

Defective suppression of Th2 cytokines by CD4⁺CD25⁺ regulatory T cells in birch allergics during birch pollen season

H. Grindebacke*, K. Wing*, A.-C. Andersson*, E. Suri-Payer†, S. Rak‡ and A. Rudin*

*Department of Rheumatology and Inflammation Research, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden, †Division of Immunogenetics, German Cancer Research Center, Heidelberg, Germany and ‡Department of Respiratory Medicine and Allergology, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden

Summary

Background CD4⁺CD25⁺ regulatory T cells suppress proliferation and cytokine production by human T cells both to self-antigens and exogenous antigens. Absence of these cells in human newborns leads to multiple autoimmune and inflammatory disorders together with elevated IgE levels. However, their role in human allergic disease is still unclear.

Objective This study aimed to evaluate the capacity of CD4⁺CD25⁺ regulatory T cells to suppress proliferation and cytokine production outside and during birch-pollen season in birch-allergic patients relative to non-allergic controls.

Methods CD4⁺ cells were obtained from blood of 13 birch-allergic patients and six non-allergic controls outside pollen season and from 10 birch-allergic patients and 10 non-allergic controls during birch-pollen season. CD25⁺ and CD25[−] fractions were purified with magnetic beads and cell fractions, alone or together in various ratios, were cultured with antigen-presenting cells and birch-pollen extract or anti-CD3 antibody. Proliferation and levels of IFN- γ , IL-13, IL-5 and IL-10 were measured by thymidin incorporation and ELISA, respectively. Numbers of CD25⁺ cells were analysed by flow cytometry.

Results CD4⁺CD25⁺ regulatory T cells from both allergics and non-allergics potently suppressed T cell proliferation to birch allergen both outside and during birch-pollen season. However, during season CD4⁺CD25⁺ regulatory T cells from allergic patients but not from non-allergic controls were defective in down-regulating birch pollen induced IL-13 and IL-5 production, while their capacity to suppress IFN- γ production was retained. In contrast, outside pollen season the regulatory cells of both allergics and non-allergic controls were able to inhibit T-helper 2 cytokine production.

Conclusion This is the first study to show differential suppression of Th1 and Th2 cytokines, with CD4⁺CD25⁺ regulatory T cells from birch-pollen-allergic patients being unable to down-regulate Th2, but not Th1 responses during birch-pollen season.

Keywords allergy, CD4⁺CD25⁺ regulatory T cells, cytokines, human, suppression

Submitted 26 November 2003; revised 05 April 2004; accepted 17 May 2004

Introduction

Depletion of CD4⁺CD25⁺ regulatory T cells (CD25⁺ Treg) leads to several different autoimmune and inflammatory conditions in mice [1, 2]. Scurfy mice with a mutation in the transcription factor *Foxp3*, which is a key gene for development of CD25⁺ Treg, develop a multitude of autoimmune disorders [3]. Children born with a dysfunctional *FOXP3* gene also succumb to several organ specific autoimmune diseases, but in addition they develop severe dermatitis, high levels of IgE and sometimes eosinophilia [4, 5]. Thus, CD25⁺ Treg cells are probably also necessary for down-regulating allergic immune reactions in humans. The immune response

in allergic patients is characterized by production of T-helper 2 (Th2) cytokines IL-4, IL-5 and IL-13 after stimulation with allergen, leading to secretion of allergen-specific IgE and recruitment of eosinophils [6]. It has been proposed that immune deviation towards Th1 may protect against allergic disease since IFN- γ inhibits the differentiation of Th2 cells. However, IFN- γ is often secreted in similar levels by T cells from allergen sensitized and as well as non-sensitized individuals in response to allergens [7]. Moreover, in mice Th1 cells cause potentiation of, rather than protection against, Th2-mediated lung inflammation [8]. Hence, other regulatory mechanisms than increased Th1 responses should be operative in suppressing both the development of allergic disease and the allergic inflammation, for example, CD25⁺ Treg.

In mice most CD4⁺CD25⁺ T cells are suppressive, whereas in humans only CD4⁺ cells with the highest expression of CD25 (CD25^{high}) have regulatory function [9]. Circulating

Correspondence: Dr Anna Rudin, Department of Rheumatology and Inflammation Research, The Sahlgrenska Academy at Göteborg University, Guldhedsgatan 10, 413 46 Göteborg, Sweden.

E-mail address: anna.rudin@microbio.gu.se

CD4⁺ T cells with intermediate expression of CD25 (CD25^{int}) are probably recently activated effector or memory cells. The CD25⁺ Treg need antigen-specific stimulation via the T cell receptor to become suppressive, but once activated they suppress antigen non-specifically [10]. The mechanism of suppression of T cell proliferation and cytokine production *in vitro* is cell-contact dependent and not mediated by IL-10 [11–15]. The role of transforming growth factor- β (TGF- β) in *in vitro* experiments is still controversial and needs to be further evaluated [11–17]. However, in animal models of inflammatory disease induced by bacteria, such as inflammatory bowel disease, IL-10 and TGF- β are clearly involved in the suppression [17, 18]. Recent *in vitro* studies of human cells show that the CD25⁺ Treg suppress T cell responses to autoantigens as well as exogenous antigens such as tetanus toxoid and food allergens [19–21]. Few studies have analysed the function of human CD25⁺ Treg after specific antigen stimulation during an ongoing immunological reaction such as in allergies naturally exposed to allergen. In addition, the vast majority of studies has examined the down-regulatory effects of CD25⁺ Treg on proliferation and only a few reports have analysed the suppression of cytokines [9, 22–24]. In this report we compared the suppressive effect of CD25⁺ Treg from birch-pollen allergies and healthy controls outside and during birch-pollen season on birch allergen-induced T cell proliferative and cytokine responses.

Methods

Subjects

This study included 23 birch-allergic patients and 12 healthy controls aged 19–50 years. All patients had a history of birch-pollen-induced moderate or severe rhinoconjunctivitis with duration of at least 4 years. Each patient also had a positive skin prick test (SPT) result to *Betula verrucosa* with a median diameter of 6.0 mm (range: 2.8–11 mm) (Soluprick SQ 10 HEP *B. verrucosa*, ALK-Abelló, Hørsholm, Denmark) and was positive for specific IgE to *B. verrucosa* with a median RAST value of 13.8 kU/mL (range: 0.7–100 kU/mL) (RAST class ≥ 2). None of the healthy controls had symptoms, positive SPT or specific IgE to *B. verrucosa*. Peripheral blood (150 mL) was obtained by venous puncture and collected into preservative-free heparin. Blood samples were collected out of season or during birch-pollen season 2003. The study was approved by the Human Research Ethics Committee of the Medical Faculty, Göteborg University.

