

Therapeutic Potential of Monoclonal IgA Antibodies in Allergic Diseases: Suppressive Effect of IgA on Immune Responses Induced By Re-exposure to Antigen in Sensitized Mice by Monoclonal IgE Antibody That Binds to a Different Epitope of the Same Antigen

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Repeated exposure to an allergen induces allergic symptoms by activating mast cells that express anti-allergen IgE, which results in further sensitization to an allergen. Considering that additional sensitization elicits more severe allergic reactions upon the next allergen challenge, suppression of the boosting phase represents an efficacious way to prevent and ameliorate allergic diseases. In this study, we investigated the therapeutic potential of allergen-specific monoclonal IgA on allergic diseases. This antibody acts by decreasing immune responses upon exposure to allergens in mice previously sensitized by a monoclonal IgE that recognizes the allergen. The lack of inhibitory effects of anti-ovalbumin monoclonal IgA (OA-4) on either the binding of anti-ovalbumin monoclonal IgE (OE-1) to ovalbumin by ELISA or on ovalbumin-induced degranulation of rat basophilic leukemia RBL2H3 cells sensitized with OE-1 indicated that OA-4 and OE-1 recognized different epitopes on ovalbumin. Immune responses (anti-ovalbumin IgG1 production and cytokine release from splenocytes) induced by intravenous ovalbumin challenge in DBA/1J mice passively sensitized with OE-1 were inhibited by intravenous injection of OA-4 15 min before challenge without affecting anaphylaxis. Moreover, OA-4 injection 1 h after ovalbumin challenge also effectively suppressed immune responses. The achievement of immunosuppression by IgA injection occurred even after allergen challenge in mice in an epitope-independent fashion. These findings suggest that monoclonal IgA administered at the time of hospitalization of a patient with allergic symptoms, who was already exposed to the allergen in the presence of IgE recognizing an undefined epitope(s) on the allergen, should effectively relieve allergic disease through its immunosuppressive effects.

Introduction

ALLERGY IS GENERALLY PROCEEDED BY SENSITIZATION that results from allergen exposure and, subsequently, allergic symptoms can develop upon re-exposure to an allergen.⁽¹⁾ Repeated exposure gradually establishes sensitization to an allergen with sufficient production of IgE by B cells and the attachment of the antibody to its receptor FcεRI on mast cells. Thereafter, occasional re-exposure to the allergen induces IgE/FcεRI-dependent mast cell activation and the release of allergic mediators, such as histamine and serotonin, and the *de novo* synthesis of leukotrienes and cytokines, which co-operatively drive allergic and anaphylactic symptoms.⁽²⁾ Therefore, drugs that suppress allergic symptoms, such as adrenalines, H1 receptor antagonists, and mast

cell degranulation inhibitors, have been developed and used widely.⁽³⁾ In addition to the primary sensitization and acute reaction steps, we must emphasize the importance of another step in which further sensitization is induced after allergen re-exposure, which elicits more severe allergic reactions upon the next allergen challenge. Allergen-specific antibodies should contribute to both the further sensitization and acute reaction steps; serum antigen-specific IgE in mice complexed with cognate antigen that is absorbed into circulation promotes immune responses directed against the antigen through CD23, which is expressed on B cells,⁽⁴⁾ and serum IgG and IgM, which act through the FcγR and complement pathways.⁽⁵⁾ Despite the potential effectiveness of controlling this step to modulate allergic diseases, studies have not focused on developing drugs to inhibit the further sensitization step

that follows allergic reactions upon allergen re-exposure. Although temporal treatment with glucocorticoids after acute anaphylactic reactions is intended to prevent late anaphylactic reactions and has the possibility to show a suppressive effect on further sensitization, the efficacy of this treatment on late anaphylactic phase reactions is not clear⁽⁶⁾; its effect on subsequent immune reactions has not yet been investigated.

