

Immunodeficiency and other clinical immunology

Gelatin-specific humoral and cellular immune responses in children with immediate- and nonimmediate-type reactions to live measles, mumps, rubella, and varicella vaccines

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Background: This study was designed to investigate the development of both cellular and humoral immune responses to gelatin in patients with vaccine-related immediate and nonimmediate reactions. Our purpose was to define the nature of the responses in the different clinical states.

Methods: Six patients with immediate reactions and 21 patients with nonimmediate reactions after inoculation of various live vaccines were studied. Measurement of gelatin-specific IgE was performed in all subjects. Gelatin-specific T-cell responses detected by an in vitro lymphocyte proliferation assay and by an assay for IL-2 responsiveness were investigated to compare the immune response in patients with the two types of reaction.

Results: All six patients with immediate reactions had IgE responses to gelatin, whereas none of the 21 patients with nonimmediate reactions had any anti-gelatin IgE. All of the six patients with immediate reactions and 17 of the 21 patients with nonimmediate reactions exhibited positive T-lymphocyte responses specific to gelatin.

Conclusions: Immediate and nonimmediate reactions are caused by different types of allergy to gelatin, and cell-mediated immunity to gelatin may play an important role in the pathogenesis of nonimmediate reactions. (*J Allergy Clin Immunol* 1997;100:130-4.)

Key words: Gelatin allergy, measles vaccine, mumps vaccine, rubella vaccine, varicella vaccine, cellular immunology, IL-2 responsiveness, lymphocyte transformation, anaphylaxis

Gelatin has long been believed to be nonimmunogenic and nonallergenic to human beings, and therefore it has been widely used as an additive to live vaccines for

Abbreviation used

SI: Stimulation index

stabilizing the viability of virus. However, an increasing number of adverse reactions in children inoculated with live attenuated virus vaccines containing gelatin have been reported by several authors during last few years.^{1,2} Two types of adverse reactions have been observed. One is an immediate reaction including anaphylaxis, which generally appears within 1 hour after inoculation of vaccine. The other is a nonimmediate reaction, which mainly consists of a generalized mild skin eruption appearing several hours or more after vaccination.

Recently, Kelso et al.³ have demonstrated gelatin-specific IgE in the serum of a child who had anaphylaxis to measles-mumps-rubella vaccine. Sakaguchi et al.⁴ also found IgE antibody to gelatin in the sera of a larger number of patients who had immediate reactions. These authors concluded that these reactions were caused by the gelatin included in the vaccine, although the possible involvement of cell-mediated immunity in these particular phenomena had not yet been studied. Although immediate allergic reactions are known to be closely correlated with the generation of specific IgE, the mechanism of nonimmediate reactions to gelatin remains controversial.

This study was designed to investigate the development of both cellular and humoral immune responses to gelatin in patients with both immediate and nonimmediate postvaccination reactions in order to characterize the nature of the response in the different clinical states.

METHODS

Patients

Six patients with immediate reactions and 21 patients with nonimmediate reactions after inoculation of various live vaccines were studied. All six patients with immediate reactions

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TABLE I. Clinical and laboratory data on patients with immediate- and nonimmediate-type reactions to various vaccines

