

Oral administration of Ag suppresses Ag-induced allergic conjunctivitis in mice: critical timing and dose of Ag

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ABSTRACT

Background/aims To investigate the effect of Ag doses and administration time points on oral tolerance induction in experimental allergic conjunctivitis (EAC). **Methods** BALB/c mice were actively sensitised twice with ovalbumin (OVA) in alum, and then challenged twice with OVA in eye drops. Twenty minutes after the last challenge, the clinical appearance was evaluated. Twenty-four hours later, the conjunctivas, spleens and blood were collected for histological analyses, cytokine production assays, and measurement of serum Ig levels, respectively. The mice were fed with a high or low dose of OVA before sensitisation (prophylactic treatment). To assess the effect of therapeutic treatment, the high-dose Ag was administered after sensitisation. Control groups received phosphate-buffered saline without OVA. To determine the role of interleukin-10 (IL-10) and transforming growth factor- β (TGF- β) in prophylactic low-dose oral tolerance, mice were injected intraperitoneally with neutralising antibodies during the entire experimental period. To assess the role of regulatory T cells, neonatally thymectomised mice were injected with depleting anti-CD25 monoclonal antibodies before the low-dose prophylactic treatment.

Results OVA-feeding was suppressive even when the mice were treated soon after Ag sensitisation. Both the low and high doses of oral OVA suppressed EAC. High-dose treatment significantly suppressed EAC-related cytokine production and serum Ig levels. IL-10 and TGF- β were less likely to be involved in prophylactic low-dose oral tolerance induction but regulatory T cells played a role.

Conclusions Prophylactic and early therapeutic treatment of OVA feeding suppressed EAC. Both high and low doses of oral OVA induced oral tolerance but with different mechanisms.

INTRODUCTION

Allergic diseases such as allergic conjunctivitis are mediated by Ag-specific acquired immune responses.¹ In clinical practices, patients with allergic conjunctivitis are often treated with anti-allergic and steroid eye drops. However, while these drugs inhibit symptoms, they are not specific for any Ags.² Once patients have been sensitised with an Ag, the Ag-specific immunological memory persists for a long time, with the result that symptoms arise every time they encounter the Ag. For this reason, Ag-specific therapy is a more attractive option than symptom-ameliorating therapies. At present allergen-specific immunotherapy,³ where the disease-causing allergens are administered

subcutaneously or sublingually, is the common disease-modifying treatment for allergy in humans.

It has been known for more than a century that when an Ag is fed, a subsequent challenge with the same Ag results in hyporesponsiveness, a phenomenon referred as 'oral tolerance'.⁴ Oral tolerance induction has been used to treat autoimmune diseases in both humans and animals, with encouraging outcomes.^{5–6} Regarding allergic diseases, the effectiveness of oral tolerance therapy has been examined quite extensively in food allergy,⁷ asthma,⁸ atopic dermatitis⁹ and allergic rhinitis.¹⁰ In contrast, only two studies investigating the efficacy of oral tolerance induction in allergic conjunctivitis have been performed. One of these was by Koizumi *et al*,¹¹ who reported that oral tolerance induction suppressed an IgE-mediated reaction in rat conjunctivas. The other was by Zemmann *et al*¹² who examined the immunological mechanisms that were involved and found that oral tolerance induction significantly suppressed conjunctival hyperaemia. These studies did not examine whether the timing of Ag-feeding altered the efficacy of oral tolerance induction. Furthermore, only one dose of oral Ag was tested. In the present study, the significance of dose and administration time point was examined by feeding an ovalbumin (OVA)-induced murine model of experimental allergic conjunctivitis (EAC) with different Ag doses prophylactically or therapeutically.

MATERIALS AND METHODS

Mice

Inbred wild-type BALB/c mice were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan), and were kept in specific pathogen-free conditions at Kochi Medical School's animal facility. Age- and gender-matched mice were used when they were 6 to 12 weeks of age. All research adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Reagents

OVA was purchased from Worthington Biochemical Corp (Lakewood, New Jersey, USA). Aluminium hydroxide (alum) was purchased from Sigma (St. Louis, Missouri, USA). Hybridomas producing neutralising antibodies to interleukin-10 (IL-10, JES5-2A5)¹³ and transforming growth factor- β (TGF- β , 1D11)¹⁴ were obtained from the American Type Culture Collection (Manassas, Virginia, USA). For *in vivo* treatments, these antibodies were

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purified from ascites by using a protein G column. The preparations contained <100 pg/ml endotoxin. Normal rat immunoglobulin G (nrIgG) was purchased from MP Biomedicals Inc. (Aurora, Ohio, USA).

