



Development and characterization of a novel anti-IgE monoclonal antibody

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ABSTRACT

IgE is the central macromolecular mediator responsible for the progression of allergic reactions. Omalizumab (Xolair) is a humanized monoclonal anti-IgE antibody directed at the FcεRI-binding domain of human IgE, which represents a novel therapeutic approach in the management of asthma. In this study, we developed a monoclonal antibody (7A5) against human IgE via hybridoma technique. Our data showed that 7A5 could inhibit free IgE molecules to bind to receptors without affecting IgE already bound to cellular receptors. Importantly, 7A5 was able to inhibit IgE-induced histamine release of basophilic leukemia cells. Next, the phage display peptide library technology was employed to select peptides binding to 7A5 and a striking peptide sequence motif was recovered, which is homologous to the sequence ³⁹¹KQR³⁹³ within the Cε3 domain of IgE-Fc. Our results further indicated that 7A5 specifically bound to the synthesized peptide “³⁸⁸KEEKQRN³⁹⁴” containing the ³⁹¹KQR³⁹³ motif in IgE-Fc. The epitope of 7A5 was found to be spatially close to the FcεRI-binding site, suggesting that 7A5 binding to IgE might block IgE binding to receptors via steric hindrance. The anti-IgE monoclonal antibody 7A5 may have the potential to be developed as a therapeutic agent for the treatment of allergic diseases.

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1. Introduction

About 10–40% of the population in industrialized countries suffers from one or more symptoms of allergy. Most allergic diseases are caused by IgE-mediated hypersensitivity reactions [1,2]. IgE, normally present in the serum at minute concentrations, is one of the five classes of antibodies in the human immune system, which is produced by IgE-secreting plasma cells. These IgE molecules circulate in the blood and bind to IgE receptors on the surface of basophils in the circulation and mast cells in various tissues [3,4]. In an allergic reaction, the allergens enter the body and bind to IgE on mast cells or basophils, crosslink the IgE molecules, aggregate the underlying receptors, and then, mediators of allergy, including histamine and serotonin, are released as a result of exposure of FcεRI-bound IgE to allergen [1,4,5].

The IgE antibody binds to specific high-affinity receptors FcεRI via its Fc region, also to low affinity receptors FcεRII (CD23) on B-lymphocytes and eosinophils [6]. The crystal structure of the human IgE-Fc bound to its high-affinity receptor (FcεRI) α-chain reveals that one receptor binds one dimeric IgE-Fc molecule asymmetrically through interactions at two sites, each involving one Cε3 domain of the IgE-Fc [7]. Because IgE is the central macromolecular mediator responsible for the progression of allergic reactions, neutralizing it and inhibiting its synthesis would appear to be a rational approach for the treatment of various allergic diseases [1]. Omalizumab (Xolair) is a developed humanized monoclonal anti-IgE antibody directed at the FcεRI-binding domain of human IgE. It can prevent binding of these molecules to FcεRI on mast cells and basophils and FcεRII on various cell types. Consequently, IgE effector functions (cross-linking IgE and triggering degranulation and synthesis of new-generated chemical mediators of IgE-sensitized cells) and the following activation of mast cells and basophils are inhibited. Omalizumab represents a novel therapeutic approach in the management of asthma and it has been approved in the United States, European Union, and several other countries for treating patients with moderate-to-severe allergic asthma [8–10].

In this study, we developed a monoclonal antibody (7A5) against human IgE via hybridoma technique. Our data showed that 7A5 bound specifically to IgE except those already bound to

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cellular receptors, thus blocking free IgE molecules to bind to soluble FcεRI and membrane FcεRI without cross-linking of IgE bound to membrane FcεRI to release histamine. Further study indicated that 7A5 dose-dependently inhibited IgE-induced histamine release of the rat basophilic leukemia cells which express a functional human FcεRI alpha subunit. The specific epitope recognized by 7A5 was identified to be ³⁹¹KQR³⁹³ within the Cε3 domain of IgE-Fc, which was spatially close to the FcεRI-binding site, suggesting that 7A5 binding to IgE might block IgE binding to receptors via steric hindrance.

