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ORIGINAL ARTICLE

Enzyme-Linked Immunosorbent Assays with High Sensitivity for Antigen-Specific and Total Murine IgE: A Useful Tool for the Study of Allergies in Mouse Models

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ABSTRACT

Background: In studies on allergies in mouse models, IgE production is an essential parameter to be evaluated. Here, we examine the effect of commercially available immunoreaction enhancer solutions and different blocking reagents in enzyme-linked immunosorbent assay (ELISA) for total or antigen-specific murine IgE in order to improve the assays.

Methods: Sera from mice immunized with recombinant house dust mite major allergens, Der f 1 and Der p 1, were used for the assays. Total IgE was measured by sandwich ELISA using monoclonal antibodies against murine IgE. Antigen-specific IgE was assayed using allergen-coated plates. Sensitivity or signal intensity in ELISA was compared among conditions differing in the use of enhancer solutions, blocking reagents, or monoclonal antibodies, and incubation time.

Results: Use of enhancer solutions improved the sensitivity of ELISA for total IgE by approximately 30-fold of that using a conventional buffer. A blocking reagent caused more unwanted enhancement of the background signal in blank wells in ELISA for total IgE compared with another blocking reagent, however, improved signal intensity in ELISA for antigen-specific ELISA without significant enhancement of the background signal. Optimal assay conditions were determined.

Conclusions: Enhancer solutions are effective in improving ELISAs for total and antigen-specific murine IgE. Selection of blocking reagents was important to decrease unwanted enhancement of background signals and was effective in enhancing signals for positive samples. The ELISAs improved in this study are useful for the study of allergies in mouse models.

KEY WORDS

allergen-specific IgE, blocking reagent, enzyme-linked immunosorbent assay, immunoreaction enhancer solution, mouse model, total IgE

INTRODUCTION

Immediate hypersensitivity is caused by the release of mediators from mast cells and basophils activated through aggregation of the high-affinity IgE receptor (FcεRI) at the cell surface and allergen-specific IgE, crosslinked by multivalent allergens such as those derived from house dust mites, and pollen.[1,2] Clini-

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cally, elevated levels of total or allergen-specific IgE in serum are a major manifestation of allergic diseases; therefore, in the study of allergies in mouse models, IgE production is an important parameter for evaluation.

Total IgE can be detected with a sandwich enzyme-linked immunosorbent assay (ELISA) using a set of 2 anti-murine IgE monoclonal antibodies (mAbs).[3] Although weak signals for antigen-specific (Ag-specific) murine IgE have been a problem, we have recently reported on the enhancement of signal intensity in ELISA for murine IgE specific, not only to ovalbumin, but also recombinant forms of clinically relevant major mite allergens, using commercially available immunoreaction enhancer solutions.[4] Ag-specific IgE ELISA simply modified by enhancer solutions has remarkable advantages over conventional methods in terms of signal intensity and handling, even over a method with pretreatment of sera with protein G-coupled beads.[4-6] However, further improvement of these assays for total IgE or Ag-specific IgE is necessary because, for example, only small volumes of sera can be collected intravenously from mice at intervals without causing death, particularly in younger mice, and the relatively long incubation time in modified Ag-specific IgE ELISA, which takes 3 days from coating plates with allergens to absorbance measurement,[4] still needs to be shortened.

Here, we examine the effect of enhancer solutions and different blocking reagents on ELISA for total or Ag-specific murine IgE, to improve the sensitivity for detection or the signal intensity, and to shorten the time required for the assay.

METHODS

RECOMBINANT Der f 1 AND Der p 1

Recombinant mature forms of house dust mite major group 1 allergens, Der f 1 and Der p 1, without (Der f 1-N53Q and Der p 1-N52Q) and with yeast-derived hyperglycosylation (Der f 1-WT and Der p 1-WT), were prepared as previously described.[7-11] Der f 1-N53Q (rDer f 1) and Der p 1-N52Q (rDer p 1) were used for immunization after incubation with *L*-cysteine.[3,4,12] Since B-cell epitopes for recombinant forms with and without hyperglycosylation are considered to be equivalent among natural Der f 1, rDer f 1, and Der f 1-WT and natural Der p 1, rDer p 1, and Der p 1-WT,[8,9] we used hyperglycosylated Der p 1-WT and Der f 1-WT to detect Der p 1- and Der f 1-specific Abs as alternatives to unglycosylated rDer p 1 and rDer f 1, respectively, the production of which is much less than that of the hyperglycosylated forms in yeast.[11]

IMMUNIZATION OF MICE

Six-to-8-week-old female CBA/J mice were purchased from Charles River Japan, Inc. (Yokohama, Japan) and immunized as previously described.[3,4,12] Mice were given 4 weekly intraperitoneal injections of 0.5 or 1 μg of rDer f 1 or 1, 2, or 2.5 μg of rDer p 1 adsorbed on Alum (ImjectAlum; Pierce, Rockford, IL, USA). Sera collected at 1 week after the last injection were stored at −20℃ before assays. Sera used for the experiment in Figures 4, 6 were collected after the mice were challenged intranasally, twice with rDer p 1 (10 μg/head) 2 months after the last intraperitoneal injection.

IMMOBILIZATION OF MONOCLONAL ANTIBODIES AND ALLERGENS AND BLOCKING

Anti-murine IgE monoclonal antibodies (mAbs) (2 μg/ml, 50 μl/well) or allergens (10 μg/ml, 50 μl/well) were diluted with phosphate-buffered saline (PBS), and 96-well microtiter plates for ELISA (MaxiSorp; Nunc, Rockilde, Denmark) were incubated overnight with the allergen solutions at 4℃. After 1 or 2 washes with PBS containing 0.05% (v/v) Tween 20 (PBST), the plates were incubated with BlockAce (BA) (DS Pharma Biomedical Co. Ltd., Osaka, Japan) diluted with pure water (1/4 dilution), or with ImmunoBlock (IB) (DS Pharma Biomedical Co. Ltd.) diluted with pure water (1/5 dilution) for 1.5 hours at 37℃ or 3 hours at room temperature (200 μl/well). After 2 washes with PBST, the plates were incubated with diluted sera.

