Effect of amino acid mixtures on nasal allergic responses induced by toluene diisocyanate in mice

Tzu-Hsiu Chen*, Keiko Takeshita†, Kentaro Sakai* Tatsushi Komatsu*, Yasuo Kishino*, and Shigeru Yamamoto*

*Department of Nutrition, The University of Tokushima School of Medicine, Tokushima Japan; and †Department of Faculty of Environmental and Symbiotic Science ; Prefectural University of Kumamoto, Kumamoto, Japan

Abstract : We studied the effect of various amino acid mixtures on nasal allergy induced by the intranasal application of toluene diisocyanate (TDI) in mice. In Experiment 1 (Exp. 1), mice were fed a 25% casein, soy protein isolate (SPI), egg white protein (EW) or gluten diet. In Experiment 2 (Exp. 2), mice were fed a 25% amino acid mixture diets patterned after casein (AA-casein), SPI (AA-SPI), EW protein (AA-EW) or gluten (AA-gluten). In Experiment 3 (Exp. 3) we modified the glutamine/glutamic acid (Gln/Glu) concentrations in the amino acid mixtures. Mice were fed a 25% AA-SPI, low Gln/Glu AA-SPI (LG-AA-SPI), AA-EW or high Gln/Glu AA-EW (HG-AA-EW) diet. At the 5 th week, mice were divided into sensitized (sen-) and non-sensitized (ns-) groups. The mice in sensitized groups were treated with two courses of intranasal application of toluene diisocyanate (TDI) in ethyl acetate for 5 consecutive days, separated by 9 days rest. The non-sensitized groups of mice were treated with a vehicle. Nine days after the second sensitization, all mice were provoked by TDI. Nasal responses and serum IgE concentration were studied. The findings of Exp. 1 showed that the sen-EW group exhibited a lower body weight gain, higher nasal symptom score and higher IgE concentration than the other sensitized groups. The findings of Exp. 2 showed that the sen-EW group had a lower body weight gain, higher nasal symptom score and higher IgE concentration than the other sensitized groups. In Exp. 3, the AA-EW group showed a higher total nasal score and IgE concentration than the HG-AA-EW group, however, the findings of LG-AA-SPI and AA-SPI were similar. These findings demonstrated that amino acid mixtures affect nasal allergy induced by the intranasal application of TDI in mice. J. Med. Invest. 47 : 128-137, 2000

Key words **:** *allergic rhinitis, protein, amino acid, mouse*

In previous studies we demonstrated that nucleosides and nucleotides enhance immunity (1, 2), however, they also aggravate nasal allergy caused by the intranasal administration of 2, 4-toluene diisocyanate (TDI) (3) and inflammatory bowel disease due to colitis by the administration of 2, 4, 6-trinitrobenzene sulphonic acid (TNBS) (4, 5). Arginine upregulates immunity (6), however, it also induces inflammatory bowel disease due to colitis by the administration of TNBS (7, 8). Glutamine (Gln) enhances immunity (9, 10) and alleviates inflammatory bowel disease induced by TNBS in rats (11), however, high supplementation aggravates the disease (12). Nucleosides, nucleotides, arginine and Gln do not possess antigenicity. From such background information, it was suggested that some amino acids or amino acid mixtures easily induce allergy.

In Exp. 1, we compared whether the 4 proteins had different effects on nasal allergy induced by TDI. In Exp. 2, we studied whether amino acid

Received for publication May 22, 2000 ; accepted June 19, 2000.

Address correspondence and reprint requests to Tzu-Hsiu Chen, MSc, Department of Nutrition, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-633-9427.

mixtures patterned after the proteins used in Exp. 1 had similar effects as the proteins. In Exp. 3, we changed the Gln and glutamic acid (Glu) concentrations to see the effect of these amino acids on nasal allergy induced by TDI. When we prepared the amino acid mixtures of the proteins, we used an amino acid composition table. Although the intact proteins contained Gln and Glu, in the table only Glu concentrations are shown. This is because Gln becomes Glu during the hydrolysis of protein for the measurement of amino acids. Gln has a strong influence on immunity (9, 10), therefore, in this study we used both Gln and Glu. Because the Gln concentration of soybean protein isolate (SPI) is high and that of egg white (EW) is low, in Exp. 3, we changed the Gln/Glu compositions of amino acid mixtures patterned after soybean protein isolate (AA-SPI) and egg white (AA-EW) to examine the effect of Gln/Glu on allergy.

Mice were administered a TDI solution into the nostrils to induce allergy. TDI is a polyurethane material than induces asthma among people working in polyurethane factories (13, 14). TDI is a very reactive chemical and easily combines with various proteins in the body, which become antigens. By the nasal administration of TDI, a nasal allergy model was produced in guinea pigs (15). We induced the nasal allergy model in mice using this method (3, 16) and studied the effects of proteins and amino acids on the allergy in mice.

MATERIALS AND METHODS

Animals and diets

Four week old female ddY mice were used for this study. The mice were kept in a room of constant temperature (25±2°C) and humidity (55±10%) with a 12 h light/dark cycle. The mice were randomly divided into 3 groups and fed diets containing 25% protein (Exp. 1) (Table 1), or 25% amino acid mixture (Exp. 2 and 3) (Tables 1, 2). In Exp. 3 mice were fed 25% AA-SPI, AA-SPI with reduced Gln/Glu (LG-AA-SPI), AA-EW or AA-EW supplemented with Gln/Glu (HG-AA-EW) (Table 3). At the 5th week, mice were divided into 2 sub-groups ; sensitized and non-sensitized. Nitrogen concentrations of dietary proteins were analyzed using the Kjeldahl method. Protein concentrations were calculated by multiplying nitrogen concentrations and protein conversion ratios (17). The other components of the diets were 5% soybean oil, 2% cellulose powder, 5% min-

