



Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc



IgG-mediated systemic anaphylaxis to protein antigen can be induced even under conditions of limited amounts of antibody and antigen

Ryosuke Ishikawa^a, Yusuke Tsujimura^{a,1}, Kazushige Obata^a, Yohei Kawano^a, Yoshiyuki Minegishi^{a,b}, Hajime Karasuyama^{a,b,*}

^a Department of Immune Regulation, Tokyo Medical and Dental University Graduate School, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

^b JST, CREST, Tokyo Medical and Dental University Graduate School, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

ARTICLE INFO

Article history:

Received 8 October 2010

Available online 26 October 2010

Keywords:

Anaphylaxis

IgE

IgG

Ovalbumin

Hapten

Allergen

ABSTRACT

Systemic anaphylaxis is an acute, severe, and potentially fatal allergic reaction. Two classes of antibodies, IgE and IgG, contribute to the development of anaphylaxis in mice, through different mechanisms with distinct usage of effector cells and chemical mediators. Larger quantities of antibody and antigen are reportedly required to induce IgG-mediated anaphylaxis than IgE-mediated one, suggesting that the former may not happen as frequently as the latter in real life. To readdress this issue, we established in the present study a novel mouse model of passive IgG-mediated systemic anaphylaxis to a native protein antigen, ovalbumin (OVA), rather than artificially haptenated protein antigens used in previous studies. Passive sensitization of mice with a cocktail of but not individual IgG1 mAbs specific to distinct OVA epitopes elicited systemic anaphylaxis in response to OVA challenge. Importantly, much smaller doses of antibody and antigen than previously reported were sufficient for the induction of IgG-mediated systemic anaphylaxis. Moreover, a relatively small dose of antigen could induce severe anaphylaxis through both IgE- and IgG-mediated mechanisms when mice had been passively sensitized with antigen-specific IgE and IgG. These results strongly suggest that IgG-mediated systemic anaphylaxis is not rare among antibody-mediated systemic anaphylaxis, in contrast to previous thought, and significantly contributes to active systemic anaphylaxis in real life, at least in mice.

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1. Introduction

Anaphylaxis is a rapid-onset, life-threatening allergic reaction, and is most commonly triggered by exposure to allergens such as foods, insect venoms, latex and medications [1–3]. Studies with mouse models have identified two types of antibody-mediated systemic anaphylaxis: one is mediated by IgE, and the other mediated by IgG, particularly IgG1 subclass [4–8]. Distinct cell types and chemical mediators are involved in the development of each type of anaphylaxis. Mast cells largely contribute to IgE-mediated systemic anaphylaxis through histamine release in response to the stimulation with IgE plus antigens. In contrast, mast cells are dispensable for the development of IgG-mediated one. Instead,

Abbreviations: CFA, complete freund's adjuvant; mAb, monoclonal antibody; OVA, ovalbumin; TNP, 2,4,6-trinitrophenol.

* Corresponding author at: Department of Immune Regulation, Tokyo Medical and Dental University Graduate School, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. Fax: +81 3 3814 7172.

E-mail address: karasuyama.mbch@tmd.ac.jp (H. Karasuyama).

¹ Present address: Laboratory of Immunoregulation and Vaccine Research, Tsukuba Primate Research Center, National Institute of Biomedical Innovation, 1 Hachimandai, Tsukuba, Ibaraki 305-0843, Japan.

either basophils or macrophages, depending on experimental systems employed, are involved in IgG-mediated systemic anaphylaxis, through release of platelet-activating factor upon stimulation with IgG-antigen immune complexes [6,9]. Moreover, IgE- and IgG-mediated systemic anaphylaxes reportedly differ in the antibody and antigen concentrations that are required to induce anaphylaxis [10,11]. It has been suggested that larger quantities of both antibodies and allergens are needed to induce IgG-mediated systemic anaphylaxis than IgE-mediated one. Indeed, 0.4–0.5 mg of IgG1 mAbs and 1 mg of allergens were commonly used to induce passive IgG-mediated anaphylaxis in mice [4,5,9,12], while as little as 0.01–0.05 mg of IgE mAbs and 0.05–0.2 mg of antigens for induction of IgE-mediated one [5,9]. This implies that IgG-mediated systemic anaphylaxis may not happen as often as IgE-mediated one in real life.