In the mucosa, IgA is the most abundant isotype and is present in the secretory IgA form,⁽⁷⁾ which is constructed with two heavy chains, two light chains, one joining chain, and one secretory component.⁽⁸⁾ The secretory form of IgA defends the body from systemic infection by invading bacteria from the mucosa and also neutralizes toxins secreted from them into the tissue. In circulation, IgA is the second most common isotype after IgG, and is predominantly present as monomeric IgA in humans or as a dimeric form in mice.⁽⁹⁾ Among the receptors for IgA identified in humans, Fc α RI mediates inhibitory and activating signals to the Fc α RI-expressing cells stimulated with monomeric IgA bound to the antigen and lattice-like IgA immune complex, respectively.⁽¹⁰⁾ Experiments with mice that genetically lack Fc α RI revealed that secretory IgA inhibits immune responses via SIGN-R1.⁽¹¹⁾ Although these studies proposed immunosuppressive functions and mechanisms for IgA, further clarification of the effect of IgA on immune responses should be conducted to understand its roles more widely and deeply.

We have therefore investigated the potential of IgA to treat allergic diseases through its immunosuppressive activities. Recently, we found that intravenous IgA attenuates primary antibody responses to an injected cognate antigen in naive mice,⁽¹²⁾ which is in contrast to the enhancing effects of other isotypes, including IgG, IgM, and IgE, on the response.^(5,12) However, the therapeutic use of IgA in allergic disease through its immunosuppressive activities should also be determined in animals that have anti-allergen IgE, since IgE against the allergens is already present in the blood of almost all allergic patients. Usually, the onset of allergic and anaphylactic symptoms after allergen re-exposure drives patients to seek medical attention, so desirable therapeutic drugs for allergic diseases would prevent further sensitization upon allergen re-exposure, even when they are administered after the onset of acute allergic reactions.

In this study, we show that anti-ovalbumin monoclonal IgA (OA-4) and anti-ovalbumin monoclonal IgE (OE-1) recognize different epitopes on ovalbumin (OVA). Using these two antibodies, the suppressive effects of intravenous administration of OA-4 prior to OVA challenge or after anaphylactic reactions induced by antigen challenge on subsequent immune responses in OE-1-sensitized mice were investigated.

Materials and Methods

Hybridomas and cell lines

Hybridomas producing anti-OVA monoclonal IgA and anti-OVA monoclonal IgE were established by the classical method using PEG1500 (Roche Diagnostics, Mannheim, Germany), following the manufacturer's instructions.^(12,13) The rat basophilic leukemia RBL2H3 cells were obtained from the Cell Resource Center for the Biomedical Research Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan.

Antibodies

OA-4 and OE-1 were purified with affinity chromatography as described previously.⁽¹²⁾ Other antibodies were purchased as follows: alkaline-phosphatase-conjugated anti-kappa light chain (Southern Biotechnology, Birmingham, AL); alkaline phosphatase-conjugated anti-mouse IgE (Bethyl Laboratories, Montgomery, TX); unlabeled anti-mouse IgG1 (Thermo Scientific, Rockford, IL); and alkaline-phosphatase-conjugated anti-biotin (Vector Laboratories, Burlingame, CA).

Animals

Female DBA/1J mice (8–12 weeks old; Charles River Laboratories, Yokohama, Japan) were maintained in a temperature-controlled environment and given standard chow and water. All animal procedures were approved by the Animal Ethics Committee of Kobe Pharmaceutical University.

Enzyme-linked immunosorbent assays to compare affinities for OVA between OA-4 and OE-1, and competitive ELISAs to determine differences in epitope recognition

For comparison of the affinities between OA-4 and OE-1 for OVA, ELISA plates were coated with 100 mg/ μ L of OVA (Sigma-Aldrich, St. Louis, MO) dissolved in PBS at 4°C for 16 h. After two washes with PBS with 0.05% Tween-20 (Sigma-Aldrich), the plates were blocked with 1% casein (Sigma-Aldrich) in PBS at room temperature for 1 h. After two washes, 100 μ L of the indicated concentrations of OA-4 and OE-1 were added to the wells. After 1 h incubation, the wells were washed four times and alkaline phosphatase-conjugated anti-mouse kappa light chain was added to detect the binding of OA-4 and OE-1 to OVA, followed by colorimetric reaction using *p*-nitrophenyl phosphate (Sigma-Aldrich).

