

CD4+ T Cells Have a Key Instructive Role in Educating Dendritic Cells in Allergy

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Key Words

T-cell response in allergy • Dendritic cells • Microarray • Rhinitis

Abstract

Background: Dendritic cells (DCs) are central in allergy as regulators of the Th1/Th2 balance. We have recently demonstrated a unique transcriptional profile of DCs in patients with ongoing allergy compared with healthy subjects and shown that crosstalk between DCs and memory T cells affects the transcriptional profile of T cells. However, the transcriptional profile of DCs educated by T cells in allergy is unknown. **Methods:** In the present study, we have examined the transcriptional profiles of DCs after stimulation with grass pollen allergens, *Phleum pratense* and coculture with autologous CD4+ memory T cells using high-density microarray. Protein analysis was performed using flow cytometry and recombinant antibody protein microarrays. Patients with allergic rhinitis and healthy subjects were compared. **Results:** The results reveal a distinct T-cell-induced DC profile in atopic individuals. Accordingly, about 170 genes were upregulated and 40 genes downregulated. For example, the chemokine receptor CXCR4 and the tumor necrosis factor receptor CD30 were upregulated in DCs derived from atopic donors, and this could also be verified at the protein level. **Conclusion:** We conclude that crosstalk between CD4+

memory T cells and autologous DCs induces transcriptional reprogramming in DCs. This finding suggests that T cells have a key instructive role in educating DCs in Th2-type responses.

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Introduction

Dendritic cells (DCs) are key regulators of the Th1/Th2 balance [1]. Accordingly, DCs respond to allergen exposure by internalizing the allergen, expressing it on the cell surface and presenting it to T cells [2]. In agreement, in material obtained from patients with ongoing allergic rhinitis, we have recently shown that crosstalk between antigen-presenting DCs and CD4+ memory T cells results in a specific transcriptional T-cell profile [3].

We have previously shown that the transcriptional profile of *Phleum pratense* (*Phl p*)-stimulated DCs is different in patients with ongoing allergic rhinitis (cf. healthy subjects), suggesting that DCs produce factors that may regulate the T-cell response in allergy [3]. However, the direct effect of allergen on DC function is not the only regulating factor. T cells also have an important role in modulating the antigen-presenting function of DCs [4]. This is emphasized by the fact that although DCs do not

upregulate costimulatory molecules upon *Phl p* stimulation, the immunological outcome is Th2 cytokine production and proliferation [3]. It has recently been shown that DCs can transfer messages from memory Th cells to naïve Th cells [5]. This finding suggests the possibility of an instructive crosstalk between DCs and memory T cells. If valid for allergy, this would add to the complexity of DC and T-cell interactions and potentially strengthen the case of viewing the DC as a treatment target. The possibility of an instructive crosstalk between DCs and memory T cells in allergy encouraged us to perform transcriptional profiling of DCs after cognate T-cell interaction.

In the present study, involving blood samples obtained from patients with ongoing allergic rhinitis and from healthy subjects, monocyte-derived DCs (MoDCs) have been produced and subjected to allergen exposure as well as to autologous CD4⁺ memory T-cell contact. We have focused our analysis on the transcriptional profile of DCs after 3 days of crosstalk in coculture and on selected proteins for verification using flow cytometry. Cell supernatants have also been analyzed using recombinant antibody microarrays [6]. We show that memory CD4⁺ T cells change the transcriptional profile of DCs. We were able to identify a panel of activated genes common in both healthy and allergic subjects. Interestingly, CD4⁺ T cells from allergic subjects also induced a transcriptional signature in autologous DCs that were not seen in the samples from healthy individuals. Allergy-specific transcripts encompass functional groups such as Toll-like receptors (TLRs), chemokine receptors, chemokine ligands and interleukin receptors, and the results suggest an instructive role for memory CD4⁺ T cells in the crosstalk between DCs and T cells in allergic rhinitis.

Material and Methods

Medium, Cytokines and Antibodies

Cell culture medium RPMI-1640 was supplemented with 5% (v/v) fetal bovine serum (Invitrogen Gibco, Carlsbad, Calif., USA), 50 µg ml⁻¹ gentamicin (Invitrogen), and 2 mM L-glutamine (Sigma-Aldrich, St. Louis, Mo., USA), and is referred to as R5 medium. Recombinant human (rh) granulocyte macrophage colony-stimulating factor was obtained from Immunex (Seattle, Wash., USA) and rhIL-4 was purchased from R&D Systems (Minneapolis, Minn., USA). The following labeled antibodies (Abs) were used for fluorescence-activated cell sorter (FACS) analysis: CD14-PE, CD1a-FITC, CD86-FITC, CD3-PeCy5, CD11c-FITC and streptavidin-PE (Dako Cytomation, Glostrup, Denmark), as well as HLADR-PE, CD80-PE and CXCR4-biotin (Becton Dickinson, San Jose, Calif., USA). Rabbit-anti-mouse-PE (Dako Cytomation)

was used as a secondary reagent to unconjugated IL17RB (R&D Systems) and CD30 Abs (Dako Cytomation). Streptavidin-PE (Dako Cytomation) was used as secondary reagent to CXCR4. Flow cytometry analyses were performed on a FACScan (Becton Dickinson), and generated data were analyzed with FlowJo (Tree Star Inc., Ashland, Oreg., USA) or CellQuest (Becton Dickinson).

Classification of Atopic and Healthy Subjects

Eight patients with allergic rhinitis and 5 healthy individuals participated in the study. The allergic donors tested positive for *Phl p* in a skin prick test and had a history of strictly seasonal allergic rhinitis. Furthermore, as inclusion criteria, these patients presented high levels of *Phl p*-specific IgE, *Phl p*-induced production of Th2 cytokines, T-cell proliferation and basophil degranulation. The healthy subjects all tested negative in a skin prick test and had no history of symptoms of allergic rhinitis. While 3 patients with allergic rhinitis and 3 healthy subjects participating in the microarray analysis were exposed to allergen during the grass pollen season, 5 atopics involved in the verification of gene expression findings were subjected to once-daily allergen challenges for 7 days, i.e. an 'artificial pollen season' [7]. The 'artificial season' was introduced in order to be able to study ongoing allergic response in atopic individuals out of season, to get homogeneous donor material and save time. Increasing doses of allergen were administered at 10-min intervals using a spray device delivering 100 µl per actuation. One actuation was administered into each nostril resulting in effective doses of 100, 300, 1,000 and 3,000 standardized quantity units per nasal cavity. This scheme was followed until the subject responded with at least 5 sneezes or recorded a symptom score of 2 or more on a scale from 0 to 3 for either of the symptoms, nasal secretion or nasal blockage, i.e. the threshold dose. In the present study, in contrast to our previous studies where this allergen has been chosen for the allergen challenge series, we chose a dose 1 dose step higher than the threshold dose in order to produce somewhat more marked symptoms. The allergen challenge was given once daily for 7 days. All donors had a history of allergic rhinitis and no infection within 4 weeks prior to blood sampling. Furthermore, no asthma, nasal polyposis or other comorbidity was allowed in the present, recent or past history. Patients were free of medication for 1 month before the study was initiated. The healthy and allergic subjects were matched with regard to gender and age. The study was approved by the regional ethics committee, and informed consent was obtained.

Allergen-Specific IgE/Basophil Degranulation Assay

Allergen-specific IgE in serum from healthy and allergic participants was determined using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions. The detection limit of the assay is 0.35 kUA l⁻¹. The BD Fastimmune assay (Becton Dickinson), using CD123-PE, HLA-DR-PerCP and CD63-FITC Abs, was performed on basophils from atopic and healthy subjects according to the manufacturer's instructions. The statistical significance was determined by Student's t test.

