

ORIGINAL ARTICLE

Experimentally induced accumulation of Foxp3⁺ T cells in upper airway allergyI. Skrindo^{1,2}, C. Scheel¹, F.-E. Johansen¹ and F. L. Jahnsen¹¹Department of Pathology, Centre for Immune Regulation, Oslo University Hospital (Rikshospitalet), University of Oslo, Oslo, Norway and ²Department of Otorhinolaryngology – Head and Neck Surgery, Division of Surgery and Neurosciences, Oslo University Hospital (Rikshospitalet), Oslo, Norway

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Summary

Background It has been suggested that Foxp3⁺ regulatory T (Treg) cells inhibit allergic inflammation in humans by suppressing the activation of allergen-specific effector T cells. Whether this occurs at the site of allergen exposure has not been determined.**Objective** To determine the occurrence of Foxp3⁺ Treg cells in the nasal mucosa of allergic rhinitis (AR) patients and non-allergic controls after a nasal allergen challenge.**Methods** Pollen-allergic patients ($n = 18$) and non-allergic volunteers ($n = 7$) were challenged locally with pollen extract or placebo for 7 days outside the pollen season. Mucosal biopsies were obtained from the inferior turbinate on days 0, 1 and 7 and subjected to multi-colour immunofluorescence and blood was drawn for eosinophil counts on days 0, 2, 5 and 7.**Results** Only AR patients receiving pollen extract experienced typical allergic symptoms and demonstrated increased levels of eosinophils in peripheral blood and nasal mucosa. In allergic patients, a transient early increase (day 1) in CD3⁺ T cells was observed in the nasal mucosa, followed by a significant increase of Foxp3^{high} T cells at day 7. No changes were found in the control group. The majority of Foxp3^{high} cells co-expressed CTLA-4, CD25 and CD4, and a substantial fraction expressed the proliferation marker Ki67.**Conclusion and Clinical Relevance** Experimentally induced inflammation in AR patients leads to an early inflammatory response followed by accumulation of Foxp3^{high} T cells in the nasal mucosa. Our findings are similar to that observed in allergic airways of experimental mice, which suggest that Treg cells are operative in allergic upper airway inflammation. It should be explored whether Treg cells accumulating in the nasal mucosa could be targets for therapeutic intervention.**Keywords** allergic rhinitis, allergy, Foxp3, human, regulatory T cells, T cells

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Introduction

Regulatory T (Treg) cells are thought to control T cell responses to both self- and non-self-antigens. Earlier studies showed that Treg cells were generated in the thymus. However, more recently it has become evident that Treg cells are also generated outside the thymus in a variety of situations. Both thymus-derived Treg cells [naturally occurring Treg cells (nTreg cells)] and Treg cells that develop in the periphery [inducible or adaptive Treg cells (iTreg cells)] express Foxp3; a transcription factor that is believed to be important for their differentiation and suppressive functions [1–4].

The importance of Foxp3⁺ Treg cells has been documented through the identification of the human X-linked inherited disease designated IPEX (immunodysregulation,

polyendocrinopathy, enteropathy, X-linked) syndrome [5]. IPEX is caused by a loss-of-function mutation in the Foxp3 gene. This leads to autoimmune diseases in several organ systems (e.g. type-1 diabetes, enteropathy and hypothyroidism) and in addition many of these patients suffer from severe atopic diseases [5–8]. Whether development of atopy is caused by lack of nTreg or iTreg cells has not been determined.

In addition, several studies have shown that other T cell populations with suppressive capacity exist which are often Foxp3 negative. These include IL-10-producing T cells – often referred to as T regulatory 1 cells (Tr1) cells as well as TGF- β -producing T cells (Th3 cells) [9–11].

There is accumulating evidence to suggest that various populations of regulatory (or suppressive) T cells are central in the control of allergy. The most intensively

studied human system involves allergy to bee sting, and clear evidence of Tr1-mediated control of ensuing immune responses has been demonstrated [12]. While many aspects of this response may be unique to venom allergy, Treg cells have also been implicated in other more common forms of allergic disease.

Seasonal allergic rhinitis is a very common and well-defined IgE-mediated disease driven by inhaled ubiquitous harmless antigen, such as pollen [13]. Several reports have suggested that Treg cells are involved in the development of allergic rhinitis (AR), but until now this information is mainly based on investigation of Treg cell function in peripheral blood. Studies of allergic airway disease in animal models demonstrate that Foxp3⁺ Treg cells accumulate in the airway mucosa during aero-allergen challenge and exert their suppressive function locally [14, 15]. Therefore, in addition to study Treg cells in peripheral blood, complementary studies of Treg cell function at the active site of human disease is clearly needed.

In this study, we used an experimentally induced human AR model, which allowed us to determine the kinetics of immune-cell trafficking (including Foxp3⁺ T cells) in the nasal mucosa during a 7-day pollen challenge period.