Table 1. Compositions of experimental diets

	Exp. 1*1				Exp. 2 & 3*2
Material	Casein	SPI	EW	Gluten	
Casein	29.7	-	-	-	-
Soybean protein isolate	-	27.8	-	-	-
Egg White	-	-	30.3	-	-
Gluten	-	-	-	33.3	-
Amino acid mixtures	-	-	-	-	25.0
α-starch	38.2	39.5	37.8	35.8	41.3
Sucrose	19.1	19.7	18.9	17.9	20.7
Oil	5.0	5.0	5.0	5.0	5.0
Cellulose	2.0	2.0	2.0	2.0	2.0
Mineral mixture*3	5.0	5.0	5.0	5.0	5.0
Vitamin mixture*4	1.0	1.0	1.0	1.0	1.0

*1 Crude protein concentrations of casein, soybean protein isolate (SPI), egg white protein (EW) and gluten were 84.3, 90.0, 82.4 and 75.0%, respectively.
*2 AA : Amino acid mixtures patterned after proteins are shown in Table 2 (Exp. 2) and 3 (Exp. 3).
*3 Obtained from Oriental Yeast Co., Tokyo. The composition was as follows : (mg/kg) $CaHPO_4 \cdot 2H_2O$, 7.280 ; KH_2PO_4, 12.860 ; NaH_2PO_4, 4.680 ; NaCl, 2.330 ; Ca • lactate, 17.550 ; Fe • citrate, 1.590 ; $MgSO_4$ 3.950 ; $ZnCO_3$, 55 ; $MnSO_4 \cdot 6H_2O$, 60 ; $CuSO_4 \cdot 5H_2O$, 15 ; Kl, 5.
*4 Obtained from Oriental Yeast Co., Tokyo. The composition was as follows : (mg/kg) thiamin-HCl, 12 ; riboflavin, 40 ; pyridoxine-HCl, 8 ; vitamin B12, 50 ; ascorbic acid, 300 ; D-biotin, 0.2 ; folic acid, 2 ; calcium pantothenate, 50 ; ρ-aminobenzoic acid, 50 ; niacin, 60 ; inositol 60 ; choline chloride, 2000 ; dl-α-tocopheryl acetate, 50 ; menadione, 52 ; and retinyl acetate, 5000 ; ergocalciferol, 1000.

Table 2. Amino acid compositions of experimental diets (Exp. 2)*

	AA-casein	AA-SPI	AA-EW	AA-gluten
	%	%	%	%
Isoleucine	5.2	4.8	5.4	3.7
Leucine	8.9	7.8	8.6	6.7
Lysine	7.6	6.5	6.6	1.7
Methionine	2.8	1.5	3.0	1.6
Cystine	0.5	1.6	3.8	2.0
Phenylalanine	4.8	5.4	5.7	5.1
Tyrosine	5.3	3.5	3.9	3.1
Threonine	3.9	3.8	4.3	2.5
Tryptophan	1.2	1.3	1.5	1.0
Valine	6.4	4.8	7.0	4.1
Histidine	2.9	3.0	2.4	2.2
Arginine	3.5	7.5	5.6	3.3
Alanine	2.9	4.3	6.0	2.6
Aspartic acid	6.7	11.9	10.1	3.3
Glutamine	10.9	8.1	3.7	33.8
Glutamic acid	9.3	9.7	9.2	1.6
Glycine	1.7	4.3	3.5	3.3
Proline	10.6	5.4	3.5	14.1
Serine	4.9	4.8	6.2	4.3

*Amino acid mixtures were patterned after casein (AA-casein), SPI (AA-SPI), EW (AA-EW) and gluten (AA-gluten).

Table 3. Amino acid compositions of experimental diets (Exp. 3)*

	AA-SPI	LG-AA-SPI	AA-EW	HG-AA-EW
	%	%	%	%
Isoleucine	4.8	5.2	5.4	5.0
Leucine	7.8	8.5	8.6	7.9
Lysine	6.5	7.1	6.6	6.1
Methionine	1.5	1.6	3.0	2.8
Cystine	1.6	1.7	3.8	3.5
Phenylalanine	5.4	5.9	5.7	5.2
Tyrosine	3.5	3.8	3.9	3.6
Threonine	3.8	4.1	4.3	3.9
Tryptophan	1.3	1.4	1.5	1.4
Valine	4.8	5.2	7.0	6.4
Histidine	3.0	3.3	2.4	2.2
Arginine	7.5	8.2	5.6	5.1
Alanine	4.3	4.7	6.0	5.5
Aspartic acid	11.9	13.0	10.1	9.3
Glutamine	8.1	4.6	3.7	5.7
Glutamic acid	9.7	5.4	9.2	14.3
Glycine	4.3	4.7	3.5	3.2
Proline	5.4	5.9	3.5	3.2
Serine	4.8	5.2	6.2	5.7

*LG-AA-SPI : Low glutamine/glutamic acid-AA-SPI
HG-AA-EW : High glutamine/glutamic acid-AA-EW

eral mixture (Oriental Co., Tokyo, Japan) and 1% vitamin mixture (Oriental Co., Tokyo, Japan). Remaining parts of the diets were carbohydrates (α-cornstarch : granule sucrose = 2 : 1). The compositions of the amino acid mixtures (Kyowa Hakko Kogyo Co. Tokyo, Japan) were made following the amino acid composition table of the Japan Science and Technology Agency (17), except Glu. The table contains Glu but not Gln, because Gln in protein form becomes Glu during the hydrolysis of the protein during amino acid analysis. However, Gln is immunologically important, therefore, we used both Gln/Glu. The ratios of Gln and Glu in the proteins used in the present study were calculated following the previous studies (18-23). Mice were fed casein, SPI, EW or gluten diet in Exp .1, amino acid mixture diet patterned after casein (AA-casein), SPI (AA-SPI), EW (AA-EW) or gluten (AA-gluten) in Exp. 2, and AA-SPI, LG-AA-SPI, AA-EW or HG-AA-EW diet in Exp. 3. After the experimental diet for 4 weeks, the mice from each dietary group were divided into 2 sub-groups, sensitized (sen-) and non-sensitized (ns-), depending upon whether they were treated with TDI (Wako Chemical Co. Tokyo, Japan) in ethyl acetate (Wako Chemical Co. Tokyo, Japan) (sensitization) or with the vehicle alone (non-sensitized). The mice were housed approximately 5 per cage under controlled environmental conditions (25±1°C, 55±10% relative humidity, 0800-2000 h lighting period). They were weighed weekly. This study was approved by the Ethical Committee of Tokushima University for Animal Studies.

