

Defects in Plasmacytoid Dendritic Cell Expression of Inducible Costimulator Ligand and IFN- α Are Associated in Asthma with Disease Persistence

To the Editor:

Plasmacytoid dendritic cells (pDCs) represent a subset of DCs specialized in antiviral defense. Originating from a different lineage than myeloid DCs and displaying a plasma cell–like morphology, they are able to secrete large amounts of type 1 IFN when activated by viruses through toll-like receptor 7 (TLR7) and TLR9 (1). In addition to their antiviral function, pDCs play a role in peripheral tolerance to allergens; in a seminal study, de Heer and colleagues showed that allergen-pulsed pDCs were able to restore tolerance in experimental ovalbumin-induced asthma (2). In addition, it was shown that pDCs from patients with asthma display impaired IFN- α secretion on TLR9 ligation by A-CpG (3). pDC-mediated tolerance is notably related to their expression of the ligand to

inducible costimulatory molecule (ICOS-L), as the ICOS/ICOS-L costimulatory pathway has been linked to the priming of IL-10–producing regulatory T cells (4) and to tumor-induced tolerance (5).

Mechanisms underlying the persistence of allergic asthma remain poorly understood. This could be related to a multitude of factors that may contribute to disease persistence, among which are persistent allergen exposure and host-related factors. IgE-mediated occupational asthma (OA) offers a unique opportunity to address this question, as one third of patients completely recover (from both symptoms and nonspecific bronchial hyper-responsiveness) after complete cessation of exposure to the causal allergen (6).

Given their tolerogenic role, we sought to explore pDCs for their expression of ICOS-L and IFN- α production in 29 patients with OA diagnosis to flour ($n = 14$) or latex ($n = 15$), according to a specific inhalation challenge (performed between 2001 and 2011). All patients had been removed from their workplace and were no longer exposed to the causal allergen. Study subjects were defined

Table 1. Clinical Characteristics of the Patients

Patient characteristics	Persistent Asthma ($n = 18$)	Cured Asthma ($n = 11$)	P Value
Causal allergen, flour/latex	10/8	4/7	0.69
Sex, M/F	9/9	4/7	0.41
Age, yr	46.5 (41.7–51.0)	52.0 (44.0–53.0)	0.38
Atopy*	13 (72)	7 (63)	0.65
Current or ex-smokers	6 (28)	2 (20)	0.54
Smoking history, pack-years (at follow-up)	7 (3–31)	19 (6–32)	0.86
Duration of exposure before removal, mo	120 (43–216)	152 (78–234)	0.59
Time elapsed since removal from exposure, yr	8.0 (5.0–11.5)	11.0 (7.0–12.0)	0.39
Initial assessment			
ICS treatment, n (%)	8 (38)	3 (27)	0.62
ICS daily dose, $\mu\text{g}^\dagger$	800 (575–950)	800 (500–2,000)	0.91
SABA use, n (%)	11 (52)	7 (64)	0.56
SABA use, puffs/wk	1 (0–14)	2 (0–14)	1.0
FEV ₁ , % predicted	90 (80–100)	100 (96–104)	0.05
FEV ₁ /FVC ratio, %	71 (64.5–79.5)	80 (77–87)	0.03
Histamine PC ₂₀ , mg/ml	1.4 (0.8–5.7)	1.4 (0.9–5.8)	0.30
Follow-up assessment			
ICS treatment†	7 (38)	0 (0)	NA
ICS daily dose, $\mu\text{g}^\dagger$	1,000 (500–1,000)	0 (0)	NA
SABA use, n (%)	6 (28)	0 (0)	NA
SABA use, puffs/wk	14 (14–21)	0 (0)	NA
FEV ₁ , % predicted	89.5 (77–98.2)	105 (97–107)	0.008
FEV ₁ /FVC ratio, %	69.5 (65.7–74.7)	77 (74–79)	0.006
Histamine PC ₂₀ , mg/ml	3.9 (1.4–7.1)	17.0 (16.0–21.0)	<0.001

Definition of abbreviations: ICS = inhaled corticosteroids; NA = not applicable; PC₂₀ = concentration of histamine inducing a 20% fall in FEV₁; SABA = inhaled short-acting β_2 -agonist.

All data are expressed as median value with 25th–75th confidence intervals unless otherwise specified.

*Atopy was defined by a positive skin-prick test response to at least one of 20 common inhalant allergens.

†Expressed as beclomethasone dipropionate equivalent; only ICS users are taken into account.

