

## Cyclosporin A reduces CD4<sup>+</sup>CD25<sup>+</sup> regulatory T-cell numbers in patients with atopic dermatitis

To the Editor:

Naturally occurring CD4<sup>+</sup>CD25<sup>+</sup> regulatory T (Treg) cells are characterized by their ability to suppress effector T cells involved in mediating inflammation. Consequently, they play a key role in balancing immune responses and maintaining tolerance against antigens and allergens. It is assumed that only the CD4<sup>+</sup>CD25<sup>high</sup> population exerts regulatory/suppressive activity. In addition, it has been shown that naturally occurring CD4<sup>+</sup>CD25<sup>high</sup> Treg cells specifically express the transcription factor forkhead box protein 3 (FOXP3). Modifying immune reactions to revert inflammatory diseases is one of the current challenges in immunology. The immunosuppressant drug cyclosporin A (CsA) is being increasingly used in the treatment of patients with severe atopic dermatitis (AD). Although it is highly efficacious and relatively safe, recent data from *in vitro* studies have suggested that CsA might induce suppression of naturally occurring CD4<sup>+</sup>CD25<sup>+</sup> Treg cell function.<sup>1-3</sup> In addition, it was recently shown that patients with liver transplant with high blood CsA levels have lower percentages of blood Treg cells compared with patients with low blood CsA levels.<sup>4</sup> These studies prompted us to investigate effects of CsA treatment on Treg cells in patients with AD.

We studied CD4<sup>+</sup> T cells expressing high levels of CD25 and the transcription factor FOXP3 in patients with AD compared with healthy controls without allergy. In addition, we studied the effects of CsA treatment on Treg cells in patients with AD.

T-cell phenotyping was performed by flow-cytometric analysis on peripheral blood from 11 patients with AD (male:female, 7:4; age, mean  $\pm$  SD, 33.9  $\pm$  10.2 years; total serum IgE level, mean  $\pm$  SD, 8781  $\pm$  3240 IU/mL). The mean  $\pm$  SD modified/objective

Scoring Atopic Dermatitis (SCORAD; without the pruritus and sleep-loss components) in the patients with AD studied was 40.0  $\pm$  9.0 (maximum score, 83). As a control group, we included 20 healthy controls without allergy (male:female, 8:12; age, mean  $\pm$  SD, 34.8  $\pm$  12.4 years).

The mean percentage of CD4<sup>+</sup>CD25<sup>high</sup> cells and CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> T cells and the relative *FOXP3* mRNA expression levels were significantly higher in patients with AD compared with healthy controls (Fig 1).

In the patients with AD (n = 11), we also analyzed T-cell populations during CsA treatment. Patients were treated with CsA (Neoral, Novartis, Switzerland) at 5 mg/kg body weight per day in 2 doses. CsA treatment resulted in a rapid improvement of disease severity (after 3 and 6 weeks of treatment) in all patients with AD. Modified SCORAD and serum thymus and activation-regulated chemokine levels decreased significantly after 3 weeks, and remained stable until week 6 of treatment (Fig 2, A and B). After 3 and 6 weeks of CsA treatment, we found a significant decrease in the numbers of CD4<sup>+</sup>CD25<sup>high</sup> and CD4<sup>+</sup>FOXP3<sup>+</sup> peripheral blood T cells (Fig 2, C and D). In addition, *FOXP3* mRNA expression levels in the CD4<sup>+</sup> T cells decreased significantly (Fig 2, E). Because it was shown that FOXP3 can be transiently expressed in activated human effector T cells *in vitro*, we also studied the coexpression of CD25 and CD69 and found that their expression remained stable during CsA therapy (data not shown). In addition, we found that the vast majority of CD4<sup>+</sup>FOXP3<sup>+</sup> T cells were CD25<sup>high</sup> and CD69<sup>low</sup> (data not shown). Routine hematology analysis showed no significant changes in total lymphocyte or leukocyte counts (data not shown). Furthermore, fluorescence-activated cell sorting analysis revealed no significant changes in the percentages of CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD4<sup>+</sup>CD25<sup>high</sup>, CD4<sup>+</sup>FOXP3<sup>+</sup>, CD45RA<sup>+</sup>, and CD45RO<sup>+</sup> T-cell populations (data not shown).

