

House dust mite-specific T cells in healthy non-atopic children

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Summary

Background T-helper type 2 (Th2) cells play an important role in the pathogenesis of allergic diseases. Recent studies have demonstrated that allergen-specific T cells can also be found in the blood of healthy individuals. Both IL-10 and IFN- γ might modulate the induction and maintenance of allergen-specific tolerance.

Aim To study the phenotype and functional characteristics of allergen-specific T cells in healthy non-atopic children.

Methods Peripheral blood mononuclear cells (PBMC) from 13 symptomatic house dust mite (HDM)-allergic children and from nine matched healthy control children were stimulated with recombinant (r)Der p 2, a major allergen from HDMs.

Results Stimulation with rDer p 2 resulted in Th2 cytokine production in cultures of PBMC from allergic but not from healthy children. In contrast, IL-10 and IFN- γ were induced in PBMC cultures from both healthy and HDM-allergic children. Intracellular staining revealed that IL-10 and IFN- γ are largely produced by the same T cells. Stimulation of T cells from healthy children with rDer p 2 also induced expression of inducible costimulator (ICOS) on a small T cell subset.

Conclusion Allergen-specific memory T cells from healthy non-atopic children produce IL-10 and IFN- γ (but not Th2 cytokines) and express ICOS upon stimulation. These cells might be responsible for a normal immune balance after allergen encounter in non-atopics.

Keywords allergen, atopy, Der p 2, ICOS, IL-10, immune regulation, Th2 cytokines

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Introduction

Recent insights into the development of allergic diseases such as allergic rhinitis, asthma and atopic eczema are based on the functional diversity of T helper type 1 (Th1) and Th2 lymphocytes [1]. Th2 cells (secreting IL-4, IL-5, IL-9 and IL-13) are considered to be responsible for the induction and for many of the manifestations of atopic diseases [1, 2]. Local overproduction of Th2 cytokines (IL-4, IL-5 and IL-13) at the site of allergic inflammation [3–5], and an intrinsic defect in the production of IFN- γ by T cells in atopic individuals have now been reported by several authors [6–8].

Th2 clones predominate among allergen-specific T cell clones from allergic individuals [9, 10]. Furthermore, allergen-specific T cells producing Th2 cytokines can be detected in blood of atopic adults as well as children [8, 11–14]. The cause of this allergen-specific Th2 predominance in atopics has not been fully explored but it could be related to a dysfunction of the so-called regulatory cytokines that modulate Th1 and Th2

responses. In particular, IL-10 has been suggested to play a modulatory role in the induction and maintenance of allergen-specific tolerance [15], and has been shown to be an essential cytokine for the induction and function of at least one subtype of regulatory T cells [16]. IL-10 also inhibits eosinophil survival and IL-4-induced IgE synthesis [17]. It has recently been shown that both healthy and allergic individuals have allergen-specific IL-10-producing T cells that can suppress the allergen-induced proliferation of T cells [17, 18]. The frequency of these IL-10-producing T cells in the blood is higher in healthy individuals than in allergic patients [17, 18]. Along the same line, our group has shown that rBet v 1 (the major allergen from birch pollen), in the presence of IL-4, induces IL-10 production by allergen-specific T cells from healthy but not from birch-pollen-allergic subjects [19]. In contrast, allergen-specific T cells from allergics produced Th2 cytokines upon stimulation by rBet v 1. It has, furthermore, been shown that cow's milk-specific T cell clones from atopic children who are tolerant to cow's milk produced higher levels of IL-10 and IFN- γ than those from non-atopic control children. Although the IL-4 production of these T cell clones is also increased, as in cow's milk-allergic children, their IL-10-producing cells are probably responsible for tolerance [20]. Finally, grass-pollen immunotherapy has been shown to induce a population of IL-10-producing T cells that

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could be responsible for the favourable outcome in these patients [17].

In search for differences in regulatory T cell cytokine production, here we have compared T cell responses to Der p 2 (a major allergen of house dust mite (HDM)) in healthy and atopic (HDM-allergic) children. As we and others could recently demonstrate the role of inducible costimulator (ICOS)⁺ regulatory T cells in tolerance [21, 22], we also studied ICOS expression after Der p 2 stimulation.

