The relevance of T_{H1} and T_{H2} cells in immediate and nonimmediate reactions to gelatin-containing vaccine

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Background: The immune mechanism of gelatin allergy, especially the participation of T_{H1} and T_{H2} cells and their cytokine secretion, has not been investigated.
Objective: We investigated the characteristics of T lymphocytes from patients allergic to gelatin-containing vaccine by secondary in vitro stimulation of circulating mononuclear cells with gelatin.
Methods: We studied 8 children with a history of immediate-type reactions and 8 with nonimmediate-type reactions after inoculation of gelatin-containing vaccine. The expression of IFN-γ (T_{H1}), IL-2 (T_{H1}), IL-4 (T_{H2}), and IL-13 (T_{H2}) mRNA was examined semiquantitatively by using a reverse transcriptase PCR. IgE antibody to bovine gelatin was measured with the fluorometric ELISA system, and gelatin-specific T-cell responses were detected by an in vitro lymphocyte proliferation assay.
Results: Patients with an immediate reaction all had gelatin-specific IgE antibody, whereas others did not. However, all patients exhibited positive T-lymphocyte responses specific to gelatin. Lymphocytes from subjects with nonimmediate-type reactions generally expressed very weak or sometimes no IFN-γ, IL-2, or IL-13 genes and especially no IL-4 gene. On the other hand, the lymphocytes of subjects with immediate-type reactions significantly expressed not only IL-4 and IL-13 but also IFN-γ and IL-2 mRNA.
Conclusion: Our observations suggest that both gelatin-specific T_{H2} and T_{H1} responses are involved in the pathogenesis of the immediate reaction to gelatin. The gelatin-specific IL-4 and/or IL-13 responses consistently observed in patients with an immediate reaction may be associated with the production of gelatin-specific IgE antibody. (J Allergy Clin Immunol 1999;103:276-81.)

Key words: *Gelatin allergy, T_{H1}, T_{H2}, IL-4, IFN-γ*

Abbreviations used
RT: Reverse transcriptase
SI: Stimulation index

Gelatin protein is derived from animal collagen tissues and is therefore present in many kinds of animal protein food. Gelatin is also used as a stabilizer for both live and inactivated vaccines. However, there is increasing evidence of allergic reactions against gelatin protein in vaccines, food, and drugs.[1,2] Occasionally an immediate "anaphylactic-like" reaction with life-threatening shock occurs in persons inoculated with gelatin-containing vaccine. More commonly, many cases with a nonimmediate reaction, consisting of a generalized mild skin eruption appearing several hours or more after vaccination, have been observed.[1,2]

Kelso et al[3] demonstrated IgE antibody to gelatin in the sera of patients experiencing an immediate reaction to mumps-measles-rubella vaccination. Sakaguchi et al[4,5] also found gelatin-specific IgE antibody in the sera of patients with an immediate reaction after inoculation with several gelatin-containing vaccines. The importance of specific IgE antibody in the immediate reaction is now established. Furthermore, Kumagai et al[6] also demonstrated gelatin-specific cell-mediated immunity not only in patients with immediate reactions but also in those with nonimmediate reactions.

Recently, human T-helper lymphocyte heterogeneity with T_{H1} and T_{H2} subclasses distinguished on the basis of their cytokine repertoire has been proposed. These subclasses may regulate the direction of the immune response.[7-11] T_{H1} cells can produce IFN-γ and IL-2 and promote cellular immunity, including delayed-type hypersensitivity, whereas T_{H2} cells are reported to produce IL-4, IL-5, IL-10, and IL-13 and to promote humoral immune responses, including IgE subclass switching. Both cell types may work together either suppressively or competitively. However, the participation profile of cytokines from primed T lymphocytes on gelatin allergy events remains unclear.

In this study we examined gelatin-specific memory T cells by in vitro antigen stimulation of PBMCs from

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patients with immediate and nonimmediate reactions to gelatin-containing vaccine. Expression of IFN-γ, IL-2, IL-4, and IL-13 mRNA and production of cytokine proteins were examined quantitatively.

METHODS

Subjects

Heparinized (50 U/mL) peripheral blood samples were obtained from 8 subjects with immediate reactions and 8 subjects with nonimmediate reactions after inoculation of various gelatin-containing vaccines. All blood samples were collected within 2 weeks after inoculation of gelatin-containing vaccine. The development of anaphylactic symptoms, such as urticaria, cough, wheezing, and dyspnea, occurred within 30 minutes after vaccination in 8 patients with immediate reactions. Nonimmediate reactions in the other 8 patients consisted of erythema and swelling at the injection site and mild systemic cutaneous reactions up to 48 hours after vaccination. Patients with nonimmediate reactions manifested neither respiratory nor cardiovascular symptoms. We also included 6 control subjects with no history of adverse reactions to vaccination.