Methods

Participants

Detailed description of the experimental set-up has been described elsewhere [16]. Twelve pollen-allergic patients, median age 24 years (range 22–29, seven males), and seven non-allergic volunteers, median age 25 years (range 22–27, five males) were challenged locally with grass or birch pollen out of season. The same individuals (11 allergic subjects and five controls) were challenged 1 year later and blood samples were obtained at days 0, 2, 5 and 7. In addition, another six grass pollen-allergic individuals, median age 26 years (range 23–36, five males) were 'challenged' with a placebo. All test subjects were skin prick tested (SPT) with a panel of eight common inhalant allergens (house dust mite, mould, horse, dog, cat, grass, mugwort and birch) (Solu Prick SQ, ALK-Abello A/S, Horsholm, Denmark), and they were all non-smoking, had no history of upper respiratory tract infection during the preceding 4 weeks, had no nasal polyps, nasal deformities or nasal surgery and had no symptoms before the allergen challenge. The allergic volunteers had a typical history of pollen-induced upper airway allergy and a positive SPT to grass or birch pollen according to the European guidelines [17]. Ten out of the 18 allergic donors had a positive SPT to one or more allergens in addition to grass or birch. All non-allergic volunteers had a history free of allergic symptoms, and a negative SPT to the same allergen panel.

The study was approved by the Norwegian Regional Committee for Medical Research Ethics, and written consent was obtained from all participating subjects.

Study design

Nasal challenge was performed with a hand-driven pump spray that delivered a defined volume (50 µL) of relevant allergen solution (Aquagen timothy or Aquagen birch, ALK-Abello A/S, Horsholm, Denmark). The dose was individualized by increasing the concentrations of allergen (10 000–100 000 SQ-U/mL) until a threshold dose that elicited typical acute rhinitis symptoms was established. This dose was applied once daily. The control subjects were exposed to the highest allergen concentration used for patients receiving Aquagen timothy. Symptoms were recorded daily on a four-point scale (0–3) for nasal blockage, nasal discharge and sneezing, with a maximum score of 9. Mucosal biopsies were obtained from the inferior turbinate in one nostril before challenge (day 0). Thereafter, the opposite nostril was challenged with the relevant allergen daily for 7 days and biopsies were obtained at days 1 and 7 from the challenged side. The placebo group was challenged with saline and mucosal tissues were obtained on days 0 and 7. All experiments were performed outside the pollen season.

Sampling of tissue specimens

Mucosal specimens were obtained from the lower edge of the inferior turbinate, 1.5–2 cm posterior to the front edge, by means of a Gerritsma forceps with a cup diameter of 2.5 mm in local anaesthesia (induced by placing a cotton wool carrier with 7 mg/mL tetracain/0.3 mg/mL adrenalin under the inferior turbinate adjacent to the biopsy site).

Samples of palatine tonsils were obtained from patients operated for recurrent tonsillitis or tonsillar hypertrophy. All specimens were immediately placed on a thin slice of carrot, embedded in OCT (Tissue-Tek, Miles Laboratories, Elkhart, IN, USA), and snap-frozen in liquid nitrogen.

Immunofluorescence staining

To enumerate Foxp3⁺ T cells, cryosections cut at 4 µm were fixed in 4% paraformaldehyde (PFA), air-dried for 2 h, boiled for 20 min in citrate buffer (pH 6.0), air-dried for 30 min and then washed in phosphate-buffered saline (PBS). Paired immunofluorescence staining was performed by applying a polyclonal rabbit anti-CD3 antibody (12 µg/mL, Dako Denmark A/S, Glostrup, Denmark) and a rat IgG2a mAb for Foxp3 (clone PCH 101, 1/20, eBioscience, San Diego, CA, USA) overnight at 4 °C. The sections were rinsed in PBS and thereafter incubated for 3 h with a combination of Alexa Fluor 488-labelled goat anti-rabbit IgG (2 µg/mL, Molecular Probes, Eugene, OR,

shows that the accumulated symptom score in the challenged AR group was significantly higher than in the control group ($P=0.001$) and in the AR group that received placebo ($P=0.001$), but there was no change in daily symptom score during the 7-day period (Fig. 1b).

Tissue eosinophilia is a hallmark of allergic airway inflammation. To determine whether our disease model elicited local allergic inflammation, we determined the density of mucosal eosinophils before and after challenge. Figure 2a shows that there was a significant increase ($P=0.001$) in the number of eosinophils at day 7 compared with baseline within the allergic group, whereas no increase was detected at day 1. There was no correlation between the overall symptom score ($r_s=0.15$, $P=0.64$) or symptom score on day 7 ($r_s=-0.15$, $P=0.67$) and the density of tissue eosinophils on day 7 in the challenged allergic individuals. In non-allergic individuals exposed to pollen and in AR patients receiving the placebo, the number of eosinophils was low at all time-points.

Using the same experimental set-up, we obtained blood samples at days 0, 2, 5 and 7. As shown in Fig. 2b, there was a significant increase in the percentage of circulating eosinophils in the allergy group at day 2 and throughout the challenge period without any increase in the total leucocyte number (not shown).

Together, these findings demonstrated that this experimental model recapitulated characteristic features of AR in season.