Materials and methods

Subjects

The study included 13 children (nine males and four females) between six and 12 years of age, sensitized to HDM, who attended the outpatient clinic of the Allergy Division of the University Hospital Gasthuisberg. All patients had chronic symptoms and positive skin prick test (SPT) to HDM extract (HAL, Haarlem, the Netherlands). Twelve out of 13 children had multiple sensitizations to one or more of the following: dog or cat dander ($n = 4$), grass pollen ($n = 9$), tree pollen ($n = 4$) and others ($n = 5$). Nine control children (six males and three females) (7–13-years-old) without a history of allergic symptoms, and negative SPT were used as controls. Control children attended the outpatient paediatric clinic for non-immunological, non-infectious, non-inflammatory problems and needed venous puncture. Parents agreed to allergy screening of the children and additional blood donation for study purposes. SPT were performed on the volar aspects of the forearms with a panel of standardized allergen extracts (HAL), including grass-pollen mixture, tree-pollen (birch, alder and hazel), weed-pollen mixture, moulds, HDM, cat and dog dander in both allergic and healthy children. Aas' criteria were used to score the SPT; a reaction was regarded as positive when the weal was identical to or larger than that of the histamine control [23, 24]. The blood was drawn between February 2001 and May 2004, equally spaced in time. The study was approved by the local ethical committee of the Faculty of Medicine, Leuven, and informed consent was obtained.

Isolation of peripheral blood mononuclear blood cells

PBMC were obtained by venous puncture on sodium–heparin vacuum tubes (10 mL). Blood was centrifuged on Ficoll–Hypaque density gradients. Interphase cells were washed three times with Dulbecco's phosphate-buffered saline (Boehringer Ingelheim Bioproducts, Heidelberg, Germany). They were resuspended at a concentration of 10×10^6 cells/mL in complete medium, consisting of RPMI 1640 (Boehringer Ingelheim Bioproducts) supplemented with 2 mM L-glutamine, penicillin (100 U/mL), streptomycin (100 µg/mL) and 10% bovine calf serum (Hyclone, Logan, UT, USA). Optimal doses of stimulatory agents and of the cytokines used, together with kinetics of cytokine secretion, were determined in an independent pilot study on four allergic and four healthy adult volunteers.

Monoclonal antibodies and cytokines

Monoclonal mouse anti-human IL-4R antibody (clone 25463.11) was bought from R&D (Abingdon, UK). Recombinant

human IL-2 was purchased from Boehringer Mannheim (Mannheim, Germany). The clone-producing mAb UCHT-1 (mouse anti-human CD3) was bought from ATCC (American Type Culture Collection, Manassas, VA, USA). Mouse anti-human ICOS mAb, F44 (IgG1), unlabelled and PE labelled was produced as reported previously [25].

Activation of T cells for cytokine production

PBMC (1×10^6) were cultured in 24-well culture plates in a 1 mL volume at 37 °C in 5% CO₂/95% air, and supernatants were collected after 3 or 7 days of stimulation as indicated in the results. For allergen-induced T cell stimulation, r Der p 2 (a gift from J.-M. Saint-Rémy, Molecular and Vascular Biology, KU Leuven, Belgium) was used at a concentration of 1 µg/mL. Human albumin was used as a control protein at a concentration of 1 µg/mL (Behring, Marburg, Germany). For recall Ag-induced T cell stimulation, PBMC were cultured with soluble tetanus toxoid (TT) (RIVM, Bilthoven, the Netherlands) (0.1 LfU/mL). T cells were also stimulated with soluble anti-CD3 mAb (UCHT-1) captured by mouse P815/CD58 mastocytoma cells, at a T/P815 ratio of 1/1. The P815 cell line is an NK-resistant DBA/2-derived mouse mastocytoma cell line that expresses mouse FcγRII and FcγRIII. P815 cells transfected with human CD58 were a gift from L. L. Lanier (then at DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA, USA). P815 cells were mitomycin-C treated and washed five times before use.

The anti-IL-4R mAb (clone 25463.11) was used at a concentration of 2.5 µg/mL in all culture conditions, aiming to measure IL-4 production [26]. rIL-2, if added, was used at a concentration of 5 ng/mL or 5 IU/mL.

