Differences in antigen-specific T-cell responses between infants with atopic dermatitis with and without cow's milk allergy: Relevance of T_H2 cytokines

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Background: Cow's milk is the most important food antigen in infancy and may lead to acute cutaneous symptoms and atopic dermatitis (AD). The role of circulating allergen-specific T cells in the pathogenesis of food-allergic skin symptoms is still under investigation.

Objective: This study was designed to analyze the cow's milk protein (CMP)–specific T-cell response at the clonal level in infants with AD and cow's milk allergy (CMA) in comparison with infants with AD without CMA.

Methods: We used an antigen-specific culturing system with autologous B cells as antigen-presenting cells to establish CMP-specific T-cell clones derived from PBMCs in infants with AD. T-cell reactivity, measured by using a lymphocyte stimulation test, and cytokine production, measured by using ELISA, was compared between infants with AD with and without CMA.

Results: Both infants with and without allergy to cow's milk had a CMP-specific T helper cell response directed against the major proteins in milk. Analysis of antigen-specific cytokine production showed that this response was T_H2 skewed in infants with CMA, with production of high levels of IL-4, IL-5, and IL-13. In contrast, infants without CMA had a T_H1-skewed response, with high levels of IFN-γ and low levels of IL-4, IL-5, and IL-13.

Conclusion: These data confirm for the first time at the clonal level that food allergy in infants with AD is associated with production of T_H2 cytokines by circulating antigen-specific $CD4^+$ T cells, whereas tolerance to food antigens is associated with low levels of these cytokines. This suggests a key role for the T helper cell–derived T_H2 cytokines in food allergy–related skin symptoms. (J Allergy Clin Immunol 2000;106:1155-62.)

Key words: *Atopic dermatitis, T-cell clone, cow's milk, casein, cytokine, food allergy, IL-4, infants, human, T_H1/T_H2 cells*

Atopic dermatitis (AD) is a chronic eczematous skin disease with early onset in infancy and is characterized by a course of remissions and exacerbations.[1] At present, the pathogenesis of AD is not fully understood. In patients with AD and allergy toward inhalation allergens, such as house dust mite, circulating allergen-specific T_H2 cells have shown to be important in the pathogenesis of allergic skin symptoms.[2-4] The infiltration into the skin of these T cells, which produce high levels of IL-4, IL-5, and IL-13 but little or no IFN-γ,[5,6] can mediate allergic skin inflammation through stimulation of IgE synthesis and eosinophil recruitment.[7]

Evidence for these mechanisms in food allergy is much less well established. Food-reactive T cells in PBMCs from patients with food allergy with AD have been shown to proliferate in the presence of food antigens.[8,9] This capacity is, however, not restricted to the patient with food allergy because PBMCs from food-tolerant individuals can also mount significant lymphoproliferative responses when stimulated in vitro.[9] Studies that determined cytokine production in food antigen–stimulated PBMCs showed that patients with food allergy have a T_H2-skewed response compared with nonatopic control subjects.[10-14] In these studies, however, cytokines were measured in bulk cultures of T cells, making it difficult to extrapolate these results to the individual T cell. Food-reactive T_H2 cells have been cloned from blood of patients with food allergy,[15-17] but the food-specific T-cell response in food-tolerant subjects has not been evaluated at the clonal level.

Cow's milk is among the first foods introduced into an infant's diet and thus one of the most common causes of food allergy in young children.[18,19] Cow's milk allergy (CMA) plays a pathogenetic role in approximately 35% to 40% of infants with AD.[20-22] In these patients ingestion of cow's milk leads to acute cuta-

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Abbreviations used
AD: Atopic dermatitis
CMA: Cow's milk allergy
CMP: Cow's milk protein
LST: Lymphocyte stimulation test
TCC: T-cell clone
[^{3}H]-TdR: Tritiated thymidine

neous symptoms, aggravation of the eczema, or disease. The aim of this study was to analyze the cow's milk protein (CMP)–specific T-cell response in infants with AD and CMA. We used an antigen-specific culturing system to establish CMP-specific T-cell clones (TCCs) from blood of infants with AD under the age of 1 year. A comparison of T-cell reactivity and cytokine production was made between infants with CMA and infants tolerant for cow's milk.

