Studies on Pharmacological Activation of Human Serum Immunoglobulin G (IgG) by Chemical Modification and Active Subfragments. VI.
Anti-allergic Activity of Carboxamidemethylated Fc (CM-Fc) Fragment from Human Serum IgG

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Immunoglobulin G from human serum was digested with papain to give an Fc fragment, which was carboxamidemethylated [carboxamidemethylated Fc (CM-Fc), M.W. about 25000]. Types I, III and IV allergic reactions were significantly depressed by the administration of CM-Fc.

Keywords—carboxamide methylation; human IgG; carboxamidemethylated Fc; anti-allergic activity; passive cutaneous anaphylaxis; complement-dependent hemolysis; immuno-complex inflammatory model; Arthus reaction; delayed-type hypersensitivity

Coombs and Gell classified allergic phenomena into four types, of which three (types I, II and III) are immediate-type, and one (type IV) is delayed-type. The former reactions are caused by humoral antibodies, while the latter is caused by a cellular antibody.

We have already reported that the carboxamidemethylated L chain and the carboxamidemethylated H and L chains show anti-ulcerogenic and anti-inflammatory activities, respectively.

In this paper, we describe the effect of carboxamidemethylated Fc (CM-Fc) on various allergic models.

Materials and Methods

Preparation of CM-Fc—According to Bennich and Turner, 2% commercially available human serum immunoglobulin G (IgG) (Globulin-Midori) dissolved in 0.1 M sodium phosphate buffer (pH 6.75) containing 2 mM ethylenediaminetetra acetic acid (EDTA) and 10 mM cysteine hydrochloride (Nakarai Chem.) was digested with 0.02% papain (type III, Sigma). After 4 h, 150 μl of 0.5 M iodoacetamide (Wako Pure Chem.) was added to the reaction mixture. The mixture was then separated according to Nishioka et al., on a Sephadex G-100 column equilibrated with 10 mM phosphate-buffered saline (PBS, pH 7.2). The fraction containing Fab and Fc from Sephadex G-100 was separated by protein A–Sepharose CL-4B (Pharmacia Fine Chemicals) affinity chromatography, and the Fc fragment was purified. This fragment was then carboxamidemethylated according to Korningsberg, resulting in a carboxamidemethylated Fc fragment (CM-Fc). The molecular weight of CM-Fc was measured by polyacrylamide–sodium dodecyl sulfate gel electrophoresis according to Laemmli.

Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats—Dinitrophenylated ascaris extract (DNP-As) was prepared according to Eisen et al., and anti-DNP-As serum was raised in rats following the method of Tada and Okumura. Male Wistar rats weighing 180–200 g had their backs shaved 24 h prior to experiments. Then 0.1 ml of anti-DNP-As serum, diluted four times with saline, was administered intradermally at three points in the left back, while 0.1 ml of saline was administered intradermally at three points in the right back. After 48 h, rats were intravenously injected with 1 ml of 0.5% Evans blue (Nakarai Chem.) in saline containing 4 mg of DNP-As. Thirty
minutes later, the rats were killed and skinned. The capillary permeability was expressed by multiplying the major and minor axes of the blue area. The sample was administered intraperitoneally 30 min prior to the injection of DNP-As.

**Heterologous PCA in Guinea Pigs**—Anti-egg albumin serum raised in the rabbit was prepared according to Eda et al. Heterologous PCA was prepared according to the method reported by Harada et al., as improved upon by Ovary. The male Hartley guinea pigs had their backs shaved 24 h prior to experiments. Then 0.1 ml of anti-egg albumin serum, diluted five hundred and twelve times with saline, was administered intradermally at two points in the left back, while 0.1 ml of saline was administered intradermally at two points in the right back. After 3.5 h, the guinea pigs were intravenously injected with 1 ml of 1% Evans blue in saline containing 5 mg of egg albumin (Nakarai Chem.). Thirty minutes later, the guinea pigs were killed and skinned. The capillary permeability was again expressed by multiplying the major and minor axes of the blue area. The sample was administered intraperitoneally 30 min prior to the injection of egg albumin.

**Complement-Dependent Hemolysis**—According to Mori et al., sheep red blood cells (SRBC; Handai Biken) were washed with gelatin veronal buffer (GVB), and suspended at $1.0 \times 10^9$ per ml. Anti-SRBC serum, which was prepared according to Globsky, was diluted 1600 times with GVB.

Five milliliters of GVB, 1 ml of the sample, 0.5 ml of SRBC suspension, 0.5 ml of anti-SRBC serum and 1 ml of the complement (from guinea pig serum, Handai Biken) were added, respectively. After incubation at 37°C for 90 min, the reaction was stopped on ice. Following centrifugation (3000 rpm × 10 min), the absorbance of the supernatant was measured at 540 nm. The result was expressed as the inhibition (percent) of hemolysis.

**Immuno-Complex Inflammatory Model**—According to Abe et al., male Balb/c mice (age 8 weeks) were immunized intravenously with $5 \times 10^8$ SRBC twice (day -19 and day -5). The right hind paw was inoculated with $2 \times 10^8$ SRBC to produce the immuno-complex (day 0). After 3 and 4 h, the amount of edema was recorded with a dial thickness gauge (Mitsutoyo Mfg. Co., Ltd.). The difference between the values after and before application was calculated. The sample was administered intraperitoneally 30 min before and 6 h after antigen challenge.