Cytokine measurements

The concentrations of IL-4, IL-5, IL-10, IL-13 and IFN-γ in culture supernatants were measured with a sandwich ELISA technique using combinations of unlabelled and biotin-coupled mAb to different epitopes of each cytokine as described previously [19]. The lower detection limit for all cytokines was <20 pg/mL.

Intracellular cytokine staining and membrane staining for inducible costimulator

PBMC (1×10^6) were cultured for 6 days with recombinant (r)Der p 2. During the last 24 h of culture, 3 mM Monensin (Sigma Diagnostics, St Louis, MO, USA) was added to the cultures. PMA (Sigma Chemical, St Louis, MO, USA) (1 ng/mL) and ionomycin (Sigma Chemical) (0.5 µg/mL) were added during the last 4 h of the culture to enhance the expression of the imprinted cytokine pattern [27]. For membrane staining, either 10 µL FITC-conjugated IgG1 isotype control (Becton Dickinson, BD Biosciences, Erembodegen, Belgium), FITC-labelled anti-CD3 mAb (Becton Dickinson), FITC-labelled anti-CD14 mAb or FITC-labeled anti-ICOS (F44) mAb in combination with either 10 µL PE-labeled IgG1 isotype control (Becton Dickinson) or PE-labeled anti-ICOS (F44) mAb and in combination with either 10 µL PerCP-labelled IgG1 isotype control (Becton Dickinson) or PerCP-labeled anti-CD3 mAb (Becton Dickinson) were added for 30 min at 4 °C. Cells were then washed with

