

Establishment and characterization of α_{s1} -casein-specific T-cell lines from patients allergic to cow's milk: Unexpected higher frequency of CD8⁺ T-cell lines

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To study cow's milk allergy at the cellular level, we assessed the reactivity of peripheral blood mononuclear cells from patients allergic to cow's milk to α_{s1} -casein, which is one of the major allergens in cow's milk. Proliferation of the cells to α_{s1} -casein activation showed a rather weak response. Therefore to understand T-cell reactivity to α_{s1} -casein in more detail, we prepared α_{s1} -casein-specific T-cell lines from patients allergic to cow's milk and established 26 T-cell lines. These T-cell lines could be classified into three groups by analyzing their surface marker expression: those containing predominantly CD4⁺CD8⁻ T cells, those containing both CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells, and those containing predominantly CD4⁻CD8⁺ T cells. The CD8⁺ T cells were obtained at an unexpectedly higher frequency from the patients. These T-cell lines produced interferon- γ and IL-4. These results suggest that CD8⁺ T cells specific for α_{s1} -casein and CD4⁺ T cells were primed by the stimulation with α_{s1} -casein in patients allergic to milk and that both T cells may play a key role in the onset, progression of, or recovery from cow's milk allergy. (J Allergy Clin Immunol 1996;97:1342-9.)

Key words: Cow's milk allergy, T-cell line, α_{s1} -casein, CD8⁺ T cell, interferon- γ

Among the complicated effects attributed to cow's milk allergy (CMA), gastrointestinal symptoms are most frequently noted as being characteristic of this allergy.¹ Another important point is that patients with CMA usually become free of the symptoms as they grow older, although CMA may be a factor for inducing another allergy. The acquisition of tolerance to cow's milk proteins in CMA appears more frequently than that in allergies to other food proteins. The immune response and tolerance of T cells and B cells specific for milk proteins may play important roles in complicated allergic reactions and natural recovery. However,

Abbreviations used

CMA:	Cow's milk allergy
EB-B cell:	Epstein Barr virus-transformed autologous B cell
IFN- γ :	Interferon- γ
MMC:	Mitomycin C
PBMCs:	Peripheral blood mononuclear cells
TCL:	T-cell line

few studies of CMA at the lymphocyte level have been performed. T-cell clones specific for house dust mite,²⁻⁴ grass pollen,⁵ cat allergen,⁶ and bee venom phospholipase A₂^{7,8} have been established from cells of patients with environmental and inhalant-allergies. The results of cytokine profile analyses of these (mostly CD4⁺) clones indicated that most of the cells from the patients secreted higher proportion of IL-4 and IL-5 than that of interferon- γ (IFN- γ) and that these T cells had the ability to promote autologous B cells to produce IgE antibodies and to differentiate eosinophils.

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The respective functions of IL-4 and IL-5 by these T cells explained well the high titer of serum IgE specific for the corresponding allergens and the induction of eosinophil differentiation in the delayed type of allergy.^{9, 10}

Characterization of the responding lymphocytes in patients with CMA has not been achieved yet, although the supernatant of peripheral blood mononuclear cells (PBMCs) stimulated with cow's milk allergens has been shown to contain IFN- γ .^{11, 12} Thus the establishment of T-cell lines (TCLs) from these patients and an elucidation of the function of these T cells are of primary importance for understanding the onset, development of, and recovery from CMA. Bovine α_{s1} -casein is one of the major allergens among milk proteins for patients with CMA. In this study we established TCLs specific for α_{s1} -casein from patients with CMA who had a high titer of serum IgE and a delayed reaction to α_{s1} -casein, and we clarified the cytokine secretion pattern and surface marker expression of these cells. The results indicate that antigenic stimulation induced the secretion of IFN- γ and IL-4 and that CD8⁺ and CD4⁺ T-cell responses had been elicited in these patients. We demonstrate that the response of the CD8⁺ T-cell repertoire to α_{s1} -casein is characteristic in patients with CMA.