METHODS

Patients and control subjects

Twelve infants (7 boys and 5 girls; age, 3.8-12.3 months; median age, 6.4 months) with AD according to the criteria of Hanifin and Rajka[23] were included in the study. The study was approved by the Medical Ethical Committee of the University Medical Center, Utrecht. After informed consent was obtained, a venous blood sample was taken. CMA was diagnosed by complete elimination of cow's milk from the infant's diet, followed by a cow's milk challenge. Infants in whom symptoms developed during challenge (Table I) that disappeared during subsequent elimination were diagnosed with CMA. Infants without CMA did not have any reactions, and no significant change in the severity of the eczema was noted throughout the entire elimination-provocation period. Age of patients with CMA was not significantly different ($P = .94$) from that of patients without CMA (range, 3.8-12.3 months; median, 6.4 months vs range, 4.4-7.3 months; median, 6.5 months).

Cow's milk antigens

Purified CMPs used at concentrations of 50 μg/mL were as follows: total casein, α-lactalbumin and β-lactoglobulin. Purified casein subfractions used at concentrations of 10 μg/mL were as follows: αS1-casein, αS2-casein, β-casein, and κ-casein. All CMPs were kindly provided by Dr E. C. H. van Beresteijn (Netherlands Institute for Dairy Research, Ede, The Netherlands). A mix of purified CMPs containing equal quantities of total casein, α-lactalbumin, and β-lactoglobulin (each at a concentration of 50 μg/mL) is referred to as CMP mix.

Culture media

Proliferation assays with PBMCs were performed with complete medium AIM V (Gibco, NY), and in lymphocyte stimulation tests (LSTs) complete medium RPMI-1640 (Gibco) was used supplemented with pooled human AB serum. EBV-transformed B cells were cultured in RPMI-1640 (Gibco) supplemented with 10% FCS (Gibco). Established TCCs were maintained in Iscove's modified Dulbecco's medium (Gibco) supplemented with 2% pooled human AB serum and 5% Yssel's medium.[24] All media were supplemented with penicillin (100 IU/mL), streptomycin (100 mg/mL), and glutamine (1 mmol/L) (Gibco).

Proliferation assays with PBMCs

PBMCs were isolated from heparinized venous blood by using Ficoll density gradient centrifugation. Recovered cells were cultured in quadruplicate (10^5 cells/well) at 37°C under 5% CO_2 for 6 days in 96-well U-bottom culture plates (Greiner, Frickenhausen, Germany) in the presence or absence of antigen. After 6 days of culturing, proliferation was measured by using tritiated thymidine ([^{3}H]-TdR) incorporation; [^{3}H]-TdR (1 μCi/well; Amersham, Aylesbury, UK) was added, and the cells were harvested after 18 hours. Thymidine incorporation was measured by using a 1205 betaplate counter (Wallac, Turku, Finland).

Lymphocyte stimulation test

LSTs were performed in triplicate in 96-well U-bottom plates (Greiner). Each well contained 4×10^4 T cells and 4×10^4 irradiated (50 Gy), autologous, EBV-transformed B cells as antigen-presenting cells. The EBV-transformed B cells were, before coculture with T cells, incubated overnight with antigen. EBV-transformed B cells incubated without antigen were used as negative controls. After 24 hours of culturing, proliferation was measured by using [^{3}H]-TdR incorporation, as described above.

Preparation of CMP-specific TCCs

CMP-specific TCCs were established from heparinized venous blood, as described previously.[25,26] In parallel to the proliferation assays, PBMCs were cultured in a 24-well flat-bottom culture plate in the presence of CMP mix. After 7 days, 50 IU/mL of both recombinant IL-2 and recombinant IL-4 (kind gift of Novartis Research Institute, Vienna, Austria) was added to the culture medium. To promote expansion of CMP-specific T cells, cultures were restimulated every 14 days with irradiated, autologous, EBV-transformed B cells that had been preincubated overnight with CMP mix as antigen-presenting cells. LST or determination of cytokine release was always done at the start of a restimulation cycle. After 2 to 3 weeks, when a polyclonal T-cell culture was obtained, CMP specificity was verified by LST. If these cultures indicated a high CMP-specific T-cell proliferation, T cells were cloned by limiting dilution at 0.3, 1, or 3 cells/well in 96-well U-bottom culture plates in the presence of IL-2, IL-4, and irradiated, autologous, EBV-transformed B cells (10^4/well) that had been preincubated overnight with CMP mix. Established clones were screened for CD4 or CD8 and T-cell receptor expression. Cells were stained with FITC-conjugated anti-CD4 (Leu-3a; Becton-Dickinson, San Jose, Calif) and phycoerythrin-conjugated anti-CD8 (Leu-2a, Becton-Dickinson) or FITC-conjugated anti-α/β T-cell receptor (WT-31, Becton-Dickinson); fixed in paraformaldehyde; and analyzed with FACScan (Becton-Dickinson). All TCCs were tested in LST to verify CMP specificity and to determine specificity for the different proteins in cow's milk.

