

ORIGINAL ARTICLE

Depletion of CD4⁺CD25⁺ T cells switches the whey-allergic response from immunoglobulin E- to immunoglobulin free light chain-dependent

B. C. A. M. van Esch^{1,2}, B. Schouten¹, B. R. J. Blokhuis¹, G. A. Hofman¹, L. Boon³, J. Garssen^{1,2}, L. M. J. Knippels², L. E. M. Willemsen¹ and F. A. Redegeld¹

¹Division of Pharmacology and Pathophysiology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands,

²Immunology, Danone Research Centre for Specialised Nutrition, Wageningen, The Netherlands and ³Bioceros B. V., CM Utrecht, The Netherlands

Clinical & Experimental Allergy

Summary

Background Symptoms of allergy are largely attributed to an IgE-mediated hypersensitivity response. However, a considerable number of patients also exhibit clinical features of allergy without detectable systemic IgE. Previous work showed that Ig-free light chains (IgLC) may act as an alternate mechanism to induce allergic responses. CD4⁺CD25⁺ T cells are crucial in the initiation and regulation of allergic responses and compromised function might affect the response to allergens.

Objective To examine the contribution of CD4⁺CD25⁺ T cells and IgLC towards the whey-allergic response.

Methods Mice were sensitized orally with whey using cholera toxin as an adjuvant. CD25⁺ T cells were depleted *in vivo* using a CD25 mAb. The acute allergic skin response to whey and *ex vivo* colon reactivity was measured in the presence or absence of F991, a specific inhibitor of IgLC. Serum whey-specific antibodies and IgLC in serum and mesenteric lymph node (MLN) supernatants were measured. Depletion of CD4⁺CD25⁺ T cells was confirmed in the spleen.

Results Anti-CD25 treatment strongly reduced whey-specific antibody levels and resulted in a partial depletion of effector T cells and a major depletion of Foxp3⁺ regulatory T cells.

Surprisingly, despite the abolished specific IgE response, the acute allergic skin response to whey was not affected. IgLC levels were enhanced in the serum and MLN supernatants of CD25-depleted sensitized mice. F991 inhibited the acute skin response and colon hyperreactivity in anti-CD25-treated mice, indicating that these responses were mainly IgLC dependent.

Conclusions Depletion of CD4⁺CD25⁺ T cells resulted in a switch from an IgE- to an IgLC-dependent acute skin response and functional hyperresponsiveness of the colon. Our data suggest that CD25⁺ T cells play a crucial role in balancing cow's milk allergy between IgE and IgE-independent responses and both mechanisms might play a role in allergic responses to the same allergen.

Keywords atopic disease, CD25 depletion, cow's milk allergy, effector T cells, food allergy, IgE, Ig-free light chain, regulatory T cells

Submitted 11 November 2009; revised 11 January 2010; accepted 9 February 2010

Correspondence:

Johan Garssen, Division of Pharmacology and Pathophysiology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands.
E-mail: j.garssen@uu.nl

Introduction

The incidence of atopic diseases is increasing world-wide. It is hypothesized that the factors responsible for this may be found in changing environmental conditions, a westernized life style, air pollution and the increased consumption of additives and preservatives with food. Similar trends have been observed for the prevalence of auto-immune diseases such as type I diabetes and multiple

sclerosis. Regulatory and effector T cells play a crucial role in balancing the immune system, and a compromised function of CD4⁺CD25⁺ T cells may provide an explanation for the increased immunological response to allergens and auto-allergens.

Cow's milk allergy is one of the leading causes of food allergy in children [1, 2]. Clinical symptoms affect the skin, respiratory and gastrointestinal tract and are mostly

caused by IgE-induced hypersensitivity [3, 4]. However, it should be realized that in atopic diseases like food allergy, rhinitis and asthma, a considerable number of patients exhibit clinical features of allergy without detectable local or systemic IgE [5–7]. Mast cells have been implicated in the pathogenesis of atopic and non-atopic diseases and several *in vivo* studies indicate that they are involved in allergic asthma and gastrointestinal allergy [8–12]. Although antigen-specific mast cell activation results from crosslinking the high-affinity IgE receptor, FcεR1, other mechanisms may also be involved in antigen-specific activation in the absence of IgE antibodies. In previous studies, a novel mechanism for the elicitation of immediate hypersensitivity-like reactions via Ig-free light chain (IgLC) has been described in the skin and airways [13–16]. Moreover, IgLC-elicited hypersensitivity responses can be inhibited by local or systemic application of the IgLC antagonizing peptide F991 [13, 15, 16]. Ig light chains are assembled with Ig heavy chains into complete antibodies. However, a substantial part of the synthesized Ig light chains is secreted as a free polypeptide. Elevated levels of IgLC are found in asthma and rhinitis patients, suggesting a role for IgLC in human atopic diseases [16–18].

In the present study, the contribution of CD4⁺ CD25⁺ T cells and IgLC towards acute allergic symptoms and intestinal responses in cow's milk allergy was assessed using *in vivo* CD25 depletion. The results indicate that after depletion or functional impairment of CD4⁺ CD25⁺ T cells, the nature of the acute allergic response to whey changed from IgE to IgLC dependent. This suggests that depending on the immunologic milieu, an IgLC- or an IgE-dependent allergic response to the same allergen can be elicited.