EAC induction

EAC was essentially induced according to the published protocol,¹⁵ albeit with a different dose of OVA and alum. The left hind-footpad and the tail base of BALB/c mice were each injected with 50 µg of OVA and 675 µg of alum. One week later, the mice were injected intraperitoneally with 100 µl of the emulsion (100 µg of OVA). Seven days after the second sensitisation, the eyes of the mice were challenged with OVA in phosphate-buffered saline (PBS) (750 µg in 5 µl per eye). Two days later, the mice were challenged again with OVA in eye drops. Twenty minutes later, the clinical early phase reactions such as lid swelling were evaluated based on previously described criteria.¹⁶ Twenty-four hours after the second challenge, the eyes, blood and spleens were harvested for histological analysis, measurement of serum Ig levels, and production of cytokines by splenocytes, respectively.

Induction of oral tolerance

For the prophylactic treatment, mice were fed every other day with 20 mg (high dose) or 500 µg (low dose) starting 10 days before the first OVA-sensitisation (five feeds in total).¹⁷ Control group mice were fed with PBS. Two days after the final feed, the mice were sensitised with OVA/alum as described above (EAC induction). For the therapeutic treatment, mice were fed daily with 20 mg of OVA starting on the day of second sensitisation (days 7–11, five feeds in total). As with the prophylactically-treated mice, these mice were challenged on days 14 and 16. Alternatively, the mice were sensitised twice and challenged with OVA in eye drops on days 14 and 16 as described above (EAC induction). One week after the first OVA challenge (day 21), the mice were fed daily with 20 mg of OVA (days 21–27, seven feeds in total). One day after the last feed (day 28), the mice were challenged with OVA in eye drops. Two days later (day 30), the mice were challenged again with OVA in eye drops and were evaluated as above.

Neutralisation of cytokines by monoclonal antibodies

The prophylactic Ag-fed mice were injected intraperitoneally with 200 µg neutralising mAb against IL-10 or TGF-β every other day, starting on the first Ag-feeding day (day -10) and ending on the last Ag-challenge day (day 16). Control group mice were injected with 200 µg nrIgG.

Thymectomy and treatment with anti-CD25mAb to deplete regulatory T cells

Neonates were thymectomised 3 days after birth as described previously¹⁸ and then injected intraperitoneally with 100 µg of mAb against CD25 (PC61, rat IgG1) or nrIgG on days 5, 7, 10, 16 and 19 after birth. Six weeks after birth, the mice underwent the prophylactic protocol. Specifically, the mice were fed once every second day with 20 mg of OVA starting 10 days before the first OVA-sensitisation (five feeds in total).

Evaluation of clinical early phase reaction

Twenty minutes after the second OVA challenge, chemosis, conjunctival redness, lid swelling and tearing were each graded on a 0–3 scale.¹⁶ The total score consisted of the sum of scores in each of these four categories. The total score ranges from 0 to the highest score of 12 which indicates severe oedema in conjunctival and lid tissue, tearing and redness in eyes.

Histological analysis

The eyes, including the conjunctivas, were harvested and fixed in 10% buffered formalin. Vertical 2 µm-thick sections were cut and stained with Giemsa. Infiltrating eosinophils in the lamina propria mucosae of the tarsal and bulbar conjunctivas throughout each section were counted by two observers in a blinded manner. The sections that were counted were those of the central portion of the eye, which included the pupil and optic nerve head. The data presented are an average ± SEM of all the mice that were examined.

Measurement of serum Ig levels

Twenty-four hours after the second OVA challenge, blood was collected and serum was prepared. Serum IgE and IgG1 were assessed by ELISA, as described before.¹⁹ The data are presented as ng/ml of total IgE or µg/ml of total IgG1.

Measurement of cytokines in the culture supernatants

Red blood cell-depleted splenocytes (10⁷ cells/ml) were cultured for 48 h with OVA (100 µg/ml) in 96-well flat-bottom plates in 0.2 ml Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal calf serum and 2-ME. The levels of IL-2, 4, 5, 10, 12p70, 13 and IFN-γ in the medium were measured by using the Bioplex system (Bio-Rad, Hercules, California, USA) according to the manufacturer's instructions. The data are shown as the average ± SEM (pg/ml).