CMP-specific cytokine release

To determine CMP-specific cytokine release, 106 cells of each TCC were incubated with 106 irradiated, autologous, EBV-transformed B cells that had been preincubated overnight with CMP mix. Control cultures of TCCs and EBV-transformed B cells preincubated without antigen were prepared in parallel. Stimulation was performed in a 24-well plate. After 24 hours of culture at 37°C, supernatants were collected and stored at –20°C. Cytokines were measured by ELISA according to the manufacturer's recommendations (IL-4, IL-13, and IFN-γ: Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands; IL-5: Endogen, Woburn, Mass). The detection limit was 0.6 pg/mL for IL-4, 5 pg/mL for IL-5, 0.5 pg/mL for IL-13, and 2 pg/mL for IFN-γ. CMP-specific cytokine release by a TCC was calculated by subtracting control-stimulated cytokine production from cytokine production after stimulation with CMP mix.

Statistical analysis

Nonparametric analysis (Wilcoxon signed-rank test and Mann-Whitney *U* test) was applied to determine significant differences between patient and control groups with regard to age, proliferative responses of PBMCs, and cytokine production by TCCs. Differences associated with *P* values of less than .05 were considered significant. Nonparametric Spearman rank correlation was used to test for correlation between production of different cytokines.

RESULTS

CMP-specific proliferation in PBMCs

Antigen-specific proliferation of PBMCs was determined for the 3 major allergens in cow's milk: total casein, α-lactalbumin, and β-lactoglobulin. Proliferative responses toward each of the 3 antigens were found in both patients with CMA and those without CMA (Fig 1). In patients with CMA, responses toward all 3 allergens were significantly higher than background responses ($P < .05$). The range of these responses, however, was quite large and overlapped extensively between patients with and without CMA. Mean T-cell proliferation in patients with CMA tended to be higher than in patients without CMA, but these differences were not significant. Also, comparison of mean stimulation index (ratio of counts per minute of antigen-stimulated cultures to unstimulated cultures) showed no significant differences in proliferative responses of PBMCs between patients with and without CMA and for each of the major cow's milk allergens (data not presented).

CMP-specific TCCs: Reactivity toward the various protein fractions in cow's milk

A total of 93 CMP-specific TCCs were obtained. Fifty TCCs were obtained from 4 patients with CMA, and 43 TCCs were obtained from 4 patients without CMA. The cloning procedure did not yield TCCs in the other 4 patients. Flow cytometric analysis showed that all clones were $CD4^+$ and expressed the α/β T-cell receptor (data not presented). Specificity of the clones was determined for the casein and whey proteins and for the protein subfractions (Table II). TCC reactivity toward the casein and whey proteins was observed in both allergic patients and patients without CMA and was not significantly different between the 2 groups.

Production of IL-4 and IFN-γ

CMP-specific cytokine release was measured in 44 CMP-specific TCCs derived from patients with CMA and 43 TCCs derived from patients without CMA. Fig 2 shows production of IL-4 and IFN-γ. In patients with CMA (Fig 2, *A*), antigen-specific TCCs with a diverse cytokine pattern were found; TCCs showed isolated production of IL-4 or IFN-γ or production of both cytokines. Clones from infants without CMA (Fig 2, *B*) showed a different pattern; none of the clones produced high levels of IL-4 (maximum, 0.36 ng/mL), and 79% of these TCCs produced even less than 0.1 ng/mL IL-4. A significant portion of the clones derived from patients without CMA produced high amounts of IFN-γ up to 30 ng/mL, and the rest (mostly TCCs from patient 11) produced very small amounts of both IL-4 and IFN-γ. To phenotype the TCCs according to their production of IL-4 and IFN-γ, an arbitrary classification was used that was adapted from a previous study with CMP-specific TCCs.[27] Table III shows phenotypes of clones from individual patients.

