

Gelatin-induced T-cell activation in children with nonanaphylactic-type reactions to vaccines containing gelatin

Kiyosu Taniguchi, MD, Takao Fujisawa, MD, Toshiaki Ihara, MD, and Hitoshi Kamiya, MD
Mie, Japan

Background: Many cases of anaphylactic or nonanaphylactic reactions have been reported to measles-mumps-rubella vaccine or its component vaccines that contain gelatin as a stabilizer. Increased levels of specific IgE antibodies to gelatin have been reported in children with anaphylactic reactions. However, IgE is not increased in cases of nonanaphylactic reaction, and the mechanisms of the reaction are still controversial. **Objective:** The study was aimed to elucidate the relationship between nonanaphylactic reaction and gelatin. **Methods:** We investigated *in vitro* induction of activated memory helper T cells (CD4⁺CD25⁺CD45RO⁺ cells) in response to gelatin in children with nonanaphylactic reactions to vaccines containing gelatin. **Results:** In patients with delayed-type sensitivity to gelatin confirmed with a positive skin test response, CD4⁺CD25⁺CD45RO⁺ cells were significantly more strongly induced in culture containing gelatin than in control cultures. However, there was no significant difference between cultures with gelatin and those with control solvent in patients without reactions after vaccination. Of 76 patients with nonanaphylactic reactions after immunization with vaccine containing gelatin, 61 had an increased lymphocyte stimulation index to gelatin versus control children. **Conclusion:** These results suggest the possibility that nonanaphylactic reactions to gelatin-containing vaccine in Japan might be mediated by delayed hypersensitivity reactions against gelatin. (*J Allergy Clin Immunol* 1998;102:1028-32.)

Key words: Vaccine, gelatin, adverse reaction, delayed-type hypersensitivity, memory T lymphocyte, CD25, CD45RO

Anaphylactic reactions rarely occur after administration of measles-mumps-rubella (MMR) vaccine or any of its component vaccines. The recent literature contains a single report on a patient with an anaphylactic sensitivity to gelatin who had an anaphylactic reaction after administration of MMR vaccine. In addition, increased levels of specific IgE antibodies to gelatin have been reported in children who have had anaphylactic reactions to measles and mumps vaccines containing gelatin.² However, nonanaphylactic reactions, such as local swelling, systemic rash, and fever, have frequently been encountered to these vaccines. Because IgE antibody to gelatin is not

Abbreviations used

MMR: Measles-mumps-rubella
DPT: Diphtheria-pertussis-tetanus
JEV: Japanese encephalitis vaccine

increased in such patients, the hypothesis that the reaction is caused by gelatin in vaccines remains controversial.

In this study, to elucidate the relationship between gelatin and nonanaphylactic reactions, we investigated the *in vitro* activation of T cells in response to gelatin in children with nonanaphylactic reactions to vaccines containing gelatin.

METHODS

Subjects

Seventy-six children who had nonanaphylactic reactions after immunization with vaccines containing gelatin were included in the study. The children were brought to the immunization department of Mie National Hospital or other hospitals because of unexpected reactions, such as local swelling, systemic rash, and fever, that occurred 3 to 48 hours after inoculation. The patients were 38 boys and 38 girls ranging in age from 5 to 85 months (mean age $\pm$ SD, 26.0 $\pm$ 17.2 months). Symptoms were characterized by local redness and swelling at the injection site only (12 patients); generalized skin rash only (17 patients); local redness, swelling, and rash (28 patients); local redness, swelling, rash, and fever (9 patients); rash and fever (6 patients); local redness, swelling, and fever (1 patient); rash with mucous membrane involvement (1 patient); and rash and stridor (2 patients). The skin rashes were characterized by fine discrete pink-red macropapules. The time from symptom onset to resolution was ranged from 1 to 5 days (median, 3 days), with one exception (20 days) with rash and mucous membrane involvement. Thirty-three patients did not require treatment. Thirty-five patients were prescribed oral antihistamines. Five subjects with fever, 1 subject with rash and mucous membrane involvement, and 2 subjects with rash and stridor were hospitalized. Forty-one of the 76 children had received measles vaccine, 12 had received rubella vaccine, 9 had received gelatin-containing DPT vaccine, 9 had received Japanese encephalitis vaccine (JEV), 4 had received varicella vaccine, and 1 had received mumps vaccine. Five children who had no reactions after immunization and 9 healthy age-matched children were included as negative controls. Peripheral blood samples were obtained from all subjects for the lymphocyte activation assay and measurement of serum gelatin-specific IgE.