Isolation of cells and flow cytometry

Lymphocytes were separated by LymphoprepTM (Nycomed, Oslo, Norway) gradient centrifugation (20 min, 850 $\times g$, room temperature). CD4⁺ cells were purified with Dynabead[®] CD4 Positive Isolation Kit (DynaL Biotech ASA, Oslo, Norway). CD4⁺ cells were incubated with anti-CD25 beads (3 μ L per 10⁷ cells), and CD25[−] and CD25⁺ fractions were recovered using an LS-magnetic column according to manufacturer's instructions (Milteny Biotech, Bergisch Gladbach, Germany). Peripheral blood mononuclear cells were depleted of CD3⁺ cells (Dynabead[®]), γ -irradiated (25 Gy) and used as antigen presenting cells (APC). Purity

of cell populations was determined by flow cytometry using the following monoclonal antibodies (mAb) obtained from Becton Dickinson (Erembodegem, Belgium): PerCP-anti-CD4 (SK3), APC-anti-CD3 (SK7) and PerCP, APC or PE-isotype control IgG1, and PE-anti-CD25 (clone M-A251) obtained from Pharmingen (San Diego, CA, USA). The purity of the CD4⁺ cells was >94% and the purity of the CD25^{high} cells was typically >85%. The percentage of CD25^{high} cells was determined according to a typical Treg phenotype based on the simultaneous expression of CD122 and intracellular CD152 [25].

Cell culture

The suppressive capacity was determined by culturing 50 $\times 10^3$ CD4⁺CD25[−] T cells with 5, 10 or 50 $\times 10^3$ CD4⁺CD25⁺ cells and 200 $\times 10^3$ APC for 6 days in round-bottomed 96-well plates in X-Vivo 15 medium (Bio Whittaker, Walkersville, MD, USA). Cultures were stimulated with 50 μ g/mL birch-pollen extract (Greer Laboratories, Lenoir, NC, USA). In addition, 10 $\times 10^3$ CD4⁺CD25[−] responder cells were cultured with 1, 5, or 10 $\times 10^3$ CD4⁺CD25⁺ cells in the presence of 100 $\times 10^3$ APC in flat-bottomed 96-well plates and stimulated with 1 μ g/mL anti-CD3 mAb (OKT-3, Ortho-McNeil Pharmaceutical, Raritan, NJ, USA) for 4 days. At the end of the culture periods cells were pulsed overnight with 1 μ Ci ³H-thymidine (Amersham Pharmacia Biotech, Little Chalfont, UK).

Elisa

Supernatants were collected at the last day of culture and stored at -20°C until analysis. Because of constraints in CD25⁺ Treg numbers, not all cytokines could be tested in every individual. A standard ELISA protocol was performed as described elsewhere [26]. All antibodies and standards were purchased from BD Pharmingen. Costar plates (Invitrogen, San Diego, CA, USA) were coated with the following anti-human capture mAb: IL-5 (TRFK5), IL-13 (JES10-5A2), IFN- γ (NIB42) and IL-10 (JES3-9D7). Standard curves were generated using recombinant human IL-5, IL-13, IFN- γ and IL-10. The following biotinylated mAb were used: IL-5 (JES1-5A10), IL-13 (B69-2), IFN- γ (4S.B3) and IL-10 (JES3-12G8). Samples, standards, biotinylated antibodies and streptavidine-HRP were diluted in high-performance ELISA dilution buffer (CLB, Amsterdam, the Netherlands).

Statistics

Data were analysed by two-tailed Mann–Whitney *U*-test using GraphPad Prism 3.00 (GraphPad, San Diego, CA, USA). *P*-values ≤ 0.05 were regarded significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Results

Allergic patients and non-allergic controls have similar numbers of CD4⁺CD25^{high} T cells, which suppress birch allergen-induced proliferation equally well

To determine if the CD25⁺ Treg from birch allergies are functional, we collected peripheral blood from 13