To determine the differences in the epitopes recognized by OA-4 and OE-1, the indicated concentrations of OA-4 were added to the OVA-coated and casein-blocked wells and incubated at room temperature for 1 h. After four washes, 1 μ g/mL of OE-1 was added to the wells. After 1 h incubation and four washes, OE-1 bound to OVA on the wells was detected by alkaline-phosphatase-conjugated anti-mouse IgE.

Degranulation assay of RBL2H3 cells

RBL2H3 cells (0.25×10^6 /mL) were cultured with RPMI1640 medium (Wako Pure Chemical Industries, Osaka, Japan) containing 10% fetal bovine serum (Biowest SAS, Nuaillé, France), penicillin (100 U/mL)-streptomycin (100 μ g/mL) (Wako Pure Chemical Industries), and 1 μ g/mL OE-1 for 24 h. These cells were washed with PIPES buffer (25 mM PIPES [pH 7.2], 119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM MgCl₂, 1 mM CaCl₂, and 0.1% (w/v) bovine serum albumin) and stimulated for 20 min with 5 μ g/mL of OVA pre-incubated with or without the indicated concentrations of OA-4 in the buffer. The ratios of the released β -hexosaminidase to the cultured supernatant from the cells were measured by a colorimetric reaction using *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide (Sigma-Aldrich Fine Chemicals).

Passive systemic anaphylaxis

Mice were passively sensitized with an intravenous injection of OE-1 (50 μ g). On the next day, OVA (50 μ g) was

injected in mice at 1 h before or 15 min after intravenous injection of OA-4 (1 mg). The decrease in rectal temperature was measured for 1 h from the OVA challenge as an indicator of anaphylaxis. The sera and spleens of mice were collected on days 11 and 14, respectively.

Measurement of anti-OVA IgG1 in sera and cytokines secreted by cultured spleen cells

Anti-OVA IgG1 levels in the sera collected on day 11 were measured using unlabeled anti-mouse IgG1-coated plates (antibody-coating method) as described previously.⁽¹²⁾

Fourteen days after OVA challenge, splenocytes (5×10^6 cells/mL) from mice were prepared. After 3 days of culture with OVA (100 μ g/mL), the concentration of cytokines in the supernatants was determined using commercially available ELISA kits (eBioscience, San Diego, CA).

Statistical analysis

The Mann-Whitney *U* test was used for comparison differences between two independent groups. A *p* value of <0.05 with the Mann-Whitney *U* test was considered significant.

Results and Discussion

Differences in epitopes recognized by OA-4 and OE-1: antibodies with similar affinities for OVA

OA-4 and OE-1 binding to OVA at concentrations of 0.003–100 μ g/mL, as determined by ELISA using anti-kappa antibody as a detection antibody, were similar, while the affinity of OE-1 was slightly higher than that of OA-4 (Fig. 1A). The binding of OE-1 to coated OVA was not affected by pre-treatment of the plates with as much as a 100-fold higher concentration of OA-4 (Fig. 1B). These results indicate that OA-4 and OE-1 have similar affinities for OVA, but bind to different epitopes on OVA.

OA-4 fails to inhibit OE-1-dependent degranulation of RBL2H3 cells and anaphylactic reactions in OE-1-sensitized mice

The pre-incubation of OVA with OA-4 did not affect OVA-induced degranulation of RBL2H3 cells sensitized by OE-1 (Fig. 1C). This observation does not contradict the finding that OA-4 and OE-1 recognize different epitopes on OVA, which we obtained by ELISA (Fig. 1B).

