

Application of Immunoreaction Enhancer Solutions to an Enzyme-Linked Immunosorbent Assay for Antigen-Specific IgE in Mice Immunized with Recombinant Major Mite Allergens or Ovalbumin

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Key Words

Enzyme-linked immunosorbent assay • Allergen-specific IgE • Immunoreaction enhancer solutions • Protein G • Recombinant mite allergens • Der p 1 • Der f 1 • Der f 2 • Ovalbumin

Abstract

Background: Weak signals for allergen-specific IgE are a problem in murine models for the study of allergies. It has been reported that the removal of IgG from murine sera enhances signal intensity. Very recently, buffer solutions designed to enhance signals in immunoassays have been developed and made commercially available. **Methods:** Sera from mice immunized either with a recombinant form of one of the major mite allergens Der p 1, Der f 1 and Der f 2, or with ovalbumin adsorbed to alum were used for the assays. Total IgE was measured by a sandwich enzyme-linked immunosorbent assay (ELISA). Allergen-specific IgE was assayed using plates coated with the allergens after the removal of IgG from sera with protein G-coupled sepharose beads in wells of other plates or with the use of commercially available enhancer solutions without the removal of IgG. IgE binding was detected with horseradish peroxidase-conjugated anti-

murine IgE monoclonal antibody as the secondary antibody. **Results:** Significant levels of total IgE were produced after the immunizations. The in-well pretreatment of diluted sera (1/10 dilution) with protein G-coupled beads enhanced the signals for allergen-specific IgE. The use of the enhancer solutions for dilution of the sera and secondary antibody and prolonged incubation remarkably enhanced the signals at a more extensive dilution of sera (1/200 or less) without the removal of IgG. **Conclusions:** An ELISA simply modified with the use of immunoreaction enhancer solutions has advantages in terms of signal intensity and ease of handling for the detection of allergen-specific murine IgE and would be useful for the study of allergies with murine models.

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Introduction

Immediate hypersensitivity is caused by the release of mediators from mast cells and basophils activated through aggregation of a high-affinity IgE receptor (FcεRI) at the cell surface and allergen-specific IgE, cross-linked by multivalent allergens derived from house dust mites, pollen and so on [1, 2]. Clinically, elevated

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levels of total or allergen-specific IgE in serum are a major manifestation of allergic diseases. For the estimation of allergen-specific IgE levels in sera, an enzyme immunoassay using a solid phase such as a cellulose sponge and paper disc to which an excess of allergen molecules can be immobilized has been used.

In mice immunized with model allergens such as ovalbumin (OVA) or clinically relevant allergens, the signal intensity for allergen-specific IgE in an enzyme-linked immunosorbent assay (ELISA) using plastic plates coated with the allergens is low, although total IgE can be easily detected with a sandwich ELISA using a set of 2 anti-murine IgE monoclonal antibodies (mAbs). The low signal intensity for allergen-specific IgE is mainly due to allergen-specific IgG, which is present in the sera from immunized mice and competitively inhibits the binding of allergen-specific IgE to the immobilized allergen. It has been reported that the removal of IgG from murine sera by passage through a packed protein G-coupled column [3] or by preincubation with protein G-coupled magnetic beads in tubes [4] enhances signal intensity. However, the loading of sera onto the column needs a relatively large volume and the process is time-consuming. Although the use of biotinylated allergens for the detection of allergen-specific IgE trapped on plates coated with anti-IgE antibody (Ab) is another way to improve signal intensity, the possibility that the modification disrupts some IgE epitopes cannot be excluded.

Very recently, buffer solutions designed to enhance signals in immunoassays have become commercially available [5–7]. In the present study, we evaluated the effects of in-well pretreatment of sera with protein G-coupled sepharose beads and the use of enhancer solutions on signals for the binding of allergen-specific IgE to plates coated with recombinant forms of the clinically relevant major house dust mite allergens [8–11] Der p 1, Der f 1 [12–17] and Der f 2 [18–20], or with OVA. We demonstrate that both methods are effective and that especially the latter has remarkable advantages in terms of signal intensity and handling.

Materials and Methods

Recombinant Der p 1, Der f 1 and Der f 2

Recombinant mature forms of Der p 1 and Der f 1 without (Der p 1-N52Q and Der f 1-N53Q) and with yeast-derived hyperglycosylation (Der p 1-WT and Der f 1-WT) were prepared as previously described [12, 13, 16]. Der p 1-N52Q (rDer p 1) and Der f 1-N53Q (rDer f 1) were used for immunization after incubation

with L-cysteine [15]. As the B cell epitopes for recombinant forms with and without hyperglycosylation are considered to be equivalent [12, 13], we used the hyperglycosylated Der p 1-WT and Der f 1-WT for detecting Der p 1- and Der f 1-specific Abs as alternatives to the unglycosylated rDer p 1 and rDer f 1, whose production is much lower than that of the hyperglycosylated forms in yeast [16]. Recombinant Der f 2 (rDer f 2) was prepared as previously described [18] and used for immunization and the coating of microtiter plates for ELISA for Der f 2-specific Abs.