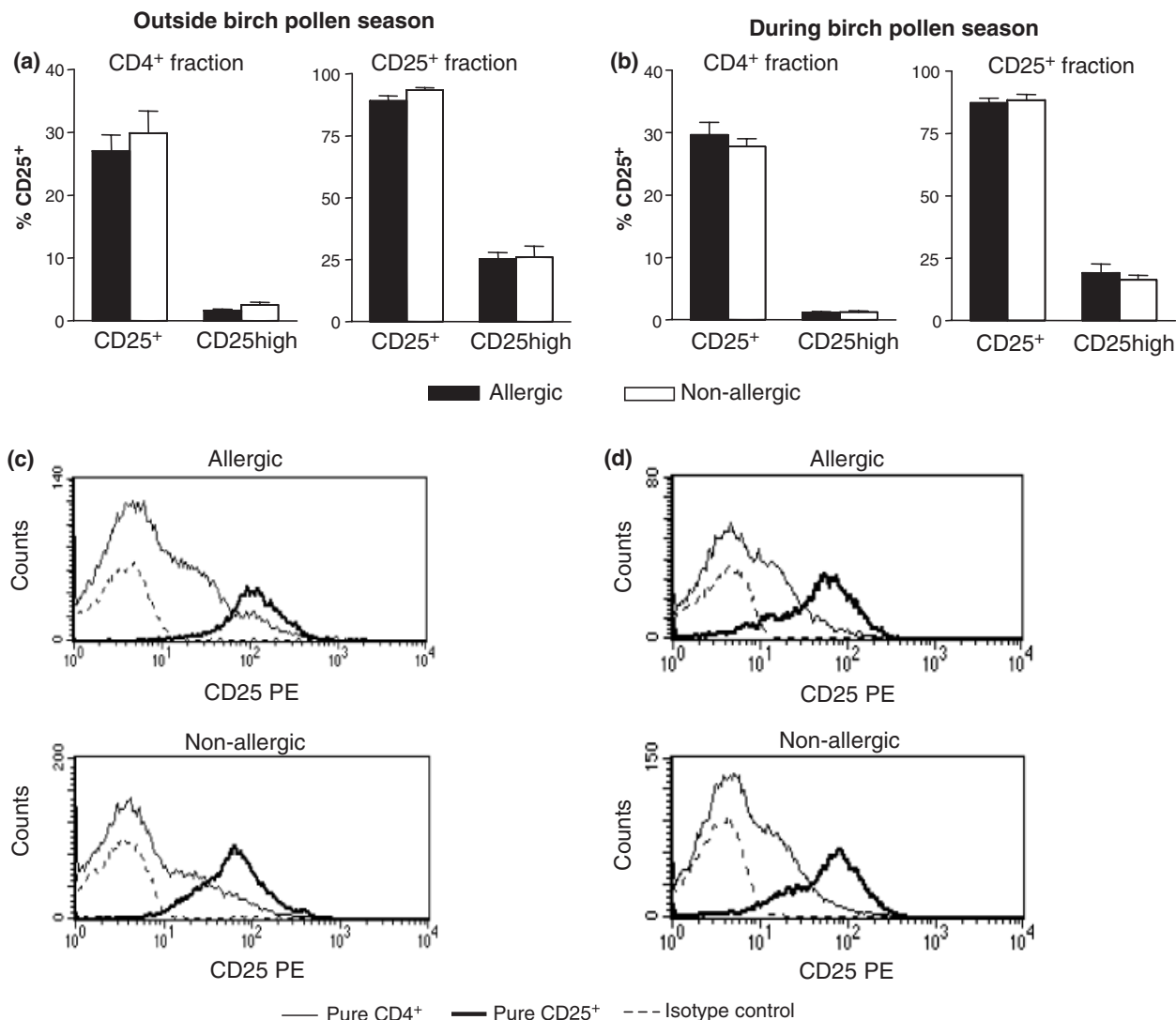


Fig. 1. Flow cytometric analysis of CD25 expression in birch allergics and non-allergics out of and during birch-pollen season. (a), Outside season and (b), during season: percentage of CD25⁺ cells and CD25^{high} cells in allergics and non-allergics after purification of CD4⁺ and CD25⁺ T cells, respectively. Bars depict mean percentage $\pm$ SEM. One representative histogram is shown for allergics and non-allergics, respectively, outside season (c) and during season (d).

birch-pollen-allergic patients and six non-allergic controls out of birch-pollen season and from 10 birch-pollen-allergic patients and 10 non-allergic controls during birch-pollen season. First, we examined if the allergics and non-allergics differed in CD25⁺ cell numbers in blood out of season or during season. Figure 1 shows percentages of CD25⁺ and CD25^{high} cells out of and during season in allergics and non-allergics after purification of CD4⁺ T cells and CD25⁺ T cells, respectively. In the CD4⁺ fraction allergics and non-allergics had similar numbers of both CD25⁺ cells and CD25^{high} cells. Also after purification of the CD25⁺ cells, the numbers remained similar between the groups. In addition, there was no difference in mean fluorescence intensity of CD25 in the two groups.

We then tested purified CD4⁺CD25⁺ T cells for their ability to inhibit the proliferation of CD4⁺CD25⁻ T cells stimulated with birch allergen. CD4⁺CD25⁻ T cells from allergic patients and non-allergic controls proliferated equally well in response to birch allergen or anti-CD3 both outside

and during season, while CD25⁺ cells showed an impaired proliferative response to both stimuli. In Fig. 2a (out of season) and b (during season), we show the decrease in absolute proliferation values in the CD25⁺:CD25⁻ coculture compared with the CD25⁻ responder cells alone for each individual after stimulation with birch allergen or anti-CD3. To be able to compare the suppressive capacity of CD25⁺ cells between groups, we calculated the proliferation in coculture wells relative to the proliferation of CD25⁻ T cells (set to 100%) for each individual and then calculated the mean percent proliferation in each group. We found that CD25⁺ cells from both allergics and non-allergics significantly suppressed the proliferation of CD25⁻ cells activated with birch allergen both out of and during season (Figs 2c and d). Similar results were obtained in parallel cultures with anti-CD3 (Figs 2e and f). Of note, the percentage of CD25^{high} cells was approximately 25% of the CD25⁺ cells (Figs 1a and b). This means that the number of CD25^{high} cells compared with CD25⁻ responder cells is four times lower than depicted

