvironment could influence the T cell response [18].

In the allergic context, we recently demonstrated that the allergic status of the donor defines the T cell response induced by relevant allergen-pulsed MDDC: MDDC, issued from

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healthy subjects pulsed with Der p 1, one of the major allergens of *Dermatophagoides pteronyssinus* (Dpt), favor a Th1 response, whereas Der p 1-pulsed MDDC issued from Dpt-sensitized allergic patients favor a Th2 response [19]. This last Th2 response was reported to be maintained in the presence of IL-12, a Th1-inducing cytokine [20, 21]. In vivo, some observations suggest that DC play a crucial role in the establishment of the allergic inflammatory reaction: Their number increases in the lung of asthmatic patients [22, 23]; bronchial epithelial DC issued from asthmatic patients activate CD4⁺ T cells with a preferential secretion of Th2 cytokines such as IL-4 and IL-5 [22]; a selective DC depletion in ovalbumin (OVA)-sensitized mice leads to a decrease in the immunoglobulin (Ig)E production, an abolition of the Th2 response and a lack of eosinophil recruitment, three elements characterizing the pulmonary allergic response [24]; and in rats, freshly isolated respiratory tract DC pulsed with OVA and reinjected in animals stimulate Th2 cells [25].

Until now, few data have considered the role of P-DC in the human allergic inflammatory response: In circulation, P-DC cell number positively correlates with serum IgE levels and blood eosinophil counts [26]; during experimental allergic rhinitis, an accumulation of P-DC in the vicinity of high endothelium venules was observed in human nasal mucosa [27], suggesting their involvement in the triggering of upper airway allergy.

To define a potential dysfunction of M-DC and/or P-DC leading to the allergic reaction, we investigated their respective response to the allergen Der p 1, one major allergen of Dpt. To this end, effects of Der p 1 on M-DC and P-DC in terms of in vitro maturation capacity and consequences on the T cell polarization were analyzed, and behaviors of DC recovered from allergic and healthy donors were compared.

MATERIALS AND METHODS

Reagents

Fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (mAb) used were anti-CD45RO, lineage cocktail (Lin)-1 containing anti-CD3, -CD14, -CD16, -CD19, -CD20, and -CD56 (Becton Dickinson, Le Pont de Claix, France) and anti-CD80 and -CD86 (PharMingen, Le Pont de Claix, France). PE-conjugated mAb included anti-CD123 (Becton Dickinson) and -CD3 (PharMingen). The CychromeTM-conjugated anti-human leukocyte antigen (HLA)-DR and anti-CD4 mAb and the allophycocyanin (APC)-conjugated anti-CD11c mAb were from PharMingen. Isotype-control mAb included FITC-, PE-, CychromeTM-, or allophycocyanin-labeled mouse IgG1 or IgG2a (PharMingen). Purified blocking anti-IL-10 mAb was from R&D Systems (Minneapolis, MN), and the purified mouse IgG_{2b} isotype control was from PharMingen. Lipopolysaccharide (LPS) and FITC-Dextran were obtained from Sigma Chemical Co. (Saint Quentin Fallavier, France). Der p 1, kindly provided by G. A. Stewart (University of Western Australia, Nedlands, Perth), was purified from lyophilized house dust mite culture supernatant by affinity chromatography using anti-Der p 1 mAb as described previously [28, 29], and the absence of endotoxin contamination was controlled for all batches.

Donors

Two groups of donors were selected: allergic patients and healthy donors. Twenty-one allergic patients without any treatment were suffering from perennial rhinitis and/or allergic asthma and exhibited positive skin prick tests toward Dpt extracts and specific IgE for Dpt (radioallergosorbent test class

≥2). Total IgE level was above 100 kU/L (between 105 and 1347 kU/L), and levels of specific IgE for Dpt were between 1.4 and 98 kU/L. Twenty-seven healthy donors displayed a total IgE level <91 kU/L (between 5 and 70.8 kU/L) without any detection of specific IgE for Dpt in their serum (<0.35 kU/L).

Rhodamine (Rhod) labeling of Der p 1

Der p 1 ($M_r=25,000$) was labeled with Rhod using Fluoreporter[®] tetramethylrhodamine protein labeling kit (Molecular Probes, Eugene, OR) following the manufacturer's instructions. Briefly, the Rhod solution was added to the Der p 1 solution (1 mg/ml) and incubated for 1 h at room temperature in the dark under stirring. The solution was further extensively dialyzed against phosphate-buffered saline (PBS). The Rhod-labeled Der p 1 (Rhod-Der p 1) solution was stored at 4°C and protected from light until use.

Cell preparations

PB mononuclear cells (PBMC) were obtained from whole blood by density gradient over Ficoll-Hypaque (Pharmacia, Saint Quentin en Yvelines, France). PBMC ($5-10 \times 10^7$) were recovered from 60 ml whole blood. PB-DC were purified with the blood DC isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). PB-DC were pre-enriched from PBMC by depletion of B cells, T cells, monocytes, natural killer (NK) cells with haptenized CD19, CD3, CD11b, and CD16 antibodies, and an anti-hapten mAb coupled to magnetic cell sorter microbeads. The magnetically labeled cells were removed in a magnetic field. PB-DC were purified from the remaining nonbound cell fraction by a positive immunoselection procedure with anti-CD4 mAb-coated magnetic microbeads. For some experiments, M-DC and P-DC were purified using the blood DC isolation kit and the BDCA-4 cell isolation kit (Miltenyi Biotec). After the B cell-, T cell-, monocyte-, and NK cell-depletion step, as described above, P-DC were purified by a positive immunoselection procedure with anti-BDCA-4 mAb-coated magnetic microbeads, which recognized P-DC specifically [30]. Then, M-DC were purified from the remaining nonbound cell fraction by a positive immunoselection procedure with anti-CD4 mAb-coated magnetic microbeads. P-DC ($2.5-7 \times 10^5$; Lin-1⁻CD123⁺HLA-DR⁺CD11c⁻) and M-DC ($0.75-3 \times 10^5$; Lin-1⁻CD123⁺HLA-DR⁺CD11c⁺) were obtained as determined by immunostaining with a FITC-labeled Lin-1, PE-labeled CD123, CychromeTM-labeled HLA-DR, and APC-labeled CD11c with a purity of 90%. Naive CD4⁺ T cells were purified by anti-CD11b/CD16/CD19/CD36/CD56/CD8/CD45RO depletion using the pan T cell isolation kit and CD45RO and CD8 beads from Miltenyi Biotec (purity >90%).