Artificially haptenated proteins are almost exclusively used as allergens in mouse models of passive systemic anaphylaxis, in which mice are passively sensitized with a single species of mAb specific to hapten, such as 2,4,6-trinitrophenol (TNP), 2,4-dinitrophenol and Penicillin V, followed by intravenous administration of the corresponding haptenated proteins [4,5,9,12]. However, most of common protein allergens do not possess such highly-repetitive epitopes on a

single molecule. Indeed, in more realistic models of active systemic anaphylaxis, mice are often primed with immunization of unmodified proteins such as ovalbumin (OVA) [13–15], which leads to production of polyclonal rather than monoclonal antibodies. This active form of systemic anaphylaxis to protein antigens can be reconstituted in naïve mice, at least in part, by passive transfer of serum from the immunized mice, and subsequent challenge with antigens [11]. However, such serum transfer experiments do not seem suitable for detailed analysis of mechanisms underlying the development of anaphylaxis, including clear discrimination between IgE- and IgG-mediated anaphylaxis. As far as we are aware, no mouse model of IgG mAb-mediated passive anaphylaxis to protein antigens has been reported.

In the present study, we sought to establish a mouse model of OVA-specific, IgG-mediated passive systemic anaphylaxis, and to address the issue regarding the quantity of antibody and antigen that is required for the elicitation of IgG-mediated anaphylaxis. Passive sensitization of mice with a cocktail of but not individual IgG1 mAbs specific to distinct OVA epitopes elicited systemic anaphylaxis in response to OVA challenge. We found that much smaller amounts of antibody and antigen than previously reported are sufficient for the induction of IgG-mediated systemic anaphylaxis, suggesting that IgG-mediated systemic anaphylaxis is not rare among antibody-mediated systemic anaphylaxis, in contrast to previous thought.

2. Materials and methods

2.1. Mice

Six to eight-week-old female C57BL/6 mice were purchased from CLEA Japan (Tokyo, Japan). All the experiments in this study were performed according to the Guidelines for Animal Use and Experimentation, as set out by Tokyo Medical and Dental University.

2.2. Establishment of OVA-specific IgG1 mAbs

A panel of hybridomas secreting OVA-specific IgG1 mAbs were prepared from splenocytes isolated from mice that had been immunized intraperitoneally 14 days before with 0.5 mg OVA (Sigma–Aldrich, Tokyo, Japan) together with either Imject Alum (Thermo Fisher Scientific, Rockford, USA) plus *B. pertussis* toxin (List Biological Laboratories, Inc., California, USA) or complete Freund's adjuvant (CFA, Sigma–Aldrich, Tokyo, Japan). Their specificity to OVA and IgG1 subclass were determined with ELISA. To select mAbs that recognize different epitopes on OVA, each mAb was biotinylated with EZ-link NHS-Biotin Reagents (Thermo Fisher Scientific, Rockford, USA), and subjected to epitope-blocking ELISA, in which OVA-coated microplate wells were incubated with serially diluted, unlabeled OVA-specific mAbs or control TNP-specific IgG1 mAb (ATCC TIB-191) for 1 h prior to incubation with 1 µg/ml each biotinylated mAb for 1 h and subsequently with streptavidin-conjugated horseradish peroxidase (GE Healthcare, Buckinghamshire, UK). Three representative OVA-specific IgG1 clones, designated OVA#1, OVA#2, and OVA#3, that recognize distinctive OVA epitopes, as judged by ELISA data, were selected and utilized in this study.