In conclusion, we have shown that although treatment of patients with AD with CsA results in good clinical improvement,

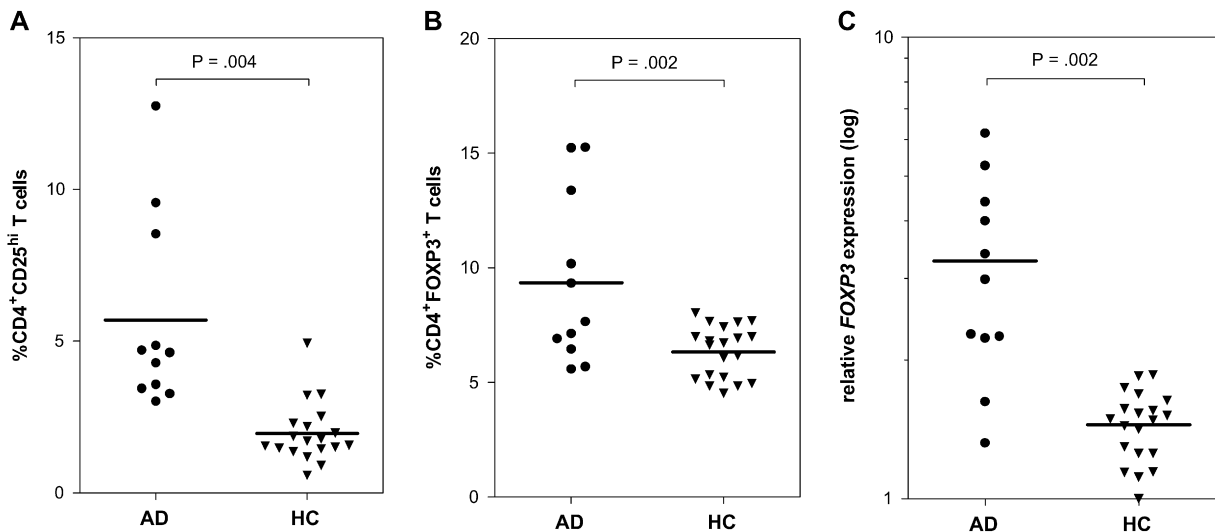


**FIG 1.** Increased percentages of CD4<sup>+</sup>CD25<sup>high</sup> T cells (A) and CD4<sup>+</sup>FOXP3<sup>+</sup> T cells (B), and relative (lowest expression level is set to 1) *FOXP3* mRNA levels in CD4<sup>+</sup> T cells (C) isolated from patients with severe AD (n=11) compared with healthy controls (HC) without allergy (n=20).