Statistical analysis

The treated and control groups of mice were compared in terms of serum Ig levels, concentrations of cytokines in the culture supernatants, and infiltrating eosinophil numbers by using unpaired t test, analysis of variance, and Fisher's Protected Least Significant Difference method. Regarding the severity of clinical early phase reaction, Mann-Whitney U test was used. p Values less than 0.05 were considered to be significant.

RESULTS

Prophylactic Ag-feeding suppresses the development of EAC

Mice were fed every other day with 20 mg or 500 µg of OVA, starting 10 days before the first OVA-sensitisation (five feeds in total). When the mice had been fed with 100 mg of OVA in total (high dose), clinical early phase reaction was significantly less in the OVA-fed group than in the PBS-fed group (figure 1A, n=10 per group). Similarly, the OVA-fed group had significantly lower numbers of conjunctival eosinophils (figure 1B) and serum IgE and IgG1 levels (figure 1C). OVA feeding also significantly suppressed the production of IL-2, 4, 5, 12p70, 13 and IFN-γ by splenocytes (figure 1D). When the mice were fed with 2.5 mg of OVA in total (low dose), their clinical early phase reaction (figure 2A, n=5 per group) was not significantly different although they had significantly fewer conjunctival eosinophils (figure 2B) than the PBS-fed group. However, these PBS- and OVA-fed groups did not differ in terms of serum Ig levels and splenocyte cytokine production (data not shown).

Therapeutic Ag-feeding soon after Ag-sensitisation suppresses the development of EAC

To examine whether therapeutic Ag-feeding can suppress EAC, Ag-feeding was performed at two different time points. (1) Starting on the day of second sensitisation, the mice were fed five times daily with 20 mg of OVA. (2) As an alternative, the mice were fed daily seven times with 20 mg of OVA starting one week after the first challenge. One day after the last feeding, the

