

Analysis of sensitization to carboxymethylcellulose: Identification of high risk group using ELISA and histamine release experiment

S. Mori¹, N. Muroi², S. Ono², H. K. Takahashi¹ and M. Nishibori¹

¹ Department of Pharmacology, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan, Fax: ++81 86 235 7140, e-mail: mbori@md.okayama-u.ac.jp

² Ako Municipal Hospital, Ako, 678-0232, Japan

Received 18 August 2003; returned for revision 29 September 2003; accepted by M. J. Parnham 10 December 2003

Abstract. *Objective and Design:* Carboxymethylcellulose (CMC) has been considered to be inert and is commonly used as an additive in medicines, foods and cosmetics. However, we experienced a patient who developed an anaphylactic reaction to CMC after an upper gastrointestinal examination using a barium meal containing CMC. Therefore, we examined the incidence of sensitization by CMC in healthy subjects, and categorized the high risk group prone to developing anaphylactic response to CMC.

Methods: An ELISA for detecting CMC-specific IgE antibody was developed using serum from the patient as a positive control. In the ten subjects exhibiting high anti-CMC IgE among 387 normal populations, histamine release from isolated leukocytes was performed.

Results: Five of ten subjects with a high IgE titer showed a significant CMC-induced histamine release from leukocyte preparations in vitro as observed in the patient, and were classified as high risk group. There was a correlation between sensitization by CMC and that by Japanese cedar pollen. The incidence of sensitization in females was 2.4 fold higher than that in males.

Conclusions: The combination of ELISA and histamine release experiment made it possible to identify the high risk group for developing anaphylactic response. The administration of high dose CMC as a suspending agent in barium sulfate or injectable corticosteroids to this group should be avoided to prevent anaphylactic reactions in the clinic.

Key words: Anaphylaxis – Carboxymethylcellulose – ELISA – High risk – Histamine release

Introduction

Gastrointestinal radiography with barium sulfate suspension as the contrast medium has been a major and widely used method for diagnosing gastrointestinal diseases in clinic. Ingestion of barium sulfate suspension is a routine practice, in which various ingredients were included. Carboxymethylcellulose (CMC) is one of such additives, providing the improvement in the coating and flow of the suspension. In a previous study [1], we reported the case of a patient who suffered an anaphylactic reaction after an upper gastrointestinal examination using a barium meal containing CMC. Skin-scratch test and in vitro histamine release experiment using a peripheral leukocyte preparation revealed that CMC was an antigen for anaphylaxis among nine additives in the suspension. The identification of CMC as allergen was particularly surprising, because CMC is considered not to be absorbed into body fluid and inert. So far, its superior features have led to the various use as suspending, thickening and emulsifying agent in pharmaceutical, food and cosmetic industry. Although the incidence of anaphylactoid reactions in barium examinations was estimated to be low [2, 3], the case reports of anaphylactic reaction after the intraarticular injection of corticosteroids containing CMC are increasing [4–8]. An anaphylactic reaction is the most severe side effect of drug treatment and anaphylactic shock is often fatal, therefore, it is important to know of the presence and the titer of antigen-specific IgE. As described in our preliminary report [9], we established an ELISA that uses the covalently immobilized CMC for the detection of IgE antibody specific for CMC and found out the presence of high anti-CMC IgE in the serum of the above mentioned patient. By using the established ELISA, we screened 387 normal healthy subjects and classified those based upon the anti-CMC IgE titer in serum. Surprisingly, ELISA elucidated a very high incidence of CMC sensitization among the normal population [9]. In this paper, we analyzed the sensitization to CMC in more detail, and found that five of ten

subjects with high IgE titer showed a significant CMC-induced histamine release from leukocyte preparations *in vitro* as observed in the patient, indicating the usefulness of combination of both methods to identify the high risk group for anaphylactic reaction. There was a correlation between sensitization by CMC and that by Japanese cedar pollen, a common allergen in Japan, the incidence of the former being about half those of sensitization by representative allergens. In final, it was concluded that the usage of medicine containing a large amount of CMC might induce an anaphylactic reaction in the high risk group, especially in the case of direct injection of drugs containing CMC into body fluid. The combination of ELISA and histamine release experiment allowed us to identify this group among healthy population. Much attention should be paid to sensitization by CMC and the medicinal use of CMC.

