

Epitope Analysis and Primary Structures of Variable Regions of Anti-human FcεRI Monoclonal Antibodies, and Expression of the Chimeric Antibodies Fused with Human Constant Regions

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The structural analysis of monoclonal antibodies (mAbs) against the α subunit of the high affinity IgE receptor (FcεRI α) is an alternative approach to obtaining information for the design of inhibitors that will block complementary interaction between IgE and FcεRI α and to analyzing the various biological effects induced by anti-FcεRI α autoantibodies in chronic urticaria. In this study, epitopes for mouse anti-human FcεRI α mAbs and primary structures of variable regions of the mAbs were analyzed. Three mAbs inhibitory for IgE-binding reacted to the deletion mutants of FcεRI α containing the whole second immunoglobulin-like domain as well as IgE did. On the other hand, two uninhibitory mAbs reacted to those containing the whole first immunoglobulin-like domain. The cDNAs for variable regions of the five mAbs were cloned and sequenced. Two inhibitory mouse/human chimeric antibodies were expressed in COS7 cells and bound to Chinese hamster ovary transfectant cells expressing FcεRI (CHO/ $\alpha\beta\gamma$), and these inhibited the binding of IgE to CHO/ $\alpha\beta\gamma$ cells.

Key words: high-affinity IgE receptor; monoclonal antibody; epitope; variable region; chimeric antibody

The high-affinity receptor for IgE (FcεRI) is the key molecule in the triggering of IgE-mediated allergic reactions. Multivalent allergens bridge the receptor-bound IgE and induce the aggregation of FcεRI

on the surface of mast cells and basophils, and in turn trigger the release of mediators responsible for allergic symptoms.^{1,2)} In humans, besides mast cells and basophils, several other types of immune effector cells and platelets have been shown to express FcεRI.^{3,4)} FcεRI consists of non-covalently associated subunits: one α chain (FcεRI α), one β chain, and two disulfide-linked γ chains.^{5,6)} The β and γ chains are required for expression of the α chain on the cell surface and for signal transduction. The binding site for the Fcε portion of IgE is located in the extracellular portion of FcεRI α .^{7,8)}

The extracellular portion of FcεRI α is highly glycosylated and composed of two immunoglobulin-like domains, D1 and D2. The binding site for IgE on FcεRI α has been investigated in studies using recombinant proteins and synthetic peptides.^{9–11)} Glycosylation of FcεRI α is not essential for IgE binding. The binding site was definitively localized to D2 by using a phage display method.¹⁰⁾ Various systems for the expression of FcεRI α have been developed for the structural analysis of FcεRI α and/or the IgE/FcεRI α complex.^{8,10,12)} Recently, the crystal structure of the extracellular portion of FcεRI α was described by Garman *et al.*¹³⁾

Analysis of antibodies (Abs) is an alternative approach to defining the binding site for ligands or receptors.^{14,15)} Sequences for variable (V) regions of two anti-human IgE monoclonal Abs (mAbs) were analyzed.^{16,17)} The binding site and amino acid

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Abbreviations: FcεRI, high affinity IgE receptor; FcεRI α , the α subunit of FcεRI; D1, the first domain; D2, the second domain; Ab, antibody; mAb, monoclonal Ab; autoAb, autoantibody; V, variable; C, constant; H, heavy; L, light; CHO, Chinese hamster ovary; CHO/ $\alpha\beta\gamma$, CHO transfectant expressing human FcεRI; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate; HRP, horseradish peroxidase; PCR, polymerase chain reaction; APC, antigen-presenting cell; CDR, complementarity determining region

residues on IgE that are critical for one of the anti-IgE mAbs were predicted by simulations of docking between molecular models of IgE and the anti-IgE mAb.¹⁸⁾ However, no amino acid sequences of V regions of anti-FcεRIα mAbs have been reported.

The IgE/FcεRI network helps to cause not only allergic diseases through allergen-specific IgE but also chronic urticaria (CU), a disease that is characterized by the recurrent and/or continuous degranulation of skin mast cells, through anti-IgE and anti-FcεRIα autoantibodies (autoAbs).^{19,20)} Specific IgG autoAbs directed against IgE or FcεRIα have been identified in 20–30% of the cases of CU studied. Although anti-IgE autoAbs are found also in normal sera, anti-FcεRIα autoAbs seem to be found in CU specifically. Therefore, IgG against FcεRIα is considered to a major factor in the pathogenesis of CU.²¹⁾ However, relationships among the activities, the structure, and the epitope-specificity of anti-FcεRIα autoAbs are still unknown.