Materials and methods

Reagents and antibodies

Whey was obtained from DMV International (Veghel, the Netherlands). Cholera toxin was purchased from Quadratech Diagnostics (Epsom, UK). Biotin-labelled rat anti-mouse IgE, IgG₁ and perCp-conjugated anti-CD4, PE-conjugated antiCD25 and isotype control mAb were from BD Biosciences (Alphen a/d Rijn, the Netherlands). APC-conjugated Foxp3 and isotype control was from eBioscience (San Diego, CA, USA). Rat anti-CD25 mAb (clone PC61) was kindly provided by Dr Louis Boon (Bioceros BV, Utrecht, the Netherlands). All other chemicals were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands).

Immunoglobulin free light chain-binding compound, F991

F991 is a 9-mer peptide and has been demonstrated to specifically inhibit IgLC-induced hypersensitivity responses [13, 15]. F991 was synthesized by Fmoc Chemistry (Ansynth, Roosendaal, the Netherlands).

Mice

Three- to 4-week-old pathogen-free female C3H/HeOuJ mice were purchased from Charles River Laboratories (Maastricht, the Netherlands), bred and raised on cow's milk protein-free standard mouse chow (AIN-93G soja, Special Diets Services, Witham, Essex, the UK) and housed in the animal facility at the Utrecht University. Animal care and use were performed in accordance with the guidelines of the Dutch Committee of Animal Experiments.

Oral sensitization and *in vivo* administration of anti-CD25 mAb

Mice were sensitized orally, using a blunt needle, on days 0, 7, 14, 21 and 28 with 20 mg whey per animal homogenized in PBS (0.5 mL, Cambrex Bio Science, Verviers, Belgium) mixed with 10 µg cholera toxin (Quadratech Diagnostics, Epsom, the UK) as an adjuvant. Non-sensitized mice received cholera toxin only. At day 14 just before the third sensitization, mice were injected intraperitoneally with 400 µg rat anti-mouse CD25 mAb (PC61). Non-sensitized mice and control mice received a non-specific IgG₁ isotype control antibody (Sigma-Aldrich-Chemie, Zwijndrecht, the Netherlands), according to the same schedule. One week after the last sensitization, the ear swelling at 1 h after intradermal whey challenge and *ex vivo* colon reactivity to carbachol were measured. Blood samples, mesenteric lymph nodes (MLNs) and spleen were collected. Blood samples were spun down for 15 min at 20 000 *g*. Sera and MLN cell supernatants were stored at –70 °C for determination of whey-specific antibodies and total IgLC.

Flow cytometer analysis of CD4⁺ CD25⁺ T cells

The efficacy of systemic CD25 depletion was confirmed in the spleen using cell surface monoclonal antibodies directed against CD4 (L3T4), CD25 (PC61) and Foxp3 (FJK-16). Single-cell suspensions prepared from the spleen were incubated for 30 min with blocking buffer (PBS containing 5% FCS, 1% BSA). Subsequently, 5 × 10⁵ cells were incubated for 20 min with 100 µL FACS buffer (PBS with 1% FCS, 1% BSA) containing perCp-conjugated anti-CD4, PE-conjugated anti-CD25 or isotype controls. For the detection of Foxp3, cells were subsequently permeabilized and incubated with anti-Foxp3 according to the manufacturer's protocol. Stained cells were analysed using a BD FACSCalibur flow cytometer.

Measurement of serum immunoglobulins

Whey-specific IgE and IgG₁ levels were measured in serum by means of ELISA. Microton plates (Greiner, Alphen a/d

Rijn, the Netherlands) were coated with 20 µg whey in coating buffer (Sigma-Aldrich) for 18 h at 4 °C. Plates were washed and blocked for 1 h with buffer containing 50 mM Tris, 2 mM EDTA and 137 mM NaCl/0.05% Tween and 5% BSA. Serum samples were incubated for 2 h at room temperature. Plates were washed and incubated with 1 µg biotin-labelled rat anti-mouse IgE or IgG₁ (Pharmingen, Alphen a/d Rijn, the Netherlands) for 1 h at room temperature. After washing, the plates were incubated with streptavidin-horse radish peroxidase (Sanquin, Amsterdam, the Netherlands) for 1 h, washed and developed with *o*-phenylenediamine (Sigma). The reaction was stopped after 10 min with 4 M H₂SO₄ and absorbance was measured at 490 nm on a Benchmark microplate reader (Biorad, Veenendaal, the Netherlands). For measuring whey-specific IgE, a serum dilution factor of 4 and for whey-specific IgG₁ a dilution factor of 50 was used for antibody detection. The whey-specific antibodies were expressed as OD.

Acute allergic skin response

An acute allergen-specific ear swelling in whey-sensitized mice was determined at 1 h after intradermal challenge with 10 µg whey in the ear pinnae. As a negative control, non-sensitized mice were challenged in the ear with whey. Ear thickness was measured in duplicate using a digital micrometer (Mitutoyo, Veenendaal, the Netherlands). The allergen-specific net ear swelling was calculated by correcting the allergen-induced increase in ear thickness with the non-specific ear swelling due to local injection in the non-sensitized mice. The delta ear swelling is expressed as µm. To investigate the contribution of IgLC towards the acute allergic skin response, F991 was applied topically in a cetomacrogol cream (1 mg F991/g cream) on the inside of the ear pinnae 3 h before the intradermal ear challenge as described earlier [15]. The cream (1 cm stripe equalling 20 mg of cream) was dispensed using an eye-cream tube and spread out using an inert spatula. Cetomacrogol cream FNA is a hydrophilic cream that is normally used in indifferent treatment of skin conditions and was readily taken up by the mouse skin.