IgE has roles in the development of anaphylactic symptoms upon antigen challenge⁽²⁾ and in promoting sensitiza-

tion to an antigen.⁽⁴⁾ In accordance with these investigative reports, OE-1 induced both anaphylaxis and promoted sensitization in response to OVA injection (Fig. 2A). Therefore, we next intended to investigate the effect of IgA on IgE-dependent anaphylaxis and following sensitization using two antibodies, OA-4 and OE-1, that recognize different epitopes on OVA (Fig. 2B, protocol). Consistent with the *in vitro* results, intravenous OVA-induced anaphylaxis in

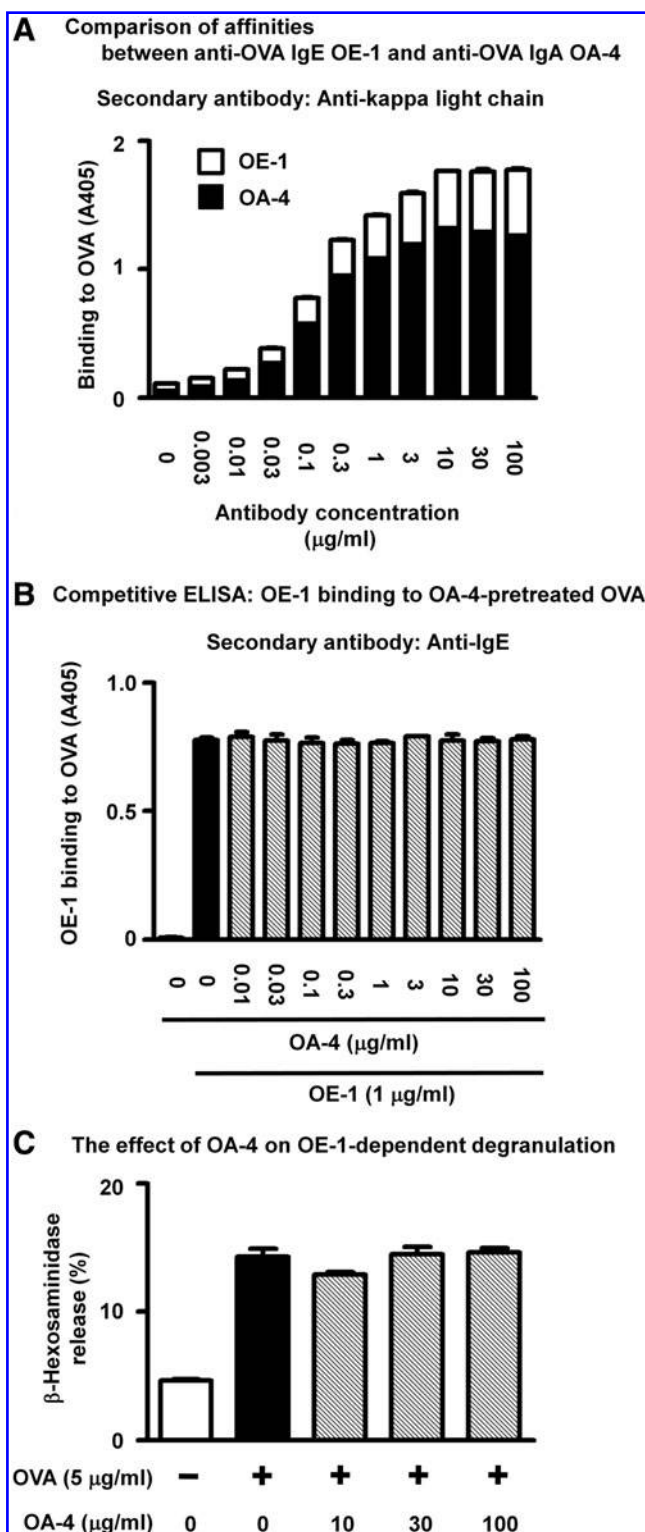


FIG. 1. OA-4 recognizes OVA epitope that is not bound by OE-1 and thus fails to inhibit OVA-induced degranulation of OE-1-sensitized RBL2H3 cells. (A) Binding affinities of OA-4 and OE-1 to OVA were compared by ELISA using an anti-kappa light chain antibody as a detection antibody. (B) Competitive binding to OVA between two antibodies, OA-4 and OE-1, was analyzed by competitive ELISA, as described in the Materials and Methods section. (C) OE-1-sensitized RBL2H3 cells were stimulated with OVA or OVA pre-incubated with the indicated concentrations of OA-4. Bars indicate the means $\pm$ SEM of either two wells (A, B) or six cultures (C). Data are representative of two independent experiments.