2. Materials and methods

2.1. Cell lines and reagents

The rat basophilic leukemia cell line (RBL-2H3) and human IgE-secreting cell line (SKO-007) were obtained from the American Type Culture Collection (ATCC). Omalizumab was purchased from Genentech. The IgE was purified from the SKO-007 cell culture supernatant by affinity chromatography on a Sepharose 4B column conjugated with omalizumab. HRP-labeled human IgE was obtained by using a HRP labeling kit (Pierce, Rockford, IL, USA). The rat basophilic leukemia cell line (RBL-2H3) was stably transfected to express a functional human FcεRI alpha subunit as described by Takagi et al. [11] and the established cell line was designated RBL-2H3/h. Next, the exodomain of the human FcεRI alpha subunit was genetically fused to the Fc portion of human IgG by overlapping polymerase chain reaction (PCR). The resulting fusion gene (FcεRI-Ig) was inserted into pcDNA3.1(+) vector (Invitrogen, San Diego, CA), yielding the expression vector pcDNA3.1–FcεRI-Ig. To express the FcεRI-Ig fusion protein, pcDNA3.1–FcεRI-Ig was transfected into Chinese hamster ovary (CHO) cells using Lipofectamine 2000 reagent (Invitrogen, San Diego, CA). After transfection, the stable transfectants were isolated by limiting dilution in the presence of 450 μg/ml G418. The cell clones producing the highest amount of recombinant protein were selected and grown in serum-free medium. Finally, the FcεRI-Ig fusion protein was purified by affinity chromatography on Protein A-Sepharose (Amersham Biosciences, Uppsala, Sweden) from the serum-free culture supernatants. HRP-conjugated goat anti-mouse IgG and HRP-conjugated goat anti-human IgG were respectively, purchased from SBA (Birmingham, AL) and Zymed (San Francisco, CA). The Ph.D.-7 Phage Display Peptide Library (PDPL) was purchased from New England BioLabs (Beverly, MA, USA). Peptides used in this study were synthesized by Yeli BioScientific Inc. (Shanghai, China). The quality of peptides was determined by analytical reverse-phase chromatography and mass spectral analysis and the purity was greater than 95%.

2.2. Preparation of monoclonal antibody specific to human IgE

The monoclonal antibody against human IgE was prepared by the standard hybridoma technique as described previously [12,13]. In brief, the myeloma cell line (NS1) was fused with spleen cells from BALB/c mice immunized with SKO-007 cells and selected in hypoxanthine/aminopterin/thymidine (HAT) selective medium. The hybridoma culture medium was detected for the presence of specific antibodies by a solid-phase ELISA using purified human IgE as antigen.

2.3. ELISA assay

A 96-well plate was coated overnight at 4 °C with 100 μl recombinant FcεRI-Ig fusion protein (5 μg/ml) in PBS. Blocking treatment was performed at 37 °C for 2 h. Before addition to the coated plate,

different dilutions of anti-IgE antibodies were incubated with 3 μg/ml of HRP-labeled human IgE in PBS. Pre-incubation mixtures were added to the coated plate for 1 h at 37 °C. 0.1 ml/well of Microwell Peroxidase Substrate (Kirkgaard & Perry Laboratories, Gaithersburg, MD, USA) was added and color was developed for 10 min. The reaction was stopped by adding an equal volume of 1 N HCl, and the absorbance measured at 450 nm.

2.4. Flow cytometry analysis

Different dilutions of anti-IgE antibodies were incubated with 5 μg/ml of FITC-labeled human IgE in PBS. RBL-2H3/h staining was performed with pre-incubation mixtures for 45 min on ice. After washing, cells were analyzed on a FACScan using CellQuest software for both data acquisition and analysis.

In order to explore whether anti-IgE antibodies bind to IgE already bound to cellular receptors, RBL-2H3/h cells were washed and resuspended at 1×10^5 cells/ml in PBS/0.1% BSA and incubated with 10 μg/ml human IgE for 45 min on ice. Thereafter cells were washed twice and different dilutions of anti-IgE antibodies were added, followed by another 45 min incubation on ice. After treated with FITC-labeled anti-human (mouse) IgG (Zymed, San Francisco, CA, USA) for 45 min on ice, cells were washed twice, resuspended in PBS/0.1% BSA and analyzed on a FACScan.

2.5. Cell degranulation assay

Cell degranulation was assessed by measuring histamine release [14,15]. Human IgE and serial dilutions of anti-IgE antibodies were incubated for 30 min at 37 °C, and then mixtures were added to the RBL-2H3/h cells for 24 h incubation at 37 °C in 5% CO₂ atmosphere incubator. Unsensitized cells served as a control. After the cells were washed, 1×10^5 cells were stimulated in the presence of 10 μg/ml anti-IgE polyclonal antibody (R&D Systems, Inc., Minneapolis, Minnesota, USA). The supernatants were collected and histamine content in supernatants was determined using a commercially available enzyme-linked immunoadsorbent (ELISA) assay kit (Biosource International, Camarillo, CA, USA) according to the manufacturer's protocol.