ELISA FOR TOTAL IgE

The total concentration of IgE in the sera was measured by sandwich ELISA using a combination of a non-labeled anti-murine IgE mAb (clone R35−72; BD Biosciences, San Jose, CA, USA) and a horseradish peroxidase (HRP)-labeled anti-murine IgE mAb (clone LO-ME-2; Technopharm Biotechnology, Paris, France) or another non-labelled anti-murine IgE mAb (clone LOME-3; Technopharm Biotechnology), and HRP-labeled mAb (clone LOME-2) with murine monoclonal IgE specific to TNP (purified mouse IgE isotype control, clone IgE-3; BD Biosciences) as the standard. Volumes of 50 μl of standard IgE or sera diluted with Solution 1 of CanGetSignal (CGS1) (Toyobo, Osaka, Japan), PBST with 10% (v/v) BA (PBST-BA), or PBST with 5% (v/v) IB (PBST-IB) were added to the wells on the plates, which were coated with each of the anti-murine IgE mAbs and blocked as described above. Serum dilution factors were 200 or 1000. After incubation for 80 minutes at 37℃ and 3−5 washes with PBST, HRP-conjugated anti-murine IgE mAb (clone LO-ME-2) diluted with Solution 2 of CanGetSignal (CGS2) (Toyobo), PBST-BA, or PBST-IB was added to the plates (50 μl/well). After incubation for 80 minutes at 37℃ and 3−5 washes with PBST, the color was developed for 20 minutes using tetramethyl benzidine (BD-OptEIA kit; BD Biosciences) (100 μl/well), and the reaction was terminated by adding 2N sulfuric acid (50 μl/well). Optical density was measured at 450 nm, from which

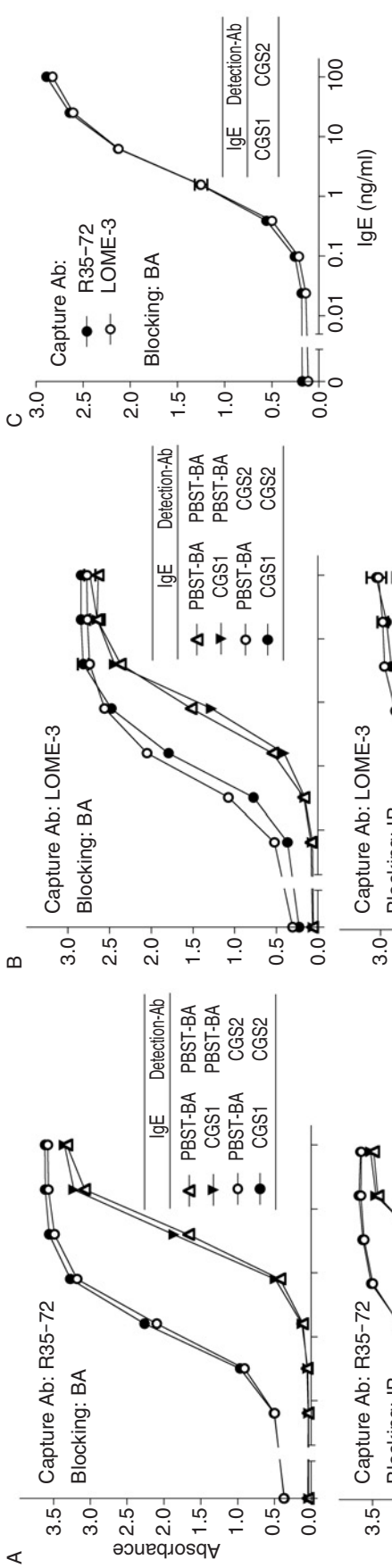

Fig. 1 Effect of blocking reagents and enhancer solutions in total IgE ELISA. **A** and **B**: Effect of blocking reagents (BA, BlockAce; IB, ImmunoBlock) and enhancer solutions (CGS1 and CGS2). **C**: Comparison of the 2 capture mAbs. Anti-murine IgE mAbs immobilized onto plates are R35−72 (**A** and **C**) and LOME-3 (**B** and **C**) (2 μg/ml). Dilution factors for detection mAb were 4000. Color was developed for 20 min (**A** and **C**) or 25 min (**B**). Data show the means ± SD of triplicate wells. Absorbance of the air blank is set at zero.