Materials and methods

Subjects

This study was approved by Review Board of Okayama University Graduate School of Medicine and Dentistry. We obtained written informed consent from the patient who experienced the anaphylactic reaction to CMC [1], with regard to the utilization of her serum as a positive control in developing an ELISA method for the detection of CMC-specific IgE. We also obtained written informed consent for the examination of CMC-specific IgE in serum from subjects who attended Ako Municipal Hospital to receive an annual health check. We screened a total of 387 subjects from February to June 2000.

CMC

The grade of CMC is described in The Japanese Pharmacopoeia (13th edition), which is recognized and guaranteed by the Ministry of Health, Labour and Welfare, Japan. The purity of CMC is 99% and the major impurities identified thus far are inorganic salts such as chlorides (less than 0.64%), sulfate (less than 0.96%) and silicate (less than 0.5%). Usually, NaCl is the major impurity. The minor impurities include heavy metals (less than 20 ppm) and arsenic (less than 10 ppm).

ELISA

CMC (0.125–200 µg/mL) was covalently fixed to the ELISA plate (Costar, EIA/RIA plate) using the coupling reagents, N-hydroxy sulfo succinimide (sulfo-NHS) (Pierce, Rockford, IL) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (Dojin, Kumamoto, Japan) at room temperature for 20 h with continuous shaking. After extensive washing with distilled water, the plate was blocked with 1% BSA in 10 mmol/L phosphate buffered saline, pH 7.4 (PBS), overnight at 4°C. The serum was diluted with PBS and 100 µL of each dilution was applied overnight at 4°C. After the plate had been washed with PBS containing 0.1% Triton X-100, biotinylated secondary antibody (anti-human IgE or anti-human IgG, Vector, Burlingame, CA) (1:200) and streptavidin peroxidase (1:300) (Vector) were applied with washing in-between. An O-phenylenediamine dihydrochloride (1 mg/mL) in 0.05 M sodium phosphate-citrate buffer (pH 5.0) was used as substrate at a volume of 100 µL and the reaction was stopped by the addition of 50 µL of 3M sulfuric acid 30 min thereafter. The plate was read at 450 nm in an ELISA reader (Tosoh, Tokyo, Japan). The intensity of the reaction was evaluated and classified into six categories; negative (ELISA OD at 1:2 dilution of serum < 1.5 × [ELISA OD at 1:16 dilu-

tion of serum]); suspected positive (ELISA OD at 1:8 dilution of serum < the mean of ELISA OD of negatives at 1:8 dilution plus 3 × SD (= 0.150)); and positive (ELISA OD at 1:8 dilution of serum ≥ the mean of ELISA OD of negatives at 1:8 dilution plus 3 × SD), positive being further classified into four groups depending on the intensity of ELISA at a 1:8 dilution of serum; positive (1+), 0.150 ≤ OD < 0.200; positive (2+), 0.200 ≤ OD < 0.250; positive (3+), 0.250 ≤ OD < 0.300; positive (4+), 0.30 ≤ OD. The classification was shown in Table 1. This was an arbitrary classification based on the ELISA value at 1:8 serum dilution, which was within the linear range of ELISA curve.

Determination of total IgE and allergen-specific IgE in serum

Total IgE in serum was determined by radio-immunosorbent assay. The allergen-specific IgE was examined by radio-allergosorbent test (RAST) on the following five common allergens in Japan: house dust I, pollen of Japanese cedar, Dermatophagoides pteronyssinus, orchard grass and ragweed. The determinations of total IgE and the five allergen-specific IgE were done in subjects who were judged to be sensitized with CMC by ELISA and fifty randomly-extracted negative controls.