METHODS

Patients

Four children (patients 1, 2, 3, and 4) with atopic dermatitis were selected; they ranged in age from 2 to 4 years. Their RAST (Pharmacia Diagnostics, Uppsala, Sweden) scores for milk were all positive (>3). ELISA indicated that they each had a higher level of anti- α_{s1} -casein-specific IgE antibodies in their sera compared with normal donors (data not shown). Milk allergy was diagnosed by clinical history and dietary records and further verified by the observation that their symptoms (erythematous and morbilliform rash, gastrointestinal symptoms, and severe atopic dermatitis) disappeared with the elimination of milk and reappeared after milk was restored to their diets. Furthermore, results of the milk challenge test, described by Sampson,¹³ confirmed the diagnosis of milk allergy.

Antigens and reagents

α_{s1} -Casein was separated from fresh raw skim milk in accordance with the method of Zittle and Custer,¹⁴ and purified by DEAE Sephacel (Pharmacia Biotech, Uppsala, Sweden) ion-exchange chromatography. Recombinant human IL-2 (rIL-2) was kindly provided by Takeda Chemical Industries (Osaka, Japan). Phycoerythrin-labeled anti-human CD4-(NU-T/H1) and fluorescein isothiocyanate-labeled anti-human CD8-(NU-Ts/c) anti-

bodies for type identification were obtained from Nichirei (Tokyo, Japan) for use in flow cytometry.

Primary response to α_{s1} -casein

The primary proliferative response of PBMCs to α_{s1} -casein was examined according to the method of Kondo et al.¹⁵ with a slight modification. PBMC samples were obtained from the patients by Ficoll/Paque (Pharmacia Biotech) gradient centrifugation and resuspended at 2×10^6 cells/ml. The cell suspension was seeded in 96-well round-bottomed plates (Costar Corp., Cambridge, Mass.) with various concentrations of α_{s1} -casein in a volume of 0.2 ml and cultured for 7 days. The incorporation of tritiated thymidine during the last 4 hours of culture was measured. The results of primary proliferation are represented by the stimulation index (SI) as follows:

SI = cpm of a stimulated sample

÷ cpm of an unstimulated sample

Preparation of α_{s1} -casein-specific TCLs

α_{s1} -Casein-specific TCLs were established according to the method described previously^{16, 17} with a minor modification. Briefly, PBMCs were resuspended at a concentration of 2×10^6 cells/ml in an RPMI-1640 culture medium (Gibco, Paisley, U.K.) supplemented with 2 mmol/L L-glutamine, 5×10^{-5} 2-mercaptoethanol, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 10% heat-inactivated human serum. They were then seeded in 96-well round-bottomed plates with 80 μ g/ml α_{s1} -casein, and 25 U/ml rIL-2 was added every 3 to 4 days over a total period of 18 to 20 days of culture. After the end of the culture, the cells in each well were divided and put into two separate wells. One part was stimulated with the antigen and mitomycin C (MMC)-treated Epstein-Barr virus-transformed autologous B cells (EB-B cells), and the other was stimulated with EB-B cells alone. Before this stimulation, the EB-B cells had been treated with 80 μ g/ml MMC for 50 minutes at 37° C. Seven to 10 days later, the T cells responding to α_{s1} -casein were selected by comparison with cells cultured in the absence of antigen and transferred to three wells of the 96-well plates. The selected cells were then restimulated with the antigen and MMC-treated EB-B cells. One day after this stimulation, TCLs were further expanded in a medium containing 25 U/ml rIL-2; TCLs reactive to α_{s1} -casein were then similarly restimulated every 10 days.

Proliferation assay

The reactivity of TCLs to α_{s1} -casein was measured by a tritiated thymidine incorporation assay. TCLs (1 to 2×10^4) were cultured in 96-well plates with autologous MMC-treated EB-B cells (1 to 2×10^4) and the indicated concentrations of α_{s1} -casein. Three days later, tritiated thymidine (1 μ Ci/well) was pulsed, and its

TABLE I. Primary proliferative responses of PBMCs to α_{s1} -casein, purified protein derivative and concanavalin A