Generation of MoDCs

In order to generate immature MoDCs, monocytes were purified (>97% purity) from peripheral blood and cultured for 7 days in R5 culture medium supplemented with rhIL-4 (50 ng ml⁻¹) and

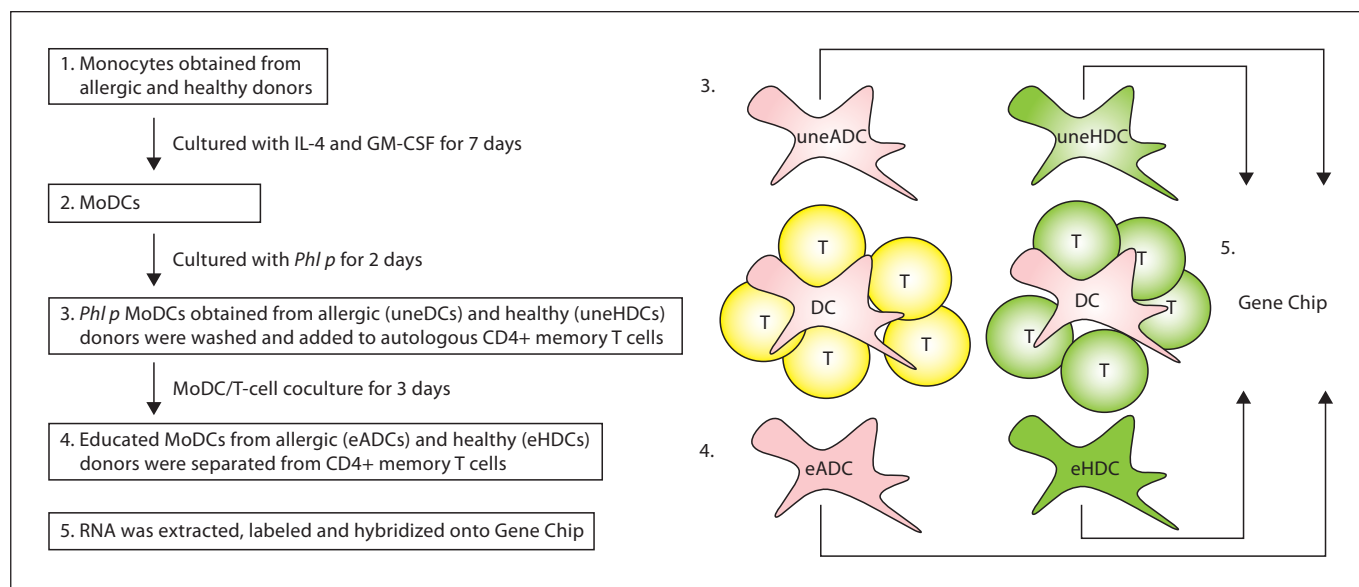


Fig. 1. Flow chart of the procedure of generating uneducated DCs and eDCs from allergic patients and healthy donors and the transcriptional analysis of these populations as described in Material and Methods. *Phl p*-stimulated DCs were derived from allergic (uneADCs) and healthy (uneHDCs) donors. These populations have not received instructive signals from memory CD4⁺ T cells. T-cell-primed *Phl p*-stimulated DCs were derived from allergic (eADCs) and healthy (eHDCs) donors. First, transcripts denoted absent in 3 out of 3 allergic samples and in 2 out of 2 healthy samples and with intensity values <100 were discarded. Second, tran-

scripts with a fold change ≥ 2 between uneADCs and eADCs were selected. This analysis was also performed using samples derived from healthy donors. These 2 generated gene lists were analyzed in a Venn diagram, and the transcripts expressed in both groups were analyzed and are presented in table 3. Furthermore, transcripts expressed in allergic DC samples after T-cell contact (eADCs) with a fold change ≥ 2 as compared with uneADCs, uneHDCs and eHDCs were selected based on the mean value of the replicates. GM-CSF = Granulocyte macrophage colony-stimulating factor.

rh granulocyte macrophage colony-stimulating factor (150 ng ml⁻¹), as described elsewhere [8]. Immature MoDCs (>90% CD14⁺/CD1a⁺/CD86⁺/HLADR^{low}) were stimulated with 25 μ g ml⁻¹ endotoxin-free *Phl p* extract, a gift from P. Adler-Würtzen (ALK-Abello, Horsholm, Denmark) for 48 h or left unstimulated. MoDCs were harvested and washed twice before coculture with memory CD4⁺/CD27⁻ T cells (fig. 1).

Isolation of Memory CD4⁺ T Cells

CD4⁺ T cells from autologous donors were purified by negative selection using the CD4⁺ T-cell isolation kit II (Miltenyi Biotec, Bergisch Gladbach, Germany). Memory CD27⁻/CD4⁺ T cells were further isolated by negative selection using anti-CD45RA, anti-CD8 and anti-glycophorin A monoclonal Abs (Dako Cytomation) and microbead-conjugated anti-CD27 Abs (Miltenyi Biotec) for 15 min at 4°C. Subsequently, the cell suspension was labeled with microbead-conjugated goat-anti-mouse monoclonal Abs (Miltenyi Biotec) for 15 min at 4°C. Depletion of contaminating cells was performed with LS columns (Miltenyi Biotec), which resulted in 80–90% memory CD4⁺ T cells enriched for CD27⁻ cells (fig. 1). Memory CD4⁺ T cells (10⁶ cells/ml) were added to already washed MoDCs (5 $\times$ 10⁴ cells/ml) and cultured for 3 days in 37°C in 5% CO₂.

Separation of DC and T-Cell Coculture

In order to determine the gene expression in MoDCs after T-cell contact, i.e. educated DCs (eDCs), *Phl p*-loaded MoDCs were separated from the T cells after 3 days of coculture. First, the cells were treated with 5 mM EDTA (Merck, Darmstadt, Germany) for 10 min at 4°C. Thereafter, the complete cell culture was incubated with microbead-conjugated anti-CD3 Abs (Miltenyi Biotec) for 15 min and separated on an LS column (Miltenyi Biotec), resulting in 2–10% contaminating T cells. DCs were lysed in Trizol reagent (Invitrogen), and the total RNA was stored at -20°C until further mRNA isolation (fig. 1).

Preparation of cRNA and Gene Chip Hybridization

Phl p-stimulated DCs from 3 allergic and 2 healthy individuals were used in the transcriptional analysis after separation from autologous memory CD4⁺/CD27⁻ T cells. Fragmentation, hybridization and scanning of the human U133Plus 2.0 arrays were performed according to the manufacturer's protocol (Affymetrix, Santa Clara, Calif., USA) at the Swegene Microarray Resource Center, Lund University, Sweden, which is an Affymetrix Service Provider. The preparation of labeled cRNA was performed according to the Two-Cycle Eukaryotic Target labeling assay protocol, using the Gene Chip Expression 3'-amplification 2-cycle labeling and control reagents kit (Affymetrix), as described previously [3].

Briefly, cDNA was generated from total RNA (20–150 ng), using Superscript II (Invitrogen) and a T7-oligo(dT) promoter primer (Affymetrix). After a second-strand cDNA synthesis, cDNA was converted to cRNA by an in vitro transcription reaction (Ambion Megascript T7 kit, Applied Biosystems, Foster City, Calif., USA). Thereafter, the cRNA was purified using RNeasy Mini kit (Qiagen, Hilden, Germany), and the yield was controlled with a spectrophotometer. A second cycle of cDNA synthesis was performed, followed by the same clean up as above, as well as a second in vitro transcription reaction cycle with biotin-labeled ribonucleotides and T7 RNA polymerase. Labeled cRNA was purified, using RNeasy Mini kit (Qiagen), quality controlled with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, Calif., USA) and denatured at 94°C before hybridization. The samples were hybridized to the Human Genome U133 Plus 2.0 array at 45°C for 16 h by rotation (60 rpm) in an oven. The arrays were then washed, stained with streptavidin-PE (Invitrogen), washed again, and scanned with a GeneArray Scanner (Affymetrix).

Transcriptional Microarray Data Analysis

Fluorescence intensities were analyzed using the Gene Chip Operating Software 1.1 (Affymetrix) and scaled to a target value of 100. Further data analysis was performed using the Gene Spring TM software 7.0 (Silicon Genetics, Redwood City, Calif., USA). First, genes with intensity values <100 and designated 'absent' in all samples were discarded. The analysis included comparisons involving 4 groups of samples. The 2 main groups consisted of allergic or healthy samples. Allergic and healthy samples were further divided into 2 subgroups: (1) DCs without T-cell contact, and (2) DCs after T-cell contact. Hence, the analysis encompassed 4 groups; uneducated, i.e. without T-cell contact, DCs in allergic individuals (uneADCs); uneducated DCs in healthy individuals (uneHDCs); educated, i.e. after T-cell contact, DCs in allergic individuals (eADCs), and eDCs in healthy individuals (eHDCs) (fig. 1).

