

Regulation by intestinal $\gamma\delta$ T cells during establishment of food allergic sensitization in mice

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Abstract

Background: Food allergy affects approximately 5% of children and is the leading cause of hospitalization for anaphylactic reactions in westernized countries. The mucosal adjuvant cholera toxin induces allergic sensitization to co-administered proteins in mice, while feeding the protein alone induces oral tolerance. Intestinal $\gamma\delta$ T cells could be of importance in the induction of oral tolerance. This study aims to investigate whether $\gamma\delta$ T cells have functional relevance in food allergic sensitization.

Methods: Changes in $\gamma\delta$ T cells on days 1, 2, 3, and 7 after initiation of food allergy were evaluated using flowcytometry. Furthermore, the anti- $\gamma\delta$ T-cell receptor (TCR) antibody UC7 was used to block the $\gamma\delta$ TCR in mice *in vivo*, followed by sensitization to peanut. After 4 weeks, peanut-specific antibodies in serum and cytokine production in spleen were measured.

Results: Induction of food allergy resulted in a profound decrease in the percentage of $\gamma\delta$ T cells in intestinal tissues and Peyer's Patches, but not in mesenteric lymph nodes or spleen. This decrease could be detected from days 1 to 2 after the initiation of food allergy and the number of $\gamma\delta$ T cells returned to normal on day 7. Blockade of the $\gamma\delta$ TCR resulted in elevated food allergic responses upon sensitization with peanut characterized by increased IgE and Th2 cytokine production in splenocytes.

Conclusion: These results demonstrate a unique regulatory role of $\gamma\delta$ T cells, suggesting that targeting $\gamma\delta$ T cells in the intestine may contribute to strategies to prevent and possibly treat food allergy.

Food allergy has emerged as a major health problem in the Western world and affects 4–6% of children, <3 years of age, and about 2% of the adult population in the United States. Allergy to peanuts and tree nuts is among the leading causes of fatal and near-fatal food-induced allergic reactions in the United States (1). In healthy individuals, the intake of food and food proteins results in an active state of nonresponsiveness, called oral tolerance. Extensive studies over the past years showed that oral tolerance is a unique feature of the (gut-associated) mucosal immune system and that defects in this process results in allergic sensitization to food proteins (1).

Remarkably, intestinal epithelial lymphocytes (IELs) are among the least studied cells in the process of allergic sensitization. Most IEL in the mouse consists of $\gamma\delta$ T cells, which are localized in the paracellular space between intestinal epithelial cells at the luminal site of the basement membrane

(2). It has been shown that treatment of mice with a T-cell receptor (TCR) δ -specific antibody resulted in impaired oral tolerance induction and oral tolerance could be transferred by means of $\gamma\delta$ T cells (3). In addition, $\gamma\delta$ T cells could also suppress Th2-dependent IgE responses without affecting parallel IgG responses to inhaled antigens (4).

Nevertheless, the role of $\gamma\delta$ T cells in the initiation of food allergy is far from understanding. The mucosal adjuvant cholera toxin (CT) induces allergic sensitization to co-administered proteins in allergy-susceptible mice, while feeding the protein alone induces oral tolerance. Therefore, CT can be used to model a pro-allergenic condition that makes it possible to follow the response and function of intestinal T cells during the establishment of allergic sensitization in mice.

In this study, we show that induction of food allergy by administration of CT results in direct effects on the pheno-

type and number of intestinal $\gamma\delta$ T cells. Furthermore, we used the anti- $\gamma\delta$ TCR antibody UC7 to study the functional role of $\gamma\delta$ T cells. We showed that treatment with UC7 results in a blockade of the $\gamma\delta$ TCR in our model, as suggested before (5). Interestingly, this blockade resulted in elevated peanut-specific responses upon sensitization to peanut extract (PE). This provides evidence for the involvement of $\gamma\delta$ T cells in the regulation of food allergic responses.

Materials and methods

Mice and reagents

Five-week-old specific pathogen-free female C57BL/6J and C3H/HeOuJ mice were purchased from Charles River (France). Experiments were approved by the Animal Experiments Committee of the Utrecht University. Peanuts were

kindly provided by Intersnack Nederland BV, and PE was prepared as previously described (6). Cholera toxin was purchased from List Biological Laboratories, Inc (Campbell, CA, USA).

Treatment protocols

For short-term CT + PE exposure, mice ($n = 4$) were orally exposed to phosphate buffer saline (PBS), PE (6 mg), CT (15 μ g) or PE mixed with CT on days 0, 1, and 2 and killed at days 1, 2, 3 and 7 (Fig. 1A).

To elicit oral sensitization toward PE, mice ($n = 8$) were orally gavaged with PE plus CT for three consecutive days (Fig. 5A), and dosing was repeated every week for 3 weeks (exposure on days 0, 1, 2, 7, 14, and 21). Control groups received PBS plus CT. All mice received a challenge of 12 mg PE on day 34 and were killed 1 day later. To investigate the

