# Changes in markers associated with dendritic cells driving the differentiation of either $T_H2$ cells or regulatory T cells correlate with clinical benefit during allergen immunotherapy

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**Background: Regulatory dendritic cell (DC) markers, such as C1Q, are upregulated in PBMCs of patients with grass pollen allergy exhibiting clinical benefit during allergen immunotherapy (AIT).**
**Objectives: We sought to define markers differentially expressed in human monocyte-derived DCs differentiated toward a proallergic (DCs driving the differentiation of $T_H2$ cells [DC2s]) phenotype and investigate whether changes in such markers in the blood correlate with AIT efficacy.**
**Methods: Transcriptomes and proteomes of monocyte-derived DCs polarized toward DCs driving the differentiation of $T_H1$ cells (DC1s), DC2s, or DCs driving the differentiation of regulatory T cells (DCreg cells) profiles were compared by using genome-wide cDNA microarrays and label-free quantitative proteomics, respectively. Markers differentially regulated in DC2s and DCreg cells were assessed by means of quantitative PCR in PBMCs from 80 patients with grass pollen allergy before and after 2 or 4 months of sublingual AIT in parallel with rhinoconjunctivitis symptom scores.**
**Results: We identified 20 and 26 new genes/proteins overexpressed in DC2s and DCreg cells, respectively. At an individual patient level, DC2-associated markers, such as CD141, GATA3, OX40 ligand, and receptor-interacting serine/threonine-protein kinase 4 (RIPK4), were downregulated after a 4-month sublingual AIT course concomitantly with an upregulation of DCreg cell–associated markers, including complement C1q subcomponent subunit A (C1QA), FcγRIIIA, ferritin light chain (FTL), and solute carrier organic anion transporter family member 2B1 (SLCO2B1), in the blood of clinical responders as opposed to nonresponders. Changes in such markers were better correlated with clinical benefit than alterations of allergen-specific $CD4^+$ T-cell or IgG responses.**
**Conclusions: A combination of 5 markers predominantly expressed by blood DCs (ie, C1Q and CD141) or shared with lymphoid cells (ie, FcγRIIIA, GATA3, and RIPK4) reflecting changes in the balance of regulatory/proallergic responses in peripheral blood can be used as early as after 2 months to monitor the early onset of AIT efficacy. (J Allergy Clin Immunol 2016;137:545-58.)**

***Key words:*** *Allergen, biomarker, dendritic cell, monocyte, sublingual immunotherapy*

Allergen immunotherapy (AIT) performed through the subcutaneous or sublingual routes is an established treatment for type I respiratory allergies.[1,2] With the aim to identify biological markers of clinical efficacy, several alterations of immune parameters have been previously documented in peripheral blood or mucosal tissues of patients receiving AIT. The latter include the downregulation of allergen-specific $T_H2$ cells and the induction of $T_H1$ and regulatory T (Treg) cells,[3-12] the upregulation of blocking $IgG_4$ antibodies concomitant with a progressive decrease in specific IgEs,[9,13-16] and the reduction of basophil recruitment and activation in target organs.[17,18] However, such immunologic changes were mostly reported in open clinical studies conducted in small cohorts of patients and consequently could not be firmly related to clinical efficacy.[10,19-21] Thus surrogate biomarkers of efficacy still remain to be established in the context of larger double-blind, placebo-controlled studies allowing to assess the clinical benefit of AIT at an individual patient level.

In our search for such biomarkers, we recently focused on blood monocyte-derived dendritic cells (MoDCs).[22] As antigen-presenting cells with a unique capacity to integrate multiple extracellular signals, dendritic cells (DCs) play a major role in the orientation of adaptive T-cell responses. A well-accepted model is that, depending on the danger signals and costimulatory molecules engaged, immature DCs become polarized as either DCs driving the differentiation of $T_H1$ cells (DC1s), DCs driving the differentiation of $T_H2$ cells (DC2s), DCs driving the differentiation of $T_H17$ cells (DC17s), or DCs driving the differentiation

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*Abbreviations used*
AIT: Allergen immunotherapy
ANR: Active nonresponder
AR: Active responder
ARTSS: Average rhinoconjunctivitis total symptom score
C1QA: Complement C1q subcomponent subunit A
Ctrl-DC: Control dendritic cell
DC: Dendritic cell
DC1: DC driving the differentiation of $T_H1$ cells
DC2: DC driving the differentiation of $T_H2$ cells
DCreg: DC driving the differentiation of regulatory T cells
FTL: Ferritin light chain
MoDC: Monocyte-derived dendritic cell
OX40L: OX40 ligand
qPCR: Quantitative PCR
ROC: Receiver operating characteristic
RIPK4: Receptor-interacting serine/threonine-protein kinase 4
SLCO2B1: Solute carrier organic anion transporter family member 2B1
Treg: Regulatory T

of regulatory T cells (DCreg cells), which subsequently support the differentiation of effector (proinflammatory) $T_H1$, $T_H2$, $T_H17$, or regulatory (tolerogenic) T cells, respectively.[22,23] After identification of molecular signatures associated with some of those polarized DC subsets, we recently showed that 2 DCreg cell markers, C1Q and Stabilin-1, are upregulated in the peripheral blood of clinical responders when compared with levels in nonresponders or placebo-treated patients in the course of sublingual AIT for grass pollen–induced rhinoconjonctivitis.[22] Although this study confirmed that clinical benefit is associated with induction of a regulatory immune response, potential alterations of circulating DC2s were not addressed at the time because of the lack of *bona fide* DC2 markers.