PBMCs were separated by flotation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) and were washed twice with RPMI 1640 medium (Nissui, Tokyo, Japan). The cell concentration was adjusted to 2.5×10^6 cells/mL in RPMI 1640 medium supplemented with 10% of each subject's own serum. Informed consent was obtained from the parents of all study subjects.

IgE measurements

IgE antibody to bovine gelatin (Wako Junyaku, Tokyo, Japan) was measured by the fluorometric ELISA system. Briefly, microplates were coated with bovine gelatin (10 μg/mL). After incubation of the serum sample with immunobilized antigens, anti-human IgE antibody conjugated with β-D-galactosidase (Pharmacia) was added. The enzyme substrate 0.1 mmol/L 4-methylumbelliferyl–β–D-galactoside (Sigma Chemical Co, St Louis, Mo) was added to each well of the microplate. The fluorescence unit was measured on a fluorometric microplate reader (Fluoroscan; Flow Laboratory, McLean, Va). To calibrate the system, positive sera were obtained from the patients with anaphylactic reactions, whereas control sera were obtained from recipients of gelatin-free vaccine under 8 months of age who experienced neither immediate nor nonimmediate reactions. Inhibition studies were performed and both specific inhibition and parallelism were demonstrated. The potential detection limit of the test was 0.05 Ua/mL.

Culture of PBMCs with gelatin antigen

PBMC cultures were established with medium alone (negative control) or with bovine gelatin (Wako Junyaku) at a final concentration of 100 μg/mL as described previously.[6] Cultures were incubated at 37°C in a 5% CO_2 atmosphere for 2, 6, 12, 24, and 48 hours. To confirm the nonspecific responsiveness to mitogen, some PBMCs were incubated with 10 μg/mL concanavalin A for 3 hours.

Cytokine assay

The culture supernatant was collected after 48 hours of incubation and stored at –20°C until assayed. Cytokine assays were done by quantitative ELISA (Biosource Institution) following the manufacturer's instructions. The detection limits of the tests were 15.6, 15.6, 7.81, and 19.5 pg/mL for IFN-γ, IL-2, IL-4, and IL-13, respectively (manufacturer's information).

Analysis of cytokine mRNA

Total cellular RNA was isolated from PBMCs by using RNAzol B and tested for IFN-γ, IL-2, IL-4, and IL-13 mRNA by using a specific reverse transcriptase (RT)-PCR as described previously.[12] For an internal control, the activity of β-actin mRNA was also determined. Fifty nanograms of the total RNA was used for RT-PCR.

For cDNA synthesis, 40 μL of RNA solution (50 ng) and 150 pmol/3 μL random hexmer (Takara, Kyoto, Japan) were heated at 70°C for 10 minutes and cooled rapidly on ice. After adding 17 μL of 5× first-strand buffer (250 mmol/L Tris-HC1, 375 mmol/L KC1, and 15 mmol/L $MgCl_2$), 9 μL of 0.1 mmol/L dithiothreitol (GIBCO BRL, Gaithersburg, Md), 17 μL of 2.5 mmol/L of each deoxynucleoside triphosphate (Takara), and 200 U of Maloney murine leukemia virus reverse transcriptase (GIBCO BRL), the mixture was stored at 37°C for 1 hour.

Sequences of the PCR primer pairs and specific probes for Southern blot analysis are shown in Table I.[13-15] The PCR reaction mixture contained 50 ng of cDNA, 10 μL of PCR buffer (500 mmol/L KCl, 10 mmol/L Tris, and 1% Triton X-100), 8 μL of 2.5 mmol/L of each deoxynucleoside triphosphate (Takara), 6 μL of 25 mmol/L $MgCl_2$, 100 pmol/L 5′ and 3′ primers, and distilled water for a total volume of 100 μL. After denaturing at 94°C for 10 minutes and cooling to 80°C, the mixture was seeded with 2.5 U of thermostable Taq polymerase (Promega, Madison, Wis). Twenty-five cycles of amplification for β-actin, 33 for IFN-γ, and 35 for IL-2, IL-4, and IL-13 were carried out by DNA thermal cycler (Perkin Elmer, Norwalk, Conn). Each cycle consisted of warming at 95°C for 35 seconds, 55°C for 2 minutes, and 72°C for 2 minutes. Finally, the preparations were incubated at 72°C for 15 minutes. Ten-microliter samples of the RT-PCR products were analyzed by electrophoresis on 2% agarose gel containing 1 μg/mL ethidium bromide, and the amplified products were visualized by UV fluorescence. The UV fluorescence signals of specific PCR products in agarose gels were quantified by using a FluorImager SI (Molecular Dynamics, Sunnyvale, Calif).