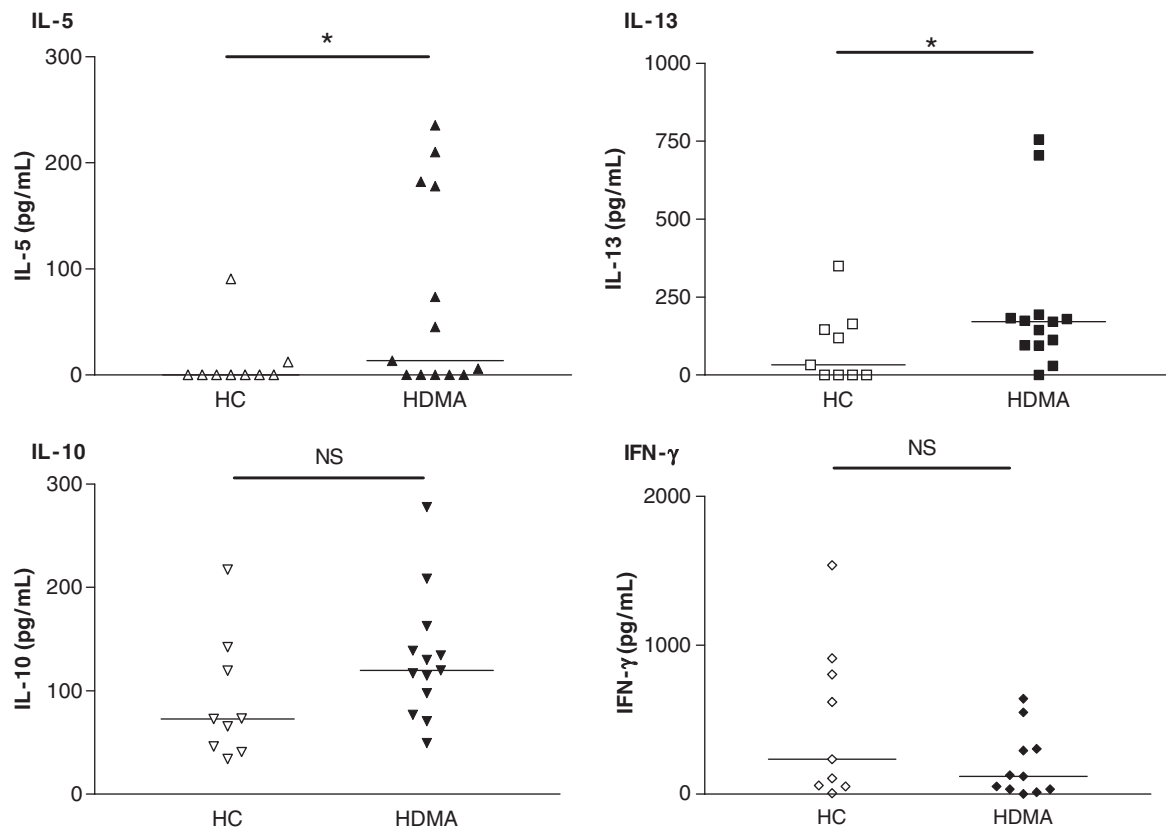


Fig. 1. Cytokine production by allergen (Der p 2) stimulated T cells. Peripheral blood mononuclear cells (1×10^6 in 1 mL) from 13 house dust mite (HDM) allergic children and nine healthy control children were cultured in medium alone or with rDer p 2 ($1 \mu\text{g/mL}$). Supernatants were collected after 7 days and the concentrations of IL-5, IL-10, IL-13 and IFN- γ in the supernatants were detected by ELISA. rDer p 2-induced production of the cytokines was calculated by subtracting the value obtained in medium conditions from its value in medium+rDer p 2. Median values obtained in medium alone for IL-5, IL-10, IL-13 and IFN- γ were respectively 5, 15, 10 and 11 pg/mL in the allergic group and 5, 18, 10 and 10 pg/mL in the control group. Mann-Whitney-*U*-test was used to compare differences in rDer p 2-induced cytokine production between healthy controls and HDM-allergic patients. * $P \leq 0.05$; NS, not significant; HC, healthy control; HDMA, house dust mite allergic.

PBS containing 0.5% bovine serum albumin (Sigma Diagnostics), collected and fixed with 500 μL paraformaldehyde (Janssen Pharmaceutica, Beerse, Belgium) 2% in PBS at 4°C for 10 min. For permeabilization, cells were washed with PBS containing 0.5% bovine serum albumin (Sigma Diagnostics) and 0.2% saponin (Sigma Diagnostics). Cells were then resuspended in 100 μL of this permeabilization buffer, and 10 μL normal mouse serum was added for 5 min at room temperature. Ten microliters of either PE-conjugated IgG1 isotype control (Pharmingen, BD Biosciences, Erembodegen, Belgium), or PE-labelled anti-human IL-10 mAb (Pharmingen) was added. For intracellular two-colour staining, 10 μL of either FITC-labelled control IgG1 mAb or FITC-labelled anti-human IFN- γ mAb (Pharmingen) was added together with anti-IL-10 PE mAb or PE-labelled isotype control. Tubes were gently mixed for 30 min in the dark at room temperature. Cells were resuspended and washed twice with permeabilization buffer, resuspended in PBS and analysed with a FACSsort flowcytometer (Becton Dickinson). Calibration was performed with CaliBRITE 3, unlabelled, FITC, PE, PerCP (Becton Dickinson). Plots were analysed with the Cell Quest software.

Statistical analysis

Results were compared with either the non-parametric Mann-Whitney *U*-test, two-tailed or one-tailed where appro-

priate, or the non-parametric Wilcoxon's paired test, where possible. A *P*-value of ≤ 0.05 was considered as statistically significant. Calculations were performed with Prism Graph 3 (GraphPad Software Inc., San Diego, CA, USA) for Macintosh.

Results

Induction of cytokine production by Der p 2 stimulation

We studied the *in vitro* rDer p 2-induced T cell cytokine profile in PBMC cultures of 13 HDM-allergic children between six and 12 years of age, with chronic symptoms related to HDM sensitization and of nine matched healthy control children. Netto allergen-induced cytokine production for both groups is shown in Fig. 1. Compared with medium conditions alone or human albumin (used as a negative control), rDer p 2-induced significant IL-5 ($P < 0.05$) and IL-13 ($P < 0.001$) production in cultures of PBMC from allergic subjects but not in those from healthy controls (see Fig. 1). However, PBMC from both healthy children and atopic children produced significant amounts of IL-10 ($P < 0.01$ in healthy children and $P < 0.001$ in atopic children) and IFN- γ ($P < 0.01$ in healthy and atopic children) upon stimulation with Der p 2 (always in comparison with medium alone or stimulation with human albumin). rDer p 2-induced produc-