Histamine release from isolated leukocytes

Peripheral blood was withdrawn from a forearm vein. The blood was layered on 4.5% Ficoll-12% meglamine iotalamate (specific gravity, 1.077) and centrifuged at 350 × g for 35 min to isolate the leukocytes. Isolated leukocytes were washed twice with Hank's balanced salt solution (NaCl, 137 mmol/L; KCl, 5.4 mmol/L; CaCl₂, 1.3 mmol/L; Na₂HPO₄, 0.3 mmol/L; KH₂PO₄, 0.4 mmol/L; MgSO₄, 0.8 mmol/L; NaHCO₃, 4.2 mmol/L; MgCl₂, 0.5 mmol/L; glucose, 5.6 mmol/L containing 0.05% BSA), resuspended in the same buffer, and then aliquoted into eppendorf tubes in a volume of 180 µL. After 5 min preincubation at 37°C, diluted sodium CMC in suspending buffer was added to the leukocyte suspension (4 × 10⁶ leukocytes) to give a final volume of 200 µL. The reaction was continued for 15 min thereafter. The reaction was stopped by cooling of the incubation mixture on ice and each tube was centrifuged at 1800 × g for 5 min at 4°C. Both the supernatant and the precipitate were assayed for histamine content according to a method described previously [10] and the results were presented as % of histamine release. The net histamine release was calculated by the subtraction of spontaneous release in the absence of CMC from total histamine release in the presence of CMC. The average spontaneous release was about 2.0%.

Statistical analysis

Statistical analysis was performed with the chi-square test for independence. The statistical analysis of histamine release was done using ANOVA followed by Dunnett's test.

Table 1. Classification of CMC-specific IgE titer in serum.

Negative:	OD at 1:2 dilution of serum < 1.5 × [OD at 1:16 dilution of serum]
Suspected positive:	OD at 1:8 dilution of serum < 0.150 ^a
Positive (1+):	0.150 ≤ OD at 1:8 dilution of serum < 0.200
Positive (2+):	0.200 ≤ OD at 1:8 dilution of serum < 0.250
Positive (3+):	0.250 ≤ OD at 1:8 dilution of serum < 0.300
Positive (4+):	0.300 ≤ OD at 1:8 dilution of serum

^a The mean OD at 1:8 dilution of Negatives plus 3 × SD was 0.150.

Results

We developed an ELISA method for the detection of CMC-specific IgE using a plate to which CMC was covalently coupled with both sulfo-NHS and EDC as coupling reagents but were unsuccessful in the non-covalent binding of CMC to the plate. As shown in Figure 1, the reaction detected by anti-human IgE was dependent on the concentration of antigen and dilution of serum from the patient but not from normal volunteers who showed no histamine release induced by CMC from peripheral leukocytes in the previous study [1]. Anti-human IgG instead of anti-human IgE did not produce any reaction. The addition of different concentrations of antigen-non-relevant human IgE (0–4 $\mu\text{g}/\text{well}$) to the incubation with biotinylated secondary anti-IgE antibody concentration-dependently inhibited the reaction (Fig. 1C), whereas the addition of the same concentration of antigen-non-relevant human IgG or IgM did not produce any effects (data not shown). This showed that anti-human IgE antibody bound to only IgE but did not crossreact with IgG or IgM. The addition of antigen-non-relevant human IgE instead of CMC-reactive patient's serum did not produce any non-specific reaction up to 10 $\mu\text{g}/\text{mL}$, which was a much higher concentration than the IgE level (2.5 $\mu\text{g}/\text{mL}$) found in serum of the patient. These results as a whole indicated that the ELISA method developed in the present study was useful for the detection of CMC-specific IgE antibody and that the serum from the patient who experienced the anaphylactic reaction to CMC contained high CMC-specific antibody of IgE class.

