Genomic and functional delineation of dendritic cells and memory T cells derived from grass pollen-allergic patients and healthy individuals

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Abstract

Dendritic cells (DCs) possess a potent ability to modulate and activate specific T-cell responses to allergens, which play a pivotal role in allergic inflammation by secreting cytokines and other mediators. However, the molecular mechanisms by which allergen-challenged DCs regulate specific T-cell responses are still not well characterized. This study aims at elucidating the molecular mechanisms underlying the DC–T-cell interaction during an allergic immune response to grass pollen, using a genomic and functional approach. Transcriptional analysis was performed on grass allergen Phleum pratense-stimulated DCs and on autologous memory CD4+ T cells co-cultured with allergen-challenged DCs from healthy and allergic donors. DCs from the allergic donors were potent inducers of T-cell proliferation and Th2 polarization, as demonstrated by high IL-4, IL-5 and IL-13, and low IFN-γ production. A gradual up-regulation of activation markers on both DCs and T cells was evident during the co-culture period, demonstrating an educational element of the DC–T-cell interaction. The global transcriptional analysis revealed a differential gene regulation in DCs and T cells derived from allergic donors after stimulation with allergen, as compared with the healthy donors. Peripheral memory CD4+ T cells from healthy and allergic donors also responded differently after stimulation with allergen-loaded DCs with respect to cytokine production, proliferation, surface marker expression and gene transcription. We found up-regulated genes involved in Th2 cell biology, such as genes important for homing, adhesion, signaling and transcription, in addition to genes previously not described in the context of allergy. The panel of differentially expressed genes in the allergic group will form the basis for an increased understanding of the molecular mechanisms in allergy.

Introduction

Allergic rhinitis is one of the most common atopic diseases, clinically characterized by inflammation of the nasal mucosa and symptoms such as rhinorrhea, sneezing and blockage (1). The immune response in subjects with allergic rhinitis as well as in other atopic diseases is characterized by circulating activated T cells producing Th2 cytokines such as IL-4, IL-5, IL-9 and IL-13 (1, 2). The secretion of these cytokines plays a major role in the inflammatory response in rhinitis, by triggering production, recruitment and activation of eosinophils, basophils and mast cells. The systemic immune response in atopic diseases, such as allergic rhinitis, is also demonstrated by data showing that local exposure of allergen, i.e. in the nose, may produce distant inflammatory changes, such as increased bronchial expression of adhesion molecules, bronchial eosinophilia and increase of serum IL-5 levels (3–5).

Dendritic cells (DCs) are professional regulators of the immune response to inhaled antigen which rapidly travel from...
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the circulation following allergen exposure to the regional lymph nodes for initiation or amplification of the allergic immune response (6). Recently, the direct effect of DCs in Th2 sensitization was shown in transfer experiments during which systemically administered allergen-loaded DCs induced Th2 immunity upon a subsequent aeroallergen challenge (7, 8). The unique capacity of DCs to initiate and regulate allergic immune responses clearly demonstrates the need to investigate the gene expression in DCs exposed to allergens and their functional properties.

Global transcriptional analysis is a superior tool for predicting verifiable phenotypic characteristics important for the understanding of disease mechanisms and, thus, for identification of potential novel drug targets. Recent studies have aimed at screening atopic and healthy subjects for differentially expressed transcripts and proteins in PBMC or biopsies (9, 10). However, PBMC as well as biopsies from nasal or bronchial airways consist of a complex mixture of cells, which makes comparative analyses very difficult. Furthermore, contribution from a specific cell type to the transcriptional signature becomes impossible to delineate, and it is therefore desirable to first isolate the different cell types from blood or biopsies before characterization of their specific expression (11) (Lindstedt et al., unpublished data). A few studies have used high-density microarrays to study the differential expression of in vitro-generated Th1 and Th2 cells from humans (12) and mice (13). However, in this study we are taking a step further, i.e. to monitor the changes in gene expression in effector T cells, following stimulation with allergen-pulsed DCs. This was performed without the addition of polarizing cytokines, utilizing cells from both healthy and atopic individuals. In addition, we screened the transcriptional profiles of allergen-stimulated DCs from healthy and allergic subjects to monitor their distinct response to the same antigens.