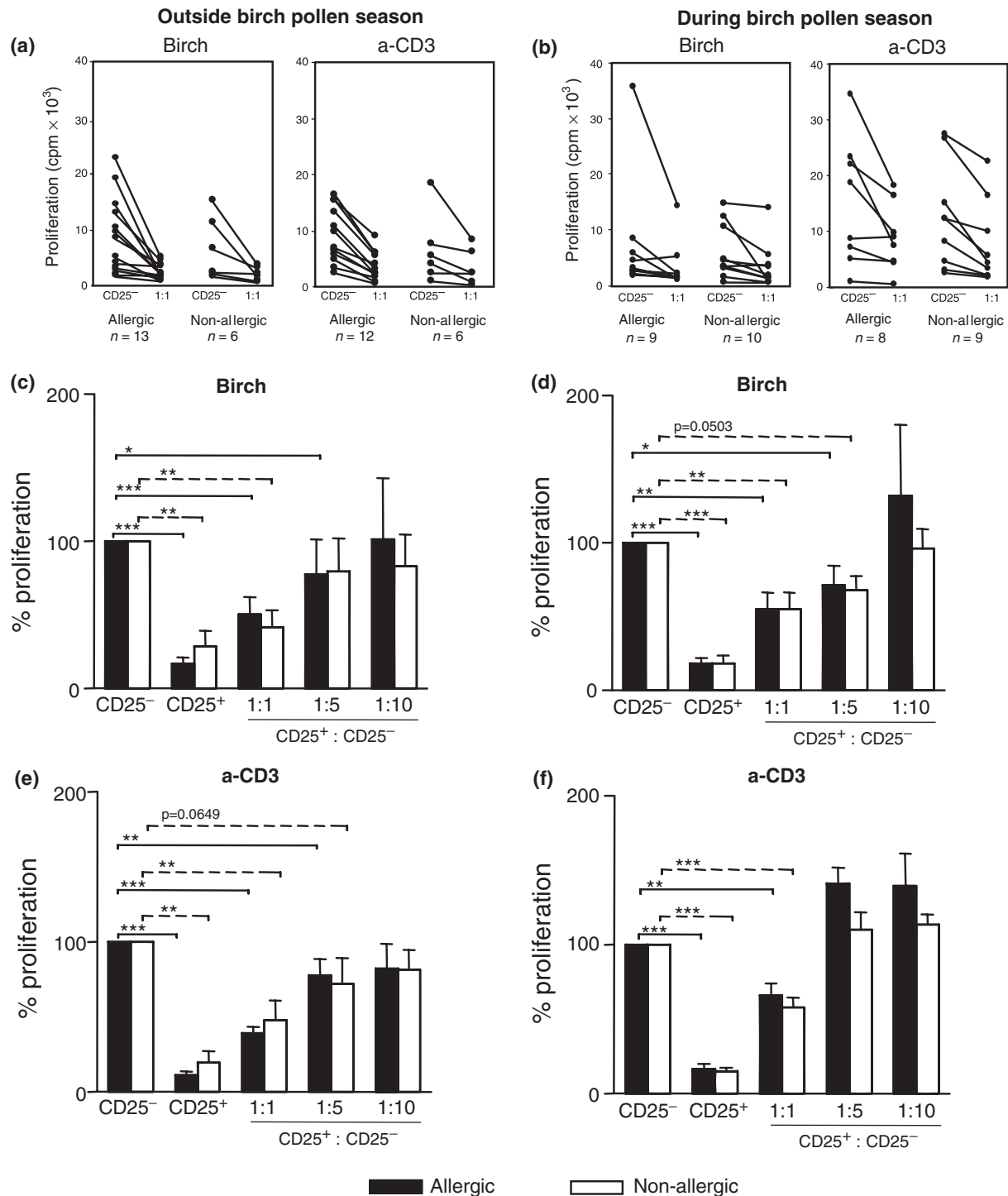


Fig. 2. CD4⁺CD25⁺ T cells suppress proliferation of CD4⁺CD25⁻ T cells in birch allergics and non-allergics both outside and during birch pollen season. (a), Outside season and (b), during season: absolute proliferation counts in birch allergic and non-allergic individuals after stimulation with birch allergen or anti-CD3 monoclonal antibodies. CD25⁻ responder cells alone and together with CD25⁺ cells in a 1 : 1 ratio are shown after stimulation with birch allergen or anti-CD3. (c), Outside season and (d), during season: proliferation of T cells after stimulation with birch allergen for 6 days. (e), Outside season and (f), during season: proliferation of T cells after stimulation with anti-CD3 for 4 days. Results in (c–f) are shown as percentage of proliferation relative to the level for CD25⁻ cells for each individual. Bars depict mean percentage proliferation ± SEM.

by the cell numbers added in each coculture, which is important to consider when comparing suppressive abilities of regulatory cells in other studies (most of which also use bead purified CD25⁺ cells). Furthermore, the smaller percentage of CD25^{high} cells also explains why proliferation

or cytokine production is often higher in the 1 : 10 coculture than in the culture with CD25⁻ cells alone. Once CD25^{high} cell numbers fall below a threshold level, suppression is abrogated and in this circumstance the CD25^{int} cells, which have been cotransferred in the CD25⁺ cell fraction, add to the

proliferation and cytokine production of the CD25⁻ cells. To conclude, there was no difference either in the numbers of CD25^{high} cells or in their capacity to inhibit allergen-induced proliferation in birch-pollen-allergic patients relative to healthy controls out of season or during season.

Birch allergen-induced interferon- γ production is potently suppressed by CD4⁺CD25⁺ T cells from allergic patients both during and out of birch-pollen season

We measured the levels of IFN- γ in the cell culture supernatants and found that CD25⁻ T cells from both allergic patients and non-allergic controls produced similar levels of IFN- γ in response to birch allergen or anti-CD3 both out of and during season (Figs 3a and b). When we compared the relative decrease in IFN- γ production mediated by the CD25⁺ Treg cells, we observed that CD25⁺ Treg cells from allergic patients significantly suppressed the IFN- γ production both outside and during season (Figs 3c and d). Similar results were observed when stimulating with anti-CD3 (Figs 3e and f). IL-10 was only detected in less than half of the individuals and differences were seen neither between the allergics and healthy controls nor between CD25⁺ and CD25⁻ T cells.

CD4⁺CD25⁺ T cells from birch-allergic patients do not suppress interleukin-13 or interleukin-5 production after birch allergen stimulation during birch-pollen season

We next investigated whether the CD25⁺ Treg could also suppress production of the Th2 cytokines IL-13 and IL-5. IL-13 was produced by CD25⁻ cells from allergic patients as well as non-allergic controls after stimulation with birch allergen both out of and during season (Figs 4a and b). Out of season the CD25⁺ Treg from both allergics and non-allergics significantly suppressed IL-13 production in cultures stimulated with birch allergen (Figs 4a and c). In contrast, the CD25⁺ Treg cells from the allergic group did not suppress the IL-13 production during season (Figs 4b and d). This difference in regulatory properties was only found after antigen-specific stimulation since cultures stimulated with anti-CD3 showed significant suppression of IL-13 in allergics during as well as out of season (Figs 4e and f). Figure 4d may give the impression that CD25⁺ Treg from allergic patients during season produce IL-13. However, the CD25⁺ fraction is contaminated by approximately 75% CD25^{int} cells that are more likely to be recently activated by natural exposure to birch allergen than the CD25-negative cells and therefore secrete higher levels of IL-13 cytokines in the allergics during season. Another reason to believe that CD25⁺ Treg by themselves do not produce IL-13 is that human CD25^{high} cells purified by flow cytometry have not been able to produce any IL-13 even after strong polyclonal stimulation [9]. When we studied the IL-5 production in the cultures, we found that only allergic patients produced IL-5 after birch stimulation (Figs 5a and b). Outside season IL-5 was suppressed by CD25⁺ Treg (Fig. 5c), whereas during season there was no suppression of IL-5 (Fig. 5d). However, when stimulating with anti-CD3, CD25⁺ Treg were able to suppress the IL-5 production (Figs 5e and f). In summary, IL-13 and IL-5 production was not suppressed in allergic patients as opposed

to healthy controls when cells were stimulated with the allergen during season. However, inhibition of Th2 responses was found out of season in both allergics and healthy controls.

Discussion

In this paper, we demonstrate that CD25⁺ Treg from allergic patients do not suppress Th2 cytokine production while retaining their inhibition of IFN- γ production when naturally exposed to allergens during birch-pollen season. Thus, cytokines can be down-regulated independent of each other. On the other hand, CD25⁺ Treg from healthy controls suppress both Th2 and Th1 cytokine production irrespective of natural allergen exposure. We also found that numbers of CD25⁺ Treg in blood, as well as their potency of suppressing allergen-specific proliferative responses, were similar in allergic patients and healthy controls outside or during season.