In order to select genes that were upregulated due to T-cell contact not depending on disease, a comparative analysis was performed between uneADCs and eADCs for each allergic donor and between uneHDCs and eHDCs for each healthy subject. Transcripts upregulated in eADCs, in 3 out of 3 samples, and eHDCs, in 2 out of 2 samples, resulted in 2 gene lists. Subsequently, transcripts upregulated in samples derived from both allergic and healthy donors due to T-cell contact were selected by a Venn diagram (table 3). Secondly, a comparative analysis was carried out to select transcripts specific to allergic subjects. The analysis encompassed all 4 groups in order to sort out transcripts expressed 2-fold in eADCs in comparison with uneADCs, uneHDCs and eHDCs. Transcripts up- or downregulated in eADCs were selected based on mean intensity values, and transcripts denoted 'present' in 3 out of 3 samples in eADCs were included (fig. 2).

Flow Cytometry Analysis

Verification of transcriptional findings at the protein level was performed using FACS. *Phl p*-stimulated and unstimulated MoDCs were cocultured with enriched CD4+/CD27- T cells for 3 days. Cells were harvested and labeled with anti-CD11c in order to separate DCs from T cells in the protein analysis. Prior to specific antibody labeling, unspecific binding was reduced by blocking the cells with Chrome Pure Mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa., USA). Thereafter, cells were

stained for 10 min at 4°C. PBS (w/o Ca/Mg), containing 1% BSA (Sigma-Aldrich), was used in all cell-labeling and washing steps. Gates were set to exclude apoptotic cells, cell debris and T cells based on cell size and granularity. Subsequently, subgates were set on CD11c+ cells to analyze DCs for CD30, IL17RB and CXCR4 expression before and after T-cell interactions. The statistical significance was determined by Student's *t* test.

Proliferation Assay

Phl p-stimulated and unstimulated MoDCs (5×10^4 cells/ml) were cocultured with enriched CD4+/CD27- T cells (10^6 cells/ml) in R5 medium supplemented with 2×10^{-5} M 2-mercaptoethanol (Merck, Whitehouse, N.J., USA) at a DC:T-cell ratio of 1:20 for 7 days. During the last 16 h, $2.5 \mu\text{Ci ml}^{-1}$ ^3H -thymidine (Amersham Biosciences, Uppsala, Sweden) was added to each well. Thymidine incorporation was measured using a Matrix 96 Direct beta scintillation counter (Packard, Meriden, Conn., USA). Samples were run in triplicates, and the statistical significance was determined with the Wilcoxon matched-pairs signed-rank test.

Intracellular Cytokine Production

Memory CD4+/CD27- T cells were cocultured with *Phl p*-stimulated and unstimulated MoDCs for 7 days. Intracellular detection of cytokines was performed after stimulation of the T cells with phorbol myristate acetate (50 ng ml^{-1}), ionomycin (500 ng ml^{-1}), and brefeldin A ($10 \mu\text{g ml}^{-1}$; Sigma-Aldrich) for 5 h. The cells were washed twice in PBS, fixed with 2% paraformaldehyde (Sigma-Aldrich) for 15 min, permeabilized using 0.5% saponin/PBS (Sigma-Aldrich) and stained with anti-IL-4 PE (Becton Dickinson), anti-interferon (IFN)- γ FITC and anti-IL-13 PE (Pharmingen, San Diego, Calif., USA) for 30 min at 4°C. Thereafter, cells were washed twice in 0.5% saponin/PBS, resuspended in 1% BSA/PBS and analyzed with a FACScan. The statistical significance was determined with the Wilcoxon matched-pairs signed-rank test.

Production and Purification of Single-Chain Variable Fragment

Eighty-eight human recombinant antibody fragments against 28 unique protein analytes (chemokines, cytokines and growth factors), selected from the n-CoDeR library [9], were kindly provided by Bioinvent International AB (Lund, Sweden). All single-chain variable fragments were produced in 100 ml *Escherichia coli* cultures and purified from the expression supernatant using affinity chromatography on Ni-NTA agarose (Qiagen).

Labeling of Cell Culture Supernatants

Cell culture supernatants of *Phl p*-stimulated DCs and cocultures of *Phl p*-stimulated DCs and autologous memory CD4+ T cells from 3 atopic and 3 healthy donors were biotinylated at a molar ratio of biotin:protein of 45:1 using the EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, Ill., USA). A labeling protocol, recently optimized for labeling of complex samples in an efficient and reproducible manner, was adopted [6]. The biotinylated samples were extensively dialyzed against PBS at 4°C to remove unreacted biotin and stored at -20°C until further use.

Fabrication and Processing of Antibody Microarrays

For production and processing of the antibody microarrays, we used a set-up previously optimized [6, 10]. Briefly, 2–25 fmol

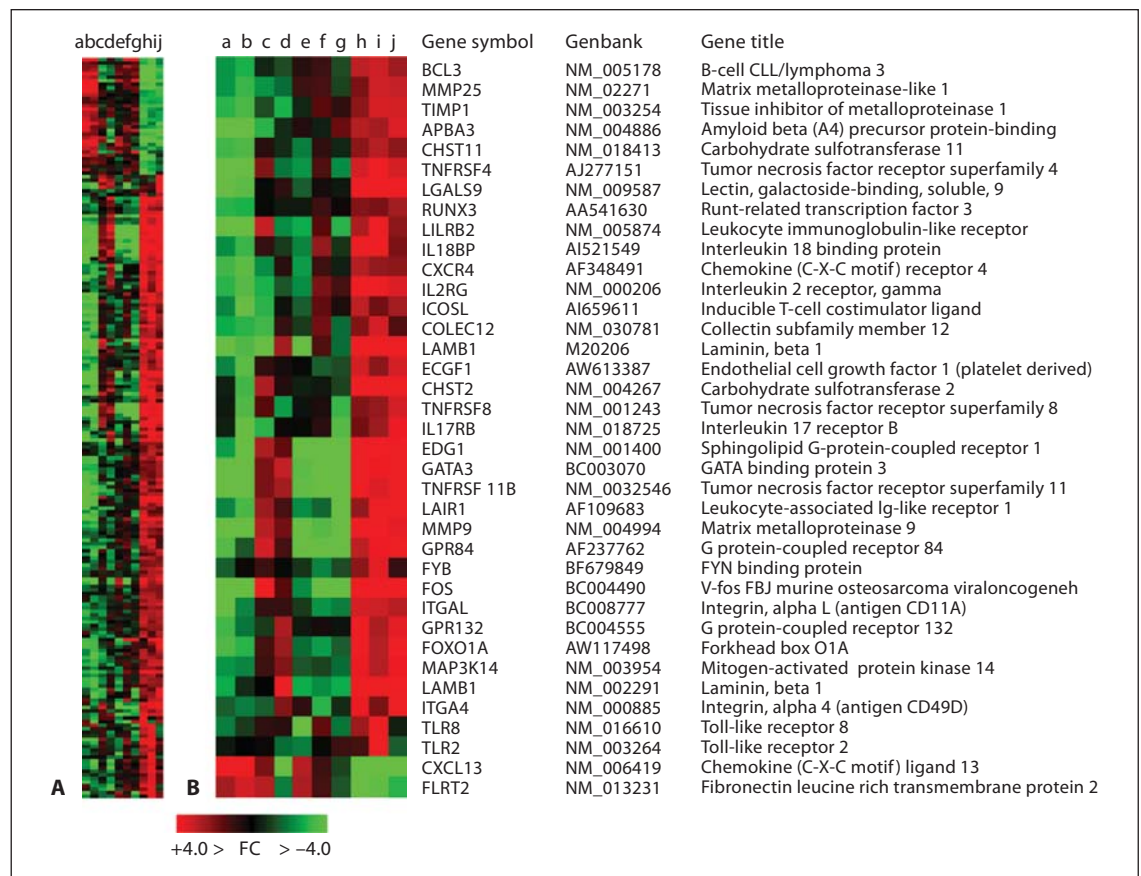


Fig. 2. Differentially expressed genes (>2 fold) in eADCs, as compared with uneADCs, uneHDCs and eHDCs (A). The results represent the effect of 2 parameters: DC/T-cell contact and the allergic status of the donors. Importantly, all genes in the list represent transcripts upregulated ≥ 2 fold in each of the 3 allergic donors and are thus considered as potential contributors to the allergic response reflected in this study (B). Each sample is represented: uneHDCs (a, b), eHDCs (c, d), uneADCs (e–g) and eADCs (h–j).