Sensitization and provocation

Sensitization was done by the method of Tanaka *et al*. (15) with slight modification. The experimental design for TDI treatment of the study is shown in Fig. 1. The mice were sensitized by dropping 2 μl of 5% TDI dissolved in ethyl acetate in Exp. 1 and 1% TDI in Exp. 2 and 3 into the nostrils under slight ether anesthesia for 5 consecutive days. This was repeated following a rest of 9 days. Non-sensitized mice were similarly treated with the vehicle. We used different concentrations of TDI in 3 experiments because mice became very weak after sensitization in Exp. 1, therefore, we reduced the TDI concentration to 1% in Exp. 2 and Exp. 3. The mice were again allowed 9 days rest and then all the mice were provoked with the same volume and concentration of TDI as the sensitization without anesthesia. Nasal responses of itching, water rhinorrhea and snorting were scored for 10 minutes by the method of Irifune *et al*. (24) (Table 4). Hair loss was scored by observing the extent of hair loss from the snout on the day of provocation (Table 4).

Serum IgE measurement

Two weeks (Exp. 1 and 2) or 1 week (Exp. 3) after the provocation with TDI, mice were anesthetized

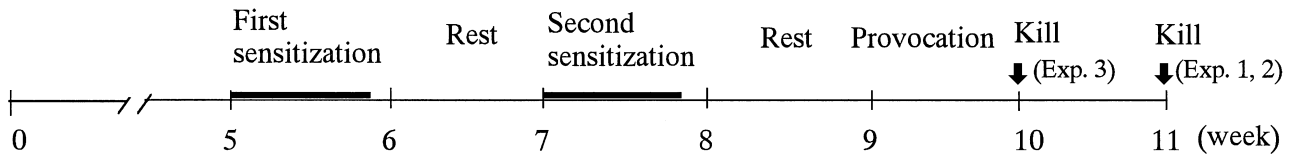

Fig. 1. Experimental design of TDI treatment. Mice were sensitized for 5 days in the 5th week, allowed to rest for 9 days, resensitized for 5 days in the 6th week. In the 9th week after 9 days rest, mice were provoked by a single administration of 5% TDI solution (Exp. 1) or 1% TDI solution (Exp. 2). Nasal responses for 10 minutes after the provocation and hair loss around the nose were observed. The mice were sacrificed 2 weeks (Exp. 1) or 1 week (Exp. 2 and 3) after the provocation.

Table 4. Criteria for scoring the nasal responses and hair loss of mice

	SCORE			
Nasal response	0	1	2	3
Itching	-	Mild	Moderate	Severe
Rhinorrhea	-	At the nostril	Between 1 & 3	Drops of discharge from the nose
Snort	-	Mild	Moderate	Severe
Hair loss	-	Mild	Moderate	Severe

Scores of itching, rhinorrhea and snort were measured for 10 minutes after provocation.
Hair loss was observed on the provocation day.

by intraperitoneal administration of pentobarbital (0.1 g/kg body weight) and killed. Serum was removed from collected blood and immediately stored at -40°C until use. Total IgE in serum was determined by a sensitive enzyme-liked immunosorbent assay (ELISA) using 96-well microtitre plates. Each well was coated with anti-mouse IgE (Yamasa Syoyu Co., Chiba, Japan) (1 μg/ml) and incubated overnight at 4°C. The wells were washed 5 times with 25 mM Tris-HCl, pH 7.4, containing 0.14 M NaCl, 5 mM KCl, 0.1% NaN_3 and 0.5% Tween 20 (TBS-T). Then the wells were blocked with 3% bovine serum albumin for 1 hour at room temperature. After washing 5 times, diluted mouse serum samples or purified anti-DNP-mouse IgE (Yamasa Syoyu Co., Chiba, Japan) (0.025-1 μg/ml) as standards were added to the wells. The standards and serum samples were diluted with 20 mM Tris-HCl, pH 7.4, containing 10 μM phenylmethyl sulphonyl fluoride. The wells were washed 5 times and incubated with biotinylated anti-mouse IgE (1 μg/ml) (Yamasa Syoyu Co., Chiba, Japan) for 1 hour at room temperature. The wells were washed with TBS-T and incubated with avidin-biotin complex solution (Vectastain ABC Elite Kit, Vector Laboratories Burlingame, CA, USA.) for 1 hour at room temperature. Then, after washing five times with TBS-T, paranitrophenyl phosphate solution was added to the wells for color development. After 5 minutes, the color development was terminated by adding 150 mM ethylene diamine tetra-acetic acid. The absorbance at 415 nm was read with an automatic ELISA reader.

Histology of nasal tissue

Samples of nasal tissue were removed for histological examination. The tissues were fixed in 10% phosphate buffered formalin at room temperature overnight. The tissues were sliced into 4-6 mm pieces, dehydrated in ethanol, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (H&E). For the examination of mast cells, the tissues were stained with toluidine blue solution. The sections were examined and photographed using a microscope (Olympus Kogyo Co., Tokyo, Japan).

Statistical analysis

Values are shown as means±SE. The statistical differences were assessed by Duncan's multiple range test. The values with different superscript letters indicate significant difference ($p<0.05$).

RESULTS

Changes in body weight in Exp. 1

The body weights of the mice were not different among the groups before the first sensitization (at 5th week) (Table 5). After the first sensitization (at 5th week), the sen-EW and sen-Casein groups lost weight, but the sen-SPI and sen-Gluten groups did not. The sen-Casein group recovered body weight after 9 weeks but the sen-EW did not.