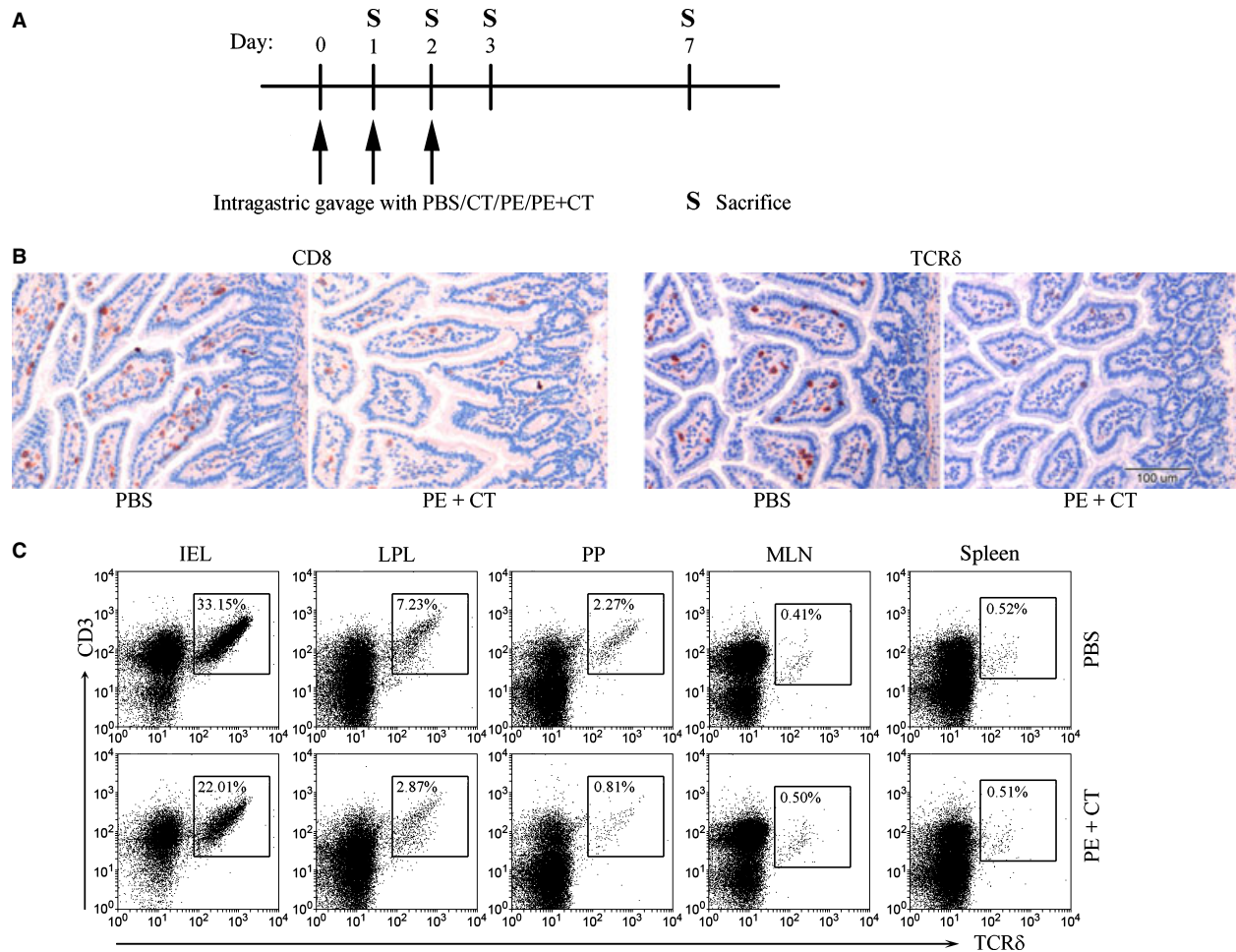


Figure 1 Effect of allergic sensitization on the number of $\gamma\delta$ T cells in various organs. (A) C57BL/6 mice ($n = 4$) were exposed to phosphate buffer saline (PBS), peanut extract (PE), cholera toxin (CT) or PE + CT for three consecutive days after which cells were isolated from intestine, MLN and spleen on the indicated days (S). (B) On day 3 cryosections of the small intestine of PBS-treated or

PE + CT-treated mice were stained for CD8 (left panels) or T-cell receptor (TCR) δ (right panels). Original magnitude $\times 100$, shown are representative tissue sections. (C) Representative dot plots of $\gamma\delta$ T cell analysis in various organs on day 3 after exposure to PBS or PE + CT. Lymphocytes were gated based on FSC-SSC pattern, followed by analysis for co-expression of CD3 and TCR δ .

role of $\gamma\delta$ T cells, mice were injected i.p. with 400 μg of the anti- $\gamma\delta$ TCR antibody UC7-13D5 (UC7) in PBS 2 days before the onset of oral sensitization. Injections with 200 μg antibody were repeated on days -1, 3, 6, 13, and 20 (Fig. 5A). No differences between PBS and hamster IgG (isotype) have been found in previous studies using the peanut sensitization model in our laboratory (7). To test the efficacy of UC7, mice ($n = 4$) received two i.p. injections of, respectively, 400 and 200 μg UC7 preceding initial oral exposure with PBS for 3 days and mice were killed at days 0, 3, 7 or 14 (Fig. 4A).

Isolation of intestinal lymphocytes

Intestinal epithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) were isolated as described previously (8, 9). In brief, the small intestine was removed from mice, and fecal contents were flushed with Hank's Balanced Salt Solution (HBSS) (Invitrogen, Breda, the Netherlands). After carefully removing Peyer's Patches (PP), the small intestine was cut into 1-cm pieces and shaken vigorously in HBSS/ethylenediaminetetraacetic acid (EDTA). The mixture was passed through a filter and remaining tissue was used to isolate LPL, whereas the supernatant was washed once, followed by isolation of IEL lymphocytes using a Percoll gradient (GE Healthcare, Hoevelaken, the Netherlands). For LPL isolation, the remaining tissue was shaken once again in HBSS/EDTA, washed, and digested with collagenase/DNAse (Roche, Almere, the Netherlands/Sigma, Zwijndrecht, the Netherlands). Cells were collected via a 100- μm filter, and tissue digestion was repeated. Both cell fractions were pooled and washed once. Thereafter, LPL cells were further enriched by a Percoll gradient (GE Healthcare). The collected PP were shaken in HBSS/EDTA, washed once, cut in small fragments, and digested at 37°C with collagenase/DNAse (Roche/Sigma). Cells were passed through a 70- μm gauze to obtain single cells. Lymphocytes from mesenteric lymph nodes (MLN) and spleen were obtained by squeezing the organs through a 70- μm gauze, after which single cells were washed once. To remove erythrocytes in spleen, cells were incubated with erythlysis buffer (containing NH_4Cl , KHCO_3 , and Na_2EDTA) for 1 min and washed once in PBS.