Measurement of immunoglobulin free light chain in the serum and mesenteric lymph node supernatants

On day 35, MLNs were incubated in RPMI 1640 (1 × 10⁶ cells/mL) without any supplements for 24 h in the absence of allergen. The supernatant was collected and analysed for IgLC. Serum samples were precipitated as described [19]. In brief, 20 µL of serum was depleted from albumin by resuspension in 80 µL ice-cold 10% TCA/acetone, followed by incubation for 90 min at -20 °C. Samples were centrifuged for 20 min at 15 000 g at 4 °C. The supernatant was removed and the pellet was washed with 1 mL ice-cold acetone and dissolved in Laemmli buffer. A small volume was run on a

12% SDS-PAGE gel, followed by electro transfer to PVDF (Biorad). Gels were run under non-reducing conditions. Membranes were blocked o/n in PBS-T with 2% non-fat dry milk. IgLC were detected with anti-mouse kappa light-chain mAb conjugated to horse radish peroxidase (Southern Biotech, Birmingham, AL, USA). Immunoreactive bands were visualized with ECL and an X-Ray film (Pierce, Etten-Leur, the Netherlands). Films were scanned and analysed on a GS710 Calibrated Imaging Densitometer equipped with Quantity One v. 4.0.3 software (Biorad).

Isometric contraction of the colon

The colon, caudal from the caecum, was dissected free of connective tissue and mesenterium. Colon parts of 1 cm length were mounted in an organ bath, using two small clamps, containing 10 mL Tyrode buffer [NaCl, 136.89; KCl, 2.68; MgCl₂, 1.05; CaCl₂ 1.77; NaH₂PO₄, 0.42; NaHCO₃, 11.9 and glucose, 5.55 (mM)]. The organ bath was maintained at 32 °C, to reduce spontaneous contraction of the colon, and was continuously gassed with a 5% CO₂ and 95% O₂ gas mixture. One clamp was attached to a fixed point in the organ bath and the other clamp was connected to an isometric transducer (Harvard Apparatus Ltd, Kent, the UK) with an analog recorder (BD 40 Kipp & Zn., Delft, the Netherlands). Contractions were measured under a constant preload of 1.00 g. The preparations were equilibrated for one hour in the organ bath before starting a log dose-response curve for carbachol (10⁻⁸ until 10⁻⁵ M). After every dosage, the organ bath was flushed two times and the tissue was allowed to recover for 10 min before addition of the next concentration.

F991 was administered by an intraperitoneal injection (50 µg/100 µL) 18 h and 1 h before removal of the colon to investigate the contribution of IgLC towards colon reactivity in anti-CD25-treated mice.

Statistics

All data, except for the isometric contractions, were analysed using one-way ANOVA and *post hoc* Dunnett's test. Isometric contraction data were analysed using repeated measures ANOVA and *post hoc* Dunnett's test. A probability value *P* < 0.05 was considered significant. Statistical analyses were conducted using GraphPad Prism software. Data are represented as mean ± SEM of six mice per group.

Results

Anti-CD25 treatment abolished the production of whey-specific immunoglobulins

In whey-sensitized mice, whey-specific serum IgE and IgG₁ levels were augmented at day 35 when compared

with non-sensitized mice. Surprisingly, administration of anti-CD25 mAb abolished the induction of whey-specific IgE and strongly reduced IgG₁ levels (Fig. 1). Previous studies showed no detectable levels of IgE at day 14 of the sensitization protocol, indicating that no whey-specific IgE was present when anti-CD25 mAb was administered. Although not significantly different from non-sensitized mice, low levels of whey-specific IgG₁ were present at day 14 (data not shown).

Depletion of CD4⁺CD25⁺ T cells

To discriminate between CD25⁺ regulatory T cells and activated CD25⁺ effector T cells, Foxp3 was used as an additional marker to identify regulatory T cells. In the current experiments, CD4⁺CD25⁺Foxp3⁺ T cells were depleted *in vivo* after administration of anti-CD25 mAb 1 h before the third sensitization ($0.25 \pm 0.091\%$ of total cells vs. $8.73 \pm 0.74\%$ of total cells for isotype controls in

the spleen, $P < 0.01$). Anti-CD25 treatment also partially depleted CD4⁺CD25⁺Foxp3⁺ T cells ($2.88 \pm 0.30\%$ of total cells vs. $4.40 \pm 0.23\%$ of the total cells for isotype controls, $P < 0.05$), indicating that PC61 treatment applied systemically at day 14 during sensitization depleted regulatory T cells but also partially depleted or affected the generation of CD4⁺CD25⁺Foxp3⁺ T cells systemically.