(5 fmol average) of each single-chain variable fragment was dispensed in 8 replicates onto black polymer Maxisorp microarray slides (Nunc, Roskilde, Denmark), using a non-contact Biochip Arrayer 1 (Perkin Elmer Life and Analytical Sciences, Wellesley, Mass., USA). The arrays were blocked with 5% (w/v) fat-free milk powder (Semper, Sundbyberg, Sweden) in PBS overnight. All incubations were conducted in a humidity chamber at room temperature. The arrays were then washed 4 times with 0.05% Tween-20 in PBS (PBS-T) and incubated with biotinylated sample diluted 1:2 in 1% (w/v) fat-free milk powder and 1% Tween in PBS (PBS-MT) for 1 h. Next, the arrays were washed 4 times with PBS-T and incubated with 1 μ g/ml Alexa Fluor 647 conjugated streptavidin diluted in PBS-MT for 1 h. Finally, the arrays were washed 4 times with PBS-T, dried immediately under a stream of nitrogen gas and scanned with a confocal microarray scanner (Scan Array Express, Perkin Elmer Life and Analytical Sciences) at a resolution of 5 μ m, using 4 different scanner settings. The Scan Array Express software version 2.0 (Perkin Elmer Life and Analytical Sciences) was used for a quantitative analysis of the intensity of each spot using

the fixed circle method. The local background was subtracted, and to compensate for possible local defects, the 2 highest and the 2 lowest replicates were automatically excluded, and each data point represents the mean value of the remaining 4 replicates.

Antibody Microarray Data Analysis and Normalization

Only intensity-unsaturated spots were used in the further analysis of the data. Chip-to-chip normalization of the data sets was performed using a semi-global normalization approach. The 14% of the analytes that displayed the lowest coefficient of variation values over all samples were identified, corresponding to 12 analytes, and used to calculate a chip-to-chip normalization factor. The normalization factor N_i was calculated by the formula $N_i = \mu / \mu_i$, where μ is the averaged signal intensity of the 12 analytes for all samples, and μ_i is the average signal intensity for the 12 analytes for each sample. Each data set generated from 1 sample was multiplied with the normalization factor. Mean intensity values for negative PBS controls from each sample were subtracted from the data set from each sample before extraction of altered

Table 1. Atopic and healthy profiles as determined by skin prick test (SPT), score for symptoms and timothy-specific IgE

Donor	History of allergic rhinitis	SPT	Allergy	Rhinor-rhea score	Conges-tion score	<i>Phl p</i> -specific IgE kUA l ⁻¹
<i>Healthy subjects</i>						
1	no	–	no	0	0	<0.35
2	no	–	no	0	0	<0.35
3	no	–	no	0	0	<0.35
<i>Atopics</i>						
1	yes	+	timothy	0	0	1.76
2	yes	+	timothy	2	2	3.99
3	yes	+	timothy, birch, dog, cat	0	0	91.2
4	yes	+	timothy, birch, mugwort	0 ^a	2 ^a	79.2
5	yes	+	timothy, birch, dog	1 ^a	2 ^a	3.32
6	yes	+	timothy, birch, dog	3 ^a	3 ^a	31.6
7	yes	+	timothy, birch, dog, cat	3 ^a	3 ^a	27.6
8	yes	+	timothy	3 ^a	2 ^a	812

Samples from healthy subjects were subjected to transcriptional analysis as well as to recombinant antibody microarray. Samples from atopics were subjected to the same tests (No. 1–3) and to FACS analysis (No. 4–8). Atopic No. 1 experienced marked symptoms of allergic rhinitis during the pollen season, but was asymptomatic on the day blood was drawn. Atopic No. 3 also had seasonal rhinitis symptoms as well as marked sneezing (score 2) and itching (score 1) on the day blood was drawn, but scored 0 for rhinorrhea and congestion on that particular day.

^a Subjects were exposed daily to allergen 7 days prior to blood sampling.

analytes. Significantly up- or downregulated analytes were identified based on 3 criteria. Analytes with a *p* value <0.05 for each donor were selected. Subsequently, up- or downregulated analytes detected in coculture as compared with *Phl p*-stimulated DC culture were based on fold change ± 1.3 . Finally, significant analytes with fold change ± 1.3 in 2 of 3 donors generated a list of up- or downregulated analytes in samples derived from allergic donors.

Results and Discussion

In this study, we report on a memory T-cell-induced reprogramming of *Phl p*-stimulated DCs that is valid for both healthy and allergic donors. Furthermore, the analysis reveals a transcriptional profile, specific to allergy, in eDCs induced by CD4⁺ memory T cells. As blood DCs are very scarce and a heterogenous population of cells, we used MoDCs in this study to enable the phenotypic, transcriptional and functional studies. MoDCs are regarded as equivalent to immature DCs in vivo and are widely used for experimental as well as for clinical purposes [2, 11].

In order to study molecular mechanisms using transcriptional analysis, it is of great importance to include well-characterized patients and to employ strict criteria to the material used. The patients included in the present study all had symptoms of allergic rhinitis, a positive skin prick test to *Phl p*, and high levels of *Phl p*-specific IgE. The material obtained was further characterized by *Phl p*-induced production of Th2 cytokines, T-cell proliferation and basophil degranulation. Accordingly, atopic donors had *Phl p*-specific IgE ranging between 2 and 800 kUA l⁻¹, whereas the levels were below the detection limit for healthy controls (table 1). The percentage of degranulated basophils was significantly higher in samples derived from atopic donors compared with healthy subjects (fig. 3A). In addition, a significant increase in T-cell proliferation was detected following stimulation with *Phl p*-conditioned DCs derived from allergic donors (ADCs) compared with unstimulated controls (from allergic donors) (fig. 3B). As would be expected, no significant increase in T-cell proliferation was detected by allergen-stimulated DCs derived from healthy donors (HDCs), compared with unstimulated controls (fig. 3B). Th2-cytokine levels were elevated in memory CD4⁺ T cells after coculture with *Phl p*-stimulated ADCs (cf. unstimulated ADCs) (fig. 3C–E). In contrast, no Th2 cytokine production was detected in memory CD4⁺ T cells after coculture using *Phl p*-stimulated HDCs compared with unstimulated HDCs (fig. 3C–E). No difference in the production of IFN- γ was observed between memory CD4⁺ T cells derived from atopic and healthy donors (fig. 3F). We conclude that the present material obtained from patients with allergic rhinitis had a clear atopic profile, and, conversely, that such features were absent in the healthy individuals. This is in line with previous studies with patient material obtained from the natural season [12]. In this context, the ‘artificial pollen season’ produced material that was indistinguishable from that obtained during the natural pollen season.

Phl p stimulation is not sufficient to induce DC maturation and, evidently, DCs require contact with autologous T cells for maturation [3]. Monitoring the kinetics of DC activation during a 7-day coculture period revealed that high levels of CD86 and CD80 were present already at day 3. Thus, in the present study, *Phl p*-loaded DCs from 3 atopic donors and 3 healthy donors were prepared for microarray analysis after a 3-day coculture period with T cells. One sample from a healthy donor had to be subsequently discarded due to a high percentage (15%) of contaminating T cells. The other 5 samples contained 5–10% T cells, which may affect the transcrip-