Immunization of Mice

Six- to 8-week-old female CBA/J, BALB/c and C57BL/6 mice were purchased from Charles River Japan Inc. (Yokohama, Japan). The mice were immunized with rDer p 1, rDer f 1, rDer f 2 or OVA (albumin from chicken egg white, grade V; Sigma, St. Louis, Mo., USA) as previously described [15], with minor modifications as follows. They were given 4 (CBA/J) or 5 (BALB/c and C57BL/6) weekly intraperitoneal injections with 0.5–10 µg of the allergens adsorbed on alum (Imject Alum; Pierce Biotechnology Inc., Rockford, Ill., USA). Sera collected 1 week after the last injections were stored at –20°C before assays. Sera used for the experiment in figure 5 were collected after the mice were challenged intranasally twice with rDer p 1 (10 µg/head) or buffer alone 2 months after the last intraperitoneal injection.

Measurement of Total IgE

The total concentration of IgE (total IgE) in the sera was measured by sandwich ELISA using a set of 2 anti-murine IgE mAbs with purified murine IgE as the standard as described previously [15].

Allergen Immobilization and Blocking

Allergens (Der p 1-WT, Der f 1-WT, rDer f 2 and OVA) were diluted with phosphate-buffered saline (PBS) and 96-well microtiter plates for ELISA (MaxiSorp; Nunc, Roskilde, Denmark) were incubated with the allergen solutions (10 µg/ml, 50 µl/well) overnight at 4°C. After 1 wash with PBS containing 0.05% (v/v) Tween 20 (PBST), the plates were incubated with BlockAce (Snow Brand, Sapporo, Japan) diluted with pure water (1/4 dilution, 200 µl/well) for 1.5 h at 37°C or 3 h at room temperature. After 2 washes with PBST, the plates were incubated with sera.

Detection of Allergen-Specific IgE by ELISA after In-Well Pretreatment of Sera with Protein G-Coupled Sepharose Beads

Sera diluted with PBST containing 10% (v/v) BlockAce were depleted of IgG with protein G sepharose 4FF (Amersham Biosciences, Uppsala, Sweden) within wells of round-bottomed microtiter plates (Corning Inc., Corning, N.Y., USA) with agitation at a speed of 1,000 rpm by a bio-shaker (Titec, Saitama, Japan) for 30 min at room temperature. The total volume of the suspension was 78 µl/well, the volume of protein G beads 18 µl/well, the volume of original serum added was 6 µl/well and the final serum dilution was 1/10. After centrifugation with a swing rotor at 1,900 g for 2 min, the supernatants were used for assays. With a multichannel micropipette, volumes of 50 µl from the supernatants in the wells were carefully moved to wells on other plates, which were coated with allergens and blocked as described above. After incubation for 1–2 h at 37°C and 3 washes with PBST, horseradish peroxidase (HRP)-conjugated rat anti-murine IgE mAb

(clone LO-ME-2; Technopharm Biotechnology, Paris, France) diluted with PBST containing 10% (v/v) BlockAce was added to the plates (1/2,000 dilution, 50 μ l/well). After incubation for 1–2 h at 37°C and 3 washes with PBST, the color was developed using tetramethylbenzidine as the substrate (100 μ l/well) and the reaction was stopped by adding 2 N sulfuric acid (Wako, Osaka, Japan) (50 μ l/well) as previously described [15]. The optical density at 450 nm, from which that at 570 nm was subtracted, was used as the signal for allergen-specific IgE.

Detection of Allergen-Specific IgE by ELISA with the Enhancer Solutions

Volumes of 50 μ l of sera diluted with solution 1 of Can Get Signal (Toyobo, Osaka, Japan), one of the commercially available immunoreaction enhancer solutions, were added to the wells on the plates which were coated with allergens and blocked as described above. After incubation for 15 h at 4°C and 3 washes with PBST, HRP-conjugated anti-murine IgE mAb (clone LO-ME-2) diluted with solution 2 of Can Get Signal was added to the plates (1/2,000 dilution, 50 μ l/well). After incubation for 5 h at room temperature and 3 washes with PBST, the color was developed for 20 min using tetramethylbenzidine (BD OptEIA kit; BD Biosciences, San Jose, Calif., USA) (100 μ l/well) and the reaction was stopped by adding 2 N sulfuric acid (50 μ l/well). The optical density at 450 nm, from which that at 570 nm was subtracted, was used as the signal for allergen-specific IgE.

Detection of Allergen-Specific IgGs by ELISA with the Enhancer Solutions

Allergen-specific IgGs were detected in the same way as allergen-specific IgE with the modification that incubation with the diluted sera and the secondary Abs was performed for 90 min at 37°C. The secondary Abs were HRP-conjugated anti-murine IgG1 mAb (clone X56; BD Biosciences), HRP-conjugated goat anti-murine IgG2a Ab (Southern Biotechnology Associates, Birmingham, Ala., USA) and HRP-conjugated rabbit anti-murine IgG2b Ab (Zymed, San Francisco, Calif., USA), which were all used at a dilution of 1/2,000.

Removal of IgE from Serum

Anti-murine IgE mAb (clone R35-72; BD Biosciences) was covalently coupled to CNBr-activated sepharose 4B beads (Amersham Biosciences) (250 μ g of mAb/approximately 250 μ l of beads) and blocked with 2-aminoethanol according to the manufacturer's instructions. The beads (approximately 250 μ l) were suspended with PBS containing 0.01% NaN₃ (total volume of the suspension: 1 ml). The anti-IgE beads (10 μ l of the suspension) were added to a PBS-diluted pooled serum (1/10 dilution, 180 μ l) from 3 mice immunized with rDer p 1 and gently agitated by rotation overnight at 4°C. The supernatant was recovered and depletion with the fresh anti-IgE beads was repeated totally 3 times. The supernatant after the third treatment with the beads was used for ELISA. Control beads, to which no protein was coupled, were also prepared by blocking of the active sites on CNBr-activated sepharose 4B beads with 2-aminoethanol.