Vaccines

Vaccines that were administered to these children were 3 measles vaccines (Kitasato Institute, Tokyo, Japan; Takeda Chemical Industries, Osaka, Japan; and Research Foundation for Microbial Diseases of Osaka University, Kagawa, Japan), 2 rubella vaccines (Research Foundation for Microbial Diseases of Osaka University and Kitasato Institute), mumps vaccine (Kitasato Institute), varicella vaccine (Research Foundation for Microbial Diseases of

From the Department of Pediatrics, Mie National Hospital, Mie.
Received for publication Aug 5, 1996; revised July 13, 1998; accepted for publication July 17, 1998.
Reprint requests: Kiyosu Taniguchi, MD, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo, 162 Japan.
Copyright © 1998 by Mosby, Inc.
0091-6749/98 \$5.00 + 0 1/1/93589

TABLE I. Results of intradermal skin test with bovine gelatin in control subjects

Subject		After 30 minutes	After 24 hours
1	Gelatin	0×0/0×0*	0×0/0×0
	Tuberculin	0×0/0×0	0×0/10×10
2	Gelatin	0×0/2×3	0×0/0×0
	Tuberculin	0×0/1×2	0×0/15×13
3	Gelatin	0×0/0×0	0×0/0×0
	Tuberculin	0×0/0×0	0×0/10×9
4	Gelatin	0×0/0×0	0×0/0×0
	Tuberculin	0×0/0×0	0×0/12×11
5	Gelatin	0×0/0×0	0×0/0×0
	Tuberculin	0×0/0×0	5×6/15×20

*Diameter of induration/diameter of redness (in millimeters).

Osaka University), 2 DPT vaccines (Research Foundation for Microbial Diseases of Osaka University and Takeda Chemical Industries), and 3 JEVs (Takeda Chemical Industries; Denka Seiken, Tokyo; and Kitasato Institute). All vaccines, except for the DPT vaccines, contained gelatin, hydrolyzed gelatin, or both as stabilizers, the amount of which ranged from 0.05 to 13.5 mg per injected dose. The DPT vaccines contained trace amounts (60 to 200 µg/mL) of gelatin that had been introduced as contaminants during the manufacturing process.

Intradermal skin test

Of the 76 patients, 5 who had been brought to the department of immunization at Mie National Hospital were recruited for skin testing with bovine gelatin. After informed consent had been obtained, 0.02 mL of 0.4% bovine gelatin solution provided by the Research Foundation for Microbial Diseases of Osaka University and control physiologic saline were applied intradermally. Five minutes, 30 minutes, and 24 hours after injection, local redness and induration were measured. In five children who had no adverse reactions after vaccination, skin testing with bovine gelatin was performed in the same manner simultaneously with Tuberculin test. All 5 subjects had received BCG from 3 to 6 months of age.

Measurement of gelatin-specific IgE antibodies

IgE antibodies to bovine gelatin were measured with the CAP system at the National Institute of Health, Tokyo.

Lymphocyte activation assay

PBMCs were isolated with Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation and washed twice with PBS and once with RPMI 1640 (Nissui, Tokyo). Cells were counted and resuspended at a concentration of 1.0×10^6 /mL in RPMI 1640 supplemented with 2 mmol/L L-glutamine and 20% autologous plasma. Cells were cultured in round-bottom plastic tubes (Becton-Dickinson, Mountain View, Calif) at 0.5 mL/tube with bovine gelatin (final concentration, 100 µg/mL) or control solvent (PBS) in a humidified atmosphere at 37°C with 5% CO₂.