In the present study, our aim was to identify molecular markers on DCs and T cells that are differentially expressed between allergic and healthy donors. For the transcriptional profiling, only donors who were either healthy or had ongoing allergic rhinitis, as defined by characteristic nasal symptoms in combination with positive skin prick test, high levels of specific IgE, allergen-induced Th2 cytokine production, T-cell proliferation, and basophil degranulation, were studied. We could demonstrate a clear differential transcriptional profile in DCs from allergic individuals in response to allergen as compared with the healthy controls. Furthermore, allergen-pulsed DCs induced differential gene expression in memory T cells derived from allergic and healthy donors. The present data delineate genomic and functional differences in allergic individuals in relation to mechanisms involved in DC–T-cell interactions and may act as a starting point for selecting potential targets for therapy.

**Methods**

**Patient inclusion criteria**

Allergic donors participating in this project tested positive for grass pollen allergen [*Phleum pratense (Phl p)*] in skin prick test. They also had a clinical history of strictly seasonal allergic rhinitis and displayed symptoms associated with seasonal allergen exposure. Patient exclusion criteria were asthma, dermatitis, nasal polyposis and infections. The healthy donors were skin prick test negative and matched with the allergic group with regards to gender and age. The study was approved by the local Ethics Committee.

**Allergen-specific IgE measurements**

Timothy-specific plasma IgE concentrations in human serum were determined with Pharmacia CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instruction. The detection limit of the assay is 0.35 kUA l⁻¹.

**Basophil degranulation assay**

The BD FastImmune assay was performed according to the manufacturer's instructions (Becton Dickinson, San Jose, CA, USA). The statistical significance was determined by Student's *t*-test.

**Generation of monocyte-derived dendritic cells**

Monocytes were purified from peripheral blood obtained during pollen season and differentiated into monocyte-derived dendritic cells (MoDCs) using R5 culture medium [RPMI 1640, 2 mM L-glutamine (Sigma–Aldrich, St Louis, MO, USA), 5% fetal bovine serum (HyClone Laboratories, Logan, UT, USA) and 50 μg ml⁻¹ gentamicin] supplemented with recombinant human granulocyte macrophage colony-stimulating factor (Novartis, Basel, Switzerland) and recombinant human IL-4 (R&D Systems, Minneapolis, MN, USA) as previously described (11). MoDCs were either stimulated with 25 μg ml⁻¹ endotoxin-free Phl p extract, a generous gift from Peter Adler Würtzen (ALK-Abello, Hørsholm, Denmark), for 48 h or left unstimulated. Cell samples of DCs were collected for microarray analysis at 48 h after grass pollen stimulation.

**T-cell purification protocols**

CD4⁺ T cells were purified by negative selection from peripheral whole blood obtained from autologous allergic and non-allergic donors 9 days after the initial blood sampling, using the CD4⁺ T-cell isolation MACS kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Memory CD27⁺ CD4⁺ T cells were isolated by first incubating the CD4⁺ cells with anti-CD45RA mAb, anti-glycophorin A mAb (DakoCytomation, Glostrup, Denmark) and microbead-conjugated anti-CD27 antibodies (Miltenyi Biotec) for 15 min at 4°C. Thereafter, microbead-conjugated goat anti-mouse antibodies (Miltenyi Biotec) were added for 15 min at 4°C to deplete naïve cells and to enrich the CD27⁻ population. The isolated memory CD4⁺ T cells contained <1% contaminating CD45RA⁺ T cells.