Nasal responses to provocation in Exp. 1

The behavior score, measured as a total of nasal responses of itching, watery rhinorrhea and snort during 10 minutes after the provocation by TDI and degree of hair loss are shown in Fig. 2. Generally, the sensitized groups showed higher total scores compared with non-sensitized groups. Significant differences between non-sensitized and sensitized groups were observed in casein, SPI and EW diet groups ($p<0.05$). The sen-EW group had a significantly higher total score than the other sensitized groups except sen-Casein group ($p<0.05$).

Total serum IgE concentrations in Exp. 1

Fig. 3 shows findings of serum concentrations

of IgE. The concentrations of sensitized groups tended to be higher than those of the non-sensitized groups without significant differences ($p>0.05$) except in the casein and EW groups ($p<0.05$). The sen-EW group showed the highest concentration of IgE ($p<0.05$). There were no significant differences among the non-sensitized groups ($p>0.05$).

Changes in body weight in Exp. 2

Changes in body weight were not significantly different among all groups ($p>0.05$), however the sen-AA-EW group lost weight after the 9th week ($p<0.05$) (Table 6).

Table 5. Changes in body weight of mice fed various protein diets and sensitized (sen-) and non-sensitized (ns-) by TDI (Exp. 1).

week	sen-Casein	ns-Casein	sen-SPI	ns-SPI	sen-EW	ns-EW	sen-Gluten	ns-Gluten
				(g)				
1	21.0±0.3	21.4±0.4	20.7±0.4	21.1±0.4	21.4±0.4	21.0±0.4	21.1±0.3	20.3±0.3
5	30.5±0.8	30.2±0.7	30.2±0.5	29.1±0.7	29.3±0.6	29.1±0.7	29.9±0.5	29.8±1.4
7	28.6 ± 0.8^{ac}	31.2 ± 0.5^{b}	31.2 ± 0.7^{b}	30.1 ± 0.8^{ab}	27.5 ± 0.6^{c}	30.2 ± 0.8^{ab}	30.4 ± 0.5^{ab}	31.1 ± 0.9^{b}
9	29.3 ± 0.7^{a}	31.3 ± 0.7^{ab}	31.3 ± 0.5^{ab}	30.5 ± 0.9^{ab}	28.1 ± 0.5^{a}	30.7 ± 0.9^{ab}	30.7 ± 0.6^{ab}	32.7 ± 2.1^{b}
11	32.4 ± 0.8^{a}	31.4 ± 1.0^{a}	31.4 ± 0.6^{a}	30.8 ± 0.7^{ab}	28.4 ± 0.5^{b}	30.1 ± 1.0^{ab}	32.3 ± 0.6^{a}	31.1 ± 1.5^{ab}

Values are mean±SE. Number of mice in each group was 5.
The statistical differences were assessed by Duncan's multiple range test.
^{a,b,c} means with different superscripts within row differ, $P<0.05$.

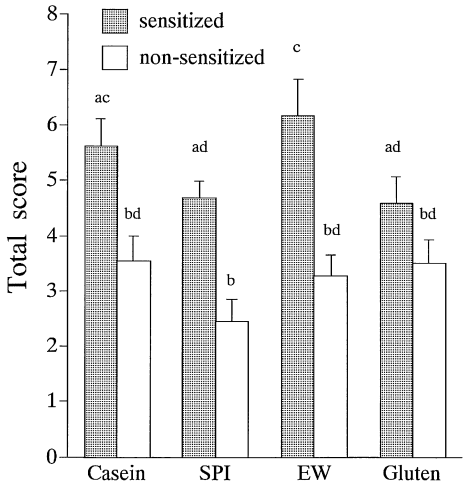

Fig. 2. Total scores of nasal symptoms of mice fed various protein diets sensitized (sen-) and non-sensitized (ns-) by TDI (Exp. 1). Bars with different letters indicate a significant difference ($p<0.05$).

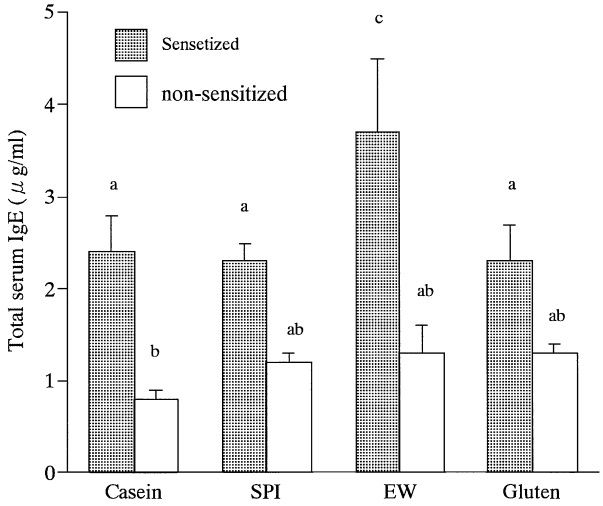

Fig. 3. Serum IgE concentrations of mice fed various protein diets sensitized (sen-) and non-sensitized (ns-) by TDI (Exp. 1). Bars with different letters indicate significant difference ($p<0.05$).