Patient No.	Age (mo)	Sex	Vaccine	Gelatin (mg/shot)*	Reaction to vaccine	Gelatin IgE (Ua/ml)
Immediate reaction						
1	59	F	Mumps	1	Wheezing, dyspnea	30.7
2	13	F	Measles	1	Urticaria, wheezing, dyspnea	3.23
3	23	M	Varicella	13.5	Wheezing, dyspnea	1.15
4	22	M	Varicella	13.5	Urticaria, wheezing, dyspnea	5.62
5	28	M	Measles	2.5	Urticaria, wheezing, dyspnea	4.7
6	28	M	Measles	2.5	Urticaria, wheezing, dyspnea	5.95
Nonimmediate reaction						
1	18	M	Measles	1.5	Mild systemic skin rash	0.35>
2	18	M	Varicella	13.5	Erythema, swelling at injection site	0.35>
3	15	M	Measles	1	Mild systemic skin rash	0.35>
4	16	M	Measles	1	Mild systemic skin rash	0.35>
5	12	F	Measles	1	Erythema, swelling at injection site	0.35>
6	35	F	Measles	1	Erythema, swelling at injection site	0.35>
7	24	M	Measles	1	Erythema, swelling at injection site	0.35>
8	23	M	Mumps	1	Mild systemic skin rash	0.35>
9	24	M	Measles	1	Mild systemic skin rash	0.35>
10	24	F	Measles	10	Mild systemic skin rash	0.35>
11	14	M	Measles	1	Mild systemic skin rash	0.35>
12	16	F	Measles	2.5	Mild systemic skin rash	0.35>
13	15	M	Measles	2.5	Erythema, swelling at injection site	0.35>
14	22	F	Rubella	2.5	Erythema, swelling at injection site	0.35>
15	18	F	Rubella	2.5	Erythema, swelling at injection site	0.35>
16	19	F	Measles	2.5	Mild systemic skin rash	0.35>
17	17	F	Varicella	13.5	Erythema, swelling at injection site	0.35>
18	12	F	Measles	†	Mild systemic skin rash	0.35>
19	20	F	Measles	2.5	Mild systemic skin rash	0.35>
20	15	F	Measles	2.5	Mild systemic skin rash	0.35>
21	12	F	Measles	2.5	Mild systemic skin rash	0.35>

*These data were obtained from the manufacturers' literature, which gives the contents of the vaccine and the volume of vaccine administered.

†Unknown.

showed anaphylactic symptoms characterized by cough, wheezing, and dyspnea occurring within 15 minutes after vaccine inoculation. Nonimmediate reactions consisted of erythema and swelling at the injection site and mild systemic cutaneous reactions, which occurred several to 48 hours after vaccination. Patients with nonimmediate reactions showed neither respiratory nor cardiovascular symptoms. The clinical characteristics of the patients are summarized in Table I. Informed consent was obtained from each patient and his or her parents.

Determination of gelatin-specific IgE antibody

IgE antibody to bovine gelatin (Wako Junyaku, Tokyo, Japan) was measured by the CAP system (Pharmacia, Uppsala, Sweden). To establish the system, positive sera were obtained from the patients with anaphylactic reactions, and control sera were obtained from vaccine recipients without any adverse reactions. Inhibition studies were performed, and specific inhibition and parallelism were demonstrated.

In vitro lymphocyte proliferation assay

Peripheral blood mononuclear cells were obtained from the patients at various time intervals (4 days to 14 months) after the onset of the reaction. The cells were cultured at a concentration of 5×10^5 cells/well in 100 μ l of RPMI-1640 medium (Gibco, Grand Island, N.Y.) containing 5% pooled AB+ serum. Trip-

licate cultures were set up with medium alone (control) or with varying concentrations of bovine gelatin (Wako Junyaku). Cultures were incubated at 37°C in a 5% CO₂ atmosphere, usually for 3 days. Then 1 μ Ci of tritiated thymidine was added to each culture, and 16 hours later, the cultures were harvested onto fiberglass filters with a multiple automated sample harvester. Incorporation of radioactive thymidine into harvested cells was determined in a Beckman LS-335 scintillation spectrometer in 5 ml of Liquiflour (New England Nuclear). The results were expressed either by actual tritiated thymidine incorporation (in counts per minute) in the cultures or by ratios of tritiated thymidine incorporation in antigen-stimulated cultures relative to that in control cultures (stimulation index [SI]). An SI exceeding 1.5 was considered to be a positive response.

Antigen-specific IL-2 responsiveness

The technique used was essentially that described by Yoshizawa et al.⁵ and Noma et al.⁶ In brief, peripheral blood mononuclear cells were obtained from the patients, then 1×10^6 cells were added to bovine gelatin antigen and suspended in 1 ml of RPMI-1640 medium containing 10% human AB+ serum in a culture tube (Falcon Plastic, 2054; Becton Dickinson Labware, Lincoln Park, N.J.). In most experiments we added 100 μ g/ml or 10 μ g/ml gelatin to the cultures, because these concentrations were found to be optimal for induction of IL-2