After 1, 3, 6, 9, or 12 days of culture, the cells were spun down, the supernatant was removed, and the cells were washed twice with PBS and immunostained for 30 minutes with FITC-conjugated anti-CD45RO, phycoerythrin-conjugated anti-CD25, and Cy-Chrome-conjugated anti-CD4 mAbs (Becton-Dickinson). After they were washed twice with PBS, cells were resuspended in 0.5 mL PBS.

Then samples were analyzed with a FACScan flow cytometer (Becton-Dickinson) that was calibrated by Autocomp software with Calibrite beads or triple-immunostained lymphocytes. Ten thousand

TABLE II. Time course of CD4⁺CD25⁺CD45RO⁺ cell percentage in control subjects

Subject		Days of culture				
		1	3	6	9	12
1	Control	—	4.39	—	6.12	NA
	Gelatin	—	4.21	—	4.92	NA
	Stimulation index	—	0.96	—	0.80	NA
2	Control	1.36	—	3.86	NA	NA
	Gelatin	1.41	—	3.31	NA	NA
	Stimulation index	1.04	—	0.86	NA	NA
3	Control	5.69	—	—	5.48	NA
	Gelatin	5.48	—	—	4.84	NA
	Stimulation index	0.96	—	—	0.88	NA
4	Control	1.41	—	2.38	NA	NA
	Gelatin	1.65	—	2.47	NA	NA
	Stimulation index	1.17	—	1.04	NA	NA
5	Control	—	—	—	13.86	8.11
	Gelatin	—	—	—	8.29	3.21
	Stimulation index	—	—	—	0.59	0.39

NA, Impossible to analyze because few live cells remained.

cells per sample were acquired and stored as list-mode data with LYSIS II software (Becton-Dickinson). Data were analyzed with Paint-A-Gate software (Becton-Dickinson). Live lymphocytes were gated on the basis of morphologic features (forward scatter for size and side scatter for granularity). Then CD4⁺CD25⁺CD45RO⁺ (triple-positive) cell percentages were calculated.

Activation responses were expressed with a stimulation index obtained by dividing the percentage of triple-positive cells from a culture with gelatin by that from a control culture. Statistical difference was evaluated by means of the Mann-Whitney U test.

RESULTS

Serum gelatin-specific IgE

Only 3 patients had anti-gelatin IgE. Each had local reactions and systemic rash 4 to 6 hours after vaccination and showed no signs or symptoms to distinguish them from other patients. The level of IgE ranged from 0.72 to 6.32 Ua/mL.

Lymphocyte activation assay and intradermal skin test in control subjects

All subjects who had no adverse reactions after vaccination had negative responses to intradermal skin testing with gelatin but had positive responses to Tuberculin (Table I). The time course of triple-positive cell percentages in samples from these subjects is shown in Table II. Triple-positive cell percentages in cultures with gelatin were similar to or less than those in control cultures. After 12 days of culture, 4 of 5 samples did not have enough live cells for analysis with FACScan. The stimulation index after 9 days ranged from 0.59 to 0.88.

The stimulation index in 9 samples from healthy control subjects ranged from 0.37 to 0.86. The geometric

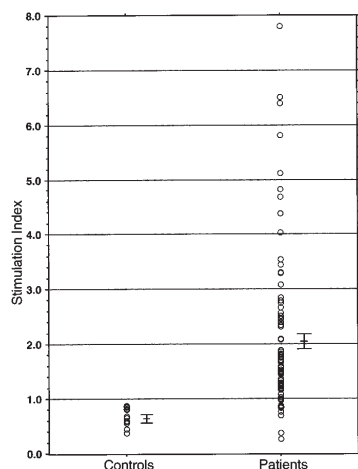


FIG 1. Distribution of stimulation index of all samples. Geometric mean and SD in control subjects was 0.65 ± 0.18 . With a cut-off value of 1.01 corresponding to mean stimulation index + 2 SDs, 61 of 76 patients with adverse reaction after vaccination had positive responses.

mean $\pm$ SD of 14 negative control patients was 0.65 ± 0.18 . The cut-off value was set at 1.01, corresponding to the mean stimulation index + 2 SDs.