The specificity of each PCR product was confirmed by Southern blot analysis. The PCR products were transferred to a nylon membrane and probed with digoxigenin 3′-end labeled internal oligonucleotide probes (Boehringer Mannheim GmbH, Mannheim, Germany).[14]

As a positive control for mRNA for each cytokine, total RNA from normal adult PBMCs incubated with concanavalin A at 10 μg/mL for 3 hours was used. To quantify relative levels of mRNA, a standard curve was obtained by titration (1/5 dilutions) of first strands obtained from 250 ng of RNA from the positive control subjects as described above (Fig 1). Relative differences in mRNA expression in the test samples were obtained from the standard curves run for the same number of cycles as the unknown samples. The intensity of fluorescence of DNA amplified from first strands obtained from 250 ng RNA was defined arbitrarily as 1000 mRNA equivalents.

The level of β-actin in each unknown sample was determined from the actin standard curve, and the levels generally varied less than 30% when the first strand was obtained from the same amount of total RNA (data not shown).

In vitro lymphocyte proliferation assay

Lymphocyte proliferation to gelatin antigen was assayed by thymidine incorporation as described previously.[6] Another set of PBMCs were incubated in 96-well microplates for 4 days. Tritiated thymidine (1 μCi) was added to each culture, and 16 hours later, the cultures were harvested onto fiberglass filters. Incorporation of radioactive thymidine was determined in a Beckman LS-335 scintillation spectrometer. The results were expressed by ratios of triti-

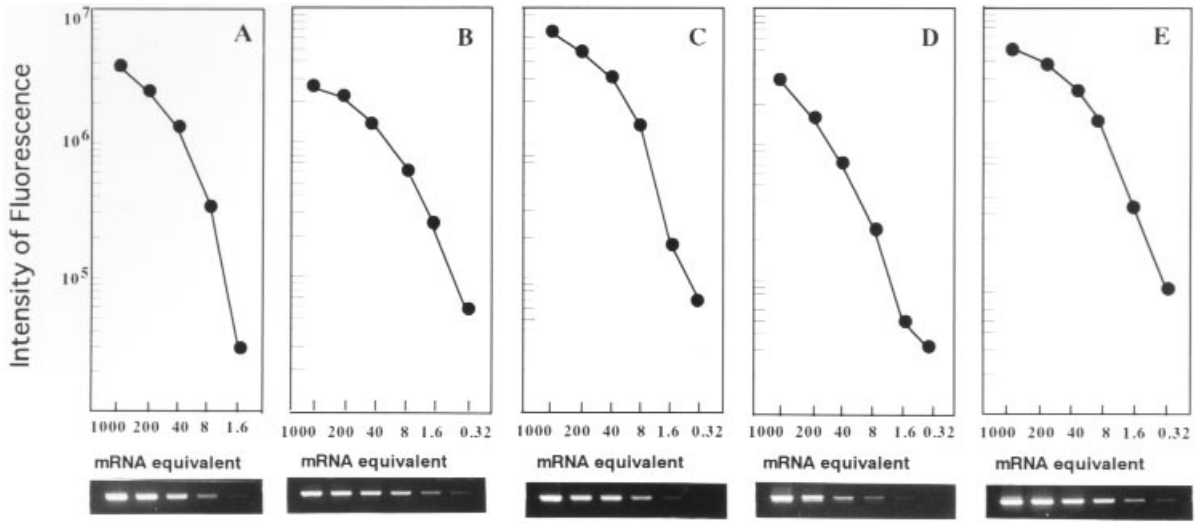

FIG 1. Ethidium bromide–stained gels and standard curves of β-actin **(A)**, IFN-γ **(B)**, IL-2 **(C)**, IL-4 **(D)**, and IL-13 **(E)** mRNA generated by RT-PCR with control RNA. A 250 ng sample of total RNA was reverse transcribed, and 5-fold dilutions of the first strand were amplified by PCR. The intensity of fluorescence of the DNA amplified from the first strand obtained from 250 ng of total RNA was defined arbitrarily as 1000 mRNA equivalents.