The structural analysis of Abs against FcεRI is an alternative approach to obtaining information for the design of inhibitors that would block complementary interaction between IgE and FcεRI and to analyzing the various biological effects induced by anti-FcεRIα autoAbs. In this study, the FcεRIα fusion protein and its deletion mutants were overexpressed in *Escherichia coli* and analyzed for binding regions for five mouse anti-human FcεRIα mAbs and human IgE. Furthermore, primary structures of V regions of the mAbs were identified, and two mouse/human chimeric Abs inhibitory for IgE-binding to FcεRI, which were composed of mouse V regions and human constant (C) regions, were expressed in COS7 cells. The inhibitory chimeric Abs are useful as prototype Abs in antibody engineering to humanize the Abs for therapy and as mimics of human autoAbs for analysis of CU.

Materials and Methods

Mouse anti-FcεRIα mAbs and rabbit anti-FcεRIα polyclonal Ab. Mice were immunized with a recombinant extracellular portion of FcεRIα secreted from recombinant CHO cells²²⁾ and five hybridoma cell lines secreting anti-human FcεRIα mAbs were established (Kuromitsu *et al.*, manuscript in preparation). The five mouse anti-human FcεRIα mAbs, CRA1, CRA2, CRA3, CRA4, and CRA5, were purified from ascitic fluid by the methods of caprylic acid (Bio-Rad, Richmond, CA, U.S.A.) precipitation (1% (v/v), pH 4.8) (supernatant was recovered), 50% saturated ammonium sulfate precipitation (precipitate was recovered) and dialysis against phosphate-buffered saline (PBS). The concentrations of purified Abs were measured with a Protein Assay Kit (Bio-Rad).

Rabbits were also immunized with the recom-

binant extracellular portion of FcεRIα secreted from recombinant CHO cells.²²⁾ The polyclonal IgG antibodies were purified from the sera with ammonium sulfate precipitation and protein A affinity column (Protein A Sepharose 4 FF: Pharmacia) chromatography.

Flow cytometry. The binding of mouse mAbs and mouse/human chimeric Abs to CHO transfectant cells expressing human FcεRI (CHO/αβγ) was detected by flow cytometry. CHO/αβγ cells (2×10^5) were incubated with the mouse mAbs or the chimeric Abs diluted with FACS buffer (PBS, 0.1% bovine serum albumin, and 10 mM sodium azide, pH 7.4) ($0.2 \mu\text{g}/150 \mu\text{l}$) at 4°C for 30 min. The cells were washed three times with FACS buffer and incubated with a fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG Ab (Fc-specific) (Caltag, San Francisco, CA, U.S.A.) or an FITC-conjugated anti-human IgG Ab (Fc-specific) (Caltag) at 4°C for 30 min. The cells were then washed and analyzed with a FAC-Scan flow cytometer (Becton Dickinson, Mountain View, CA, U.S.A.).

Activities of the mouse mAbs and the chimeric Abs to inhibit the binding of human IgE to the CHO/αβγ cells were analyzed as follows. CHO/αβγ cells were incubated with the mouse mAbs or the chimeric Abs ($1 \mu\text{g}/100 \mu\text{l}$) at 4°C for 10 min. Then, FITC-conjugated human IgE ($0.2 \mu\text{g}/50 \mu\text{l}$) was added to the cell suspension. The cells were incubated at 4°C for 30 min, washed, and analyzed with the flow cytometer.