Antigen uptake assay

Purified PB-DC were resuspended in the uptake buffer [45% RPMI, 50% PBS, 5% fetal calf serum (FCS)] at a density of $1-3 \times 10^6$ cells/ml. PB-DC were prewarmed at 37°C or kept on ice for 20 min. Before use, Rhod-Der p 1 and FITC-Dextran were spun down in a microfuge to eliminate aggregates. On the basis of previous observations on MDDC [31], PB-DC were incubated for 1 h with 50 µg/ml Rhod-Der p 1, dialyzed Rhod, or 50 µg/ml FITC-Dextran at 4°C or 37°C. Then, PB-DC were collected and washed three times with ice-cold PBS. Rhod-Der p 1- and Rhod-pulsed cells were triple-stained with anti-Lin-1 FITC, anti-HLA-DR CychromeTM, and anti-CD11c APC, and FITC-Dextran-pulsed cells were triple-stained with anti-Lin-1 PtdEtn, anti-HLA-DR CychromeTM, and anti-CD11c APC. Antigen uptake by M-DC and P-DC was immediately determined by flow cytometry by gating cells on Lin-1⁺HLA-DR⁺CD11c⁺ (M-DC) and Lin-1⁺HLA-DR⁺CD11c⁻ (P-DC) and measuring the mean Rhod-Der p 1 or the FITC-Dextran fluorescence intensity.

PB-DC culture

Purified PB-DC, M-DC, or P-DC were cultured in RPMI-1640 medium containing 10% FCS, 2.5 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells were seeded at a density of 0.5×10^6 /ml in 24-well culture plates (Costar, Brumath, France). Cell suspensions were cultured for 1 day in three different conditions: without any additive stimulus as negative control, after addition of 1 µg/ml Der p 1, or 1 µg/ml LPS as positive control for DC

maturation. In some experiments, blocking anti-IL-10 mAb (10 $\mu\text{g/ml}$) or isotype-matched control Ab (10 $\mu\text{g/ml}$) was added to the culture. Then, cells were immediately analyzed or cocultured with allogeneic naive CD4⁺ T cells from healthy subjects for 5 days (T cell:DC ratio was 20:1).

Cell-surface marker analysis

For four-color immunolabeling, cells were washed twice in ice-cold PBS and incubated in 100 μl PBS containing appropriate fluorochrome-labeled mAb for 30 min on ice. At least 20,000 cells were analyzed on a FACScanTM (Becton Dickinson). The analysis of cell-surface markers (CD80, CD86, HLA-DR) on PB-DC was performed by gating cells on HLA-DR⁺CD11c⁺CD123^{low} (M-DC) and HLA-DR⁺CD11c^{low}CD123⁺ (P-DC) as described previously [32].

Evaluation of cytokine secretion

IL-10 and IL-12 (p70) production by M-DC and P-DC were measured in supernatants by enzyme-linked immunosorbent assay (ELISA; Diaclone, Besançon, France). IL-4, IFN- γ , and IL-10 production by T cells cocultured with M-DC or P-DC was measured in supernatants by ELISA (Diaclone).

Statistical analysis

Nonparametric statistical analysis of cell-surface molecule expression and cytokine production by M-DC and P-DC was performed using the Mann-Whitney test. Values of $P \leq 0.05$ were considered statistically significant.

RESULTS

Der p 1 is preferentially taken up by M-DC in allergic and healthy donors

In a first series of experiments, the Der p 1 uptake by M-DC and P-DC subsets in allergic and healthy donors was investigated. As expected, no capture of Der p 1 occurred at 4°C. **Figure 1, A and B**, shows that at 37°C, M-DC and P-DC took up Der p 1 without statistical differences between allergic and

healthy donors. However, in both groups, M-DC displayed a twofold higher Der p 1 uptake capacity than P-DC. It is interesting that FITC-Dextran, an antigen mainly taken up by MDDC via the mannose receptor [31], did not bind to M-DC and P-DC from allergic as well as healthy donors (Fig. 1, C and D). This last observation correlated with the absence of the mannose receptor expression on the surface of the two DC subsets (data not shown).

HLA-DR expression on M-DC and P-DC in response to Der p 1

To investigate whether M-DC and P-DC mature in response to Der p 1, we performed a comparative HLA-DR expression analysis on M-DC and P-DC recovered from allergic patients and from healthy donors. Freshly isolated M-DC and P-DC displayed a weak HLA-DR expression in both groups (**Fig. 2**). After 1 day of culture in medium, M-DC from allergic patients strongly increased HLA-DR expression (Fig. 2A). In response to Der p 1, HLA-DR expression increased, as observed with LPS used as positive control for DC maturation. In healthy donors, similar observations were done: In the absence of stimulus, M-DC overexpressed HLA-DR, and in response to Der p 1 or LPS, HLA-DR was also increased (Fig. 2B). However, M-DC from healthy donors displayed a lower level of HLA-DR expression than M-DC from allergic patients. It is worthy of note that in the allergic group, a heterogeneity was observed for values obtained in all culture conditions in contrast to the healthy group that displayed some homogeneity. On P-DC from allergic patients, HLA-DR expression remained at a low level in three culture conditions (Fig. 2C). On P-DC from healthy donors, HLA-DR expression was slightly increased in response to Der p 1 and LPS (Fig. 2D). Thus, on the basis of HLA-DR expression, it appears that Der p 1 induced a higher maturation of M-DC from allergic patients than M-DC from healthy donors, and Der p 1 did not induce maturation of P-DC from allergic and healthy donors.

Costimulatory molecule expression on M-DC and P-DC is differently modulated by Der p 1 in allergic and healthy donors

As costimulatory molecules, such as CD80 and CD86, are involved in the initiation of the immune response and the T cell polarization, their presence on the surface of each PB-DC subset, M-DC and P-DC, was analyzed in response to Der p 1. After Der p 1 incubation, M-DC from allergic patients slightly overexpressed CD86 but not CD80 (**Fig. 3A**; 268.65 ± 30.83 and 35.76 ± 6.51 , respectively) compared with medium (230.67 ± 100.26 and 21.55 ± 14.71 , respectively). However, in response to LPS, only CD80 was significantly increased on M-DC (86.37 ± 13.99). In healthy subjects, in response to Der p 1, only CD80 was significantly overexpressed on M-DC (57.58 ± 11.96) compared with medium (18.55 ± 9.02), whereas in the presence of LPS, CD80 and CD86 expressions were up-regulated (Fig. 3C; 78.33 ± 16.85 and 354.21 ± 64.17 , respectively) compared with medium (18.55 ± 9.02 and 190.03 ± 63.06 , respectively). Concerning P-DC from allergic

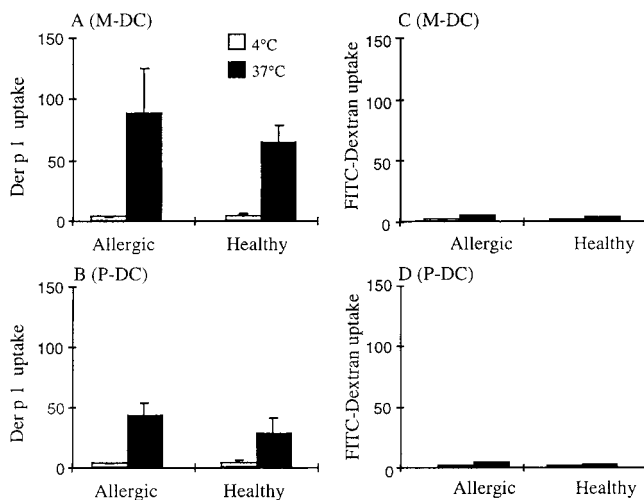


Fig. 1. Der p 1 is taken up more by M-DC than P-DC. After purification, PB-DC from allergic patients or from healthy subjects were incubated for 1 h with 50 $\mu\text{g/ml}$ Rhod-Der p 1 (A and B) or FITC-Dextran (C and D) at 4°C (open bars) or 37°C (solid bars). The Der p 1 uptake is given as the fluorescence intensity geometric mean of Rhod-Der p 1 minus dialyzed Rhod of individual subpopulations M-DC (A) or P-DC (B). The FITC-Dextran uptake is given as the fluorescence intensity geometric mean of FITC-Dextran of individual subpopulations M-DC (C) or P-DC (D). Mean $\pm$ SEM values obtained in four independent patients are shown.