Tissue staining

Acetone-fixed cryostat sections of the small intestine were incubated for 1 h with anti TCR δ (GL3, hamster antibody) or anti CD8 α (53-6.7, rat antibody) diluted in PBS, 1% Bovine Serum Albumin (BSA). The antibodies were then visualized by first applying biotinylated anti-hamster or anti-rat antibodies (DAKO, Glostrup, Denmark) for 1 h, followed by 1 h incubation with horseradish peroxidase-labeled streptavidin (DAKO) and aminoethylcarbazole as a substrate. Sections were counterstained with Mayer's hematoxylin (DAKO) and mounted with DAKO Faramount Aqueous mounting medium. Images were acquired using an Olympus BX60 microscope with colorview III digital camera and CELL IMAGING software (Olympus, Zoeterwoude, the Netherlands).

Flow cytometry

For flow cytometric analysis, cells were preincubated with anti-CD16/CD32 (2.4G2) to block FcR, stained with fluorescent-labeled antibodies, and stored in 0.4% formaldehyde until acquisition. Following antibodies were used (obtained from e-Bioscience, San Diego, CA, USA or BD-Biosciences, Erembodegem, Belgium): CD3 (145-2C11); CD8 α (53-6.7); TCR β (H57-597); TCR δ (GL3). Data were acquired by means of FACSCalibur (Becton Dickinson, Breda, the Netherlands).

Measurement of PE-specific antibodies and mMCP-I in serum

Peanut extract-specific IgG1, IgG2a, and IgE in serum were measured by enzyme-linked immunosorbent assay (ELISA) as described previously (6). Serum was collected within 45 min after oral challenge, and mMCP-I was determined using a specific ELISA kit (Moredun scientific Ltd, Midlothian, Scotland). Enzyme-linked immunosorbent assay was performed according to instructions of the manufacturer.

Cell culture and cytokine measurement

Spleen and MLN single-cell suspensions ($2.5 \times 10^6/\text{ml}$) were incubated in the presence or absence of PE for 96 h at 37°C, after which culture supernatants were harvested and stored at -20°C until analysis. Levels of IFN- γ , IL-4, IL-5, IL-13, and IL-10 in culture supernatants were determined by sandwich ELISA according to the instructions of the manufacturers (BD-Pharmingen; e-Bioscience).

Statistical analysis

Data are presented as means $\pm$ standard error of the mean (SEM) and analyzed using GRAPHPAD PRISM software (La Jolla, CA, USA). One-way ANOVA was performed followed by Bonferroni as a *post hoc* test.

Results

Establishment of food allergy by CT treatment reduced $\gamma\delta$ T cells in the intestine, but not in the MLN and spleen

To investigate the role of $\gamma\delta$ T cells during the initiation of allergic sensitization, we studied whether sensitization to peanut by CT exposure changed the frequency of intestinal IEL and $\gamma\delta$ T cells in particular. Administration of CT + PE (and CT alone, data not shown) to C57BL/6 mice resulted in a decrease in CD8 α + cells in intestinal mucosa and lamina propria compared to control treatment, as shown by immunohistochemical analysis of the small intestine taken 3 days after the initial administration of PE + CT (Fig. 1B). This decrease in CD8 α cells was accompanied by a decrease in $\gamma\delta$ T cells, in line with the fact that a large proportion of CD8 α cells in the intestine are $\gamma\delta$ T cells. These changes in the number of $\gamma\delta$ T cells in the intestine were further quantified by flow cytometry and revealed a reduction in (CD8+)

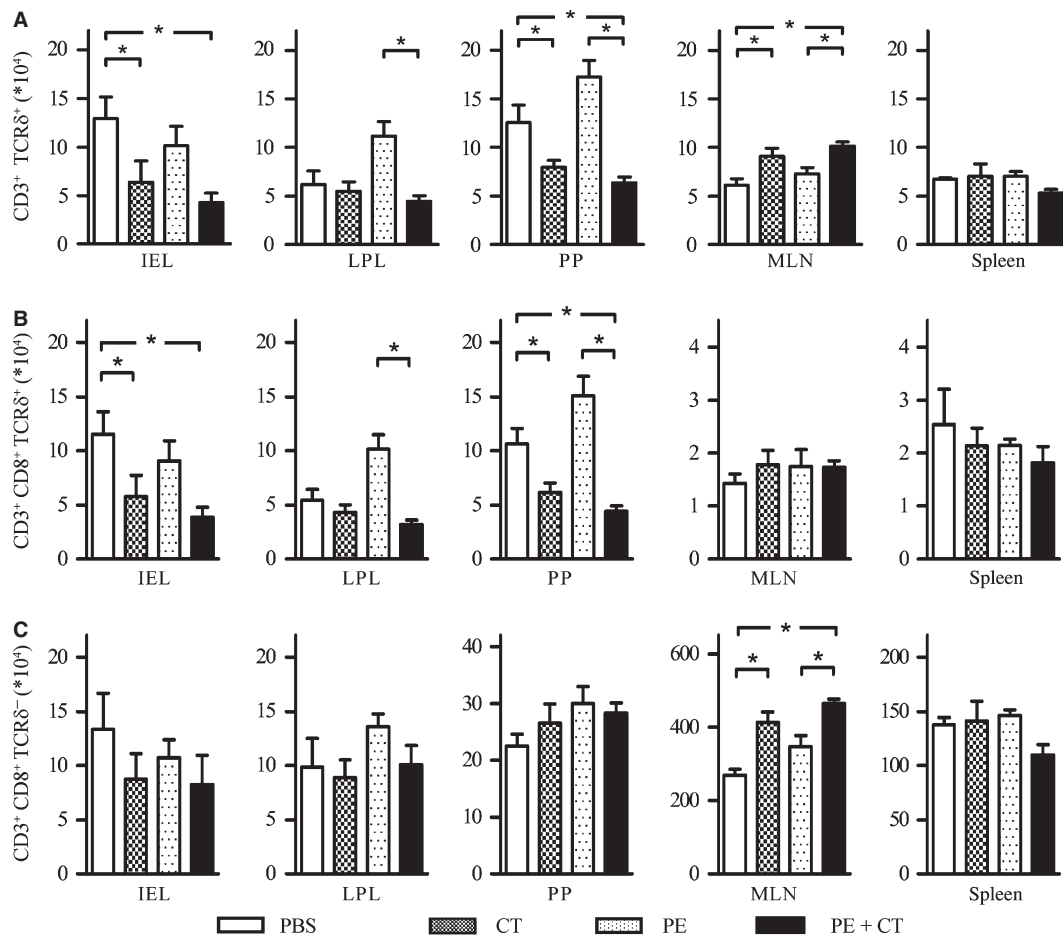


Figure 2 Effect of allergic sensitization on total $\gamma\delta$ T cell numbers and CD8⁺ $\gamma\delta$ T cell numbers in various organs. Mice were treated as described in Fig. 1A. (A–C): Flow cytometric analysis on day 3. Lymphocytes were gated based on FSC-SSC pattern and analyzed for the co-expression of indicated membrane staining.