Construction of the expression vector for the FcεRIα fusion protein. The DNA fragment encoding the extracellular portion of the human FcεRIα with a stop codon was generated by the polymerase chain reaction (PCR) incorporating unique restriction sites to assist with subcloning. The PCR was done as described previously.²³⁾ The oligonucleotides, hα1F and hα172B, were used as primers for the PCR (Table 1). PCR products with an *Eco*RI site and a *Nde*I site at the 5' end and with a stop codon and a *Sal*I site at the 3' end were generated. The amplified fragments were digested with *Eco*RI and *Sal*I, and ligated to the pMAL-c vector,²⁴⁾ which was digested with *Eco*RI and *Sal*I. As a result, the DNA fragment encoding the extracellular portion of human FcεRIα with a stop codon was fused to the maltose binding protein gene. The DNA sequence of the inserted fragment was analyzed using a 373A DNA sequencer and Dyedexy Primer Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.) and confirmed to be the same as the corresponding region in a previous report.²⁵⁾

Construction of deletion mutants of the FcεRIα fusion protein. The DNA fragments encoding the deleted peptides of the extracellular portion of hu-

Table 1. PCR Primers Used for the Construction of Expression Vectors of the Extracellular Region of the Human FcεRI α Chain and Its Deletion Mutants

Name	Sequence	Mutant constructed using the primer
	<i>EcoRI</i> <i>NdeI</i>	
hα1F	5'-GCGAATTCCATATGGTCCCTCAGAAACCTAAGGTC-3'	1-172, 1-165, 1-155, 1-145, 1-128, 1-84, 1-60, 1-35
hα43F	5'-GCGAATTCCATATGGGCAGCCTTTCAGAAGAGACA-3'	43-172, 43-128
hα85F	5'-GCGAATTCCATATGAGTGACTGGCTGCTCCTTCAG-3'	85-172
hα100F	5'-GCGAATTCGGCCAGCCCCTCTTCCTC-3'	100-172
hα115F	5'-GCGAATTCGTGTACAAGGTGATCTAT-3'	115-172
	<i>SalI</i> stop	
hα35B	5'-CCGTCGACATTAACTGACTTCAAAGAAATT-3'	1-35
hα60B	5'-CCGTCGACATTAATAATTGGCATTACAAT-3'	1-60
hα84B	5'-GCCGTCGACACGTTAGAAGACTTCCAGGTACAC-3'	1-84
hα128B	5'-GCCGTCGACACGTTACTTGAGAGCTTCAACCATC-3'	1-128, 43-128
hα145B	5'-CCGTCGACATTAGTCTTCAACTGTGGCATT-3'	1-145
hα155B	5'-CCGTCGACATTACACTTGGCCGTACAGTA-3'	1-155
hα165B	5'-CCGTCGACATTAGAGGGGCTCAGACTCATA-3'	1-165
hα172B	5'-GCCGTCGACACGTTAAGCTTTATTACAGT-3'	1-172, 43-172, 85-172, 100-172, 115-172

The recognition sequences for *EcoRI*, *SalI*, and *NdeI* are underlined, or in italics. The sequences coding for stop codons are in bold.

man FcεRIα were generated by PCR and subcloned into the pMAL-c vector as was the DNA fragment encoding the whole extracellular portion. The oligonucleotides shown in Table 1 were used as primers for the PCR. The inserted fragments were sequenced and confirmed that they had the same sequences as the corresponding regions.

Expression of the FcεRIα fusion protein and its deletion mutants in *E. coli*. *E. coli* TB1 transformed with each expression vector was cultivated at 37°C in a 15-ml test tube containing 5 ml of LB medium with 100 µg/ml of ampicillin until the optical density at 600 nm of the culture reached 0.4. Then, isopropyl β-D-thiogalactoside was added to 1 mM and the incubation was continued for a further 4 hr.^{24,26} The cells were harvested by centrifugation, suspended in 500 µl of a buffer containing 10% (v/v) glycerol, 0.5% (w/v) SDS, and 5 mM Tris/HCl (pH 6.8) and boiled for 5 min.

Immunoblotting. Cell lysates were electrophoresed on sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) (4–20%) under non-reduced conditions.²⁷ After electrophoresis, the gel was used for immunoblotting as described previously but with some modifications.^{28,26} Proteins transferred onto nitrocellulose membranes (Bio-Rad) were incubated with the rabbit anti-human FcεRIα polyclonal Ab, mouse anti-human FcεRIα mAbs, or human myeloma IgE (Yamasa Co, Choshi, Japan), washed, and detected by a horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG Ab (Cappel, Malvern, PA, U.S.A.), an HRP-conjugated goat anti-mouse IgG Ab (Bio-Rad), or an HRP-conjugated goat anti-human IgE Ab (ε-specific, Biosource International, Camarillo, CA, U. S. A.) respectively, with diaminobenzidine (Wako, Osaka, Japan) and H₂O₂.