Using this ELISA method, we examined the CMC-specific IgE of 387 subjects who attended the Ako Municipal Hospital to receive an annual health check. The results of screening are summarized in Figure 2. Among the 387 subjects, we found 36 positive individuals (9%), six of whom had a high CMC-specific IgE (4+) comparable to that of the patient. Then, in vitro histamine release from isolated leukocytes was performed in ten positive subjects (3+–4+) including the six strongly positive individuals. CMC at the concentration ranging from 10^{-5} to $10^{-2.5}\%$ significantly induced the release of histamine from leukocytes in five subjects out of ten (Fig. 3A), suggesting the presence of high risk group for anaphylactic reaction. The CMC concentration required for inducing maximal histamine release differed depending on the individual. Figure 3B represents the plots of CMC-specific IgE titer in an individual determined by ELISA vs. the maximal CMC-induced histamine release in vitro from isolated leukocytes from the ten subjects with high CMC-specific IgE (the closed and open circles represent the subjects showing the significant and non-significant histamine release, respectively). An asterisk indicates the patient who developed the anaphylactic response to CMC. There was no correlation between CMC-specific IgE titer and the extent of maximal histamine release among the ten subjects. The three CMC-specific IgE-negative subjects did not experience a CMC-induced release of histamine at any concentration (data not shown) as observed in the previous study [1]. Therefore, it was suggested that the extent of in vitro histamine release induced by CMC was not solely

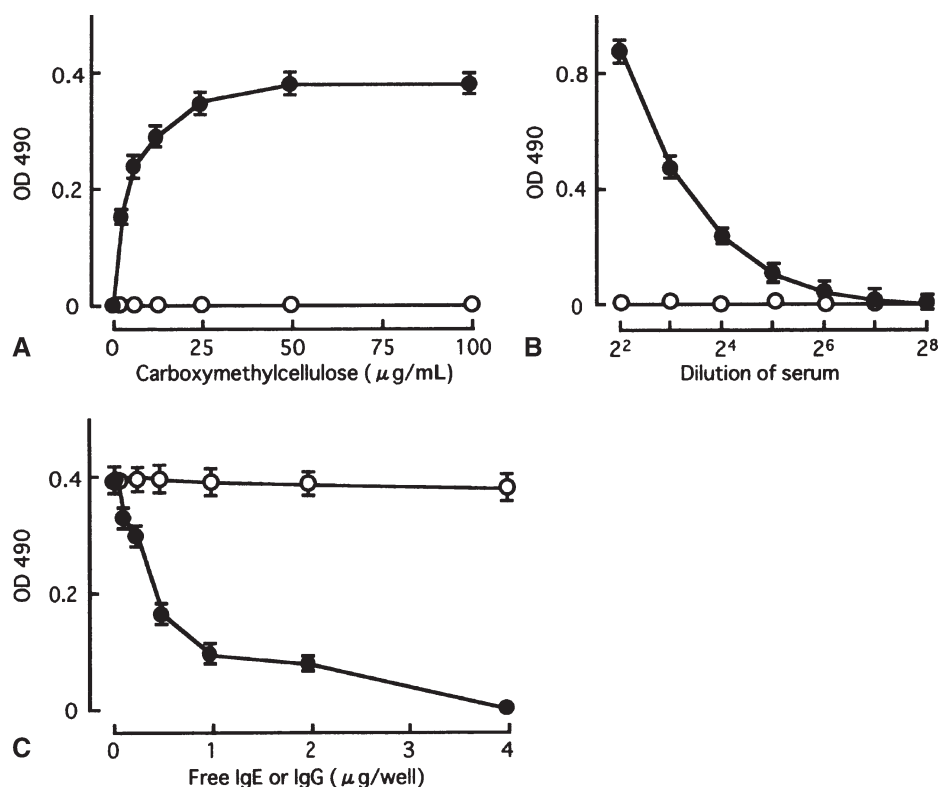


Fig. 1. ELISA for the detection of the specific IgE antibody against CMC. A, Dependency of the reaction on the concentration of antigen using sera from the patient (●) and normal volunteers (○). The dilution of serum was 1:8. B, Dependence of the reaction on the dilution of serum from the patient (●) and normal volunteers (○). C, Inhibition of the reaction in the presence of non-relevant human IgE (●) or IgG (○) with secondary biotinylated anti-IgE antibody. The dilution of the serum from patient was 1:8.