Table 1. Allergen-induced IFN- γ production (pg/mL) in the absence and presence of IL-2*

	Healthy controls (n = 8)		House dust mite allergic patients (n = 11)	
	PBMC+ Der p 2	PBMC+ Der p 2+rIL-2	PBMC+ Der p 2	PBMC+ Der p 2+rIL-2
Experiment				
1	1538	1581	33	296
2	235	487	292	1646
3	619	7790	ND	26
4	52	1933	549	910
5	58	427	127	ND
6	805	3500	640	4490
7	105	1037	304	1878
8	913	3643	13	356
9			119	496
10			32	125
11			50	385
Median (pg/mL)	427	** 1757	119	** 385
	NS		*	

* 1×10^6 PBMC were cultured either in medium alone or in the presence of rDer p 2 (1 μ g/mL) in the absence or presence of rIL-2 (5 IU/mL). Supernatants were collected after 7 days and the concentration of IFN- γ in the supernatants was detected by ELISA. RDer p 2-induced production of IFN- γ was calculated by subtracting the value obtained in medium conditions from its value in medium+rDer p 2. Effect of IL-2 was tested by Wilcoxon's paired test and the differences between healthy controls and house dust mite-allergic patients were tested by the use of Mann-Whitney U-test. ND, not detectable; * $P \leq 0.05$, ** $P \leq 0.01$ and NS, not significant.

tion of IL-10 and IFN- γ did not differ significantly between both groups (Fig. 1). Der p 2-induced IL-4 production was below the detection limit of the ELISA assay in both groups.

Spontaneous cytokine secretion by PBMC did not differ significantly between healthy control children and atopic children (data not shown).

IL-2 has been shown to increase the production of both Th1 and Th2 cytokines by PBMC [28]. In order to study whether we could reinforce the allergen-induced T cell cytokine profile, we stimulated PBMC from allergic patients and healthy controls with rDer p 2 in the absence or presence of rIL-2. Adding rIL-2 significantly increased Der p 2-induced IFN- γ production both in healthy controls and atopic children (Table 1). The increase in rDer p 2-induced IFN- γ production was more pronounced in healthy individuals, resulting in significantly higher levels of Der p 2-induced IFN- γ production in healthy individuals when compared with atopic children (Table 1). Somewhat surprisingly, no significant effect on Der p 2-induced Th2 cytokine or IL-10 production was observed after adding rIL-2 to the PBMC cultures from either atopic children or healthy control children (data not shown). The data suggest a defect in IL-2-enhanced IFN- γ production by allergen-specific T cells in allergic children.

Cytokine production in response to recall antigen and to polyclonal stimulation

To study whether the observed difference in Th2 cytokine production was restricted or not to allergenic stimulation,

cells were stimulated with a microbial recall protein: TT. IL-5, IL-13 and IL-4 as well as IL-10 and IFN- γ were equally induced by TT in allergic patients and in control subjects (data not shown), and the Th2/Th1 ratios upon stimulation with TT were similar in both groups (Fig. 2a).

T cells were also polyclonally stimulated with anti-CD3 mAb presented on a P815 cell-line transfected with human CD58 (the ligand for CD2 on T cells). We have previously shown that T cells stimulated by anti-CD3 and CD58 mainly produce IFN- γ and IL-10 [29]. No significant differences in polyclonally induced IL-4, IL-5, IL-13, IFN- γ or IL-10 production could be observed between HDM-allergic patients and healthy controls. However, the polyclonally induced Th2/Th1 ratios were significantly higher in the atopic children as compared with healthy control children (Fig. 2b). This probably reflects the preferential Th2 pattern that is related to the atopic state of the donors [1]. The IL-10/IFN- γ ratios were similar in both groups (data not shown).

Intracellular cytokine production in response to Der p 2

In order to study the source of the allergen-induced IL-10 production, 1×10^6 PBMC from five healthy children were cultured in the absence or presence of rDer p 2. In the gated CD14⁺ population (monocytes), intracellular IL-10 was only detectable in one out of four donors tested (data not shown). A rise in IL-10-positive CD3⁺ cells was detected upon stimulation with rDer p 2 (Table 2). Double staining for IL-10 and IFN- γ revealed that up to 95% of the IL-10-producing cells also expressed IFN- γ , whereas only a small proportion of IFN- γ -positive cells produced IL-10 (data not shown).