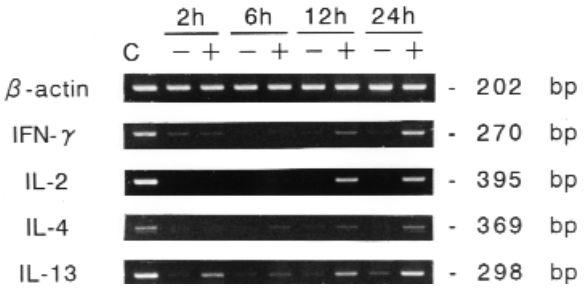

FIG 2. Time course of induction of cytokine mRNA (IFN-γ, IL-2, IL-4, and IL-13) expression in PBMCs of patients with immediate reactions. mRNA expression was assessed at 2, 6, 12, and 24 hours after incubation without *(–)* and with *(+)* gelatin antigen. *Lane C,* Positive control.

ated thymidine incorporation in antigen-stimulated culture relative to that in control culture (stimulation index [SI]). SI of control subjects ranged from 0.9 to 1.1. Therefore we considered an SI equal to or exceeding 1.5 as a positive response.

RESULTS

IgE antibody specific to gelatin

All subjects with immediate reactions showed positive IgE responses specific to gelatin, which ranged from 0.12 to 10.57 Ua/mL (Table II). In contrast, neither the subjects with nonimmediate reactions nor the normal control subjects had detectable levels of gelatin-specific IgE.

Induction kinetics of cytokine mRNA expression in PBMCs exposed to gelatin

The time course of the expression of mRNA for IFN-γ, IL-2, IL-4, and IL-13 in a representative case with an immediate reaction is shown in Fig 2. Although the IL-13 gene appeared transiently as early as 2 hours after incubation, expression of mRNA for all 4 cytokines was observed at 12 hours and further increased up to 24 hours

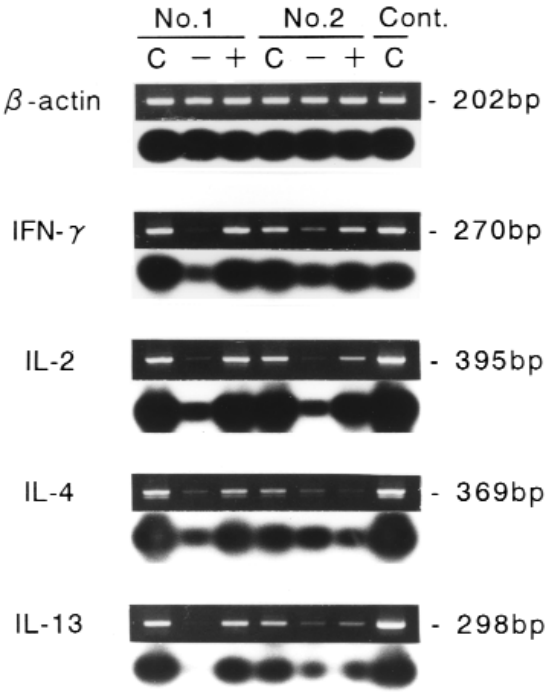

FIG 3. Induction of IFN-γ, IL-2, IL-4, and IL-13 genes by gelatin antigen in PBMCs of subjects with immediate-type *(No. 1)* or nonimmediate-type *(No. 2)* reactions. RT-PCR *(upper panels)* and Southern blot *(lower panels)* analysis of mRNA expression in PBMCs incubated without *(–)* and with *(+)* gelatin antigen. Nonspecific induction of cytokine genes by concanavalin A *(C)* is shown, and positive control was also included *(Cont./C)*.

TABLE I. Sequence of PCR primers and specific probes used in these experiments

Cytokine (size)	Sequence (5′-3′)	Probe
β-Actin	5′: CCTTCCTGGGCATGGAGTCCTG	5′: AAAGACCTGTACGCCAACA
(202 bp)	3′: GGAGCAATGATCTTGATCTTC	
IFN-γ	5′: AATGCAGGTCATTCAGATG	5′: TGCAGAGCCAAATTGTCTC
(270 bp)	3′: TTGGACATTCAAGTCAGTT	
IL-2	5′: TACAGGATGCAACTCCTGTCTTGCATTGCA	5′: AGCTAAATTTAGCACTTCCTCCAG
(395 bp)	3′: GTTGCTGTCTCATCAGCATATTCACACATG	
IL-4	5′: CTGCTAGCATGTGCCGGCAACTTTGTCCAC	5′: GAGCAGAAGACTCTGTGCACCGAGTTGACC
(369 bp)	3′: GAAGTTTTCCAACGTACTCTGGTTGGCTTC	
IL-13	5′: CCCAGAACCAGAAGGCTCCGCTCT	5′: CGAGACACCAAAATCGAGGTGGCC
(278 bp)	3′: GTTGAACCGTCCCTCGCGAA	