Anti-CD25 treatment did not affect the acute allergic skin response

In the next experiments, it was determined whether the reduced IgE and IgG₁ production in anti-CD25-treated mice also resulted in an impaired allergic skin reaction after challenge with the allergen as well. As expected, intradermal skin challenge with whey induced a significant ear swelling at 1 h in sensitized mice compared with non-sensitized mice (142.1 ± 16.2 vs. 30.3 ± 14.5 μm ; Fig. 2). Unexpectedly, in the anti-CD25-treated animals, no statistically significant reduction in the acute ear-swelling response was observed (142.1 ± 16.2 vs. 120.2 ± 9.7 μm ; Fig. 2). These results indicated that the reduction in specific IgE and IgG₁ levels did not correlate with the ear-swelling response in the CD25-depleted mice and suggested that other mechanisms causing an allergic skin response may be involved.

Immunoglobulin free light chain levels in serum and mesenteric lymph node supernatants

In previous studies, IgLC was shown to mediate antigen-specific mast cell-dependent responses. Therefore, it was

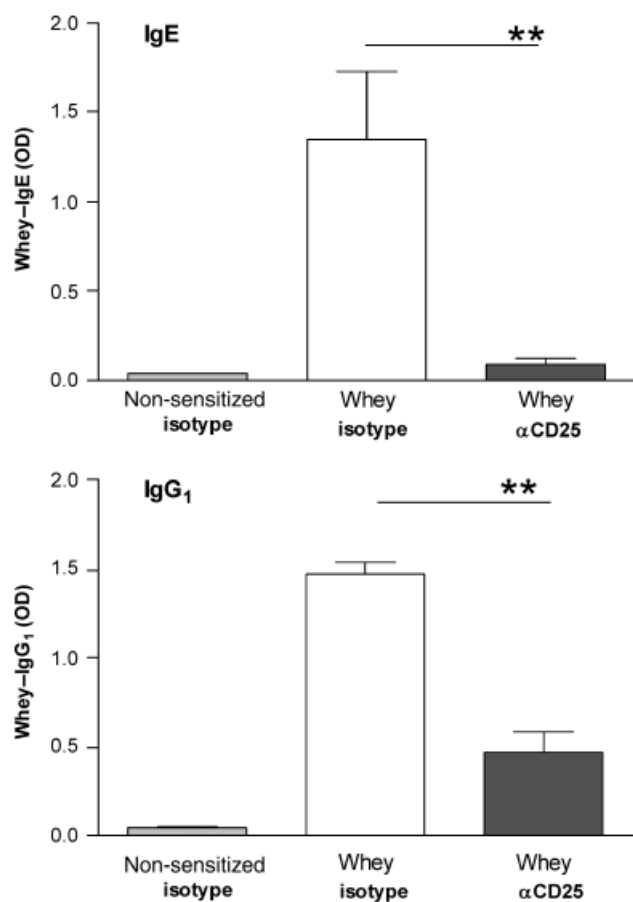


Fig. 1. Whey-specific antibodies were found in mice after sensitization to whey compared with non-sensitized mice. Administration of anti-CD25 mAb (α CD25) significantly reduced whey-specific IgE (four times dilution) and IgG₁ (50 times dilution) levels compared with isotype controls (** $P < 0.01$, $n = 6$). Whey-sensitized mice received 400 μg α CD25 mAb or isotype control mAb. Non-sensitized mice received an isotype control mAb.

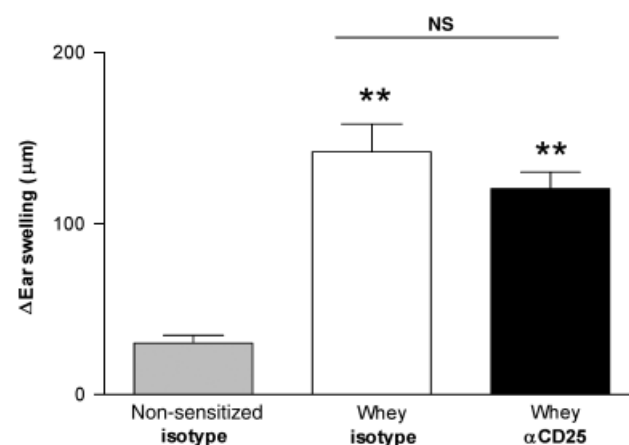


Fig. 2. The acute allergic skin response after local whey challenge was not affected in anti-CD25 mAb (α CD25)-treated whey-sensitized mice ($P > 0.27$). Local whey challenge (intradermal in ear) in both anti-CD25 mAb-treated and isotype control-treated whey-sensitized animals was enhanced compared with non-sensitized mice (** $P < 0.01$, $n = 6$). Whey-sensitized mice received 400 μg α CD25 mAb or isotype control mAb. Non-sensitized mice received an isotype control mAb.

examined whether IgLC levels in serum and MLN supernatants from non-sensitized and whey-sensitized mice were detectable. Immunoblot analysis showed that IgLC protein expression was strongly increased in serum and MLN supernatants of whey-sensitized mice after anti-CD25 mAb treatment, while no increase in IgLC levels was found in the whey-sensitized control group as compared with the non-sensitized controls (Fig. 3).