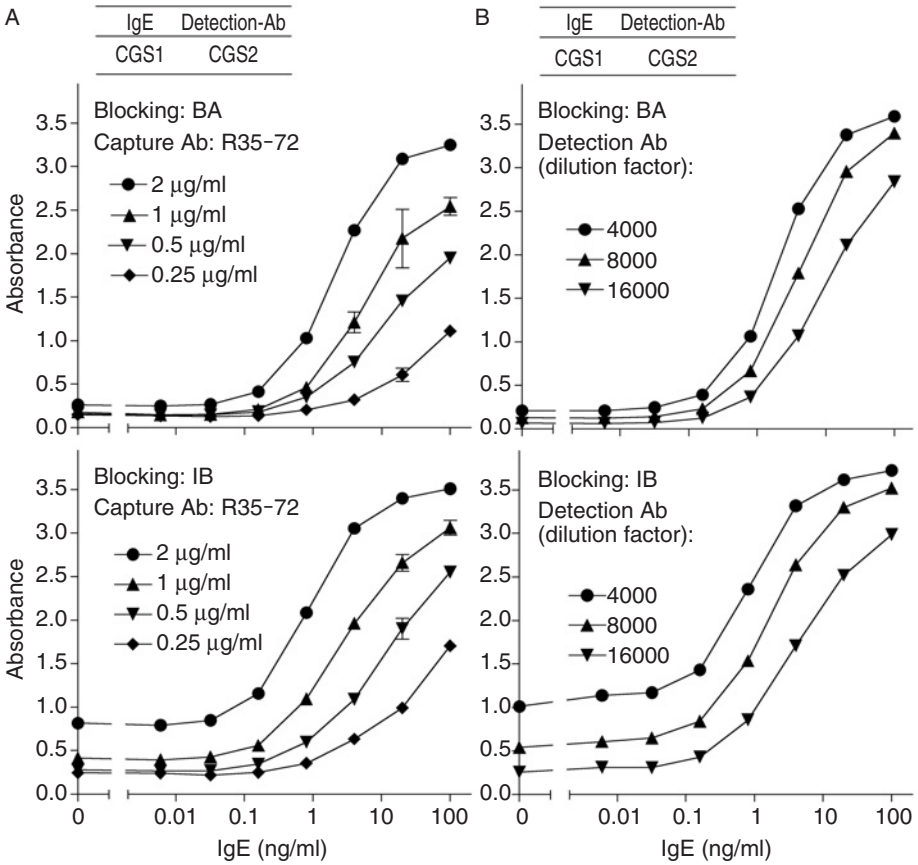

Fig. 2 Effect of concentrations of capture and detection mAbs in total IgE ELISA. Anti-murine IgE mAb, clone R35－72, was immobilized onto the plates at concentrations indicated (**A**) or at 2 μg/ml (**B**). BlockAce (BA) or ImmunoBlock (IB) was used for blocking. Dilution factors for detection mAb were 4000 (**A**) or as indicated (**B**). Data show the means ± ranges of duplicated wells. Absorbance of the air blank is set at zero.

570 nm was subtracted. Detailed conditions of the assay are described in the figure legends.

ELISA FOR Ag-specific IgE

Volumes of 50 μl of sera diluted with CGS1, PBST-BA, or PBST-IB were added to the wells on the plates, which were coated with allergens and blocked as described above. After incubation and 3–5 washes with PBST, HRP-conjugated anti-murine IgE mAb (clone LO-ME-2) diluted with CGS2, PBST-BA, or PBST-IB was added to the plates (50 μl/well). After incubation and 3–5 washes with PBST, color was developed and the reaction was terminated in a manner similar to the total IgE ELISA described above. Optical density at 450 nm, from which 570 nm was subtracted, was used as the signal for Ag-specific IgE. Detailed conditions of the assay are described in the figure legends.

REMOVAL OF IgE FROM SERUM

Anti-IgE beads were prepared by covalent coupling of anti-murine IgE mAb (clone R35–72) to CNBr-activated Sepharose 4B beads and used to remove IgE from sera, as described previously.[4] Sera treated with control beads, with no protein coupling, were also prepared.

RESULTS

EFFECT OF BLOCKING REAGENTS AND ENHANCER SOLUTIONS IN TOTAL IgE ELISA

The effect of blocking reagents (BA and IB) and enhancer solutions (CGS1 and CGS2) in sandwich ELISA for murine IgE was examined (Fig. 1, 2). The use of CGS1 to dilute standard IgE did not affect the detection sensitivity and background absorbance in blank wells (Fig. 1A, B, triangles). CGS2 to dilute the detection mAb improved detection sensitivity by approximately 30-fold but increased the background absorbance in blank wells (Fig. 1A, B, circles). The increase in background absorbance was higher when using IB for blocking (Fig. 1A, B, lower panels) than BA (Fig. 1A, B, upper panels). Similar effects were observed when using both the 2 capture mAbs, R35–72 (Fig. 1A) and LOME-3 (Fig. 1B). The dose-

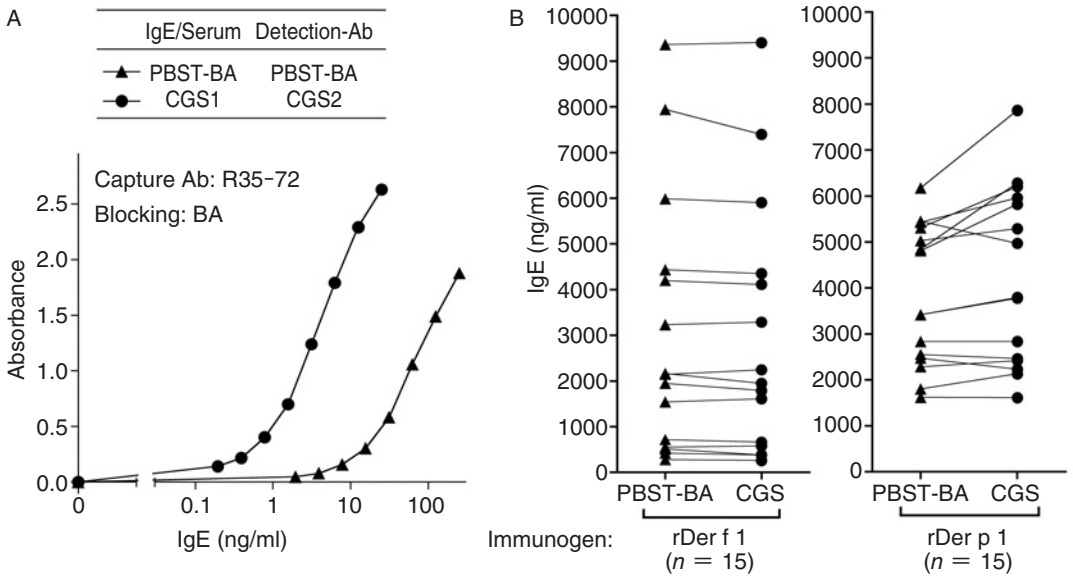

Fig. 3 Comparison of IgE concentrations determined by total IgE ELISAs using conventional buffers and enhancer solutions. **A**: Standard curves. Anti-murine IgE mAb, clone R35－72, was immobilized onto the plates at 2 μg/ml. BlockAce (BA) was used for blocking. Dilution factor for detection mAb was 4000. Data show the means ± ranges of duplicated wells. Absorbance of blank wells with no addition of standard IgE is set at zero. **B**: IgE concentration determined by using conventional buffer (PBST-BA) or enhancer solutions (CGS). Individual sera from 15 mice intraperitoneally immunized with rDer f 1 (0.5 or 1 μg/head) or rDer p 1 (1 or 2 μg/head), respectively, were used for the assay.

dependent curves in the 2 capture mAbs almost completely overlapped when using BA for blocking (Fig. 1C). The results shown in Figure 1 indicate that using CGS2 to dilute the detection mAb is strikingly effective, that BA for blocking exhibits lower background absorbance in blank wells than blocking reagent IB, and that the 2 capture mAbs have similar potential.