**Proliferation assay**

Proliferation assays were performed with enriched CD27⁻ CD4⁺ memory T cells by co-culturing with autologous DCs that were either unstimulated or had been pulsed with Phl p 48 h prior to the T-cell isolation. The assays were performed with a DC/T cell ratio of 1/20, using R5 culture medium, supplemented with 2 × 10⁻⁵ M 2-mercaptoethanol (Merck, Whitehouse, NJ, USA). After 7 days, 2.5 μCi ml⁻¹ [³H]thymidine (Amersham Biosciences, Uppsala, Sweden) was added.
to each well for 16 h. Thymidine incorporation was measured in a beta scintillation counter (Matrix 96 Direct beta counter, Packard, Meriden, CT, USA). Samples were run in four replicates and the statistical significance was determined by Student’s t test.

**Cytokine production by memory CD4⁺ T cells**

T cells were co-cultured with autologous unstimulated or allergen-stimulated DCs for 7 days. For intracellular cytokine detection, cells were incubated with phorbol myristate acetate (50 ng ml⁻¹), ionomycin (500 ng ml⁻¹) and brefeldin A (10 μg ml⁻¹) (all from Sigma–Aldrich) for 5 h, washed twice in PBS and fixed with 2% PFA for 15 min. Cells were permeabilized with 0.5% saponin/PBS (Sigma–Aldrich) and stained with IL-4 PE (Becton Dickinson), IFN-γ FITC and IL-13 PE antibodies (Pharmingen, San Diego, CA, USA) for 30 min at 4°C. Thereafter, cells were washed twice in 0.5% saponin/PBS, re-suspended in 1% BSA/PBS and analyzed with a FACSScan (Becton Dickinson). For soluble cytokine detection, the human T₅/₁T₅/₂ cytokine bead array kit was used, according to the manufacturer’s instruction (Becton Dickinson).

**Preparation of cRNA and gene chip hybridization**

*Phl p*-stimulated DCs and T cells from allergic and non-allergic individuals were co-cultured (1:20) for 7 days as above. During the last 5 h, T cells were activated with plate bound anti-CD3 mAb (10 μg ml⁻¹) (OKT3, Ortho Biotech, Raritan, NJ, USA) and soluble anti-CD28 mAb (0.5 μg ml⁻¹) (Pharmingen). Cell cultures from allergic donors A4–A6 and non-allergic donors H1, H3 and H7 were lyzed in TRIzol Reagent (Invitrogen, Paisley, UK) and stored at −20°C until further RNA isolation. Fragmentation, hybridization and scanning of the human U133A arrays were performed according to the manufacturer’s protocol (Affymetrix Inc., Santa Clara, CA, USA) and as previously described (11). For the DC-co-cultured T cells, preparation of labeled cRNA was performed according to the small-sample labeling protocol v7 (Affymetrix Inc.).

**Microarray data analysis**

The analysis was performed at the S(Http://academic.oup.com/intimm/article-abstract/17/4/401/680426 by guest on 18 January 2019)wgene MicroArray Resource Center (MARC) at Lund University, which is an Affymetrix Service Provider. MARC is a Swedish reference laboratory for DNA microarray analysis and is minimum information about a microarray experiment compliant. The fluorescence intensities were analyzed, using the Microarray Suite Software 5.0 (Affymetrix Inc.) and scaled to a target value of 100. Further data analysis was performed with GeneSpring™ 5.0 software (Silicon Genetics, Redwood City, CA, USA). The median of the signal intensity in three samples in each group was calculated and thereafter, based on fold change, genes were selected that were either up- or down-regulated in the allergic donors A4–A6 compared with the non-allergic donors H1, H3 and H7. In the up-regulated list of genes, the transcripts were denoted present (P) in A4–A6. Conversely, the down-regulated transcripts were denoted present (P) in H1, H3 and H7. Primary expression data were submitted to the Array Express database (http://www.ebi.ac.uk/arrayexpress/).