Immunoglobulin free light chain-dependent acute allergic skin response in anti-CD25-treated whey-sensitized mice

To investigate whether the increase in IgLC was responsible for the sensitization of anti-CD25-treated mice, the whey-induced ear-swelling response was analysed in the absence or presence of the IgLC antagonist F991 (topical application); vehicle cream was applied as a control. F991 is a 9-mer peptide, which has been demonstrated to specifically inhibit IgLC-induced hypersensitivity responses. As expected, F991 did not inhibit the ear-

swelling response induced in whey-sensitized mice treated with the isotype control antibody (112.5 ± 6.0 vs. $103.7 \pm 5.0 \mu\text{m}$; Fig. 4), indicating that no IgLC was involved here and this response was mediated by IgE and/or IgG₁. However, the acute ear swelling in anti-CD25 mAb-treated whey-sensitized mice was significantly inhibited by F991 (90.8 ± 4.2 vs. $33.3 \pm 6.9 \mu\text{m}$; Fig. 4), suggesting that indeed the increased IgLC was responsible for systemic sensitization and the whey-induced allergic skin response in these mice.

Immunoglobulin free light chain-dependent colon hyperreactivity in anti-CD25-treated whey-sensitized mice

To investigate whether gastrointestinal symptoms were also affected by CD25 depletion, the *ex vivo* isometric contraction of the colon in response to carbachol was measured. In previous experiments, it was shown that no differences in colon reactivity were detected in whey-

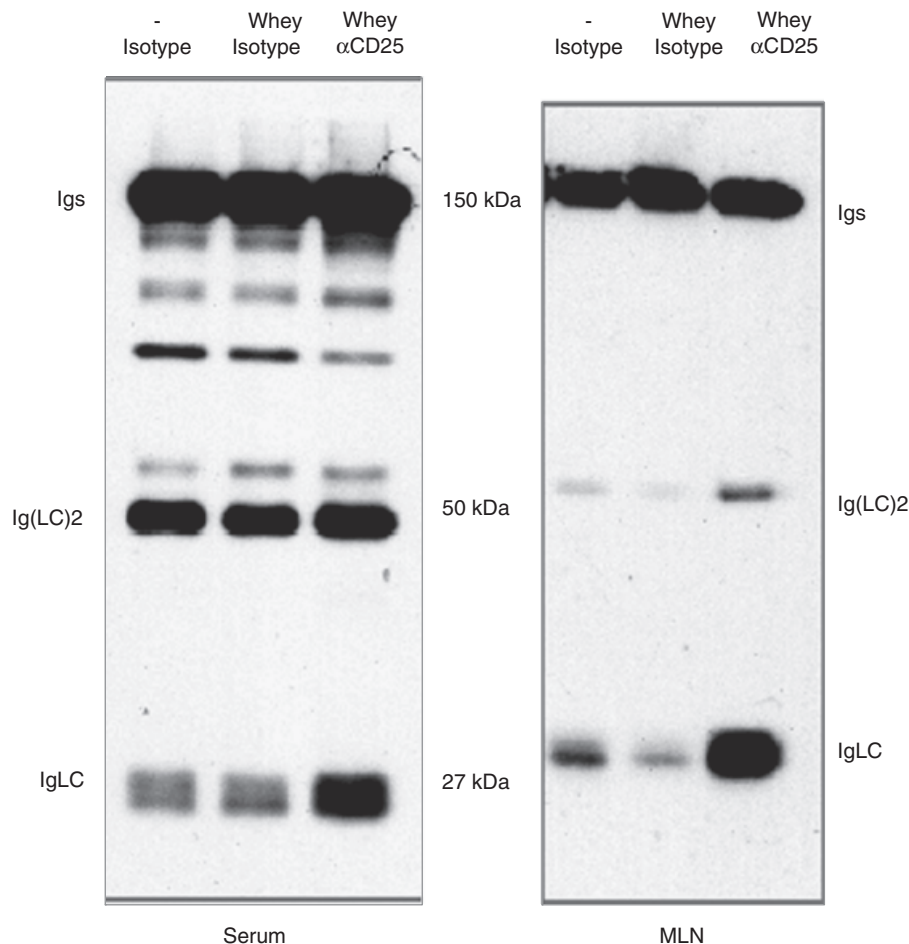


Fig. 3. Anti-CD25 mAb (αCD25) treatment in whey-sensitized mice increased the IgLC levels in serum and mesenteric lymph node (MLN) supernatants. Whey-sensitized mice received $400 \mu\text{g}$ αCD25 mAb or control mAb on day 14 during sensitization. Non-sensitized mice received an isotype control mAb. Representative blots are shown from $n = 3$ independent experiments. Gels were run under non-reducing conditions. IgLC, monomer free light chain; Ig(LC)₂, dimer IgLC.