Concentrations of capture and detection mAbs when using CGS1 to dilute standard IgE, and CGS2 to dilute detection mAb, were examined (Fig. 2). The background absorbance in blank wells to which no standard IgE was added (Fig. 2, IgE: 0 ng/ml) decreased, corresponding to the decrease in the concentrations of capture (Fig. 2A) and detection mAbs (Fig. 2B) particularly when using IB for blocking (Fig. 2, lower panels). The results shown in Figure 2 suggest that the increase in background absorbance when using CGS2 to dilute detection mAb (Fig. 1A, B) could be due to the enhancement of direct binding of detection mAb to immobilized capture mAb.

COMPARISON OF IgE CONCENTRATIONS DETERMINED BY TOTAL IgE ELISAs USING CONVENTIONAL BUFFERS AND ENHANCER SOLUTIONS

According to the results shown in Figures 1, 2, we considered the following conditions for total IgE ELISA to be the optimum: 2 μg/ml for the concentration of capture mAb R35–72, BA for blocking, CGS1 for dilution of standard IgE and sera, and CGS2 for dilution (1/4000 dilution) of the capture mAb. We examined whether these conditions were suitable to measure total IgE in mouse sera by comparing with conventional assay conditions previously described[3] (Fig. 3). Detection sensitivity improved by approximately 30-fold when using the enhancer solutions (Fig. 3A). The determined IgE concentrations in 30 individual sera from mice immunized with rDer f 1 or rDer p1 were almost identical for both conventional (Fig. 3B, PBST-BA) and improved assay conditions (Fig. 3B, CGS). The results indicate that the improved assay condition is suitable for measurement of total IgE.

EFFECT OF BLOCKING REAGENTS AND BUFFERS ON DILUTION OF SERA AND DETECTION mAb IN Ag-specific IgE ELISA

The effect of blocking reagents and enhancer solutions in ELISA for murine Ag-specific IgE in pooled serum from mice immunized with rDer p 1, used in our previous study[4] (Fig. 4), or from mice immunized with rDer f 1 (Fig. 5), was examined. Previously, we reported that the use of enhancer solutions, CGS1 and CGS2, significantly enhanced the signal intensity in Ag-specific IgE ELISA, when BA was used for blocking.[4] When using CGS2 to dilute the detection mAb (Fig. 4), signal intensity was increased when us-