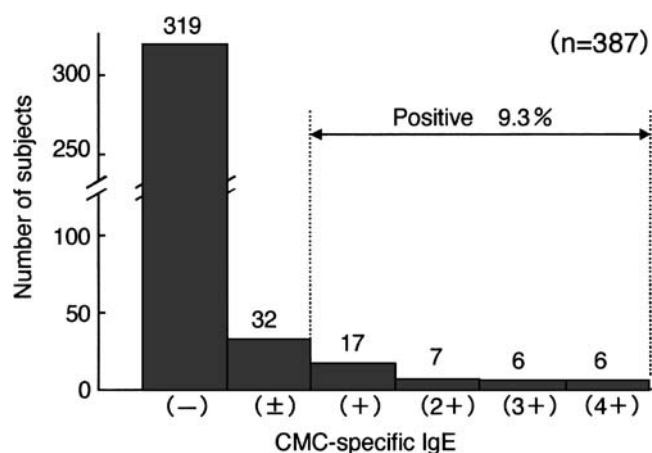


Fig. 2. Distribution of CMC-specific IgE in 387 subjects. ELISA was performed as described in Fig. 1 and the methods. The CMC-specific IgE titer in each patient was classified into six categories according to the definition described in the methods. There were 36 positive subjects among 387 subjects examined.

dependent on the CMC-specific IgE titer detected by ELISA.

Interestingly, we found a 2.4-fold difference in the incidence of sensitization to CMC between female and male subjects (Table 2). Moreover, there was a significant difference in the allergic history between CMC-specific IgE-negative and -positive groups. In thirty six CMC-specific IgE-positive and fifty negative subjects, we also determined the specific IgE antibodies against each of five common allergens in Japan; house dust I, pollen of Japanese cedar, Dermatophagoides pteronyssinus, orchard grass and ragweed. The analysis is summarized in Table 3. There was a significant correlation between sensitization with CMC and that

Table 2. Age, sex, and allergic history of CMC-specific IgE – negative and -positive groups.

	CMC-specific IgE		P value
	Negative (n = 351)	Positive (n = 36)	
Age	48.7 $\pm$ 2.0	51.0 $\pm$ 2.0	NS
Sex (Male/Female)	254/97	18/18	0.02
Allergic history ^a	36 (10.1%)	10 (31.3%)	0.05

^a Allergic history was evaluated by a questionnaire on allergic rhinitis, allergic conjunctivitis, atopic dermatitis and food allergies.

Table 3. Comparison of the sensitization with five common allergens between CMC-specific IgE – negative and -positive groups.

	CMC-specific IgE		P value
	Negative (n = 50)	Positive (n = 36)	
Allergens			
Orchard grass	8 (16%)	10 (28%)	NS
Ragweed	4 (8%)	6 (17%)	NS
Japanese cedar	11 (22%)	17 (47%)	0.02
Dermatophagoides pteronyssinus	11 (22%)	6 (17%)	NS
House dust I	11 (22%)	7 (19%)	NS
Serum total IgE (IU/mL)	185 $\pm$ 358	231 $\pm$ 385	NS