after incubation. No further increase in mRNA expression was observed thereafter (data not shown). Therefore we report the induction of cytokine genes after 24 hours of incubation.

Profile of cytokine mRNA expression

Fig 3 shows the ethidium bromide–stained RT-PCR products and Southern blot analysis of 2 representative patients with immediate- or nonimmediate-type reactions. Nonspecific induction of cytokine mRNA by concanavalin A is also displayed in each case, and a positive control for cytokine mRNA is also included. In PBMCs of subjects with immediate reactions, all 4 cytokine genes were significantly induced by gelatin antigen. In contrast, in PBMCs of subjects with nonimmediate reactions, although IFN-γ and IL-2 genes were moderately expressed, IL-4 gene was not induced at all, and IL-13 mRNA was only slightly induced.

Semiquantitative analysis of cytokine mRNA

Induction of IFN-γ, IL-2, IL-4, and IL-13 mRNA by gelatin antigen was semiquantitatively determined, with standard curves obtained from positive control subjects. The minimum detectable mRNA equivalents in this analysis were 0.32. None of the 6 control subjects had detectable expression of these 4 cytokine genes against gelatin (data not shown).

Of the 8 patients with an immediate reaction, 6 showed significant expression of the gene for IFN-γ, 7 for IL-2, 6 for IL-4, and 7 for IL-13 (Table II). Of the nonimmediate group, 4 of the 8 subjects expressed none of the 4 cytokine genes in response to gelatin. Of the remainder, 3, 3, and 4 subjects expressed generally low levels of IFN-γ, IL-2, and IL-13 genes, respectively. No IL-4 gene expression was observed in this group (Table II).

Lymphocyte proliferation to gelatin

The 8 patients with immediate reactions showed generally high responses in lymphocyte proliferation assay specific to gelatin (Table II). The 8 patients with nonimmediate reactions also showed positive responses to gelatin (Table II). However, all 6 control subjects had an SI of less than 1.5, which was considered to be a negative response (data not shown).

Cytokine production

The secretion of these cytokines from PBMCs was assayed by ELISA (Table III). No cytokine production was detected in PBMC cultures of subjects with nonimmediate reactions nor in PBMC cultures from normal control subjects (data not shown). However, all PBMCs from subjects with immediate-type reactions exhibited moderate amounts of IL-2 production, including 1 subject with no detectable IL-2 mRNA. However, IFN-γ, IL-4, and IL-13 cytokine production were only weakly detected in just 1 or 2 PBMC samples among the subjects.

DISCUSSION

In this study we detected IL-2 protein production in PBMC cultures of all subjects with an immediate-type reaction, although little IFN-γ, IL-4, and IL-13 was detected in spite of moderate mRNA expression observed in some subjects. These findings may be explained by the short half-life of some cytokine proteins, especially IL-4,[16] in addition to the lower sensitivity of the assay. Poor or insignificant in vitro IL-4 production against specific allergens has been observed in studies of PBMCs from patients with egg or mite allergy with a positive RAST score.[16,17] Therefore estimations of the induction of T_{H1} and T_{H2} cytokine genes, but not their protein production, may be more valuable to clarify the repertories of gelatin-specific memory T cells.

In this article, on the basis of RT-PCR amplification of the mRNA of IFN-γ, IL-2, IL-4, and IL-13 induced by in vitro stimulation with gelatin protein, we provide the first report of the characteristics of gelatin-specific T cells in subjects with an allergic reaction to gelatin-containing vaccines.