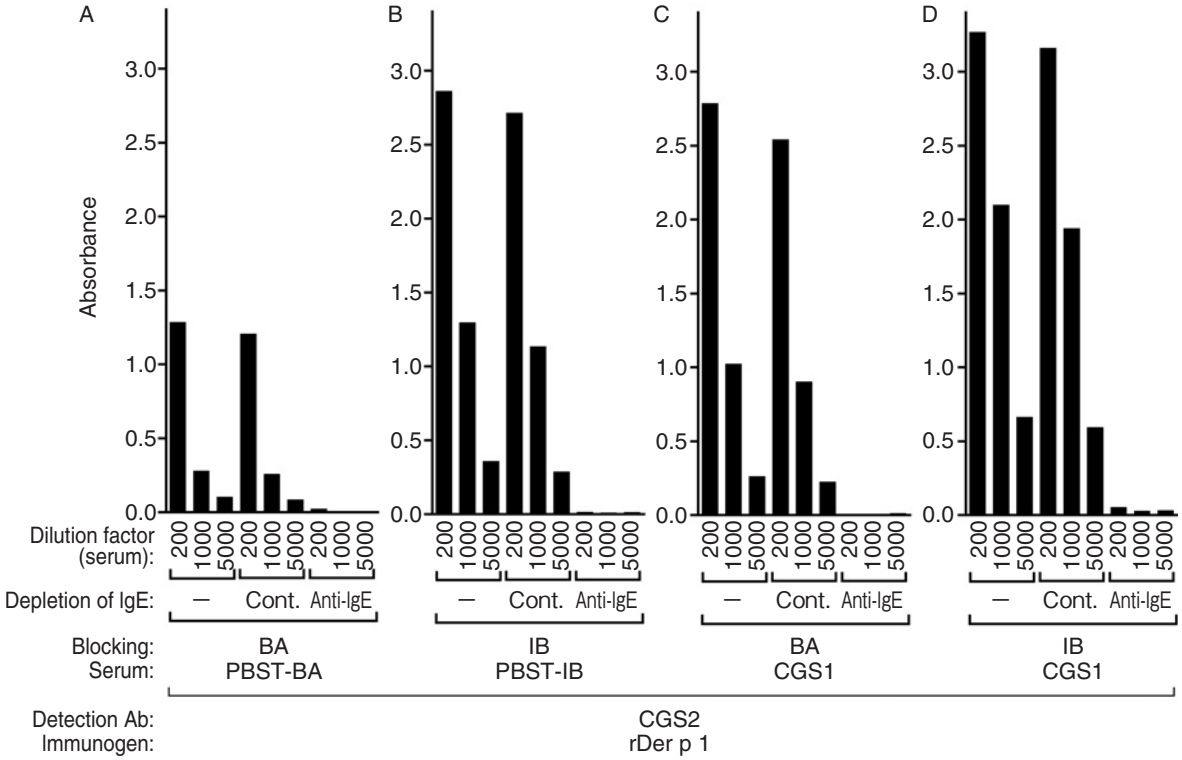

Fig. 4 Effect of blocking reagents and enhancer solutions for serum dilution in Ag-specific IgE ELISA and confirmation of IgE isotype specificity. Der p 1-WT was immobilized onto the plates at 10 μg/ml. BlockAce (BA) (**A** and **C**) or ImmunoBlock (IB) (**B** and **D**) was used for blocking. Sera were anti-rDer p 1 pooled serum from mice intraperitoneally immunized with rDer p 1 (2.5 μg/head) and then intranasally administrated with rDer p 1 (10 μg/head) (rDer p 1) and those treated with anti-IgE beads (Anti-IgE) or control beads (Cont.), and diluted with PBST-BA (**A**), PBST-IB (**B**), or CGS1 (**C** and **D**). Incubation with sera was for 15 hours at 4℃. Incubation with detection mAb diluted with CGS2 was for 5 hours at room temperature. Dilution factor for detection mAb was 2000. Data show the values for single wells. Absorbance of triplicate blank wells with no addition of sera (means ± SD: 0.143 ± 0.006 in **A**, 0.255 ± 0.002 in **B**, 0.233 ± 0.008 in **C**, and 0.344 ± 0.001 in **D**) is set at zero.

ing IB for blocking (Fig. 4B), CGS1 for serum dilution (Fig. 4C), or a combination of both (Fig. 4D). Compared with previously published data,[4] CGS2 for dilution of detection mAb was effective to enhance the signal intensity even when using BA for blocking and PBST-BA for serum dilution (Fig. 4A). Isotype specificity for IgE was confirmed using a pooled serum treated with anti-IgE beads (Fig. 4, Anti-IgE).

When using IB for blocking (Fig. 5), CGS2 used to dilute detection mAb was extremely effective in increasing signal intensity (Fig. 5C, D). However, compared with the marked enhancement observed by using CGS2 to dilute the capture mAb, CGS1 for serum dilution was not so effective.

EFFECT OF BLOCKING REAGENTS AND INCUBATION TIME IN Ag-specific IgE ELISA USING ENHANCER SOLUTIONS

As IB for blocking was more effective for signal enhancement than BA (Fig. 4, 5), we further examined the effect of IB to shorten the incubation time (Fig. 6, 7). Using the anti-Der p 1 pooled serum from Figure 4, the effect of IB (Fig. 6B, C) in 2 incubation schedules was compared with that of BA in a previous condition (Fig. 6A). When following the same schedule for the duration of incubation (overnight for serum and 5 hours for detection mAb), blocking with IB (Fig. 6B) enhanced signal intensity more than blocking with BA (Fig. 6A), although nonspecific binding of IgE to the plate was also enhanced by adding anti-TNP IgE (10 μg/ml and 100 μg/ml) to the pooled serum from vehicle-treated mice (Fig. 6B, Vehicle). Blocking with IB under a shortened incubation schedule (2 hours for serum and 2 hours for detection mAb) (Fig. 6C), showed similar signal intensity to blocking with BA under the longer schedule (Fig. 6A) without enhancement of nonspecific IgE binding to the plate.

Using 35 individual sera from mice immunized with rDer f 1 (Fig. 7A) or rDer p 1 (Fig. 7B), we examined whether the conditions using IB for blocking are suitable for detection of Ag-specific IgE by com-