Cloning of V region genes for anti-FcεRIα mAbs. Poly A-RNAs were prepared from CRA1, CRA2, CRA3, CRA4, and CRA5 hybridoma cells by the method of Badley *et al.*²⁹ Using a ScFv Module Kit (Pharmacia, Uppsala, Sweden), double-stranded cDNAs were synthesized and the cDNAs coding the mouse V regions were specifically amplified by PCR by the methods described in the manufacturer's instructions. The cDNAs were cloned and sequenced. VL and VH family usages were identified by sequence similarity with mouse germline V and J genes registered in the GenBank database using the program GENETYX (Software Development Co., Tokyo, Japan). The complementarity determining regions (CDRs) of the mAbs were identified by comparison of their Vκ and VH with the IgG compendium of Kabat *et al.*³⁰

Construction of expression vectors for heavy (H) and light (L) chains of mouse/human chimeric anti-FcεRIα Abs. Expression vectors (pMHgpt, pSRL, and pSRH) were kindly provided by Dr. Hidetoshi Kanda, Eiken Chemical Co., Ltd., and Dr. Takeshi Watanabe, Kyushu University.³¹ Each of the pSRL and pSRH vectors has an SRα promoter.³² The vectors for expression of the chimeric L chains were constructed as follows. The genomic region coding for the signal sequence for the H chain of the mouse anti-human cALLA mAb on the pMHgpt vector³³ was modified so as to include a *XhoI* site at the 5' end, and a *PvuII* site at the 3' end by PCR. The cDNAs coding for the mouse Vκ of CRA2 or CRA4 were modified so as to contain a *PvuII* site at the 5' ends, and a splice donor sequence³⁴ and an *EcoRI* site at the 3' end by PCR. These Vκ cDNAs and the genomic DNA fragment coding for the signal sequence were treated with the restriction enzymes, and then linked to the gene coding for the human κ constant region³⁵

in a pSRL expression vector. The resulting vectors for expression of the chimeric L chains were designated pSRL-CRA2 and pSRL-CRA4.

The vectors for expression of the chimeric H chains were constructed as follows. The genomic region coding for the signal sequence for the H chain of the anti-cALLA mAb was modified so as to contain a *Xho*I site at the 5' end, and a *Pst*I site at the 3' end by PCR. The cDNAs coding for the mouse VH of CRA2 or CRA4 were modified so as to include *Pst*I sites at the 5' ends, and a splice donor sequence and *Eco*RI sites at the 3' ends by PCR. These VH cDNAs and the genomic DNA fragment coding for the signal sequence were treated with the restriction enzymes, and then linked to the gene coding for the human IgG1 constant region³⁶ in a pSRH expression vector. The resulting vectors for expression of the chimeric H chains were designated pSRH-CRA2 and pSRH-CRA4.

Expression of mouse/human chimeric anti-Fc ϵ RI α Abs in COS7 cells. Appropriate L and H chain expression vectors were co-transfected into COS7 cells using LipofectAMINE reagent (Life Technologies, Grand Island, NY, U.S.A.). GIT medium (Nihon Pharmaceutical Co., Tokyo, Japan) without fractions of IgG and IgM was used for expression of the mouse/human chimeric Abs. After culture for 5 to 15 days, the culture supernatants were harvested. The concentrations of fully assembled recombinant Abs in the supernatants were monitored by a sandwich ELISA using a goat anti-human IgG Ab (Fc-specific) (Cappel) and an HRP-conjugated goat anti-human κ chain Ab (Binding Site, Birmingham, U.K.).

Purification of mouse/human chimeric anti-Fc ϵ RI α Abs. Purification of the mouse/human chimeric Abs was done by the methods of caprylic acid (Sigma) precipitation, ammonium sulfate precipitation, protein A affinity column (Protein A Sepharose 4 FF: Pharmacia) chromatography, and size-exclusion gel filtration (Superdex200:Pharmacia). The concentrations of purified recombinant Abs were measured with a Protein Assay Kit (Bio-Rad).