Fig. 2. HLA-DR expression by cultured PB-DC. After purification, PB-DC from allergic patients (open symbols, A and C) or from healthy subjects (solid symbols, A and C) were immediately analyzed by three-color flow cytometry (NS, circles) or incubated in unsupplemented medium (diamonds), supplemented with Der p 1 (1 μ g/ml; squares) or with LPS (1 μ g/ml; triangles) for 1 day. Data represent the mean fluorescence intensity (MFI) $\pm$ SEM for HLA-DR of M-DC (A and B) or P-DC (C and D). Data of individual donors are shown; means are indicated by bold lines. *, $P < 0.05$.

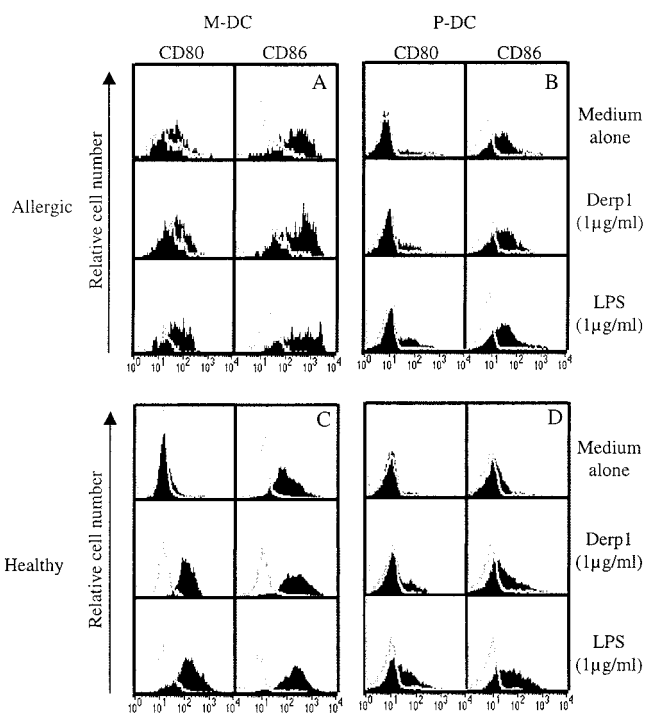
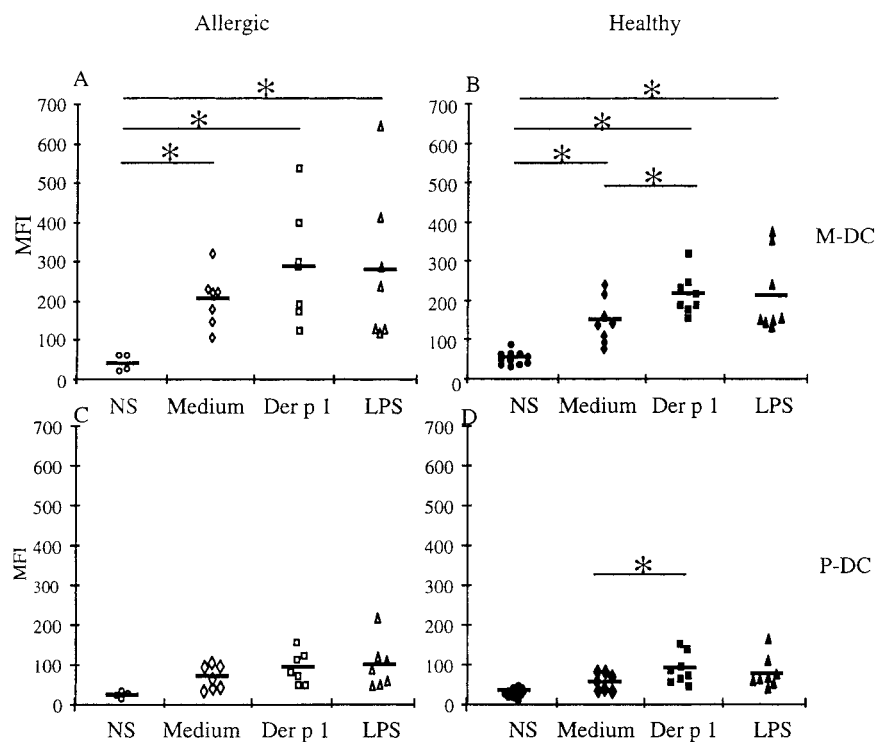


Fig. 3. Costimulatory molecule expression pattern of cultured PB-DC subsets issued from allergic patients (A and B) or healthy subjects (C and D). After purification, PB-DC were incubated in unsupplemented medium, supplemented with Der p 1 (1 μ g/ml) or with LPS (1 μ g/ml) for 1 day, and then analyzed by four-color flow cytometry. Closed histograms represent the anti-CD80 and the anti-CD86 immunoreactivity of M-DC (A and C) or P-DC (B and D). The reactivities of isotype-matched control mAb with the same PB-DC subpopulations are shown by open histograms. One representative independent patient of three (allergic) and five (healthy) is shown.

patients, Der p 1 did not modify CD80 and CD86 expression (2.63 ± 1.33 and 13.73 ± 7.3 , respectively), whereas LPS slightly induced the CD80 expression (Fig. 3B; 8.12 ± 4.34) compared with medium (2.63 ± 1.03 and 14.07 ± 7.05 , respectively). In healthy subjects, CD80 and CD86 expression slightly increased after Der p 1 incubation (2.1 ± 0.95 and 9.52 ± 1.69) as well as in response to LPS (8.02 ± 4.24 and 21.55 ± 7.9 ; Fig. 3D) compared with medium (0.68 ± 0.22 and 7.33 ± 1.07).