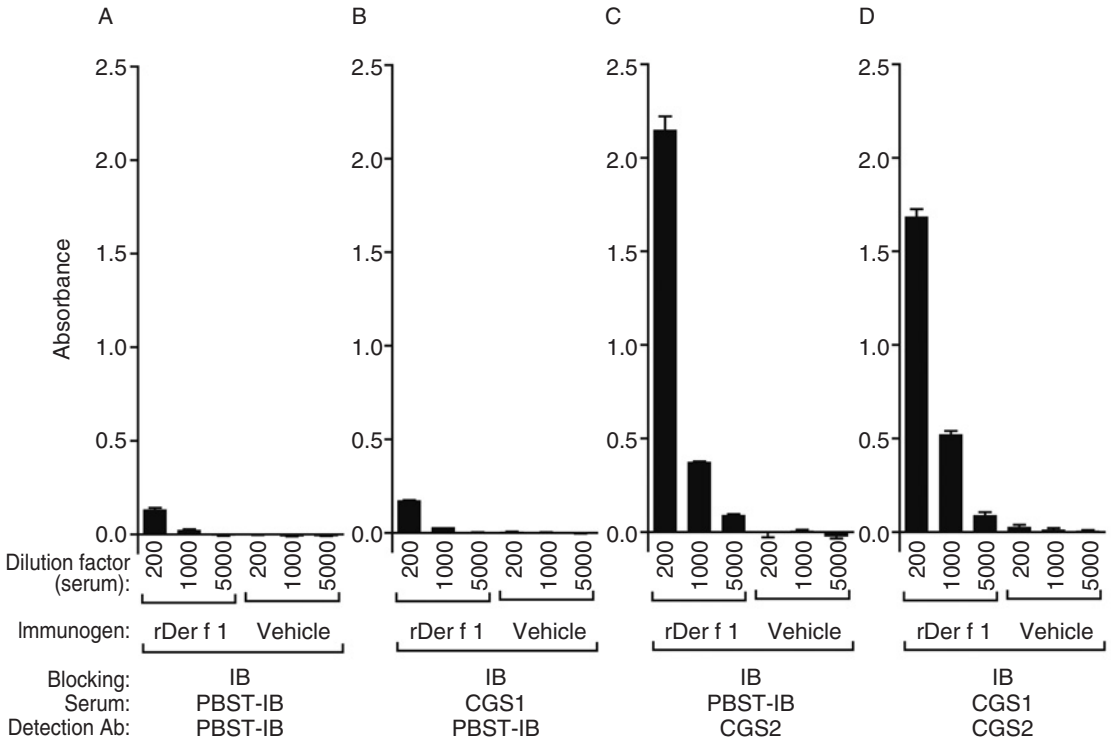

Fig. 5 Effect of enhancer solutions in Ag-specific IgE ELISA. Der f 1-WT was immobilized onto the plates at 10 μg/ml. ImmunoBlock (IB) was used for blocking. Sera were anti-rDer f 1 pooled serum from mice intraperitoneally immunized with rDer f 1 (1 μg/head) (rDer f 1) and control pooled serum (Vehicle), and diluted with PBST-IB (**A** and **C**) or CGS1 (**B** and **D**). Incubation with the pooled sera was for 15 hours at 4℃. Incubation with detection mAb diluted with PBST-IB (**A** and **B**) or CGS2 (**C** and **D**) (1/2000 dilution) was for 5 hours at room temperature. Data show the means ± SD of triplicate wells. Absorbance of triplicate blank wells with no addition of sera (means ± SD: 0.054 ± 0.007 in **A**, 0.049 ± 0.006 in **B**, 0.255 ± 0.031 in **C**, and 0.264 ± 0.023 in **D**) is set at zero.

paring them with assay conditions using BA. Overall, the order of signals for the sera was similar among the conditions tested (Fig. 7). Signal intensity was at its highest when blocking with IB, a 15-hour incubation with sera, and a 5-hour incubation with detection mAb. In addition, 2 other conditions (blocking with BA, 15-hour incubation with sera, and 5-hour incubation with detection mAb; and blocking with IB, 15-hour incubation with sera, and 2-hour incubation with detection mAb) showed a similar signal intensity, and the shortest condition (blocking with IB, 2-hour incubation with sera, and 2-hour incubation with detection mAb) showed a similar or slightly less signal intensity. The results indicate that the assay conditions using IB for blocking are most suited to detect Ag-specific IgE.

DOSE-DEPENDENCY OF ABSORBANCE WITH Ag-concentration IN THE Ag-immobilization STEP

Using the anti-Der p 1 pooled serum from Figures 4, 6, we examined the dose-dependency of absorbance with Ag-concentration in the Ag-immobilization step (Fig. 8). Absorbance increased in a dose-dependent manner in the short assay condition (blocking with IB, 2-hour incubation with sera, and 2-hour incubation with detection mAb) using the enhancer solutions (Fig. 8, solid dots with solid lines). Without the use of enhancer solutions, a dose-dependent increase of absorbance was very small (Fig. 8A, triangles). Pooled serum from vehicle-treated mice (Fig. 8B, Vehicle, circles with broken lines) showed similar absorbance levels to that when no additions of serum were made (Fig. 8B, No serum).

DISCUSSION

When studying allergies in mouse models, IgE production is an important parameter for evaluation. In this study, we examined the effect of enhancer solutions and different blocking reagents on ELISA for total or Ag-specific murine IgE, in order to improve the detection sensitivity or signal intensity or to shorten the time of the assay. The application of enhancer solutions was effective in improving ELISAs for total (Fig. 1–3)and Ag-specific murine IgE (Fig. 4–8). Selection of blocking reagents was important to de-

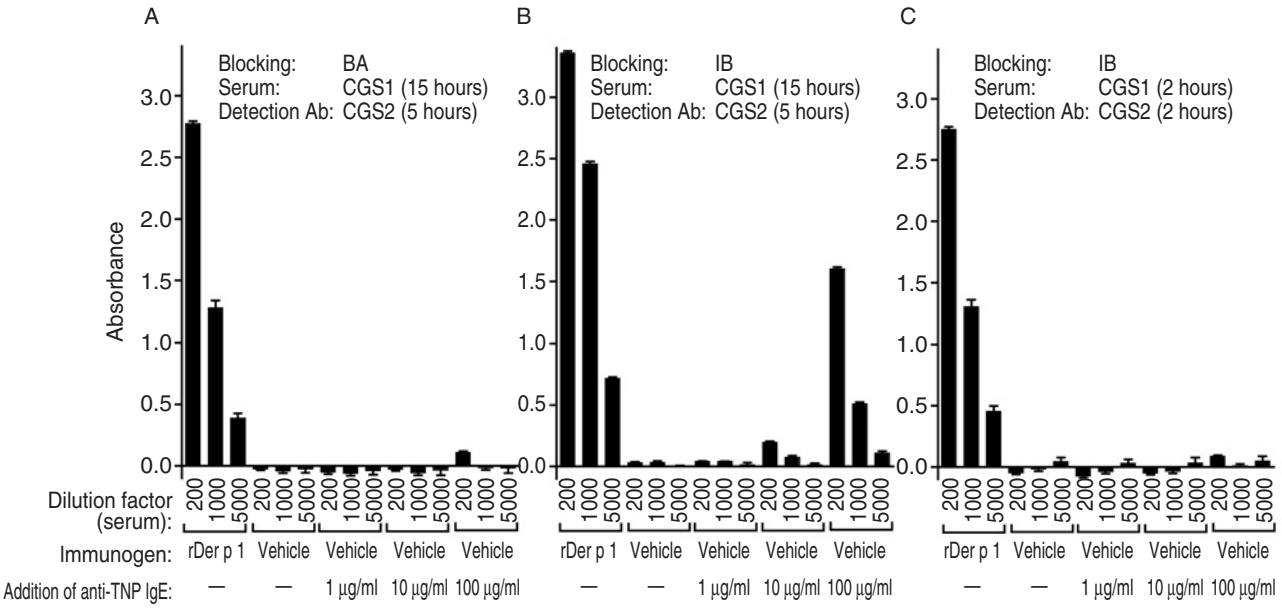

Fig. 6 Effect of blocking reagents, enhancer solutions, and incubation time in Ag-specific IgE ELISA and evaluation of nonspecific binding of IgE to the plate. Der p 1-WT was immobilized onto the plates at 10 μg/ml. BlockAce (BA) (**A**) or ImmunoBlock (IB) (**B** and **C**) was used for blocking. Sera were anti-rDer p 1 pooled serum from Fig. 4 and control pooled serum (Vehicle), and diluted with CGS1. Pooled serum of the vehicle was added to 1, 10, or 100 μg of monoclonal IgE specific to TNP/ml of serum. Incubation with the pooled sera was for 15 hours at 4℃ (**A** and **B**) or 2 hours at 37℃ (**C**). Incubation with detection mAb diluted with CGS2 (1/2000 dilution) was for 5 hours at room temperature (**A** and **B**) or 2 hours at 37℃ (**C**). Data show the means ± SD of triplicate wells. Absorbance of blank wells with no addition of sera in each assay condition is set at zero.