Four of 8 subjects with nonimmediate reactions showed no expression of these 4 types of mRNA. IFN-γ and IL-2 mRNA were detected in 3 of 8 patients, although no IL-4 mRNA was found. The group with nonimmediate reactions are likely to represent a heterogeneous group of subjects, some of whose reactions are not immune-mediated, although the T_{H1}-dominant pattern may be associated with a nonimmediate reaction to gelatin protein. However, mRNA of IL-13, considered to

TABLE II. Semiquantitative analysis of amplified PCR products of 8 subjects with immediate reactions and 8 subjects with nonimmediate reactions

Patient		IFN-γ		IL-2		IL-4		IL-13		Lymphocyte proliferation	Gelatin IgE
no.	Age	–	+	–	+	–	+	–	+	(SI)	(Ua/mL)
Immediate reactions											
1	6 mos	ND	40.0	ND	8.60	0.56	1.40	ND	2.32	5.6	0.12
2	2 y 11 mos	ND	ND	ND	6.80	ND	0.80	ND	ND	2.7	0.24
3	1 y 9 mos	ND	3.12	ND	31.2	0.84	5.20	ND	0.40	6.9	10.57
4	3 y 3 mos	ND	40.0	0.76	34.4	0.40	23.2	ND	4.40	31.3	1.52
5	2 y	ND	5.28	ND	4.60	ND	2.72	ND	1.92	12.4	4.13
6	2 y 5 mos	1.30	35.2	ND	17.6	ND	0.36	ND	5.00	10.2	0.82
7	1 y 4 mos	ND	ND	ND	0.52	ND	ND	ND	2.80	3.7	0.13
8	2 y 6 mos	2.28	3.92	ND	ND	ND	ND	0.40	3.64	25.1	0.24
Nonimmediate reactions											
1	1 y	0.40	32.0	ND	5.44	ND	ND	ND	0.32	10.8	<0.05
2	8 mos	0.60	7.40	1.20	3.36	ND	ND	ND	0.52	10.5	<0.05
3	2 y	ND	ND	ND	ND	ND	ND	1.3	1.92	3.6	<0.05
4	2 y 7 mos	2.72	3.82	ND	0.60	ND	ND	0.56	0.96	5.2	<0.05
5	7 mos	ND	ND	ND	ND	ND	ND	ND	ND	2.4	<0.05
6	9 mos	ND	ND	ND	ND	ND	ND	ND	ND	5.4	<0.05
7	8 mos	ND	ND	ND	ND	ND	ND	ND	ND	3.0	<0.05
8	1 y 4 mos	ND	ND	ND	ND	ND	ND	ND	ND	2.7	<0.05

Fifty nanograms of total RNA were analyzed by RT-PCR. Relative quantities of mRNA obtained from a standard curve derived from positive controls are shown. mRNA expression was assessed 24 hours after incubation without *(–)* and with *(+)* gelatin antigen. The intensity of fluorescence of DNA amplified from the first strand obtained from 250 ng RNA was defined arbitrarily as 1000 mRNA equivalents. Lymphocyte proliferation specific to gelatin was expressed as SI. Serum gelatin–specific IgE antibody titers are also shown.
ND, Equivalents are under 0.32.

TABLE III. Production of cytokines in vitro by PBMCs of 8 subjects with immediate reactions (pg/mL)

		IFN-γ		IL-2		IL-4		IL-13	
Patient no.	Age	–	+	–	+	–	+	–	+
1	6 mos	ND	ND	ND	90.0	ND	ND	ND	ND
2	2 y 11 mos	ND	ND	ND	62.5	ND	ND	ND	ND
3	1 y 9 mos	ND	ND	ND	86.0	ND	ND	ND	ND
4	3 y 3 mos	ND	ND	100	450	ND	7.80	ND	ND
5	2 y	ND	ND	72.0	140	ND	ND	ND	125
6	2 y 5 mos	ND	46.0	ND	53.0	ND	ND	ND	30.0
7	1 y 4 mos	ND	ND	ND	76.0	ND	ND	ND	ND
8	2 y 6 mos	ND	ND	ND	70.0	ND	ND	ND	ND

Culture supernatants were collected 48 hours after incubation without *(–)* and with *(+)* gelatin antigen, and cytokine activities were assessed by ELISA.
ND, Not detected.

be involved in IgE synthesis, was weakly detected in 4 of 8 subjects with nonimmediate reactions, although they had no gelatin-specific IgE. Huang et al[18] observed upregulation of IL-13 in the bronchoalveolar lavage obtained 18 to 24 hours after ragweed challenge in patients with asthma and proposed the association of IL-13 with allergen-induced late-phase inflammation. The IL-13 mRNA expression induced by gelatin antigen may be involved in the nonimmediate reaction (ie, the late-phase reaction to gelatin).