Lymphocyte activation assay and intradermal skin test in patients

Results of intradermal skin tests of the patients are shown in Table III. Four of 5 patients had negative results 30 minutes after intradermal injection and strongly positive results at 24 hours. One patient had positive results at both 30 minutes and 24 hours. The time course of triple-positive cell percentages in culture from patients are shown in Table IV. The percentage of triple-positive cells in coculture with gelatin increased gradually. The values were much higher than those in control cultures. The stimulation index after 9 days ranged from 2.11 to 6.50. The color of the medium in most cultures became completely yellow after 9 days. Therefore the duration of culture was set at 9 days.

Lymphocyte activation assay

All results of lymphocyte activation assays are shown in Fig 1. The geometric mean $\pm$ SEM of the stimulation index was 0.65 ± 0.05 in the control group and 2.16 ± 0.17 in patients. With a cut-off value of 1.01, 61 of 76 (80.2%) patients with adverse reactions after receiving gelatin-containing vaccines had positive results.

The distribution of the stimulation index in each vaccine group is shown in Fig 2. The geometric mean $\pm$ SEM of the stimulation index in patients with nonanaphylactic reactions after vaccination was 1.59 ± 0.39 with gelatin-containing DPT vaccine, 2.15 ± 0.56 with JEV, 2.31 ± 0.25 with measles, 2.47 ± 0.0 with mumps, 1.87 ± 0.35 with rubella, and 2.72 ± 0.70 with varicella.

Positive results were obtained in 4 of 9 (44.4%) children with nonanaphylactic reactions after vaccination with gelatin-containing DPT vaccine, in 7 of 9 (77.7%) with

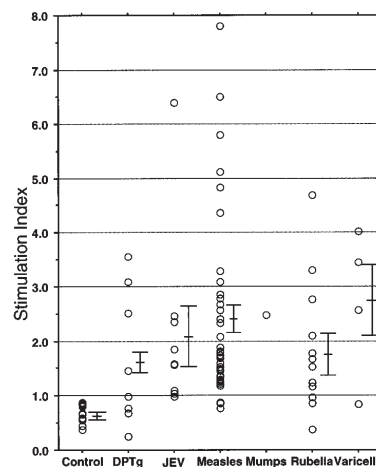


FIG 2. Distribution of stimulation index among the vaccine group. The mean stimulation index and SEM was 1.59 ± 0.39 with gelatin-containing DPT vaccine, 2.15 ± 0.56 with JEV, 2.31 ± 0.25 with measles, 2.47 with mumps, 1.87 ± 0.35 with rubella, and 2.72 ± 0.70 with varicella.

TABLE III. Results of intradermal skin test with bovine gelatin in the patients

Patient	After 30 minutes	After 24 hours
A	0×0/0×0*	12×12/17×21
B	0×0/0×0	20×22/32×30
C	0×0/0×0	20×18/50×45
D	0×0/15×15	5×7/25×21
E	0×0/0×0	17×18/22×21

*Diameter of induration/diameter of redness (in millimeters).

JEV, in 37 of 41 (90.2%) with measles, in 1 of 1 (100%) with mumps, in 9 of 12 (75%) with rubella, and in 3 of 4 (75%) with varicella.

Relationship between clinical reactions and the amount of gelatin in vaccines

We compared the amount of gelatin present in vaccines between the group with only redness and swelling at the injection site and the group with systemic reactions including rash or fever. The mean value was 2.39 mg/shot in the systemic group versus 1.82 mg/shot in the local group ($P = .0152$). There was no statistical correlation between the stimulation index and the gelatin content of vaccines (Table V).