Der p 1 exclusively induces an IL-10 production by M-DC from healthy donors and does not induce any IL-12 expression

As IL-10 and IL-12 are known to play a key role in the modulation of the T cell response, their production by each unpulsed Der p 1- and LPS-pulsed PB-DC subset was evaluated. IL-12, a key cytokine in the development of the Th1 response, was not detected in any culture supernatant tested from healthy as well as allergic donors (data not shown). M-DC from allergic patients pulsed with Der p 1 produced low levels of IL-10 (3.16 ± 3.53), whereas M-DC from healthy donors secreted significantly higher amounts of IL-10 (42.21 ± 35.55 ; Fig. 4A). However, in the presence of LPS, the production of IL-10 was highly increased in both donor groups, and an IL-10 level was more elevated in healthy (1942 ± 1216.15) rather than allergic groups (775.55 ± 166.80). Concerning P-DC, the incubation with Der p 1 induced a low production of IL-10 in allergic (25.14 ± 25.51) and healthy donors (3.27 ± 4.01 ; Fig. 4B). P-DC from healthy subjects incubated with LPS produced IL-10 higher (107.90 ± 80.11) than P-DC from allergic donors (23.45 ± 16.08).

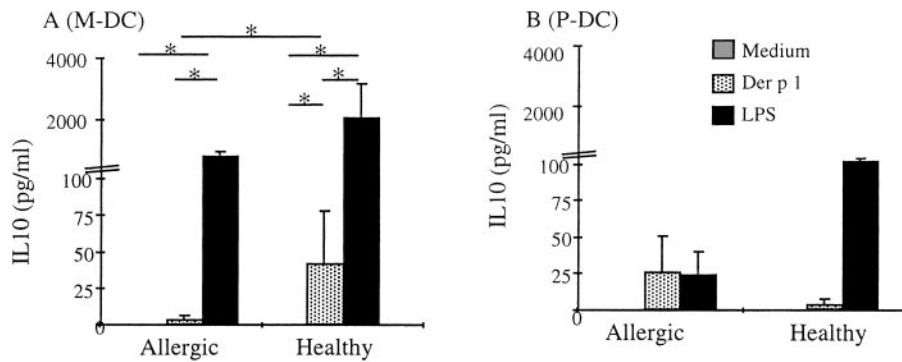


Fig. 4. IL-10 production by each cultured PB-DC subset. After purification, M-DC (A) or P-DC (B) from healthy donors (n=5) or from allergic patients (n=4) were incubated in unsupplemented medium (light shaded bars), supplemented with Der p 1 (1 μ g/ml; dotted bars) or with LPS (1 μ g/ml; solid bars) for 1 day. Supernatants were collected, and the amounts of IL-10 were measured by ELISA. Results shown are expressed as the mean $\pm$ SEM. *, $P < 0.05$.

Der p 1-pulsed PB-DC subsets from Dpt-sensitized allergic patients polarize the primary allogeneic T cell response but not Der p 1-pulsed PB-DC subsets from healthy donors

We then examined the polarization of the primary allogeneic T cells induced by each PB-DC subset measuring IFN- γ and IL-4 production in supernatants of coculture with DC from allergic or healthy donors and allogeneic naive CD4⁺ T cells from healthy donors. Naive T cells cultured with Der p 1-pulsed M-DC from allergic patients secreted pronounced IFN- γ amounts and low levels of IL-4, corresponding to a Th1-response profile, similar to T cells stimulated with LPS-pulsed M-DC (**Fig. 5, A and B**). In contrast, in healthy subjects, Der p 1-pulsed M-DC did not significantly modify IFN- γ and IL-4 production observed with unpulsed M-DC (**Fig. 5, C and D**). LPS-pulsed M-DC induced a weak increase in

IFN- γ production and no increase in IL-4 secretion. It is interesting that a different profile response was obtained with P-DC. Der p 1-pulsed P-DC from allergic patients led to a low production of IFN- γ and an increase in IL-4 production, corresponding to a Th2-response profile (**Fig. 5, A and B**). If P-DC were previously pulsed with LPS, then the IL-4 and IFN- γ production was increased. Control experiments indicated that IL-4 was produced during coculture only when T cells were incubated with P-DC (data not shown). In addition, P-DC stimulated with Der p 1 or LPS did not secrete IL-4, suggesting that the IL-4 production observed in P-DC:T cell coculture was produced by T cells. In healthy subjects, in response to Der p 1, P-DC led to a low secretion of IL-4 and IFN- γ production (**Fig. 5, C and D**). Thus, in response to Der p 1, M-DC from allergic patients polarized the T cell response toward a type 1 profile, and P-DC primed the polarization of the T cell response