Consequently, we proceeded to identify a molecular signature associated with DC2s and investigated whether AIT had an effect on such proallergic DCs. As a first step, we defined optimal culture conditions to differentiate immature MoDCs toward a DC2 profile. Using cDNA microarrays together with label-free quantitative proteomics, we identified novel markers differentially regulated in DC2s and DCreg cells. We demonstrate that a combination of 5 markers predominantly expressed by DCs or shared with lymphoid cells can be used to monitor peripheral blood AIT efficacy as early as after 2 months of treatment.

## METHODS

### DC1, DC2, and DCreg cell polarization of blood MoDCs

The polarization of MoDCs into DC1, DC2, and DCreg cell subsets is described in the Methods section in this article's Online Repository at www.jacionline.org. Markers differentially expressed in the DC2 and DCreg cell subsets were identified by using combined transcriptomic and proteomic analyses and confirmed by using quantitative PCR (qPCR), flow cytometry, or both, as described in detail elsewhere[22] and as summarized in the Methods section in this article's Online Repository.

### Clinical samples from the VO56.07A pollen chamber study

Details of the double-blind, placebo-controlled clinical trial VO56.07A (ClinicalTrials.gov NCT00619827) have been published elsewhere.[24] Briefly, 89 patients with grass pollen allergy were randomized to receive sublingually once daily either a grass pollen or a placebo tablet (Stallergenes SA, Antony, France) for 4 months. Patients were exposed outside of the pollen season to grass pollens in a challenge chamber at baseline (visit 3, before treatment) and after 2 (visit 6) and 4 (visit 7) months of treatment.[24] Percentages of improvement in average rhinoconjunctivitis total symptom scores (ARTSSs) were calculated between baseline and each challenge for all patients.[24] The analysis of DC markers was performed on PBMCs from 80 patients (n = 42 and 38 in the active and placebo groups, respectively) collected at baseline (visit 3), as well as after 2 (visit 6) and 4 (visit 7) months of immunotherapy. The median percentage of ARTSS improvement at visit 7 in the active group (corresponding to a 43.9% decrease in ARTSSs) was used arbitrarily as a threshold to distinguish responder from nonresponder patients. As a result, patients were classified in 4 subgroups, including active responders (ARs; n = 21), active nonresponders (ANRs; n = 21), placebo responders (n = 7), and placebo nonresponders (n = 31). RNA was isolated from PBMCs, as previously described,[22] and qPCR analyses were performed as described in the Methods section in this article's Online Repository. Samples were coded, and all biological analyses were conducted in a blind manner by the operators.

### Statistical analyses

Data are expressed as means ± SEMs. Statistical differences between groups were assessed by using 2-tailed Wilcoxon or Mann-Whitney nonparametric tests to compare paired or independent data, respectively, with $P$ values of less than .05 considered significant. Correlation analyses were performed by using the nonparametric Spearman test, and receiver operating characteristic (ROC) curves were produced by using an empiric model. Statistical and graphic analyses were performed with Prism 6 software (GraphPad Software, La Jolla, Calif). ROC analyses of combinations of markers were performed with the mROC program.[25]

## RESULTS

### Polarization and characterization of DC2s

We first defined optimal culture conditions to polarize immature MoDCs toward a DC2 profile capable of promoting the differentiation of naive $CD4^+$ T lymphocytes into $T_H2$ cells. After screening more than 30 biological or synthetic pro-$T_H2$ molecules, a mixture of histamine plus IL-25, IL-33, LPS, prostaglandin $E_2$, and thymic stromal lymphopoietin, subsequently termed the DC2 cocktail, was selected to differentiate MoDCs into DC2s. Immature MoDCs were treated in parallel with either medium, LPS, or dexamethasone to obtain control dendritic cells (Ctrl-DCs), DC1s, and DCreg cells, respectively,[22,26] which were used to perform comparative studies.

We characterized the pattern of cytokines secreted by DC2s generated from 12 independent donors in comparison with other DC subsets. Although DC2s secrete proinflammatory cytokines, such as IL-6, IL-8, IL-10, and TNF-α, in the same range as DC1s, they only produce background levels of the $T_H1$ cytokines IL-12p70 and IFN-γ (Fig 1, *A*). Notably, DC2s specifically secrete high amounts of IL-13, a cytokine driving $T_H2$ polarization (Fig 1, *A*, right panel). In contrast, only very low amounts, if any, of these proinflammatory cytokines were secreted by DCreg cells (Fig 1, *A*).

Subsequently, using qPCR, we assessed the expression of various genes upregulated in either effector (proinflammatory) or regulatory (tolerogenic) DCs in those DC subsets, as described in our previous study.[22] Similar to DC1s, DC2s significantly overexpress genes associated with effector DCs (ie, *IRF4, FSCN1*, and *MX1*) while downregulating genes encoding regulatory DC markers, such as *C1Q, CATC, GILZ,* and *STAB1* (Fig 1, *B*).[22]

Importantly, as shown in Fig 1, *C*, allogeneic naive $CD4^+$ T cells cocultured for 5 days with DC2s secrete IL-5 and high levels of IL-13 but no IFN-γ and limited amounts of IL-10 when compared