2.3. Induction of passive systemic anaphylaxis

For IgG-mediated anaphylaxis, C57BL/6 mice were passively sensitized with an intravenous injection of indicated amounts of OVA-specific IgG1 mAb in 0.2 ml PBS. Two hour later, they were intravenously challenged with indicated amounts of OVA or TNP-conjugated OVA (TNP₅-OVA) in 0.2 ml PBS. For IgE-mediated ana-

phylaxis, C57BL/6 mice were passively sensitized with an intravenous injection of 8 µg TNP-specific IgE mAb (IGELb4, ATCC, TIB-141). One day later, they were intravenously challenged with 8 µg TNP₅-OVA. Their rectal temperature was monitored with a digital thermometer (Shibaura Electronics Co., Ltd. Saitama, Japan).

2.4. Measurement of OVA-specific IgG1 in mouse sera

Two hour after the intravenous injection of indicated amounts of OVA-specific IgG1 mAbs, sera were prepared from their peripheral blood. Sera were also collected from mice that had been immunized 14 days before with 0.5 mg OVA together with either Imject Alum plus *B. pertussis* toxin or CFA. Sera were subjected to ELISA, in which OVA-coated microplate wells were incubated with serially-diluted serum samples for 1 h, followed by incubation with horseradish peroxidase-conjugated anti-mouse IgG1 antibody (SouthernBiotech, Birmingham, USA) for 1 h.

2.5. Statistical analysis

The statistical significance was determined by Student's *t*-test. *P*-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Establishment of a mouse model of OVA-specific, IgG-mediated systemic anaphylaxis through passive sensitization with a cocktail of mAbs

We established a panel of OVA-specific mAbs of IgG1 subclass by immunizing mice with OVA, and selected three representative clones (OVA#1, OVA#2, and OVA#3) that recognize distinct epitopes on OVA, based on the data from the epitope-blocking ELISA (Supplementary Fig. 1). When mice were passively sensitized with each mAb alone (up to 1 mg) and then challenged with OVA (up to 1 mg), none of them developed systemic anaphylaxis, as judged by no significant drop in their rectal temperature (Fig. 1A and D). We also obtained comparable results by using 5 additional IgG1 mAbs (data not shown). This was in sharp contrast to the observations in the previous models, in that sensitization of mice with a single species of IgG1 mAb specific to hapten such as TNP, 2,4-dinitrophenol, and Penicillin V was sufficient for induction of anaphylaxis to haptened proteins [4,5,9,12]. Of note, sensitization with any combination of two OVA-specific IgG1 mAbs (total 1 mg per mouse, containing 0.5 mg each mAb) enabled mice to develop systemic anaphylaxis with maximum drop of 4–5 °C in their rectal temperature, when mice were challenged with 1 mg OVA (Fig. 1B and D). Moreover, the combination of three mAbs (total 1 mg per mouse, containing 0.33 mg each mAb) could induce even severer anaphylaxis with maximum drop of ~7 °C in rectal temperature that last for longer time, compared to the combination of two mAbs (Fig. 1C and D).

3.2. Comparison of OVA-specific IgG1 levels in sera from mice passively sensitized with the mAbs and those immunized with OVA

We next examined the possible relevance of this novel model of OVA-specific, IgG-mediated, passive systemic anaphylaxis to active systemic anaphylaxis in which mice are immunized with OVA in adjuvants and 14 days later challenged with OVA. In the model of passive anaphylaxis, the concentration of serum IgG1 specific to OVA was ~1 mg/ml at the time of OVA challenge, that is, 2 h after mice were passively sensitized with 1 mg OVA-specific IgG1 mAb, regardless of the mAb clones utilized (Fig. 2A). On the other hand, the serum concentration of OVA-specific IgG1 was 0.06–0.07 and 0.3–0.4 mg/ml, 14 days after mice were immunized with OVA