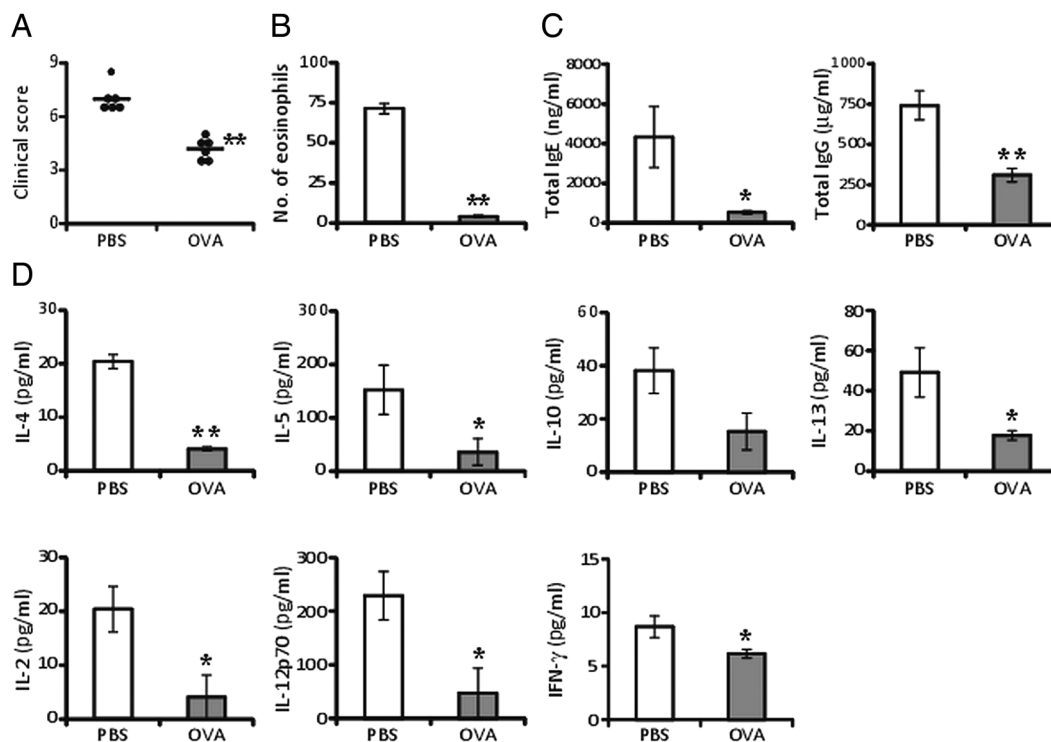


Figure 1 Prophylactic high-dose Ag-feeding suppresses the development of experimental allergic conjunctivitis (EAC) and EAC-related immune responses. Starting 10 days before the first ovalbumin (OVA)-sensitisation, mice were fed every other day with 20 mg of OVA or PBS (five feeds in total, n=10 per group). Two days after the final feeding, the mice were sensitised with OVA/alum twice on days 0 and 7, and EAC was induced by two OVA challenges in eye drops on days 14 and 16. Twenty minutes after the second challenge, clinical early phase reactions were evaluated (A). Twenty-four hours after the second challenge, the eyes, blood and spleens were harvested for histological analysis to count the numbers of eosinophils in the conjunctiva (B), for measurements of serum total IgE and IgG1 levels (C), and for measurements of the production of cytokines by splenocytes (D), respectively. *p<0.05, **p<0.01.

mice were challenged with OVA in eye drops and 2 days later, the mice were challenged again.

Initiation of therapeutic feeding immediately after the second sensitisation significantly suppressed the clinical appearance (figure 3A, n=6 per group), conjunctival eosinophil numbers (figure 3B), serum IgE but not IgG1 levels (figure 3C) and cytokine production (figure 3D). This early therapeutic Ag-feeding protocol suppressed the conjunctival eosinophilia less markedly (68% inhibition, figure 3A) than the prophylactic Ag-feeding

protocol (89% inhibition, figure 1A). By contrast, when feeding was initiated 1 week after the first challenge, it did not affect the clinical appearance (figure 4A, n=6 per group), conjunctival eosinophil numbers (figure 4B), serum Ig levels, or cytokine production (data not shown).

Blocking of IL-10 and TGF- β does not affect the effects of prophylactic Ag-feeding

Next, the mechanism by which low-dose prophylactic Ag-feeding suppresses the development of EAC was investigated. It has been demonstrated that IL-10 and TGF- β are the major cytokines that are induced by Ag-feeding and participate in oral tolerance. Therefore, the mice were injected with neutralising Abs against IL-10 and TGF- β every other day, starting on the day of the first Ag-feeding and continuing until the last Ag-challenge with eye drops. As with the data in figure 2, prophylactic Ag-feeding significantly suppressed the conjunctival eosinophil numbers (figure 5A). Treatment of the PBS-fed mice with anti-IL-10 (n=4) or anti-TGF- β Abs (n=5) also significantly decreased their conjunctival eosinophil numbers compared to the control group (n=4) (figure 5A). However, treatment with anti-IL-10 (n=5) or anti-TGF- β Abs (n=4) did not significantly affect the OVA-feeding-induced suppression of conjunctival eosinophil numbers compared to the control group (n=5) (figure 5A).

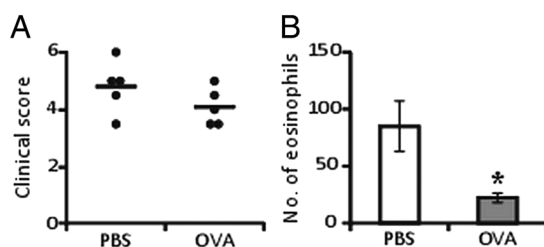


Figure 2 Prophylactic low-dose Ag-feeding suppresses the development of experimental allergic conjunctivitis (EAC). Starting 10 days before the first ovalbumin (OVA)-sensitisation, mice were fed every other day with 500 µg of OVA or PBS (five feeds in total, n=5 per group). Two days after the final feeding, the mice were sensitised with OVA/alum twice on days 0 and 7, and EAC was induced by two OVA challenges in eye drops on days 14 and 16. Twenty minutes after the second challenge, clinical early phase reactions were evaluated (A). Twenty-four hours after the second challenge, the eyes were harvested for histological analysis to count the numbers of eosinophils in the conjunctiva (B). *p<0.05.

Thymectomy plus anti-CD25 Ab treatment abrogates oral tolerance induction.