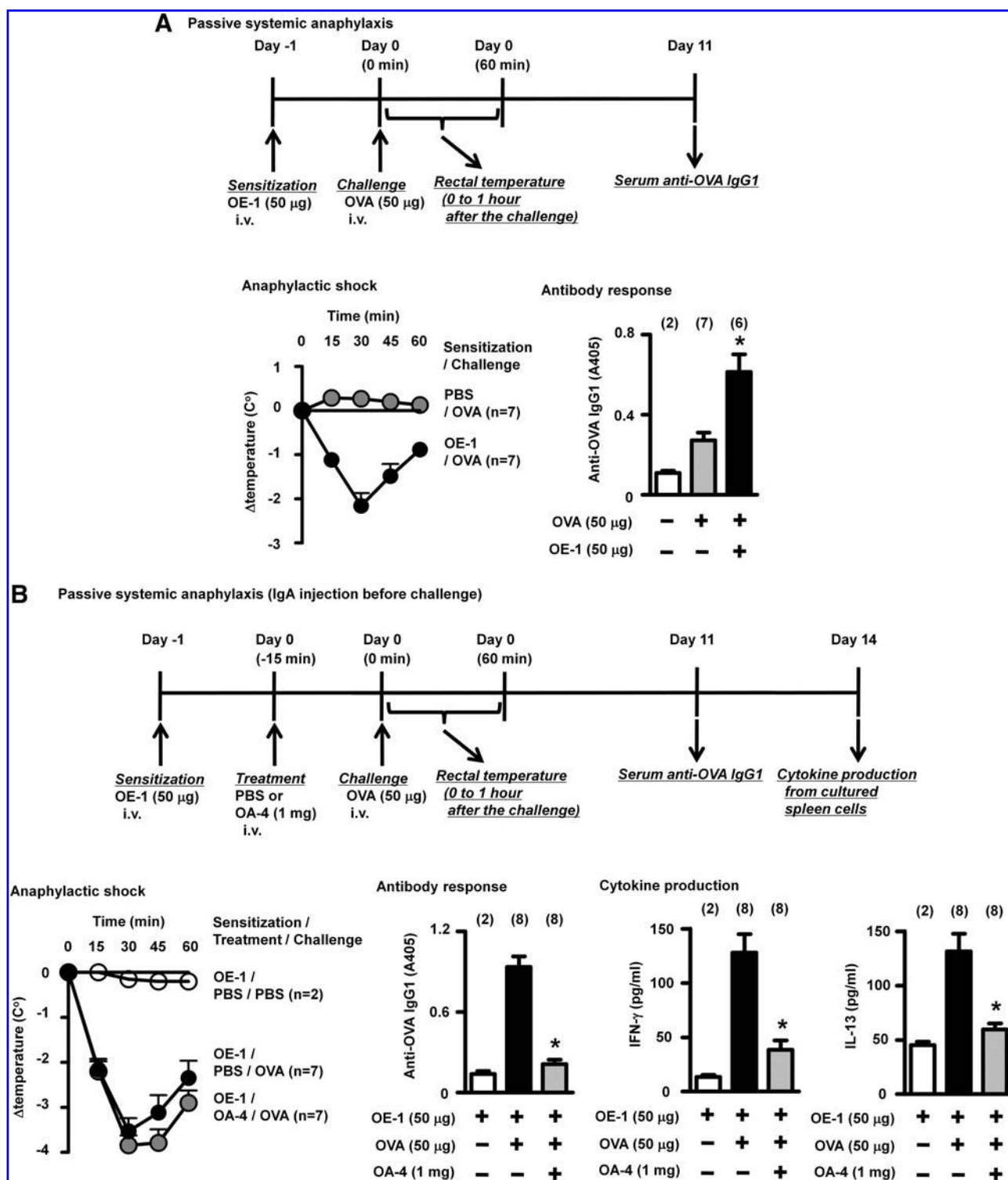


FIG. 2. Effects of OA-4 injection before or after OVA challenge on anaphylaxis and subsequent immune responses in OE-1-sensitized mice. DBA/1J mice were passively sensitized with OE-1 and challenged with OVA without OA-4 treatment (**A**), or with OA-4 treatment administered 15 min before (**B**) or 1 h after (**C**) OVA injection, according to the protocol shown. Rectal temperature after OVA challenge was monitored for 1 h and serum anti-OVA IgG1 levels were measured on day 11. Splenocytes obtained on day 14 were cultured with OVA (100 µg/mL) for 3 days to measure secreted IFN- γ and IL-13 in the supernatants. Symbols and bars indicate the means $\pm$ SEM for the indicated numbers of mice (rectal temperature and IgG1 level) or three cultures of cells pooled from the indicated numbers of mice (cytokine concentration). Data are representative of two independent experiments; * $p < 0.05$ vs. OVA group (Mann-Whitney U test).