Statistical Analysis

Group data were statistically analyzed with Prism version 4.0 (GraphPad, San Diego, Calif., USA). The unpaired Student t test (two-tailed) or the Tukey post hoc test followed by one-way

ANOVA were used to evaluate the significance of differences. $p < 0.05$ was regarded as statistically significant.

Results

In-Well Pretreatment of the Sera with Protein G-Coupled Sepharose Beads Enhanced the Signals for Allergen-Specific IgE

In this experiment, immunizing CBA/J mice 4 times with rDer f 1 activated with L-cysteine (0.5 μ g/head), rDer f 2 (0.5 μ g/head) or OVA (2.5 μ g/head) resulted in elevated levels of total IgE in the sera (fig. 1a). Using 1/10-diluted sera pretreated with protein G-coupled sepharose beads (fig. 1b), a significant strengthening of signals for the allergen-specific IgE was obtained (fig. 1c). Without the pretreatment with protein G beads, the signals were much weaker or not detected at all (unpubl. data). Although the total concentration of IgE on immunization with rDer f 1 or rDer f 2 was higher than or similar to that on immunization with OVA (fig. 1a), the signals for Der f 1- or Der f 2-specific IgE were weaker than those for OVA-specific IgE (fig. 1c).

Use of the Enhancer Solutions Enhanced the Signals for Allergen-Specific IgE without Removal of IgG

In this experiment, immunizing CBA/J mice 4 times with activated rDer p 1 (2.5 μ g/head) or OVA (2.5 μ g/head) resulted in elevated levels of total IgE in the sera (fig. 2a). The effect of the commercially available enhancer solutions on the detection of allergen-specific IgE was evaluated. We determined conditions suitable for the detection of allergen-specific IgE signals (fig. 2b), including a prolonged incubation with the sera and secondary Ab and a more extensive dilution of sera, but not the removal of IgG with protein G beads. Surprisingly, even at dilutions of 1/1,000 and 1/5,000, respectively, Der p 1- and OVA-specific IgE signals were of significant intensity (fig. 2d, e). Although the total concentration of IgE was higher on rDer p 1 immunization than OVA immunization (fig. 2a), the titer evaluated from the titration curves for Der p 1-specific IgE was lower than that for OVA-specific IgE (fig. 1d, e).

For the detection of allergen-specific IgGs, prolonged incubation was not needed (fig. 2c). IgG1 showed the highest titer among the 3 subclasses tested, IgG1, IgG2a and IgG2b (fig. 2f, g). Without the use of the enhancer solutions, signals for allergen-specific IgGs were detectable, although less dilution was necessary to obtain the same signal levels (unpubl. data).

ELISA Using the Enhancer Solutions Exhibited Specificity to Immunized Allergens and High Sensitivity for Detection

In this experiment, immunizing CBA/J mice 4 times with activated rDer f 1 (0.5 $\mu\text{g}/\text{head}$), rDer f 2 (0.5 $\mu\text{g}/\text{head}$) or OVA (1 $\mu\text{g}/\text{head}$) increased the total concentration of IgE in the sera (fig. 3a). In the ELISA system using the enhancer solutions (fig. 2b), IgE from mice immunized with rDer f 1 bound to the plates coated with Der f 1-WT but not to the plates coated with rDer f 2 or OVA (fig. 3b). Similarly, IgE from mice immunized with rDer f 2 or OVA selectively bound to the plates coated with rDer f 2 (fig. 3b) or OVA (fig. 3b), respectively. With the same procedure (fig. 2b) – except that a conventional buffer, PBST containing the blocking reagent, was used for dilution of the sera and secondary Ab – the intensity of signals for the allergen-specific IgE was remarkably decreased, although specificity to the allergen was retained (fig. 3c). Although the total concentration of IgE on immunization with rDer f 1 or rDer f 2 was higher than or similar to that on immunization with OVA (fig. 3a), the signals for Der f 1- or Der f 2-specific IgE were weaker than those for OVA-specific IgE (fig. 3b, c).

ELISA Using the Enhancer Solutions Could Effectively Detect Allergen-Specific IgE in Other Strains of Mice

In this experiment, immunizing BALB/c and C57BL/6 mice 5 times with activated rDer f 1 (10 $\mu\text{g}/\text{head}$) resulted in a rise and no change, respectively, in the total concentration of IgE relative to the vehicle control group (fig. 4a). However, in the ELISA system using the enhancer solutions (fig. 2b), significant levels of Der f 1-specific IgE were detected in the rDer f 1-immunized groups in both strains (fig. 4b).