Foxp3⁺ T cells accumulate in nasal mucosa of allergic patients after challenge

To determine the occurrence of mucosal Treg cells during the challenge period, we first tested various Treg cell markers on tonsillar tissue. To date, Foxp3 is the best

marker to identify Treg cells, and we applied two different antibodies that gave a similar result (not shown). When co-staining for Foxp3 and CD3 in tonsils, we could clearly distinguish between cells with bright Foxp3 nuclear staining and cells with faint positivity (Fig. 3). Co-staining with anti-CD4 and the Treg cell markers CD25 and CTLA-4, we found that the vast majority of Foxp3^{high} cells expressed CD4, and the majority were strongly positive for CD25 and CTLA-4 (not shown). Based on these results, only Foxp3^{high} cells were enumerated in samples from the nasal mucosa.

Figure 4a shows that in all test subjects, there were high numbers of resident CD3⁺Foxp3⁺ T cells in the mucosa at baseline (day 0) with no difference between the allergic and the control group. After the first challenge (day 1), a small and transient increase ($P=0.012$) in the total number of CD3⁺Foxp3⁺ T cells was observed in challenged pollen allergics, but not in either control group. Approximately 3% of the CD3⁺ T cells at day 0 were Foxp3^{high} with no difference between the groups (not shown). The number of CD3⁺Foxp3^{high} cells was unchanged at day 1, but after 7 days of pollen exposure we observed a fivefold increase ($P=0.009$) in the density of CD3⁺Foxp3^{high} cells in the allergic group (Figs 4b and 5a and b), but not in the control group. Similarly, the ratio of CD3⁺Foxp3^{high}/CD3⁺Foxp3⁺ cells increased in the allergic group at day 7 (Fig. 4c), but not in the control group. Co-staining with CD4 revealed no significant change in the numbers of the CD3⁺CD4⁺ or CD3⁺CD4⁺ (presumably CD8⁺) subset (Fig. 4d and not shown), suggesting that the transient increase in CD3⁺ T cells at day 1 was caused by a slight increase of both subsets, which was not able to be detected when counted separately. The vast majority of Foxp3^{high} cells co-expressed CD4, whereas Foxp3⁺ cells were both CD4⁺ and CD4⁺ (CD8⁺). Moreover, most CD3⁺ T cells expressed

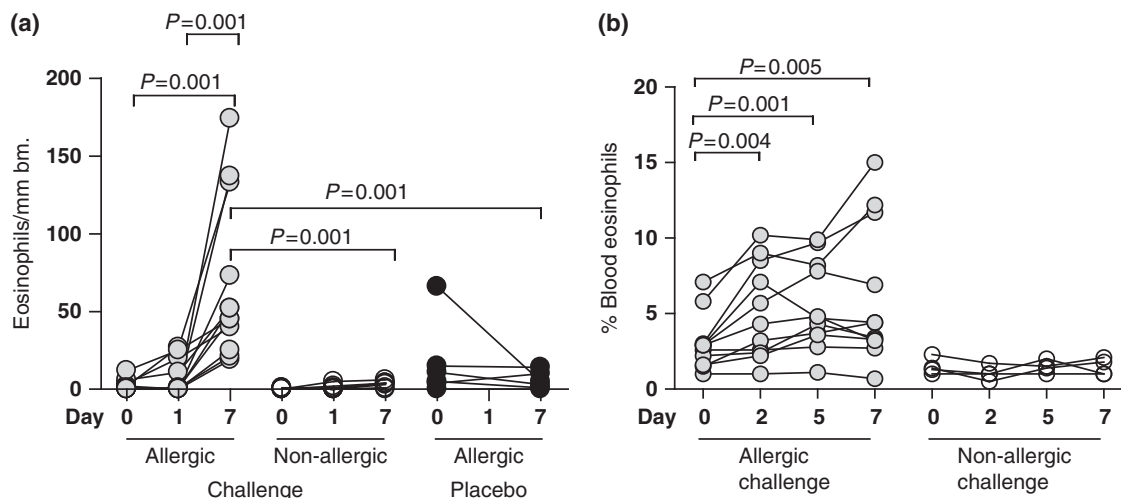


Fig. 2. Nasal allergen challenge of allergic individuals increased numbers of tissue and blood eosinophils. (a) The numbers of eosinophils in the epithelium and 250 μm subepithelial region at days 0, 1 and 7 after allergen challenge (bm, basement membrane). (b) The percentage of the eosinophils in the blood at days 0, 2, 5 and 7.