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as cured if they had no asthma symptoms, no need for medication, and no nonspecific bronchial hyper-responsiveness (histamine PC₂₀ > 8 mg/ml) and as having persistent disease when failure to meet any of these criteria was observed. Subjects underwent a leukapheresis, and pDCs were isolated from peripheral blood mononuclear cells using the Diamond Plasmacytoid Dendritic Cells purification kit (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were stained for HLA-DR, CD123 (IL-3 receptor α -chain), Lin 1 cocktail (CD3, CD14, CD16, CD19, CD20, and CD56),

and ICOS-L. IFN- α and IL-10 production after pDC stimulation by type A CpG was measured by ELISA. (See the online supplement for detailed information regarding materials and methods.)

At follow-up, 11 patients were cured and 18 had persistent disease. When comparing their characteristics at the time of the initial

assessment (Table 1), the two groups did not differ regarding the causal allergen (flour or latex), age, sex, atopy, smoking history, or duration of exposure before asthma onset. The only difference was that patients with persistent disease at follow-up had significantly decreased airflow indices, whereas histamine PC₂₀ was similar.

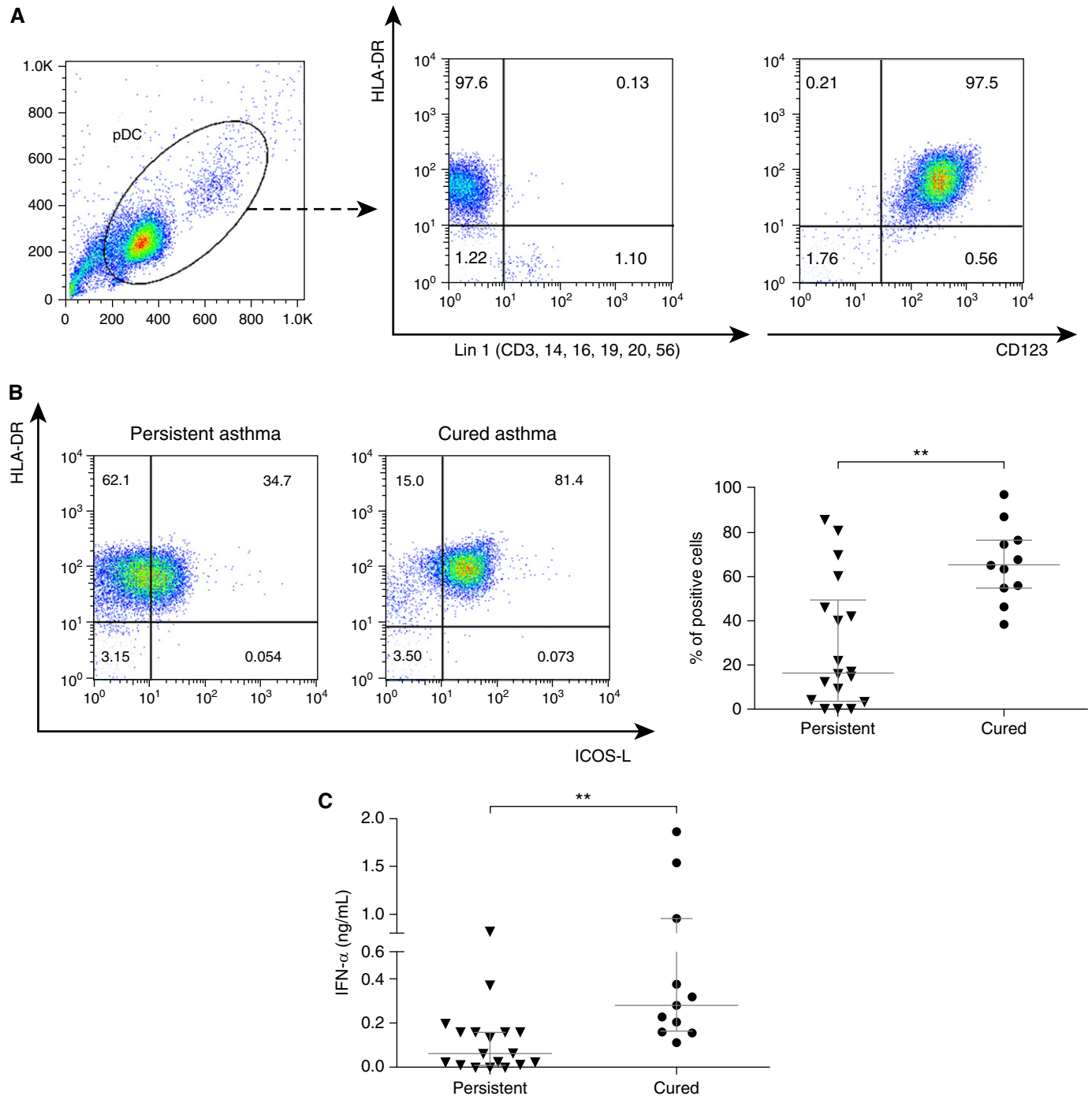


Figure 1. (A) Plasmacytoid dendritic cells are HLA-DR⁺, CD123⁺, and Lin⁻ cells. The purity was 97% or higher. (B) Plasmacytoid dendritic cells from patients with persistent asthma have a lower expression of the ligand to inducible costimulatory molecule compared with cured patients (statistics: Mann-Whitney *U* test, ***P* < 0.01); two representative dot plots and pooled data are shown. (C) After overnight stimulation with type A CpG-DNA, plasmacytoid dendritic cells from patients with persistent asthma display decreased interferon α production compared with cured patients (statistics: Mann-Whitney *U* test, ***P* < 0.01). ICOS-L = ligand to inducible costimulatory molecule; pDC = plasmacytoid dendritic cell.

After purification, we obtained more than 97% pDCs that were HLA-DR⁺, CD123⁺, and Lin 1⁻ (Figure 1A). We found out that pDCs from patients with persistent disease had a significantly lower expression of ICOS-L compared with cured patients (median, 16.5% [interquartile range, 4.1–49.4%] vs. median, 64.9% [interquartile range, 54.2–76.5%]; $P = 0.003$; Figure 1B). Mean fluorescence intensity for ICOS-L expression also significantly differed between both groups (median, 432 [interquartile range, 276–497] vs. median, 135 [interquartile range, 78–169]; $P = 0.012$). In