An early report showed that patients with diabetes type 1 had lower total numbers of CD25⁺ cells in peripheral blood compared with healthy controls and patients with diabetes type 2 (CD25^{high} cells were not enumerated) [27]. In more recent studies of patients with rheumatoid arthritis and of patients with allergic rhinoconjunctivitis, no difference in numbers of CD25⁺ Treg was found in comparison with healthy controls, which is in line with the results of the present study [24, 28]. However, these findings do not exclude that differences in numbers of cells could still be seen in the target organs. There are few studies comparing the effect of CD25⁺ Treg in allergic patients and healthy controls. One of these shows that CD25⁺ Treg were able to suppress proliferation equally well in cow milk-allergic patients and in healthy controls after both antigen-specific and polyclonal activation [20]. Another study reported the function of CD25⁺ Treg in pollen-allergic patients [24]. In accordance with our results, they showed that CD25⁺ Treg from the majority of the grass or birch-allergic patients as well as the healthy controls suppressed allergen-induced proliferation. In contrast, a recent report indicated that CD25⁺ Treg cells from nickel-allergic patients were deficient in suppressing nickel-specific T cell proliferation relative to CD25⁺ Treg from healthy individuals [15]. However, since the immunologic mechanisms leading to pollen allergy and nickel allergy are very different, it is not surprising that results from these two patient groups are discordant.

Interestingly, while CD25⁺ Treg from allergic patients and healthy controls down-regulated proliferation and Th1 cytokine production equally well, CD25⁺ Treg from birch-pollen-allergic patients were deficient in suppressing Th2 responses to birch-pollen allergen during season. This lack of suppression was not seen outside season when CD25⁺ Treg from both allergics and healthy controls suppressed Th2 cytokine responses. Hitherto, levels of allergen-induced cytokine production after coculture of human CD25⁻ responder cells with CD25⁺ Treg have only been shown by Bellinghausen et al. [24]. They concluded that CD25⁺ Treg were able to suppress both Th1 and Th2 cytokine production in pollen-allergic patients with rhinoconjunctivitis. However,

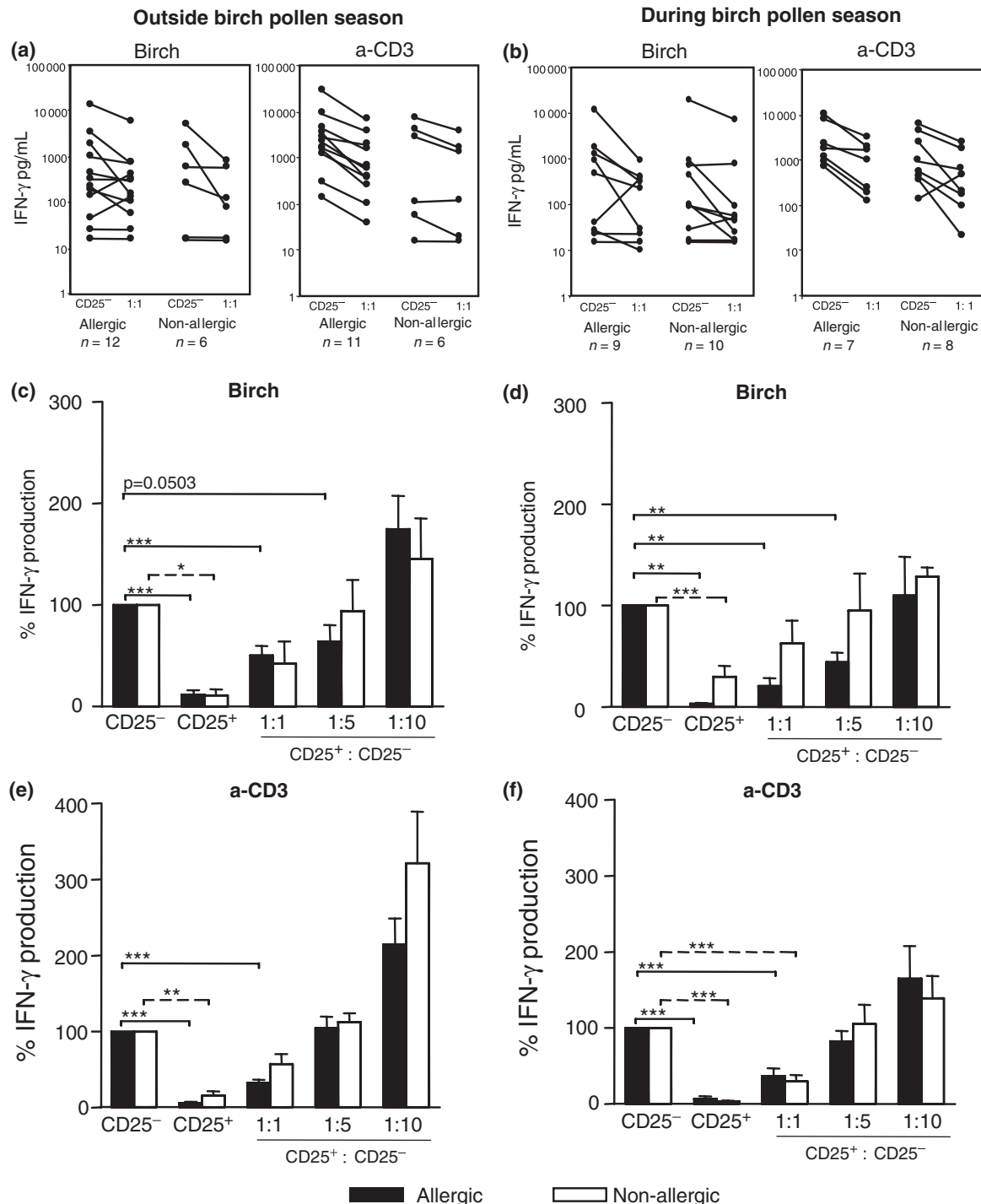


Fig. 3. CD4⁺CD25⁺ T cells from birch-allergic patients inhibit birch allergen and anti-CD3 induced IFN- γ production from CD4⁺CD25⁻ T cells outside and during birch pollen season. (a), Outside season and (b), during season: absolute levels of IFN- γ production in birch-allergic and non-allergic individuals after stimulation with birch allergen or anti-CD3 monoclonal antibodies. CD25⁻ responder cells alone and together with CD25⁺ cells in a 1 : 1 ratio are shown after stimulation with birch allergen or anti-CD3. (c), Outside season and (d), during season: percentage of IFN- γ production for each group after stimulation with birch allergen. (e), Outside season and (f), during season: percentage of IFN- γ production for each group after stimulation with anti-CD3. Results in (c-f) are shown as percentage of IFN- γ production relative to the production from CD25⁻ cells for each individual. Bars depict mean percentage IFN- γ production $\pm$ SEM.