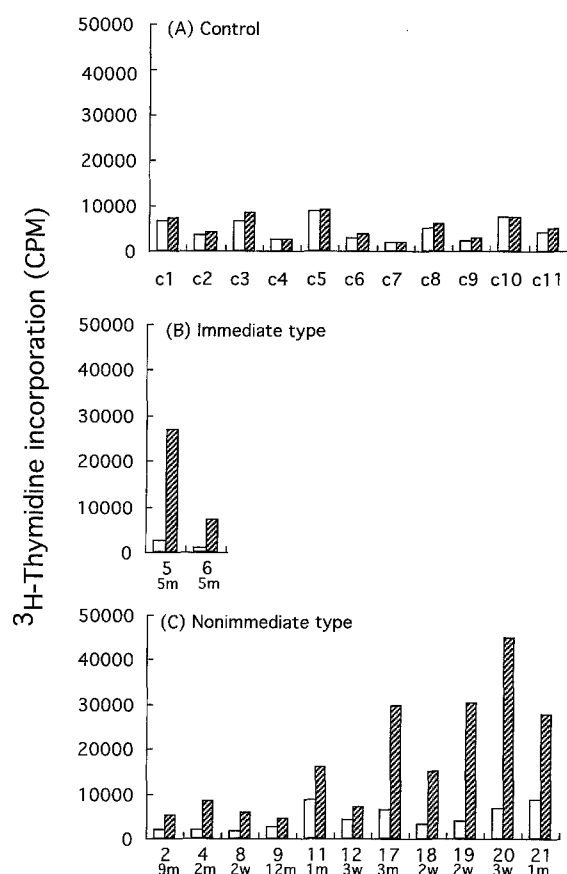


FIG. 1. Gelatin-specific lymphoproliferative activity. **A**, Control subjects. **B**, Patients with immediate-type reactions and positive responses. **C**, Patients with nonimmediate-type reactions and positive responses. Open bar shows tritiated thymidine incorporation in unstimulated culture. Case numbers below bars in **B** and **C** correspond to patient numbers in Table I. Numbers below the case numbers indicate interval between clinical reaction and lymphocyte testing. *m*, Months; *w*, weeks; *d*, days.

responsiveness. After 5 days of culture (37°C in 10% CO_2), cells were washed three times to remove the antigen, readjusted to 2×10^5 in $200\ \mu\text{l}$ of medium, and cultured for an additional 3 days in the presence of $0.5\ \text{U/ml}$ (optimal concentration) recombinant IL-2 (Takeda Co. Ltd., Tokyo, Japan) in 96-well flat-bottom microplates (Nunc Laboratories, Roskilde, Denmark). Cell growth was measured by enumerating the number of viable cells with a propidium iodide enumerating as described by Bruning et al.⁷ At the end of this culture, $50\ \mu\text{l}$ of lysing-staining quenching (LSQ) medium was added to the cell culture, and the mixture was incubated for 2 hours at 37°C . The addition of propidium iodide [2,7-diamino-9-phenyl-10 (diethyl aminopropyl) phenanthridinium iodide methiodide] to the lysed lymphocytes produced staining of double-stranded DNA. The medium's fluorescence was quenched by adding drawing ink to the LSQ medium. Leitz PATIMED equipment (Leica) was used to determine the absorbance at $620\ \text{nm}$ for each well. IL-2 responsiveness was evaluated as follows: IL-2 responsiveness (SI) = Absorbance with IL-2 - Background absorbance $\div$ Absorbance without IL-2 - Background absorbance.

Absorbance with LSQ medium alone was taken as background. For actual evaluation of each patient, the SI increment

was determined by subtracting the SI of unstimulated culture from the SI of gelatin antigen-stimulated culture.

RESULTS

IgE antibody specific to gelatin

The positive/negative cutoff level value was set at $0.35\ \text{Ua/ml}$ for IgE. All patients with immediate reactions showed positive IgE responses specific to gelatin, which ranged from 1.15 to $30.7\ \text{Ua/ml}$ (Table I). The levels of IgE did not seem to correlate with the severity of the clinical reactions. In contrast to the patients with immediate-type reactions, none of the patients with nonimmediate reactions had positive IgE responses.