Relationship between clinical reactions and previous immunization history with gelatin-containing vaccines

All but 1 subject with nonanaphylactic reactions to vaccines had at least 1 previous immunization with a gelatin-containing vaccine before the current episode. One subject who had a generalized skin rash after primary immunization had no increase in lymphocyte stimulation index in response to gelatin. The mean number of

TABLE IV. Time course of CD4⁺CD25⁺CD45RO⁺ cell percentage and stimulation index in patients with positive skin test responses

Patient		Days of culture				
		1	3	6	9	12
A	Control	1.41	—	—	4.85	NA
	Gelatin	2.64	—	4.64	31.54	48.21
	Stimulation index	1.87	—	—	6.50	—
B	Control	2.21	—	—	5.11	NA
	Gelatin	2.38	—	—	13.65	35.72
	Stimulation index	1.07	—	—	2.67	—
C	Control	3.21	—	3.64	NA	NA
	Gelatin	2.20	—	6.04	8.97	16.80
	Stimulation index	0.68	—	1.65	—	—
D	Control	—	—	4.38	9.10	—
	Gelatin	—	—	6.28	19.23	—
	Stimulation index	—	—	1.43	2.11	—
E	Control	2.38	—	—	3.91	NA
	Gelatin	2.23	—	—	18.51	26.50
	Stimulation index	0.94	—	—	4.73	—

NA, Impossible to analyze because few live cells remained.

doses of previous immunization with gelatin-containing vaccines was 3.6 (range, 1 to 8 doses). There was no statistical difference in the number of doses of prior vaccines between the local reaction group and the systemic reaction group (4.8 vs 3.5 doses, $P = .1246$).

Subjects who had more previous immunization doses tended to have higher stimulation indices (Table VI), but a statistical difference was found only between groups of 1 to 2 and over 5 doses.

DISCUSSION

Recently, many cases of anaphylactic or nonanaphylactic reactions to MMR vaccine or its component vaccines have been reported. According to reports from the Immunization Research Group granted by the Ministry of Health and Welfare of Japan, anaphylactic reactions to measles vaccine occur after 1 of every 467 vaccinations, and nonanaphylactic reactions occur after 1 of every 59 vaccinations. In addition, anaphylactic reactions to rubella vaccine containing gelatin occur after 1 of every 50 vaccinations, and nonanaphylactic reactions occur after 1 of every 17 vaccinations. However, neither anaphylactic nor nonanaphylactic reactions have occurred with rubella vaccine without gelatin.³

The MMR vaccine and some of its component vaccines, varicella, JEV, and some of the DPT vaccines in Japan contain gelatin or hydrolyzed gelatin as a stabilizer. Two DPT vaccines contain trace amounts of gelatin as a result of the manufacturing process.

One report from the United States describes a child with an anaphylactic reaction to MMR vaccine whose serum contained specific IgE antibody to gelatin.¹ A

TABLE V. Stimulation index in 3 groups by gelatin content in vaccine

	Gelatin content (mg/shot)		
	<1.0	1.0-2.0	≥2.0
Stimulation index (mean ± SE)	1.87 ± 0.39	2.00 ± 0.41	2.17 ± 0.26

<1.0 vs 1.0-2.0, $P = .6908$.

1.0-2.0 vs ≥2.0, $P = .2519$.

<1.0 vs ≥2.0, $P = .2033$.

TABLE VI. Stimulation index in 3 groups by doses of previous immunization with gelatin containing vaccines

	Doses of immunization		
	1-2	3-4	≥5
Stimulation index (mean ± SE)	1.31 ± 0.24	2.33 ± 0.34	2.18 ± 0.28

1-2 vs 3-4, $P = .1975$.

3-4 vs ≥5, $P = .5547$.

1-2 vs ≥5, $P = .0361$.

report from Japan describes increased levels of specific IgE antibody to gelatin in children with anaphylactic reactions to measles and mumps vaccines containing gelatin.²

In nonanaphylactic reactions IgE antibody is not detected.^{2,3} The mechanism of the reaction and its relationship to gelatin are not clear. A report from the Immunization Research Group⁴ suggests that type IV hypersensitivity to gelatin might play a role in nonanaphylactic reactions on the basis of positive gelatin-induced lymphocyte proliferation assays.