Confirmation of Isotype Specificity to IgE and Evaluation of Nonspecific Binding of IgE to the Plate

In this experiment, CBA/J mice were immunized 4 times with activated rDer p 1 (2.5 $\mu\text{g}/\text{head}$). Two months later, the mice were intranasally challenged twice with rDer p 1 (10 $\mu\text{g}/\text{head}$). The sera from the 3 immunized mice were mixed as a pooled serum and used for the assays. Total IgE concentrations of the pooled serum from rDer p 1-immunized mice and from the vehicle control were determined as 3.5 and 0.11 $\mu\text{g}/\text{ml}$, respectively, by a sandwich ELISA. After the removal of IgE by treatment with anti-IgE beads, the titer for Der p 1-specific IgE was decreased to 1/125–1/25 compared with the serum treated with the control beads (fig. 5a) but the signals for Der p 1-specific IgGs were unchanged (fig. 5b), demonstrat-

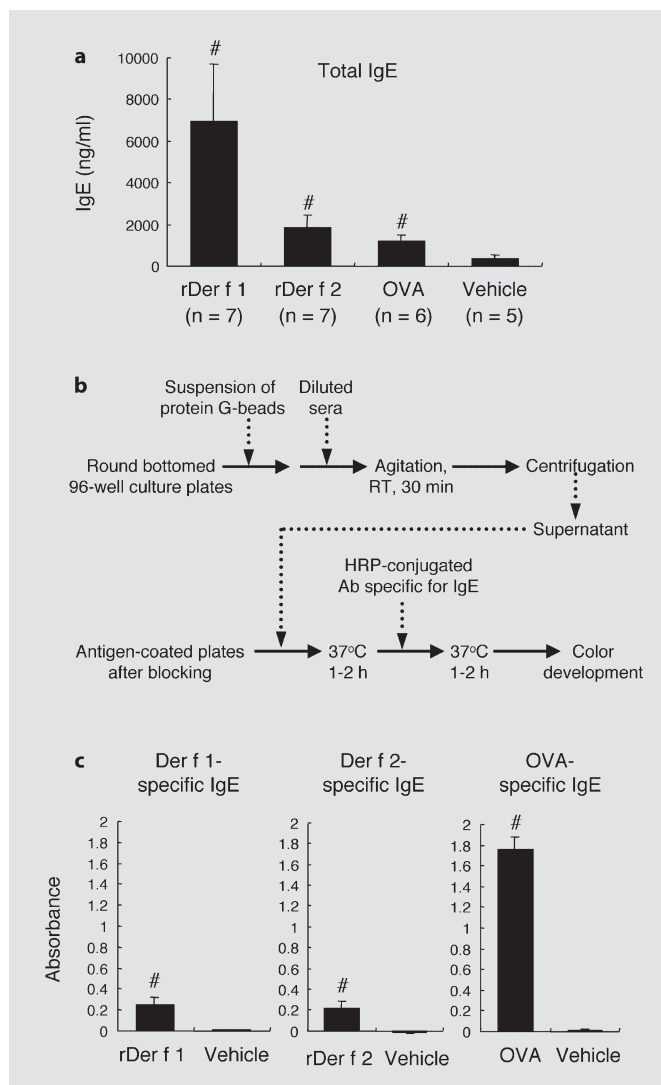


Fig. 1. In-well pretreatment of sera from immunized mice with protein G-coupled sepharose beads enhanced the signals for allergen-specific IgE. Activated rDer f 1 (Der f 1-N53Q, 0.5 $\mu\text{g}/\text{head}$), rDer f 2 (0.5 $\mu\text{g}/\text{head}$), OVA (2.5 $\mu\text{g}/\text{head}$) or buffer alone was mixed with alum and injected into CBA/J mice. **a** Total IgE. **b** Procedure for the pretreatment of sera (1/10 dilution) and detection of allergen-specific IgE. **c** Signals detected for Der f 1-, Der f 2- or OVA-specific IgE. Data shown represent the means $\pm$ SD of 7 (rDer f 1 and rDer f 2), 6 (OVA) or 5 mice (vehicle). # $p < 0.001$ vs. vehicle control by unpaired t test (two-tailed). RT = Room temperature.

ing an isotype specificity to IgE. The signals detected at dilutions of 1/40 and 1/200 (fig. 5a) are considered due to residual Der p 1-specific IgE, because less than 1% of total IgE remained after the treatment with anti-IgE beads (unpubl. data).