Gelatin-specific cell-mediated immunity

Lymphoproliferation assays specific to gelatin were performed in sera of all six patients with immediate reactions and also in peripheral blood mononuclear cells of all of the 21 patients with nonimmediate reactions. Eleven control subjects, who had no clinical reaction to the vaccine, had an SI of less than 1.5 , which was therefore considered a negative response (Fig. 1, A). The results of the lymphocyte proliferation assay in the peripheral blood mononuclear cells of patients with immediate and nonimmediate reactions are shown in Fig. 1, B and C, respectively (only the results of patients with positive responses are depicted). Two of six patients (33%) with immediate reactions had a significant lymphoproliferative response in which SIs ranged from 8.1 to 11.2 (Fig. 1, B), whereas 11 of 21 patients (52%) with nonimmediate reactions showed positive responses with SIs ranging from 1.72 to 7.7 (Fig. 1, C). The mean intervals between the clinical reactions and the time of study in patients with immediate and nonimmediate reactions and positive responses in lymphoproliferation assay were 5 months and 2.6 months, respectively (data shown in Fig. 1). In those with negative responses, the intervals were 7.3 months and 5.0 months, respectively (data not shown).

Gelatin-specific IL-2 responsiveness assays were performed in peripheral blood mononuclear cells of all patients (Fig. 2). The positive/negative cutoff value in this assay system was determined on the basis of a comparison with the distribution of the SI values obtained with 11 healthy control samples (Fig. 2, A). An SI increment of 0.08 or more was regarded as a positive response. Positive responses ranging from 0.32 to 0.69 in SI increments were obtained in all six patients (100%) with immediate reactions (Fig. 2, B), whereas positive responses ranging from 0.09 to 0.22 in SI increments were obtained in 10 of 21 patients (48%) with nonimmediate reactions (Fig. 2, C). The mean intervals between the clinical reactions and the time of study in patients with immediate and nonimmediate reactions and positive responses in the IL-2 responsiveness assay were 5.0 months and 2.3 months, respectively (data shown in Fig. 2). In those with negative responses in nonimmediate reactions, the interval was 7.3 months (data not shown).

In the patients with nonimmediate-type reactions, 17 of 21 showed a positive response in the lymphocyte proliferation assay, in the IL-2 responsiveness assay, or in both assays.

Thus not only in patients with immediate-type reactions but also in those with nonimmediate-type reactions, a significant number of children showed positive cell-mediated immune reactions as determined by the lymphocyte proliferation assay and/or the assay for IL-2 responsiveness. There was no correlation between the degree of cell-mediated immune responses and gelatin-specific IgE concentrations.

DISCUSSION

There has been an increasing number of reports regarding anaphylactic reactions to measles or measles-mumps-rubella vaccines in children without allergy to eggs,⁸⁻¹³ and components of the vaccine other than egg protein have been implicated as the cause.¹⁴ In 1986, Miyake et al.¹⁵ found specific IgE antibody to measles vaccine by means of radioimmunoassay in the sera of patients who had episodes of immediate local allergic reactions. Subsequently, the actual antigen was identified as gelatin by an inhibition test.¹⁶ An identical result was reported by Kelso et al.³ in a patient with anaphylaxis to measles-mumps-rubella vaccine, in whom they detected gelatin-specific IgE in the serum by means of protein immunoblotting. Sakaguchi et al.⁴ also found gelatin-specific IgE in a larger number of patients with immediate postvaccination reactions. This study reconfirms these observations and shows that sera from all patients with immediate reactions are positive for IgE specific to gelatin. In contrast, IgE was not detected in sera from patients with nonimmediate reactions. The combined data indicate that immediate reactions observed in patients with vaccine-related allergic symptoms are mediated by humoral immunity.

Because there have been no studies of the mechanism of nonimmediate reactions, we examined the cell-mediated immune response to gelatin by using an in vitro lymphocyte proliferation assay and an assay for antigen-specific IL-2 responsiveness. In vitro lymphocyte proliferation assays have been used widely to demonstrate the presence of antigen-sensitized lymphocytes in allergic patients.¹⁷⁻²² Recently, Noma et al. developed an assay for antigen-specific IL-2 responsiveness, which has a greater sensitivity and specificity in the research field of allergology.^{5, 6, 23, 24} We argued that the sensitivity to identify patients with positive cell-mediated immune responses would be increased by running these two assay methods simultaneously.