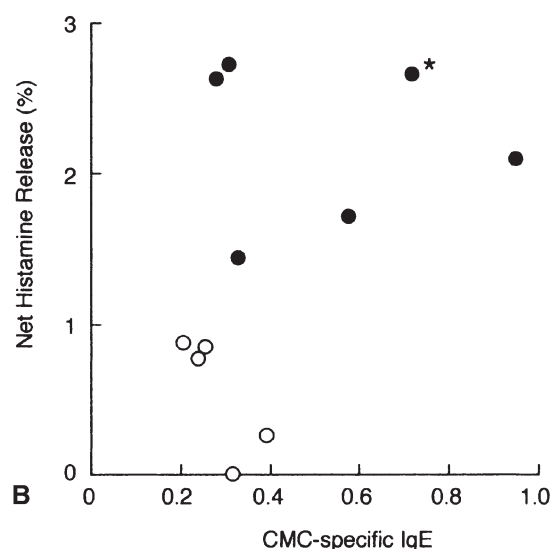
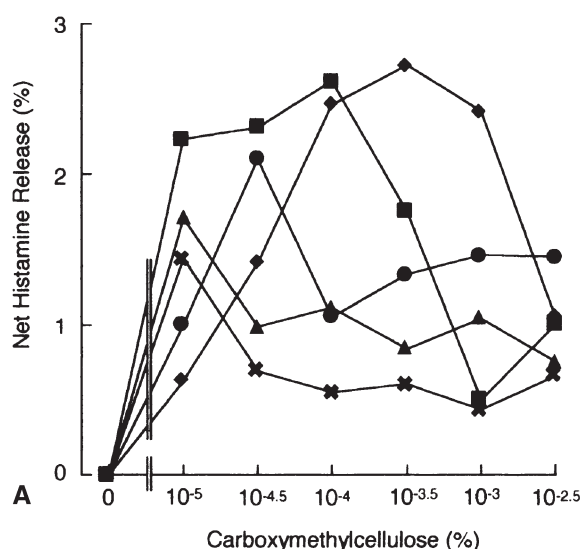


Fig. 3. Histamine release induced by CMC from isolated leukocytes of individuals with high CMC-specific IgE. The results were obtained from five subjects who showed a significant histamine release induced by carboxymethylcellulose (Panel A). Each value represents the mean of three replicates. The standard errors were within 10% of the means. The correlation between CMC-induced histamine release and CMC-specific IgE detected by ELISA was evaluated (Panel B). Closed and open circles represent the subjects who showed a significant and non-significant histamine release induced by CMC, respectively. An asterisk represents the patient who developed an anaphylactic response to the CMC in a barium sulfate suspension.

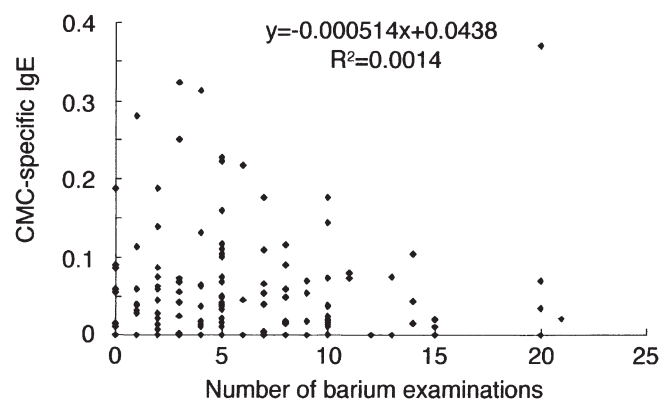


Fig. 4. Lack of correlation between the CMC-specific IgE and frequency of examinations using barium sulfate suspensions containing CMC. One hundred and eighty seven subjects, who received gastrointestinal examinations only in Ako Municipal Hospital using a barium sulfate suspension containing CMC, were selected from 387 subjects.

with Japanese cedar. Total IgE levels in the CMC-sensitized group were not higher than those in the non-sensitized control. On the other hand, the frequencies of examinations using barium sulfate suspensions containing CMC did not correlate with either the titer of CMC-specific IgE or the incidence of sensitization with CMC (Fig. 4).