TABLE I. Characteristics of infants with AD with and without CMA

Patient No.	Symptoms*	IgE	SPT
1†	Erythema, AD	–	–
2‡	–	–	–
3†	Erythema, UR, AD	+	+
4†	Erythema, AD	–	+
5‡	–	–	–
6†	Erythema, AD	–	+
7‡	–	–	–
8†	Erythema, UR	+	+
9‡	–	–	–
10†	Erythema, UR, AD	+	+
11‡	–	–	+
13‡	–	–	–

IgE, Cow's milk–specific IgE detected by using RAST; *SPT*, skin prick test for cow's milk; *AD*, worsening of atopic dermatitis; *UR*, urticaria.
*Symptoms developed on challenge.
†Patient with CMA.
‡Patient without CMA.

Comparison of the CMP-specific cytokine response in infants with and without CMA

To compare the overall T_H1/T_H2 skewing of the CMP-specific T-cell response of infants with clinical reactions to cow's milk and infants without CMA for each cytokine, mean production by all CMP-specific TCCs from each patient was calculated (Table IV). In infants with CMA, mean IL-4 production was markedly higher than that found in infants without CMA, who showed a very low mean production of IL-4. This difference was statistically significant ($P < .05$, Mann Whitney *U* test). Mean production of IL-5 and IL-13 was also markedly higher in infants with CMA than in infants without CMA. In contrast, mean production of IFN-γ was markedly higher in infants without CMA compared with the infants with CMA. Although the differences in production of IL-5, IL-13, and IFN-γ did not reach statistical significance, when all clones from patients with CMA (n = 44) were pooled and compared with the mean cytokine production of all clones from patients without CMA (n = 43), these differences were all statistically significant ($P < .001$, Mann Whitney *U* test; data not presented).

Correlation between IL-4 release and release of IL-5 and IL-13

To determine the association between CMP-specific production of IL-4 and release of other T_H2-type cytokines during stimulation with cow's milk, correlation analysis was performed (Fig 3). Production of both IL-5 (Fig 3, *A*) and IL-13 (Fig 3, *B*) was significantly correlated with IL-4 production.

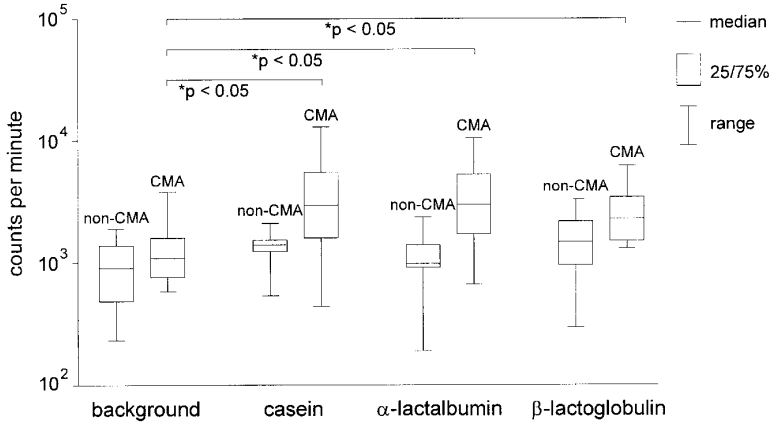

FIG 1. Proliferative responses of PBMCs from 12 infants with atopic dermatitis, 6 with CMA and 6 without CMA *(non-CMA)*, to the 3 major cow's milk allergens. [^{3}H]-TdR incorporation was measured 6 days after stimulation with the allergens.