Significant IL-4 mRNA expression occurred in 6 of 8 subjects with an immediate reaction to gelatin-containing vaccine. IL-4 has been considered to be central to the induction of IgE secretion from PBMCs from patients atopic to many inhaled or food allergens.[19-21] Our study confirmed that gelatin allergy with specific IgE antibody was also associated with the activation of IL-4–producing T lymphocytes. In addition, moderate IL-13 gene expression was also observed in almost all subjects with an immediate reaction to gelatin. The role of IL-13 in inducing IgE synthesis has also been confirmed in cases of allergy to mite or ragweed.[21-23] Therefore gelatin-specific IL-13 may play a role in induction of IgE antibody to gelatin.

We also observed strong induction of IFN-γ and IL-2 (ie, T_{H1} cytokines in addition to T_{H2} cytokines in PBMCs) from subjects with immediate reactions. These 4 T_{H1}- and T_{H2}-cytokine gene expressions have never been observed in PBMCs from normal control subjects, which suggests that sensitization to gelatin antigen was either

not established or not significant as is also confirmed by the in vitro lymphocyte proliferation assay. Therefore gelatin allergy may differ from allergy to universal inhaled allergens, such as mite, house dust, and grass pollen, in which suppression of antigen-specific or whole IFN-γ activity compared with that of control subjects was considered to be an essential factor for the appearance of clinical allergy, as was a high antigen-inducible IL-4 expression.[24-27]

In contrast, in PBMCs from subjects with milk or egg allergy, antigen-specific IFN-γ activity was equally or predominantly expressed compared with IL-4 activity.[16,28] These findings were also confirmed by analysis in antigen-specific T-lymphocyte clones or T-cell lines.[29-31] The characteristics of gelatin-sensitized lymphocytes with both intense T_{H1} and T_{H2} signals may resemble those sensitized by food allergens.

A positive correlation was suspected between food-specific activation of IFN-γ–secreting T lymphocytes and a worsening of atopic dermatitis in response to oral provocation.[28] However, subjects with gelatin allergy have no chronic lesions resembling the atopic dermatitis associated with an intake of gelatin. Therefore the role of T_{H1} cytokines induced by gelatin might not be related to chronic pathology.

The suppressive effect of IFN-γ to T_{H2} activity was confirmed in the response to inhaled allergens.[24-27] The strong induction of IFN-γ and IL-2 in gelatin-specific T lymphocytes might reflect a suppressive or feedback mechanism in the immune system.