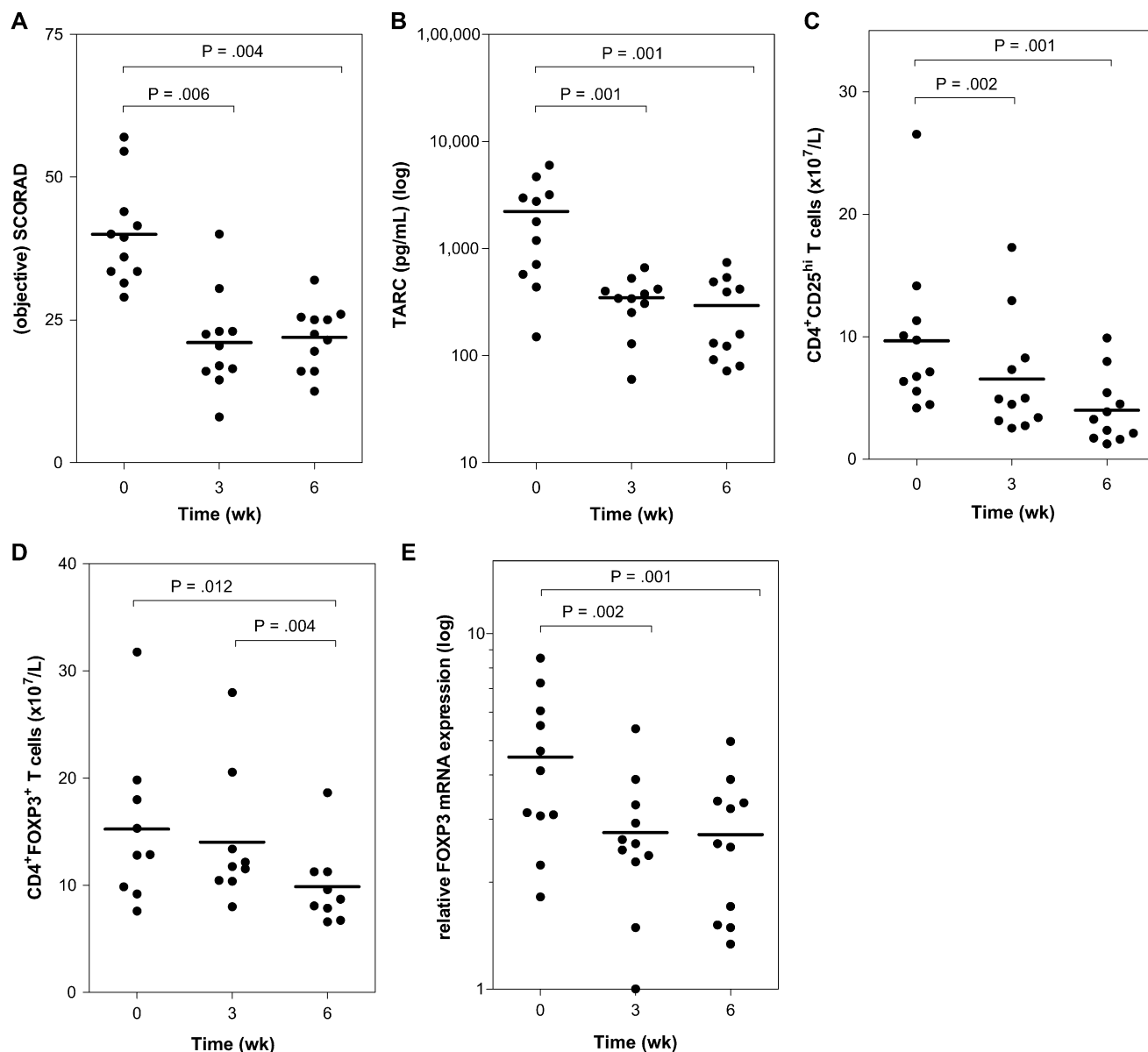


**FIG 2.** CsA treatment resulted in a significant improvement of disease severity, reflected by a decrease in objective SCORAD level (**A**) and decreased serum thymus and activation-regulated chemokine (TARC) levels (**B**). In addition, we found decreasing numbers of CD4<sup>+</sup>CD25<sup>hi</sup> (**C**) and CD4<sup>+</sup>FOXP3<sup>+</sup> T cells (**D**) (note that n=9; 2 samples were lost because of technical errors), and a decrease in FOXP3 mRNA expression in peripheral blood CD4<sup>+</sup> T cells (**E**).

there is a significant decrease of peripheral blood Treg cells. This decrease results in percentages of Treg cells comparable to healthy controls.

Our results are in agreement with Ou et al,<sup>5</sup> who have also shown increased percentages of CD25<sup>+</sup> T cells in patients with AD compared with healthy controls. In addition, we show that the percentages of CD4<sup>+</sup>FOXP3<sup>+</sup> cells are equally increased. Unfortunately, the number of lymphocytes obtained in this study was not sufficient to perform T-cell suppression assays to assess the suppressive capacity of the regulatory cells. We hypothesize that the increased numbers of Treg cells found in the peripheral blood of patients with AD is the result of a strong inflammatory signal. Although Treg cell numbers are increased, they do not

prevent chronic inflammation. This seems to be similar in rheumatoid arthritis, in which increased numbers of FOXP3<sup>+</sup> regulatory T cells were found in synovial fluid and peripheral blood.<sup>6</sup> Van Amelsfort et al<sup>7</sup> hypothesized that in rheumatoid arthritis, Treg cell suppressive activity was subverted by proinflammatory mediators.

However, we cannot yet reach a conclusion on the exact mechanism by which CsA is affecting CD4<sup>+</sup>CD25<sup>high</sup> and FOXP3<sup>+</sup> T cells in AD. This might be a direct effect of CsA on Treg cells, as was shown *in vitro*,<sup>2</sup> or indirect effects, as a result of decreased inflammation by CsA. Our results contrast with a recent report by Brandt et al<sup>8</sup> showing an increase in Treg cells during low-dose CsA treatment. Whether this

difference is exclusively the result of a lower CsA dose (2 mg/kg) used in the study of Brandt et al<sup>8</sup> needs to be investigated.

In conclusion, our study provides the first *in vivo* evidence that the increased numbers of CD4<sup>+</sup>CD25<sup>high</sup> T cells, as well as CD4<sup>+</sup>FOXP3<sup>+</sup> T cells, in patients with AD are reduced by treatment with CsA. Although this may have important implications for graft survival in patients undergoing transplantation procedures, the clinical relevance of this effect in patients with AD treated with CsA remains unclear.

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