Finally, to investigate whether regulatory T cells play a role in oral tolerance induction with low Ag dose, thymectomised mice

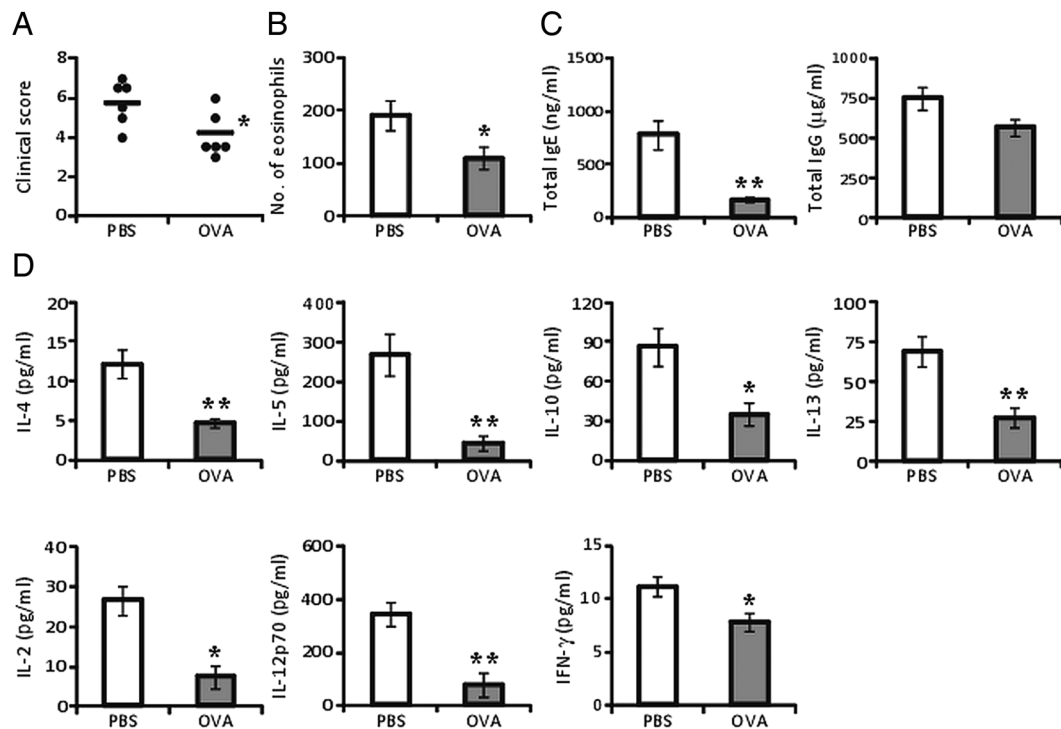


Figure 3 Therapeutic Ag-feeding soon after Ag-sensitisation suppresses the development of experimental allergic conjunctivitis (EAC) and EAC-related immune responses. Mice were sensitised with ovalbumin (OVA)/alum twice on days 0 and 7, and EAC was induced by two OVA challenges in eye drops on days 14 and 16. The mice were fed daily with 20 mg of OVA or PBS (n=6 per group) starting on the day of second sensitisation (days 7–11, five feeds in total). Twenty minutes after the second challenge, clinical early phase reactions were evaluated (A). Twenty-four hours after the second challenge, the eyes, blood and spleens were harvested for histological analysis to count the numbers of eosinophils in the conjunctiva (B), for measurements of serum IgE and IgG1 levels (C), and for measurements of the production of cytokines by splenocytes (D), respectively. * $p < 0.05$, ** $p < 0.01$.

were treated with anti-CD25 Abs to deplete the regulatory T cells (n=6 in PBS-fed group and n=7 in OVA-fed group).¹⁸ The controls were thymectomised mice that were not treated with anti-CD25 Abs (n=6 per group). Prophylactic low-dose Ag feeding was initiated and the mice were immunised and challenged with OVA as described in figure 2. OVA feeding

significantly inhibited the conjunctival eosinophilia in the control mice but not the regulatory T cell-depleted mice (figure 5B).

DISCUSSION

Oral tolerance to EAC in animals has been investigated by only two studies.^{11 12} The present study confirmed that Ag-feeding effectively suppresses EAC. Analysis of the effect of the timing and dose of Ag-feeding revealed that (i) the suppressive effects of Ag-feeding were obtained even when treatment was initiated after Ag sensitisation, and (ii) both low and high doses of fed Ag suppressed EAC.