in a subgroup of their patients with T cells producing more IL-4, the secretion of IL-5 in response to pollen allergen was clearly not suppressed by CD25⁺ Treg. This finding is similar to the results obtained from the cells of the majority of our allergic patients sampled during pollen season. Thus, when

the allergic patients are stimulated with allergen *in vivo* during season, IL-4 is produced in higher levels and CD25⁺ Treg fail to suppress Th2 cytokine production. Interestingly, Cosmi et al. [29] recently showed that addition of IL-4 or IL-9 into the costimulation culture reduced the suppressive capacity of

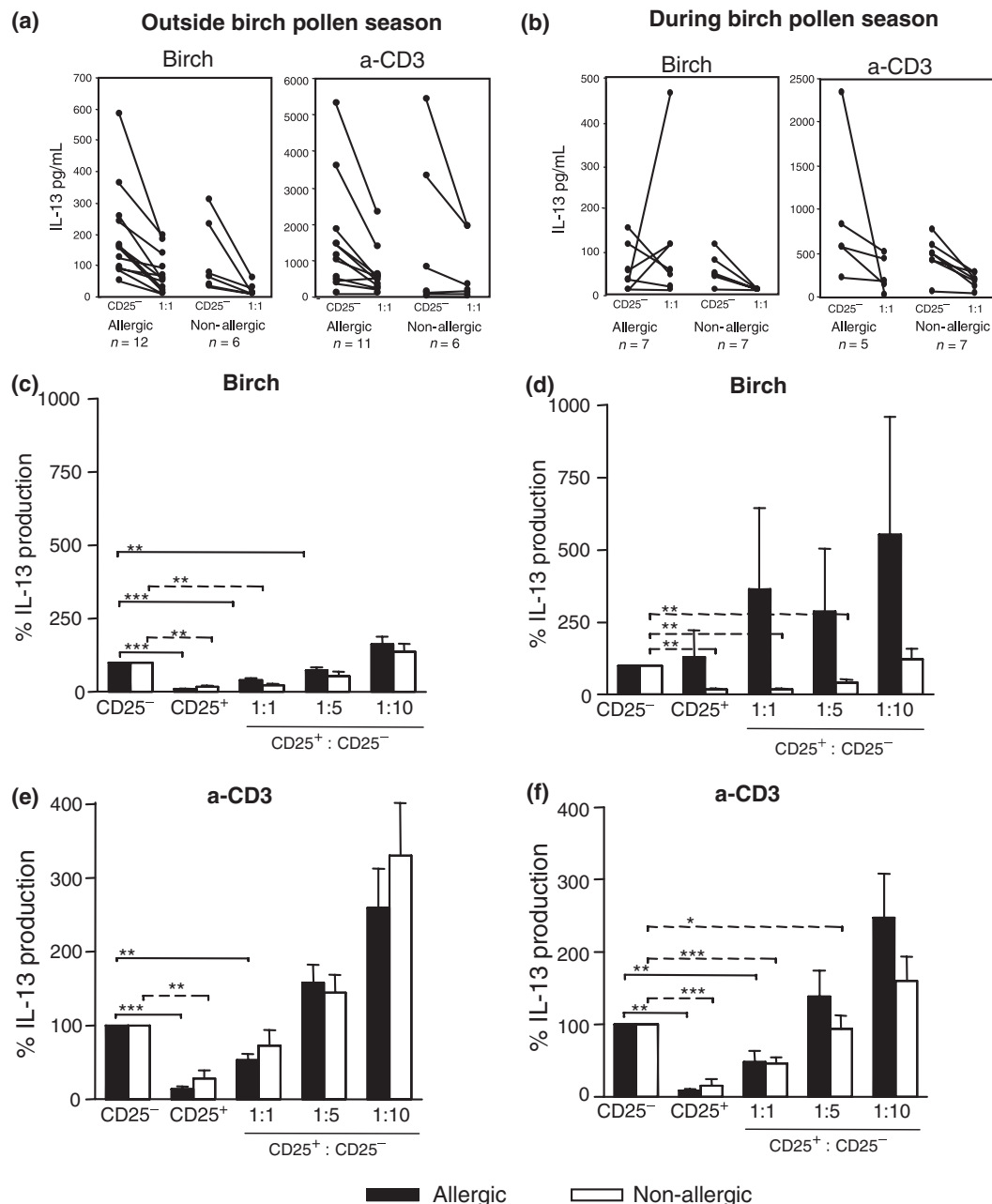


Fig. 4. Capacity of $CD4^+CD25^+$ T cells to suppress birch allergen or anti-CD3-induced IL-13 production from $CD4^+CD25^-$ T cells in birch allergics and non-allergics outside and during birch-pollen season. (a), Outside season and (b), during season: absolute levels of IL-13 production in birch allergic and non-allergic individuals after stimulation with birch allergen or anti-CD3 monoclonal antibodies. $CD25^-$ responder cells alone and together with $CD25^+$ cells in a 1 : 1 ratio are shown after stimulation with birch allergen or anti-CD3. (c), Outside season and (d), during season: percentage of IL-13 production for each group after stimulation with birch allergen. (e), Outside season and (f), during season: percentage of IL-13 production for each group after stimulation with anti-CD3. Percentage of IL-13 production in (c–f) is calculated in relation to IL-13 production from $CD25^-$ cells for each individual. Bars depict mean percentage cytokine production $\pm$ SEM.

human thymocyte-derived $CD25^+$ Treg cell lines on Th2 cell lines. They also showed that Th2 clones were harder to suppress than Th1 clones by $CD25^+$ Treg. Moreover, Jonuleit et al. [13] showed that IL-4 could reverse the anergic state of human beads-sorted $CD25^+$ Treg cells. An attractive explanation to our results might, therefore, be that IL-4 and IL-9 produced after natural allergen exposure inhibit the capacity of $CD25^+$ Treg cells to suppress IL-13 and IL-5 production.