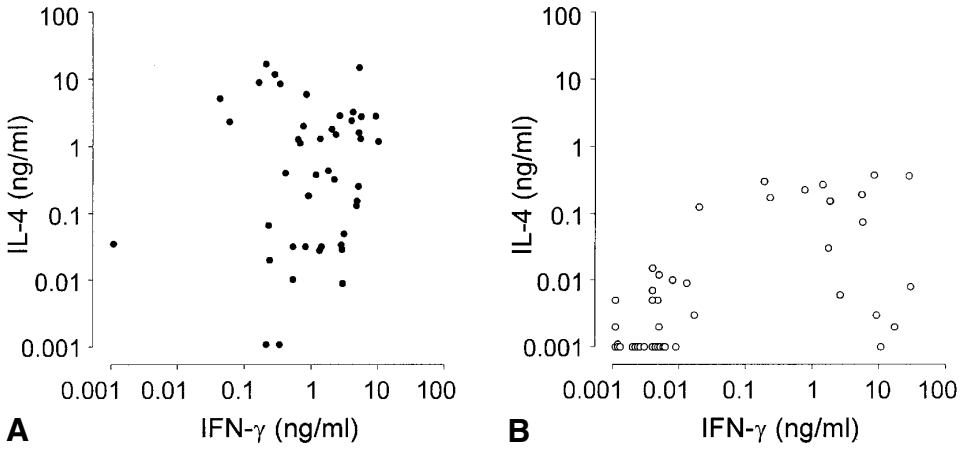

FIG 2. Antigen-specific production of IL-4 and IFN-γ by cow's milk–specific TCCs established from infants with AD with CMA **(A)** and without CMA **(B)**. Cytokine production was measured in culture supernatants 24 hours after stimulation with irradiated, autologous, EBV-transformed B cells preincubated overnight with CMPs.

DISCUSSION

In this study we analyzed the CMP-specific T-cell response in infants with AD and allergy for cow's milk and in infants with AD tolerance for cow's milk.

The results from the proliferation assays confirmed that lymphoproliferative responses to the major allergens in cow's milk exist in PBMCs of infants with AD. Although within the patients with CMA, proliferation to the cow's milk allergens was higher than background proliferation, the range of proliferative responses overlapped extensively between patients with and without allergic reactions to cow's milk. It is generally accepted that because of this capacity of both allergic and control subjects to mount lymphoproliferative responses to food allergens, it is not possible to distinguish between individual patients with and without food allergy on the basis of proliferation assays with PBMCs.[9,28]

To analyze the CMP-specific T-cell response at the clonal level, CMP-specific TCCs were established. We show that infants with AD have a CMP-specific T helper cell response, irrespective of their (cow's milk) allergic state. This response may be directed against all the major proteins in cow's milk and is not restricted to a particular subfraction.

Determination of cytokine release showed a clear dichotomy between the production of IL-4 in infants with and without CMA. In TCCs from infants with CMA, production of IL-4 was generally high, whereas none of the 43 clones derived from milk-tolerant infants produced significant amounts of IL-4 (Fig 2).

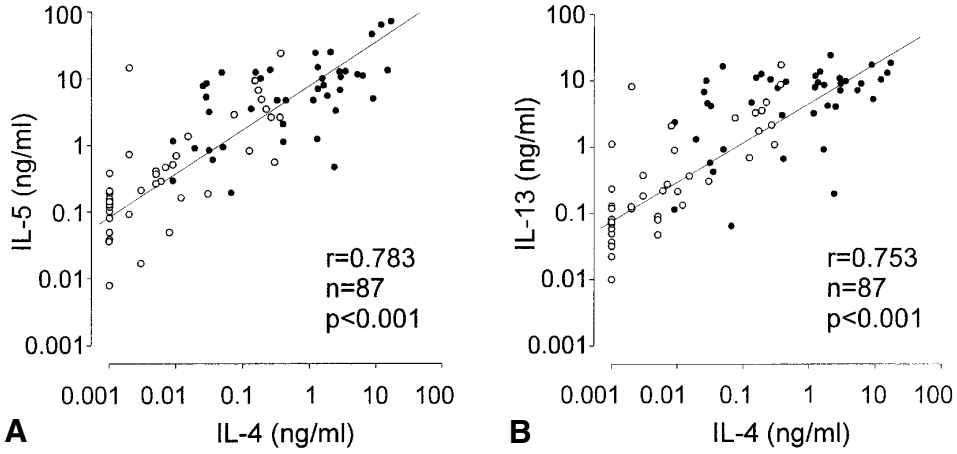

FIG 3. Correlation between antigen-specific production of IL-4 and IL-5 **(A)** and IL-4 and IL-13 **(B)** by cow's milk–specific TCCs established from infants with AD with and without CMA. *Filled circles* represent clones from allergic infants, and *open circles* represent clones from nonallergic infants. Cytokine production was measured in culture supernatants 24 hours after stimulation with irradiated, autologous, EBV-transformed B cells preincubated overnight with CMPs.