Discussion

In a previous study, we reported a patient who showed an anaphylactic reaction to the CMC in a barium sulfate suspension after an upper gastrointestinal examination [1]. The antigen was identified by skin scratch test and *in vitro* histamine release induced by possible antigen [1]. After obtaining informed consent, we examined the optimal condition of an ELISA for the determination of CMC-specific IgE by using serum from the patient as a positive anti-serum. Because non-covalent binding of CMC to the ELISA plate was not successful, the covalent fixation of CMC with sulfo-NHS and EDC through a hydroxyl group in CMC was performed. The ELISA utilized biotinylated anti-human IgE, streptavidin peroxidase and an enzyme reaction with *O*-phenylenediamine and hydrogen peroxide as substrates. The reaction was dependent on not only the concentration of antigen used for the fixation on the plate but also the dilution of the serum. When biotinylated anti-human IgG was used instead of anti-human IgE, the specific reaction did not develop. Non-relevant human IgE but not IgG or IgM inhibited the reaction by interfering with the recognition of biotinylated secondary antibody. Moreover, non-relevant human IgE at a concentration corresponding to the plasma IgE level of the patient did not produce any reaction at all. These results as a whole indicated that the ELISA specifically detected CMC-specific IgE in the serum. We already demonstrated that CMC in a certain concentration range induced the release of histamine from isolated leukocytes from this patient [1]. In our histamine release assay, we paid much attention not to stimulate leukocyte unnecessarily during the preparation of leukocytes and obtained low level of spontaneous release around 2% constantly in both patient

and normal subjects. In the case of the patient, the maximal net release of histamine was 2.7% [1], which was relatively low level as compared with those stimulated with other stimulants such as anti-IgE antibody. However, this patient developed severe anaphylactic symptoms after oral administration of barium suspension and skin reaction after topical application. Thus, this amount of histamine release observed *in vitro* study was thought to have the significant meaning under *in vivo* condition. The detection of CMC-specific IgE in the serum confirmed the presence and the involvement of specific IgE bound to the surface of basophils in the CMC-triggered release of histamine. We found that five of ten subjects with the high IgE titer showed a significant CMC-induced histamine release from leukocyte preparations *in vitro*. In particular, two subjects showed the highest histamine release comparable to that of the patient. These findings suggested the presence of high risk group for anaphylactic reaction. The combination of both ELISA and histamine release experiments may enable us to identify this group. On the other hand, the extent of *in vitro* histamine release from isolated peripheral leukocytes induced by CMC challenge was not necessarily correlated to the specific IgE level (Fig. 3B). Although the subjects classified as high risk group have not experienced an anaphylactoid reaction to a barium meal containing CMC, this may happen if a considerable amount of CMC enters into the circulation as was supposed to have occurred in the patient [11].

There was no correlation between sensitization by CMC and the frequency of barium examination, suggesting that the examination is not the main route of sensitization by CMC. Interestingly enough, there was a difference in the incidence of sensitization between males and females. Since there was no difference in the sensitization with other common allergens between males and females, this strongly suggested that most of the sensitization to CMC takes place through contact with cosmetics or domestic detergents containing CMC. Another source of CMC is the food additives in ice cream, cake, cheese, margarine and many kinds of processed frozen foods. People who have a western life style are expected to come into contact with CMC daily. Therefore, it is quite likely that the incidence of sensitization by CMC in randomly extracted populations in countries with western life styles may be as high as that found in the present study. A mass surveillance study on populations in different countries is needed to address this point.

There are increasing reports describing anaphylactic reaction after intraarticular injection of corticosteroids containing several mg of CMC, i.e., prednisolone acetate, triamcinolone acetonide and halopredone acetate [4–8]. In this case, CMC is administered directly into body fluid. Once a patient with arthritis is sensitized to CMC through cosmetics, detergents, foods or the injection itself, later injection of the same steroid containing CMC may produce a severe anaphylactic response. Judging from the high incidence (9%) of sensitization by CMC and the presence of a high titer group (1.6%) [9], it is speculated that among arthritis patients receiving injectable steroids containing CMC there is a considerable number of individuals at high risk for developing an anaphylactic response. Other injections containing CMC available in Japan include estradiol and oxendolone.