**Results**

The allergic patient inclusion criteria were history and symptoms of rhinitis of grass pollen exposure, positive skin prick test and elevated levels of grass pollen-specific IgE in serum (Table 1). Furthermore, to confirm that the allergic donors were actually responding to the allergen, a basophil degranulation test was performed (Fig. 1A). This tool has proven to be highly efficient for detection of IgE-mediated allergy to inhalant allergens (14). Altogether, the differences between healthy and allergic donors, in terms of responsiveness to grass pollen allergens, were highly significant ($P < 0.005$).

Culturing blood monocytes from healthy and allergic donors for 7 days with granulocyte macrophage colony-stimulating factor and IL-4 resulted consistently in CD1α⁺, CD14⁺, CD80⁻ and CD86⁻ immature DCs (data not shown). To investigate whether *Phl p* could induce maturational reprogramming of these DCs, cells were stimulated with *Phl p* extract for 48 h and analyzed phenotypically. However, no up-regulation of activation and co-stimulatory markers was detected. In addition, DC supernatants were analyzed for the production of inflammatory cytokines, such as IL-1β, tumor necrosis factor-α (TNF-α), IL-6 and IL-12. Again, no detectable cytokine production was induced by the allergens (data not shown). Despite the fact that no phenotypical changes were observed in DCs stimulated with allergen, samples were collected after 48 h of culture in the presence of *Phl p* and snap frozen for global transcriptional analysis.

Autologous memory CD4⁺ T cells were co-cultured for 7 days with unstimulated and *Phl p*-stimulated DCs. The expression of co-stimulatory markers CD80 and CD86 on *Phl p*-stimulated DCs was analyzed during the T-cell co-culture period, on days 0, 3, 5 and 7 (Fig. 1B). Of note, both unstimulated and *Phl p*-stimulated DCs derived from healthy and allergic subjects up-regulated the CD80 and CD86 following the cognate T-cell interaction. However, no up-regulation of these DCs, cells were stimulated with *Phl p* extract for 48 h and analyzed phenotypically. However, no up-regulation of activation and co-stimulatory markers was detected. In addition, DC supernatants were analyzed for the production of inflammatory cytokines, such as IL-1β, tumor necrosis factor-α (TNF-α), IL-6 and IL-12. Again, no detectable cytokine production was induced by the allergens (data not shown). Despite the fact that no phenotypical changes were observed in DCs stimulated with allergen, samples were collected after 48 h of culture in the presence of *Phl p* and snap frozen for global transcriptional analysis.

Even more intriguing, the gated CD3⁺ memory T cells evidently also up-regulated the co-stimulatory markers CD80 and CD86 following the cognate T-cell interaction. Since DCs from both healthy and allergic subjects displayed a similar kinetics of activation, this clearly demonstrated an antigen-independent activation of DCs, induced only by the presence of T cells in the culture.

**Table 1. Donor parameters**

<table>
<thead>
<tr>
<th>Donor</th>
<th>History</th>
<th>Scorea (0–6)</th>
<th>Specific IgE (kUA l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>−</td>
<td>0</td>
<td>&lt;0.35</td>
</tr>
<tr>
<td>H2</td>
<td>−</td>
<td>2</td>
<td>&lt;0.35</td>
</tr>
<tr>
<td>H3</td>
<td>−</td>
<td>0</td>
<td>&lt;0.35</td>
</tr>
<tr>
<td>H4</td>
<td>−</td>
<td>0</td>
<td>&lt;0.35</td>
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<tr>
<td>H5</td>
<td>−</td>
<td>2</td>
<td>&lt;0.35</td>
</tr>
<tr>
<td>H6</td>
<td>−</td>
<td>0</td>
<td>&lt;0.35</td>
</tr>
<tr>
<td>H7</td>
<td>−</td>
<td>0</td>
<td>&lt;0.35</td>
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<tr>
<td>A1</td>
<td>Rhinitis</td>
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<td>1.16</td>
</tr>
<tr>
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<td>Rhinitis</td>
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<td>36.4</td>
</tr>
<tr>
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<td>Rhinitis</td>
<td>3</td>
<td>55.2</td>
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<td>Rhinitis</td>
<td>2.5</td>
<td>&gt;100</td>
</tr>
<tr>
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<td>Rhinitis</td>
<td>1</td>
<td>24.7</td>
</tr>
<tr>
<td>A6</td>
<td>Rhinitis</td>
<td>4.5</td>
<td>12.8</td>
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aSymptoms of rhinitis at time of blood sampling.
memory T cells was progressively up-regulated during the 7-day culture period. In this case, however, a clear difference in T-cell response between the allergic and healthy donors was observed.