(A) Number of CD3⁺ T-cell receptor (TCR)δ⁺ lymphocytes after treatment with phosphate buffer saline, cholera toxin (CT), peanut extract (PE) or PE + CT. * $P < 0.05$ (B) As in C for CD3⁺ CD8⁺ TCRδ⁺ lymphocytes (C). As in C for CD3⁺ CD8⁺ TCRδ⁻ lymphocytes.

$\gamma\delta$ T cells after treatment with CT or PE + CT in IEL and LPL fractions and PP, but not in spleen. In contrast, the number of $\gamma\delta$ T cells in MLN was increased upon CT or PE + CT treatment, although this increase was not observed within the CD8⁺ $\gamma\delta$ T cell fraction (Figs 1C and 2A,B).

The kinetics of the change in $\gamma\delta$ T cells in the intestine was further investigated (supplemental Fig. S1). The decrease in $\gamma\delta$ T cells could be detected from days 1 to 2 after administration of PE + CT or CT alone, and the number of cells returned to normal on day 7. Importantly, the observed decrease was specific for $\gamma\delta$ T cells, as expression of TCRδ within the total CD3⁺ cell population decreased (Fig. 3A), as did the ratio between TCRδ⁺ and TCRβ⁺ expression within the CD3⁺ T cell population after CT and PE + CT administration (Fig. 3B). Furthermore, the number of $\gamma\delta$ -CD8⁺ T cells was not altered upon CT or PE + CT treatment except for an increase within the MLN (Fig. 2C). Treatment with PE alone did not change the percentage or number of $\gamma\delta$ T cells. Comparable results were obtained

when using C3H/HeOuJ mice instead of C57BL/6 mice (data not shown).

Treatment with anti- $\gamma\delta$ TCR antibodies blocks the $\gamma\delta$ TCR

We investigated the functional role of $\gamma\delta$ T cells in the intestine by treating mice with a $\gamma\delta$ TCR-specific antibody (UC7). Controversy exists about the effect of treatment with this antibody on $\gamma\delta$ T cells *in vivo*. In our studies, administration of UC7 antibody during two consecutive days resulted in the inability to detect $\gamma\delta$ T cells in intestine and lymphoid organs, which lasted for at least 7 days (Fig. 4B–C). Most $\gamma\delta$ T cells in intestine are CD8α⁺ cells, implicating that depletion of $\gamma\delta$ T cells would also lead to reduced numbers of CD3⁺ CD8α⁺ (TCRβ⁻) cells. In contrast, the number of CD3⁺ CD8α⁺ cells and of CD3⁺ TCRβ⁻ cells remained unaltered in our model (Fig. 4D–E). Together, these findings confirm that treatment with UC7 blocks the $\gamma\delta$ TCR rather than depletes $\gamma\delta$ T cells.

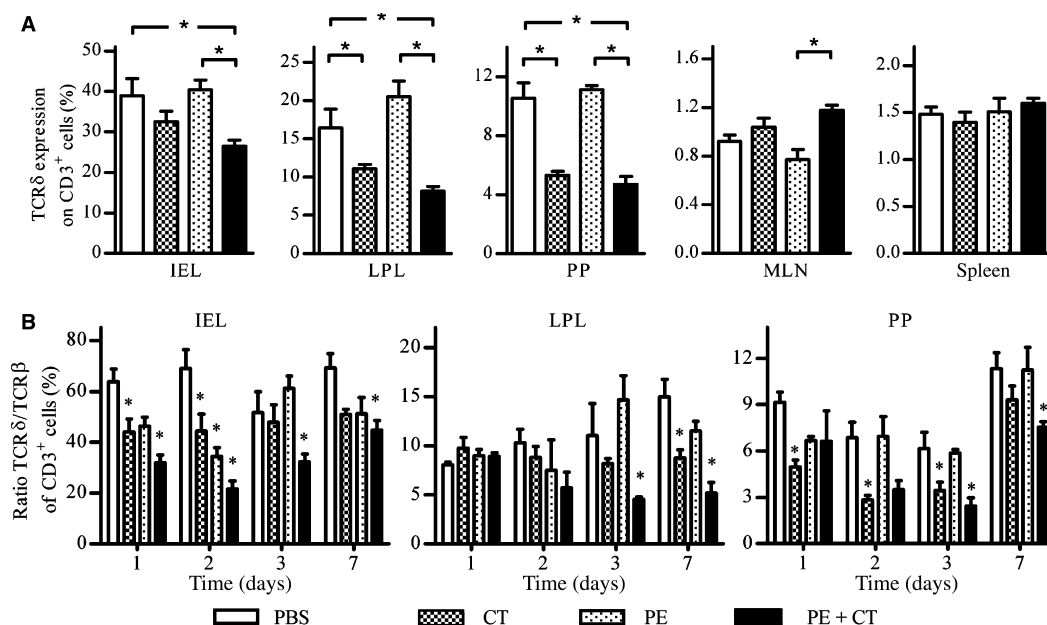


Figure 3 The effect of allergic sensitization is specific for $\gamma\delta$ T cells. C57BL/6 mice ($n = 4$) were treated with phosphate buffer saline (PBS), peanut extract (PE), cholera toxin (CT) or PE + CT for three consecutive days as indicated in Fig. 1A and isolated cells from intestine, MLN, and spleen were analyzed by flow cytometry. Lymphocytes were gated based on FSC-SSC pattern and analyzed for the co-expression of indicated membrane staining.