2.6. Bio-panning and selection of phages

The segment of the Ph.D.-C7C library, containing a repertoire of 1.2×10^9 sequences of random 7-amino acid peptides, is flanked by a pair of cysteine residues, which are oxidized during phage assembly to a disulfide linkage, resulting in the displayed peptides being presented to the target as loops [16,17]. Bio-panning of PDPLs with anti-IgE antibody was performed as described previously [13,18]. Briefly, the antibody 7A5 was immobilized on 96-well plates. Then the wells were blocked with 3% bovine serum albumin and incubated with phages (1×10^{11} pfus) from the Ph.D.-C7C PDPL at 25 °C for 1 h. The wells were washed for five times. Then, the phages that bound with the antibody were amplified by direct infection with *Escherichia coli* ER2738. Three rounds of selection were routinely performed.

2.7. Phage ELISA

Ninety-six-well plates were coated with anti-IgE antibody (10 μg/well). After two washings and blockade of free protein-binding sites, 100 μl of supernatant containing amplified particles from each phage clone were added to wells and incubated for 2 h at 37 °C. The plates were then washed for five times, and HRP-conjugated anti-phage M13 monoclonal antibody (Amersham Biosciences, Uppsala, Sweden) was added and incubated for 1 h at 37 °C. After washing, 3,3',5,5'-tetramethylbenzidine (TMB) was

added as a substrate and the absorbance was read at 450 nm using an immunoreader. Finally, phage clones highly reactive in ELISA were purified from supernatants of positive clones by using a Spin M13 Kit (Jierui, Shanghai, China) and subjected to DNA sequence analysis (Jierui, Shanghai, China).

2.8. Western blot

For Western blot analysis, purified phage particles (1×10^{10} pfus/lane) were loaded onto 10% polyacrylamide gels, fractionated by SDS–PAGE and transferred electrophoretically onto polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences, Uppsala, Sweden). After blockade of its free protein active-binding site, the PVDF membranes were treated with an appropriate dilution of antibody 7A5, followed by incubation with HRP-conjugated goat anti-mouse IgG (Zymed, San Francisco, CA, USA). Finally, the bands were visualized by 3,3'-diaminobenzidine (Sigma, St. Louis, MO, USA) as a peroxidase substrate.

2.9. Immunochemical assay

The peptide “³⁸⁸KEEKQRN³⁹⁴” containing the ³⁹¹KQR³⁹³ motif in human IgE-Fc was synthesized and denoted as GP. The reactivity of antibody 7A5 with GP-peptide was determined by ELISA. Briefly, 96-well plates were coated with KLH-GP peptide. Following washing and blockade of free protein-binding sites, different concentrations of antibody 7A5 was added to each well. After a period of 4 h incubation, mAbs binding to peptide were detected by sequential addition of an appropriate dilution of HRP-conjugated goat anti-mouse IgG. After the addition of TMB and stop solution, absorbance was read at 450 nm by an immunoreader.

For the blocking assay, 7A5 was incubated with different concentrations of GP-peptides for 1 h. Then the mixture was added to 96-well plates pre-coated with human IgE. After 2 h incubation, the wells were washed and detected with an appropriate dilution of HRP-conjugated goat anti-mouse IgG. After the addition of TMB and stop solution, absorbance was read at 450 nm by an immunoreader. The percentage of inhibition was calculated using the following formula: $\% \text{inhibition} = (A450_{\text{in the absence of inhibitor}} - A450_{\text{in the presence of inhibitor}}) / A450_{\text{in the absence of inhibitor}} \times 100$.

3. Results

3.1. Characterization of anti-IgE mAb

In present study, we have obtained a monoclonal antibody against human IgE named 7A5 by hybridoma technique and then evaluated the potential of 7A5 to inhibit IgE binding to FcεRI. Similar to omalizumab, 7A5 was able to dose-dependently block the binding of FcεRI-Ig fusion protein and IgE. Pre-incubation of IgE with 7A5 resulted in 100% IgE binding inhibition at a concentration of 50 μg/ml (Fig. 1A). To investigate whether the mAb 7A5 could block the binding between IgE and membrane FcεRI, we performed an IgE binding inhibition assay using the rat basophilic leukemia cell line (RBL-2H3) transfected with a functional human FcεRI alpha subunit (RBL-2H3/h). 7A5 was demonstrated to be able to inhibit IgE binding to cell surface FcεRI (Fig. 1B). IgE previously bound to the membrane receptors was unable to bind 7A5, indicating that 7A5 did not lead to cross-linking of IgE bound to membrane FcεRI to release histamine (Fig. 1C). These results clearly demonstrate that 7A5 decreases the amount of soluble IgE but has no effect on IgE bound to membrane FcεRI.