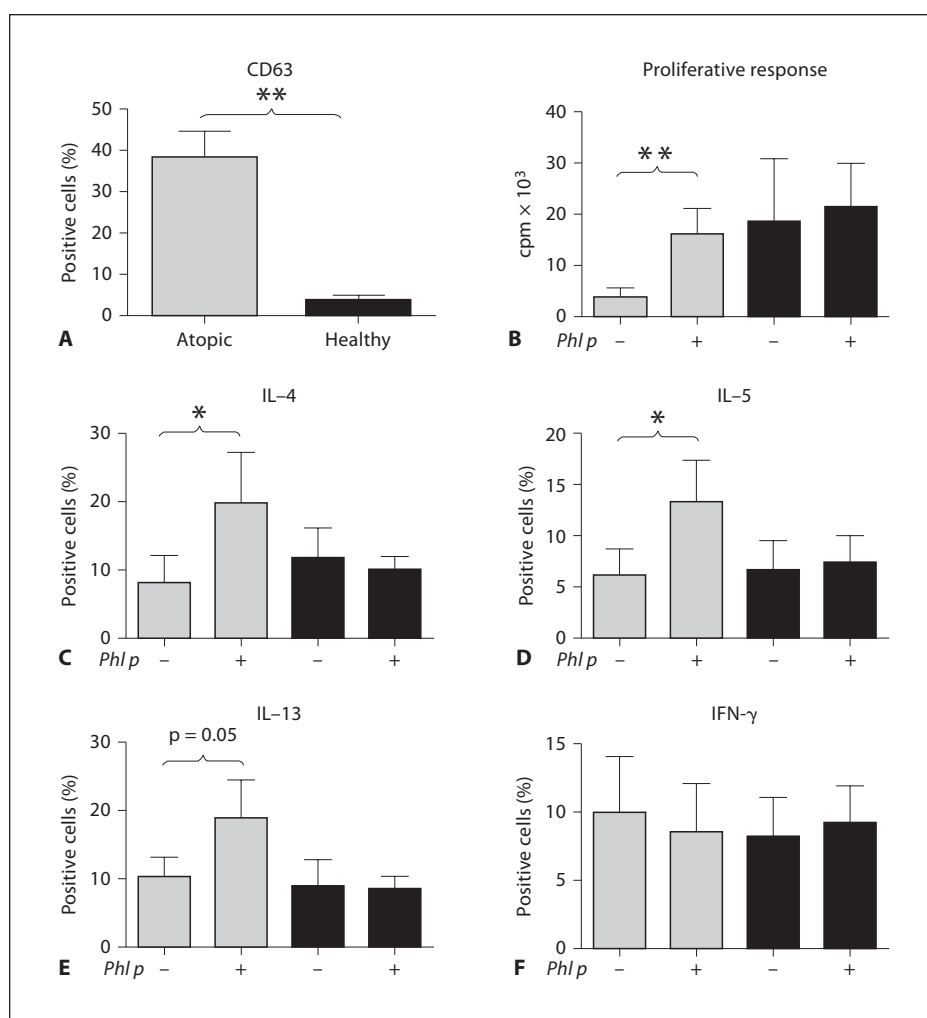


Fig. 3. Profiling atopic and healthy donors by assessment of basophil degranulation (A), T-cell proliferation (B) and intracellular T-cell cytokine production (C–F). Degranulation of basophils was measured by flow cytometry and resulted in a significantly higher percentage of CD63+ basophils in atopic donors as compared with healthy subjects ($p = 0.001$) (A). *Phl p*-stimulated DCs induced significant T-cell proliferation in atopic but not in healthy donors ($p = 0.002$ and 0.75 , respectively) (B). The production of IL-4 (C) and IL-5 (D) was significantly elevated in atopic donors as determined by flow cytometry ($p = 0.02$ and 0.008 , respectively) in contrast to healthy donors ($p = 0.75$ and 0.5 , respectively). The production of IL-13 (E) was elevated in *Phl p*-stimulated cells derived from allergic donors as compared with unstimulated cells

($p = 0.05$), and no elevation was detected between samples derived from healthy donors ($p = 1.0$). The production of IFN- γ was detected in cells derived both from atopic and healthy donors, without any significant differences (F). Unstimulated ADCs and HDCs were used as controls in all experiments. Atopic donors are represented by gray bars and healthy donors by black bars. *Phl p*-stimulated samples are marked (+) and unstimulated samples are marked (-). Data are presented as mean values $\pm$ SEM. Student's *t* test was applied to determine statistical significance of basophil degranulation and the Wilcoxon matched-pairs signed-rank test was applied for Th2 cytokine production and proliferation * $p < 0.05$, ** $p < 0.005$.

tional analysis. To secure the absence of transcripts derived from the contaminating cells, the presence of typical T-cell transcripts was determined. Out of 16 T-cell transcripts, only 1 was expressed in eHDCs (CD3D) and 3 in eADCs (CD3D, CD3Z and IL-9) (table 2). None of

the 16 T-cell transcripts were expressed in uneHDCs and uneADCs, and thus, the contribution of contaminating T cells in the subsequent analysis was regarded as negligible.

Table 2. Intensity values for typical T-cell transcripts

Transcript	Genbank	uneHDC	eHDC	uneADC	eADC
TCRA	L34703	–	–	–	–
TCRB	AF009787	–	–	–	–
CD3D	NM_000732	–	144.9	–	188.1
CD3E	NM_000733	–	–	–	–
CD3G	NM_000073	–	–	–	–
CD3Z	J04132	–	–	–	178.3
CD4	S79267	–	–	–	–
CD8A	AW006735	–	–	–	–
CD8B1	NM_172100	–	–	–	–
CD28	NM_006139	–	–	–	–
CTLA4	U90273	–	–	–	–
IL-4	NM_000589	–	–	–	–
IL-5	NM_000879	–	–	–	–
IL-9	NM_000590	–	–	–	277.0
IL-10	NM_000572	–	–	–	–
IL-13	NM_002188	–	–	–	–

The mean value of 3 atopic and 2 healthy donors is presented. Transcripts denoted 'absent' in at least 1 sample or with intensity values <100 are marked (–).

Transcriptional Activity Induced in eDCs Generated from Healthy and Allergic Subjects

To evaluate general effects of DC/T-cell crosstalk, the transcriptional activity of *Phl p*-loaded DCs in response to coculture with T cells was first studied in allergic as well as in healthy subjects. Autologous CD4+ memory T cells induced a wide range of genes in *Phl p*-stimulated DCs, and transcripts that were similarly expressed in both groups were extracted. Accordingly, 1,033 transcripts were upregulated and 426 downregulated in eADCs as well as in eHDCs. Examples of upregulated transcripts include proteins involved in key DC functions (table 3), such as antigen processing (*LAMP1*, *LAMP3*), adhesion (*ICAM1*, *ICAM2*), chemotaxis (*CX3CR1*, *CXCR6*, *CCR7*, *CXCL2*, *CXCL9*, *CXCL10*, *CXCL11*, *CCL5*, *CCL17*, *CCL19*, *CCL22*) and T-cell costimulation (*CD80*, *CD83*, *ICOS*). The upregulation of CD80 expression on the cell surface was verified by flow cytometry (data not shown). In addition, transcripts corresponding to regulatory molecules such as interleukins (*IL1B*, *IL-8*, *IL12B*, *IL-15*), interleukin receptors/binding proteins (*IL2RA*, *IL2RB*, *IL7R*, *IL9R*, *IL15RA*, *IL18RAP*, *IL21R*, *IL18BP*), tumor necrosis factor (TNF)-induced proteins (*TNFAIP2*, *TNFAIP3*, *TNFAIP6*), TNF receptors (*RANK*, *OPG*, *CD137*) and TNF ligands (*TRAIL*, *OX40L*) were upregulated. Examples of downregulated transcripts comprised such encoding for pro-

teins involved in antigen presentation (*DC-SIGNR*), migration (*CD226*) and chemotaxis (*CCL18*, *CCL23*, *CCR5*). Transcripts upregulated but with unknown relevance to DC function included, e.g., *CD33L3* and *RGS1*, and downregulated transcripts comprised *CD36*, *CD59* and *CD99*. Taken together, our observations indicate, for the first time, that memory T cells initiate a general transcriptional activity in *Phl p*-stimulated DCs. This effect is potentially relevant for our understanding of the regulatory role of DCs in immune responses.

Allergy-Specific Transcriptional Activities in eDCs

To explore differences between transcriptional profiles in DCs from atopic and healthy subjects upon allergen and T-cell contact, the transcriptomes of eADCs were compared with uneADC, uneHDC and eHDCs. Genes with a >2-fold higher expression level in eADC, compared with the other samples, were selected. The analysis resulted in 175 upregulated and 46 downregulated genes in eADCs (fig. 2A). Relative intensity values of selected biomarkers which may influence the Th1/Th2 balance are visualized in a heatmap (fig. 2B). Accordingly, differences between atopic and healthy subjects included a range of molecules of potential importance to allergic inflammation: for example, TLRs, CXC ligands/receptors, interleukin receptors, G-protein-coupled membrane receptors, costimulatory molecules, TNF receptors, matrix metalloproteinases (MMPs) and transcription factors regulating the immune response (see below). The results demonstrate that reprogramming of *Phl p*-pulsed DCs occurs after contact with memory T-cell contact and that this T-cell-dependent education is different in DCs from allergic individuals as compared with healthy controls. The following genes are specifically upregulated in eADCs.

Toll-Like Receptors

TLRs are predominantly expressed on antigen-presenting cells and leukocytes in tissues exposed to the external environment [13]. In the present study, *TLR2* and *TLR8* were upregulated in eADCs compared with uneADCs, indicating a T-cell-induced change that is specific to allergy. Previous studies suggest that distinct TLR ligands can activate DCs differently to elicit diverse signaling pathways and cytokine profiles and, thereby, to regulate the Th1/Th2 balance [14–17]. Furthermore, increased tissue expression of TLRs, including *TLR2*, have been shown in allergic rhinitis [18]. Although TLRs are recognized primarily as receptors of key importance to the innate immune system, the above observations suggest a role for specific TLRs also in allergy/adaptive defense.