Table 6. Changes in body weight of mice fed amino acid mixture diets and sensitized (sen-) and non-sensitized (ns-) by TDI (Exp. 2).

week	sen-AA-Casein	ns-AA-Casein	sen-AA-SPI	ns-AA-SPI	sen-AA-EW	ns-AA-EW	sen-AA-Gluten	ns-AA-Gluten
				(g)				
1	22.2±0.4	22.8±0.7	22.3±0.5	22.4±0.4	22.2±0.5	22.3±0.4	22.5±0.5	22.4±0.7
5	31.7±0.9	33.5±1.2	33.8±1.2	33.9±1.1	33.7±1.6	31.4±1.5	33.5±1.5	32.2±1.0
7	31.2±1.0	32.2±1.1	32.9±1.3	34.3±1.5	33.1±1.3	30.9±1.2	32.8±1.5	30.8±0.9
9	32.1 ± 1.2^{ab}	33.1 ± 1.1^{a}	34.1 ± 1.6^{a}	36.2 ± 1.6^{a}	27.9 ± 2.2^{b}	32.8 ± 1.4^{a}	34.6 ± 2.2^{a}	32.8 ± 0.8^{a}
11	33.9 ± 1.2^{a}	33.7 ± 1.0^{a}	35.8 ± 1.9^{a}	36.7 ± 1.6^{a}	27.3 ± 2.1^{b}	33.7 ± 1.9^{a}	36.5 ± 2.6^{a}	33.6 ± 1.1^{a}

Values are mean±SE. Number of mice in each group was 6~13.
The statistical differences were assessed by Duncan's multiple range test.
^{a,b,c} means with different superscripts within row differ, $P<0.05$.

Nasal responses to provocation in Exp. 2

Fig. 4 shows the total score of nasal responses to provocation in Exp 2. Total score of the sen-AA-EW group was higher than the scores of the other groups ($p<0.05$).

Serum IgE concentrations in Exp. 2

Fig. 5 shows the serum concentrations of IgE. The sen-AA-EW group showed higher IgE concentration than the other groups ($p<0.05$). Differences in the other groups were not observed ($p>0.05$).

Changes in body weight in Exp. 3

Before the first sensitization (5th week), the body weights of the mice were not different among the groups (Table 7). After sensitization, the sen-AA-EW and sen-HG-AA-EW groups lost weight significantly, however, the sen-AA-SPI and sen-LG-AA-SPI did not. After the provocation at the 9th week, all the sensitized groups lost weight. The loss was greatest in the sen-AA-EW and sen-HG-AA-EW groups.

Nasal responses to provocation in Exp. 3

Fig. 6 shows the total score of nasal responses to provocation in Exp. 3. The total score of the sen-AA-EW group was significantly higher than those of the other groups ($p<0.05$). The scores of the sen-AA-SPI and sen-LG-AA-SPI groups tended to be lower than those of sen-HG-AA-EW group, however, the difference was not significant ($p>0.05$).

Serum IgE concentrations in Exp. 3

Fig. 7 shows the serum IgE concentrations. The sen-AA-EW group showed significantly higher values than the other groups ($p<0.05$). The concentration of the sen-HG-AA-EW group was not different from the concentrations of the sen-AA-SPI and sen-LG-AA-SPI groups ($p>0.05$).

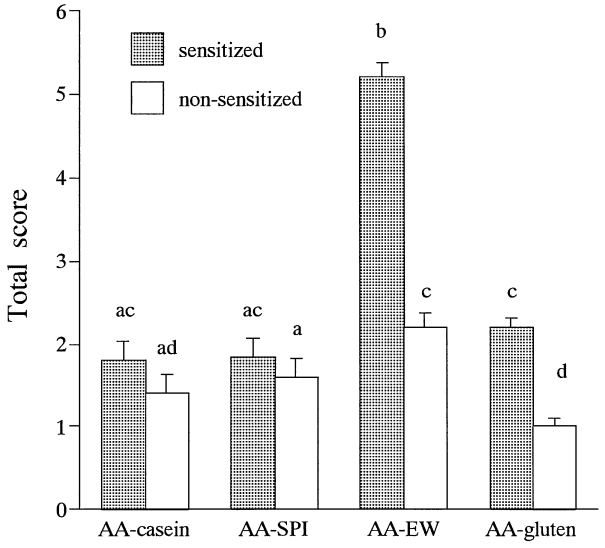

Fig. 4. Total scores of nasal symptoms of mice fed amino acid mixture diets and sensitized (sen-) and non-sensitized (ns-) by TDI (Exp. 2). Bars with different letters indicate a significant difference ($p<0.05$).

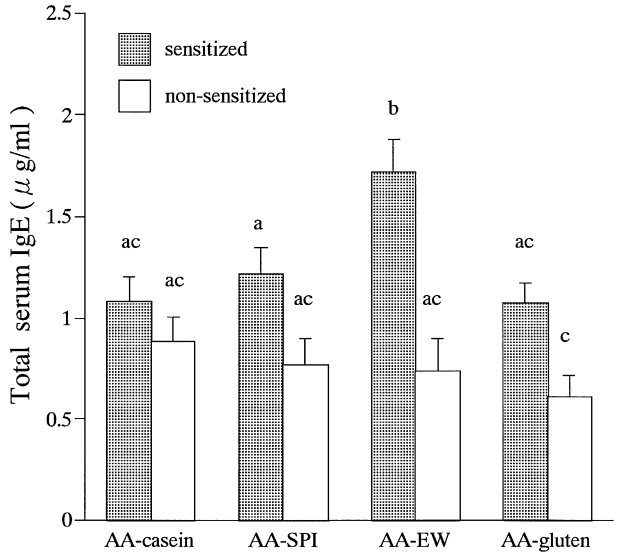

Fig. 5. Serum IgE concentrations of mice fed amino acid mixture diets and sensitized (sen-) and non-sensitized (ns-) by TDI (Exp.2). Bars with different letters indicate a significant difference ($p<0.05$).