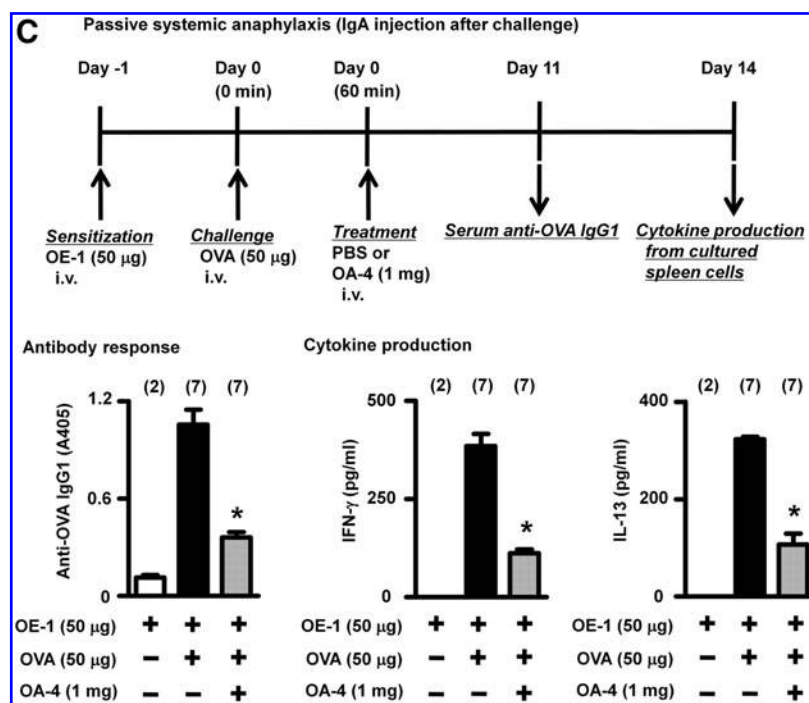


FIG. 2. (Continued).

mice sensitized with OE-1 was not affected by the pre-injection of OA-4 (Fig. 2B, rectal temperature), perhaps because of the lack of competition between OA-4 and OE-1 in binding to OVA.

Injection of OA-4 before or after OVA challenge similarly suppressed immune responses to OVA in OE-1-sensitized mice

In contrast to the absence of an inhibitory effect on rectal temperature reduction upon OVA challenge, pre-injection of OA-4 strongly reduced anti-OVA IgG1 levels in the serum on day 11 (Fig. 2B, antibody production) and Th1 cytokine, IFN- γ , and Th2 cytokine, IL-13, production by antigen-restimulated cultured splenocytes collected on day 14 in OE-1-sensitized mice (Fig. 2B, cytokine production).