The capacity of allergen-stimulated DCs to regulate the cytokine production of autologous memory T cells was explored with flow cytometry after 7 days of culture (Fig. 2). The production of cytokines varied considerably between the donors and allergic donors. A4–A6 displayed a clear Th2 profile after stimulation, with the production of IL-4, IL-5 and IL-13. No differences in IFN-γ production could be detected between the healthy and allergic group. DCs were also compared for their ability to induce memory T-cell proliferation (Fig. 3). T cells stimulated with Phl p-pulsed DCs from the allergic donors displayed a significantly higher level (P < 0.05) of proliferation as compared with T cells cultured with unstimulated DCs.

**Fig. 1.** Flow cytometric analysis of basophils, DCs and T cells after allergen challenge. (A) Allergic individuals displayed higher levels of surface CD63 expression, a measure of basophil degranulation, after allergen challenge, as compared with the healthy individuals. Gates were set to include viable HLA-DR+/CD123+ cells. Allergic donors are shown in gray bars while healthy donors are shown in black bars. (B) Allergen stimulation was not sufficient to induce DC activation and, evidently, DCs required contact with autologous T cells for maturation. DCs from both healthy and allergic subjects were either left unstimulated (open diamond) or cultured with Phl p for 48 h (0 day) (closed square) prior to co-culture with CD27+/CD28-enriched CD4+ memory T cells. The kinetics of up-regulation of CD80 and CD86 on DCs were assayed on days 0, 3, 5 and 7. (C) Net expression on memory T cells induced by allergen-loaded DCs from allergic (closed square) and healthy donors (open diamond), i.e. with expression induced by unstimulated DCs subtracted. Gates were set to include only viable CD3+ cells. The average values are represented in (B) and (C) of the healthy and allergic donors ± standard deviations.

**Fig. 2.** IL-4, IL-5, IL-13 and IFN-γ production by CD27+ enriched memory CD4+ T cells after stimulation with allergen-loaded DCs. DCs were stimulated with grass pollen extract or medium alone for 48 h. Isolated memory T cells were thereafter co-cultured with DCs for 7 days and the intracellular cytokine production was analyzed by flow cytometry. Results represent net cytokine production induced by allergen-loaded DCs subtracted by the production induced by the unstimulated DCs. Atopic donors are shown in gray bars while healthy donors are shown in white bars.
To further dissect the molecular mechanisms involved in the DC/T cell response to allergen, high-density oligonucleotide arrays (with >12,500 transcripts) were used to produce a comprehensive picture of gene regulation following allergen stimulation in both DCs and memory T cells. Only the donors exhibiting either complete absence of allergic response or a strong ongoing response were chosen for transcriptional analysis. This selection was based on the overall results of the skin prick test, specific IgE levels, allergen-induced Th2 cytokine production, T-cell proliferation, and basophil degranulation. Consequently, cells from the allergic donors A4–A6, as well as the healthy donors H1, H3 and H7, were selected for transcriptional analysis. The differential transcriptional profiles of DCs challenged for 48 h with Phl p were compared between the above donors (Fig. 4A). In addition, we monitored the gene regulation in response to Phl p-pulsed DCs in the expanding memory T cells from the same individuals (Fig. 4B). A selection of the differentially expressed genes is displayed in Fig. 4, functionally grouped according to their gene ontology. The global transcriptional analysis of Phl p-challenged DCs clearly revealed that grass pollen induced differential transcription profiles in DCs from allergic as compared with healthy donors. Accordingly, many genes coding for adhesion and signaling molecules were differentially expressed. For example, in DCs derived from allergic donors TNFRSF11A (RANK), SPN (CD43), CDH2 and LAMC1 were up-regulated, whereas IL2RG, IL7R, IL8RB, CD209L, and CLEC1 were found to be down-regulated. Other examples of genes differentially regulated were the up-regulated membrane tetraspanin TM4SF2 and the down-regulated TM4SF7 and TM9SF4. The divergence of transcription in allergen-pulsed DC/T cells in healthy and allergic individuals was also extensive and may explain many of the important molecular features of the interaction between DCs and T cells in the allergic response. Among signaling molecules and receptors IL1A, IL1RAP, HRH4, MS4A2 (FCER1B) and FCGR2B were up-regulated in the allergic group, whereas IL1R2, IL7R, LTA, LTb, LTBP1, MRC1 and IGF6 were down-regulated. It was evident that well-established marker genes for a cellular Th2 response or atopic disease, such as IL-9 (15), TNFRSF8 (CD30) (16) and VEGF (9), were differentially expressed in healthy and allergic donors as well as genes not previously described in the context of allergy, such as TNFRSF11B, BCL-3, INSR and CD11E.