Table 7. Changes in body weight of mice fed expeerimental diets and sensitized (sen-) and non-sensitized (ns-) by TDI (Exp.3).

week	sen-AA-SPI	ns-AA-SPI	sen-LG-AA-SPI	ns-LG-AA-SPI	sen-AA-EW	ns-AA-EW	sen-HG-AA-EW	ns-HG-AA-EW
				(g)				
1	22.3±0.6	22.6±0.6	21.9±0.4	22.8±0.5	22.4±0.4	22.3±0.4	22.4±0.5	22.6±0.7
5	30.6±0.4	31.3±0.8	30.3±0.7	31.8±1.1	31.5±1.0	32.0±0.5	30.2±1.1	30.2±0.8
7	32.3 ± 0.7^{a}	32.5 ± 1.6^{a}	31.4 ± 0.8^{ac}	30.3 ± 1.9^{ab}	28.3 ± 1.3^{bc}	32.7 ± 0.9^{a}	26.9 ± 1.0^{a}	32.0 ± 0.4^{b}
9	29.9 ± 2.1^{ab}	31.1 ± 0.5^{ac}	30.2 ± 1.0^{ab}	32.6 ± 2.0^{a}	26.7 ± 0.7^{bc}	27.6 ± 1.2^{bc}	25.7 ± 1.3^{b}	29.0 ± 1.5^{ab}
10	29.1 ± 1.8^{ab}	31.0 ± 1.3^{ac}	29.6 ± 1.0^{ab}	32.4 ± 1.6^{a}	26.5 ± 1.0^{b}	26.9 ± 0.9^{bc}	25.5 ± 1.9^{b}	29.2 ± 1.0^{ab}

Values are mean±SE. Number of mice in each group was 6～13.
The statistical differences were assessed by Duncan's multiple range test.
^{a,b,c} means with different superscripts within row differ, $P<0.05$.

Pathologic findings

Fig. 8 shows typical examples of the microscopic views of nasal membrane in the sensitized and non-sensitized mice.

Fig. 9 shows typical examples of the mast cells of nasal tissue in the sensitized and non-sensitized

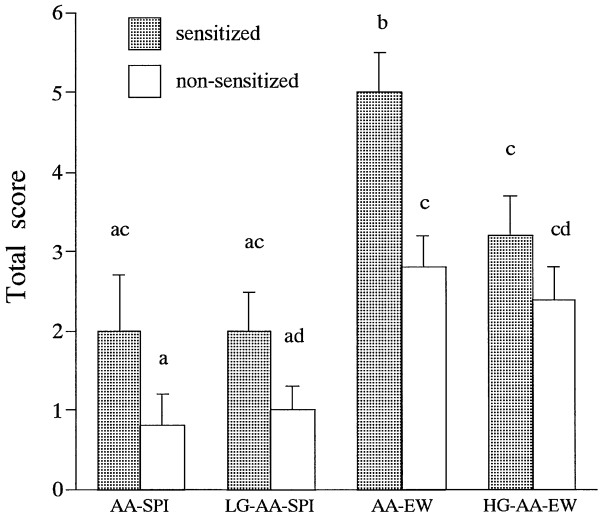

Fig. 6. Total scores of nasal symptoms of mice fed AA-SPI, LG-AA-SPI, AA-EW, HG-AA-EW diets and sensitized (sen-) and non-sensitized (ns-) by TDI (Exp. 3). Bars with different letters indicate a significant difference ($p<0.05$).

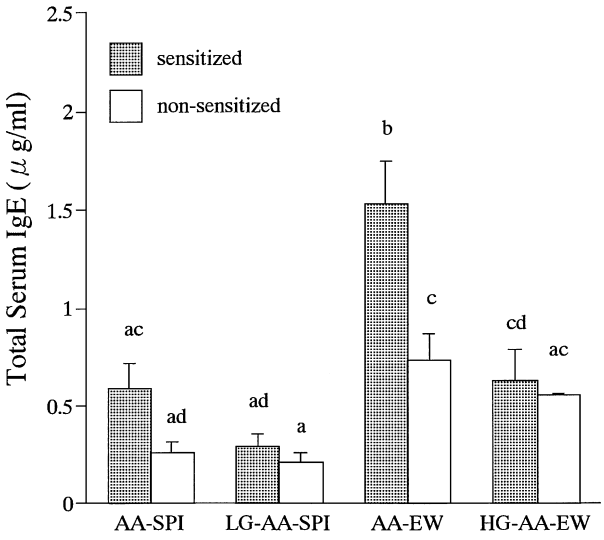

Fig. 7. Serum IgE concentrations of mice fed AA-SPI, LG-AA-SPI, AA-EW, HG-AA-EW diets and sensitized (sen-) and non-sensitized (ns-) by TDI (Exp. 2). Bars with different letters indicate a significant difference ($p<0.05$).

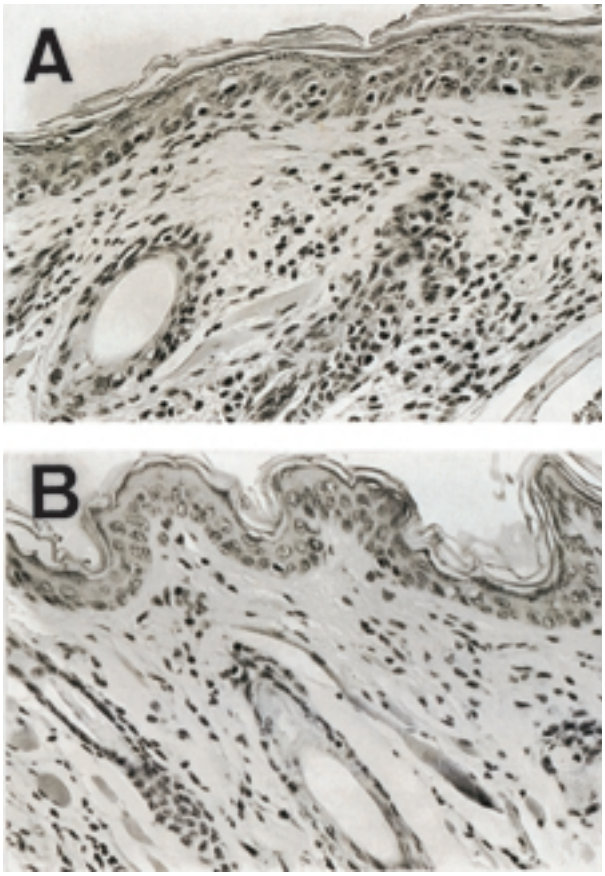

Fig. 8. Microscopic views of nasal tissue in TDI sensitized (A) and non-sensitized (B) mice. Marked infiltration of inflammatory cells was present in the sensitized group (A). H&E stain ×200.