Studies on the suppressive effects of $CD25^+$ Treg *in vitro* have shown that the suppression of $CD25^-$ T cells by $CD25^+$ Treg is cell-contact dependent and independent of IL-10 or TGF- β [13, 14, 30–33]. This was also recently shown when studying suppression against nickel allergy in human subjects [15]. Also, clones of suppressive human $CD25^+$ Treg did not produce any IL-10 and not more TGF- β than the non-suppressive T cells [34]. In accordance with this, we could only detect very low levels of IL-10 in our cultures with no

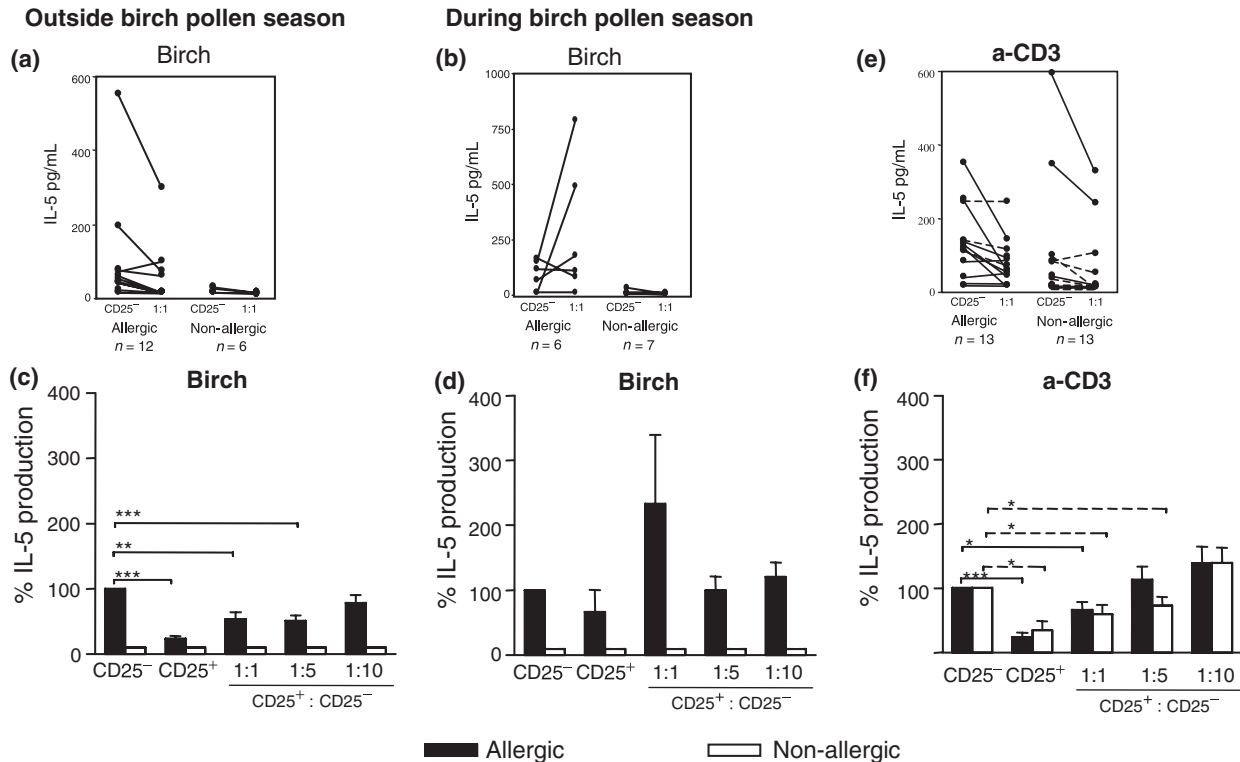


Fig. 5. Capacity of CD4⁺CD25⁺ T cells to suppress birch allergen-induced IL-5 production from CD4⁺CD25⁺ T cells in birch allergics and non-allergics outside and during birch-pollen season. (a), Outside season and (b), during season: absolute levels of IL-5 production in birch allergics and non-allergics after stimulation with birch allergen. CD25⁺ responder cells alone and together with CD25⁺ cells in a 1 : 1 ratio are shown after stimulation with birch allergen. (c), Outside season) and (d), during season: percentage of IL-5 production after stimulation with birch allergen. (e), Absolute levels of IL-5 in birch allergics and non-allergics outside (filled lines) and during season (dashed lines). (f), Percentage of IL-5 production after stimulation with anti-CD3 outside and during season combined. Percentage of IL-5 production in (c, d and f) is calculated in relation to IL-5 production from CD25⁺ cells for each individual. Bars depict mean percentage cytokine levels $\pm$ SEM. IL-5 levels of non-allergics after birch allergen stimulation were all below detection level [shown by white bars in (c) and (d)].

relation to the allergic status of the individuals (data not shown). We therefore regard it unlikely that IL-10 plays an inhibitory role in our *in vitro* suppression assays. However, future studies in which IL-10 is blocked will be needed to verify this notion. This is important since production of IL-10 has been suggested to play a role in the inhibition of T cell activity after systemic immunotherapy [35]. Thus, further work should address whether IL-10-producing Tr1 cells are induced or if CD25⁺ Treg are activated by this treatment [35, 36]

In conclusion, this paper does not support a general deficiency in functional CD25⁺ Treg in adult allergic patients compared with healthy controls. However, during season the CD25⁺ Treg are deficient in suppressing Th2 cytokine production. This might be explained by the fact that increased levels of the Th2 growth factor IL-4 inhibit the capacity of CD25⁺ Treg to suppress Th2 clones in the same fashion as the Th1 growth factor IL-2 inhibit suppression of Th1 cells. Blocking IL-4 might therefore restore the suppressive capacity of the CD25⁺ Treg on IL-5 and IL-13 production in allergic patients during season. Indeed, preclinical anti-IL-4 treatment was shown to inhibit IL-5 production and Th2 cell activation [37].

Acknowledgements

We are grateful to all volunteers for donating blood to the study and for the skilful assistance of study nurse Yvonne

Bäckberg. We also thank Esbjörn Telemo for critically reading the manuscript. This work was supported by The Swedish Research Council (grant K2002-745X-14455-01A), The Medical Faculty, Göteborg University, The Vårdal Foundation, and The Swedish Asthma and Allergy Association Research Foundation.

References

- Shevach EM. CD4⁺CD25⁺ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002; 2:389–400.
- Suri-Payer E, Amar AZ, Thornton AM, Shevach EM. CD4⁺CD25⁺ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J Immunol* 1998; 160:1212–8.
- Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor *Foxp3*. *Science* 2003; 299:1057–61.
- Baud O, Goulet O, Canioni D et al. Treatment of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) by allogeneic bone marrow transplantation. *N Engl J Med* 2001; 344:1758–62.
- Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of *FOXP3*, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 2003; 15:430–5.
- Akbari O, Stock P, DeKruyff RH, Umetsu DT. Role of regulatory T cells in allergy and asthma. *Curr Opin Immunol* 2003; 15:627–33.