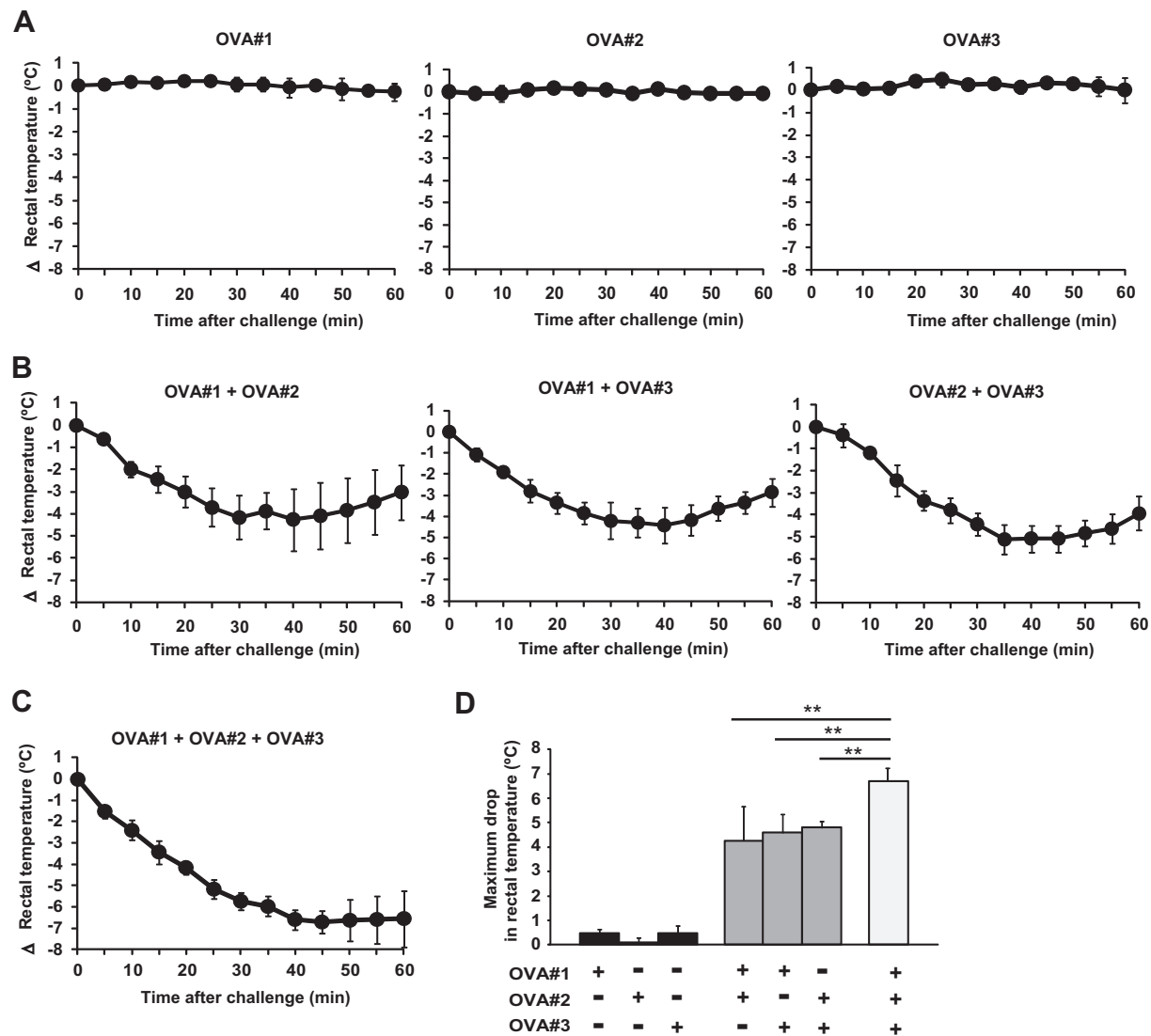


Fig. 1. Establishment of a mouse model of OVA-specific, IgG-mediated, passive systemic anaphylaxis. Mice were first sensitized passively with intravenous administration of three different OVA-specific IgG1 mAbs (OVA#1, OVA#2, and OVA#3), individually (A), in combination of two of them (B), or in combination of all three (C). The dose of IgG1 injected was 1 mg per mouse, containing equal amounts of each mAb in case of combination. Change in rectal temperature over time after challenge with intravenous administration of 1 mg OVA is shown in A–C. In D, maximum drop in rectal temperature in each group is summarized for comparison. Data are expressed as the mean $\pm$ SD ($n = 3$ each) and are representative of three independent experiments. $^{**}P < 0.01$.