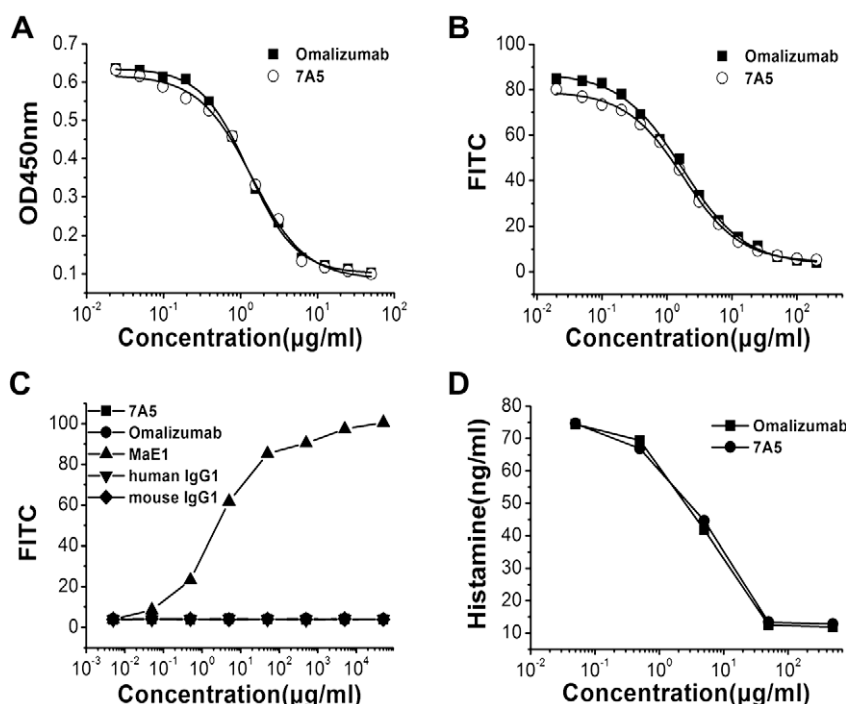


Fig. 1. Characterization of anti-IgE mAb. (A) Inhibition of FcεRI-Ig fusion protein binding to IgE. Different dilutions of anti-IgE antibodies were added to 96-well plates pre-coated with recombinant FcεRI-Ig fusion protein in the presence of HRP-labeled human IgE. (B) The inhibition of IgE binding to membrane FcεRI by anti-IgE mAbs. Different dilutions of anti-IgE antibodies were added to RBL-2H3/h in the presence of FITC-labeled human IgE. Data are presented as means $\pm$ SD of triplicate experiments. (C) Anti-IgE antibodies binding to IgE already bound to cellular receptors. Different dilutions of anti-IgE antibodies were added to RBL-2H3/h pre-incubated with human IgE. After stained with FITC-labeled anti-human (mouse) IgG, RBL-2H3/h was analyzed by flow cytometry. Human IgG1 and mouse IgG1 were used as unrelated control. MaE1, mouse anti-human IgE antibody, served as positive control. Each point represents means $\pm$ SD of the data from three independent experiments. (D) The inhibition of histamine release by anti-IgE mAbs. RBL-2H3/h were treated with human IgE for 24 h in the presence of anti-IgE antibodies at the indicated concentrations, stimulated with anti-IgE polyclonal antibody, and then determined histamine content in supernatants by ELISA assay kit. Each point represents means $\pm$ SD of the data from three independent experiments.