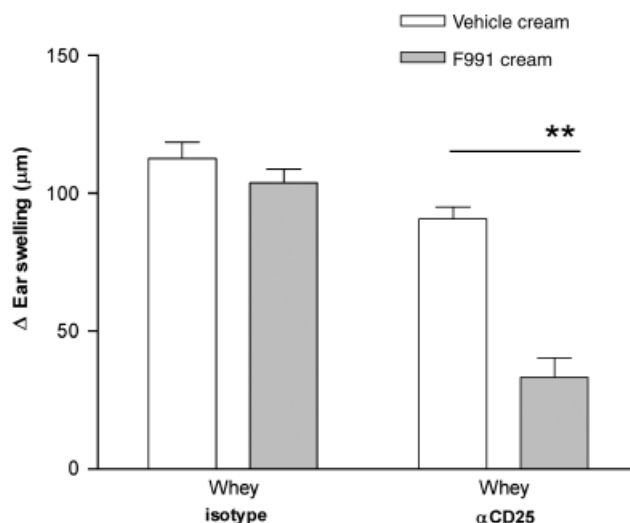


Fig. 4. The acute allergic skin response was measured in the presence or absence of the IgLC inhibitor F991. In mice treated with anti-CD25 mAb (α CD25), F991 inhibited the acute allergic skin response to whey when compared with vehicle cream-treated mice ($^{***}P < 0.01$, $n = 6$). Whey-sensitized mice received 400 μ g α CD25 mAb or isotype control mAb.

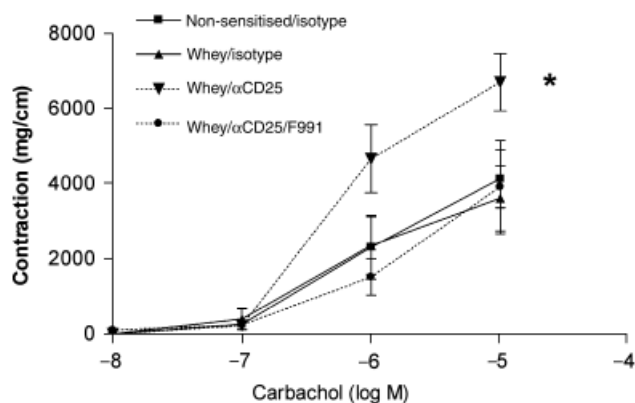


Fig. 5. Hyperreactivity of the colon in sensitized mice was observed after anti-CD25 mAb (α CD25) treatment compared with isotype controls ($^{*}P < 0.05$, $n = 6$). This hyperreactivity was abolished after administration of the IgLC inhibitor F991. Whey-sensitized mice received 400 μ g α CD25 mAb or isotype control mAb. F991 or PBS was injected intraperitoneally 24 h and 1 h before dissection of the colon.

sensitized mice compared with non-sensitized mice [20]. CD25 depletion resulted in a significant hyperreactivity of the colon when compared with control allergic mice (Fig. 5). To investigate whether IgLC was also involved in the induction of the colon hyperreactivity after CD25 depletion, whey-sensitized mice were treated *in vivo* with the IgLC inhibitor F991. Indeed, the carbachol-induced hyperreactivity of the colon was abolished after treatment of the anti-CD25-treated mice with F991, demonstrating the IgLC dependency of this response (Fig. 5).

Discussion

Acute allergic symptoms are generally closely associated with IgE-induced hypersensitivity; however, IgE-independent mechanisms may also be involved [21]. Previously, it was shown that in mice orally sensitized to whey, allergic responses correlated with whey-specific IgE and IgG₁ levels in serum [20]. The current study describes the involvement of CD4⁺CD25⁺ T cells in balancing the immune system between IgE and IgE-independent effector responses in allergic disease. Depletion of CD4⁺CD25⁺ T cells resulted in an abolished specific IgE response. Surprisingly, the allergic skin response to whey was not affected and was explained by the presence of IgLC in CD25-depleted mice. This study suggests that CD25⁺ T cells may determine the nature of allergic responses and could modulate a switch from an IgE- to an IgLC-driven response.

In the past few years, a novel mechanism for the elicitation of immediate hypersensitivity-like reactions via IgLC has been described. Transfer of antigen-specific IgLC into naïve mice sensitizes them to the respective antigen, resulting in local mast cell activation upon challenge, leading to oedema formation after skin challenge or acute bronchoconstriction after an intranasal challenge [13, 16]. IgLC-elicited hypersensitivity responses can be inhibited by local or systemic application of a specific antagonist F991 [13, 15]. In the current study, F991 was used to investigate whether the acute allergic skin response (ear swelling upon allergen challenge) in the CD25-depleted mice could be ascribed to IgLC. F991 does not interfere with IgE- or IgG-mediated mast cell activation [13]. Indeed, the allergic skin reaction was strongly inhibited by F991. An involvement of IgG₁ in the remaining part of the acute skin response cannot be excluded because CD25 depletion did not completely reduce whey-specific IgG₁ levels. In accordance with the indicated Ig switch, it was observed that the IgLC concentration in the serum and production by MLN cells upon *in vivo* anti-CD25 mAb treatment were significantly increased. Recent studies have shown enhanced IgLC serum concentrations in humans affected with immune disorders, like rheumatoid arthritis [22, 23] and multiple sclerosis [24, 25]. Moreover, elevated IgLC concentrations are found in rhinitis and asthmatic patients, suggesting a role for IgLC in human allergic disease [16, 17].