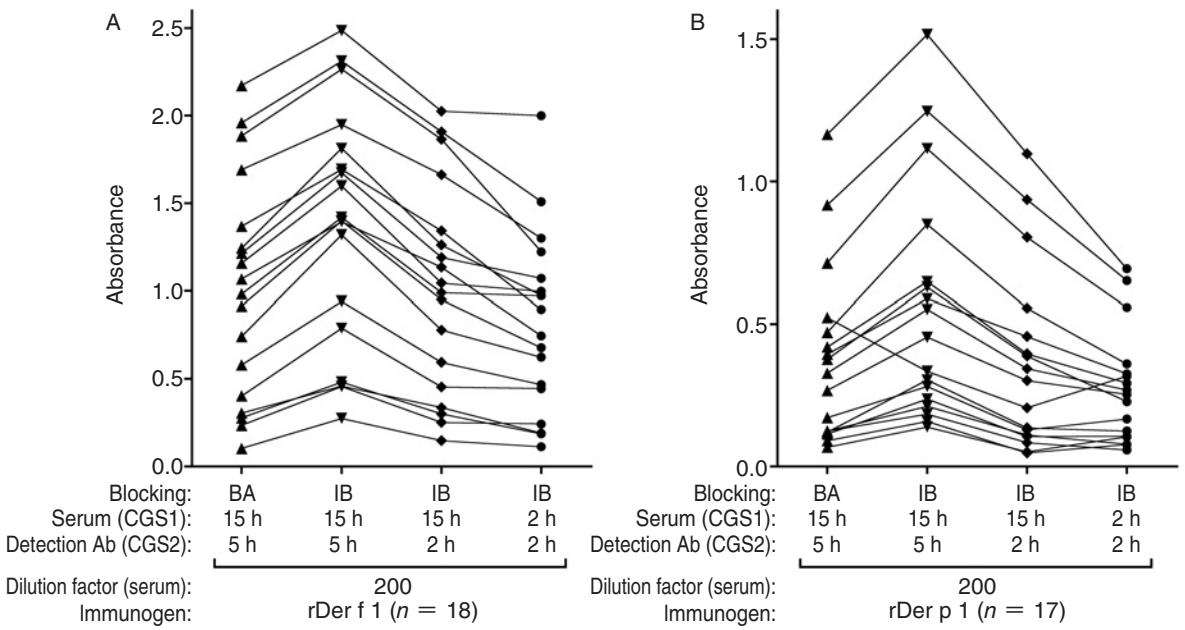

Fig. 7 Comparison of signal intensity among different assay conditions in Ag-specific IgE ELISA. Der f 1-WT (**A**) or Der p 1-WT (**B**) was immobilized onto the plates at 10 μg/ml. BlockAce (BA) or ImmunoBlock (IB) was used for blocking as indicated. Individual sera from 18 or 17 mice intraperitoneally immunized with rDer f 1 (0.5 or 1 μg/head) or rDer p 1 (1 or 2 μg/head), respectively, diluted with CGS1 (1/200 dilution) were used. Incubation with the sera was for 15 hours at 4℃ or 2 hours at 37℃. Incubation with detection mAb diluted with CGS2 (1/2000 dilution) was for 5 hours at room temperature or 2 hours at 37℃. Absorbance of blank wells with no addition of sera in each assay condition is set at zero.