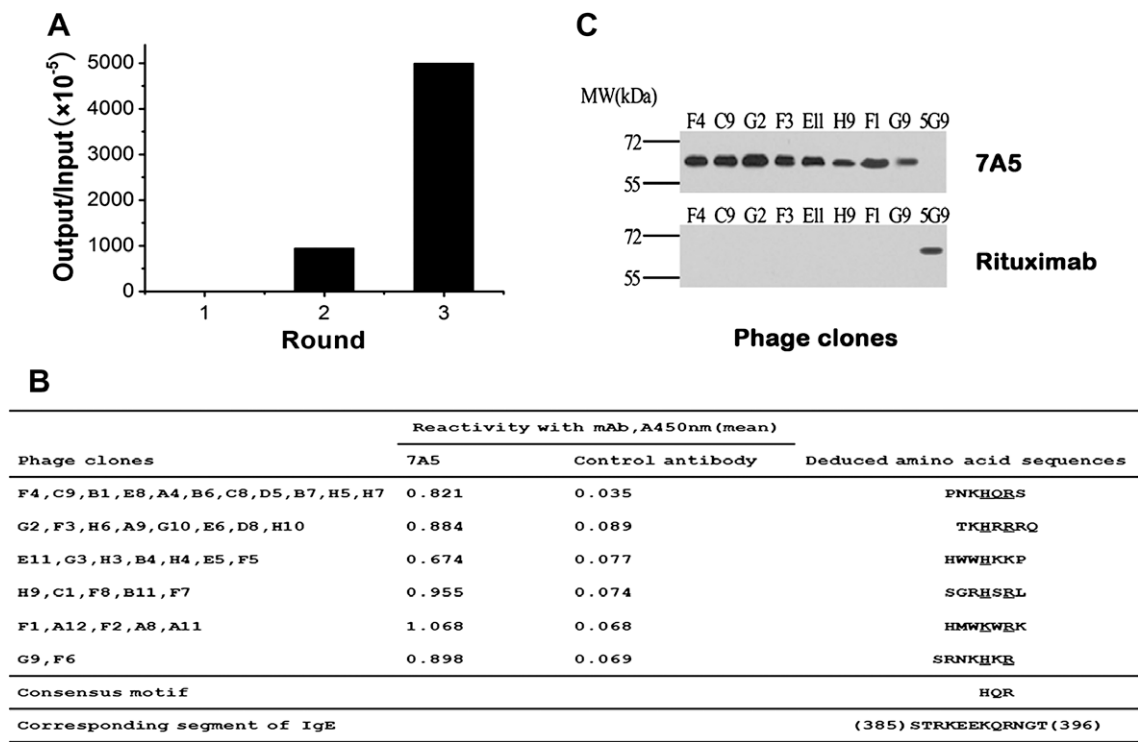


Fig. 2. Enrichment and analysis of 7A5-specific phages by bio-panning. (A) Output/input ratios of phages by titration were indicated in each round of bio-panning. (B) Reactivity and deduced amino acid sequences of inserts of selected phage clones. Phage clones were isolated by panning the Ph.D.-C7C PDPL with 7A5. After three rounds of panning, reactivity of positive phage clones with 7A5 was measured by ELISA as described in Section 2. Phage clones highly reactive in ELISA were subjected to DNA sequence analysis. Deduced amino acid sequences of inserts from selected phage clones were aligned for the consensus motif, which is indicated by underlined letters. (C) Western blot analysis of the specific reactivity of 7A5 with phage PIII protein-fused peptides from the positive ELISA phage clones. Purified phage particles (1×10^{10} pfus/lane) isolated by bio-panning 7A5 were run onto a 10% PAGE under nonreducing conditions and transferred to PVDF membranes. The membranes were incubated with 7A5 or control mAb rituximab (an anti-CD20 mouse/human chimeric antibody), followed by incubation with HRP-conjugated goat anti-mouse IgG (for detection of 7A5) or HRP-conjugated goat anti-human IgG (for detection of rituximab). Finally, the bands were visualized by DAB as a peroxidase substrate. A rituximab-specific phage clone (5G9) was used as control. MW, molecular weight.

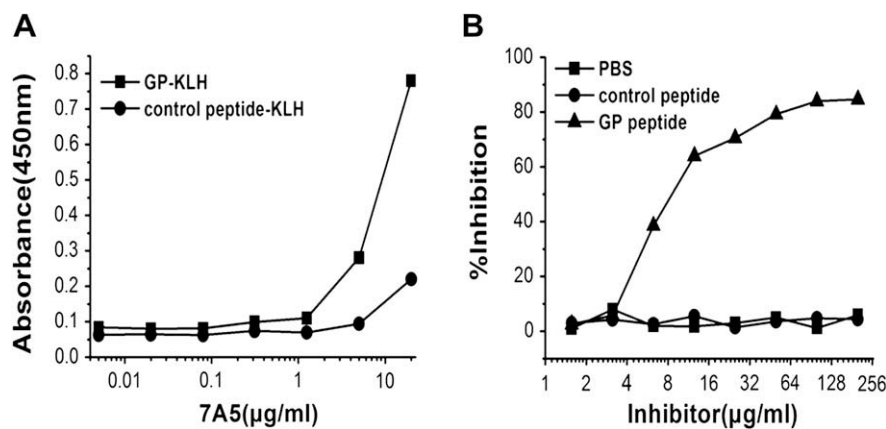


Fig. 3. Reactivity of synthetic epitope peptides with 7A5. (A) Specificity of the reactivity of 7A5 with GP-peptide. Ninety-six-well polyvinyl-chloride microtiter plates were incubated with KLH-GP. After washing and blockage of free protein-binding sites, different concentrations of antibody 7A5 was added to the plate and their binding to peptide was detected with HRP-conjugated goat anti-mouse IgG. (B) Competitively inhibition assay on the binding of 7A5 to human IgE by peptide GP. 7A5 was incubated with GP or control peptide in the indicated concentrations for 1 h at 37 °C, and then transferred to human IgE-coated plates. Bound 7A5 was detected with HRP-conjugated goat anti-mouse IgG.