Der p 2 stimulation induces inducible costimulator expression on T cells

ICOS is expressed on activated T cells [25], and the interaction of ICOS with its ligand on the APC, ICOS-ligand, is especially important for the induction of IL-10 production by CD4⁺ T cells [30]. We therefore studied whether HDM-specific T cells from non-atopic children might be induced to express ICOS. PBMC from four healthy children were cultured in the absence or presence of rDer p 2 for 6 days and subsequently stained for CD3 and ICOS. As indicated in Table 3, Der p 2 stimulation resulted in the expression of ICOS on a substantial number of CD3⁺ cells. Unfortunately, as the study of intracellular IL-10 required restimulation with PMA and ionomycin, no double staining with anti-ICOS mAb and anti-IL-10 mAb could be performed, as these activating agents also affect ICOS induction.

Discussion

We demonstrate here that Der p 2-specific T cells circulate in the blood of healthy control children, and that they produce IL-10 and IFN- γ but no Th2 cytokines upon *in vitro* stimulation with allergen. This contrasts with the allergen-specific T cells of HDM-allergic children, which, besides IL-10 and IFN- γ , also produce IL-5 and IL-13 upon stimulation with Der p 2. It has been shown previously by Kimura et al.

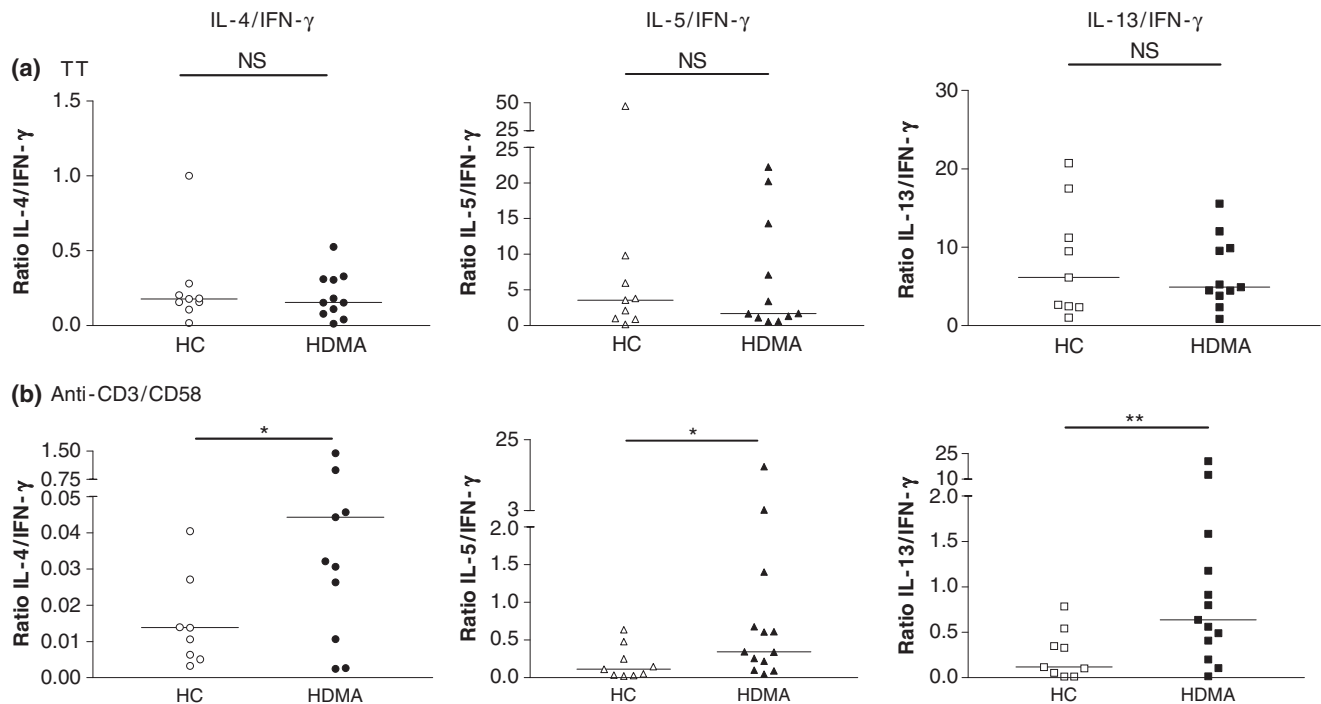


Fig. 2. Cytokine production by tetanus toxoid (TT)- and polyclonally activated T cells. PBMC (1×10^6 in 1 mL) were stimulated with TT (0.1 LfU/mL) (a) or anti-CD3 (UCHT-1; 2 μ g/mL) presented by a CD58-transfected P815 cell line (b). Supernatants were collected after 7 (A) or 3 (B) days and the concentrations of IL-4, IL-5, IL-13 and IFN- γ in the supernatants were detected by ELISA. The ratios were calculated by dividing the amount (expressed in pg/mL) of the Th2 cytokines IL-4, IL-5 and IL-13 by the amount of IFN- γ (also expressed in pg/mL). The differences between healthy controls and HDM-allergic patients were tested by the use of Mann–Whitney *U*-test. Median values are indicated by horizontal black line. * $P \leq 0.05$; ** $P \leq 0.01$; NS, not significant; HC, healthy control, HDMA, house dust mite allergic.