Table 3. Downregulated (*italics*) and upregulated (*regular*) genes similarly regulated in eADCs and eHDCs by a fold change (FC) in expression levels of at least 2

Gene symbol	FC A1	FC A2	FC A3	FC H1	FC H2	Genbank	Gene title
CCL17	14.9	3.2	12.4	16.0	17.7	NM_002987	chemokine (C-C motif) ligand 17
<i>CCL18</i>	-6.6	-5.9	-5.3	-3.1	-2.6	<i>Y13710</i>	<i>chemokine (C-C motif) ligand 18</i>
CCL19	139.5	41.2	54.2	47.3	119.1	U88321	chemokine (C-C motif) ligand 19
CCL22	4.6	4.0	8.1	16.3	23.6	NM_002990	chemokine (C-C motif) ligand 22
<i>CCL23</i>	-28.2	-16.2	-25.7	-18.7	-8.5	<i>U58913</i>	<i>chemokine (C-C motif) ligand 23</i>
CCL5	26.9	5.6	54.5	109.4	161.9	M21121	chemokine (C-C motif) ligand 5
CCR5	-2.0	-2.0	-2.2	-3.9	-2.3	<i>NM_000579</i>	<i>chemokine (C-C motif) receptor 5</i>
CCR7	45.4	17.0	43.4	39.4	122.5	NM_001838	chemokine (C-C motif) receptor 7
CD137	22.7	15.7	67.2	37.9	70.9	NM_001561	tumor necrosis factor receptor superfamily, member 9
<i>CD226</i>	-2.9	-3.7	-2.2	-3.2	-4.8	<i>NM_006566</i>	<i>CD226 antigen</i>
CD33L3	4.7	4.0	4.4	3.2	3.0	AK025833	CD33 antigen-like 3
<i>CD36</i>	-3.8	-2.1	-8.2	-9.1	-5.6	<i>M98399</i>	<i>CD36 antigen</i>
<i>CD59</i>	-3.6	-3.5	-5.5	-4.2	-3.4	<i>NM_000611</i>	<i>CD59 antigen</i>
CD80	3.4	2.5	2.8	4.3	7.0	BC042665	CD80 antigen
CD83	3.7	2.5	3.7	2.9	4.1	NM_004233	CD83 antigen
<i>CD99</i>	-2.7	-2.9	-2.8	-3.0	-3.3	<i>NM_002414</i>	<i>CD99 antigen</i>
CX3CR1	72.4	61.4	26.4	59.8	328.8	U20350	chemokine (C-X3-C motif) receptor 1
CXCL10	220.0	641.6	464.7	178.7	218.1	NM_001565	chemokine (C-X-C motif) ligand 10
CXCL11	20.1	23.1	8.4	24.9	58.5	AF030514	chemokine (C-X-C motif) ligand 11
CXCL2	16.8	11.8	2.6	8.0	3.5	M57731	chemokine (C-X-C motif) ligand 2
CXCL9	1,753.0	391.6	213.2	587.3	464.1	NM_002416	chemokine (C-X-C motif) ligand 9
CXCR6	16.9	7.2	3.2	2.2	9.8	NM_006564	chemokine (C-X-C motif) receptor 6
<i>DC-SIGNR</i>	-3.1	-12.4	-29.6	-5.0	-4.5	<i>NM_014257</i>	<i>C-type lectin domain family 4, member M</i>
ICAM1	4.4	2.7	3.6	3.4	4.9	NM_000201	intercellular adhesion molecule 1 (CD54)
ICAM2	3.1	2.3	3.6	5.8	7.9	AA126728	intercellular adhesion molecule 2
ICOS	74.0	171.5	100.7	22.6	153.1	AB023135	inducible T-cell costimulator
IL12B	58.1	4.5	29.3	59.0	71.4	NM_002187	interleukin 12B
IL-15	19.1	16.7	28.2	21.9	21.0	NM_000585	interleukin 15
IL15RA	19.3	10.0	7.3	9.4	28.2	NM_002189	interleukin 15 receptor, alpha
IL18BP	8.8	3.6	2.5	2.4	2.3	A1521549	interleukin 18 binding protein
IL18RAP	19.6	12.3	9.8	15.9	79.4	NM_003853	interleukin 18 receptor accessory protein
IL1B	22.3	6.7	26.1	16.5	21.9	NM_000576	interleukin 1, beta
IL21R	11.1	4.5	3.6	3.9	81.2	NM_021798	interleukin 21 receptor
IL2RA	7.7	5.4	166.6	61.5	22.4	K03122	interleukin 2 receptor, alpha
IL2RB	90.1	19.2	83.3	8.4	8.6	NM_000878	interleukin 2 receptor, beta
IL7R	12.8	8.6	31.1	22.8	29.5	NM_002185	interleukin 7 receptor
IL-8	2.9	4.9	34.4	18.4	2.7	NM_000584	interleukin 8
IL9R	7.6	6.3	15.1	12.1	6.6	L39064	interleukin 9 receptor
LAMP1	3.3	2.7	2.5	6.7	6.2	NM_005561	lysosomal-associated membrane protein 1
LAMP3	16.6	10.1	25.4	10.0	32.9	NM_014398	lysosomal-associated membrane protein 3
OPG	21.2	130.1	725.0	17.6	106.7	NM_002546	tumor necrosis factor receptor superfamily, member 11b
OX40L	4.1	3.8	6.5	24.4	21.1	NM_003326	tumor necrosis factor (ligand) superfamily, member 4
RANK	7.2	2.2	4.1	3.3	6.5	AW026379	tumor necrosis factor receptor superfamily, member 11a
RGS1	74.1	51.5	52.2	16.1	120.7	NM_002922	regulator of G-protein signaling 1
TNFAIP2	13.1	12.0	16.2	15.5	23.1	NM_006291	tumor necrosis factor, alpha-induced protein 2
TNFAIP3	11.8	7.8	11.1	5.0	16.1	NM_006290	tumor necrosis factor, alpha-induced protein 3
TNFAIP6	83.9	15.6	40.3	43.9	101.7	NM_007115	tumor necrosis factor, alpha-induced protein 6
TRAIL	4.8	9.5	12.5	15.9	12.5	NM_003810	tumor necrosis factor (ligand) superfamily, member 10

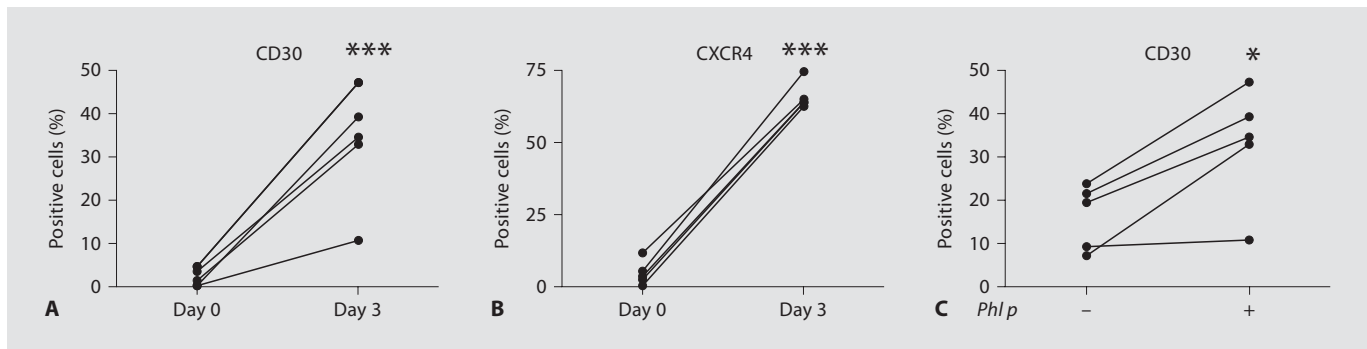


Fig. 4. Cell surface receptors expressed by *Phl p*-stimulated DCs were detected using flow cytometry. CD30 and CXCR4 expression was significantly upregulated on eADCs after 3 days of coculture with memory CD4⁺ T cells (**A**, **B**). In addition, CD30 was significantly upregulated in an allergen-specific manner on the cell surface of *Phl p*-stimulated eADCs after 3 days of culture with memory T cells as compared with the unstimulated eADCs (**C**). *Phl p*-stimulated samples are marked with (+) and the unstimulated samples are marked with (-). Each line represents 1 donor (A4-8). Statistical significance was determined by paired Student's t test. * $p < 0.05$, *** $p < 0.0005$.