There are two possible mechanisms by which oral tolerance suppresses the immune system.^{20 21} One is anergy/deletion which is essentially induced when high dose of Ag is administered.^{20 21} The other is active suppression, when a low dose of Ag is administered; it is mediated by inhibitory cytokines such as IL-10 and TGF- β .^{20 21} A recent study has suggested that the two forms of tolerance are not mutually exclusive and may overlap.²² In the present study, high-dose prophylactic feeding uniformly suppressed the Ag-specific cytokine production by splenocytes and profoundly inhibited IgG1 and IgE production. These data indicate that suppression of EAC by high-dose Ag-feeding is likely to be mediated by the clonal anergy/deletion of OVA-reactive T cells rather than active suppression.^{20 21}

Similar to the effect of 20 mg of prophylactic OVA-feeding (high dose), 500 μ g of prophylactic OVA-feeding (low dose) suppressed the clinical early phase reaction and conjunctival eosinophilia. However, unlike the high dose, the low dose did not affect the serum Ig levels and splenocyte cytokine production. Thus, Ag-specific oral tolerance was induced by low-dose

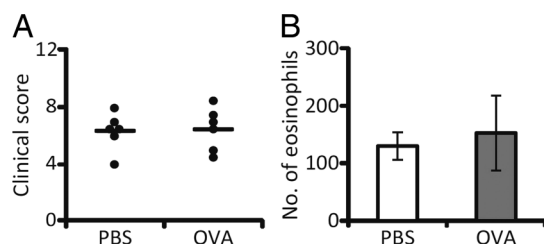


Figure 4 Therapeutic Ag-feeding one week after Ag-sensitisation does not affect the development of experimental allergic conjunctivitis (EAC). Mice were sensitised with ovalbumin (OVA)/alum twice on days 0 and 7, and EAC was induced by two OVA challenges in eye drops on days 14 and 16. Starting one week after the first challenge, the mice were fed daily with 20 mg of OVA or PBS (days 21–27, seven feeds in total, n=6 per group). One day after the last feeding (day 28), the mice were challenged with OVA in eye drops and two days later (day 30), the mice were challenged again. Twenty minutes after the second challenge, clinical early phase reactions were evaluated (A). Twenty-four hours after the second challenge, the eyes were harvested for histological analysis to count the numbers of eosinophils in the conjunctiva (B).

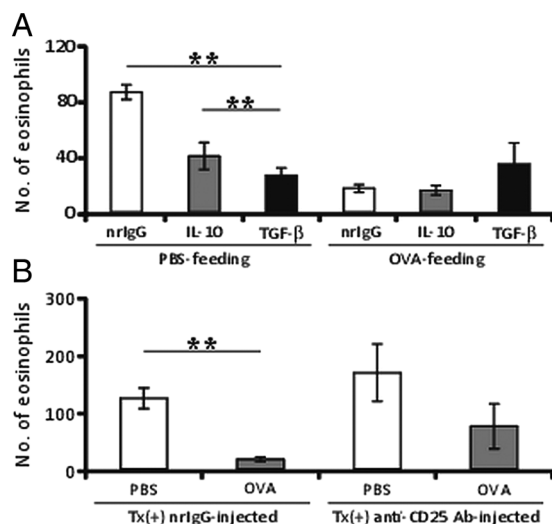


Figure 5 Analyses of the oral tolerance mechanism induced by low-dose ovalbumin (OVA)-feeding. (A) Blocking of interleukin-10 (IL-10) and transforming growth factor- β (TGF- β) does not affect the suppressive effects of prophylactic low dose OVA-feeding. Starting 10 days before the first OVA-sensitisation, mice were fed every other day with 500 μ g of OVA (five feeds in total) or PBS. Two days after the final feeding, the mice were sensitised with OVA/alum twice on days 0 and 7, and experimental allergic conjunctivitis (EAC) was induced by two OVA challenges in eye drops on days 14 and 16. The mice were injected with neutralising Abs against IL-10, TGF- β or the control Ab (n=4–5 in each group) every other day starting on the day of the first OVA-feeding (day -10) and continuing to the second OVA-challenge in eye drops (day 16). Twenty-four hours after the second challenge, the eyes were harvested for histological analysis to count the numbers of eosinophils in the conjunctiva. **p<0.01. (B) Depletion of regulatory T cells abrogates the suppressive effects of prophylactic low-dose OVA-feeding. Mice were thymectomised (Tx) neonatally, divided into two groups, and injected with anti-CD25 Ab (PC61) or control rat IgG on days 5, 7, 10, 16 and 19 after birth. Six weeks after birth, the mice were fed every other day with 500 μ g of OVA or PBS starting 10 days before the first OVA-sensitisation (five feeds in total, n=6–7 in each group). Two days after the final feed, the mice were sensitised with OVA/alum twice on days 0 and 7, and EAC was induced by two OVA challenges in eye drops on days 14 and 16. Twenty-four hours after the second challenge, the eyes were harvested for histological analysis to count the numbers of eosinophils in the conjunctiva. **p<0.01.