(A) Changes in the percentage of $\gamma\delta$ T-cell receptor (TCR) expression on CD3 $^{+}$ T cells on day 3 after indicated treatments. Numbers are depicted as mean $\pm$ standard error of the mean (SEM) from four mice. (B) Changes in the ratio of TCR δ + CD3 $^{+}$ vs TCR β + CD3 $^{+}$ T cell numbers in time. Percentages are depicted as mean $\pm$ SEM. * $P < 0.05$ compared to PBS treatment of the same day.

Treatment with anti- $\gamma\delta$ TCR antibodies resulted in enhanced sensitization to peanut

To elucidate the functional role of $\gamma\delta$ T cells in food allergy, C57BL/6 mice were sensitized with PE + CT and PE-specific responses were determined 4 weeks later (Fig. 5A). As expected, exposure to PE + CT resulted in the induction of PE-specific IgG1, IgG2a, and IgE. Interestingly, treatment with anti- $\gamma\delta$ TCR antibodies significantly increased PE-specific IgE levels without affecting IgG1 and IgG2a (Fig. 5B). Mice that were exposed to PE in the absence of CT did not develop PE-specific antibody responses, irrespective of anti- $\gamma\delta$ TCR treatment (data not shown). In addition, anti- $\gamma\delta$ TCR antibody treatment of C3H/HeOuJ mice, which are more prone to developing allergic manifestations in the food allergy model used here, also resulted in increased PE-specific IgE (Fig. 5C). Moreover, in these mice, *in vivo* mast cell degranulation upon oral PE challenge was detected by measuring the levels of mMCP-I in serum. Interestingly, blockade of $\gamma\delta$ TCR during sensitization increased mast cell degranulation *in vivo* (Fig. 5D).

To investigate PE-specific T cell responses, splenocytes and MLN-derived lymphocytes from sensitized C57BL/6 and C3H/HeOuJ mice were cultured for 96 h in the presence or absence of PE. Exposure to PE + CT resulted in the induction of IFN- γ , IL-13, IL-10, and IL-5 (Fig. 6). Treatment with anti- $\gamma\delta$ TCR antibodies significantly enhanced cytokine

production in C3H/HeOuJ mice, but not so profound in C57BL/6 mice. Together, these data demonstrate an increase in allergic manifestations, including IgE and mast cell degranulation level, upon $\gamma\delta$ TCR blockade.

Discussion

The present study shows that sensitization to PE (using CT as a mucosal adjuvant) was accompanied by a decrease in $\gamma\delta$ T cells in the intestine and PP and an increase in $\gamma\delta$ T cells in the MLN. In addition, we showed that blocking of the $\gamma\delta$ TCR during allergic sensitization to PE resulted in enhanced allergic manifestations to PE. Together these data suggest a regulatory role for $\gamma\delta$ T cells in allergic sensitization.

In mammals, $\gamma\delta$ T cells are most abundant in IEL and predominantly located in the paracellular space between intestinal epithelial cells on the luminal side of the basement membrane. Because of this location, they are ideally situated for crosstalk with intestinal epithelial cells and other immune cells (e.g. dendritic cells, B, and T cells) in the lamina propria (10). Several reports indicate that $\gamma\delta$ T cells are involved in the induction of oral tolerance to soluble food proteins (11, 12). In general, $\gamma\delta$ T cells are able to recognize and eliminate stressed or damaged cells (13). For instance, a study by Cardoso et al. described that sensitization to peanut followed by peanut challenge was accompanied by the induction of intestinal epithelial damage and reduced numbers of