The present study suggested a correlation between sensitization by CMC and Japanese cedar pollen. Moreover, CMC-specific IgE-positive subjects experienced allergic symptoms at higher rate than CMC-specific IgE-negative subjects (Table 2). Therefore, it would be reasonable at this moment not to prescribe CMC-containing injections to atopic patients to prevent anaphylactic reactions in the clinic. On the other hand, it must be pointed out that sensitization by CMC does not necessarily mean that all sensitized subjects will develop anaphylaxis when the antigen enters the body fluid, because the intensity of CMC-induced histamine release varied dependent on individuals among subjects with high CMC-specific IgE. It is noteworthy that the frequency of anaphylactoid reactions during barium examinations has been increasing [12]. We speculate that some of the reactions to barium sulfate suspensions reported previously were mediated by CMC-specific IgE antibody [2, 3, 12]. Anaphylactic reactions are often severe and sometimes fatal. Therefore, it is important to know whether the patients are sensitized to CMC or not, to avoid the risk of antigen being ingested through drug treatment or barium examination, especially in those at high risk. In this study, we developed an ELISA for the detection of CMC-specific antibody of IgE class. The combination of ELISA and histamine release make it possible to identify the high risk group for anaphylactic reaction, and the information obtained will be helpful for both patients and physicians.

Acknowledgments. This work was supported by grants from Suzuken Memorial Foundation, Kurozumi Medical Research Foundation, Mishimakaiun Memorial Foundation, and Protein 3000 Project, Japan.

References

- [1] Muroi N, Nishibori M, Fujii T, Yamagata M, Hosoi S, Nakaya N et al. Anaphylaxis from the carboxymethylcellulose component of barium sulfate suspension. *N Engl J Med* 1997; 337: 1275–7.
- [2] Gelfand DW, Sowers JC, Deponte KA, Sumner TE, Ott DJ. Anaphylactic and allergic reactions during double contrast studies. Is glucagon or barium suspension the allergen? *Am J Roentgenol* 1985; 144: 405–6.
- [3] Janower ML. Hypersensitivity reactions after barium studies of upper and lower gastrointestinal tract. *Radiology* 1986; 161: 139–40.
- [4] Montoro J, Valero A, Elices A, Rubira N, Serra-Baldrich E, Amat P et al. Anaphylactic shock after intra-articular injection of carboxymethylcellulose. *Allergol Immunopathol (Madr)* 2000; 28: 332–3.
- [5] Schuster C, Wuthrich B, Hartmann K, Kuhn M. Anaphylaxis to E466. *Allergy* 2000; 55: 303–4.
- [6] Mace S, Vadas P, Pruzanski W. Anaphylactic shock induced by intraarticular injection of methylprednisolone acetate. *J Rheumatol* 1997; 24: 1191–4.
- [7] Aitken MM. Induction of hypersensitivity to carboxymethylcellulose in cattle. *Res Vet Sci* 1975; 9: 110–3.
- [8] Patterson DL, Yunginger JW, Dunn WF, Jones RT, Hunt LW. Anaphylaxis induced by the carboxymethylcellulose component of injectable triamcinolone acetonide suspension. *Ann Allergy Asthma Immunol* 1995; 74: 163–6.
- [9] Muroi N, Mori S, Ono S, Takahashi HK, Fujii T, Hosoi S et al. Allergy to carboxymethylcellulose. *Allergy* 2002; 57: 1212–3.
- [10] Itoh Y, Oishi R, Adachi N, Saeki K. A high sensitive assay for histamine using ion-pair HPLC coupled with postcolumn fluorescent derivatization: Its application to biological specimens. *J Neurochem* 1992; 58: 884–9.
- [11] Nishibori M, Muroi N, Fujii T. Correspondence: Anaphylaxis from carboxymethylcellulose component of barium sulfate suspension. *N Engl J Med* 1998; 338: 623.
- [12] Feczko PJ. Increased frequency of reactions to contrast materials during gastrointestinal studies. *Radiology* 1990; 174: 367–8.



To access this journal online:
<http://www.birkhauser.ch>