It has been shown that anti-CD25 mAb (PC61) selectively depletes naturally occurring CD4⁺CD25⁺Foxp3⁺ regulatory T cells in autoimmune disease and more recently in allergic disease [26, 27]. Selective depletion of CD4⁺CD25⁺Foxp3⁺-regulatory T cells using the PC61 mAb was found to enhance allergen-specific IgE and allergic symptoms in mouse models of peanut allergy, allergic asthma [28] and allergic conjunctivitis [29]. In contrast to our study, where the CD25 mAb was administered at day 14 only, the CD25 mAb was applied before

sensitization and on day 14 of the sensitization protocol. In the latter study, the mice were also thymectomized to further exclude the presence of thymus-derived regulatory T cells. However, CD25 is not a unique marker for regulatory T cells as it is also transiently expressed by allergen-specific effector T cells, which drive the allergic response after sensitization. CD25⁺-regulatory T cells counteract these effects and *in vivo* both cells are important players in the adaptive immune response. Parallel to the present study, *in vivo* application of anti-CD25 mAb in a mouse model of chronic *Toxoplasma gondii* infection was found to deplete both regulatory and effector T cells [30].

Depletion of CD25⁺ T cells during sensitization resulted in a combined depletion of CD4⁺Foxp3⁺ regulatory and to a lesser extent CD4⁺ effector T cells. Different mechanisms can underlie the induced IgLC-dependent acute allergic responses. Regulatory CD25⁺ T cells might directly interact with B cells [31, 32], and loss of these cells may lead to aberrant Ig responses. On the other hand, allergen-specific CD25⁺ effector T cells are required for B cell isotype switch and differentiation of the B cell into IgE/IgG₁-secreting plasma cells. *In vitro* polyclonal stimulation of mouse spleen cells showed that the release of IgLC vs. the amount incorporated into complete Igs depends on the strength of stimulation [33], suggesting that the suboptimal function of CD25⁺ effector T cells might affect the differentiation of B cells, resulting in the production of IgLC upon sensitization rather than inducing whey-specific antibodies. Hence, the compromised function of regulatory T cells combined with dysfunctional effector T cell responses might have been responsible for induction of IgLC rather than IgE/IgG₁.

Apart from investigating the acute allergic skin response, the effects on intestinal motility using *ex vivo* carbachol-induced muscle contraction were investigated. These reactions are generally not associated with specific serum IgE [34] and it is known that food allergy may have adverse effects on gut health, causing motility disturbances [35, 36]. In previous studies, it was shown that motility of the colon was not affected in mice sensitized to whey [20]. In this study, it is shown that depletion of CD25⁺ T cells results in a hyperresponsiveness of the colon. Development of this hyperresponsiveness could be inhibited by *in vivo* treatment with the specific IgLC antagonist F991, showing the involvement of IgLC in this change in motility response. Hence, this suggests that CD4⁺CD25⁺ T cells may contribute to the mechanism underlying non-IgE-mediated motility disorders of the intestine in food allergy. In line with these results, the development of tracheal hyperreactivity in a mouse model for non-atopic asthma was shown to be IgLC dependent [16].

The current study showed that CD4⁺CD25⁺ T cells may play a role in balancing between IgE-dependent and IgE-independent responses in allergic disease. Depletion of

CD25⁺ T cells changed the nature of the allergic response to cow's milk proteins in mice from a specific-IgE-dependent acute allergic response to an IgLC-dependent response. Moreover, depletion of CD25⁺ T cells induced an IgLC-dependent colonic hyperresponsiveness. This suggests that depending on the immunologic milieu, an IgLC- or an IgE-dependent allergic response to the same allergen can be elicited. Further studies are needed to gain an insight into the underlying mechanisms and the contribution of regulatory and/or effector T cells in the balance between IgE-dependent and IgE-independent allergic responses and whether these two independent mechanisms may contribute to each other's allergic response.

References

- 1 Tariq SM, Matthews SM, Hakim EA, Stevens M, Arshad SH, Hide DW. The prevalence of and risk factors for atopy in early childhood: a whole population birth cohort study. *J Allergy Clin Immunol* 1998; 101:587–93.
- 2 Osterballe M, Hansen TK, Mortz CG, Host A, Bindslev-Jensen C. The prevalence of food hypersensitivity in an unselected population of children and adults. *Pediatr Allergy Immunol* 2005; 16:567–73.
- 3 Host A. Frequency of cow's milk allergy in childhood. *Ann Allergy Asthma Immunol* 2002; 89 (Suppl. 1):33–7.
- 4 Rhodes HL, Sporik R, Thomas P, Holgate ST, Cogswell JJ. Early life risk factors for adult asthma: a birth cohort study of subjects at risk. *J Allergy Clin Immunol* 2001; 108:720–5.
- 5 Host A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy* 1990; 45:587–96.
- 6 Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* 2002; 57:643–8.
- 7 Shek LP, Bardina L, Castro R, Sampson HA, Beyer K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. *Allergy* 2005; 60:912–9.
- 8 Peterson CG, Hansson T, Skott A, Bengtsson U, Ahlstedt S, Magnusson J. Detection of local mast-cell activity in patients with food hypersensitivity. *J Invest Allergol Clin Immunol* 2007; 17:314–20.
- 9 Amin K, Ludviksdottir D, Janson C *et al.* Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. *Am J Respir Crit Care Med* 2000; 162:2295–301.
- 10 Raithel M, Pacurar A, Winterkamp S, Dalbay S, Ell C, Hahn EG. Analysis and characteristics of mast cell tryptase and eosinophilic cationic protein from human gut mucosa in gastrointestinal allergy. *Monogr Allergy* 1996; 32:143–56.
- 11 Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* 2002; 57:677–82.
- 12 Kraneveld AD, van der Kleij HP, Kool M *et al.* Key role for mast cells in nonatopic asthma. *J Immunol* 2002; 169:2044–53.