Similar to injection prior to challenge, injection of OA-4 1 h after OVA challenge (Fig. 2C, protocol) also effectively suppressed immune responses to OVA in OE-1-sensitized mice, as evidenced by reductions in anti-OVA IgG1 levels (Fig. 2C, antibody production) and cytokine production by splenocytes (Fig. 2C, cytokine production).

Our findings established the potential of IgA to treat allergic diseases by suppressing further sensitization upon allergen exposure. Three distinctive features of the immunosuppressive effects of IgA should be mentioned. First, IgA can attenuate sensitization to the cognate antigen in mice sensitized with IgE that binds to different epitopes on the same antigen (i.e., in an epitope-independent fashion). Second, IgA could attenuate sensitization without affecting IgE-induced mast cell activation and anaphylactic reactions (i.e., in a mast cell-independent fashion). Third, IgA administration after anaphylaxis also inhibited sensitization to antigen challenge, suggesting the potential utility of IgA in the clinical setting.

Commonly, sensitization to T cell-dependent antigens, including OVA, is initiated by the uptake of antigens by antigen-presenting cells, such as B cells; antigen then activates B and T cells through interaction between antigen-major histocompatibility complex class II complex molecules and the T cell receptor and co-stimulatory molecules.⁽¹⁴⁾ Additionally, sensitization to antigen can be greatly enhanced by the binding of IgE-antigen immune complexes to CD23 expressed on B cells through increasing antigen uptake by B cells in mice,⁽⁴⁾ and possibly in allergic patients. Thus, in the mice in our experiments, IgA might not only inhibit immune responses to free OVA, but could also impede CD23-dependent B cell antigen uptake through OVA-IgE complexes. These events might be followed by reductions in *de novo* antibody and cytokine production by allergen-stimulated splenocytes. Because our anti-OVA monoclonal IgE and IgA antibodies recognized different epitopes of the allergen, the inhibitory effect of IgA on the immunostimulatory functions of IgE was not caused by competitive neutralization of the allergen, namely epitope masking,⁽¹⁵⁾ preventing its binding to IgE associated with CD23 on B cells. Rather, the inclusion of IgA in IgE-allergen immune complexes might alter the stimulatory effect of the complex on B cells. One possibility is that the reduction of the density of IgE in immune complexes by IgA might reduce the stimulatory efficacy of the complex on B cells through CD23. Indeed, attachment of monomeric IgE on another IgE receptor, Fc ϵ RI, and IgE immune complexes bridges the receptors to differentially affect mast cell functions.⁽¹⁶⁾ Alternatively, IgA might induce immunosuppressive signaling through defined or undefined IgA receptors on B cells to compete for IgE/CD23-dependent immunoactivating signaling, such as ITIM-bearing receptors that include Fc γ RIIB.⁽¹⁷⁾ Regulatory T (Treg) cell induction could represent another possible mechanism whereby the

immunosuppressive effects of OA-4 are mediated. However, our preliminary study could not identify a contribution of immunosuppressive molecules, namely, transforming growth factor (TGF)- β ^(18,19) and latency-associated peptide (LAP),⁽²⁰⁾ that are expressed by Treg cells to the immunosuppressive effect of IgA (data not shown). Future studies should analyze other readouts of Treg cell function, such as IL-10 production, cytotoxic T-lymphocyte-associated protein 4 expression, and glucocorticoid-induced tumor necrosis factor receptor expression,⁽²¹⁾ as well as more detailed analysis of TGF- β and LAP. Elucidating the mechanisms that underlie the immunosuppressive effects of IgA on IgE-dependent immunoactivation should be meaningful for revealing unknown immunosuppressive machinery and accelerate the application of IgA to the treatment of immune diseases. Attenuation of immune responses in the presence of IgE by IgA that recognizes a different epitope on an allergen is a favorable characteristic for its use as a drug, because it may enable us to treat allergic patients with an antibody without determining the epitopes recognized by IgE within and among allergic patients. Further experiments are required to reveal the immunosuppressive effects of IgA in mice actively sensitized with antigen injections.