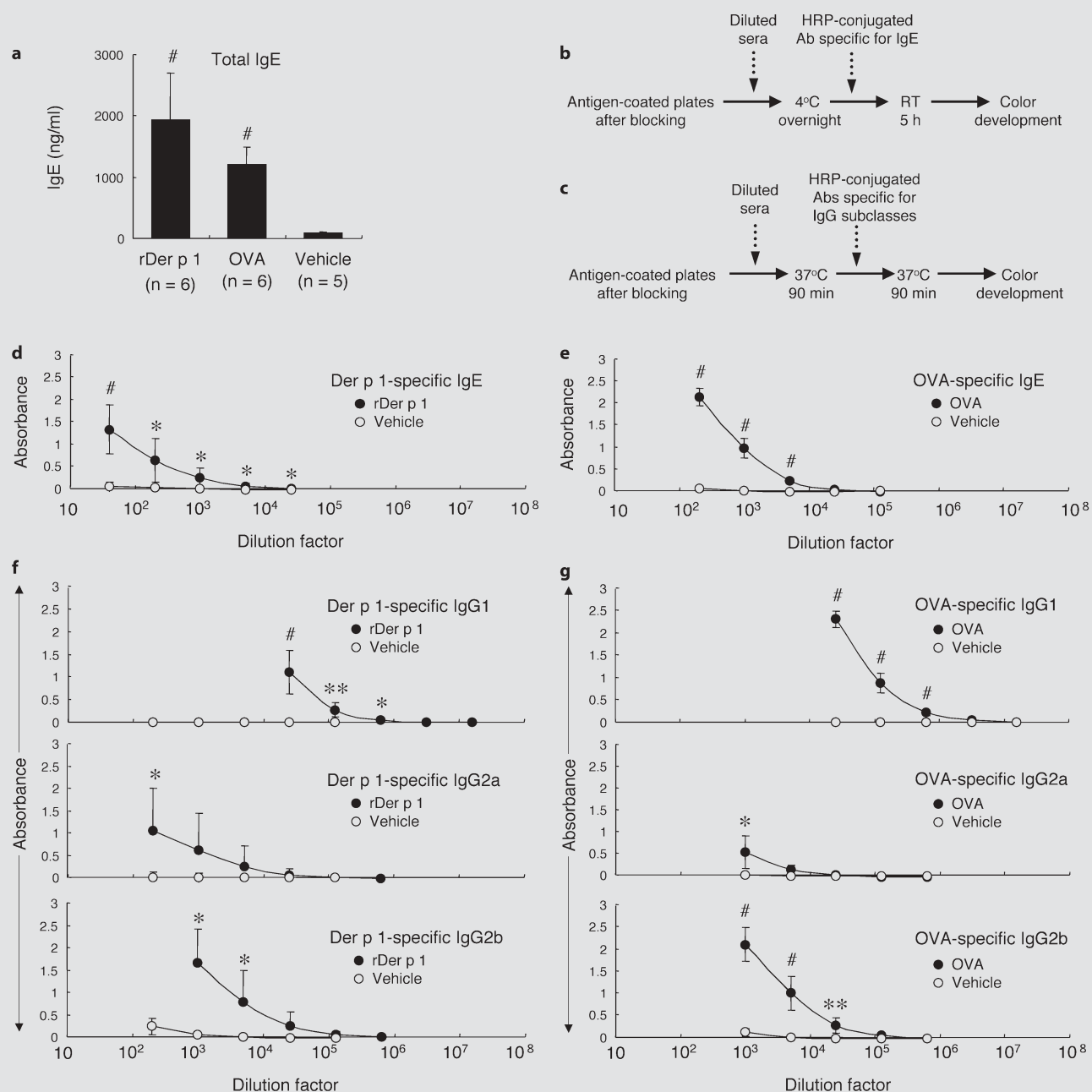


Fig. 2. Use of the immunoreaction enhancer solutions strengthened the signals for allergen-specific IgE without the removal of IgG. Activated rDer p 1 (Der p 1-N52Q, 2.5 μ g/head), OVA (2.5 μ g/head) or buffer alone was mixed with alum and injected into CBA/J mice. Enhancer solutions (Can Get Signal; Toyobo) were used for the dilution of the sera and secondary Abs. **a** Total IgE. Procedures for the detection of allergen-specific IgE (**b**) and IgGs (**c**). Signals detected for IgE specific to Der p 1 (**d**) and OVA (**e**) in serially diluted sera. Signals detected for IgG subclasses specific to Der p 1 (**f**) and OVA (**g**). Dilution factor: factors for serum dilution. Data shown represent the means $\pm$ SD of 6 (rDer p 1 and OVA) or 5 mice (vehicle). * $p < 0.05$, ** $p < 0.01$ and # $p < 0.001$ vs. vehicle control by unpaired t test (two-tailed). RT = Room temperature.