To confirm the differences in cytokine production in the healthy and allergic in vitro cell systems, we performed cytometric bead array analysis of memory T-cell supernatants 2 days after collecting samples for transcriptional profiling (Fig. 5). TNF-α was highly expressed in the healthy group, whereas more IL-4 and IL-5 were produced in the allergic group, demonstrating an expected skewing of effector T-cell responses.

**Discussion**

We have studied factors of importance for the interaction between DCs and memory T cells during an immune response relevant to allergic airway inflammation, such as in allergic rhinitis and asthma. Furthermore, we analyzed the regulatory mechanisms of DCs in response to Phl p, in both healthy and allergic individuals. These results reveal differential transcriptional profiles relevant for both a basic understanding of the allergic reaction as well as for the quest of finding novel therapeutic targets.

The lack of up-regulation of the co-stimulatory molecules CD80 and CD86 on DCs after Phl p stimulation was intriguing, especially since both CD80 and CD86 were induced after T-cell contact in both healthy and allergic individuals. Surprisingly little is still known about the role of DCs in human T2 effector choice and there is a great need for further investigations. Interestingly, it was recently shown in a mice model that DCs can discriminate between Th1- and Th2-polarizing antigens even when presented simultaneously (17). Both antigens were internalized by discrete pathways and entered non-overlapping cellular compartments. Furthermore, many maturation-associated changes in DCs were reportedly only seen with the Th1-inducing antigen, which is in agreement with our finding that allergen stimulation did not induce any phenotypical changes in DCs. We observed that the allergen-stimulated DCs need the presence of T cells for their maturational process, which then could be mediated either indirectly by mediators or directly by a cognate interaction. This phenomenon is likely to enable allergen-challenged DCs to maintain their activity levels within defined limits in tissues of allergic inflammation, such as nasal and bronchial airways, and postpone the maturational trigger until an effector T-cell contact is established.

Our kinetic study also clearly showed that T cells became activated after stimulation with allergen-loaded DCs from allergic donors. A gradual increase of CD80 and CD86 expression on T cells was evident. CD86 expression on T cells has been shown in *vivo* to be induced by long-term CD3 and IL-2R stimulation (18) and down-regulated when the cells become quiescent. Such CD86*+* T cells represent effector memory cells, which are functional in co-stimulating other resting T cells into proliferation and cytokine production. These results are intriguing since it indicates that T cells can themselves amplify and regulate their own responses through proteins mainly referred to as antigen-presenting cell markers. The differences observed between allergic and healthy donors.
Transcript analysis of Phl p-pulsed DCs and T cells

A

B

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in the present study demonstrate that an antigen-specific signal is required for the T cells to become CD80, CD86 and HLA-DR positive.