- 7 Epton MJ, Hales BJ, Thompson PJ, Thomas WR. T cell cytokine responses to outer membrane proteins of *Haemophilus influenzae* and the house dust mite allergens Der p 1 in allergic and non-allergic subjects. *Clin Exp Allergy* 2002; 32:1589–95.
- 8 Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyper-reactivity but cause severe airway inflammation. *J Clin Invest* 1999; 103:175–83.
- 9 Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4⁺CD25^{high} regulatory cells in human peripheral blood. *J Immunol* 2001; 167:1245–53.
- 10 Thornton AM, Shevach EM. Suppressor effector function of CD4⁺CD25⁺ immunoregulatory T cells is antigen nonspecific. *J Immunol* 2000; 164:183–90.
- 11 Thornton AM, Shevach EM. CD4⁺CD25⁺ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J Exp Med* 1998; 188:287–96.
- 12 Takahashi T, Kuniyasu Y, Toda M et al. Immunologic self-tolerance maintained by CD25⁺CD4⁺ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int Immunol* 1998; 10:1969–80.
- 13 Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, Enk AH. Identification and functional characterization of human CD4⁺CD25⁺ T cells with regulatory properties isolated from peripheral blood. *J Exp Med* 2001; 193:1285–94.
- 14 Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G. Ex vivo isolation and characterization of CD4⁺CD25⁺ T cells with regulatory properties from human blood. *J Exp Med* 2001; 193:1303–10.
- 15 Cavani A, Nasorri F, Ottaviani C, Sebastiani S, De Pita O, Girolomoni G. Human CD25⁺ regulatory T cells maintain immune tolerance to nickel in healthy, nonallergic individuals. *J Immunol* 2003; 171:5760–8.
- 16 Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4⁺CD25⁺ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med* 2001; 194:629–44.
- 17 Nakamura K, Kitani A, Fuss I et al. TGF-β1 plays an important role in the mechanism of CD4⁺CD25⁺ regulatory T cell activity in both humans and mice. *J Immunol* 2004; 172:834–42.
- 18 Maloy KJ, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F. CD4⁺CD25⁺ T_R cells suppress innate immune pathology through cytokine-dependent mechanisms. *J Exp Med* 2003; 197:111–9.
- 19 Taams LS, Vukmanovic-Stejić M, Smith J et al. Antigen-specific T cell suppression by human CD4⁺CD25⁺ regulatory T cells. *Eur J Immunol* 2002; 32:1621–30.
- 20 Tiemessen MM, Van Hoffen E, Knulst AC, Van Der Zee JA, Knol EF, Taams LS. CD4⁺CD25⁺ regulatory T cells are not functionally impaired in adult patients with IgE-mediated cow's milk allergy. *J Allergy Clin Immunol* 2002; 110:934–6.
- 21 Wing K, Lindgren S, Kollberg G et al. CD4 T cell activation by myelin oligodendrocyte glycoprotein is suppressed by adult but not cord blood CD25⁺ T cells. *Eur J Immunol* 2003; 33:579–87.
- 22 Xu D, Liu H, Komai-Koma M et al. CD4⁺CD25⁺ regulatory T cells suppress differentiation and functions of Th1 and Th2 cells, *Leishmania major* infection, and colitis in mice. *J Immunol* 2003; 170:394–9.
- 23 George TC, Bilsborough J, Viney JL, Norment AM. High antigen dose and activated dendritic cells enable Th cells to escape regulatory T cell-mediated suppression *in vitro*. *Eur J Immunol* 2003; 33:502–11.
- 24 Bellinghausen I, Klostermann B, Knop J, Saloga J. Human CD4⁺CD25⁺ T cells derived from the majority of atopic donors are able to suppress T_H1 and T_H2 cytokine production. *J Allergy Clin Immunol* 2003; 111:862–8.
- 25 Wing K, Ekmark A, Karlsson H, Rudin A, Suri-Payer E. Characterization of human CD25⁺CD4⁺ T cells in thymus, cord and adult blood. *Immunology* 2002; 106:190–9.
- 26 Karlsson H, Hessle C, Rudin A. Innate immune responses of human neonatal cells to bacteria from the normal gastrointestinal flora. *Infect Immun* 2002; 70:6688–96.
- 27 Kukreja A, Cost G, Marker J et al. Multiple immuno-regulatory defects in type-1 diabetes. *J Clin Invest* 2002; 109:131–40.
- 28 Cao D, Malmstrom V, Baecher-Allan C, Hafler D, Klareskog L, Trollmo C. Isolation and functional characterization of regulatory CD25^{bright}CD4⁺ T cells from the target organ of patients with rheumatoid arthritis. *Eur J Immunol* 2003; 33:215–23.
- 29 Cosmi L, Liotta F, Angeli R et al. Th2 cells are less susceptible than Th1 cells to the suppressive activity of CD25⁺ regulatory thymocytes because of their responsiveness to different cytokines. *Blood* 2004; 103:3117–21.
- 30 Levings MK, Sangregorio R, Roncarolo MG. Human CD25⁺CD4⁺ regulatory cells suppress naive and memory T cell proliferation and can be expanded *in vitro* without loss of function. *J Exp Med* 2001; 193:1295–302.
- 31 Ng WF, Duggan PJ, Ponchel F et al. Human CD4⁺CD25⁺ cells: a naturally occurring population of regulatory T cells. *Blood* 2001; 98:2736–44.
- 32 Taams LS, Smith J, Rustin MH, Salmon M, Poulter LW, Akbar AN. Human anergic/suppressive CD4⁺CD25⁺ T cells: a highly differentiated and apoptosis-prone population. *Eur J Immunol* 2001; 31:1122–31.
- 33 Walker M, Kaspirowicz DJ, Gersuk VH et al. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4⁺CD25⁺ T cells. *J. Clin. Invest* 2003; 112:1437–43.
- 34 Levings MK, Sangregorio R, Sartirana C et al. Human CD25⁺CD4⁺ T suppressor cell clones produce transforming growth factor β, but not interleukin 10, and are distinct from type 1 T regulatory cells. *J Exp Med* 2002; 196:1335–46.
- 35 Francis JN, Till SJ, Durham SR. Induction of IL-10⁺CD4⁺CD25⁺ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; 111:1255–61.
- 36 Jutel M, Akdis M, Budak F et al. IL-10 and TGF-β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; 33:1205–14.
- 37 Hart TK, Blackburn MN, Brigham-Burke M et al. Preclinical efficacy and safety of pascolizumab (SB 240683): a humanized anti-interleukin-4 antibody with therapeutic potential in asthma. *Clin Exp Immunol* 2002; 130:93–100.