TABLE II. TCC reactivity toward the major CMPs and protein subfractions in infants with AD with and without CMA

			Casein proteins					Whey proteins	
Patient No.	**TCC reactivity**	**No. of TCCs**	**αS1-casein**	**αS2-casein**	**β-casein**	**κ-casein**	**Undifferentiated***	**α-Lactalbumin**	**β-Lactoglobulin**
1†	Casein-whey	23		8		2	1	3	9
3†	Casein-whey	17	2	1		4	3	5	2
4†	Casein	3		3					
8†	Casein	7	2	3	1		1		
2‡	Casein	4		1			3		
5‡	Casein	3	1			1	1		
7‡	Casein-whey	7				2			5
11‡	Casein-whey	29					1		28

*TCCs specific for total casein, but specificity toward the casein-subfractions could not be determined.
†Patient with CMA.
‡Patient without CMA.

The obtained TCCs were phenotyped according to their production of IL-4 and IFN-γ on the basis of criteria that were previously used to designate T_H2, T_H0, and T_H1 subsets in CMP-specific TCCs.[27] According to this arbitrary classification, the CMP-specific T cells from infants with CMA were predominantly from the T_H2 type and T_H0 type, whereas the infants without CMA did not have any CMP-specific T_H2 cells but had predominantly CMP-specific T cells from the T_H1 type (Table III).

Studies that evaluate human TCCs generally designate distinct TCC phenotypes similar to the T_H1 and T_H2 subsets that were originally found in murine models.[29,30] It has become clear, however, that human T cells producing a clear-cut T_H1 or T_H2 cytokine profile, as can be observed in mice, form 2 ends of a continuous spectrum in which most T cells produce a more heterogeneous cytokine profile.[31-33] Criteria for human T_H2-T_H0-T_H1 subsets vary considerably between reports, and each classification remains arbitrary.[31,34] For this reason, we analyzed differences between cytokine profiles of antigen-specific T cells from infants with CMA and cow's milk–tolerant infants also by comparison of the absolute amounts of each individual cytokine (Table IV). This showed that in the infants with allergic reactions to milk, mean production of IL-4 was much higher than in the nonallergic infants. In contrast, mean production of IFN-γ was higher in the infants that were tolerant for cow's milk. Three of these patients had a high IFN-γ production, whereas one patient had a cytokine response dominated by very low production of both IL-4 and IFN-γ.

In addition to IL-4, we determined the CMP-specific production of IL-5 and IL-13, 2 cytokines that are closely associated with the T_H2 response and that are potent inducers of allergic inflammation through recruitment of eosinophils and the induction of IgE synthesis.[35,36] In the

TABLE III. Phenotype of cow's milk–specific TCCs from infants with AD with and without CMA

Patient No.	No. of TCCs	T_H2*	T_H0*	T_H1*
1†	20	2 (10%)	6 (30%)	12 (60%)
3†	15	3 (20%)	9 (60%)	3 (20%)
4†	3	2 (67%)	1 (33%)	0
8†	6	4 (66%)	1 (17%)	1 (17%)
2‡	4	0	0	4 (100%)
5‡	3	0	0	3 (100%)
7‡	7	0	1 (14%)	6 (86%)
11‡	29	0	29 (100%)	0

*T_H2 phenotype was defined as follows: IL-4 ≥0.4 ng/mL and IFN-γ <1 ng/mL. T_H0 phenotype was defined as follows: IL-4 <0.4 ng/mL and IFN-γ <1 ng/mL or IL-4 ≥0.4 ng/mL and IFN-γ ≥1 ng/mL. T_H1 phenotype was defined as follows: IFN-γ ≥1 ng/mL and IL-4 <0.4 ng/mL.
†Patient with CMA.
‡Patient without CMA.

TABLE IV. Mean cytokine production by cow's milk–specific TCCs from infants with AD with and without CMA