Experience from our previous investigations has demonstrated the importance of having clearly defined patient parameters as well as purified cell populations for the transcriptional profiling. Consequently, using isolated cell populations, we could demonstrate that Phl p clearly induced differential transcriptional profiles in both DCs and memory T cells from allergic and healthy donors (Fig. 6). Many transcriptional changes associated with functions in the allergic immune reaction were identified, in addition to novel molecular mechanisms.

The presence of specialized T cells at the site of inflammation is associated with the pathology of allergic airway disease (19). Chemokines directly affect the retention and relocation of DCs and T cells in the allergic inflammation. Thus, a breakdown of the underlying control mechanisms of leukocyte migration might contribute to the atopic disease (20). Interestingly, in the present study, a wide range of chemokines were found to be differentially expressed in Phl p-challenged DC/T cells from allergic as compared with healthy individuals, suggesting a highly regulated migratory response in the two panels of donors. In the allergic donors, the chemokines CCL17 (TARC) and CCL22 (MDC), active on CCR4+ T_{r}2 cells, were both expressed, as well as CCL2 (MCP-1), which plays an important role in the activation of mast cells and induction of histidine decarboxylase mRNAs (21). Interestingly, the allergic donors also displayed 2.5-fold increased transcript levels of histidine decarboxylase, as compared with the healthy group (data not shown). CCL17 and CCL22 levels have also been demonstrated to increase in bronchoalveolar lavage after allergen challenge in patients with allergic pulmonary inflammation (22), and our data suggest that memory T_{r}2 cells and DCs were the main sources of chemokines in that study. In addition, CXCR4 mRNA, a chemotactic component involved in allergic airway disease (23), was detected in the allergic individuals, suggesting a switch in highly specific migratory properties. Furthermore, a diverse set of chemokines were down-regulated in the allergic group, among others CXCL13 (BLC) and CCL18 (DC-CK1), which have previously been shown to be down-regulated after CD40–CD40 ligand interaction (24, 25), and XCL1 (lymphotactin), which is expressed in activated Th1 cells (26).

**Fig. 4.** Differential transcript profiles of Phl p-stimulated DCs (A) and their effect on transcription in autologous CD4+ memory T cells (B), from allergic (A4–A6) and healthy donors (H1, H3, H7). Genes were considered differentially expressed if a change of at least 1.8 fold-change in expression level was observed and the raw values were above 10 in signal intensity. Also, all genes had to be denoted as present in the triplicate samples. The level of fluorescent intensity is indicated by color; high signal (>500) is yellow, medium signal (100–500) is red and low signal (<100) is blue. GenBank accession numbers as well as short gene name are indicated and positive fold change represents higher signal intensity in the allergic group, as compared with the healthy group. Based on information from the Gene Ontology™ Consortium, genes are grouped according to their known functions: (i) adhesion, migration, cell surface, (ii) miscellaneous, (iii) signaling and transcriptional regulation, (iv) apoptosis and cell cycle, (v) transport and (vi) cytokines, cytokine receptors and growth factors.

**Fig. 5.** Production of soluble TNF-α, IL-4 and IL-5 by memory T cells after stimulation with Phl p-loaded DCs. Supernatants were harvested 48 h after collecting samples for microarray analysis. White bars correspond to cytokine levels in the healthy donors, whereas gray bars correspond to atopic donors. Results show average cytokine levels (in ng) in cell culture supernatants ± standard deviation.