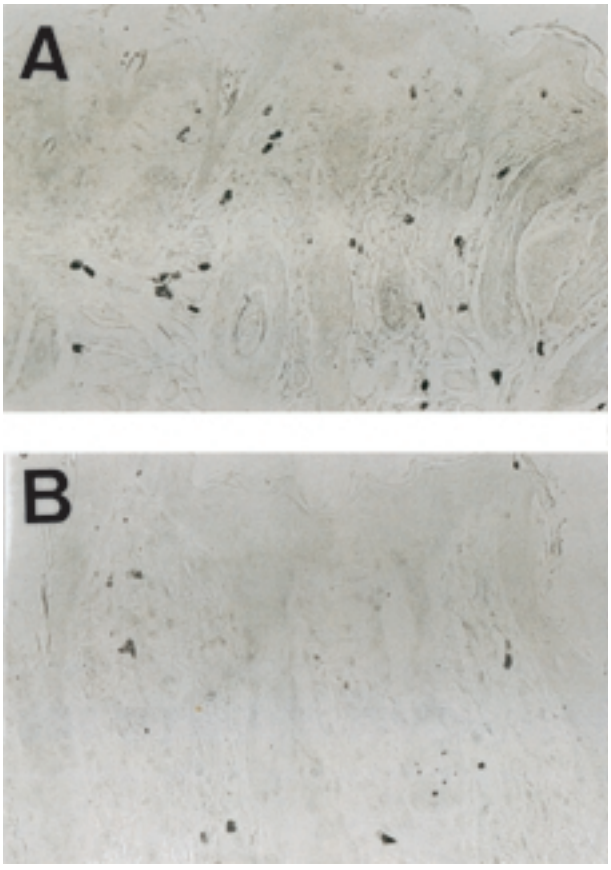

Fig. 9. Microscopic views of nasal tissue in TDI sensitized (A) and non-sensitized (B) mice. A marked increase in mast cells was present in sensitized group (A).

mice.

DISCUSSION

We studied whether different amino acid mixtures have different effects on nasal allergy induced by TDI and found that mice fed AA-EW had low body weight (Table 6), high nasal scores (Fig. 4) and high serum IgE concentrations (Fig. 5). The findings were similar to those of Exp. 1 in which various proteins were compared. These findings suggest that amino acid mixtures composed of EW, easily induce nasal allergy by TDI sensitization compared with amino acid mixtures, composed of other proteins. The present findings support the hypothesis that some amino acid mixtures may easily induce allergy and hypersensitivity. This finding must be important for the control of allergy and immune hypersensitivity.

Some mechanisms can be suggested to explain the above findings. One is the high intake of high quality proteins. Poor protein nutrition reduces immunity and then resistance against infection (25, 26), indicating that good protein nutrition enhances immunity. We found that nutrients which enhance immunity also easily induced hypersensitivity by the over intake as discussed in the introduction of this paper (1-10). Protein quality of EW is the highest of all food types or groups. For example, the biological values reported by Food and Agriculture Organization and World Health Organization are 94 for EW, 82 for casein, 65 for SPI and 48 for gluten (27). Therefore, EW may be prone to induce hypersensitivity because of its high protein quality. This hypothesis can easily explain why the incidence of allergy is very high in Japan and other developed countries. From the greatly increased consumption in protein rich foods during the last half a century in Japan, protein intake has increased greatly as shown in the report of the annual nutrition survey by the Ministry of Health and Welfare (28). More than half of the protein is supplied by animal foods, indicating that the quality and quantity of amino acids derived from the ingested proteins are very high in Japan.

Another possible factor of allergy aggravated by EW and AA-EW diets is the specific amino acids. Gln upregulates immunity (9, 10). We have found that Gln supplementation alleviates inflammatory bowel disease induced by TNBS in rats (17), although high supplementation aggravates it (12). Those studies (9-12) indicated that Gln plays an important role in immunity, however, the effect on allergy is not known. The concentrations of Gln were 33.7% in gluten, 10.9% in casein, 8.1% in SPI and 3.7% in EW (Table 2). EW has the lowest concentration of Gln. Therefore, in Exp. 3 we examined whether the low concentration of Gln was a factor in the severe allergy aggravated by EW. We decreased Gln in AA-SPI and increased Gln in AA-EW. Gln supplementation to AA-EW (HG-AA-EW) was effective in alleviating the nasal symptoms and to reducing the IgE level, but was not effective in preventing body weight loss. However, Gln reduction in the SPI groups showed no effects. The findings of Exp. 3 suggest that low Gln in EW is one of the factors of severe allergy in mice fed amino acid mixtures patterned after egg white protein. However, for a definitive clarification more studies are required. Further studies are required as the effects of other amino acids, for example, cystine which is very high in EW.

From the present study we can conclude that amino acid mixtures affect allergy. This suggests that for the control of allergy it is necessary to pay more attention to the pattern and the concentration of amino acids in diets.

ACKNOWLEDGEMENT

We thank FANCL Co. for their financial support to this research and Kyowa Hakko Kogyo Co. for their donation of amino acids.

REFERENCES

1. Adjei AA, Yamamoto S, Kulkarni A : Nucleic acids and/or their components : a possible role in immune function. J Nutr Sci Vitaminol 41 : 1-16, 1995
2. Yamauchi K, Adjei AA, Ameho CK, Chan YC, Kulkarni AD, Sato S, Okamoto K, Yamamoto S : A nucleoside-nucleotide mixture and its components increase lymphoproliferative and delayed hypersensitivity responses in mice. J Nutr 126: 1571-1577, 1996
3. Ameho CK, Adjei AA, Yamauchi K, Asato L, Kakinohana S, Kulkarni AD, Hiroi Y, Yamamoto S : Dietary nucleoside-nucleotide mixture aggravates nasal allergic responses induced by toluene diisocyanate in mice. Nutr Res 17 : 1597-1605, 1997
4. Adjei AA, Morioka T, Ameho CK, Yamauchi K, Kulkarni AD, Almansouri HM, Kawajiri A, Yamamoto S : Nucleoside-nucleotide free diet protects rat colonic mucosa from damage by trinitrobenzene sulphonic acid. Gut 39 : 428-33,