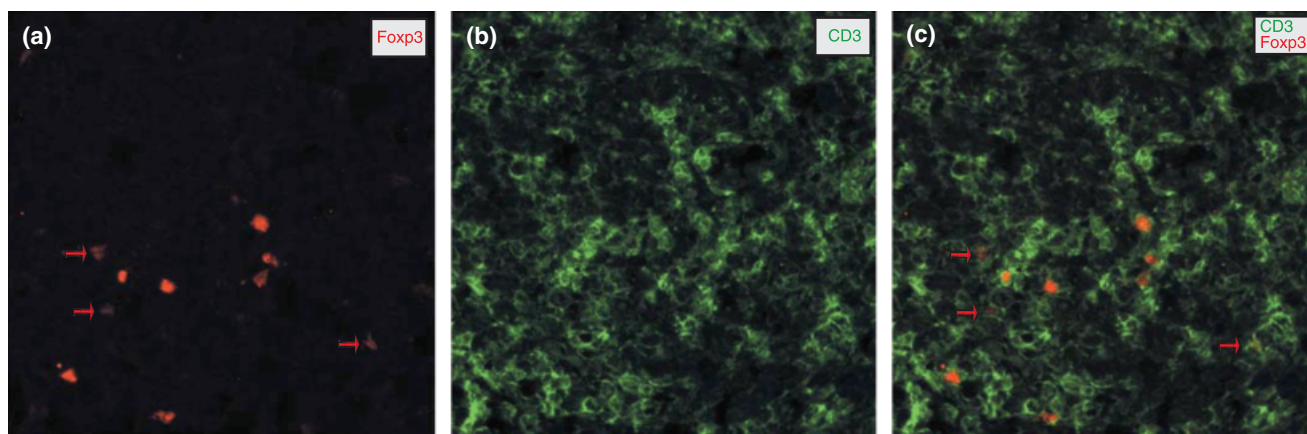


Fig. 3. CD3Foxp3 staining in tonsil tissue. (a) Foxp3. (b) CD3. (c) CD3 and Foxp3 merged. Arrows indicate weak Foxp3⁺ cells not included. Original magnification $\times 400$.

CD45RO at all time-points (not shown), compatible with being memory/effector cells.

To substantiate a Treg cell phenotype of the Foxp3^{high} cells, we performed additional immunostainings. Most Foxp3^{high} cells co-expressed CTLA-4 (Fig. 5c), and more than 70% (median values) expressed CD25 at all time-points (Figs 4e and 5d). These findings strongly suggested that Foxp3^{high} cells accumulating in the nasal mucosa at day 7 were Treg cells.

Foxp3^{high} Treg cells proliferate locally

The increased density of Foxp3^{high} CD25⁺CTLA-4⁺ T cells at day 7 could be caused by increased recruitment and/or increased local proliferation. To examine the latter possibility, we co-stained for Foxp3 and the proliferation marker Ki-67. Samples with the highest number of Foxp3^{high} cells were chosen ($n = 3$) to obtain reliable figures for co-expression. Interestingly, 46–66% of all Foxp3^{high} T cells co-expressed Ki-67 (Fig. 6). This finding strongly suggested that accumulation of Foxp3^{high} T cells in the allergic inflammation to a large extent was caused by local proliferation.

Discussion

An increasing number of reports from human studies suggest that Treg cells play an important role in the suppression of allergic reactions by their action on allergen-reactive effector T cells. However, these results are primarily based on *in vitro* experiments studying Treg cells and effector T cells isolated from peripheral blood. Very little is known about the presence or function of Treg cells at tissue sites where the immune system is exposed to allergens *in vivo*. We used a unique human experimental model of upper airway allergy to explore the kinetics of T cell trafficking in the nasal mucosa during a 7-day challenge period. Before the challenge (day 0), high

numbers of both CD4⁺ and CD8⁺ T cells were found in the nasal mucosa, with no apparent difference between AR patients and controls. At day 1, a transient, small but significant increase in the total number of T cells was observed in the mucosa of allergic patients. Although it is well established that CD4⁺ T effector cells play an important role in AR [13, 20–23], several studies have, similar to this study, shown that there is no or only marginal increase in the accumulation of CD4⁺ T cells, both in season and in experimentally induced inflammation [23, 24]. However, our data clearly show an early inflammatory response to the allergen challenge documented by a rapid twofold increase in the number of circulating eosinophils. Based on the current knowledge, it is reasonable to suggest that the observed blood eosinophilia is an indirect effect caused by early activation of allergen-specific T cells and/or mast cells at the mucosal site. Moreover, evidence for local allergen-dependent inflammation was demonstrated by a dramatic accumulation of mucosal eosinophils at day 7.

The frequency of Foxp3^{high} T cells in uninflamed nasal mucosa was approximately 3% of all CD3⁺ T cells in both groups, comparable to levels in peripheral blood [25, 26]. A significant increase in Foxp3^{high} T cells was observed at day 7 in the allergic group. This finding is in accordance with a recent study by Radulovic et al. [24] who found that the number of Foxp3⁺ cells increased in nasal mucosa of grass-pollen AR patients in season. Moreover, in agreement with their study we showed that the density of mucosal Foxp3^{high} T cells was similar in allergic and non-allergic individuals outside season. However, in contrast to Radulovic and colleagues, we were able to follow the kinetics of immune-cell trafficking (T cell subsets and eosinophils) in the tissue during the course of challenge in both allergic and non-allergic individuals. Recent reports have shown that tolerance to allergens depends on the Treg/T effector cell ratio. However, we found no increase in the density of Foxp3^{high} T cells (or increased Foxp3^{high}/