**Fig. 6.** Potential mechanisms involved in the specific memory T cell response induced by allergen-triggered DCs. Gene names in red text represent up-regulated genes in the atopic donors, whereas the genes in green are down-regulated.
Two cytokines expressed in inflammatory airway diseases, IL-17 (27) and leukemia inhibitory factor (28), were up-regulated in the DC-stimulated T cells of the present allergic donors. Furthermore, IL-9 and BCL-3 were both up-regulated in the DC/T cells of these subjects, as compared with the healthy donors. IL-9 has been shown to regulate BCL-3 expression, and thus nuclear factor-κB (NF-κB) activity, in activated T cells and mast cells and represses TNF-dependent transcriptional activation, suggesting different regulatory pathways for the NF-κB transcription factors (29). Interestingly, we observed reduced TNF-α in the DC/T cell co-culture supernatants derived from the allergic donors, as compared with the healthy donors.

Adenosine as well as adenosine metabolites and their receptors have powerful immunoregulatory effects and evidence is accumulating on their role as potent features of allergic inflammation. The allergen-challenged DC/T cells displayed up-regulated mRNA levels of two of these receptors, namely adenosine A2b receptor (ADORA2B) and purinergic receptor P2X (P2RX5). Recently, an adenosine–IL-13 amplification pathway was described in IL-13 transgenic mice, defining a clear role for adenosine in Th2-2-mediated inflammation in respiratory tissues (30). The transcription of ADORA2B in the allergic setting of this study suggests that the human DC/T cells stimulated with allergen contribute to the response induced by adenosine. In addition, signaling through ATP receptors such as P2RX5, in parallel to LPS-induced DC maturation, results in the inhibition of IL-6, IL-12, TNF-α and IL-1β production and thus lower IFN-γ and higher IL-4 and IL-5 production by DC-co-cultured T cells (31), in addition to strong up-regulation of CXCR4 and CCL22 production (32).

Inhibition of the endo/lysosomal enzyme cathepsin L in *Leishmania major*-infected BALB/c mice potentiates Th2 responses with up-regulated IL-4 production (33). Our findings of CTSL (cathepsin L) down-regulation in allergic donors suggest that cathepsin L has a function in the degradation of endocytosed antigens leading toward a Th1-type instead of a Th2-type response. Similarly, up-regulation of LAMP1 and LAPTMM4B mRNAs encoding for two lysosomal proteins, in DCs stimulated with *Phl p* in allergic donors suggests that they respond differently in terms of endocytosis and processing of the same antigen.

Of note, it was evident that markers expressed by other cells involved in the allergic reactions were also present in our DC/T cells, such as prostaglandin receptor EP3 (PTGER3) involved in IgE/allergen-induced degranulation in mast cells (34) and transforming growth factor-α, induced by IL-4, IL-13 and allergen Der p in bronchial epithelial cells (35). This suggests that DCs and T cells may be more involved in other processes of allergic inflammation than previously acknowledged.

In summary, we have studied allergen-pulsed DCs, derived from allergic and healthy donors, and their interaction with Tn cells, without the addition of exogenous lymphocytes. This probably more closely mirrors an allergic immune response, as compared with T-cell polarization in vitro studied under the influence of e.g. IL-4 and IL-12. The rational is that allergic rhinitis is associated with various local and systemic inflammatory processes and that several cell types, such as airway epithelial cells, mast cells, basophils, eosinophils and infiltrating mononuclear cells, are involved (36). However, the recognition and initiation of the allergic immune response is mediated by DCs (6) and in the present study we report the presence of specific gene regulation in *Phl p* pulsed DCs derived from atopic donors. We have also followed the impact of these DCs on gene transcription in memory T cells, reflecting the first recognition of allergens in tissues and the subsequent process of T-cell activation in tissues of allergic rhinitis, respectively. The data demonstrate a complexed immunoregulatory network of potential gene products, comprising both known as well as previously not described components, which will pave the way for further functional studies on the molecular mechanisms of allergy.

**Acknowledgements**

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**Abbreviations**

| DC | dendritic cells |
| MARC | MicroArray Resource Center |
| MoDC | monocyte-derived dendritic cells |
| NF-κB | nuclear factor-κB |
| *Phl p* | Phleum pratense |
| TNF | tumor necrosis factor |

**References**

transcript analysis of phi p-pulsed DCs and T cells