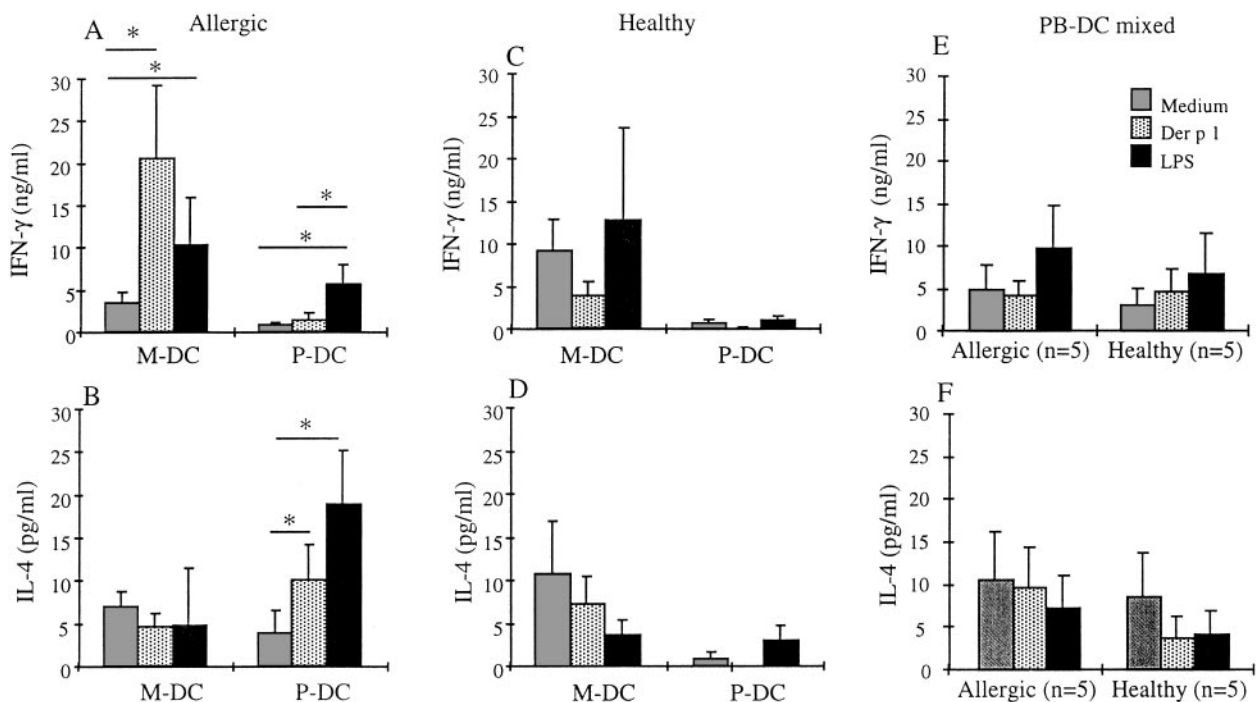


Fig. 5. Cytokine secretion profile of naive T cells stimulated with M-DC or P-DC depends on the allergic status of the donor. M-DC, P-DC, and mixed PB-DC from allergic patients (A, B, E, and F; n=5) or from healthy donors (C, D, E, and F; n=5) were incubated in unsupplemented medium (light shaded bars), supplemented with Der p 1 (1 μ g/ml; dotted bars), or with LPS (1 μ g/ml; solid bars) for 1 day. Then, they were cocultured with naive CD4⁺ T cells for 5 days. Supernatants were harvested and (A, C, and E) IFN- γ or (B, D, and F) IL-4 was measured by ELISA. Results shown are given as mean $\pm$ SEM values. *, $P < 0.05$.

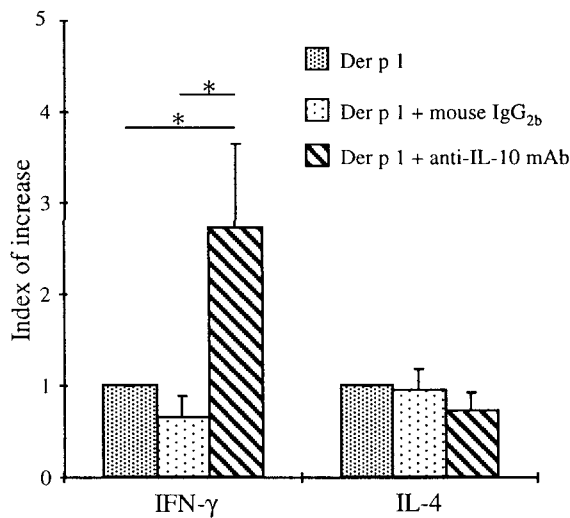


Fig. 6. Endogenous IL-10 inhibits the capacity of Der p 1-pulsed M-DC from healthy subjects to initiate a Th1 response. M-DC from healthy donors ($n=6$) were incubated with Der p 1 in the absence (densely dotted bars), in the presence of mouse IgG_{2b}, isotype control (spaced, dotted bars), or blocking anti-IL-10 mAb (hatched bars) for 1 day. Then, they were cocultured with naive CD4⁺ T cells for 5 days. Supernatants were harvested, and IFN- γ and IL-4 were measured by ELISA. Results shown are given as an index of increase corresponding to the mean $\pm$ SEM values of ratio of cytokine amount in tested coculture supernatant over cytokine amount in Der p 1-pulsed M-DC:T cell coculture supernatant. *, $P < 0.05$.

toward a type 2 profile. In contrast in healthy subjects, none of M-DC and P-DC was able to polarize T cell response in response to Der p 1.

To be closer to the physiological situation, the polarization of T cells induced by PB-DC populations mixed was examined. In allergic patients as well as in healthy subjects, Der p 1-pulsed, mixed PB-DC did not induce any increase in IFN- γ and IL-4 production by T cells (Fig. 5, D and F). This result suggests that in allergic patients, one DC subset might repress the function of the other DC subset.

The absence of T cell polarization in response to Der p 1-pulsed M-DC from healthy subjects is IL-10-dependent

As IL-10 secreted by DC was reported playing a critical role in the establishment of tolerance, the eventual role of IL-10 produced by Der p 1-pulsed M-DC from healthy subjects in the absence of T cell polarization was determined by neutralizing IL-10 production. The IL-10 neutralization did not affect the expression of DC maturation markers such as HLA-DR, CD40, CD80, and CD86 (data not shown). In contrast, the addition of the blocking anti-IL-10 mAb to Der p 1-pulsed M-DC led to an increase in the IFN- γ but not in the IL-4 production by naive T cells, characterizing a Th1 profile (Fig. 6). It is worthy of note that in this neutralization condition, the IFN- γ amount reached the same levels as those observed with Der p 1-pulsed M-DC from allergic patients (data not shown). In contrast, the isotype-matched control Ab did not modify the cytokine secretion profile of T cells stimulated by Der p 1-pulsed M-DC. In allergic patients, the anti-IL-10 mAb did not modify the Th1 response induced by M-DC (data not shown). These results

suggest that IL-10 secreted by Der p 1-pulsed M-DC from healthy subjects should be responsible for the lack of Th1 response by naive T cells.

IL-10 produced by Der p 1-pulsed M-DC from healthy subjects induced IL-10-producing T cells

As IL-10 produced by T cells is considered to play a crucial role in tolerance process, we then investigated if Der p 1-pulsed M-DC from healthy donors induced IL-10 secretion by T cells. Naive T cells cultured for 5 days with Der p 1-pulsed M-DC secreted pronounced amounts of IL-10 (Fig. 7). It is interesting that when the IL-10 secreted by Der p 1-pulsed M-DC was neutralized, T cells did not secrete IL-10 (Fig. 7). Flow cytometry analysis confirmed that IL-10 detected in coculture supernatants was secreted by T cells (data not shown). These results suggest that IL-10 secreted by Der p 1-pulsed M-DC from healthy donors should be responsible for the generation of IL-10-producing T cells.

DISCUSSION

M-DC and P-DC are two DC subsets reported to play a distinct role in orchestrating the initiation of the immune response and the determination of the Th polarization. In vitro, the degree of the Th polarization may differ according to the maturation state, the dose and nature of the antigen, and the ratio of DC:T cells. In the context of allergic diseases, characterized by a Th2 profile, the function of each PB-DC subset has not yet been defined. In the present study, we showed that in allergic patients, in response to Der p 1, M-DC induced a T cell polarization toward a type 1, and P-DC primed a T cell polarization toward a type 2. In contrast, in healthy donors, none of PB-DC subsets stimulates naive T cells. These findings argue for the existence of potential dysfunctions of each PB-DC

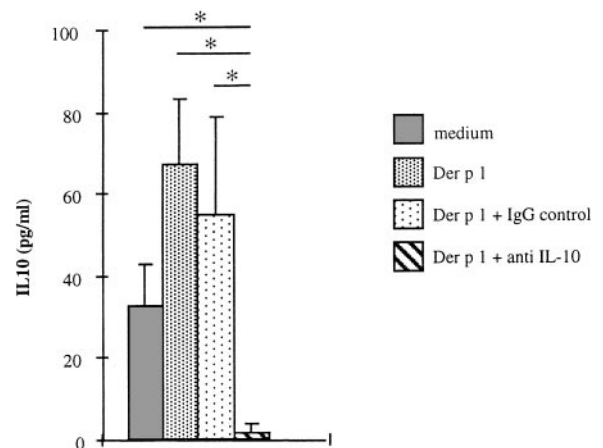


Fig. 7. The IL-10 produced by Der p 1-pulsed M-DC from healthy subjects generates IL-10-producing T cells. M-DC from healthy donors ($n=5$) were incubated in unsupplemented medium (light shaded bars), with Der p 1 in the absence (densely dotted bars) or in the presence of mouse IgG_{2b}, isotype control (spaced, dotted bars), or blocking anti-IL-10 mAb (hatched bars) for 1 day. Then, they were cocultured with naive CD4⁺ T cells for 5 days. Supernatants were harvested, and IL-10 was measured by ELISA. Results shown are given as mean $\pm$ SEM values. *, $P < 0.05$.

subset in response to Der p 1 in allergic patients who develop an allergen-specific inflammatory response.