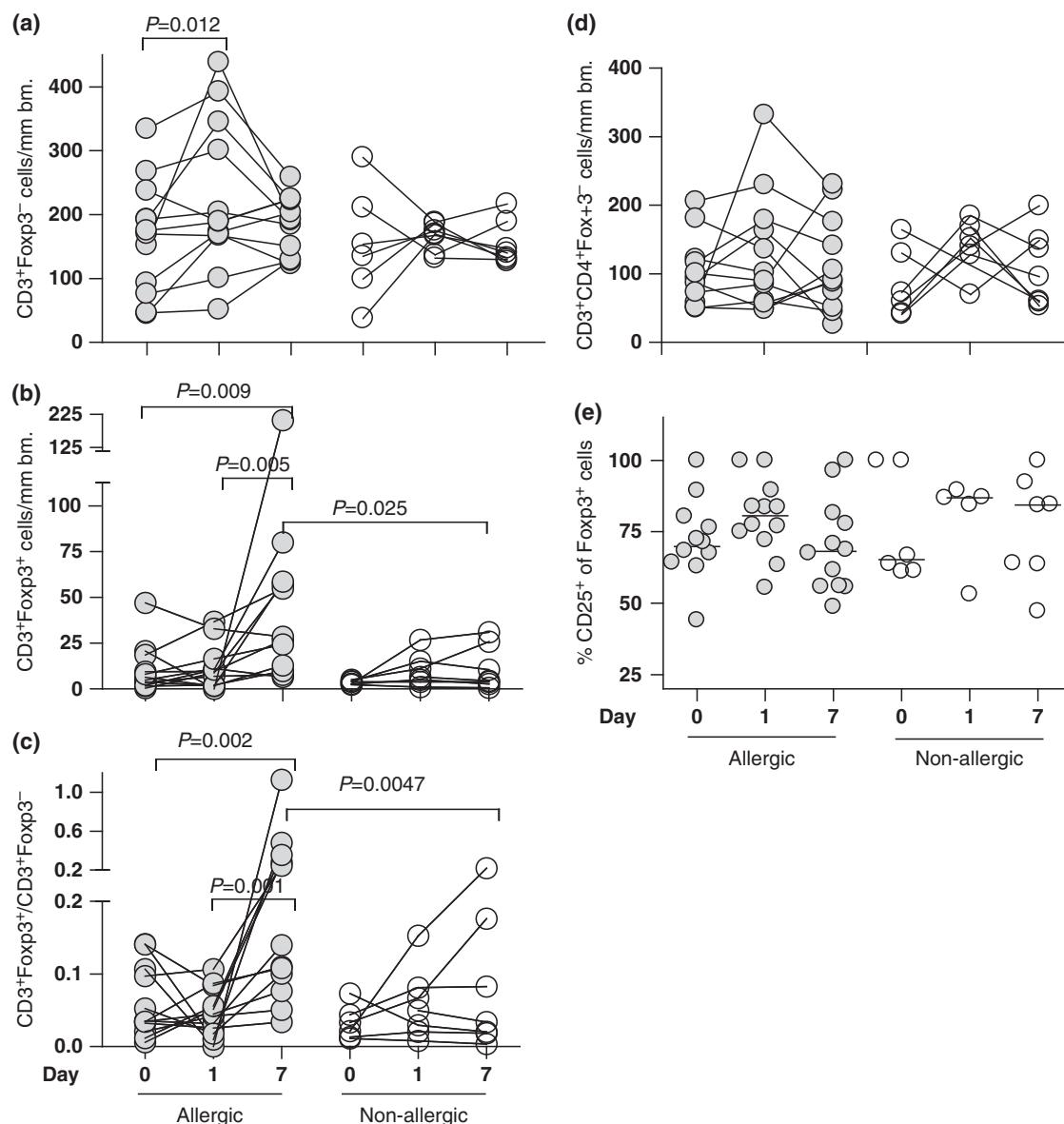


Fig. 4. Nasal allergen challenge of allergic individuals increased numbers of tissue T cells. The numbers of (a) CD3⁺Foxp3⁻ cells, (b) CD3⁺Foxp3⁺ cells and (d) CD3⁺CD4⁺Foxp3⁻ cells in the epithelium and 250 μ m subepithelial region in challenged allergic and non-allergic individuals at days 0, 1 and 7 after allergen challenge (bm, basement membrane), (c) CD3⁺Foxp3⁺/CD3⁺Foxp3⁻ cells, (e) percentage of Foxp3⁺ cells that are CD25⁺.

Foxp3⁻ T cell ratio) during the course of challenge in the non-allergic individuals. In fact, an increased Foxp3^{high}/Foxp3⁻ T cell ratio was only found in the challenged allergics. These results may indicate that low numbers of Treg cells are sufficient to keep allergen-specific T cells in check in non-allergic individuals or that other mechanisms (in addition to local Treg cells) are involved in mucosal tolerance to aeroallergens.

It is well known that Foxp3⁺ T cells accumulate in a variety of human inflammatory disorders [27, 28]. Based on studies in experimental animals, it is believed that these Treg cells limit the intensity and duration of the inflammatory reaction to avoid unnecessary tissue damage. However, it is very difficult to test this hypothesis in

human models *in vivo* because a comparison of inflammatory insults with or without accumulating Treg cells is not possible. In a mouse asthma model similar to our human model, Curotto de Lafaille et al. [14] reported that inducible Foxp3⁺ Treg cells accumulated in inflamed airways. These Treg cells did not inhibit acute eosinophil inflammation, but in the chronic phase they reduced the severity of the inflammation, reduced remodelling and prevented the dissemination of disease to other organs. Translation of these results to our human model suggests that even though there is a concomitant accumulation of eosinophils with the Foxp3^{high} T cell accumulation in the nasal mucosa, the Foxp3^{high} T cells have an anti-inflammatory effect in the chronic phase of the reaction.