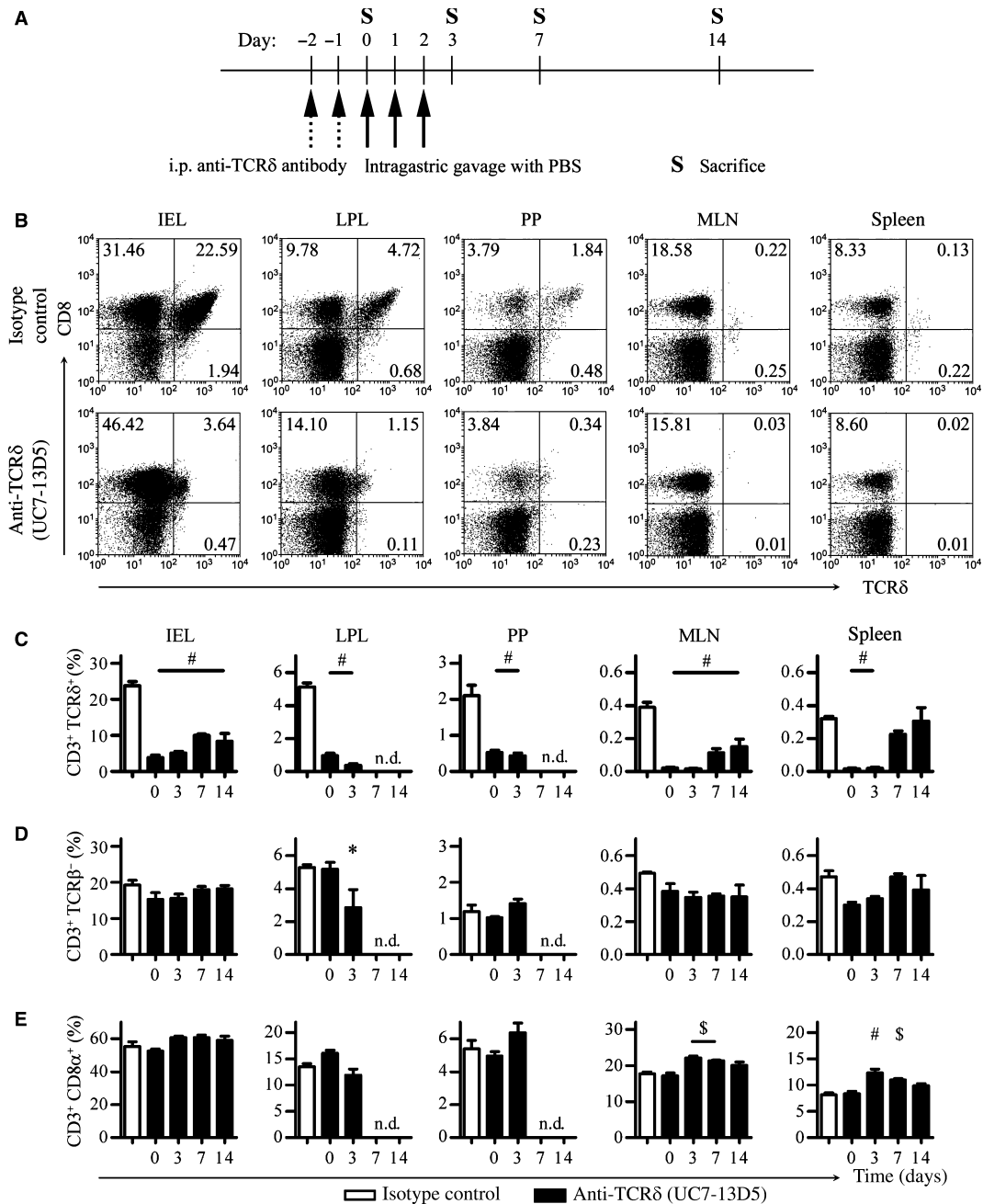


Figure 4 Effect of administration of UC7 on $\gamma\delta$ T cells in the intestine and lymphoid organs. (A) To test the efficacy of UC7 treatment, C57BL/6 mice ($n = 4$) received two i.p. injections of 400 and 200 μg UC7 respectively on day -2 and -1, oral PBS for 3 days and were sacrificed at days 0, 3, 7 or 14 (S). T cell subsets were analyzed by means of flow cytometry. (B) Representative dot-plots of T-cell receptor (TCR) δ expression (stained with clone GL3) in various organs after treatment with UC7. T cells were selected based on

FSC/SSC pattern and CD3 expression, after which they were analyzed for CD8 and TCR δ . Numbers represent percentage of total lymphocytes expressing TCR δ and/or CD8 α . (C) Percentage of CD3⁺ TCR δ ⁺ lymphocytes in time. (D) Percentage of CD3⁺ lymphocytes that lack TCR β expression in time. (E) Percentage of CD3⁺ CD8 α ⁺ lymphocytes in time. Data are depicted as mean $\pm$ standard error of the mean of four mice; * $P < 0.05$; \$ $P < 0.01$; # $P < 0.001$ compared to isotype control. n.d. = not determined.

$\gamma\delta$ T cells in the intestine (14). These and our data indicate that establishment of food allergy is accompanied by a decrease in intestinal $\gamma\delta$ T cells. As this decrease in $\gamma\delta$ T cells

in the intestine upon CT administration was accompanied with a decrease in CD8 α cells, our data indicates the actual disappearance of $\gamma\delta$ T cells during allergic sensitization,

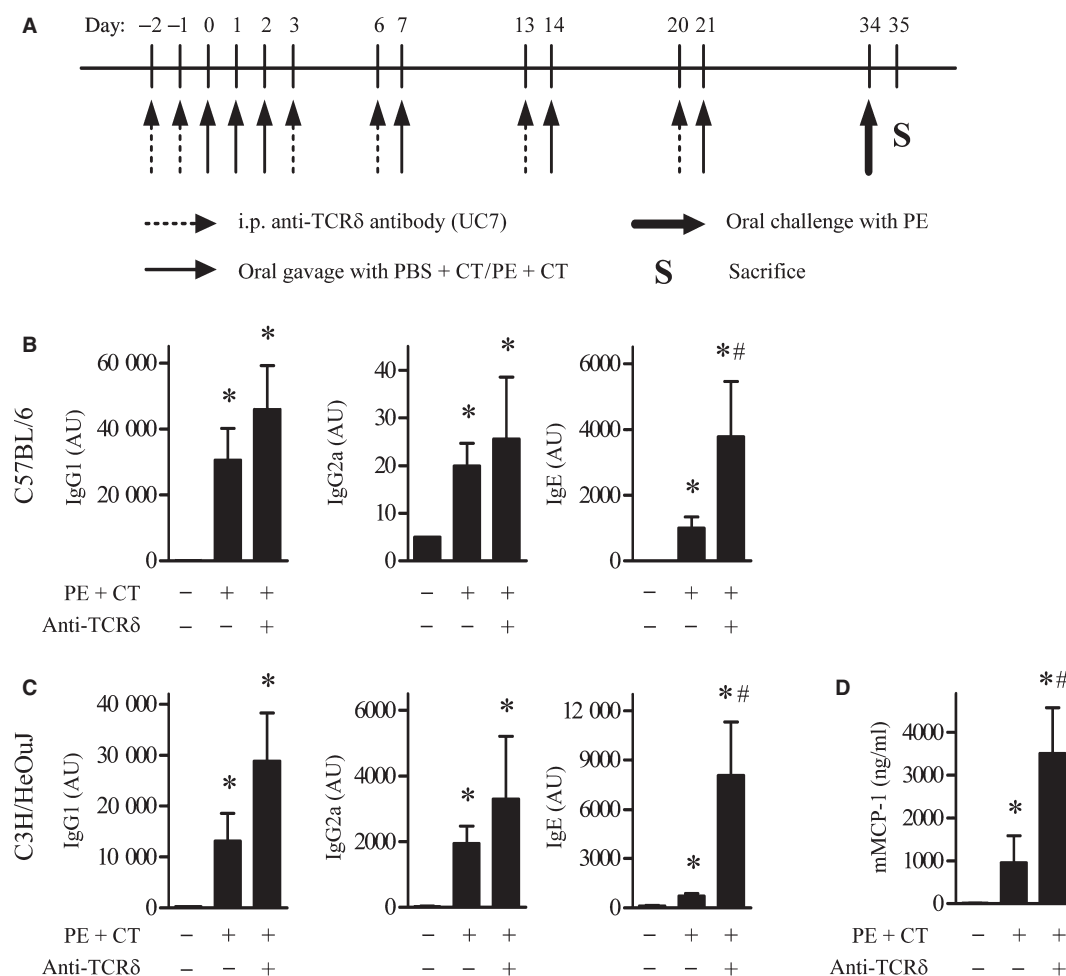


Figure 5 Effect of anti- $\gamma\delta$ T-cell receptor (TCR) treatment on peanut extract (PE)-specific antibody responses. (A) Mice were repeatedly exposed to PE + cholera toxin and PE-specific responses were analyzed after 4 weeks. Prior to and during sensitization, indicated groups received the anti- $\gamma\delta$ TCR antibody UC7. (B) PE-specific IgG1, IgG2a, and IgE levels in serum of C57BL/6 mice were determined by enzyme-linked immunosorbent assay. (C) As in B for