Several methods for detecting the response of lymphocytes to allergens or other antigens have been reported for investigating the role of cellular immunity in hypersensitivity reactions or host defense to viruses. The blastogenesis reaction, IL-2 responsiveness, and phenotypic change with activation of lymphocytes are used to detect the response of lymphocytes to specific antigens.⁵⁻¹⁰

The cells that play a major role in delayed-type hypersensitivity reactions seems to be activated, memory, CD4⁺ T lymphocytes.¹¹ CD4⁺ T lymphocytes have been found to be the dominant cell type in delayed-type hypersensitivity reactions induced by purified protein derivative.¹² Several studies show that CD4⁺CD45RO⁺ lymphocytes infiltrate inflamed tissue in vivo.¹³ After intradermal allergen injections, analysis of skin biopsy specimens at 24 and 48 hours reveals that most CD3⁺ cells are CD4⁺ and express an increased level of IL-2 receptors (CD25) and HLA-DR antigens.¹⁴

Therefore we hypothesized that immunologic memory selects and activates the clone responsive to gelatin when peripheral blood lymphocytes are cultured with gelatin, whereas other clones undergo cell death. We studied the induction of activated CD4⁺CD45RO⁺ cells by coculture with gelatin in patients with nonanaphylactic reactions after injection of vaccines containing gelatin.

Patients with positive skin test responses at 24 hours seemed to have a delayed-type hypersensitivity to gelatin. With the *in vitro* lymphocyte activation assay, CD4⁺CD25⁺CD45RO⁺ cells were significantly more strongly induced in coculture with gelatin than in control cultures with PBS. Only 2 of 36 (5.5%) patients tested for β -lactoglobulin-induced lymphocyte activation had positive responses, which indicated that these responses to gelatin in patients were not nonspecific reactions (data not shown). We speculate that a T-cell clone with immunologic memory to gelatin causes positive skin test responses to gelatin.

A correlation between a positive skin test response and a positive *in vitro* lymphocyte activation assay result was not confirmed in all patients. However, on the other hand, subjects without any reaction to a vaccine showed negative skin test responses to gelatin and positive Tuberculin test results. These control subjects showed no increase in CD4⁺CD25⁺CD45RO⁺ cells in gelatin cultures compared with control cultures. From these observations we infer this assay system will be of use for detecting delayed-type hypersensitivity to gelatin.

In this study approximately 80% of patients with nonanaphylactic reactions to vaccines containing gelatin had positive lymphocyte activation responses against gelatin. It seems that the gelatin contained in vaccines plays some role in nonanaphylactic reactions to vaccine. In the systemic reaction group the amount of gelatin administered was more than in the local reaction group.

Although we did not study whether an increased lymphocyte stimulation index to gelatin will predict who will have a nonanaphylactic reaction to vaccine, 2 patients suggested such a clinical implication. Both children showed positive lymphocyte-activation responses to gelatin after they had experienced nonanaphylactic reactions after measles vaccination. One individual later received immunization with rubella vaccine containing gelatin at another clinic and experienced fever and generalized skin rash. By contrast, another child who received nongelatin-containing rubella vaccine at our clinic had no adverse reaction. We believe that children who have experienced a nonanaphylactic reaction to a gelatin-containing vaccine should be evaluated for lymphocyte stimulation assay and gelatin-specific IgE before another immunization. Because 1 subject found in another clinic had an anaphylactic reaction after skin testing with gelatin, we do an *in vitro* assay first. Skin testing is then applied if the result of the *in vitro* assay is unclear. In children with positive test responses to gelatin, gelatin-free vaccine should be considered.

Vaccines contain many antigens that might evoke allergic reaction. Actually, about 20% of patients had no evidence of gelatin hypersensitivity. Eighteen of 40 (45.0%) subjects tested for ovalbumin-induced lymphocyte activation assay showed positive responses. Recent studies tend to agree that egg sensitivity is unrelated to adverse reactions to vaccine. More investigation will be required to clarify the allergen evoking hypersensitivity reactions.