Ag-feeding even though there was no significant effect on systemic cellular and humoral immune responses. This suggests that the suppressive mechanism induced by low-dose OVA-feeding differed from the mechanism induced by high-dose OVA-feeding.

Given the association between active suppression and IL-10 and TGF- β ,^{20–21} it is possible that the low-dose OVA-feeding suppresses EAC by upregulating the production of these two cytokines. To test this, neutralising Abs against IL-10 or TGF- β were administered during the entire experimental period. However, this treatment did not affect the suppression of conjunctival eosinophilia induced by OVA-feeding. This indicates that neither IL-10 nor TGF- β is the suppressive cytokine in EAC in its own right. This result is also in good agreement with our previous report demonstrating that IL-10 and TGF- β augment the infiltration of eosinophils into the conjunctiva during the effector phase of EAC.²³

Our previous study showed that EAC was exacerbated when the CD4 CD25 regulatory T cells in thymectomised mice were

depleted by anti-CD25 Ab treatment.¹⁸ This suggested that CD4 CD25 regulatory T cells regulate the development of EAC. To investigate whether these regulatory T cells play a role in the suppression of EAC by low-dose OVA-feeding, thymectomised mice treated either anti-CD25 Abs or nrIgG underwent EAC induction and OVA-feeding. When the thymectomised mice were treated with anti-CD25 Abs, OVA-feeding no longer significantly suppressed EAC. This is a strong indication that the suppression of EAC by low-dose OVA-feeding is mediated by CD4 CD25 regulatory T cells. However, data should be interpreted with caution, since the anti-CD25 depleted mice had a trend towards reduced eosinophil numbers. This may be due to the fact that CD25 ablation is likely to result in a more variable disease response, and therefore it may require larger group sizes for an effect to be observed.

Specific immunotherapy (SIT) is a common therapeutic approach for treating a number of allergic diseases where the allergen is known, and has been used successfully for treating rhinoconjunctivitis.^{9–10} In fact, Wessel *et al*²⁴ have reported its high safety by using an SQ-standardised grass allergy sublingual immunotherapy tablet (GRAZAX) when taken over a 3-year period. Further experiments will be necessary to discern whether GRAZAX is effective in a ragweed pollen-induced model of EAC.

Recently, an approach to use staple food as a source of Ag was developed.²⁵ This approach is quite reasonable, as we consume staple foods every day and thus the Ag will be supplied continuously through the gastrointestinal tract. An example of this approach is the transgenic rice that expresses murine dominant T cell-epitope peptides of the Cry j I and Cry j II allergens of Japanese cedar pollen.²⁶ This approach effectively suppresses the pollen-induced clinical symptoms in an experimental sneezing mouse model. Our next study will examine the effects of this transgenic rice in a Japanese cedar pollen-induced mouse allergic conjunctivitis model. The present study delineated optimal timing and Ag-feeding dose regimens, providing valuable information for future studies in this field.

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REFERENCES

- Friedlaender MH. Immunologic aspects of diseases of the eye. *JAMA* 1992;268:2869–73.
- Bielory L. Update on ocular allergy treatment. *Expert Opin Pharmacother* 2002;3:541–53.
- Valenta R, Campana R, Marth K, *et al*. Allergen-specific immunotherapy: from therapeutic vaccines to prophylactic approaches. *J Intern Med* 2012;272:144–57.
- Weiner HL. Oral tolerance. *Proc Natl Acad Sci USA* 1994;91:10762–5.
- Weiner HL, Friedman A, Miller A, *et al*. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu Rev Immunol* 1994;12:809–37.
- Faria AM, Weiner HL. Oral tolerance: therapeutic implications for autoimmune diseases. *Clin Dev Immunol* 2006;13:143–57.
- Brozek JL, Terracciano L, Hsu J, *et al*. Oral immunotherapy for IgE-mediated cow's milk allergy: a systematic review and meta-analysis. *Clin Exp Allergy* 2012;42:363–74.
- Nelson HS. Advances in upper airway diseases and allergen immunotherapy. *J Allergy Clin Immunol* 2006;117:1047–53.
- Pajno GB, Finegold I. SIT beyond respiratory diseases. *Ann Allergy Asthma Immunol* 2011;107:395–400.