C3H/HeOuj mice. (D) Mast cell degranulation as measured by mMCP1 levels in C3H/HeOuj mice were determined in blood samples that were collected within 45 min after oral PE challenge. Data are presented as mean $\pm$ standard error of the mean from eight mice. * $P < 0.05$ compared to no sensitization; # $P < 0.05$ compared to no antibody pretreatment.

rather than lack of staining or disappearance of the $\gamma\delta$ TCR on these cells after activation (5). We also observed an increase in $\gamma\delta$ T cells in the MLN within the same time frame, but this coincided with an overall increase in T cells. Therefore, we cannot prove that this increase of $\gamma\delta$ T cells is the result of migration from the intestine.

There is controversy about the effect of *in vivo* treatment with the UC7 antibody on $\gamma\delta$ T cells. We demonstrate in the present study that UC7, like GL3, is blocking the $\gamma\delta$ TCR, as also suggested by Koenecke (5). Whether blockade results in internalization of the TCR, followed by activation of $\gamma\delta$ T cells remains unsolved. Further *in vitro* and *in vivo* studies need to be carried out to investigate possible activation of these cells upon treatment with UC7.

Increased allergic sensitization to PE after functional blocking of the $\gamma\delta$ TCR as found in the present study dem-

onstrates a protective role for $\gamma\delta$ T cells in allergic sensitization. This was also indirectly demonstrated in a study using c-kit deficient mice, possessing very low numbers of $\gamma\delta$ TCR-expressing IEL and displaying high susceptibility to allergic sensitization (15). In addition, after $\gamma\delta$ T cell reconstitution, these mast-cell-deficient mice displayed diminished allergic responses (16). Accordingly, there is strong evidence that $\gamma\delta$ T cells are involved in the decision making between tolerance and immunity in the intestine. This is supported by other studies showing that transfer of splenic $\gamma\delta$ T cells from ovalbumin-tolerized mice resulted in tolerance to ovalbumin in recipients (4) and that pretreatment with anti-TCR δ antibody resulted in abrogation of tolerance induction (12).

How $\gamma\delta$ T cells and the $\gamma\delta$ TCR are involved in the regulation of adaptive immune responses is not clear. Ligands for these cells in the intestine in the mouse are still not known.

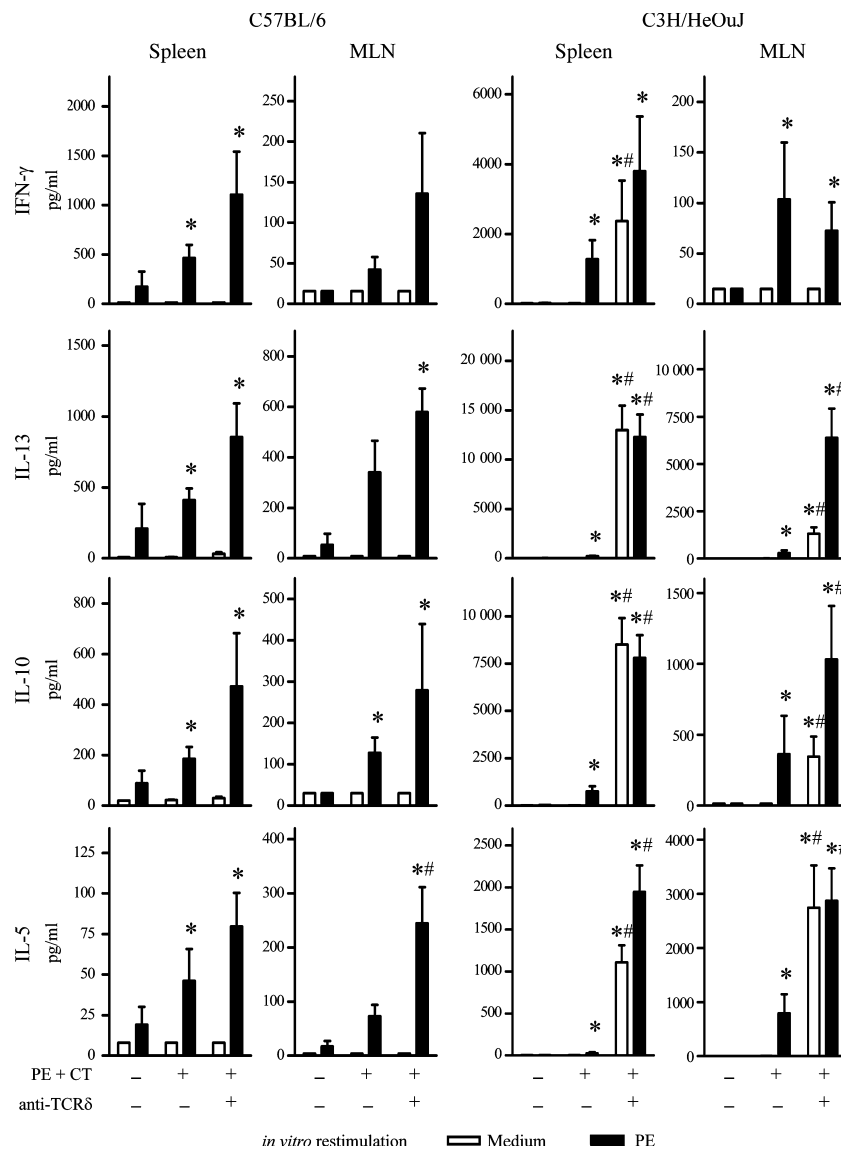


Figure 6 Effect of anti- $\gamma\delta$ T-cell receptor (TCR) antibody treatment on cytokine production by lymphocytes from spleen and MLN. Mice were exposed to peanut extract + cholera toxin as described in Fig. 4A. Prior to and during sensitization indicated groups received the anti $\gamma\delta$ TCR antibody UC7. Lymphocytes from spleen and MLN from C3H/HeOuJ mice or C57BL/6 mice were cultured in

the presence or absence of peanut extract for 96 h. Cytokine levels were determined in supernatant by means of specific enzyme-linked immunosorbent assay. Data are presented as mean $\pm$ standard error of the mean for eight mice per group. * P < 0.05 for C57BL/6 and P < 0.001 for C3H, compared to no sensitization; # P < 0.001 (<0.05 for IL-5) compared to no antibody treatment.

