

Letter to the Editor

Serum 25-dihydroxyvitamin D levels correlate with CD4⁺Foxp3⁺ T-cell numbers in moderate/severe asthma*To the Editor:*

Asthma is a chronic inflammatory disease characterized by airways hyperresponsiveness, mucus hyperplasia, and airways remodeling. The immune and inflammatory processes that underpin asthmatic disease are complex and include evidence for impaired immune regulation by both Foxp3⁺ and IL-10⁺ regulatory T (Treg) cells (reviewed in Lloyd and Hawrylowicz¹).

The major treatment for asthma is corticosteroids (steroids), enabling most asthmatic patients to achieve some control of their symptoms (steroid sensitive [SS]). However, a proportion of asthmatic patients fail to gain any clinical benefit in terms of lung function and are termed steroid resistant (SR), representing those most at risk from their asthma. Corticosteroids enhance the production of the antiinflammatory cytokine IL-10 by T cells *in vitro*, but notably this response is impaired in cultures from SR asthmatic patients, implying an association between impaired IL-10 response and poor asthma control.² This defect in steroid-induced IL-10 can be restored by the addition of the active form of vitamin D₃ (1,25-hydroxyvitamin D₃) into the culture, thus suggesting that vitamin D may play a role in controlling steroid responsiveness.³ These data are complemented by a number of independent clinical studies that highlight a high prevalence of vitamin D deficiency and insufficiency worldwide and its association with an increased incidence, severity, and poor control of asthma.^{4,5}

The importance and status of a well-defined subset of Treg cells, as defined by the expression of the transcription factor forkhead box P3 (Foxp3), in SS and SR asthma are less well understood.^{1,6} Although 1,25-hydroxyvitamin D₃ has been shown to enhance the frequency of human Foxp3⁺ Treg cells *in vitro*, no *in vivo* correlates of these data exist.⁷ The aim of the present study was therefore to investigate whether differences exist in the frequency of Foxp3⁺ Treg cells in the peripheral blood of SS and SR adult asthma patients, and the relationship between vitamin D status and the Foxp3⁺ Treg-cell compartment.

Adults aged 18 to 75 years with moderate to severe asthma for at least 6 months on therapy step 3 or 4 of the British Thoracic Society guidelines on management of asthma, and who remained clinically poorly controlled, were recruited. All patients had a prebronchodilator FEV₁ of less than 80% of the predicted value with reversibility of more than 12% following 400 µg of short-acting bronchodilator and had undergone detailed assessment to exclude an incorrect diagnosis and comorbidities affecting asthma control. SR asthma was defined as an increase of less than 10% in FEV₁ following a 2-week course of prednisolone of 40 mg/1.73 m² body surface area. Smokers and participants who had suffered from a respiratory tract infection or asthma exacerbation during or 4 weeks prior to enrolling for the study were excluded. This study was approved by the NHS research ethics committee London Bridge (08/H0804/84), and all patients gave written informed consent. Fourteen SR and 8 SS patients with mean ages of 54 and 50 years, respectively, were assessed. Mean prebronchodilator FEV₁ was 2.06 L (65.6%) and 1.99 L (64.6%) in the SR patients and 1.80 L (61.6%) and 2.29 L (77.6%) in the SS patients

TABLE I. Patient characteristics

	Steroid sensitive	Steroid resistant
Age (y)	50 (21-64)	53 (30-67)
Sex ratio (female/male)	4/4	5/9
FEV ₁ (L)	1.8 (1.33-2.21)	2.06 (1.17-2.65)
FEV ₁ (L) poststeroids	2.29 (1.77-3.08)	1.99 (1.06-2.53)
FEV ₁ (% predicted)	61.6% (45-76)	65.6% (40-79)
FEV ₁ (%) poststeroids	77.6% (68-87)	64.6% (36-80)
FENO	26.5 (18-42)	27.6 (12-70)
Atopy (yes/no)	7/1	12/2
Race		
Caucasian	6 (75%)	9 (64%)
Afro-Caribbean	2 (25%)	4 (29%)
Asian	0 (0%)	1 (7%)
Average inhaled corticosteroid dose (BDP equivalent) (µg)	1225 (800-2000)	1228 (800-2000)
Serum 25(OH)D	37.54 (20-91)	40.63 (27-57)

Data shown as mean and range.

BDP, Beclometasone dipropionate; FENO, fraction of exhaled nitric oxide.

before and after the course of oral corticosteroid, respectively (poststeroid FEV₁ SR vs SS: $P < .001$; 95% CI for difference 0.27-0.78 L; analysis of covariance with presteroid FEV₁ as covariate). The patients did not significantly differ in their mean body mass index (28.6 vs 31.1), inhaled corticosteroid dose (beclometasone dipropionate equivalent: 1228 µg vs 1225 µg), or race (9 Caucasian, 5 Afro-Caribbean/Asian origin vs 6 Caucasian, 2 Afro-Caribbean origin) as can be seen in Table I.

