A HUMORAL LIPID PREVENTING FROM MOUSE ANAPHYLACTIC DEATH

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Abstract : Anaphylaxis gives mice severe stress killing them. They could produce adaptogenic substance for coping the stress. In the present study, the adaptogenic substance preventing from mouse anaphylactic death was examined. Six-week-old male ddY mice were initially immunized with 1 mg/kg of ovalbumin and second immunized with the same dosage 10 days after the initial immunization. The mice further immunized with the same dosage at 5 days or 15 days after the second immunization. All of the former were killed by the anaphylactic response, but none of the latter were. Although reactivity of fucosylated antibodies inducing anaphylactic response was not different in the former sera and the latter sera, a lipid preventing from anaphylactic death was found in fraction eluted with 300 mM NaCl of the latter sera. The present findings suggest that the lipid had an adaptogenic activity against severe stress induced by anaphylactic response, and was produced in late phase of the second immune response.

Keywords : anaphylaxis, immune response, adaptogenic activity, humoral lipids.

Introduction

Some of botanical anti-stress substances, which are neither protein nor peptide, have stress-coping activity on animals. They are named adaptogens[1,2]. Animals could also produce original adaptogenic substances in stressful situation. In the fact, the authors found an adaptogenic lipid in sera of mice given forced-swimming stress[3]. As anaphylaxis also induces severe stress killing animals, they could produce a humoral lipid coping the stress. In the present study, the authors examine mouse humoral lipid preventing from the anaphylactic death.

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Materials and Methods

1. Animal

Male 6 week-old ddY mice purchased from Japan SLC Co. Tokyo were used. All experiments were conditioned in accordance with animal research regulations at Akita University School of Medicine.

2. Immunizing condition

Mice were intraperitoneally injected with 1 mg/kg of ovalbumin (OVA, Grade V, Sigma-Aldrich Co, MO, USA) dissolved in physiological saline (PS). Ten days later, the second immunization was performed with 1 mg/kg of OVA. The mice was divided into two groups. Group A mice were immunized with 1 mg/kg of OVA 5 days after the second immunization. Group B mice were immunized with 1 mg/kg of OVA 15 days after the second immunization. As a control, group C mice were intraperitonally injected with PS 10 days after the initial immunization of OVA 1 mg/kg and were immunized with 1 mg/kg

of OVA 5 days after the PS injection.

3. Detection of death rate

Five mice of these groups were individually detected the anaphylactic death within one h after the final immunization.

4. Reactivity of fucosylated anti-OVA antibodies in sera

Group A sera, group B sera or group C sera was collected and pooled from group A mice, group B mice or group C mice just before the final immunization. Forty μl of each sera was diluted with PS to 4%. One hundred μl of the diluted sera was poured into a well of a 96 holes plastic plate (Sumitomo Bakelite Co. Osaka) previously coated with 10 mg/ml of OVA, and left for two hours at room temperature (RT). The well was washed with PS 5 times and was blocked with 5% bovine serum albumin (BSA, Sigma-Aldrich Co. MO. USA) for 30 min at RT. The well was washed with PS 5 times and was poured biotinized lectins DSA recognizing Gal$\beta_{1\text{-}4}$GlcNAc in which all antibodies have, or AAL recognizing Fuc$\alpha_{1\text{-}3}$GlcNAc (Seikagaku Co. Tokyo). Then, the well was left for one h at RT. The well washed with PS 5 times and was poured horseradish peroxidase-conjugated avidin (Seikagaku Co.) and was left for 5 min at RT. The degree of color change obtained by a peroxidase-coloring kit (Sumitomo Bakelite Co.) was measured at dual wave length 455/600 nm.

5. Humoral lipid fraction

The fractioning was initially performed using methanol-chloroform method previously described (4). Briefly, 10 ml of group A sera, group B sera or group C sera was added 12.5 ml of chloroform and 25 ml of methanol. The solution was agitatedly shaken for 2 min, and left for 10 min at RT. Then, 12.5 ml of chloroform was added to the solution, and was agitatedly shaken for 30 s. Furthermore, 12.5 ml of water was added to the solution, and agitatedly shaken for 30 s. The solution was centrifuged with 150$\times$g for 5 min at RT. The lower chloroform layer was collected and evaporated. Then, the extracted lipids were dissolved in water. The solution was applied on an ion-exchanger DE-52 (Whatman Co., Maidstone, UK) which had been saturated with a buffer of 10 mM $NaHCO_3$ (pH 8.3), and eluted with 50, 100, 150, 200, 250 and 300 mM NaCl. These fractions were prepared to 10 ml with water.