The diversity of the $\gamma\delta$ TCR is limited, and it might act as a pattern recognition receptor to mediate innate immune responses (17, 18). In addition, $\gamma\delta$ T cells, like natural killer (NK) cells, express the NKG2D receptor, which may contribute to effective stress-responses and immune surveillance (13, 19). Interestingly, immune surveillance may be of relevance in the induction of food allergy as well. Intestinal $\gamma\delta$ T cells are an important source of IL-10 and TGF- β (20), two important mediators of immune regulation and food allergy. A decrease in $\gamma\delta$ T cells caused by CT exposure or blockade of $\gamma\delta$ TCR function using specific antibodies could

lead to a diminished suppressive environment in the intestine, thereby facilitating allergic sensitization. Another explanation for the observed effects might come from the regulatory role of $\gamma\delta$ T cells on epithelial turnover and epithelial restitution in the intestine by the production of keratinocyte growth factor and TGF- β (21). A decrease in $\gamma\delta$ T cells or $\gamma\delta$ T cell function could impair the recovery of epithelial damage such as caused by CT. This may have decreased the epithelial barrier function leading to a breakdown of oral tolerance (11) and enhanced sensitization to food allergens as observed in our experiments.

A recent *in vitro* study showed that mouse peripheral $\gamma\delta$ T cells are capable of expressing Major Histocompatibility Complex (MHC)II molecules, turning them into antigen presenting cell (22). This increase in MHCII expression was accompanied by internalization of the $\gamma\delta$ TCR, but whether this also occurs in response to CT exposure *in vivo* remains to be established. Expression of MHCII and antigen-presenting functions were also found in human (23) and bovine (24) $\gamma\delta$ T cells in response to bacterial products, although this remains controversial in murine $\gamma\delta$ T cells (19). In addition, human peripheral $\gamma\delta$ T cells were shown to have strong regulatory potential in suppressing T helper cells *in vitro* (25). These findings may all link to growing evidence that activated $\gamma\delta$ T cells are directly involved in antigen presentation and regulation of T helper responses (26), which may contribute to the role of these cells on allergic sensitization.

In summary, the mucosal adjuvant CT induces allergic sensitization to co-administered proteins in mice, while feeding the protein alone induces tolerance. In this way, sensitization using CT can be used as a tool to model the induction

of food allergy. In the present study, we found that blocking the $\gamma\delta$ TCR during allergic sensitization resulted in enhanced allergic manifestations. In addition, we show that the establishment of allergic sensitization causes a decrease in $\gamma\delta$ T cells in the intestine. Present data suggest a regulatory role for $\gamma\delta$ T cells, implicating that modulation of $\gamma\delta$ T cells (and other innate immune cells) in the intestine may contribute to strategies to prevent and possibly treat food allergy.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Kinetics of changes in $\gamma\delta$ T cells in various organs after allergic sensitization.

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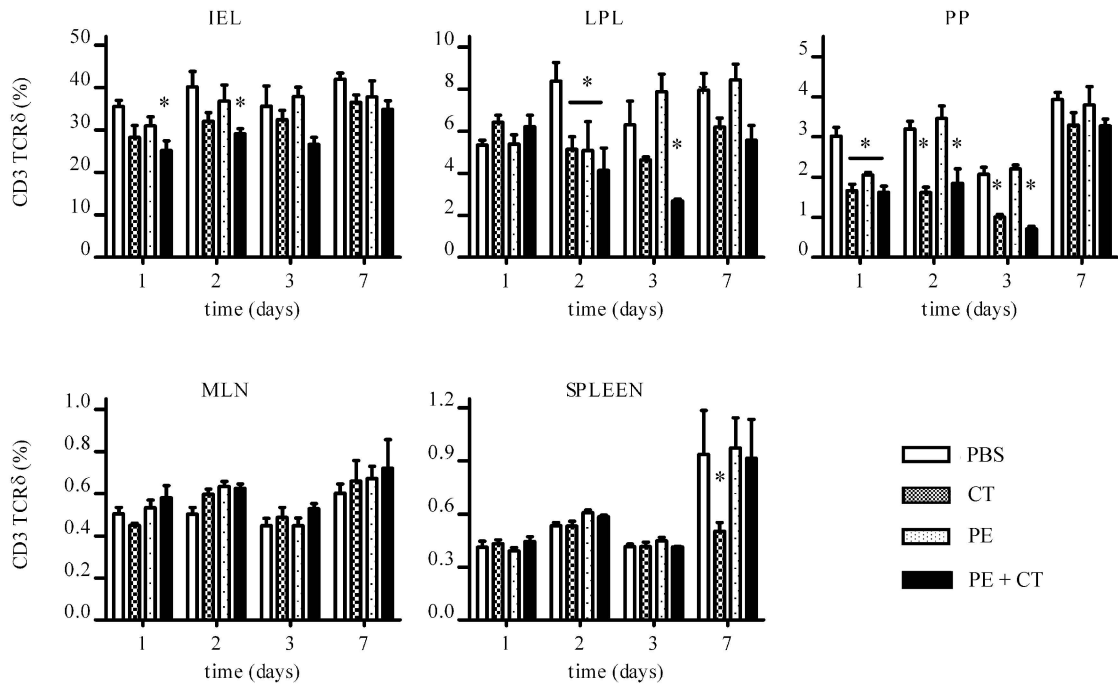


Figure S1