CXC Ligands/Receptors

CXC ligands/receptors are involved in the migration of DCs to sites of action. In atopics, but not in healthy subjects, DC expression of CXCR4 was upregulated in response to DC and T-cell coculture (this study). Moreover, the finding was verified at the protein level (fig. 4B). Our observations indicate a T-cell-induced DC action that is specific to allergy. CXCR4 has also been shown to be upregulated on T cells following exposure to the Th2-type cytokine IL-4 [19]. Furthermore, increased levels of CXCR4 mRNA have been shown in nasal biopsies obtained from patients with symptomatic seasonal allergic rhinitis (compared with preseasonal observations) [20]. Taken together, the above observations suggest a role for CXCR4 and its ligands in allergy/allergic airway disease.

Interleukin Receptors

IL-2 is a cytokine of importance to T-cell proliferation. IL2RA, IL2RB and IL2RG constitute the high-affinity IL-2 receptors [21]. Among the IL-2 receptors, IL2RG was elevated in eADCs (cf. uneADCs), whereas IL2RA and IL2RB were upregulated in both atopic and healthy subjects. Our observation suggests that IL2RG (and its ligand) may be of importance in Th2-skewed allergic responses. Overexpression of IL17E in transgenic mice has been reported to increase the production of Th2 cytokines and immunoglobulins [22]. Receptor ligand-binding studies have shown that IL17E binds specifically to IL17RB [23]. In the present study, upregulated transcriptional levels of IL17RB in eADCs as compared with

uneADCs suggest a role for this receptor in allergy. Moreover, expression of IL17RB was examined in 5 atopic donors, and all 5 were positive for this receptor, whereas only 1 presented upregulated levels compared with uneADCs (data not shown). IL-18 is a Th1-polarizing cytokine that is neutralized by IL-18 binding protein (IL18BP) [24]. In the present study, IL18BP was upregulated in eADCs as well as in eHDCs following T-cell contact. However, this change was approximately 2-fold greater in atopics. The fact that IL18BP suppresses Th1 responses [24], and thereby affects the Th1/Th2 balance, makes IL18BP a candidate drug target. Taken together, our observations provide support to the hypothesis that specific interleukin receptors and interleukin-binding proteins are of interest in allergy [24]. In this context, the novelty of the present observations lies in the fact that these molecules have been demonstrated in DCs specifically.

G-Protein-Coupled Receptors

Once a DC T-cell synapse is established, signals delivered and received by each cell type are prolonged by cell membrane receptors including G-protein-coupled receptors (GPCRs) [25]. This characteristic makes GPCRs attractive as treatment targets [26]. In the present study, activator GPCR (GPR84) was upregulated exclusively in eADCs, whereas others (EDG1, GPR132, P2RY13) were upregulated in eADCs as well as in eHDCs. However, for the latter 3, the change was greater in atopics than in healthy subjects. Our observations indicate that the regulation of GPCRs is finely tuned and suggest that GPR84,

GPR132, EDG1 and P2RY13 may be targets for drug discovery relevant to allergy.

Costimulatory Molecules

The ligand ICOSL (CD275) is a costimulatory molecule expressed on DCs involved in the interaction with T cells [27]. A regulatory role for ICOSL in Th1/Th2 responses has previously been discussed [28]. Observations in man on ICOSL in allergy are conflicting, but upregulation of other costimulatory molecules, e.g., B7 (CD80), has been shown in allergic rhinitis [3]. Also, Blocki et al. [29] recently demonstrated changes in gene expression in DCs activated by an IgM antibody that crosslinked B7 and DCs. In the present study, *CD80* was upregulated in eADCs as well as in eHDCs. In contrast, upregulation of *ICOSL* occurred exclusively in eADCs. Our observations suggest a specific role for ICOSL in Th2 responses.

TNF Receptors

Previous studies have shown greater expression of the TNF receptor OX40 on Th2 cells in patients with allergic rhinitis as compared with healthy controls [30]. In the present study, we report an upregulation of the TNF receptor CD30 (TNFRSF8) in DCs from atopics subjected to memory T-cell coculture. *CD30* was upregulated in an allergen-dependent manner on *Phl p*-stimulated eADCs (compared with unstimulated eADCs). Moreover, CD30 expression was verified to be elevated at the protein level (fig. 4A). *OX40* was upregulated in atopics, but this also occurred in healthy subjects. Taken together, the above observations support the hypothesis that TNF receptors, e.g., CD30, are involved in the regulation of the Th1/Th2 balance [31, 32].

Matrix Metalloproteinases

The role of MMPs in allergic disease is currently under investigation [33]. Increased levels of MMP9 have been shown in lavage fluids and sputum obtained from patients with allergic rhinitis and asthma, respectively [33, 34]. Moreover, the ratio between MMP9 and tissue inhibitor of metalloproteinase (TIMP)-1, a natural MMP inhibitor, may correlate to disease severity [33, 35]. MMP9 may be involved in the migration of DCs into the airways [36, 37]. In the present study, *TIMP1* was upregulated in eADCs exclusively, whereas *MMP9* was upregulated in eADCs as well as in eHDCs. Our data suggest that DCs may be a source for these proteins and that MMP9/TIMP1 may be of importance to DC activity. *MMP25* was exclusively upregulated in eADCs (cf. uneADCs), but this MMP has not been previously discussed in the context of

allergy. Carbohydrate sulfotransferases are involved in the activation of MMPs [38]. In this study, carbohydrate sulfotransferases (*CHST2*, *CHST11*) were upregulated in eADCs (cf. uneADCs). Our results indicate that increased expression of *MMP9*, *MMP25*, *TIMP1*, *CHST2* and *CHST11* can be a result of DC and T-cell crosstalk and suggest that this may be relevant to inflammatory activities.

Transcription Factors Regulating the Immune Response

In the present study, the transcription factors *RUNX3*, *GATA3*, *BCL3* and *FOXO1A* were upregulated in eADCs as compared with uneADCs. *GATA3* has recently been implicated in Th1/Th2 regulation [39] and *RUNX3* in DC migration [40]. In addition, *RUNX3* has been associated with increased levels of lymphocyte function-associated antigen-1 (ITGA4/CD11a, ITGA4/CD49d) [41], suggesting a regulatory effect of *RUNX3* relevant to trans-endothelial migration, antigen presentation and T-cell-stimulatory capabilities of mature DCs [41]. The present results support the view that *RUNX3* may be of importance in T-cell and DC crosstalk. T-cell expression of *BCL3* has previously been shown to be increased by IL-9 [42]. In the present study, we report, for the first time, on upregulation of *BCL3* in eADCs following memory T-cell and DC crosstalk. However, *FOXO1A* has neither been previously reported in DCs nor been demonstrated in the context of allergy.

In this study, we have utilized microarray technology to describe cellular mechanisms resulting in allergic reactions and we present 37 markers that may be involved in skewing the Th1/Th2 balance. Some of the corresponding proteins have previously been described in the literature to be involved in allergic responses. In addition, we present several markers, previously not described in this context, which are likely to be involved in the regulation of the Th1/Th2 balance (fig. 5).