together with alum plus pertussis toxin, and CFA, respectively (Fig. 2B). Thus, the experimental conditions used in Fig. 1 for induction of passive anaphylaxis were artificial, and do not seem relevant to those of active anaphylaxis, particularly in mice immunized with OVA together with alum plus pertussis toxin, which protocol is frequently used to induce allergic Th2 responses. Accordingly, we next determined the dose of sensitizing mAbs that is necessary to obtain 0.06–0.07 mg/ml of serum concentration 2 h after injection. As shown in Fig. 2A, the sensitization of mice with 0.04 instead of 1 mg IgG1 mAbs, regardless of single use or their combination, provided optimal conditions relevant to active systemic anaphylaxis in terms of serum concentration of OVA-specific IgG1 antibodies.

3.3. IgG-mediated systemic anaphylaxis can be induced with limited doses of antibody and antigen

To make the mouse model of IgG-mediated systemic anaphylaxis more realistic and relevant to active systemic anaphylaxis,

we titrated down the doses of both antibodies for sensitization and antigens for challenge, that are necessary for elicitation of IgG-mediated passive anaphylaxis in our model (Fig. 3). The sensitization of mice with a single mAb in the doses ranging between 1 and 0.008 mg failed to induce anaphylaxis. The combination of two mAbs at 1:1 ratio with the total amount of 0.2 but not 0.04 mg induced anaphylaxis (Fig. 3A). Importantly, a cocktail of three mAbs even at the dose of as little as 0.04 mg enabled mice to develop anaphylaxis in response to challenge with 0.04 mg OVA (Fig. 3A). We further titrated down the dose of antigen, and found that as little as 0.008 mg OVA was still sufficient for induction of systemic anaphylaxis with maximum drop of $\sim 4^{\circ}\text{C}$ in rectal temperature in mice sensitized with 0.04 mg of a cocktail of three mAbs (Fig. 3B).

3.4. Even a small dose of antigen can induce severe anaphylaxis in mice sensitized with both IgG and IgE

The requirement of much smaller amounts of antibody and antigen than previously thought in the induction of IgG-mediated

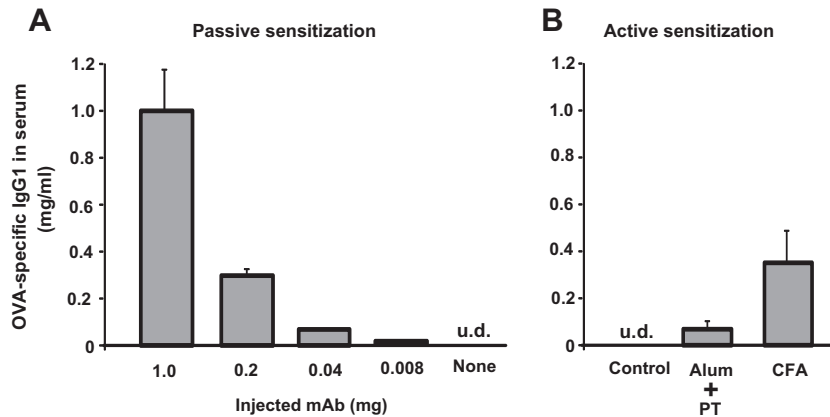


Fig. 2. Comparison of OVA-specific IgG1 levels in sera from mice passively sensitized with the mAbs and those immunized with OVA. (A) Mice were passively sensitized with intravenous administration of indicated doses of OVA-specific IgG1 (OVA#1). Its serum concentration 2 h after the administration in each group is shown. Data are expressed as the mean $\pm$ SD ($n = 5$ each) and are representative of four independent experiments. Comparable results were obtained when OVA#2 or OVA#3 was used for sensitization (data not shown). (B) Mice were immunized with OVA together with alum plus pertussis toxin (PT) or CFA, or left unimmunized (cont.). The concentration of OVA-specific IgG1 in their sera 14 days after immunization is shown (the mean $\pm$ SD, $n = 3$ in each group, representative of 3 independent experiments). u.d., undetectable.