Total ^3H thymidine incorporation (stimulation index)*								
Experiment 1					Experiment 2			
	Antigen concentration ($\mu\text{g/ml}$)	α_{s1} -Casein	PPD	Con A	Antigen concentration ($\mu\text{g/ml}$)	α_{s1} -Casein	PPD	Con A
Patient 1	10	0.54	99.53	10.50	10	1.99	83.10	10.03
	30	3.26			30	1.19		
	100	4.50			100	2.00		
Patient 2	10	1.24	10.40	7.78	10	4.33	67.52	4.66
	30				30	5.03		
	100	1.93			100	3.50		
Patient 3	10	0.34	4.26	2.63	10	0.77	6.43	1.06
	30	0.82			30	0.72		
	100	1.19			100	2.17		

Background counts per minute data for each experiment in each patient were as follows: 862 (experiment 1) and 1006.3 (experiment 2) in patient 1, 1828.0 (experiment 1) and 2606.0 (experiment 2) in patient 2, and 16703.5 (experiment 1) and 2274.5 (experiment 2) in patient 3.

PPD, Purified protein derivative; Con A, concanavalin A.

*Stimulation index: 2×10^5 PBMCs were stimulated with the respective antigens. Seven days later, tritiated thymidine incorporation was measured, and the stimulation index was calculated.

incorporation into DNA was assessed by liquid scintillation counting after 16 to 20 hours.

Quantification of cytokines in the TCL supernatants

Seven to 10 days after their previous stimulation, α_{s1} -casein-specific TCLs at a concentration of 1×10^6 cells/ml were stimulated again with 80 $\mu\text{g/ml}$ α_{s1} -casein in the presence of 1 to 1.5×10^6 cells/ml MMC-treated EB-B cells. Twenty-four hours after this restimulation, the supernatants were collected and stored at -20°C until they were needed for testing. IL-4 and IFN- γ secreted in the culture supernatant were quantified by using Intertest ELISA kits (Genzyme, Cambridge, Mass.).

Immunofluorescence analysis

Cells of each TCL were collected 7 to 10 days after being stimulated with α_{s1} -casein and MMC-treated EB-B cells. They were stained with phycoerythrin-labeled anti-human CD4-specific and fluorescein isothiocyanate-labeled anti-human CD8-specific mouse monoclonal antibodies, and an immunofluorescence analysis was performed by using FACSsort (Becton Dickinson, Mountain View, Calif.).

RESULTS

Analysis of the primary response to α_{s1} -casein

We first analyzed the primary proliferative response to α_{s1} -casein and the surface marker expression of PBMCs from three patients with CMA.

As shown in Table I, PBMCs from patients 1 and 2 showed obvious proliferation to α_{s1} -casein in both experiments I and II, respectively, whereas the response of PBMCs from patient 3 to α_{s1} -casein was rather weak. These PBMCs proliferated strongly in the presence of tuberculin purified protein derivatives and concanavalin A in patients 1 and 2. In patient 3, PBMCs proliferated in response to purified protein derivatives and concanavalin A but not in response to α_{s1} -casein in a 5-day culture (data not shown).

Establishment of α_{s1} -casein-specific TCLs

To clarify in more detail the characteristic and functions of T cells reacting to α_{s1} -casein, we established 26 α_{s1} -casein-specific TCLs from PBMCs of four patients allergic to milk, including the three we had already studied. These TCLs proliferated strongly in response to the antigen, specifically with autologous MMC-treated EB-B cells (Fig. 1).

Subsets of TCLs

We next studied the surface marker expression of these TCLs (Fig. 2). The sum of each percentage of $\text{CD4}^+\text{CD8}^-$ and $\text{CD4}^-\text{CD8}^+$ T cells for some TCLs was less than 100%, indicating that some cells in these TCLs were $\text{CD4}^-\text{CD8}^-$. We