REFERENCES

1. Mochizuki Y, Nakayama T. Studies of adverse reactions mainly composed of skin eruptions in the patients inoculated with measles and mumps vaccine [in Japanese]. J Jpn Pediatr Soc 1995;99:873-4.
2. Ogura H, Ogura Y, Tornoda T, Kurashige T, Sakaguchi M, Inouye S. Anaphylaxis to measles vaccine in a case with gelatin allergy [in Japanese]. Infect Immun Child 1995;7:115-8.
3. Kelso JM, Jones RT, Yunginger JW. Anaphylaxis to measles, mumps and rubella vaccine mediated by IgE to gelatin. J Allergy Clin Immunol 1993;91:867-72.
4. Sakaguchi M, Ogura H, Inouye S. IgE antibody to gelatin in children with immediate-type reactions to measles and mumps vaccines. J Allergy Clin Immunol 1995;96:563-5.
5. Sakaguchi M, Yamanaka T, Ikeda K, et al. IgE-mediated systemic reaction reactions to gelatin included in the varicella vaccine. J Allergy Clin Immunol 1995;84:246-55.
6. Kumagai T, Yamanaka T, Wataya Y, et al. Gelatin-specific humoral and immediate- and nonimmediate-type reactions to live measles, mumps, rubella, and varicella vaccines. J Allergy Clin Immunol 1997;100:130-4.
7. Mossmann TR, Cherwinsky H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. J Immunol 1986;136:2348-57.
8. Robinson DS, Kay AB. Role of Th1 and Th2 cells in human allergic disorders. Chem Immunol 1996;63:187-203.
9. Romaganani S. TH1 and TH2 in human diseases. Clin Immunol Immunopathol 1996;80:225-35.
10. Umetsu DT, DeKruyff RH. Th1 and Th2 CD4 cells in the pathogenesis of allergic diseases. Proc Soc Exp Biol Med 1997;215:11-20.
11. Mossmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996;17:138-46.
12. Takeuchi R, Tsutsumi H, Osaki M, Sone S, Imai S, Chiba S. Respiratory syncytial virus infection of neonatal monocytes stimulates synthesis of interferon regulatory factor 1 and interleukin-1β (IL-1β)-converting enzyme and secretion of IL-1β. J Virol 1998;72:837-40.
13. Tsai SL, Lai MY, Chen DS. Analysis of rearranged T cell receptor (TCR) V β transcripts in livers of primary biliary cirrhosis: preferential V β usage suggests antigen-driven selection. Clin Exp Immunol 1996;103:99-104.
14. Tsutsumi H, Matsuda K, Sone S, Takeuchi R, Chiba S. Respiratory syncytial virus-induced cytokine production by neonatal macrophages. Clin Exp Immunol 1996;106:442-6.
15. Van Voorhis WC, Barrett LK, Koelle DM, Nasio JM, Plummer FA, Lukehart SA. Primary and secondary syphilis lesions contain mRNA for Thl cytokines. J Infect Dis 1996;173:491-5.
16. Shinbara M, Kondo N, Agata H, et al. Interferon-γ and interleukin-4 production of ovalbumin-stimulated lymphocytes in egg-sensitive children. Ann Allergy Asthma Immunol 1996;77:60-6.
17. Kubota Y, Koga T, Imayama S, Hori Y. Mite-antigen-stimulated cytokine production by peripheral blood mononuclear cells of atopic dermatitis patients with positive mite patch tests. Contact Dermatitis 1994;31:217-9.
18. Huang SK, Xiao HQ, Kleine-Tebble J, et al. IL-13 expression at the sites of allergen challenge in patients with asthma. J Immunol 1995;155:2688-94.
19. Del Prete G, Maggi E, Parronchi P, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. J Immunol 1998;140:4193-8.
20. Gauchat JF, Lebmann DA, Coffman RL, Gascan H, de Vries JE. Structure and expression of germline ε transcripts in human B cells induced by interleukin 4 to switch to IgE production. J Exp Med 1990;172:463-73.
21. Dolecek C, Steinberger P, Susani M, Kraft D, Valenta R, Boltz-Nitulescu G. Effect of IL-4 and IL-13 on total and allergen specific IgE production by cultured PBMCs from allergic patients determined with recombinant pollen allergen. Clin Exp Allergy 1995;25:879-89.
22. Katagiri K, Itami S, Hatano Y, Takayasu S. Increased levels of IL-13 mRNA, but not IL-4 mRNA, are found in vivo in peripheral blood mononuclear cells (PBMCs) of patients with atopic dermatitis (AD). Clin Exp Immunol 1997;108:289-94.
23. Levy F, Kristofic C, Heusser C, Brinkmann V. Role of IL-13 in CD4 T cell-dependent IgE production in atopy. Int Arch Allergy Immunol 1997;112:49-58.
24. Chang JH, Chan H, Quirce S, et al. In vitro T-lymphocyte response and house dust mite-induced bronchoconstriction. J Allergy Clin Immunol 1996;98:922-31.
25. Li Y, Simons ER, Jay FT, HayGlass KT. Allergen-driven limiting dilution analysis of human IL-4 and IFN-γ production in allergic rhinitis and clinically tolerant individuals. Int Immunol 1996;8:897-904.
26. Gagnon R, Akoum A, Hebert J. Lop p I-induced IL-4 and IFN-γ production by peripheral blood mononuclear cells of atopic and nonatopic subjects during and out of the pollen season. J Allergy Clin Immunol 1993;91:950-6.
27. Byron KA, O'Brien RM, Varigos GA, Wootton AM. *Dermatophagoides pteronyssinus* II-induced interleukin-4 and interferon-γ expression by freshly isolated lymphocytes of atopic individuals. Clin Exp Allergy 1994;24:878-83.
28. Kondo N, Fukutomi O, Agata H, et al. The role of T lymphocytes in patients with food-sensitive atopic dermatitis. J Allergy Clin Immunol 1993;91:658-68.
29. Werfel T, Ahler G, Schmidt P, Boecker M, Kapp A, Neumann C. Milk-responsive atopic dermatitis is associated with a casein-specific lymphocyte response in adolescent and adult patients. J Allergy Clin Immunol 1997;99:124-33.
30. Reekers R, Beyer K, Niggemann B, et al. The role of circulating food antigen-specific lymphocytes in food allergic children with atopic dermatitis. Br J Dermatol 1996;135:935-41.
31. Nakajima H, Hachimura S, Nishiwaki S, et al. 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. J Allergy Clin Immunol 1996;97:1342-9.