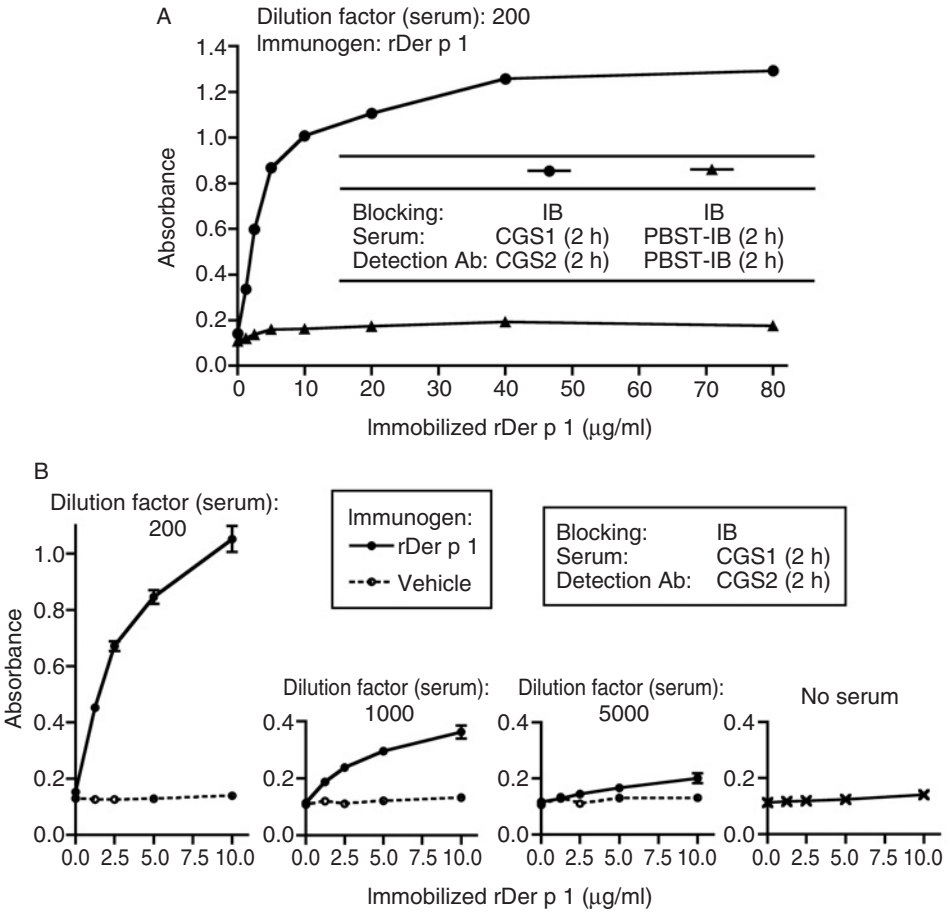

Fig. 8 Dose dependency of absorbance with Ag-concentration in the Ag-immobilization step. Der p 1-WT was immobilized onto the plates at 1.25 − 80 μg/ml. ImmunoBlock (IB) was used for blocking. **A**: Anti-rDer p 1 pooled serum from Figure 4, 6 was diluted with CGS1 or PBST-IB (1/200 dilution). Incubation with the sera was for 2 hours at 37℃. Incubation with detection mAb diluted with CGS2 (1/4000 dilution) or PBST-IB (1/2000 dilution) was for 2 hours at 37℃. **B**: Sera were the anti-rDer p 1 pooled serum (rDer p 1) and control pooled serum (Vehicle), and diluted with CGS1 (1/200, 1/1000, or 1/5000 dilution). No serum: CGS1 with no addition of serum was added to wells. Data show the values for single wells in **A** and the means ± ranges of duplicated wells in **B**. Differently from Figure 4 − 7, absorbance of air blank is set at zero.

crease unwanted enhancement of the background signal in total IgE ELISA (Fig. 1, 2) and was effective in enhancing signals for positive samples in Ag-specific IgE ELISA (Fig. 4, 6, 7). The sensitivity of total IgE ELISA was improved to approximately 30-fold with enhancer solutions (Fig. 1A, B, 3A). Previously, we reported on signal enhancement in Ag-specific IgE ELISA using enhancer solutions, yet the assay took 3 days from coating plates with allergens to absorbance measurement.[4] However, by combining with another blocking reagent, IB, in the present study, we achieved similar signal intensity levels even under assay conditions with a shorter incubation time (Fig. 6–8).

In total IgE ELISA, sensitivity was improved by approximately 30-fold compared with our previous assay[4] (Fig. 1A, B, 3A). The use of CGS2 to dilute the detection mAb critically contributes to improvement, and BA for blocking shows lower background absorbance in blank wells than another blocking reagent IB (Fig. 1). The increase of background absorbance with CGS2 to dilute detection mAb (Fig. 1A, B) could be due to enhanced binding of the detection mAb to the immobilized capture mAb (Fig. 2). The sensitivity improvement in the present study using HRP-labeled anti-murine IgE mAb for detection is equivalent to using a combination of biotinylated anti-murine IgE mAb and streptavidin/alkaline phosphatase conjugate for detection.[13]

Although weak signals for Ag-specific murine IgE

have been a problem, we recently reported the enhancement of signal intensity in ELISA for murine IgE specific not only to ovalbumin but also recombinant forms of clinically relevant major mite allergens using enhancer solutions.[4] CGS2 to dilute detection mAb was strikingly effective in increasing signal intensity (Fig. 5). Compared with the marked enhancement using CGS2 to dilute detection mAb, the effects observed using CGS1 for serum dilution were limited (Fig. 4, 5). A combination of enhancer solutions and another blocking reagent, IB, achieved equivalent signal intensity even under assay conditions with a shorter incubation time (Fig. 6, 7). Results using serum depleted of IgE (Fig. 4) or supplemented with IgE (Fig. 6) support isotype specificity to IgE and indicate that the nonspecific binding of IgE to the plate is low under the conditions in Figure 6A, C, and that titration is useful for the appropriate evaluation of Ag-specific IgE, even at higher IgE concentrations, under the conditions shown in Figure 6B. Blocking with IB under a shortened incubation schedule (2 hours for serum and 2 hours for detection mAb) (Fig. 6C) showed a similar signal intensity to blocking with BA under a longer incubation schedule (Fig. 6A) without significant enhancement of nonspecific IgE binding to the plate.

Dose dependency of absorbance with Ag-concentration in the Ag-immobilization step (Fig. 8) along with Figure 6C supports the fact that the binding of IgE to the plates is Ag-specific. Unwanted direct binding of the detection Ab to Ag proteins immobilized onto the plates differs among Ag proteins (unpublished data). In the present study, we showed data using sera from CBA/J mice because the strain indicated a higher total and Ag-specific IgE response even at a relatively low dose.[4] The use of enhancer solutions was also effective for signal enhancement in Ag-specific IgE within sera from BALB/c and C57BL/6 mice (Ref. 4, in addition to unpublished data). We recommend optimization of Ag-concentration in the immobilization process, incubation time, and blocking reagents according to the Ags and Ag-specific IgE titers of sera.

The results using individual sera from mice immunized with recombinant house dust mite major allergens before and after improvement indicate that the improved assay conditions are suitable for measurement of total IgE (Fig. 3) or Ag-specific IgE (Fig. 7). Since only small amounts of sera can be collected intravenously from mice at intervals without causing death, particularly in younger mice, improvement in detection sensitivity and signal enhancement, even with extensive serum dilution, is beneficial. The IgE response specific to clinically relevant allergens other than ovalbumin, which has been frequently used in animal models of allergies, is an important issue to investigate because recent studies have suggested that allergen sources have specific properties to promote Th2 sensitization to IgE production or exacerbation processes.[3,6,12,14-30] Ag-specific IgE ELISA with enhanced signal intensity for clinically relevant major allergens[4] (Fig. 4–8) is a strong tool for addressing this issue. The conditions recommended in this report are useful for studying allergies using mouse models.

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