The correlation between serum levels of allergen-specific IgE in allergic patients and the severity of their allergic symptoms⁽²²⁾ or the effectiveness of anti-IgE (Omalizumab),⁽²³⁾ mast cell stabilizers,⁽²⁴⁾ and H₁ receptor antagonists⁽²⁵⁾ for allergic diseases indicate the importance of the IgE–mast cell–histamine axis in human anaphylaxis. Similarly, the IgE–mast cell–histamine axis^(26,27) is thought to be a critical pathway that mediates anaphylaxis in mice, even after accounting for the potential of the IgG–macrophage–platelet activating factor,⁽²⁸⁾ IgG–neutrophil,⁽²⁹⁾ and IgG–basophil axes⁽³⁰⁾ to induce an acute anaphylactic reaction. By contrast, the role of mast cells in sensitization remains controversial; mast cells have been proposed to either promote⁽³¹⁾ or show no⁽³²⁾ activities, depending on the cellular context. Moreover, the mechanism whereby anaphylactic reactions are induced by IgE-antigen-dependent mast cell activation affecting subsequent sensitization against antigen is not yet known.

In this present study, we showed that the attenuation of immune responses upon allergen challenge in IgE-sensitized mice by IgA recognition of that same allergen occurs without affecting the anaphylactic response *in vivo*. This finding was in accordance with the lack of inhibition of IgE-dependent mast cell degranulation by IgA *in vitro*. This indicated that IgE-induced acute phase reactions mainly occur because of mast cell activation upon allergen re-exposure, which is not closely associated with the sensitization phase. This direct evidence of the small role that mast cells play in the late sensitization using the same allergen (i.e., OVA) for inducing anaphylaxis and subsequent immune responses was strengthened by the observation in our previous study that sensitization to phosphorylcholine–keyhole limpet hemocyanin was induced similarly, irrespective of the presence or absence of anaphylactic responses triggered by the simultaneous injection of another allergen, OVA, in OVA-sensitized mice.⁽³³⁾ Commonly, mast cell degranulation inhibitors and H₁ receptor antagonists are used to prevent and treat allergic diseases, respectively.⁽³⁾ However, based on our results, these medications are thought to be good for patients only in at-

tenuating allergic symptoms and are insufficient for preventing further sensitization to provide relief from allergic diseases. We propose here that novel drugs, such as allergen-specific IgA, to modulate the sensitization phase should be administered to patients with allergic and anaphylactic symptoms in addition to drugs to suppress acute reactions. It will be worthwhile to investigate the effectiveness of other drugs normally used for anaphylaxis, such as epinephrine and glucocorticoids, on the later sensitization phase more deeply in mice and humans, since they also have immunosuppressive activities.^(34–36)

Typically allergic patients, especially those with anaphylaxis, are treated medically by medical personnel soon after the onset of symptoms. The effectiveness of IgA to suppress sensitization in mice when administered 1 h after allergen exposure suggests that IgA could be administered at favorable dosing times as a therapeutic drug for allergic diseases in the clinic. Considering our results with sensitized mice and the preventive effect of IgA on sensitization in naive mice reported previously,⁽¹²⁾ IgA could be applicable to a wide range of the human population, such as for preventative purposes in healthy, non-sensitized persons and for therapeutic purposes in allergic patients and previously sensitized persons. A limitation of our study presented here is that the immunosuppressive potential of IgA has only been established in mice with mouse IgA. To advance this finding toward the invention of new anti-allergic drugs, studies are needed to determine whether the immunosuppressive response induced by IgA is conserved among a variety species, including humans.

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Author Disclosure Statement

The authors have no financial interests to disclose.

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