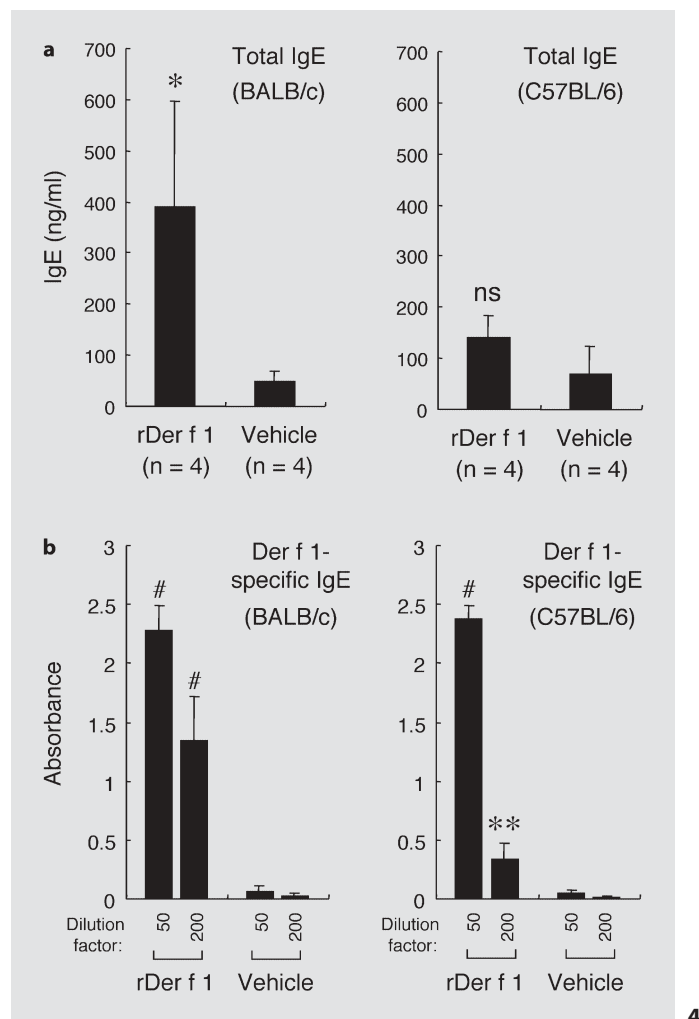
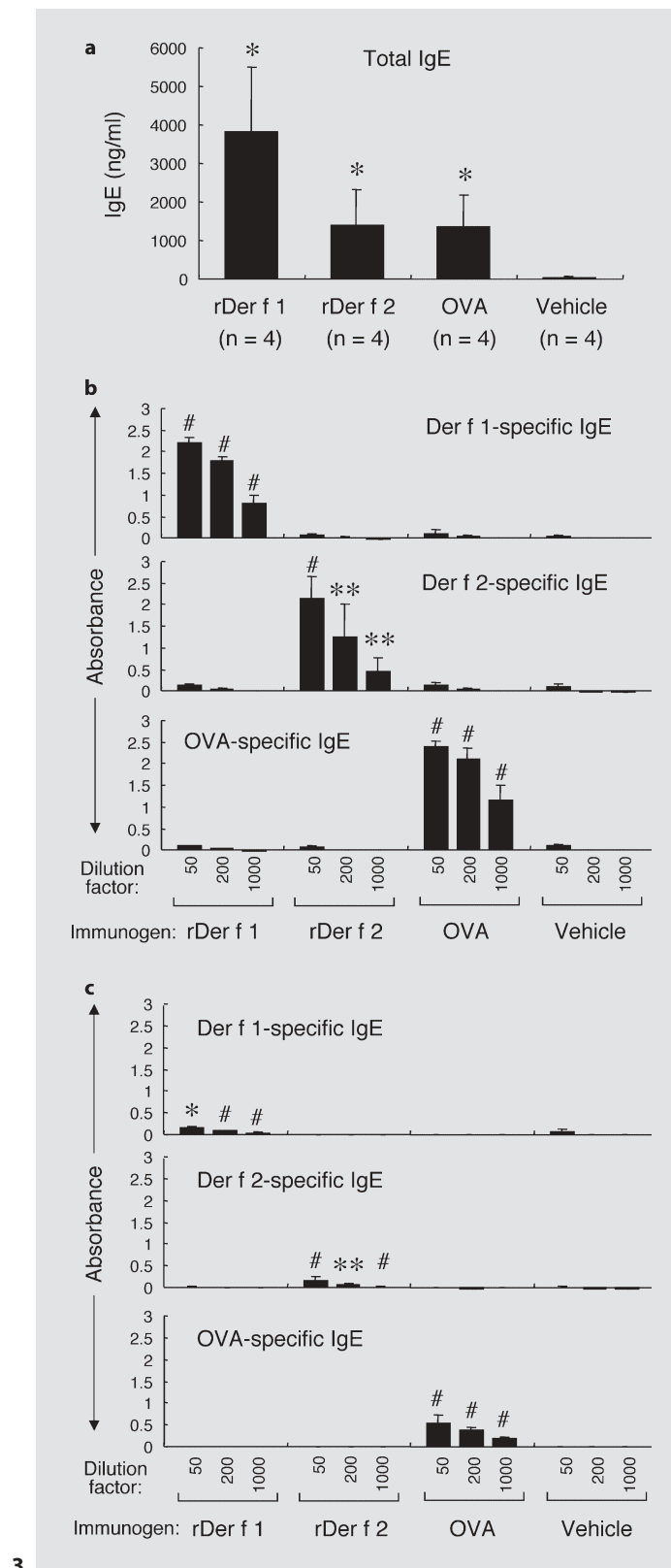


Fig. 3. ELISA using the immunoreaction enhancer solutions exhibited specificity to immunized allergens and high sensitivity for detection. Activated rDer f 1 (Der f 1-N53Q, 0.5 µg/head), rDer f 2 (0.5 µg/head), OVA (1 µg/head) or buffer alone was mixed with alum and injected into CBA/J mice. **a** Total IgE. IgE specific to Der f 1, Der f 2 and OVA detected using enhancer solutions (Can Get Signal; Toyobo) (**b**) or PBST containing a blocking reagent (**c**) for the dilution of the sera and secondary Ab by the procedure shown in figure 2b. Dilution factor: factors for serum dilution. Data shown represent the means ± SD of 4 mice. * $p < 0.05$, ** $p < 0.01$ and # $p < 0.001$ vs. vehicle control by unpaired t test (two-tailed) (**a**) and by the Tukey post hoc test followed by one-way ANOVA (**b**, **c**).

Fig. 4. ELISA using the immunoreaction enhancer solutions effectively detected allergen-specific IgE in other strains of mice. Activated rDer f 1 (Der f 1-N53Q, 10 µg/head) or buffer alone was mixed with alum and injected into BALB/c and C57BL/6 mice. **a** Total IgE. **b** Signals for Der f 1-specific IgE detected by the procedure shown in figure 2b using enhancer solutions (Can Get Signal; Toyobo) for the dilution of the sera and secondary Ab. Dilution factor: factors for serum dilution. Data shown represent the means ± SD of 4 mice. * $p < 0.05$, ** $p < 0.01$ and # $p < 0.001$ vs. vehicle control by unpaired t test (two-tailed). n.s. = Not significantly different ($p > 0.05$).