Patient No.	No. of TCCs	IL-4 (ng/mL)	IL-5 (ng/mL)	IL-13 (ng/mL)	IFN-γ (ng/mL)
1†	20	0.76 ± 0.29	7.96 ± 1.23	7.85 ± 0.87	2.71 ± 0.64
3†	15	1.86 ± 0.60	4.37 ± 1.20	3.97 ± 1.03	2.39 ± 0.53
4†	3	14.53 ± 1.46	50.62 ± 18.78	14.28 ± 2.42	1.92 ± 1.67
8†	6	3.08 ± 1.41	16.83 ± 7.13	11.09 ± 3.90	0.70 ± 0.15
2‡	4	0.1 ± 0.1	6.15 ± 6.07	5.23 ± 4.11	14.50 ± 5.09
5‡	3	0.04 ± 0.02	1.14 ± 0.90	1.11 ± 0.84	3.34 ± 1.18
7‡	7	0.2 ± 0.04	9.26 ± 4.33	6.12 ± 2.21	7.85 ± 4.04
11‡	29	0.02 ± 0.01	0.29 ± 0.06	0.19 ± 0.05	0.01 ± 0.01
Patients with CMA, total (n = 4)		5.06 ± 3.19*	19.94 ± 10.55	9.30 ± 2.21	1.93 ± 0.44
Patients without CMA, total (n = 4)		0.09 ± 0.04*	4.21 ± 2.12	3.16 ± 1.47	6.43 ± 3.14

Results are expressed as mean ± SEM.
*Differences between patients with and without CMA are significant ($P < .05$).
†Patient with CMA.
‡Patient without CMA.

infants with CMA, production of IL-5 and IL-13 was high, whereas it was markedly lower in patients without CMA. Correlation analysis showed that release of both IL-5 and IL-13 was strongly correlated with IL-4 production, indicating that in CMP-specific T cells, release of T_H2 cytokines is closely related.

Taken together, these results show that CMA in infants with AD is associated with a T_H2-skewed, CMP-specific, T helper cell response in blood, whereas tolerance to cow's milk is associated with much lower levels of these cytokines and a T_H1-skewed cytokine response.

The elaborate process of establishing antigen-specific TCCs hampers the evaluation of large groups of patients. In most studies with TCCs, comparison of TCC reactivity between patient and control groups is done by pooling of all clones from each group to achieve a larger number of data. To make a true comparison, however, results from TCCs should be regarded as representative for an individual patient, and clones established from different patients should not be equated. For this reason, we analyzed differences by pooling of all TCCs from each individual patient. Because of the small sample size of 4 patients in each group, only a trend could be observed, and statistical significance was not reached for all differences. However, when the TCCs from all allergic patients were pooled and mean cytokine production was compared with all clones from patients without CMA, differences that were observed at the patient level between both groups were all statistically significant. This strongly suggests that these findings represent real differences that exist between infants with CMA and cow's milk–tolerant infants with AD.

Thus far, only 2 previous studies have analyzed isolated TCCs specific for CMPs.[27,37] In both studies only the casein fraction of cow's milk was used for T-cell culturing, and, as a consequence, only casein-specific TCCs were generated. Reekers et al[37] described 14 casein-specific TCCs established from 3 children with AD and allergic reactions to hen's egg and cow's milk. These TCCs had been obtained by using mitogenic polyclonal stimulation, a method that may dramatically affect the primary responding T-cell population[14] and may have resulted in only 43% of the obtained TCCs belonging to the $CD4^+$ subset. Cytokine release by TCCs is also often determined after exposure to mitogenic stimuli, such as anti-CD3/anti-CD28 antibodies[17] or concavalin A.[27,37] However, in vitro cytokine release by TCCs activated

through these nonspecific stimuli can be markedly different from cytokine production after antigen-specific stimulation.[15,17] For these reasons, we propagated and activated the CMP-specific TCCs in our study by using an antigen-specific culture system with autologous B cells as antigen presenters and did not use nonspecific mitogens. We believe that in this antigen-specific in vitro system, the observed reactivity of T cells is a good representation of the in vivo reactivity.

In summary, reports that evaluate the T-cell response against food antigens at the clonal level are scarce.[15-17,27,37] The present study is the first to analyze food-specific TCCs from food-tolerant individuals and to directly compare T-cell reactivity and cytokine profiles from allergic and age-matched food-tolerant subjects. The results show that although food allergy is associated with high production of T_H2 cytokines, the food-specific T helper response in food-tolerant individuals is characterized by low levels of these cytokines. In particular, the minute production of IL-4 in these infants, the hallmark cytokine of the allergic T_H2 response, suggests a key role for the T helper cell–derived T_H2 cytokines in food allergy–related skin symptoms. More studies on the mechanisms that determine T_H1/T_H2 skewing of the food antigen–specific T-cell response[38] and the precise mechanisms through which circulating food-reactive T_H2 cells mediate allergic inflammation in the skin[39] are needed to give future directions for the development of therapy in food allergy and AD.

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