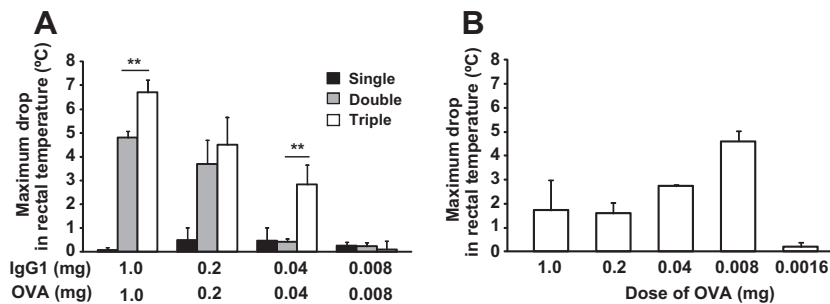


Fig. 3. IgG-mediated systemic anaphylaxis can be induced with limited doses of antibody and antigen. (A) Mice were first sensitized with indicated doses of three OVA-specific IgG1 mAbs, either individually (single), or in combination of two (double) or three (triple), and subsequently challenged with indicated doses of OVA. Maximum drop in rectal temperature in each group is shown (the mean $\pm$ SD, $n = 3$), in that we used OVA#2 as single, and OVA#2 plus OVA#3 as double. $^{**}P < 0.01$. Comparable results were obtained when other combinations of the mAbs were used (data not shown). (B) Mice sensitized with 0.04 mg of a cocktail of three mAbs were challenged with indicated doses of OVA. Maximum drop in rectal temperature in each group is shown (the mean $\pm$ SD, $n = 3$). Data shown in A and B are representative of three independent experiments.

anaphylaxis prompted us to examine the possibility of simultaneous induction of IgG- and IgE-mediated anaphylaxis upon challenge with relatively small amounts of antigen. When mice were passively sensitized with 0.008 mg TNP-specific IgE mAb, the challenge with 0.008 mg TNP-OVA induced systemic anaphylaxis with maximum drop of 2–3 °C in rectal temperature (Fig. 4). In accordance with the observation in mice challenged with OVA (Fig. 3), the challenge with 0.008 mg TNP-OVA in mice sensitized with 0.04 mg of a cocktail of three OVA-specific IgG1 mAbs induced anaphylaxis with maximum drop of 3–4 °C in rectal temperature (Fig. 4). Of note, mice sensitized with both IgE and a cocktail of IgG1 mAbs developed severer (maximum drop of 5–6 °C in rectal temperature) and longer lasting anaphylaxis, compared to mice sensitized with either IgE or IgG1 alone, in response to challenge with 0.008 mg TNP-OVA (Fig. 4).

4. Discussion

We have established in the present study a novel mouse model of IgG-mediated, passive systemic anaphylaxis. In previous models, artificially haptenated proteins were commonly utilized for convenience [4,5,9,12]. Because haptenated proteins possess highly-repetitive epitopes (haptens), they can readily form large immune complexes with even a single species of IgG1 mAb specific to