6. Fraction preventing from anaphylactic death

Other mice were treated with 1 mg/kg of OVA, and also treated with 1 mg/kg of OVA 10 days after the initial immunization. Five days after the second immunization, five of these mice were intrateritoneally injected with 400 μl of fraction eluted with 50, 100, 150, 200, 250 or 300 mM NaCl of group A, group B or group C sera, and with 1 mg/kg of OVA. One h after the injection, anaphylactic death of these mice was detected.

Other mice were treated with 1 mg/kg of OVA, and also treated with 1 mg/kg of OVA 10 days after the initial immunization. Five days after the second immunization, the five mice were intraperitoneally injected 500, 300 or 100 μl of fraction eluted with 300 mM NaCl of group B sera, group C sera or 500 μl of PS as a control, and with 1 mg/kg of OVA. One h after the injection, anaphylactic death of these mice was detected.

7. Glycosylation of the effective humoral lipid

Glycosylation of the fraction eluted with 300 mM NaCl was detected using 50% ethanol lectin ELISA (3). Briefly, 50 μl of the fraction separated from group A, group B or group C sera, and 50 μl of ethanol was poured into a well of a 96 holes plastic plate (Sumitomo Bakelite Co.) and left for two h at RT. Each well was blocked with 5% BSA for 30 min at RT. After 5 times-washing with PS, the well was reacted with biotinized lectins : AAL recognizing Fuc$\alpha_{1\text{-}3}$GlcNAc, DBA recognizing GalNAc$\alpha_{1\text{-}3}$GalNAc, MAM recognizing Sial$\alpha_{2\text{-}3}$Gal, PNA recognizing Gal$\beta_{1\text{-}3}$GalNAc and SBA recognizing GalNAc $\alpha_{1\text{-}3}$Gal (Seikagaku Co.) for 45 min at RT. After 5 times-washing with PS, horseradish peroxidase-conjugated avidin (Seikagaku Co.) was poured into each well and was left for 5 min at RT. The degree of color change obtained by a peroxidase-coloring kit (Sumitomo Bakelite Co.) was measured at dual wave length 455/600 nm.

8. Fusocylation of lipid in the fraction

Fucosylation of lipid in the fraction eluted with 50, 100,

150, 200, 250 or 300 mM NaCl of group A, group B or group C sera, which was previously obtained under the method of "5. Humoral lipid fraction", was detected using 50% ethanol lectin ELISA (3). Briefly, 50 μl of the fraction and then 50 μl of ethanol was poured into a well of a 96 holes plastic plate (Sumitomo Bakelite Co.) and left for two h at RT. Each well was blocked with 5% BSA for 30 min at RT. After 5 times-washing with PS, the well was reacted with biotinized lectin AAL (Seikagaku Co.) for 45 min at RT. After 5 times-washing PS, the horseradish peroxidase-conjugated avidin (Seikagaku Co.) was poured to each well and was left for 5 min at RT. The degree of color change obtained by a peroxidase-coloring kit (Sumitomo Bakelite Co.) was measured at dual wave length 455/600 nm.

Results

1. OVA immunization schedule

OVA immunization was performed as shown in Table 1.

2. Anaphylactic death

All mice of group A were dead within one h after the final OVA immunization. On the other hand, none of group B and group C mice were dead after the final OVA immunization (Table 2).

3. Fucosylated anti-OVA antibodies

Although reactivity of glycosylated anti-OVA antibodies in the group A sera was slightly higher than that in group B or group C sera, reactivity of fucosylated anti-OVA antibodies was not different in group A, group B and group C sera (Table 3).

4. Humoral lipid fraction preventing from anaphylactic death

None of fractions of group A sera prevented from anaphylactic death of the treated mice. On the other hand, fraction eluted with 300 mM NaCl of group B or group C sera prevented from the anaphylactic death (Table 4). Dose-dependent effect of the fraction eluted with 300 mM NaCl of group B or group C sera was shown in Table 5.