Table 2. Percentage of IL-10-positive cells among CD3⁺ T cell population*

	Medium (%)	Medium+Der p 2 (%)
Donor		
1	4.5	23
2	1.0	8.8
3	0.7	2.1
4	2	2.3
5	1	1.1

* 1×10^6 PBMC were cultured for 5 days in the absence or presence of rDer p 2 (1 μ g/mL). After 5 days, monensin was added for 15 h. To measure IL-10, cells were restimulated for 4 h at day 6 with PMA (1 ng/mL) and Ionomycin (0.5 μ g/mL). Afterwards, cells were membrane-stained by either anti-IgG2a FITC or PerCP mAb or anti-CD3 FITC or PerCP mAb and anti-IL-10 PE mAb. 50,000 cells were then analysed on the FACS. The number of IL-10-producing cells among CD3⁺ cells is represented in percentage. The results of five independent experiment with PBMC from different non-atopic children are shown.

[8] that non-allergic children develop HDM-specific IFN- γ -producing cells starting from 3 years on, while atopic children do not. However, cells from both allergic and non-allergic children in the study by Kimura et al. produced the Th2 cytokines IL-4 and IL-5 upon *in vitro* stimulation with HDM. A possible explanation for the difference between their findings and ours might be the stimulatory agent used, as they used a crude Der f extract, whereas we used a recombinant Der p 2. Macaubas et al. [31] studied the *in vitro* cytokine production by PBMC from healthy and HDM-allergic children (aged 5 years) upon stimulation with aqueous

Table 3. Percentage of ICOS⁺ T cells amongst CD3⁺ population*

	Donor 1	Donor 2	Donor 3	Donor 4
Medium (%)	1.82	1.69	1.69	2.19
Medium+Der p 2(%)	12.39	6.63	2.42	5.19

* 1×10^6 PBMC were cultured for 6 days in the absence or presence of rDer p 2 (1 μ g/mL). Cells were membrane stained by either anti-IgG2a PE mAb or anti-ICOS PE mAb and by anti-CD3 PerCP mAb, and 50,000 cells were analysed on the FACS. Four independent experiments with PBMC from different non-atopic children are shown.

extract of lyophilized *Dermatophagoides pteronyssinus*. In parallel to what we found in 6–12 years old, PBMC from healthy 5-year-olds produced IL-6, IL-10 and IFN- γ in response to HDM, whereas PBMC from allergic 5-year-olds produced, besides IL-6, IL-10 and IFN- γ , and also IL-5 and IL-13 [31].