After treating with the combination of IgE and serial dilutions of 7A5, RBL-2H3/h cells were triggered by cross-linking of FcεRI by the addition of anti-IgE polyclonal antibody. Release of histamine into cell supernatant was monitored as a measure of degranulation. In the absence of mAb 7A5, stimulation of cells with IgE induced a marked increase of histamine release, which was up to sixfold in IgE stimulated cells compared to the cells non-treated with IgE (spontaneous release). In addition, the levels of released

histamine showed a marked decrease in cells treated with mAb 7A5 (Fig. 1D).

3.2. Selection of phage-displayed peptides reactive with mAb 7A5

To identify the peptides recognized by mAb 7A5, phage clones were isolated by panning the Ph.D.-C7C PDPL with mAb 7A5. The ratio of the output versus input phages (output/input ratio) in each

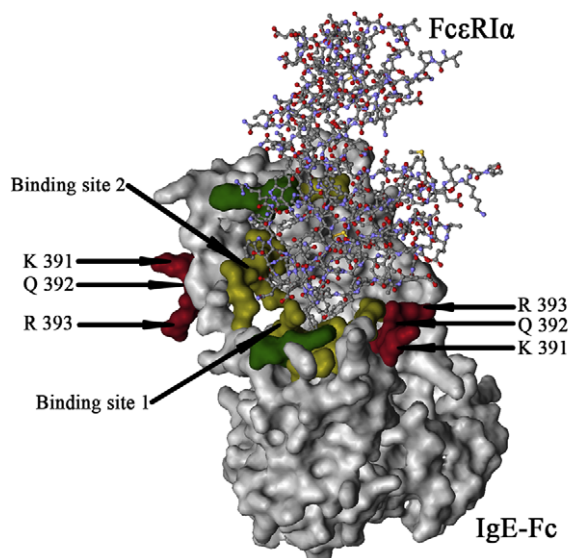


Fig. 4. Model of FcεRIα-binding to IgE-Fc. The model was established using the software Discovery Studio 2.0 based on the previously reported crystal structure of FcεRIα in complex with IgE-Fc. IgE-Fc is shown in a surface representation and FcεRIα is depicted in ball and stick form. The IgE-Fc residues within 5 Å of FcεRIα are defined as FcεRIα-binding site residues and colored yellow. The omalizumab epitope is colored green and the 7A5 epitope comprising amino acids “³⁹¹KQR³⁹³” is colored red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

round of panning was shown in Fig. 2A. After the third round of panning, the binding of the cloned phages to mAb 7A5 was tested in an ELISA. The results showed that 38 out of the 56 isolated phages specifically bound to 7A5 (Fig. 2B). The results of Western blot analysis also indicated that 7A5 could bind to these 38 phage clones (representative results are shown in Fig. 2C). The reactivity was specific since 7A5 did not react with the anti-CD20 chimeric antibody rituximab-specific phage clone 5G9, nor did 7A5-specific phage clones react with rituximab (Fig. 2C). All 38 positive clones were subjected to DNA sequence analysis.

3.3. Mapping of 7A5-specific epitope on human IgE

A total of 10 sequences were found and listed in Fig. 2B. Alignment of the amino acid sequences of these clones resulted in the motif HQR and this could be aligned with the sequence ³⁹¹KQR³⁹³ located on the Cε3 domain of human IgE (Fig. 2B), suggesting that ³⁹¹KQR³⁹³ was involved in the binding of IgE-Fc to 7A5. The results of Western blotting also indicated that 7A5 could specifically bind to these phage clones (Fig. 2C).

3.4. Reactivity of synthetic peptides with mAb 7A5

The ability of synthetic peptides to block the interaction between the antibody 7A5 and human IgE was also investigated. The ELISA results showed that the binding of 7A5 to KLH-conjugated GP-peptide (GP-KLH) is dose-dependent, which is also specific because 7A5 did not bind to control peptide-KLH (Fig. 3A) nor did GP-KLH react with rituximab (data not shown). The capability of the GP-peptide to block the binding of 7A5 to human IgE was also performed to investigate whether GP resembled the epitope of IgE. The results demonstrated that GP-peptide effectively inhibited the binding of 7A5 to human IgE in a dose-dependent manner. In contrast, irrelevant control peptide was ineffective in inhibiting their interaction (Fig. 3B). These results further revealed that the ³⁹¹KQR³⁹³ motif was the 7A5 epitope.