At first, we showed that M-DC were able to capture Der p 1. In allergic patients, the capacity to capture the allergen was not disturbed when compared with healthy donors. In addition, no differences in the phenotype (CD80, CD86, HLA-DR) of freshly isolated M-DC were observed between the two groups. Among maturation markers analyzed, HLA-DR and CD86 were similarly up-regulated in both groups. However, a major difference in the CD80 expression was detected in response to Der p 1 between allergic and healthy donors: Only M-DC from healthy donors significantly increased in their CD80 expression. It is interesting that similar observations were done with Der p 1-pulsed DC derived from monocytes: Those from healthy donors display CD80 up-regulation, whereas those from allergic patients maintain a low CD80 expression level [19]. In allergic patients, the lack of CD80 up-regulation is in keeping with other data obtained *in vivo*, which showed that following an allergen challenge in asthmatic patients, CD80 was not up-regulated on alveolar macrophages and on B cells [33, 34].

Surprisingly, the up-regulation of CD80 expression on Der p 1-pulsed M-DC from healthy subjects did not lead to a T cell polarization. However, unlike allergic patients, Der p 1-pulsed M-DC from healthy donors secreted IL-10, which inhibits the capacity of M-DC to polarize naive T cells and promotes M-DC to generate IL-10-secreting T cells. In mice, T regulatory cells (Tr1), which are involved in the tolerance process, are characterized by a high production of IL-10 [35]. In humans, also, *in vitro* studies show that MDDC-secreting IL-10 generates T cells that display similar characteristics to Tr1 cells [36–38]. So, IL-10 should be a crucial cytokine in healthy donors to convert M-DC into tolerogenic DC. In the allergic context, pretreatment with IL-10 of MDDC from allergic patients incubated with the appropriate allergen inhibits the production of Th1 and Th2 cytokines by T cells [39]; in mouse, pulmonary DC from mice exposed to OVA transiently produced IL-10, which is critical for the induction of tolerance [40]; and IL-10^{-/-} mice have more eosinophilic airway inflammation [41]. So, IL-10 plays a critical role in the establishment of tolerance against an allergen, and the IL-10 production by Der p 1-pulsed M-DC could be an important source of IL-10 involved in the establishment of the tolerance against Der p 1 in healthy donors. It is worthy of note that the tolerance process is accompanied with a M-DC maturation, as it was described in a mouse model of asthma with pulmonary DC [40]. Thus, the tolerance against an allergen in healthy donors would be induced by mature DC in contrast to other tolerance mechanisms involving immature DC expressing low amounts of costimulatory molecules [38].

In contrast, in allergic patients, it appears that the lack of IL-10 secretion by Der p 1-pulsed M-DC correlated with the capacity to activate allogeneic T cells and prime naive T cells to a more prominent Th1 polarization. It was already shown that the neutralization of IL-10 secreted by MDDC allows the induction of a Th1 response [36]. So, in allergic patients, the absence of IL-10 secretion by Der p 1-pulsed M-DC should favor the development of a Th1 response. Recently, Salvi et al. [42] claimed that “the Th2 hypothesis for asthma” is too

simplistic, as evidence argues for the presence of a Th1 response. The presence of IFN- γ , a Th1 cytokine, in bronchoalveolar lavage and in blood correlates with asthma severity [42–45]. In addition, in a mouse model of asthma, Randolph et al. [45] demonstrated that antigen-specific Th1 cells, which predominate early in the response, are not protective but rather potentiate the allergic inflammatory response. Thus, our results suggest that M-DC from allergic patients could dysregulate the tolerance against an allergen and initiate the Th1 response involved in an allergic inflammatory response. Surprisingly, M-DC from allergic patients did not secrete IL-12, a major cytokine inducing a Th1 response. DC need at least two signals, such as CD40 ligand (CD40-L) and IFN- γ , to produce IL-12 [46]. Here, M-DC received only one signal, LPS or Der p 1, which should not be enough for inducing IL-12 production.

It is interesting that in another experimental model, we have previously reported that Der p 1-pulsed MDDC amplify the Th2 response in a syngeneic total T cell population [19]. In this last study, we elucidated the recall response mechanism, whereas here, we analyze the initiation of the allergic-immune response with freshly isolated blood M-DC and naive T cells. The comparison of our experimental human models allows us to suggest that also in humans, a Th1 response could occur during the initiation of the allergic response with the involvement of M-DC, and then, a Th2 response takes place, characterizing the recall response. In addition, we have recently observed that the mannose receptor was involved in the Der p 1 uptake by MDDC [31]. The inability of M-DC to uptake FITC-Dextran and their lack of mannose receptor expression suggest that M-DC should capture Der p 1 in a different way than MDDC. Recent data suggest that the internalization pathway influences the T cell polarization process induced by DC [47]. Thus, MDDC and M-DC induce different T cell responses, probably as a result of their different Der p 1 capture pathways.

With P-DC, a low Der p 1 capture was observed in healthy and allergic donors. Also, after 24 h incubation with Der p 1, no maturation marker expression, such as HLA-DR, CD80, or CD86, was detected in allergic as well as healthy donors. However, unlike healthy donors, Der p 1-pulsed P-DC from allergic patients in contact with naive T cells led to the priming of a Th2 polarization. It is well known that *in vitro*, in response to CD40-L, P-DC mature and polarize naive T cells toward a type 2 profile [4]. Thus, Der p 1-pulsed P-DC from allergic patients may induce a Th2 response in contact with naive T cells via the interaction CD40-CD40-L. Recent studies described that in allergic patients, the P-DC number positively correlates with serum IgE levels and blood eosinophil counts [24], and *in vivo* allergen challenge induces P-DC recruitment in the nasal mucosa [27], where they are in contact with the allergen. Our results suggest that if they migrate into the lymph node, P-DC would be responsible for the induction of an allergen-specific Th2 response.

In addition, it is worthy of note that in response to LPS, P-DC from allergic patients induced a Th2 response. Until now, such a response to LPS was not reported as a result of the absence of Toll-like receptor 4 (TLR-4) expression on the surface of P-DC [48]. This unusual response to LPS allows us to hypothesize that in allergic patients, the LPS receptor pro-

file, such as TLR expression, might be different in comparison to healthy donors.