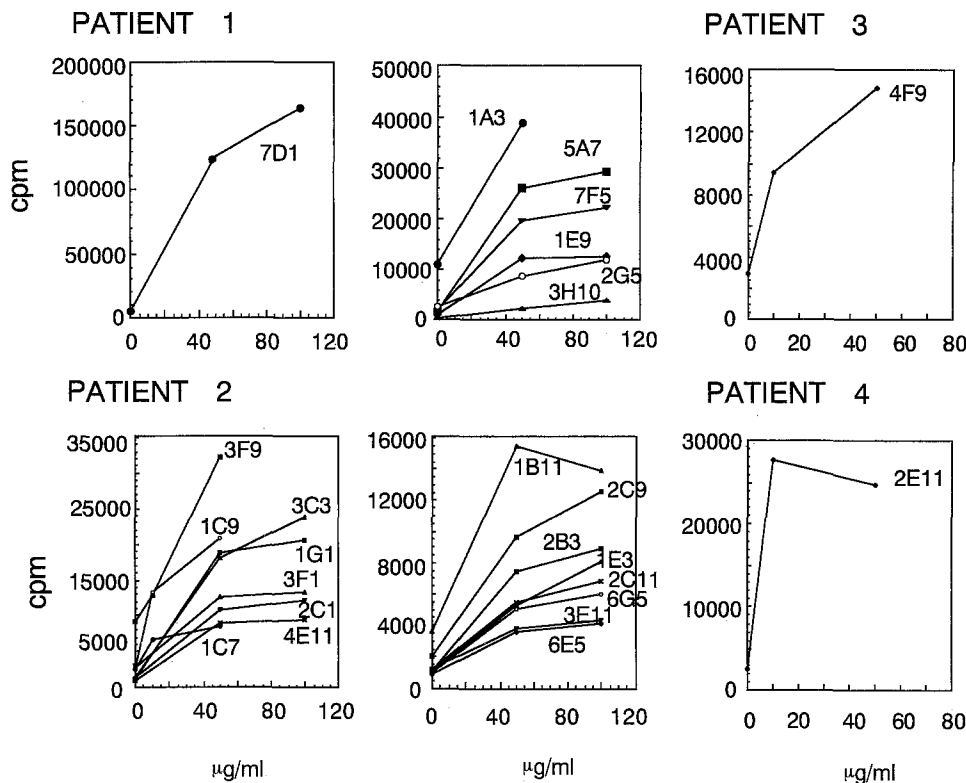


FIG. 1. Proliferative response to α_{s1} -casein of 26 α_{s1} -casein-specific TCLs from four patients with CMA (patients 1, 2, 3, and 4). TCLs (1 to 2×10^4) were stimulated with the indicated concentration of α_{s1} -casein, and tritiated thymidine incorporation was measured after 72 hours.

determined the ratio (percent) of $\text{CD4}^+\text{CD8}^+$ T cells to the fraction of $\text{CD4}^+\text{CD8}^-$ and $\text{CD4}^-\text{CD8}^+$ T cells for each TCL. TCLs could be classified into three groups: one group containing 0% to 8% of CD8^+ T cells (CD4-dominant TCLs), another containing 35% to 60% of CD8^+ T cells (CD4/8 TCLs), and the third with more than 83% of CD8^+ T cells (CD8-dominant TCLs). The CD8-dominant and CD4/8 TCLs contained 13 TCLs, which is more than half of those analyzed for CD4 and CD8 expression. In contrast, the CD4 TCLs comprised 10 lines, which is less than half of the 23 TCLs.

IL-4 and IFN- γ production profiles in the supernatants of α_{s1} -casein-specific TCLs

We analyzed the cytokine secretion pattern of these TCLs by measuring IL-4 and IFN- γ by ELISA (Table II). In response to stimulation with α_{s1} -casein, 18 lines secreted detectable amounts of IL-4. Seven lines of these IL-4-secreting TCLs (1A3, 1E9, 7D1, 7F5, 1G1, 2B3, and 2C11) produced both IL-4 and IFN- γ , whereas two lines (1E9 and 2B3) produced more IFN- γ than IL-4. The others secreted only IL-4 or did not secrete a detectable amount of either of these cytokines. These results indicate that α_{s1} -case-