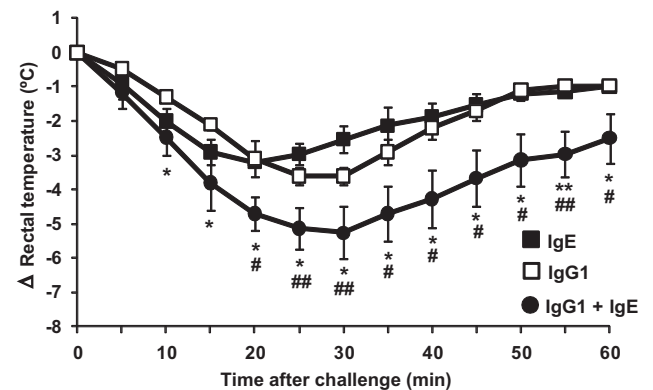


Fig. 4. Even a small dose of antigen can induce severe anaphylaxis in mice sensitized with both IgG and IgE. Mice were first sensitized with TNP-specific IgE (0.008 mg administered one day before antigen challenge, closed squares) alone or OVA-specific IgG1 (0.04 mg of cocktail of three mAbs administered 2 hr before antigen challenge, open squares) alone or both of IgE and IgG1 with sequential administration (closed circle), and subsequently challenged with 0.008 mg TNP-OVA. Change in rectal temperature over time after challenge is shown (the mean $\pm$ SD, $n = 3$), as a representative of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ between IgG1 alone and the combination of IgE and IgG1. $^{*}P < 0.05$, $^{##}P < 0.01$ between IgE alone and the combination of IgE and IgG1. Co-administration of irrelevant Penicillin V-specific IgG1 with TNP-specific IgE did not show any significant effect on IgE-mediated systemic anaphylaxis (data not shown).

haptens, and such immune complexes trigger anaphylactic reactions. In our model, we used a common protein antigen OVA, instead of haptenated proteins. Only a cocktail of but not individual IgG1 mAbs specific to OVA successfully sensitized mice for induction of anaphylaxis. This is in sharp contrast to the observation in previous models using haptenated proteins, and appears to mimic more faithfully the setting of active systemic anaphylaxis, in which mice produce antigen-specific polyclonal rather than monoclonal antibodies. To our knowledge, this is the first mouse model of IgG mAb-mediated, passive systemic anaphylaxis to protein antigen.

Previous studies suggested that IgG-mediated systemic anaphylaxis might not happen as frequently as IgE-mediated one in real life, due to the requirement of larger quantities of both antibody and antigen for the induction of the former than the latter [10,11]. We demonstrated in our model that relatively small doses of antibody (0.04 mg) and antigen (0.008 mg) were sufficient for elicitation of IgG-mediated systemic anaphylaxis. Under these experimental conditions, serum concentration of OVA-specific IgG1 was comparable to that in mice immunized with OVA. Thus, IgG-mediated systemic anaphylaxis can be induced under conditions of conventional sensitization of mice with antigens, and therefore may not be rare among antibody-mediated systemic anaphylaxis, in contrast to previous thought.

It has been shown that IgG1 antibodies can function as blocking reagents to inhibit IgE-mediated systemic anaphylaxis when antigen-specific IgE and IgG1 coexist [10,11]. Large quantities of IgG1 are needed to elicit IgG1-mediated anaphylaxis in the previous models. Therefore, smaller quantities of IgG1 may not induce anaphylaxis and rather suppress IgE-mediated anaphylaxis by intercepting antigens and/or by cross-linking IgE receptor FcεRI to inhibitory IgG receptor FcγRIIb [11]. However, under our experimental conditions, sensitization of mice with relatively small amounts of IgG1 (0.04 mg) and IgE (0.008 mg), followed by challenge with a small dose (0.008 mg) of antigen, induced severer and longer lasting anaphylaxis than sensitization with either IgE or IgG1 alone. This suggests that IgG- and IgE-mediated systemic anaphylaxes can be elicited simultaneously under conditions of conventional sensitization of mice with antigens.

In conclusion, we have developed a novel mouse model of protein antigen-specific, IgG-mediated, passive systemic anaphylaxis, which appears more natural than the previous models. We demonstrated in this model that IgG-mediated anaphylaxis can be induced even under conditions of limited amounts of antibody and antigen, in contrast to previous thought. Thus, our model casts new light on the significance and reality of IgG-mediated systemic anaphylaxis.

Acknowledgments

This work was supported by research grants from JST, CREST, the Japanese Ministry of Education, Culture, Sports, Science and

Technology, Takeda Science Foundation, the Mitsubishi Foundation, the Naito Foundation, and the Uehara Memorial Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.10.098.

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