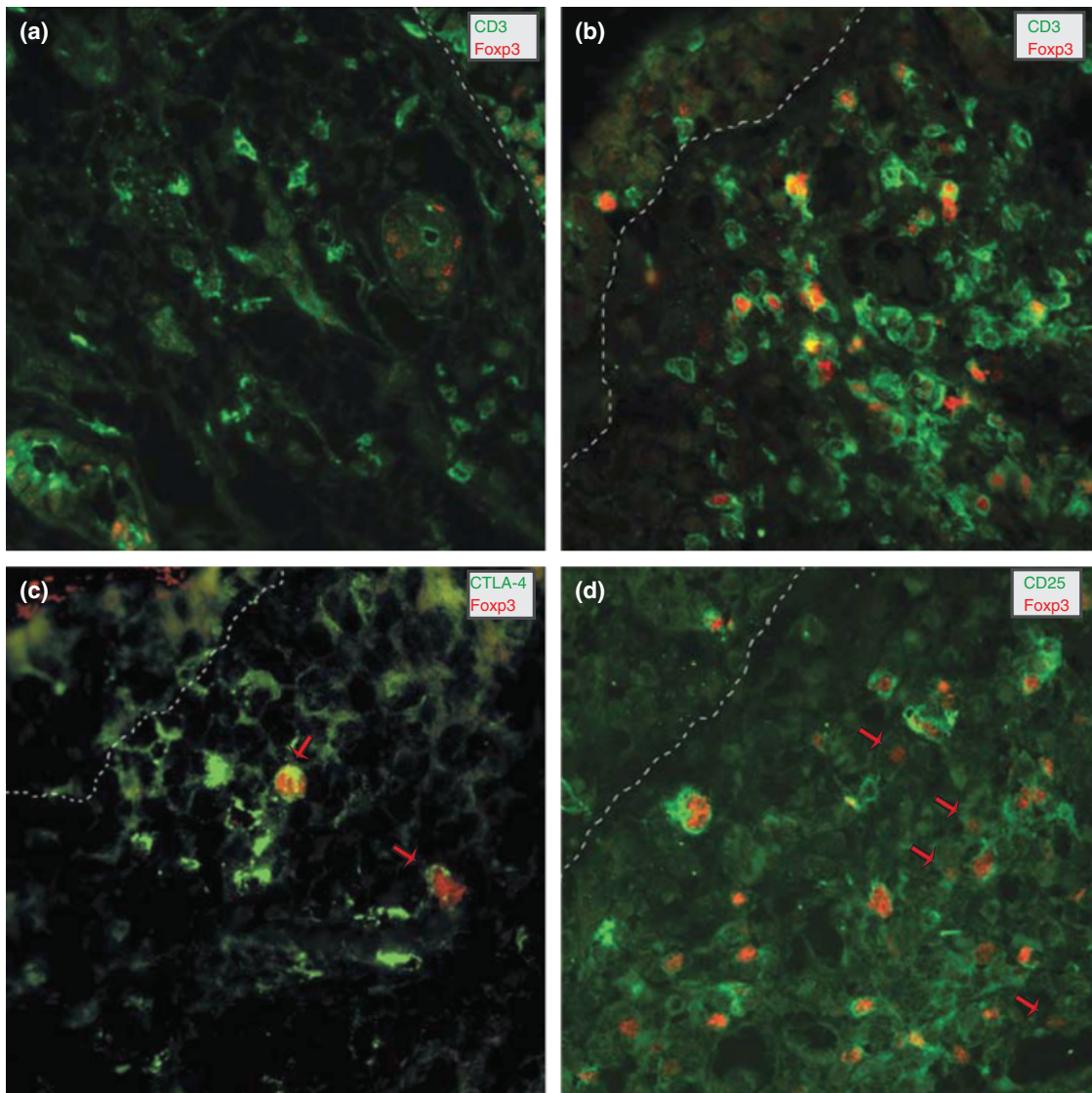


Fig. 5. Co-expression of Foxp3⁺ with CD25 and CTLA-4 suggests a regulatory phenotype. Immunohistochemical staining. (a) CD3 and Foxp3, allergic patient day 0, (b) CD3 and Foxp3, allergic patient day 7, (c) CTLA-4 and Foxp3, allergic patient day 7 (arrows indicate Foxp3^{high} cells), (d) CD25 and Foxp3, allergic patient day 7 (arrows indicate Foxp3^{low} cells). Basement membrane of surface epithelium is indicated by the broken line. Original magnification $\times 400$.