P-DC also act as type 1 IFN-producing cells to alert the immune system to dangers, in response to viruses such as influenza virus or HSV [12, 15]. The IFN- α , known to favor a Th1 response [15], was detected at very low levels in culture supernatants (data not shown) in response to Der p 1 in allergic as well as in healthy donors. Indeed, Der p 1-pulsed P-DC from allergic patients did favor a Th2 cytokine profile. So, in healthy donors, Der p 1 did not activate any known function of P-DC, initiation of a Th2 response, and production of type 1 IFN, suggesting that P-DC should contribute to maintain the steady state in response to allergens, whereas in allergic patients, they should contribute to establish the allergic reaction by inducing an allergen-specific Th2 response.

When M-DC and P-DC from allergic patients were mixed and then pulsed with Der p 1, their capacity to polarize T cell responses toward a type 1 and a type 2, respectively, was lost. IL-4 produced by T cells stimulated by Der p 1-pulsed P-DC might inhibit IFN- γ production as already shown [49]. However, this lack of in vitro T cell polarization does not reflect the physiological situation where a sequential T cell polarization has been emphasized [45]. Indeed, in vivo, M-DC and P-DC are present in blood and could be recruited in tissues and/or in lymph nodes by different routes [50]. Thus, in response to an allergen, M-DC and P-DC could separately polarize T cells toward type 1 and type 2, respectively, in space and/or in time.

In conclusion, we have demonstrated that compared with healthy donors, in allergic patients, M-DC and P-DC display dysfunctions in response to Der p 1 with a capacity to induce a T cell polarization toward a type 1 and a type 2, respectively. Thus, M-DC and P-DC should play a critical role in the establishment of the balance between tolerance and allergic status against an allergen. In healthy donors, in response to Der p 1, P-DC and M-DC do not stimulate naive T cells and might contribute to the steady state, whereas in allergic patients, they dysregulate the T cell response in Dpt-sensitized allergic patients, leading to allergic status.

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REFERENCES

- Steinman, R. M. (1991) The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* **9**, 271–296.
- Banchereau, J., Steinman, R. M. (1998) Dendritic cells and the control of immunity. *Nature* **392**, 245–252.
- Holt, P. G., Stumbles, P. A., McWilliam, A. S. (1999) Functional studies on dendritic cells in the respiratory tract and related mucosal tissues. *J. Leukoc. Biol.* **66**, 272–275.
- Rissoan, M. C., Soumelis, V., Kadowaki, N., Grouard, G., Brière, F., de Waal Malefyt, R., Liu, Y.-J. (1999) Reciprocal control of T helper cell and dendritic cell differentiation. *Science* **283**, 1183–1186.
- Kohrgruber, N., Halanek, N., Groger, M., Winter, D., Rappersberger, K., Schmitt-Egenolf, M., Stingl, G., Maurer, D. (1999) Survival, maturation, and function of CD11c⁺ and CD11c⁺ peripheral blood dendritic cells are differentially regulated by cytokines. *J. Immunol.* **163**, 3250–3259.
- Banchereau, J., Pulendran, B., Steinman, R., Palucka, K. (2000) Will the making of plasmacytoid dendritic cells *in vitro* help unravel their mysteries. *J. Exp. Med.* **192**, F39–F44.
- Grouard, G., Durand, I., Filgueira, L., Banchereau, J., Liu, Y. J. (1996) Dendritic cells capable of stimulating T cells in germinal centers. *Nature* **384**, 364–367.
- Grouard, G., Rissoan, M. C., Filgueira, L., Durand, I., Banchereau, J., Liu, Y. J. (1997) The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J. Exp. Med.* **185**, 1101–1111.
- Lanzavecchia, A., Sallusto, F. (2000) Dynamics of T lymphocyte response: intermediates, effectors, and memory cells. *Science* **290**, 92–97.
- Whelan, M., Harnett, M. M., Houston, K. M., Patel, V., Harnett, W., Rigley, K. P. (2000) A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *J. Immunol.* **164**, 6453–6460.
- Ruedl, C., Bachmann, M. F., Kopf, M. (2000) The antigen dose determines T helper subset development by regulation of CD40 ligand. *Eur. J. Immunol.* **30**, 2056–2064.
- Cella, M., Facchetti, F., Lanzavecchia, A., Colonna, M. (2000) Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent Th1 polarization. *Nat. Immunol.* **1**, 305–310.
- Tanaka, H., Demeure, C. E., Rubio, M., Delespessis, G., Sarfati, M. (2000) Human monocyte-derived dendritic cells induce naive T cell differentiation into T helper cell type 2 (Th2) or Th1/Th2 effectors: role of stimulator/responder ratio. *J. Exp. Med.* **192**, 405–412.
- Langenkamp, A., Messi, M., Lanzavecchia, A., Sallusto, F. (2000) Kinetics of dendritic cell activation: impact on priming of Th1, Th2 and nonpolarized T cells. *Nat. Immunol.* **1**, 311–316.
- Kadowaki, N., Antonenko, S., Lau, J. Y. N., Liu, Y. J. (2000) Natural interferon α/β -producing cells link innate and adaptive immunity. *J. Exp. Med.* **192**, 219–226.
- Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, A., Colonna, M. (1999) Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat. Med.* **5**, 919–923.
- Siegal, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P. A., Shah, K., Ho, S., Antonenko, S., Liu, Y.-J. (1999) The nature of the principal type 1 interferon-producing cells in human blood. *Science* **284**, 1835–1837.
- Everson, M. P., McDuffies, D. S., Lemak, D. G., Koopman, W. J., McGhee, J. R., Beagley, K. W. (1996) Dendritic cells from different tissues induce production of different T cell cytokine profiles. *J. Leukoc. Biol.* **59**, 494–498.
- Hammad, H., Charbonnier, A. S., Duez, C., Jacquet, A., Stewart, G. A., Tonnel, A. B., Pestel, J. (2001) Der p 1 pulsed-dendritic cells derived from allergic patients sensitive to house dust mite favor a Th2 response. *Blood* **98**, 1135–1141.
- de Wit, D., Amraoui, Z., Vincart, B., Michel, O., Michils, A., van Overvelt, L., Willens, F., Goldman, M. (2000) Helper T-cell responses elicited by Der p 1-pulsed dendritic cells and recombinant IL-12 in atopic and healthy subjects. *J. Allergy Clin. Immunol.* **105**, 346–352.
- Bellinghausen, I., Brand, U., Knop, J., Saloga, J. (2000) Comparison of allergen-stimulated dendritic cells from atopic and nonatopic donors dissecting their effect on autologous naive and memory T helper cells of such donors. *J. Allergy Clin. Immunol.* **105**, 988–996.
- Bellini, A., Vittori, E., Marini, M., Ackerman, V., Mattoli, S. (1993) Intraepithelial dendritic cells and selective activation of Th2-like lymphocytes in patients with atopic asthma. *Chest* **103**, 997–1005.
- Moller, G. M., Overbeek, S. E., Van Helden Meeuwse, C. G., Van Haarst, J. M., Prens, E. P., Mulder, P. G., Postma, D. S., Hoogsteden, H. C. (1996) Increased numbers of dendritic cells in the bronchial mucosa of atopic asthmatic patients: downregulation by inhaled corticosteroids. *Clin. Exp. Allergy* **26**, 517–524.
- Lambrecht, B. N., Salomon, B., Klatzmann, D., Pauwels, R. A. (1998) Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J. Immunol.* **160**, 4090–4097.
- Stumbles, P., Thomas, J. A., Pimm, C. L., Lee, P. T., Venaille, T. J., Proksch, S., Holt, P. G. (1998) Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require