Table 1. Ovalbumin-immunizing condition

OVA immunization (1 mg/kg)		
Group A	Group B	Group C
1st immunization	1st immunization	1st immunization
↓ 10 days	↓ 10 days	↓ 10 days
2 nd immunization	2 nd immunization	2 nd immunization (PS)
↓ 5 days	↓ 15 days	↓ 5 days
3 rd immunization	3 rd immunization	3 rd immunization

PS : physiological saline

Table 2. The anaphylactic death-rate within 1 hour after the 3rd immunization

Death rate (Dead / Total)		
Group A	Group B	Group C
5 / 5	0 /5	0 / 5
(100 %)	(0 %)	(0 %)

Table 3. Reactivity of fucosylated anti-OVA antibodies in the sera

	Light absorbance			
Lectin	Group A	Group B	Group C	Blank
DSA	0.143	0.115	0.110	0.010
AAL	0.084	0.086	0.086	0.018

DSA recognizes sugar chain structure Gal $\beta_{1\text{-}4}$GlcNAc, which all antibodies have. AAL does Fuc$\alpha_{1\text{-}3}$GlcNAc.

Table 4. Humoral lipid fraction preventing from anaphylactic death

	Death rate (%)					
	Fraction eluted with NaCl (mM)					
	50	100	150	200	250	300
A	100	100	100	100	100	100
B	100	100	100	100	100	0
C	100	100	100	100	100	20

Table 5. Dose-dependent effect of the fraction eluted with 300 mM NaCl obtained from group B or group C sera

Group	Death rate (%)			
	Dose (μl eq. sera) of the fraction			
	500	300	100	500 μl of PS
B	0	60	100	100
C	0	80	100	

PS : physiological saline

Table 6. Lipid-glycosylation in the fraction eluted with 300 mM NaCl obtained from the sera

	Light absorbance			
Lectin	Group A	Group B	Group C	Blank
AAL	0.172	0.348	0.257	0.077
DBA	0.122	0.117	0.108	0.022
MAM	0.185	0.125	0.110	0.048
PNA	0.136	0.079	0.088	0.023
SBA	0.153	0.086	0.066	0.022

AAL recognizes Fuc$\alpha_{1\text{-}3}$GlcNAc. DBA does GalNAc$\alpha_{1\text{-}3}$GalNAc.
MAM does Sial$\alpha_{2\text{-}3}$Gal. PNA does Gal $\beta_{1\text{-}3}$GalNAc.
SBA does GalNAc$\alpha_{1\text{-}3}$Gal.

Humoral lipid in the fraction eluted with 300 mM NaCl of group B and group C sera was more fucosylated than that of group A sera (Table 6).

5. Fucosylation of lipid in the fractions

Lipid-fucosylation in fraction eluted with 50 mM or 100 mM NaCl of group A sera was not different from that of group B or group C sera. But, lipid-fucosylation in the fraction eluted with 150 mM, 200 mM or 250 mM NaCl of group A sera was higher than that of group B or group C sera (Table 7).

Table 7. Lipid-fucosylation in fraction eluted with NaCl obtained from the sera

Group	Light absorbance						
	Fraction eluted with NaCl (mM)						
	50	100	150	200	250	300	Blank
A	0.038	0.069	0.210	0.160	0.233	0.122	
B	0.043	0.053	0.051	0.049	0.175	0.298	0.023
C	0.054	0.066	0.072	0.068	0.178	0.207	

Discussion

It was previously reported that fucosylated antibody IgG increases after repeated immunization and the fucosylated IgG induces mouse anaphylactic response[5,6]. But the present findings suggest that mouse anaphylactic death was related not with the fucosylated antibodies, but with a humoral lipid produced in late phase of the second immunization. The lipid that might be fucosylated was suggested to be also produced in the first immune response. As animals produce adaptogenic substance for coping stress, the humoral lipid found in the present study is suggested to be an internal adaptogenic substance coping immunization stress.

Anaphylactic response is induced with enlargement of blood capillary caused by antibody IgE and chemical mediators. The humoral lipid might decrease activities of the chemical mediators. But, regretfully, mechanism of the humoral lipid on anaphylactic response is not clear in the present study. Immunization gives mice another stress that is invaded by antigen. Lipid-fucosylation found in the other fractions of Group A sera might be related with antigen exclusion in early phase of second immunization. In the second immune response, this antigen exclusion might be prior to production of the adaptogenic lipid, nevertheless, mechanism of the lipid-fucosylation is also unclear.

The authors are interested in humoral lipids having physical activities[3,4]. Although mechanism of the adaptogenic lipid on stress induced by anaphylactic response has not been clarified, the lipid may have a clinical potential on Type I allergy diseases.

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