Laboratory science

- 10 Calderon MA, Simons FE, Malling HJ, *et al.* Sublingual allergen immunotherapy: mode of action and its relationship with the safety profile. *Allergy* 2012;67:302–11.
- 11 Koizumi T, Abe T. Induction of oral tolerance to experimental allergic conjunctivitis in rats. *Nihon Ganka Gakkai Zasshi* 1995;99:515–20.
- 12 Zemmann B, Schwaerzler C, Griot-Wenk M, *et al.* Oral administration of specific antigens to allergy-prone infant dogs induces IL-10 and TGF-beta expression and prevents allergy in adult life. *J Allergy Clin Immunol* 2003;111:1069–75.
- 13 Marchant A, Bruyns C, Vandenabeele P, *et al.* Interleukin-10 controls interferon-gamma and tumor necrosis factor production during experimental endotoxemia. *Eur J Immunol* 1994;24:1167–71.
- 14 Dasch JR, Pace DR, Waegell W, *et al.* Monoclonal antibodies recognizing transforming growth factor-beta. Bioactivity neutralization and transforming growth factor beta 2 affinity purification. *J Immunol* 1989;142:1536–41.
- 15 Shoji J, Sakimoto T, Muromoto K, *et al.* Comparison of topical dexamethasone and topical FK506 treatment for the experimental allergic conjunctivitis model in BALB/c mice. *Jpn J Ophthalmol* 2005;49:205–10.
- 16 Magone MT, Chan CC, Rizzo LV, *et al.* A novel murine model of allergic conjunctivitis. *Clin Immunol Immunopathol* 1998;87:75–84.
- 17 Haneda K, Sano K, Tamura G, *et al.* TGF-beta induced by oral tolerance ameliorates experimental tracheal eosinophilia. *J Immunol* 1997;159:4484–90.
- 18 Sumi T, Fukushima A, Fukuda K, *et al.* Thymus-derived CD4+ CD25+ T cells suppress the development of murine allergic conjunctivitis. *Int Arch Allergy Immunol* 2007;143:276–81.
- 19 Fukushima A, Yamaguchi T, Azuma M, *et al.* Involvement of programmed death-ligand 2 (PD-L2) in the development of experimental allergic conjunctivitis in mice. *Br J Ophthalmol* 2006;90:1040–5.
- 20 Faria AM, Maron R, Ficker SM, *et al.* Oral tolerance induced by continuous feeding: enhanced up-regulation of transforming growth factor-beta/interleukin-10 and suppression of experimental autoimmune encephalomyelitis. *J Autoimmun* 2003;20:135–45.
- 21 Min SY, Hwang SY, Park KS, *et al.* Induction of IL-10-producing CD4+CD25+ T cells in animal model of collagen-induced arthritis by oral administration of type II collagen. *Arthritis Res Ther* 2004;6:213–19.
- 22 Fadok VA, Bratton DL, Konowal A, *et al.* Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest* 1998;101:890–8.
- 23 Fukushima A, Sumi T, Fukuda K, *et al.* Interleukin 10 and transforming growth factor beta contribute to the development of experimentally induced allergic conjunctivitis in mice during the effector phase. *Br J Ophthalmol* 2006;90:1535–41.
- 24 Wessel F, Chartier A, Meunier JP, *et al.* Safety and tolerability of an SQ-standardized GRAss Allergy immunotherapy tablet (GRAZAX®) in a real-life setting for three consecutive seasons—the GRAAL trial. *Clin Drug Investig* 2012;32:451–63.
- 25 Takaiwa F. Seed-based oral vaccines as allergen-specific immunotherapies. *Hum Vaccin* 2011;7:357–66.
- 26 Takagi H, Hiroi T, Yang L, *et al.* A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. *Proc Natl Acad Sci USA* 2005;102:17525–30.



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