Flow cytometry was performed on peripheral blood from each patient obtained pre-oral corticosteroid. Treg cells were defined as Foxp3⁺ cells within the CD4⁺CD3⁺ lymphocyte population as shown in Fig 1. Additional staining demonstrated that between 90% and 95% of the Foxp3⁺ cells were CD127^{lo}CD25^{hi}, markers that distinguish Treg cells from recently activated effector T cells.⁸ SR asthma patients had a significantly lower frequency of Foxp3⁺ T cells as compared to SS asthma patients at baseline (mean SR, 7.18%; mean SS, 9.77%; $P = .015$). Serum 25-hydroxyvitamin D (25(OH)D) concentrations were assessed by using a 2-dimensional high performance liquid chromatography system - tandem mass spectrometry.⁹ Although patients were recruited all year round, all but 1 patient was vitamin D insufficient (<75 nmol/L). No correlation was observed between body mass index and serum 25(OH)D or inhaled steroid dose and serum 25(OH)D. There were significantly lower levels of serum 25(OH)D in patients who were of Afro-Caribbean origin than in Caucasians (mean Caucasian origin 38.55 nmol/L vs Afro-Caribbean/Asian origin 23.13 nmol/L; $P = .045$). There was also a trend toward seasonal differences observed, with highest levels of serum 25(OH)D seen in the summer and autumn and lowest levels seen in the winter and spring, but this did not achieve statistical significance.

A strong positive correlation between serum 25(OH)D levels and Foxp3⁺ T-cell number in all asthmatic patients was observed (Pearson correlation $r = 0.7$; $P = .0003$).

This cross-sectional study demonstrates that the frequency of circulating Foxp3⁺ CD4⁺ Treg cells is significantly lower in steroid resistant than in SS asthmatic patients with comparable disease severity. A majority of patients with severe asthma had serum

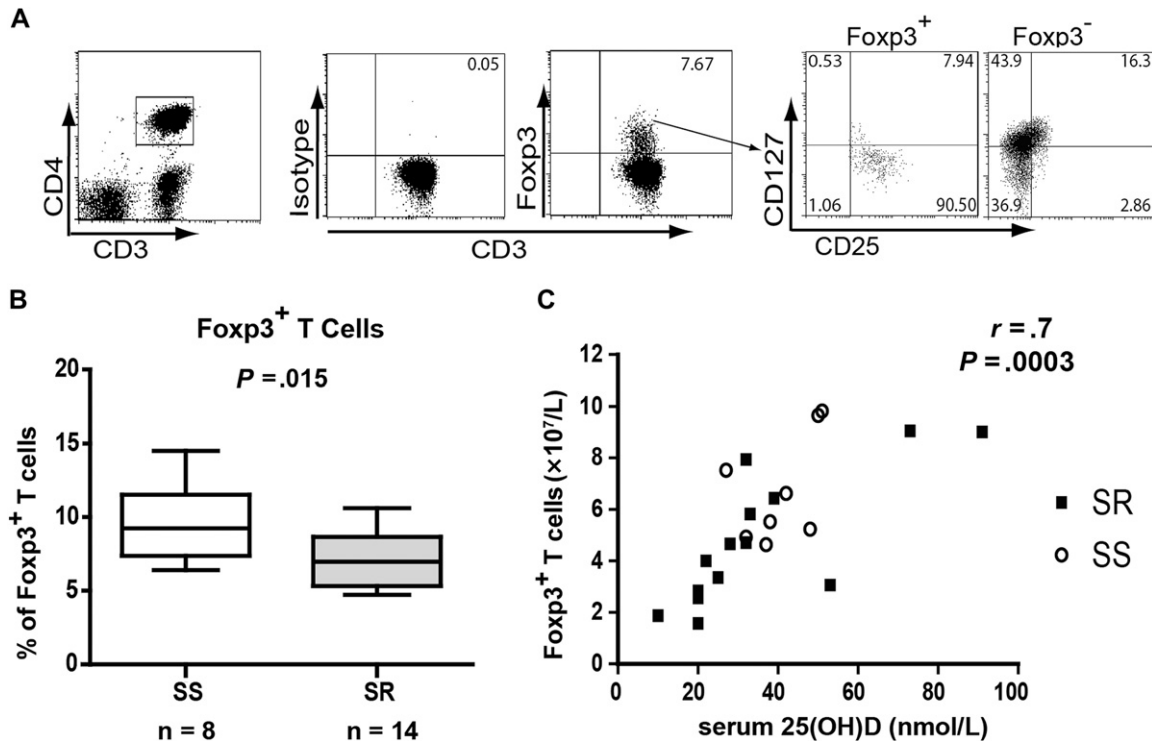


FIG 1. SR asthmatic patients have a lower frequency of Foxp3⁺ Treg cells than do SS asthmatic patients, and serum 25(OH)D concentration correlates positively with Foxp3⁺ Treg cells in the peripheral blood. **A**, Representative dot plots demonstrating the gating strategy to define Treg cells. Values represent % of gated live CD4⁺CD3⁺ lymphocyte population. **B**, Frequency of Foxp3⁺ Treg cells in SS and SR asthmatic patients. Data shown as mean, 5%-95% CI, assessed by *t* test. **C**, Correlation of Foxp3⁺ Treg cells with serum 25(OH)D in all the patients with moderate to severe asthma. Assessed by Pearson correlation test.

concentrations of 25(OH)D within the deficient range, and this strongly correlated with a paucity of Foxp3⁺ Treg cells in the peripheral blood. These data suggest a strong functional correlation between vitamin D status, assessed as circulating 25(OH)D, circulating CD4⁺Foxp3⁺ Treg-cell numbers, and corticosteroid responsiveness in chronic asthma. They also provide important *in vivo* validation of *in vitro* studies with human cells and evidence from animal models suggesting that the vitamin D pathway plays an important role in enhancing the frequency of the CD4⁺Foxp3⁺ Treg-cell compartment in humans.

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