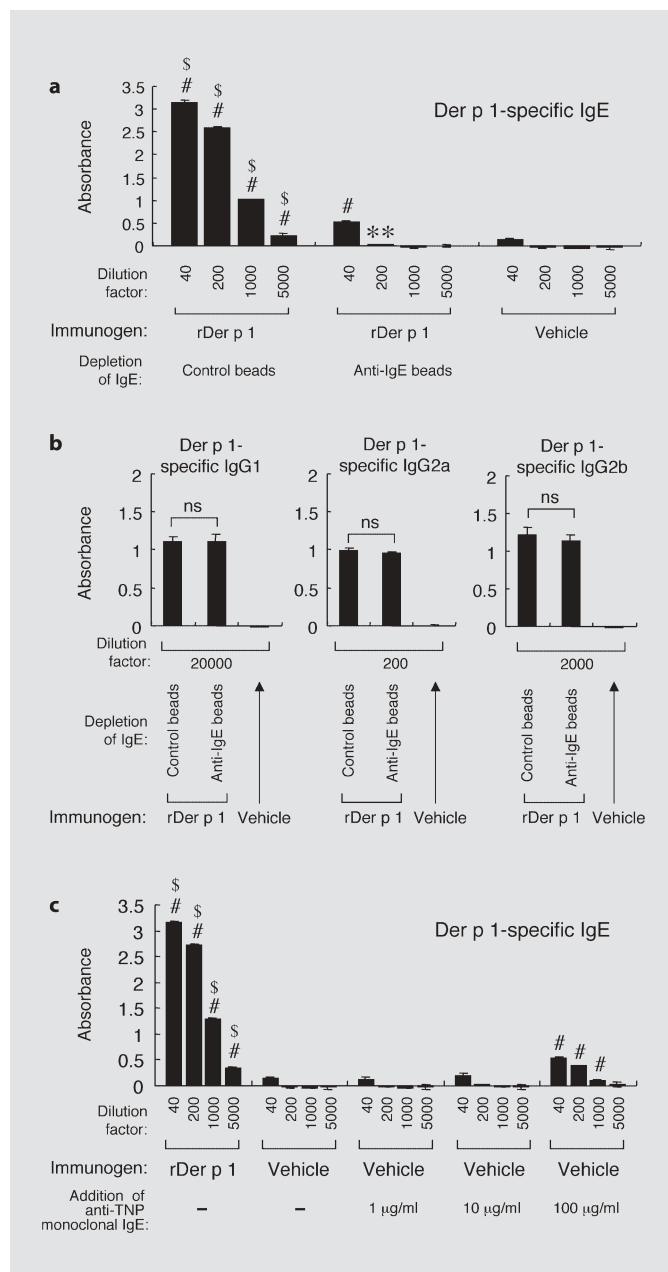


Fig. 5. Confirmation of isotype specificity to IgE and evaluation of nonspecific binding of IgE to the plate. Activated rDer p 1 (Der p 1-N52Q, 2.5 µg/head) or buffer alone was mixed with alum and injected into CBA/J mice. Two months after the last intraperitoneal injection, the mice were challenged intranasally twice with rDer p 1 (10 µg/head) or buffer alone. Enhancer solutions (Can Get Signal; Toyobo) were used for the dilution of the sera and secondary Abs. Signals for Der p 1-specific Abs detected by the procedures shown in figure 2b for IgE (**a**, **c**) and figure 2c for IgGs (**b**). rDer p 1-immunized pooled serum was treated with anti-IgE beads or control beads. **c** Pooled serum of the vehicle was added to 1, 10 or 100 µg of monoclonal IgE specific to TNP (purified mouse IgE isotype control, clone IgE-3; BD Biosciences)/ml of serum. Dilution factor: factors for serum dilution. Data shown

Addition of monoclonal IgE, which is not specific for Der p 1, to the pooled serum of the vehicle control did not significantly increase the signals at 1 and 10 µg of IgE/ml of serum compared with the vehicle control without additional IgE (fig. 5c). Although the signals at 100 µg of IgE/ml of serum were stronger than for the vehicle control, the titer was much lower (approximately 1/25) than with the rDer p 1-immunized serum, which contained a total of 3.5 µg/ml of IgE.

Discussion

A number of methods have been employed to estimate concentrations of allergen-specific IgE in immunized mice, including passive cutaneous anaphylaxis, skin testing [21], degranulation assay using a rat basophilic leukemia cell line [22], immunoblotting, and ELISA using plastic microtiter plates. ELISA for the detection of allergen-specific IgE using allergen-coated plates has the advantage of high throughput, reproducibility of results and simple handling. However, low signal intensity for allergen-specific murine IgE has been a problem, although the total concentration of IgE in serum can be measured by sandwich ELISA (fig. 1a, 2a, 3a, 4a). In the present study, we demonstrated that the in-well pretreatment of murine sera with protein G-coupled sepharose beads (fig. 1c) or the use of commercially available enhancer solutions (fig. 2d, e, 3b, 4b) enhances the signals for the binding of allergen-specific IgE to allergen-coated plates. As the in-well pretreatment of sera with protein G beads needs only a small volume of serum (1/10 dilution, 50 µl of diluted and pretreated serum/well) (fig. 1b) and achieves the simultaneous treatment of many sera, there are advantages over a method using a packed protein G column for the removal of IgG [3]. In the latter system, by simply using enhancer solutions for the dilution of the sera and secondary Ab, a more extensive serum dilution (1/50, 1/200 or less dilution; 50 µl of diluted serum/well) was possible for the detection of the signal and the removal of IgG was not necessary, although the incubation

represent the means $\pm$ SD for 3 wells of each of the pooled sera. ** $p < 0.01$ and # $p < 0.001$ vs. vehicle (**a**, **c**), \$ $p < 0.001$ (control beads) vs. anti-IgE beads (**a**) and \$ $p < 0.001$ (rDer p 1) vs. vehicle supplemented with 100 µg/ml of IgE (**c**) by the Tukey post hoc test followed by one-way ANOVA. n.s. = Not significantly different ($p > 0.05$) by unpaired t test (two-tailed) (**b**).