4. Discussion

The present study shows that a new anti-IgE antibody, 7A5, tightly binds to free circulating IgE molecules and prevents binding of these molecules to receptors. Degranulation is the formation after cross-linking of multiple receptor molecules on the cell surface which can occur directly by complexing receptor bound IgE molecules with anti-IgE antibodies [19]. Our results provide first evidence that in IgE-sensitized RBL-2H3/h cells the mAb 7A5 efficiently and dose-dependently inhibits anti-IgE-triggered degranulation.

The crystal structure of human IgE-Fc in complex with its high-affinity receptor FcεRIα reveals that one dimeric IgE-Fc molecule asymmetrically interacts with one receptor molecule at two sites, each involving one Cε3 domain of the IgE-Fc [7]. In our previous study, the omalizumab epitope has been identified to be the ⁴²⁴HLP⁴²⁶ motif within the Cε3 domain of IgE-Fc, which overlaps with the high-affinity IgE receptor-binding site [18]. Here, the specific epitope ³⁹¹KQR³⁹³ recognized by 7A5 was defined through phage display and bio-panning technology. Next, we compared the binding site of FcεRI α-chain to IgE-Fc with the localization of the 7A5 epitope identified in this study. Fig. 4 illustrates the structure of FcεRI in complex with IgE-Fc. The IgE-Fc residues within 5 Å of FcεRIα are defined as FcεRIα-binding site residues and colored yellow. The omalizumab epitope is colored green and the 7A5 epitope “³⁹¹KQR³⁹³” is colored red. Fig. 4 shows that the 7A5 epitope is spatially close to the FcεRI-binding site, which explains why 7A5 can inhibit free IgE molecules to bind to receptors without affecting IgE already bound to cellular receptors. As a consequence of the inhibition, the expression of FcεRI on basophils, mast cells and dendritic cells is also markedly downregulated [20–24]. Thus, IgE effector functions are inhibited since the binding of IgE to FcεRI on IgE effector cells and the following activation of mast cells and basophils are blocked [19]. Moreover, because 7A5 and omalizumab epitope are also spatially close, 7A5 could compete with omalizumab in binding to human IgE (data not shown). Without the cross-linking of anti-IgE antibody, 7A5 and IgE incubated with RBL-2H3/h could not lead to histamine release (data not shown), so it does not crosslink FcεRI-bound IgE and sensitize mast cells and basophils.

In conclusion, the ³⁹¹KQR³⁹³ motif within the Cε3 domain of IgE-Fc was identified as the 7A5 epitope in the present study and found to be spatially close to the FcεRI-binding site, suggesting that 7A5 binding to IgE might block IgE binding to receptors via steric hindrance. Taken together, the anti-IgE monoclonal antibody 7A5 may have the potential to be developed as a therapeutic agent for the treatment of allergic diseases.

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References

- [1] T.W. Chang, The pharmacological basis of anti-IgE therapy, *Nat. Biotechnol.* 18 (2000) 157–162.
- [2] B. Lundbäck, Epidemiology of rhinitis and asthma, *Clin. Exp. Allergy* 28 (1998) 3–10.
- [3] H. Metzger, G. Alcaraz, R. Hohman, J.P. Kinet, V. Pribluda, R. Quarto, The receptor with high affinity for immunoglobulin E, *Annu. Rev. Immunol.* 4 (1986) 419–470.
- [4] F.M. Davis, L.A. Gossett, K.L. Pinkston, R.S. Liou, L.K. Sun, Y.W. Kim, N.T. Chang, T.W. Chang, K. Wagner, J. Bews, V. Brinkmann, H. Towbin, N. Subramanian, C. Heusser, Can anti-IgE be used to treat allergy?, *Springer Semin Immunopathol.* 15 (1993) 51–73.