The clinical observations that the symptoms of nonimmediate reactions always appear after the injection of gelatin containing vaccine and that none of the patients have anti-egg IgE suggest that these reactions are also related to gelatin allergy, although the mechanisms might be different from those of immediate reactions.⁴ Furthermore, this study demonstrates that significant numbers of not only patients with immediate reactions

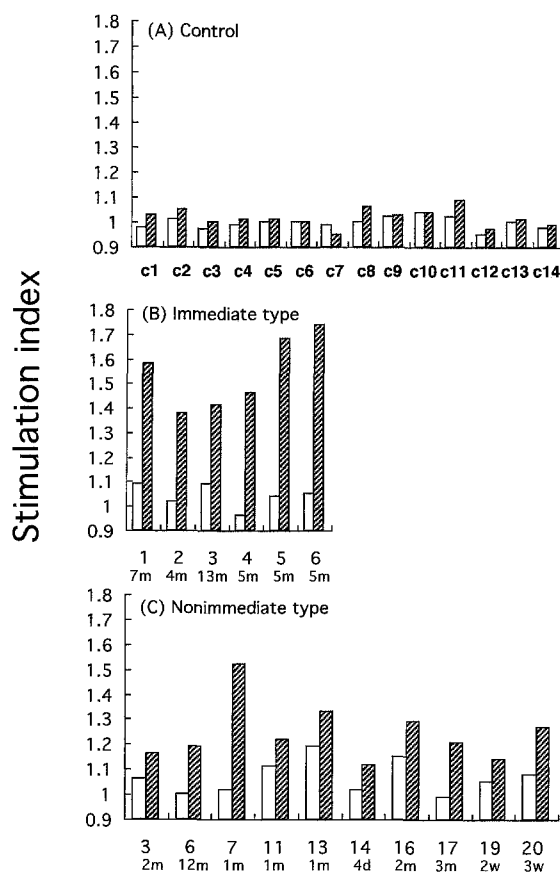


FIG. 2. Gelatin-specific IL-2 responsiveness. **A**, Control subjects. **B**, Patients with immediate-type reactions and positive responses. **C**, Patients with nonimmediate-type reactions and positive responses. Open bar shows SI of control culture, and cross-hatched bar shows SI of gelatin antigen-stimulated culture. Case numbers in **B** and **C** correspond to patient numbers in Table I. Numbers below the case numbers indicate interval between clinical reaction and lymphocyte testing. *m*, Months; *w*, weeks; *d*, days.

but also those with nonimmediate reactions have a positive cell-mediated immune response specific to gelatin. The finding that the patients who had positive lymphocyte proliferation in the tritiated thymidine assay are not the same patients as those who had antigen-induced IL-2 responsiveness may be explained by the fact that these two assays measure two fundamentally different parameters of the cell population. The tritiated thymidine assay detects an increase in the proportion of cells in S phase within the target population, whereas the IL-2 responsiveness assay detects an increase in total cell number.

The variable time interval between the clinical reaction and the lymphocyte testing (from 4 days to 14 months) might be questioned. There was a trend suggesting that the shorter the time elapsed, the higher were the levels of gelatin-specific cell-mediated immunity. However, the fact that a positive cell-mediated immune response was observed, even 13 months after the adverse reaction in one patient, suggests that allergy

to gelatin persists for a long period. Thus we believe that the variation in interval between reaction and study time does not contradict the overall findings of this investigation.

In any case, our data provide the first laboratory-based evidence that nonimmediate reactions are also caused by allergy to gelatin and that cell-mediated immunity may play a crucial role in the pathogenesis of these reactions.

Functional characterization, including investigation of the cytokine profile of the T-cell clones derived from the peripheral blood of these patients, should be attempted in the future to clarify the differences between sensitized lymphocytes from the patients with immediate reactions and from those with nonimmediate reactions.

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