was prolonged (fig. 2b). The former and latter systems take 2 and 3 days, respectively, from the coating of plates with allergens to measurements of absorbance. Although both methods are effective, the latter has remarkable advantages over conventional methods in terms of signal intensity and handling.

Although the total concentration of IgE on immunization with rDer p 1, rDer f 1 or rDer f 2 was higher than or similar to that on immunization with OVA (fig. 1a, 2a, 3a), the signal for IgE specific to Der p 1, Der f 1 or Der f 2 was weaker than that for OVA-specific IgE (fig. 1c, 2d, e, 3b, c). Although one possible explanation for the results is that the efficiency of the immobilization of Der p 1-WT, Der f 1-WT and rDer f 2 could be lower than that of OVA, other explanations could also be envisaged, such as the fact that the ratio of total to specific IgE could be influenced by the nature of the administered protein. Very recently, we have reported on the commitment of proteolytic activity of rDer p 1 to sensitization towards IgE and IgG responses [23]. Both methods, in-well pretreatment of the murine sera with protein G beads and the use of enhancer solutions, are considered applicable to various allergens.

Pretreatment of the sera with protein G beads enhances signals for allergen-specific IgE because of the removal of allergen-specific IgG, which competitively inhibits the binding of allergen-specific IgE to the allergen immobilized on the plates. In our preliminary experiments, more than 95% of the allergen-specific IgG1 and approximately 20% of the total IgE were removed by the pretreatment (unpubl. data). However, in the case of the mice used for the experiment in figure 5, some individual sera showed strong signals for Der p 1-specific IgE even without the treatment with protein G beads and the signals were equivalent in intensity to those for sera with the treatment, suggesting that a different administration protocol might influence the affinity and amount of allergen-specific IgE and the ratio of allergen-specific IgE versus allergen-specific IgG, which are considered to affect the intensity of the signal for allergen-specific IgE.

The enhancer solutions remarkably strengthened the signals for allergen-specific IgE in spite of the presence of abundant allergen-specific IgG (fig. 2f, g). The composition of the enhancer solutions and the mechanism for the signal enhancement have not been revealed by the manufacturer. Ionic strength or some additives might be designed to stabilize the molecular interaction between the antigen and Ab and between the Ab and secondary Ab to decrease the dissociation rate in the enhancer solutions. In our experience, the use of the second of the enhancer

solution 2 seems critical to the enhancement of signals for allergen-specific IgE, although both solution 1 and solution 2 contribute to the enhancement (unpubl. data). The IgE in mice immunized with an allergen did not bind to allergens with which the mice were not immunized, indicating that the binding of IgE to the plates is allergen-specific and nonspecific binding is negligible (fig. 3b, c). Results using serum depleted of (fig. 5a, b) or supplemented with IgE (fig. 5c) also support the isotype specificity of IgE and indicate that the nonspecific binding of IgE to the plate is negligible below a total IgE concentration of 10 µg/ml and that titration will be useful for the appropriate evaluation of allergen-specific IgE even at higher concentrations such as 100 µg/ml. The ELISA system using the enhancer solutions is applicable to sera from 3 mouse strains with differences in genetic background, the strength of the IgE response and the optimal dose of antigen to induce a maximum IgE response. Upon rDer f 1 immunization, CBA/J mice (H-2^k) showed a higher IgE response even at a relatively low dose (fig. 1–3), and BALB/c (H-2^d) and C57BL/6 mice (H-2^b) showed a lower response (fig. 4). Furthermore, even though no significant increase in the total concentration of IgE, which includes nonspecific IgE and allergen-specific IgE, was observed in C57BL/6 mice immunized with rDer f 1 (fig. 4a, C57BL/6), a significant increase in the level of Der f 1-specific IgE was detected (fig. 4b, C57BL/6), implying that the signal for allergen-specific IgE measured by the ELISA system is highly sensitive and more reliable as an indicator of whether the murine model tested is suitable for the study of allergies than total IgE.

In summary, we evaluated the effects of the in-well pretreatment of sera with protein G-coupled sepharose beads and the use of immunoreaction enhancer solutions, which have recently become commercially available, on the enhancement of the signals for the binding of allergen-specific IgE to plates coated with recombinant major house dust mite allergens or OVA. Although we demonstrated that both methods are effective and did not compare them under the same conditions, the use of enhancer solutions is more convenient because it allows the detection of the signals at more extensive dilutions of sera and needs no protein G treatment. An ELISA simply using the enhancer solutions, which obtains remarkably strengthened signals for allergen-specific IgE, would be useful for the study of allergies with murine models. In addition, this paper showed that recombinant forms of the major mite allergens Der p 1, Der f 1 [12–17, 23] and Der f 2 [18–20, 24] can elicit a significant allergen-specific IgE response in mice.

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