- [5] C.E. Owen, Immunoglobulin E: role in asthma and allergic disease: lessons from the clinic, *Pharmacol. Ther.* 113 (2007) 121–133.
- [6] L. Presta, R. Shields, L. O'Connell, S. Lahr, J. Porter, C. Gorman, P. Jardieu, The binding site on human immunoglobulin E for its high affinity receptor, *J. Biol. Chem.* 269 (1994) 26368–26373.
- [7] S.C. Garman, B.A. Wurzburg, S.S. Tarchevskaya, J.P. Kinet, T.S. Jardetzky, Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc epsilonRI alpha, *Nature* 406 (2000) 259–266.
- [8] L.G. Presta, S.J. Lahr, R.L. Shields, J.P. Porter, C.M. Gorman, B.M. Fendly, P.M. Jardieu, Humanization of an antibody directed against IgE, *J. Immunol.* 151 (1993) 2623–2632.
- [9] G. D'Amato, A. Salzillo, A. Piccolo, M. D'Amato, G. Liccardi, A review of anti-IgE monoclonal antibody (omalizumab) as add on therapy for severe allergic (IgE-mediated) asthma, *Ther. Clin. Risk Manag.* 3 (2007) 613–619.
- [10] D. Nowak, Management of asthma with anti-immunoglobulin E: a review of clinical trials of omalizumab, *Respir. Med.* 100 (2006) 1907–1917.
- [11] K. Takagi, R. Nakamura, R. Teshima, J. Sawada, Application of human Fc epsilonRI alpha-chain-transfected RBL-2H3 cells for estimation of active serum IgE, *Biol. Pharm. Bull.* 26 (2003) 252–255.
- [12] B. Shopes, M. Weetall, D. Holowka, B. Baird, Recombinant human IgG1-murine IgE chimeric Ig. Construction, expression, and binding to human Fc gamma receptors, *J. Immunol.* 145 (1990) 3842–3848.
- [13] Z. Cao, J. Dai, K. Fan, H. Wang, G. Ji, B. Li, D. Zhang, S. Hou, W. Qian, J. Zhao, H. Wang, Y. Guo, A novel functional motif of osteopontin for human lymphocyte migration and survival, *Mol. Immunol.* 45 (2008) 3683–3692.
- [14] M. Kulka, C.H. Sheen, B.P. Tancowny, L.C. Grammer, R.P. Schleimer, Neuropeptides activate human mast cell degranulation and chemokine production, *Immunology* 123 (2008) 398–410.
- [15] I. Mirkina, T. Schweighoffer, F. Kricek, Inhibition of human cord blood-derived mast cell responses by anti-Fc epsilonRI mAb 15/1 versus anti-IgE omalizumab, *Immunol. Lett.* 109 (2007) 120–128.
- [16] M. Binder, F.N. Vögtle, S. Michelfelder, F. Müller, G. Illerhaus, S. Sundararajan, R. Mertelsmann, M. Trepel, Identification of their epitope reveals the structural basis for the mechanism of action of the immunosuppressive antibodies basiliximab and daclizumab, *Cancer Res.* 67 (2007) 3518–3523.
- [17] F. Perosa, E. Favoino, M.A. Caragnano, F. Dammacco, Generation of biologically active linear and cyclic peptides has revealed a unique fine specificity of rituximab and its possible cross-reactivity with acid sphingomyelinase-like phosphodiesterase 3b precursor, *Blood* 107 (2006) 1070–1077.
- [18] L. Zheng, B. Li, W. Qian, L. Zhao, Z. Cao, S. Shi, J. Gao, D. Zhang, S. Hou, J. Dai, H. Wang, Y. Guo, Fine epitope mapping of humanized anti-IgE monoclonal antibody omalizumab, *Biochem. Biophys. Res. Commun.* 375 (2008) 619–622.
- [19] G. Gomez, S. Jogie-Brahim, M. Shima, L.B. Schwartz, Omalizumab reverses the phenotypic and functional effects of IgE-enhanced Fc epsilonRI on human skin mast cells, *J. Immunol.* 179 (2007) 1353–1361.
- [20] L.A. Beck, G.V. Marcotte, D. MacGlashan, A. Togias, S. Saini, Omalizumab-induced reductions in mast cell Fc epsilonRI expression and function, *J. Allergy Clin. Immunol.* 114 (2004) 527–530.
- [21] H. Lin, K.M. Boesel, D.T. Griffith, C. Prussin, B. Foster, F.A. Romero, R. Townley, T.B. Casale, Omalizumab rapidly decreases nasal allergic response and Fc epsilonRI on basophils, *J. Allergy Clin. Immunol.* 113 (2004) 297–302.
- [22] S.S. Saini, D.W. MacGlashan Jr., S.A. Sterbinsky, A. Togias, D.C. Adelman, L.M. Lichtenstein, B.S. Bochner, Down-regulation of human basophil IgE and FC epsilonRI alpha surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo, *J. Immunol.* 162 (1999) 5624–5630.
- [23] T. Feuchtinger, H. Bartz, A. von Berg, F. Riedinger, J. Brauburger, S. Stenglein, U. Wahn, U. Schauer, Treatment with omalizumab normalizes the number of myeloid dendritic cells during the grass pollen season, *J. Allergy Clin. Immunol.* 111 (2003) 428–430.
- [24] C. Prussin, D.T. Griffith, K.M. Boesel, H. Lin, B. Foster, T.B. Casale, Omalizumab treatment downregulates dendritic cell Fc epsilonRI expression, *J. Allergy Clin. Immunol.* 112 (2003) 1147–1154.