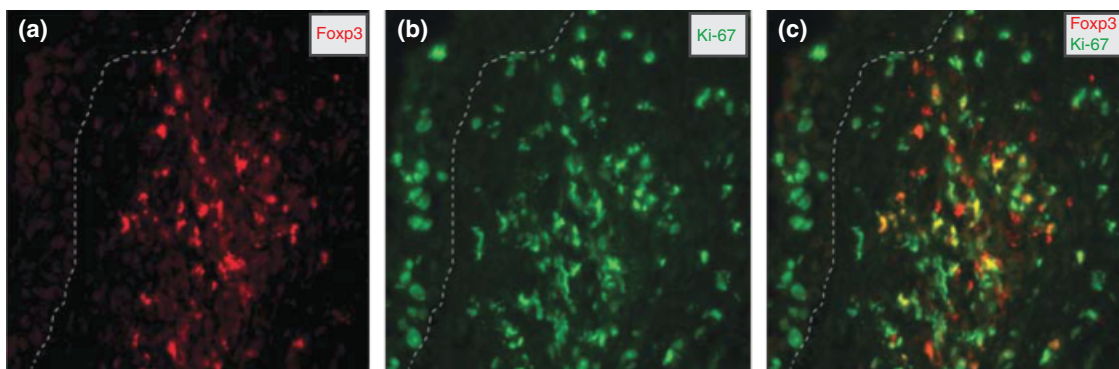


Fig. 6. Most Foxp3⁺ cells are proliferating. Immunohistochemical staining for Foxp3 and Ki-67 in cryosection of nasal mucosa from allergic patient at day 7. (a) Foxp3, (b) Ki-67, (c) Foxp3 and Ki-67 merged. Basement membrane of surface epithelium is indicated by the broken line. Original magnification $\times 200$.

Although Foxp3 is the best marker to identify Treg cells to date, it has become evident that the Foxp3 expression is not unique to Treg cells, and the transient expression of Foxp3 is also observed in effector T cells activated *in vitro* [29, 30]. In both tonsillar and nasal mucosal tissue, we detected both weak and strong nuclear staining for Foxp3. Because Treg cells express high levels of Foxp3 [31], we included only those cells that were strongly positive. Moreover, the vast majority of these Foxp3^{high} cells expressed CTLA-4 and CD25, markers that are constitutively expressed on Treg cells. Together, these findings strongly suggest that the Foxp3^{high} T cell population accumulating in inflamed nasal mucosa indeed are Treg cells.

Recent studies have suggested that dysregulation in the fine-tuned balance between Treg cells and effector T cells lies at the core of human allergic responses. Some studies have shown that the balance between Tregs and effector T cells is mainly a matter of numbers [32]. However, other reports have shown that Treg cells in allergic patients, compared with normal non-atopic controls, have reduced suppressive capacity [33, 34]. In a third variety of this inflammatory theme, it has been demonstrated that Treg cells may function normally but the effector T cells are refractory to suppression [35]. Thus, the effect of Treg cells on inflammatory reaction is not only a matter of the relative ratio between Treg and effector T cells but also depends both on the suppressive capacity of Treg cells and on the responsiveness of the antigen-specific effector T cells.

We found that a high fraction of Foxp3⁺ cells in nasal mucosa after allergen challenge were Ki-67⁺. Similarly, it was recently shown that when tuberculin-purified protein derivate was injected into human skin, Foxp3⁺ Treg cells accumulated at the lesional site reaching maximum levels at day 7 [36]. Interestingly, in the cutaneous model, a

significant fraction of Foxp3⁺ Treg cells also proliferated locally as defined by their expression of Ki-67. This strongly suggests that accumulation of Foxp3⁺ cells, at least in part, depends on local proliferation. It has been shown that Treg cells specific for non-self-antigens that develop in the periphery are short-lived unless antigen persist [4]. This idea is compatible with the present study, which shows that a substantial fraction of Treg cells proliferate within the effector tissue. To gain further insight into the function of Treg cells accumulating in the mucosa in our *in vivo* model, it is necessary to isolate T cells from the tissue and examine the suppressive capacity of Treg cells as well as the responsiveness of effector T cells to suppressive signals. Such studies would require larger tissue biopsies, or more efficient isolation and analysis of tissue T cells than currently available and is therefore outside the scope of the present study.

In conclusion, nasal challenge with pollen extract increased the number of Foxp3^{high} T cells at the inflammatory site in AR patients, suggesting that Treg cells are accumulating in upper airway allergy. There is a large body of evidence to suggest that Treg cells are able to suppress allergic inflammation. It should therefore be explored whether Foxp3^{high} T cells accumulating in the nasal mucosa of AR patients could be targets for therapeutic intervention.