1996
5. Adjei AA, Ameho CK, Erick HK, Yamauchi K, Kulkarni A, Kawajiri A, Yamamoto S : Nucleoside-nucleotide-free diet suppresses cytokine production and contact sensitivity responses in rats with trinitrobenzene sulphonic acid-induced colitis. Am J Med Sci 314 : 89-96, 1997
6. Barbul A, Rettura G : Arginine: a thymotrophic and wound-healing Promoting agent. Surgical Forum 28 : 101-103, 1997
7. Kamen M, Senkal M, Hoffman HH : Influence of arginine, RNA and Omega-3-fatty acid supplemental enteral nutrition on postoperative humoral immunity in cancer patients undergoing major upper gastrointestinal surgery. Clin Nutr 11: S123, 1992
8. Neilly PJ, Kirk SJ, Gardiner KR, Anderson NH, Rowlands BJ : Manipulation of L-arginine-nitric oxide pathway in experimental colitis. Br J Surg 82 : 1188-1191, 1995
9. Ko TC, Beauchamp RD, Townsend CM, Thompson JC : Glutamine is essential for epidermal growth factor-stimulated intestinal cell proliferation. Surgery 114 : 147-54, 1993
10. Wilmore DW, Shabert JK : Role of glutamine in immunologic response. Nutrition14 : 618-626, 1998
11. Ameho CK, Adjei AA, Harrison EK, Takeshita K, Morioka T, Arakaki Y, Ito E, Suzuki I, Kulkarni AD, Kawajiri A, Yamamoto S Ameho CK, Adjei AA, Harrison EK, Takeshita K, Morioka T, Arakaki Y, Ito E, Suzuki I, Kulkarni AD, Kawajiri A, Yamamoto S : Prophylactic effect of dietary glutamine supplementation on interleukin 8 and tumor necrosis factor α production in trinitrobenzene sulphonic acid induced colitis. Gut 41: 487-493, 1997
12. Shinozaki M, Saito H, Muto T : Excess glutamine exacerbates trinitrobenzenesulfonic acid-induced colitis in rats. Dis Colon Rectum 40 : 59S-63S, 1997
13. Banks DE, Rando RJ, Barkman HW : Persistence of toluene diisocyanateinduced asthma despite negligible workplace exposures. Chest 97:121-125, 1990
14. Huang J, Wang XP, Ueda A, Aoyama K, Chen BM, Matushita T : Allergologic evaluation for workers exposed to toluene diisocyanate. Ind Health 29 : 85-92, 1991
15. Tanaka K, Okamoto Y, Nagaya Y, Nishimura F, Takeoka A, Hanada S : A nasal allergy model developed in the guinea pig by intranasal application of 2.4-toluene diisocyanate. Int Arch Allergy Appl Immunol 85 : 312-317, 1988
16. Almansouri HM, Yamamoto S, Kulkarni AD, Ariizumi M. Adjei AA. Yamauchi K : Effect of dietary nucleosides and nucleotides on murine allergic rhinitis. Am J Med Sci 312 : 202-205, 1996
17. Japan Science and Technology Agency : Japanese standard of food amino acid composition table. Japan Science and Technology Agency (in Japanese) Tokyo, 1990.
18. McReynolds L, O Malley BW, Nisbet AD, Fothergill JE, Givol D, Fields S, Robertson M, Brownlee GG : Sequence of chicken ovalbumin mRNA. Nature 273 : 723-728, 1978
19. Brantl V, Teschemacher H, Henschen A, Lottspeich F : Novel opioid peptides derived from casein (beta-casomorphins). I. Isolation from bovine casein peptone. Hoppe Seylers Z Physiol Chem 360 : 1211-1216, 1979
20. Nisbet AD, Saundry RH, Moir AJ, Fothergill LA, Fothergill JE : The complete amino-acid sequence of hen ovalbumin. Eur J Biochem115: 335-345, 1988
21. Hirano H, Fukazawa C, Harada K. The complete amino acid sequence of the A 3 subunit of the glycinin seed storage protein of the soybean (Glycine max(L.) Merrill). J Biol Chem 259: 1431-1437, 1984
22. Momma T, Negoro T, Hirano H, Matsumoto A, Udaka K, Fukazawa C : Glycinin $A_5A_4B_3$ mRNA : cDNA cloning and nucleotide sequencing of a splitting storage protein subunit of soybean. Eur J Biochem 149 : 491-496, 1985
23. Wrigley CW, Bietz JA : Proteins and amino acids. In : Pomeranz Y, ed, Wheat : chemistry and technology. The American Association of Cereal Chemists Inc, Washington, USA, 1988. pp. 159-276
24. Irifune M, Ogino S, Harada T, Mtsunaga T : Acute and chronic effects of unilateral cervical sympathectomy on nasal allergy-like symptoms by toluene diisocyanate in guinea-pigs. Acta Otolaryngol (Suppl 501) : 97-99, 1993
25. Chandra RK, Newberne PM : Nutritional, metabolic, and immunologic effects of infection. In : Chandra RK, Newberne PM, eds, Nutrition, immunity and infection mechanisms of interactions, Plenum Press, New York, 1979, pp.47-66
26. Baqui AH, Black RE, Bradley SR, Chowdhury HR, Yunus M, Siddique AK : Malnutrition, cell-mediated immune deficiency, and diarrhea: a community-based longitudinal study in rural

Bangladeshi children. Am J Epid 137:355-365, 1993
27. World Health Organization Technical Report Series No.522. Energy and protein requirements. In : FAO and WHO ed, FAO Nutrition Meetings Report Series. No 52. FAO and WHO Press, USA, 1973
28. Ministry of Health and Welfare National nutrition survey (in Japanese), Daichi Publishing Co., Tokyo, 1998