The proteomic profile may not mirror the transcriptional profiles due to posttranslational modifications. Therefore, the corresponding protein levels of markers identified with transcriptional analysis were investigated. Thus, the TNF receptor CD30, the chemokine receptor CXCR4 and the interleukin receptor IL17RB were selected for flow cytometric analysis. In addition to the fact that transcription levels of these markers were elevated in eADCs (above), they have all been implicated in the Th1/Th2 regulation as discussed above. Significant upregulation of cell surface-expressed CD30 and CXCR4 was detected on eADCs as compared with uneADCs (fig. 4A,

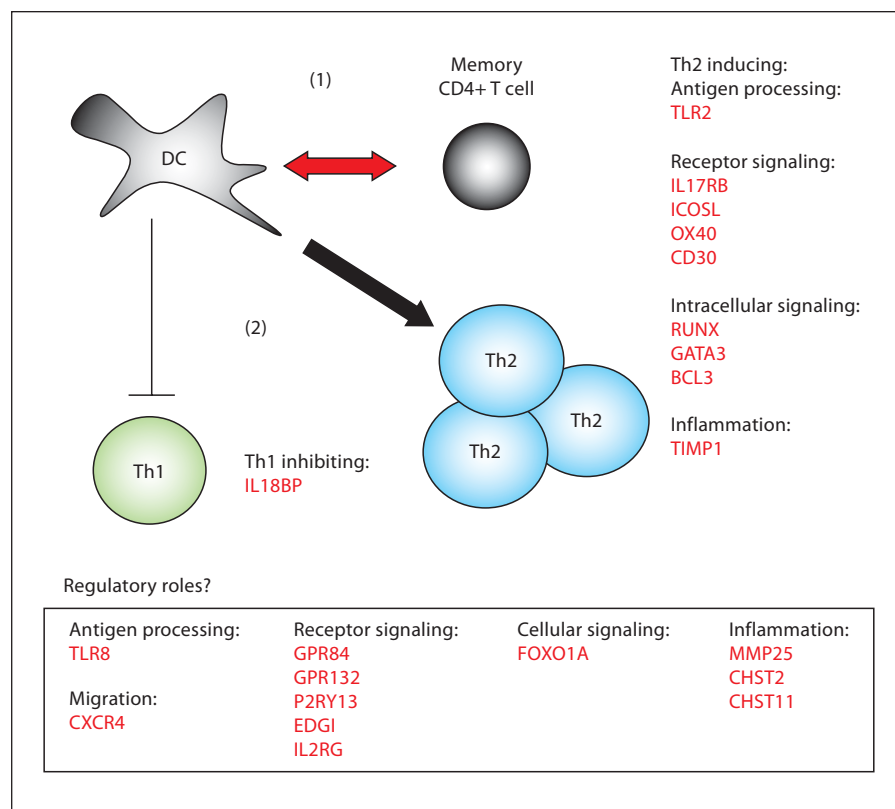


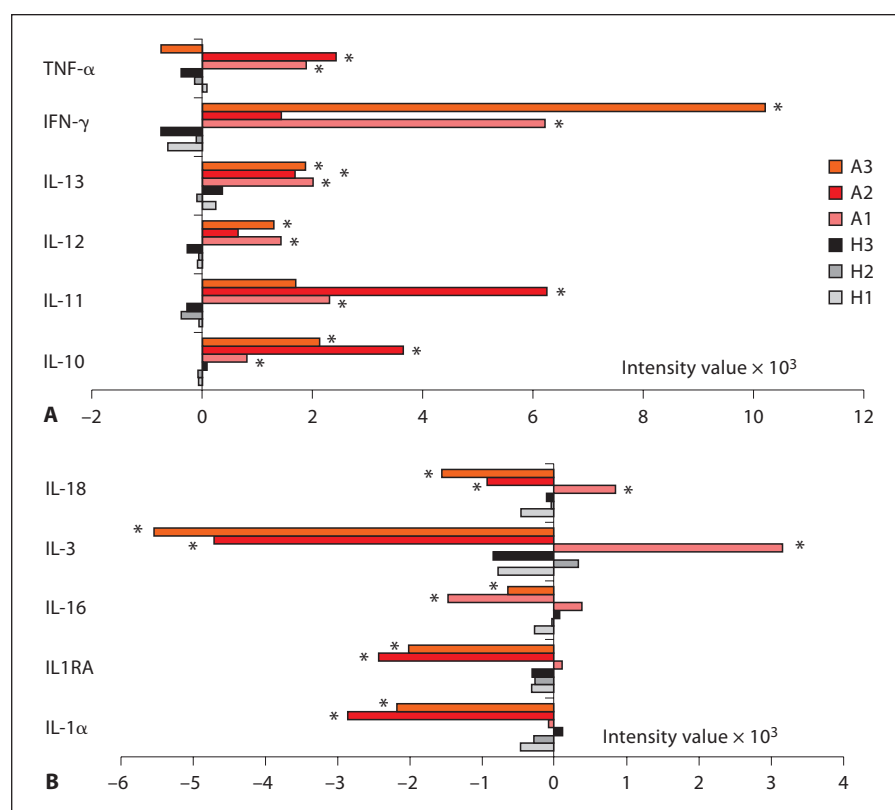
Fig. 5. Reprogramming of the DC transcriptional profile after memory CD4+ T-cell interaction results in initiation of regulatory mechanisms. (1) DC and T-cell education takes place. (2) eADCs generate a profile of Th1-inhibitory or Th2-inducing transcripts. In addition, genes with a potential to be involved in the allergic reaction are summarized. Transcripts in red represent upregulated genes.

B). Furthermore, IL17RB was detected on eADCs, which has not been demonstrated for monocyte-derived DCs before, but no significant upregulation was detected compared with uneADCs. Also, CD30 was significantly upregulated in allergen-stimulated eADCs after 3 days of coculture, as compared with eADCs without *Phl p* stimulation (fig. 4C). This observation was not seen for CXCR4 and IL17RB. Thus, CD30 is suggested to be important in the DC/T-cell crosstalk resulting in an allergic reaction, while signaling through CXCR4 is allergen independent. From studies in asthmatic mouse models, IL-17 is suggested to function as a negative regulator in the established stage of the disease by inhibiting the production of the Th2-attracting DC-produced chemokine thymus and activation-regulated chemokine [43]. The role of IL-17 and its receptors in human asthma/rhinitis remains to be determined. The protein analysis of these markers was performed on cells from allergic donors due to the use of an artificial pollen season, and thus, we cannot exclude their influence on cells from healthy donors. Nevertheless, it is clear that CD30 and CXCR4 are important mediators in the augmentation of Th2 responses. In conclusion, protein verification corresponding to mRNA data

was provided for CD30 and CXCR4, supporting the conclusions as outlined above, but not for IL17RB.

To further investigate differential protein expression, a recombinant antibody microarray analysis was performed on cell supernatants obtained before and 3 days after *Phl p*-stimulated DC and T-cell crosstalk using samples from atopics as well as from healthy subjects. The analysis revealed increased levels of IL-10, IL-11, IL-12, IL-13, TNF- α and IFN- γ in atopic individuals following DC and T-cell crosstalk, as well as reduced levels of IL-1 α , IL-R α , IL-3, IL-16 and IL-18 (fig. 6). IL-10, IL-11 and IL-13 are recognized as Th2-inducing cytokines [44–46], and TNF- α plays a critical role in the pathogenesis of allergic responses [47, 48]. The observed reduction in IL-1 α is also in agreement with a Th2-type response since IL-1 α has been shown to promote Th1 differentiation [49]. Similarly, IL-16 preferentially induces a Th1 response [50]. IL-18 has been reported to have differential roles in the allergic response by promoting both Th1 and Th2 polarization [51]. The presence of IL18BP mRNA and the subsequent production of IL18BP protein may be the reason for the low amounts of IL-18 recorded in the present study. The results were generated from cell su-

Fig. 6. Up- (A) and downregulation (B) of secreted analytes in cell supernatants of *Phl p*-stimulated and T-cell eDCs, as compared with only *Phl p*-stimulated DC cultures, determined by recombinant antibody microarrays. Analytes with a p value <0.05 and FC ± 1.3 are marked (*) and selected as described earlier. Healthy donors: H1–H3; atopic donors: A1–A3.



pernatant in which 95% of the cells in the coculture were T cells.

The interactions between DCs and allergen-specific T cells are affected by other T-cell subsets such as regulatory and anergic T cells in peripheral tissues of allergic individuals [52]. In this study, we have used an enriched population of CD27-/CD4+ memory T cells instead of T-cell clones in order to study signaling from the whole effector T-cell population instead of signals from 1 single clone. Therefore, the differences in frequency of allergen-specific T-cell subsets in allergic and healthy donors impact the transcriptional activity in the DCs. Moreover, the setup does not distinguish between the effects of cell contact and the release of soluble mediators. In future studies, it would be highly valuable to identify the contact-dependent and/or the cytokine-dependent factors involved in the interaction between DCs and Th2 cells.

In summary, we conclude that crosstalk between *Phl p*-stimulated DCs and memory T cells induces a transcriptional activity in DCs that is specific to allergy. Accordingly, our data suggest that T cells educate DCs in allergy. For some proteins identified by the present study as being specific to allergy, supporting data already exist

in terms of human in vivo observations. The novelty of the present findings in that context lies in the fact that a specific cell type has been studied in association with a relevant intervention (i.e., DC and T-cell crosstalk). For other novel proteins, human in vivo confirmation is as yet lacking. Finally, we conclude that the present coculture system may serve as a model for pharmacological and other interventions relevant to allergy.

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