In this study all subjects except 1 had at least 1 previous immunization with a gelatin-containing vaccine before the current episode. In other words, there were

few cases with adverse reaction at their primary immunization in the group with nonanaphylactic reactions. This implies some role of previous immunization in adverse reactions. Because it is known that many pre-cooked baby foods contain gelatin, how children are sensitized by gelatin is still a subject of debate.

Our findings show that 80% of patients with nonanaphylactic reactions to vaccines containing gelatin have positive lymphocyte responses against gelatin *in vitro*. Although the mechanism of nonanaphylactic-type reactions to vaccine is still controversial, gelatin should be considered as one of the antigens evoking delayed-type adverse reactions after immunization.

We thank the physicians for their help in sample collection. We also thank Dr Masahiro Sakaguchi, National Institute of Health, Japan, for measurement of specific IgE to gelatin, and the Research Foundation for Microbial Diseases of Osaka University for providing us with bovine gelatin solution.

REFERENCES

1. 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.
2. Sakaguchi M, Ogura H, Inoue S. IgE antibody to gelatin in children with immediate-type reactions to measles and mumps vaccines. *J Allergy Clin Immunol* 1995;96:563-5.
3. Yamanaka T, Kumagai T, Wataya Y, et al. Gelatin allergy in cases with adverse reaction after immunization [in Japanese]. In: Takenaka K, editor. Reports of immunization research group. Immunization Research Center and Ministry of Health and Welfare of Japan; 1996. p. 346-54.
4. Kumagai T, Yamanaka T, Wataya Y, et al. Gelatin specific immune reaction in cases with adverse reaction after immunization [in Japanese]. In: Takenaka K, editor. Reports of immunization research group. Immunization Research Center and Ministry of Health and Welfare of Japan; 1996. p. 341-5.
5. Agata H, Kondo N, Fukutomi O, Shinoda S, Orii T. Interleukin-2 production of lymphocytes in food sensitive atopic dermatitis. *Arch Dis Child* 1992;67:280-4.
6. Agata H, Kondo N, Fukutomi O, Shinoda S, Orii T. Effect of elimination diets on food-specific IgE antibodies and lymphocyte proliferative responses to food antigens in atopic dermatitis patients exhibiting sensitivity to food allergens. *J Allergy Clin Immunol* 1993;91:668-79.
7. 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.
8. Noma T, Mori A, Yoshizawa I. Induction of allergen-specific IL-2 responsiveness of lymphocytes after respiratory syncytial virus infection and prediction of onset of recurrent wheezing and bronchial asthma. *J Allergy Clin Immunol* 1996;98:816-26.
9. Ito M, Nakano T, Kamiya T, et al. Activation of lymphocytes by Varicella-Zoster virus (VZV): expression of interleukin-2 receptor on lymphocytes cultured with VZV antigen. *J Infect Dis* 1992;165:158-61.
10. Mizutani K, Ito M, Nakano T, Kamiya H, Sakurai M. Impaired expression of interleukin 2 receptor and CD45RO antigen on lymphocytes from children with acute lymphoblastic leukemia in response to cytomegalovirus and Varicella-Zoster virus. *Clin Diagn Lab Immunol* 1995;2:381-4.
11. Werfel S, Massey W, Lichtenstein LM, Bochner BS. Preferential recruitment of activated, memory T lymphocytes into skin chamber fluids during human cutaneous late-phase allergic reactions. *J Allergy Clin Immunol* 1995;96:57-65.
12. Bos JD, Kapsenberg ML. The skin immune system: progress in cutaneous biology. *Immunol Today* 1993;15:75-8.
13. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature* 1991;349:796-9.
14. Frew AJ, Kay AB. The relationship between infiltrating CD4⁺ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late-phase cutaneous reaction. *J Immunol* 1988;141:4158-64.