**Delayed-Type Hypersensitivity to Picryl Chloride**—According to Tajima et al., male ddy mice weighing 20—30 g were immunized with 100 µl of 1% picryl chloride (Nakarai Chem.) solution in ethanol on the surface of the right ear. After 7 d, 100 µl of 1% picryl chloride solution in olive oil was applied to the surface of the left ear. Twenty-four hours later, the thickness of the left ear was measured with a dial thickness gauge, and the difference between the values before and after application was calculated. The sample was administered intraperitoneally 30 min before and 6 h after antigen challenge.

**Delayed-Type Hypersensitivity to Methylated Bovine Serum Albumin**—According to Baba et al., male ddy mice weighing 20—30 g were immunized subcutaneously with 0.2 ml of emulsion prepared from equal volumes of 0.25% methylated bovine serum albumin (MBSA, Sigma) in saline and Freund's complete adjuvant (Nakarai Chem.). After 6 d, 0.04 ml of 0.1% MBSA in saline was subcutaneously administered into the left hind paw of mice. Twenty-four hours later, the thickness of the foot pad was measured with a dial thickness gauge. The difference between the values immediately after and 24 h after the injection was calculated. The sample was administered intraperitoneally 30 min before and 6 h after antigen challenge.

**Statistics**—Student's t-test was employed to assess the significance of differences between the mean values for the control group and the sample-administered groups.

### Results

**Type I Allergy**

The effects of CM-Fc and Fc on homologous PCA in rats are summarized in Table I. CM-Fc significantly depressed the colored area at a dose of 20 mg/kg by 38.8%, while Fc showed no depression at the same dose.

The effect of CM-Fc on heterologous PCA in guinea pigs was also examined. CM-Fc at a dose of 20 mg/kg significantly depressed the colored area (27.4% inhibition; Table II).

**Type II Allergy**

The effects of CM-Fc and Fc on complement-dependent hemolysis are shown in Fig. 1. Fc inhibited hemolysis dose-dependently; the inhibition reached 40% at a dose of 100 µg/ml. On the other hand, CM-Fc did not inhibit hemolysis at all.

**Type III Allergy**

We examined the effects of CM-Fc and Fc using the immuno-complex inflammatory model for the active Arthus reaction. The maximum swelling of the hind pad was observed after 3 h. Fc at a dose of 20 mg/kg did not depress the pad swelling at the indicated times,
although CM-Fc at doses of 10 and 20 mg/kg significantly depressed the swelling (Fig. 2).

Type IV Allergy

As regards delayed-type hypersensitivity response to picryl chloride, CM-Fc significantly depressed the ear swelling at the dose of 20 mg/kg when the reagent was administered twice,
The effects of CM-Fc and Fc on delayed-type hypersensitivity to MBSA are shown in Table IV. CM-Fc had a significant inhibitory capability at a dose of 20 mg/kg, and Fc slightly depressed the swelling at the dose of 20 mg/kg when the reagents were administered twice.

### Discussion

Recently, we reported that bovine serum IgG L chain,\(^{18}\) carboxamidemethylated Bence Jones protein\(^{19}\) and human serum IgG L chain (Fr. I-L)\(^{21}\) show anti-ulcerogenic activity based on their inhibition of gastric juice secretion. Moreover, Fr. I-L and Fr. I-H also had anti-inflammatory activity.\(^{3}\) In this investigation, we prepared an Fc subfragment without the variable region for the purpose of obtaining a more homogenous structure from human serum IgG, and we obtained CM-Fc with a molecular weight of about 25000 (data not shown).

Coombs and Gell\(^{1}\) classified allergic phenomena into four types. First, CM-Fc was examined for type I, an immediate allergy caused by an IgE antibody. Homologous PCA reaction, generally used as a type I allergic model, was depressed by CM-Fc, but not by Fc. Heterologous PCA was also depressed by CM-Fc.

Type II allergy is characterized by cell destruction resulting from a combination of the surface antigen of plasma membrane and its antibody, which depends on the complement.\(^{20}\) CM-Fc did not inhibit complement-dependent hemolysis at all (Fig. 1), and also showed little inhibition of hypotonic hemolysis (data not shown). As Fc inhibited complement-dependent hemolysis, it was assumed that the complement-binding site was lost after carboxamide-methylation.

Opie\(^{21}\) showed that type III allergy was due to antibody and antigen reacting in the tissue. Culbertson\(^{22}\) and later Cannon and Marshall\(^{23}\) demonstrated that the severity of this...
phenomenon closely paralleled the level of precipitin in the blood. In the present experiments, we used the active Arthus phenomenon as a type III allergy model. CM-Fc significantly depressed this phenomenon at doses of 10 and 20 mg/kg. The Arthus phenomenon is divided into first and second stages characterized by slight and transient enhancement and strong and sustained enhancement of vascular permeability.24) It was suggested that CM-Fc inhibited the latter stage to depress the Arthus phenomenon.

Type IV allergy is the phenomenon caused by lymphokines, which are released on contact of the antigen and sensitized T cells.25) CM-Fc depressed the delayed-type hypersensitivities to both picryl chloride and MBSA. Therefore, we supposed that CM-Fc affected the cellular immune system.

Thus, CM-Fc is a potentially useful drug for three allergy types (I, III and IV) whereas Fc is not. This finding that anti-allergic activity was not acquired on carboxamidemethylation is consistent with our previous findings.2,3,18,19) The next step will be a more detailed examination of the mechanism of action of CM-Fc.

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References

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