addition, although resting pDCs did not produce detectable levels (data not shown), pDCs from these patients also displayed impaired secretion of IFN- α on TLR9 stimulation by type A CpG-DNA (median, 0.07 ng/ml [interquartile range, 0.01–0.2 ng/ml] vs. median, 0.3 ng/ml [interquartile range, 0.2–0.9 ng/ml]; $P = 0.001$; Figure 1C). Of note, IFN- α production and ICOS-L levels did not correlate (Spearman $r = 0.05$; $P = 0.8$), and (resting or activated) pDCs did not produce detectable levels of IL-10.

Our findings indicate defects in tolerogenic features of pDCs from patients with OA with persistent disease, despite strict allergen avoidance, in the setting of complete cessation of occupational exposure to the culprit allergen. Although the potential link between ICOS-L expression and IFN- α production is not established and was not found in a previous study in experimental asthma (7), it has been shown that several factors, including viruses (8) and cytokines (9), do influence these features, hence interfering with tolerance in asthma. To our knowledge, this is the first study establishing a link between pDC function and asthma outcome. Mechanisms underlying asthma remission have been poorly explored for several reasons: first, current asthma therapies, although usually highly effective, are unable to induce sustained remission, with the exception of allergen immunotherapy in very selected patients (10); second, there is a lack of a proper experimental model to study disease persistence; third, published longitudinal studies used a very broad definition of remission, notably without taking the potential persistence of nonspecific bronchial hyper-responsiveness into account (11).

In the present study, we used as model a carefully selected population of IgE-mediated OA that progressed after strict allergen avoidance toward remission or not, and correlated pDC function with clinical outcome. Although highlighting a potential role for pDCs in asthma remission, our study has some limitations. First, it was not prospective, and therefore it could not be excluded that pDCs could already differ between the two groups at baseline. Second, we did not study airway pDCs, which would have integrated the putative role of local factors (e.g., epithelial cytokines) for regulating pDCs locally, even though it was shown that lung pDCs are recruited from blood on allergen exposure (12). Finally, investigations should further address pDC functionality, for example, in coculture with naïve CD4⁺ T cells, to decipher impaired mechanisms of T-cell tolerance in these patients.