Both IL-10 and IFN- γ can originate from myeloid and (or) T cells. We demonstrate here that the cells from healthy children who produce IL-10 and IFN- γ are CD3⁺ cells. The majority of CD3⁺ T cells-producing IL-10 also produced IFN- γ ; however, a large population of IFN- γ -producing cells did not produce IL-10. This contrasts with the findings of Akdis et al., showing that only a minority of the allergen-specific IL-10-producing cells produces IFN- γ upon restimulation after *in vitro* expansion. *In vitro* expanded IFN- γ -producing allergen-specific T cells in their study did produce some IL-10 upon restimulation [18] IL-10/IFN- γ double-positive cells have also been described by Saloga et al. in some patients receiving venom immunotherapy, and they suggested

that those cells could be T regulatory 1 cells [15, 32]. Further experiments are needed to explore whether the IL-10/IFN- γ double-positive cells induced by Der p 2 in our study are classical Th1 cells, known to produce IL-10 in humans [33], or could have regulatory capacity [16]. There recently has been considerable interest in IL-10 and IFN- γ as cytokines that might inhibit allergic reactions [15, 34]. The induction of IFN- γ -producing CD4⁺ T cells after exposure to house dust containing endotoxin was found to correlate with the prevention of allergic diseases [35]. Furthermore, IL-10 production by regulatory T cells (Treg) might be required to balance the immune system [16, 36]. The expression of IL-10 may also play a role in the induction and maintenance of allergen-specific tolerance in allergic disorders [17, 18, 37].

Here we also showed that stimulation of PBMC with Der p 2 induces ICOS expression on a subpopulation of T cells. ICOS–ICOS–ligand interaction strongly promotes IL-10 production by CD4⁺ T cells [30]. Thus, ICOS expression might have relevance for the function of these cells in prevention of allergic disease. In mice, allergic airway inflammation can be suppressed by allergen-specific IL-10-producing T cells that have been primed *in vitro*. Both the development and the inhibitory function of these regulatory cells were dependent on the presence of IL-10 and on ICOS–ICOS–ligand interactions [22]. ICOS-deficient mice are more prone to develop experimental allergic encephalomyelitis [38]. Although ICOS can be induced on all activated T cells, its ligation superinduces the synthesis of IL-10 [25, 30, 39, 40]. We have previously shown that if T cells are stimulated by costimulation-deficient APC, ICOS is induced on a T cell subset that produces IL-10 depending on the ICOS–ligand–ICOS interaction [21]. Whether the ICOS-positive cells induced by Der p 2 stimulation have regulatory capacities has not yet been studied. A hint in that direction is our finding that healthy children, although they can mount a Th2 response upon polyclonal stimulation or recall-antigen stimulation, do not produce Th2 cytokines upon stimulation by an allergen. We were not able to study the relation of these ICOS-expressing cells with the natural CD4⁺CD25⁺ regulatory T cells, as effector T cells also become CD25-positive upon stimulation. In mice, CD4⁺CD25⁺ regulatory T cells exploit IL-2 for the induction of IL-10 production [41], and IL-2 signalling in regulatory T cells is essential for their protective function [42]. In parallel, preculture of human CD4⁺CD25⁺ regulatory T cells in the presence of IL-2 and anti-CD3-induced potent suppressor activity in those cells [43]. Addition of IL-2 to our culture system increased the IFN- γ but not the IL-10 production of the allergen-specific T cells. This somehow argues against specific triggering of the natural regulatory CD4⁺CD25⁺ T cell population. However, IFN- γ production by CD4⁺CD25⁺ regulatory T cells has recently been described as being essential for their function [44].

The induction of ICOS upon stimulation could help to identify the allergen-specific T cells among the total T cell population in healthy children, and help to isolate them and to further identify their function and cytokine production pattern. These studies are ongoing. It would furthermore, be interesting to compare isolated allergen-specific T cells from healthy donors with those from allergic individuals, and to study whether other allergens also induce ICOS-positive cells

in those individuals. ICOS expression on allergen-specific T cells from allergic children has not yet been studied, but, as ICOS may have a particularly important role in development of murine Th2 cells [45], one might speculate that allergen-specific Th2 cells should also express ICOS in these children. Indeed, ICOS expression on T cells from atopic donors cultured in the presence of autologous allergen-pulsed DC has been described [46].

In summary we demonstrate here that healthy control children have HDM-specific T cells that, upon *in vitro* T cell receptor triggering, mainly produce IL-10 and IFN- γ and little or no Th2 cytokines, and become ICOS positive. These cells might be important in the prevention of adverse immune reactions against harmless allergens.

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