in-specific T cells in patients allergic to milk secreted not only IL-4 but also IFN- γ . The cytokine production profiles for CD4/8 TCLs indicate that three lines (3F9, 3C3, and 2E11) secreted only IL-4, whereas two lines (1E9 and 1G1) secreted both IL-4 and IFN- γ . Nine of the CD4-dominant TCLs (1A3, 5A7, 7D1, 7F5, 1C7, 1C9, 4E11, 3E11, and 2B3) secreted IL-4, and four of them (1A3, 7D1, 7F5, and 2B3) secreted IFN- γ , as well as IL-4. TCLs 1A3, 5A7, 7D1, 1C7, and 1B11 contained CD4^+ T cells but no CD8^+ T cells. From these results, it is concluded that some CD4^+ T cell clones secreted only IL-4, whereas other CD4^+ T cells secreted only IFN- γ or IL-4 plus IFN- γ . Among the CD8-dominant TCLs, 2C11 secreted IFN- γ , as well as IL-4, whereas 2C9 only secreted IL-4. Among these α_{s1} -casein-specific TCLs, neither of the two cytokines was detected in cultures of eight lines. Analysis of IL-2 production by bioassay indicated that two of them secreted IL-2 (3F1 and 2C1), whereas another (6E5, 6G5 and 3E11) did not (data not shown).

DISCUSSION

To study the reactivity of lymphocytes in CMA, we examined the proliferation of PBMCs from