In conclusion, we demonstrated in the setting of IgE-mediated occupational asthma that the disease outcome after allergen avoidance is correlated with pDC function, pDCs from patients with persistent disease displaying defective ICOS-L expression, and IFN- α response. Further research should determine the molecular determinants of these pDC defects and whether restoring tolerogenic properties of pDCs could promote asthma remission. ■

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Functionally Relevant Cut Point for Isometric Quadriceps Muscle Strength in Chronic Respiratory Disease

To the Editor:

The recently updated American Thoracic Society/European Respiratory Society statement on limb muscle dysfunction in chronic obstructive pulmonary disease (COPD) (1) highlighted important clinical implications of quadriceps muscle weakness, including reduced exercise tolerance (2), sarcopenia (3), and increased mortality (4). Quadriceps weakness is present in more than a quarter of stable outpatients with COPD, including those with only mild abnormalities in spirometry (5). Although quadriceps muscle strength can be readily measured by volitional and nonvolitional techniques in the research setting, none have been incorporated into clinical practice. The American Thoracic Society/European Respiratory Society statement suggests that isometric quadriceps maximal voluntary contraction (QMVC) shows the most potential for being “implemented in clinical practice to provide reliable and reproducible measurements” (1). Although normal values for QMVC have been described (5), clinically relevant cut points have not been determined. This is important, as the relationship between QMVC and functional performance is not linear (6). With several putative anabolic agents entering phase 2 and phase 3 studies, there is a need to validate outcome measures that can directly assess the effects of interventions of lower limb muscle strength. The aim of the current study was to determine and validate sex-specific QMVC cut points to provide clinical context for clinicians and patients.

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Participants were recruited from respiratory and pulmonary rehabilitation assessment clinics at Harefield Hospital, London, United Kingdom. Inclusion criteria for the derivation cohort included age older than 35 years, a diagnosis of COPD according to Global Initiative for Chronic Obstructive Lung Disease criteria (7), and stable state (no exacerbation requiring change of medication within the previous 4 weeks). Exclusion criteria included a predominant joint or neurological limitation to standing or walking.

QMVC of the dominant leg was measured using a specially adapted chair and strain gauge (8), with hips and knees at 90° flexion, and was recorded as the maximal force that could be sustained over the course of 1 second. The best of three reproducible maneuvers was reported. QMVC was also normalized to weight (kilograms), body mass index (kilograms/meters squared), height (meters), and height squared (meters squared). As the ability to stand from a sitting position is fundamental to functional independence, we anchored QMVC measurements to sit-to-stand ability. Participants sitting, with upper limbs folded, on a straight-backed armless chair with floor to seat height of 48 cm, were instructed to stand up, all the way, without using the upper limbs. This was performed by an assessor blinded to QMVC measurements. Other assessments included comorbidity burden using the COPD-specific comorbidity test index (9), Medical Research Council dyspnea score, incremental shuttle walk, and COPD Assessment Test (10).

Men and women were analyzed separately. We plotted receiver operating curves and calculated area under the curve for raw and normalized QMVC values that best identified patients who were unable to stand from a sitting position, giving equal weighting to sensitivity and specificity.

A total of 437 patients with COPD were recruited (277 men, 160 women), with a mean age of 70 years (standard deviation, 10 years); FEV₁, 47% predicted (standard deviation, 19% predicted); FEV₁/FVC ratio, 0.48 (standard deviation, 0.12); Medical Research Council dyspnea score, 3.2 (standard deviation, 1.1); body mass index, 27.1 kg/m² (standard deviation, 5.9 kg/m²); COPD-specific comorbidity test score, 1.4 (standard deviation, 2.0); incremental shuttle walk, 236 m (standard deviation, 154); COPD Assessment Test score, 21.0 (standard deviation, 8.0); and QMVC, 25.7 kg (standard deviation, 10.3 kg; men, 29.5 kg [standard deviation, 9.6 kg], and women, 19.0 kg [standard deviation, 7.7 kg]). Twenty-three women and 26 men were unable to stand unaided (14 and 9%, respectively); two of the men were receiving long-term systemic corticosteroids. Table 1 describes the clinical characteristics of those who could and those who could not stand unaided. Table 2 shows the area under the curve, sensitivity, and specificity for sex-specific cut points for raw and normalized QMVC values in predicting failure to stand in patients with COPD. QMVC normalized to height squared produced the highest area under the curve, with 8.30 and 5.99 kg/m² identified as the cut points for men and women that best identified failure to stand.

To validate these results, we recruited a separate cohort of 208 patients (98 men, 110 women) with non-COPD chronic respiratory disease (62 patients with asthma, 61 with bronchiectasis, 76 with interstitial lung disease, and nine with extrathoracic restriction) from outpatient respiratory clinics. Baseline characteristics were mean age, 68 years (standard deviation, 12 years); FEV₁, 68% predicted (standard deviation, 24% predicted), FEV₁/FVC ratio, 0.73 (standard deviation, 0.13); Medical Research Council dyspnea score, 3.0 (standard deviation, 1.0); body mass index, 28.5 kg/m² (standard deviation, 5.8 kg/m²), incremental shuttle walk, 285 m

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