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References

- 1 Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3⁺ regulatory T cells: more of the same or a division of labor? *Immunity* 2009; **30**:626–35.
- 2 Feuerer M, Hill JA, Mathis D, Benoist C. Foxp3⁺ regulatory T cells: differentiation, specification, subphenotypes. *Nat Immunol* 2009; **10**:689–95.
- 3 Zhou L, Chong MM, Littman DR. Plasticity of CD4⁺ T cell lineage differentiation. *Immunity* 2009; **30**:646–55.
- 4 Akbar AN, Vukmanovic-Stejić M, Taams LS, Macallan DC. The dynamic co-evolution of memory and regulatory CD4⁺ T cells in the periphery. *Nat Rev Immunol* 2007; **7**:231–7.
- 5 Bennett CL, Christie J, Ramsdell F *et al*. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001; **27**:20–1.
- 6 Wildin RS, Freitas A. IPEX and FOXP3: clinical and research perspectives. *J Autoimmun* 2005; **25** (Suppl.): 56–62.
- 7 Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: forkhead box protein 3 mutations and lack of regulatory T cells. *J Allergy Clin Immunol* 2007; **120**: 744–50; quiz 751–742.
- 8 Chatila TA, Blaeser F, Ho N *et al*. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J Clin Invest* 2000; **106**:R75–81.
- 9 Akdis CA. New insights into mechanisms of immunoregulation in 2007. *J Allergy Clin Immunol* 2008; **122**:700–9.
- 10 Bacchetta R, Gambineri E, Roncarolo MG. Role of regulatory T cells and FOXP3 in human diseases. *J Allergy Clin Immunol* 2007; **120**:227–35; quiz 236–227.
- 11 Larche M. Regulatory T cells in allergy and asthma. *Chest* 2007; **132**:1007–14.
- 12 Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med* 2008; **205**:2887–98.
- 13 Kay AB. Allergy and allergic diseases. First of two parts. *N Engl J Med* 2001; **344**:30–7.

- 14 Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3⁺ regulatory T cell-dependent and - independent control of allergic inflammation. *Immunity* 2008; 29:114–26.
- 15 Strickland DH, Stumbles PA, Zosky GR *et al.* Reversal of airway hyperresponsiveness by induction of airway mucosal CD4+CD25⁺ regulatory T cells. *J Exp Med* 2006; 203:2649–60.
- 16 Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P. Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. *J Immunol* 2000; 165:4062–8.
- 17 Dreborg SF A: position paper: allergen standardization and skin tests. *Allergy* 1993; 47:48s–82.
- 18 Jahnsen FL, Farstad IN, Aanesen JP, Brandtzaeg P. Phenotypic distribution of T cells in human nasal mucosa differs from that in the gut. *Am J Respir Cell Mol Biol* 1998; 18:392–401.
- 19 Jahnsen FL, Haraldsen G, Aanesen JP, Haye R, Brandtzaeg P. Eosinophil infiltration is related to increased expression of vascular cell adhesion molecule-1 in nasal polyps. *Am J Respir Cell Mol Biol* 1995; 12:624–32.
- 20 Woodfolk JA. T-cell responses to allergens. *J Allergy Clin Immunol* 2007; 119:280–94; quiz 295–286.
- 21 Bohle B. T cell responses during allergen-specific immunotherapy of Type I allergy. *Front Biosci* 2008; 13: 6079–85.
- 22 Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. *J Allergy Clin Immunol* 2009; 123:735–46; quiz 747–738.
- 23 Varney VA, Jacobson MR, Sudderick RM *et al.* Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992; 146:170–6.
- 24 Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+CD25⁺ cells in the nasal mucosa. *J Allergy Clin Immunol* 2008; 121: 1467–72; e1461.
- 25 Seddiki N, Santner-Nanan B, Martinson J *et al.* Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med* 2006; 203:1693–700.
- 26 Skrido I, Farkas L, Kvale EO, Johansen FE, Jahnsen FL. Depletion of CD4+CD25+CD127lo regulatory T cells does not increase allergen-driven T cell activation. *Clin Exp Allergy* 2008; 38: 1752–9.
- 27 Joosten SA, Ottenhoff TH. Human CD4 and CD8 regulatory T cells in infectious diseases and vaccination. *Hum Immunol* 2008; 69:760–7.
- 28 Christodoulou MI, Kapsogeorgou EK, Moutsopoulos NM, Moutsopoulos HM. Foxp3+T-regulatory cells in Sjogren's syndrome: correlation with the grade of the autoimmune lesion and certain adverse prognostic factors. *Am J Pathol* 2008; 173:1389–96.
- 29 Tran DQ, Ramsey H, Shevach EM. Induction of FOXP3 expression in naive human CD4+FOXP3⁺ T cells by T-cell receptor stimulation is transforming growth factor-beta dependent but does not confer a regulatory phenotype. *Blood* 2007; 110:2983–90.
- 30 Pillai V, Ortega SB, Wang CK, Karandikar NJ. Transient regulatory T-cells: a state attained by all activated human T-cells. *Clin Immunol* 2007; 123:18–29.
- 31 Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3(+) regulatory T cells in the human immune system. *Nat Rev Immunol* 2010; 10:490–500.
- 32 Akdis M, Verhagen J, Taylor A *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199:1567–75.
- 33 Ling EM, Smith T, Nguyen XD *et al.* Relation of CD4+CD25⁺ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004; 363:608–15.
- 34 Grindebacke H, Wing K, Andersson AC, Suri-Payer E, Rak S, Rudin A. Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergics during birch pollen season. *Clin Exp Allergy* 2004; 34:1364–72.
- 35 Schneider A, Rieck M, Sanda S, Pihoker C, Greenbaum C, Buckner JH. The effector T cells of diabetic subjects are resistant to regulation via CD4+FOXP3⁺ regulatory T cells. *J Immunol* 2008; 181: 7350–5.
- 36 Vukmanovic-Stejic M, Agius E, Booth N *et al.* The kinetics of CD4+Foxp3⁺ T cell accumulation during a human cutaneous antigen-specific memory response in vivo. *J Clin Invest* 2008; 118: 3639–50.