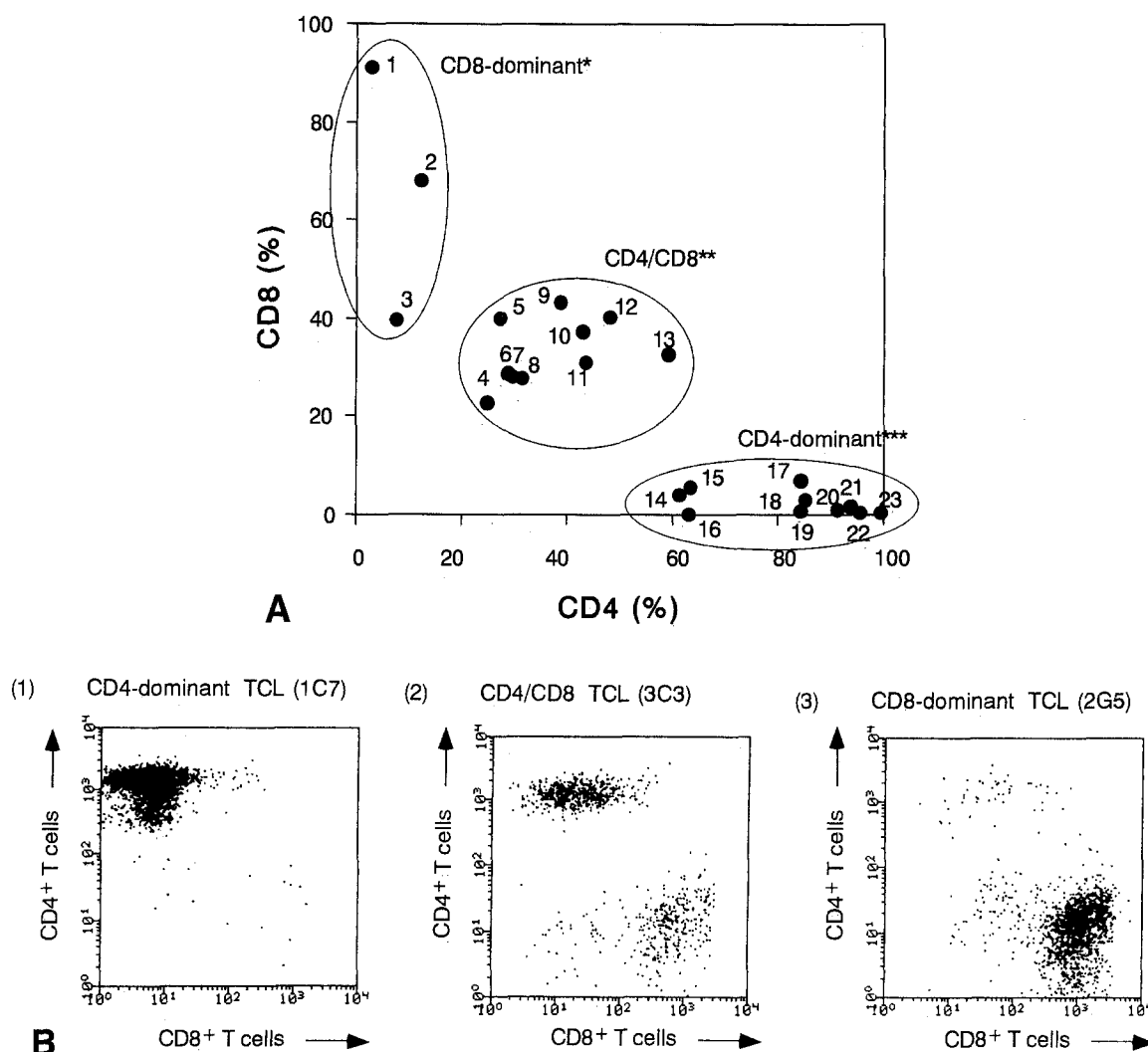


FIG. 2. A, Subsets of α_{s1} -casein-specific TCLs. Surface markers of these TCLs were analyzed by FACSsort; each number indicates each TCL. TCLs were classified into three groups according to the ratio of CD4⁺CD8⁺ T cells, which was calculated as follows: Ratio (%) of CD4⁺CD8⁺ T cells = $\frac{\text{CD4}^+\text{CD8}^+\text{ T cells}}{(\text{CD4}^+\text{CD8}^- + \text{CD4}^+\text{CD8}^+ \text{ T cells})} \times 100$. *CD8-dominant: TCLs containing more than 83% of CD8⁺ T cells (1, 2G5; 2, 2C9; and 3, 2C11). **CD4/CD8: TCLs containing 35% to 60% of CD8⁺ T cells (4, 2E11; 5, 1E3; 6, 6E5; 7, 6G5; 8, 3F1; 9, 3F9; 10, 5F2; 11, 1G1; 12, 1E9; and 13, 3C3). ***CD4-dominant: TCLs containing (0% to 8% of CD8⁺ T cells (14, 4E11; 15, 3E11; 16, 1A3; 17, 2B3; 18, 7F5; 19, 1B11; 20, 5A7; 21, 1C9; 22, 7D1; and 23, 1C7). B, FACSsort profiles of the representative TCLs of each group. (1), CD4-dominant TCL (1C7); (2), CD4/CD8 TCL (3C3); (3), CD8-dominant TCL (2G).

three patients allergic to bovine α_{s1} -casein. PBMCs from two patients proliferated in response to α_{s1} -casein in only one of the experiments, whereas those from the third patient did not respond in any experiments. These results suggest that α_{s1} -casein-specific T cells were elicited in the patients with CMA. However, because PBMCs did not show a strong response to α_{s1} -casein in all repeated experiments, this strategy did not seem to be appropriate for characterizing those T cells specific for the allergen. Accordingly, we attempted to establish

α_{s1} -casein-specific TCLs from four patients with CMA by stimulating PBMCs several times with α_{s1} -casein in the presence of rIL-2, and we finally succeeded in establishing 26 α_{s1} -casein-specific TCLs from cells from the same four children. We could not establish any α_{s1} -casein-specific TCLs from normal donors by this method (data not shown). α_{s1} -Casein-specific TCLs from patients with CMA proliferated strongly in response to α_{s1} -casein (Fig. 1), and this is the first report showing human TCLs specific for α_{s1} -casein. Al-

though casein-specific CD4⁺ T-cell clones have been established by stimulation with diluted cow's milk,¹⁸ the authors could not demonstrate the casein (α_{s1} -, α_{s2} -, β -, or κ -) for which these clones were specific or which cytokines they secreted. In this study we can demonstrate that TCLs were specific for α_{s1} -casein and that these secreted either, both, or neither IL-4 and IFN- γ .