- obligatory cytokine signals for induction of Th1. *J. Exp. Med.* **188**, 2019–2031.
26. Uchida, Y., Kurasawa, K., Nakajima, H., Nakagawa, N., Tanabe, E., Sueishi, M., Saito, Y., Iwamoto, I. (2001) Increase of dendritic cells of type 2 (DC2) by altered response to IL-4 in atopic patients. *J. Allergy Clin. Immunol.* **108**, 1005–1011.
27. Jahnsen, F. L., Lund-Johansen, F., Dunne, J. F., Farkas, L., Haye, R., Brandtaeg, P. (2000) Experimentally induced recruitment of plasmacytoid (CD123^{high}) dendritic cells in human nasal allergy. *J. Immunol.* **165**, 4062–4068.
28. Lombardero, M., Heymann, P. W., Platts-Mills, T. A. E., Fox, J. W., Chapman, M. D. (1990) Conformational stability of B cell epitopes on group I and group II *Dermatophagoides* spp. allergens. *J. Immunol.* **144**, 1353–1360.
29. King, C., Simpson, R. J., Moritz, R. L., Reed, G. E., Thompson, P. J., Stewart, G. A. (1996) The isolation and characterization of a novel collagenolytic serine protease allergen (Der p 9) from the dust mite *Dermatophagoides pteronyssinus*. *J. Allergy Clin. Immunol.* **98**, 739–747.
30. Dzionek, A., Fuchs, A., Schmidt, P., Cremer, S., Zysk, M., Miltenyi, S., Buck, D. W., Schmitz, J. (2000) BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J. Immunol.* **165**, 6037–6046.
31. Deslée, G., Charbonnier, A. S., Hammad, H., Angyalosi, G., Tillie-Leblond, I., Mantovani, A., Tonnel, A. B., Pestel, J. (2000) Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells. *J. Allergy Clin. Immunol.* **110**, 763–770.
32. Willmann, K., Dunne, J. F. (2000) A flow cytometry immune function assay for human peripheral blood dendritic cells. *J. Leukoc. Biol.* **67**, 536–544.
33. Balbo, P., Silvestri, M., Rossi, G. A., Crimi, E., Burastero, S. E. (2001) Differential role of CD80 and CD86 on alveolar macrophages in the presentation of allergen to T lymphocytes in asthma. *Clin. Exp. Allergy* **31**, 625–636.
34. Hofer, M. F., Jirapongsananuruk, O., Trumble, A. E., Leung, D. Y. (1998) Upregulation of B7.2 but not B7.1 on B cells from patients with allergic asthma. *J. Allergy Clin. Immunol.* **101**, 96–102.
35. Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J. E., Roncarolo, M. G. (1997) A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389**, 737–742.
36. Corinti, S., Albanesi, C., la Sala, A., Pastore, S., Girolomoni, G. (2001) Regulatory activity of autocrine IL-10 on dendritic cell functions. *J. Immunol.* **166**, 4312–4318.
37. Steinbrink, K., Wölfl, M., Jonuleit, H., Knop, J., Enk, A. (1997) Induction of tolerance by IL-10-treated dendritic cells. *J. Immunol.* **159**, 4772–4780.
38. Jonuleit, H., Schmitt, E., Schuler, G., Knop, J., Enk, A. H. (2000) Induction of interleukin 10-producing, nonproliferating CD4⁺ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J. Exp. Med.* **192**, 1213–1222.
39. Bellinghausen, I., Brand, U., Steinbrink, K., Enk, A. H., Knop, J., Saloga, J. (2001) Inhibition of human allergic T-cell responses by IL-10-treated dendritic cells: differences from hydrocortisone-treated dendritic cells. *J. Allergy Clin. Immunol.* **108**, 242–249.
40. Akbari, O., DeKruyff, R. H., Umetsu, D. T. (2001) Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat. Immunol.* **2**, 725–731.
41. Tournay, K. G., Kips, J. C., Pauwels, R. A. (2000) Endogenous interleukin-10 suppresses allergen-induced airway inflammation and nonspecific airway responsiveness. *Clin. Exp. Allergy* **30**, 775–783.
42. Salvi, S. S., Babu, K. S., Holgate, S. T. (2001) Is asthma really due to a polarized T cell response toward a helper T cell type 2 phenotype? *Am. J. Respir. Crit. Care Med.* **164**, 1343–1346.
43. Cembrzynska-Nowak, M., Szklarz, E., Inglot, A. D., Teodorczyk-Injeyan, J. A. (1993) Elevated release of tumor necrosis factor- α and interferon- γ by bronchoalveolar leukocytes from patients with bronchial asthma. *Am. Rev. Respir. Dis.* **147**, 291–295.
44. Krug, N., Madden, J., Redington, A. E., Lackie, P., Djukanovic, R., Schauer, U., Holgate, S. T., Frew, A. J., Howarth, P. H. (1996) T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am. J. Respir. Cell. Mol. Biol.* **14**, 319–326.
45. Randolph, D. A., Carruthers, C. J. L., Szabo, S. J., Murphy, K. M., Chaplin, D. D. (1999) Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J. Immunol.* **162**, 2375–2383.
46. Mosca, P. J., Hobeika, A. C., Clay, T. M., Nair, S. K., Thomas, E. K., Morse, M. A., Lyerly, H. K. (2000) A subset of human monocyte-derived dendritic cells expresses high levels of interleukin-12 in response to combined CD40 ligand and interferon- γ treatment. *Blood* **96**, 3499–3504.
47. Nigou, J., Zelle-Rieser, C., Gilleron, M., Thurnher, M., Puzo, G. (2001) Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: evidence for a negative signal delivered through the mannose receptor. *J. Immunol.* **166**, 7477–7485.
48. Kadowaki, N., Ho, S., Antonenko, S., de Wall Malefyt, R., Kastelein, R. A., Bazan, F., Liu, Y.-J. (2001) Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* **194**, 863–869.
49. Vercelli, D., Jabara, H. H., Lauener, R. P., Geha, R. S. (1990) IL-4 inhibits the synthesis of IFN- γ and induces the synthesis of IgE in human mixed lymphocyte cultures. *J. Immunol.* **144**, 570–573.
50. Patterson, S. (2000) Flexibility and cooperation among dendritic cells. *Nat. Immunol.* **1**, 273–274.