- 13 Redegeld FA, van der Heijden MW, Kool M *et al*. Immunoglobulin-free light chains elicit immediate hypersensitivity-like responses. *Nat Med* 2002; **8**:694–701.
- 14 Paliwal V, Tsuji RF, Szczepanik M *et al*. Subunits of IgM reconstitute defective contact sensitivity in B-1 cell-deficient xid mice: kappa light chains recruit T cells independent of complement. *J Immunol* 2002; **169**:4113–23.
- 15 van Houwelingen AH, Kaczynska K, Kraneveld AD, Kool M, Nijkamp FP, Redegeld FA. Topical application of F991, an immunoglobulin free light chain antagonist, prevents development of contact sensitivity in mice. *Clin Exp Allergy* 2007; **37**:270–5.
- 16 Kraneveld AD, Kool M, van Houwelingen AH *et al*. Elicitation of allergic asthma by immunoglobulin free light chains. *Proc Natl Acad Sci USA* 2005; **102**:1578–83.
- 17 Groot Kormelink T, Thio M, Blokhuis BR, Nijkamp FP, Redegeld FA. Atopic and non-atopic allergic disorders: current insights into the possible involvement of free immunoglobulin light chains. *Clin Exp Allergy* 2009; **39**:33–42.
- 18 Powe DG, Kormelink TG, Sisson M *et al*. Evidence for the involvement of free light chain immunoglobulins in allergic and non-allergic rhinitis. *J Allergy Clin Immunol* 2010; **125**:139–45.
- 19 Chen YY, Lin SY, Yeh YY *et al*. A modified protein precipitation procedure for efficient removal of albumin from serum. *Electrophoresis* 2005; **26**:2117–27.
- 20 Schouten B, van Esch BC, Hofman GA, van den Elsen LW, Willemsen LE, Garssen J. Acute allergic skin reactions and intestinal contractility changes in mice orally sensitized against casein or whey. *Int Arch Allergy Immunol* 2008; **147**:125–34.
- 21 Johansson SG, Bieber T, Dahl R *et al*. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004; **113**:832–6.
- 22 Low JM, Chauhan AK, Moore TL. Abnormal kappa:lambda light chain ratio in circulating immune complexes as a marker for B cell activity in juvenile idiopathic arthritis. *Scand J Immunol* 2007; **65**:76–83.
- 23 Gottenberg JE, Aucouturier F, Goetz J *et al*. Serum immunoglobulin free light chain assessment in rheumatoid arthritis and primary Sjogren's syndrome. *Ann Rheum Dis* 2007; **66**:23–7.
- 24 Presslauer S, Milosavljevic D, Brucke T, Bayer P, Hubl W. Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis. *J Neurol* 2008; **255**:1508–14.
- 25 Rinker JR II, Trinkaus K, Cross AH. Elevated CSF free kappa light chains correlate with disability prognosis in multiple sclerosis. *Neurology* 2006; **67**:1288–90.
- 26 van Wijk F, Wehrens EJ, Nierkens S *et al*. CD4+CD25+ T cells regulate the intensity of hypersensitivity responses to peanut, but are not decisive in the induction of oral sensitization. *Clin Exp Allergy* 2007; **37**:572–81.
- 27 Long TT, Nakazawa S, Onizuka S, Huaman MC, Kanbara H. Influence of CD4+CD25+ T cells on *Plasmodium berghei* NK65 infection in BALB/c mice. *Int J Parasitol* 2003; **33**:175–83.
- 28 Lewkowich IP, Herman NS, Schleifer KW *et al*. CD4+CD25+ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function. *J Exp Med* 2005; **202**:1549–61.
- 29 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.
- 30 Couper KN, Lanthier PA, Perona-Wright G *et al*. Anti-CD25 antibody-mediated depletion of effector T cell populations enhances susceptibility of mice to acute but not chronic *Toxoplasma gondii* infection. *J Immunol* 2009; **182**:3985–94.
- 31 Lim HW, Hillsamer P, Banham AH, Kim CH. Cutting edge: direct suppression of B cells by CD4+CD25+ regulatory T cells. *J Immunol* 2005; **175**:4180–3.
- 32 Bystry RS, Aluvihare V, Welch KA, Kallikourdis M, Betz AG. B cells and professional APCs recruit regulatory T cells via CCL4. *Nat Immunol* 2001; **2**:1126–32.
- 33 Hannam-Harris AC, Smith JL. Induction of balanced immunoglobulin chain synthesis in free light chain-producing lymphocytes by mitogen stimulation. *J Immunol* 1981; **126**:1848–51.
- 34 De Boissieu D, Wagué JC, Dupont C. The atopy patch tests for detection of cow's milk allergy with digestive symptoms. *J Pediatr* 2003; **142**:203–5.
- 35 Crowe SE, Perdue MH. Gastrointestinal food hypersensitivity: basic mechanisms of pathophysiology. *Gastroenterology* 1992; **103**:1075–95.
- 36 Cummings JH, Antoine JM, Azpiroz F *et al*. PASSCLAIM – gut health and immunity. *Eur J Nutr* 2004; **43** (Suppl. 2):II118–73.