When the surface marker expression of the cells was studied in these TCLs, the TCLs were classified into three groups as mentioned in the Results section (i.e., CD4-dominant TCLs, CD4/CD8 TCLs, and CD8-dominant TCLs). The most unexpected and important result is that we established 10 CD8/CD4 and three CD8-dominant TCLs; the TCLs containing CD8⁺ T cells formed almost half of all the TCLs. Because the fraction from PBMCs freshly separated from the patients' blood had less than 25% CD8⁺ T cells, we consider that the larger content of CD8⁺ T cells compared with that in fresh PBMCs (CD8-dominant and CD4/8 TCLs) had resulted from the expansion of this subset by stimulation with α_{s1} -casein in vitro. In addition, although a few studies have shown that a phospholipase A₂-specific TCL contained a CD8⁺ T-cell population and that a Der p 2-specific CD8⁺ T-cell clone was isolated,^{2,8} the frequency of CD8⁺ T cells was much less than that in α_{s1} -casein-specific TCLs. We established ovalbumin-specific TCLs from the same patients, the results indicating that all of ovalbumin-specific TCLs tested were composed of CD4-dominant TCLs, as classified in this study (Katsuki et al. Unpublished data). Thus our study indicates that CD8⁺ T cells were obtained at a higher frequency in the TCLs after stimulation with α_{s1} -casein when compared with the other allergen-specific T cells. This suggests that α_{s1} -casein induced a significant number of CD8⁺ T cells in the patients with CMA.

A study about immune response to α_{s1} -casein in mice has shown that a CD8⁺ T-cell response was elicited by injection of α_{s1} -casein.^{19,20} It is notable that the CD8⁺ T-cell repertoire responded to α_{s1} -casein in both mice and human beings, and we suspect that this may be attributable to the context of the allergenic molecules. A study on the immune response to ovalbumin in mice has shown that denatured ovalbumin induced priming of CD8⁺ T cells, whereas native ovalbumin did not.²¹ α_{s1} -Casein can be regarded as sharing the characteristics of denatured globular proteins rather than those of native molecules in that these have an extended, flexible, and disordered but not compact or rigid conformation.²² Thus it is demonstrable

TABLE II. Cytokine production profiles of α_{s1} -casein-specific TCLs and their subset classification

	TCL*	IL-4 (pg/ml)	IFN- γ (pg/ml)	Subset
Patient 1				
	1A3	596.8	312.8	CD4
	5A7	482.1	†	CD4
	7D1	3389.2	168.6	CD4
	7F5	1031.0	355.6	CD4
	1E9	267.2	514.7	CD4/CD8
	2G5	†	†	CD8
	3H10	227.2	†	ND
Patient 2				
	1C7	403.0	†	CD4
	1C9	385.2	†	CD4
	4E11	278.2	†	CD4
	3E11	211.4	†	CD4
	2B3	220.4	249.3	CD4
	1B11	†	†	CD4
	1G1	218.2	108.0	CD4/CD8
	3C3	265.0	†	CD4/CD8
	3F9	518.8	†	CD4/CD8
	6E5	†	†	CD4/CD8
	6G5	†	†	CD4/CD8
	3F1	†	†	CD4/CD8
	1E3	†	†	CD4/CD8
	5F2	†	†	CD4/CD8
	2C11	287.2	160.4	CD8
	2C9	242.8	†	CD8
	2C1	†	†	ND
Patient 3				
	4F9	2572.0	†	ND
Patient 4				
	2E11	293.8	†	CD4/CD8

CD4, CD4-dominant TCLs; CD4/CD8, CD4/CD8 TCLs; CD8, CD8-dominant TCLs; ND, not determined.

*TCLs (1×10^6 cells/ml) were stimulated with 80 μ g/ml α_{s1} -casein and 1 to 1.5×10^6 cells/ml autologous MMC-treated EB-B cells. Supernatants were collected after 24 hours and assessed by commercial ELISA kits.

†Production of cytokines was less than 200 pg/ml (IL-4) or less than 100 pg/ml (IFN- γ).

that these molecular characteristics shared by both α_{s1} -casein and denatured proteins are responsible for inducing the CD8⁺ T-cell response in mice and human beings, although the precise mechanism for this activation has not yet been clarified.

To understand which roles those CD4⁺ and CD8⁺ T cells responding to α_{s1} -casein would play in CMA, we assessed the IL-4 and IFN- γ production pattern of the TCLs. The secretion of IL-4 and IFN- γ was detected in 18 lines responding to α_{s1} -casein, and the pattern of these TCLs was independent of the predominance of each CD4⁺

or CD8⁺ T-cell subset. The cytokine production profiles of the CD4-dominant TCLs in this experiment show that five lines secreted only IL-4, whereas four lines secreted both IL-4 and IFN- γ . Because it is well known that IL-4 induces IgE class switching and synthesis of this isotype antibody, the IL-4-secreting T cells are considered to be T_{H2}-type and to accelerate IgE production specific for α_{s1} -casein. On the other hand, the type of CD4⁺ T cells producing IFN- γ is not obvious at present. CD4-dominant TCLs that produced both of the cytokines may have contained either a mixture of T_{H1}- and T_{H2}-type T cells producing either IFN- γ or IL-4, respectively, or T_{H0}-type cells producing both cytokines. It is possible that CD8⁺ T-cell clones in these TCLs secreted IL-4, IFN- γ , or both, although the cytokines from the CD8-dominant TCLs may have been from a small number of CD4⁺ T cells in these lines. Some reports, apart from this study have also suggested that IFN- γ produced by PBMCs was likely to function in allergic reactions to milk.^{11, 12} In this study we verified that IFN- γ in the patients with CMA originated from a CD4⁺ T-cell response to α_{s1} -casein, and it seems to have also been secreted by CD8⁺ T cells specific for α_{s1} -casein. Although T_{H1}- or T_{H0}-type clones primed with Der p or Lol p 1^{3, 5} have been isolated, many reports about allergen-specific T-cell clones have indicated that T_{H2}-type cells were related to allergic response. In addition, ovalbumin-specific TCLs established from cells of patients with CMA, which were evaluated in this experiment according to the same procedures, produced only IL-4 and never secreted IFN- γ (Katsuki et al. Unpublished data). This result may show that ovalbumin cannot elicit any response from the T cells producing IFN- γ , whereas α_{s1} -casein did this in the same patients. However, because T cells from normal donors have not been characterized in this or any other study, the function of IFN- γ produced by TCLs from patients with CMA remains to be clarified.

It is one of the most important characteristics of CMA that in accordance with their growth, patients usually acquire and maintain tolerance to cow's milk allergens. This natural recovery appears more clearly with CMA than with other food allergies. In our established TCLs, some of the CD8⁺ T cells may exert a suppressive activity on the allergic reactions of different cell populations or may influence the acquisition of tolerance. The function inhibiting IgE synthesis of CD8⁺ T cells has also been indicated in the mouse model and in patients with allergy.^{19, 20, 23, 24} However, in this

case it must be assumed that these suppressive CD8⁺ T cells cannot function efficiently or that this population would not be sufficiently dominant in the patients with CMA who were included in this study. Competition between the T cells that facilitate an allergic reaction and those that inhibit development of the disease may be an important selection pressure that determines the manifestation of CMA.²⁵ When the suppressor T cells are able to achieve a sufficient inhibiting effect or to function efficiently, patients may recover from CMA. On the other hand, according to the results of our study, it can be expected that at the onset of CMA, α_{s1} -casein would enter a patient's system through the gastrointestinal epithelium and induce a CD8⁺ T-cell response at a higher frequency. These CD8⁺ T cells may influence the development of inflammation by producing cytokines and/or by their cytotoxic activity in the gastrointestinal tract.²⁶ A recent study has shown that Peyer's patch CD8⁺ T cells could help immunoglobulin secretion, suggesting that CD8⁺ T cells primed with α_{s1} -casein in the gastrointestinal tract may function to help antibody production.²⁷ In any of the cases of suppressor and effector CD8⁺ T cells, both of these may have an important role in CMA, and elucidation of the function of this subset is awaited.

We established, in this study, different subsets of TCLs reacting to α_{s1} -casein from cells of four patients with CMA. CD8⁺ T cells and CD4⁺ T cells were demonstrated to be primed with α_{s1} -casein in the patients with CMA. The induction of CD8⁺ T cells responding to α_{s1} -casein is one of the most notable characteristics among the peripheral immune responses in the patients, although two contrary functions (effector or suppressor) in CMA have been assumed for our established CD8⁺ T cells at present. The inhibition or activation of this T-cell repertoire may be one of the critical targets for preventing or recovering from CMA.

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