Results

Activities of five mouse anti-human Fc ϵ RI α mAbs to bind to CHO transfectant cells expressing human Fc ϵ RI (CHO/ $\alpha\beta\gamma$) and to inhibit binding of human IgE to the cells

Five mouse anti-human Fc ϵ RI α mAbs, CRA2, CRA3, CRA4, CRA1, and CRA5, bound to CHO/ $\alpha\beta\gamma$ cells as well as FITC-conjugated human IgE (Fig. 1A). Three mAbs, CRA2, CRA3, and CRA4, significantly inhibited binding of FITC-conjugated human IgE but the other two mAbs, CRA1 and CRA5, did not (Fig. 1B).

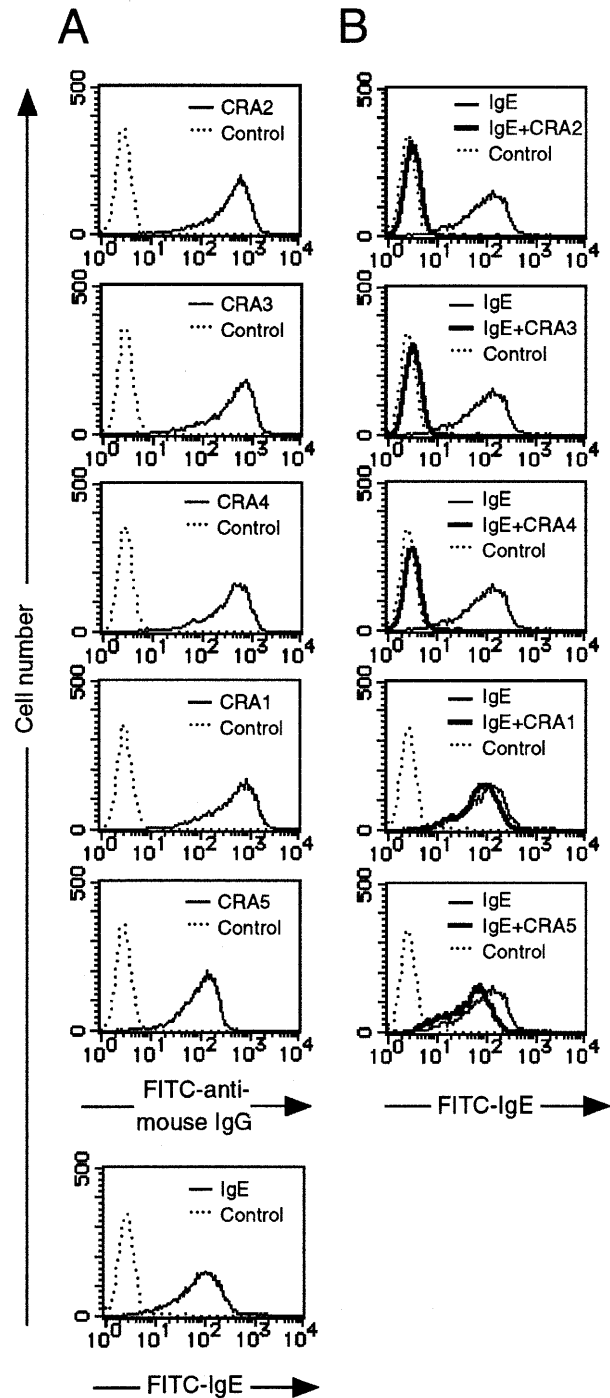


Fig. 1. Activities of Five Mouse Anti-human Fc ϵ RI α mAbs to Bind to CHO/ $\alpha\beta\gamma$ Cells Expressing Human Fc ϵ RI and to Inhibit Binding of Human IgE to the Cells.

(A) Binding of five mouse mAbs (CRA2 CRA3 CRA4, CRA1, and CRA5) and human IgE to CHO/ $\alpha\beta\gamma$ cells (solid line). Histograms for CHO/ $\alpha\beta\gamma$ cells incubated only with an FITC-conjugated anti-mouse IgG Ab are also shown as negative controls (dashed line). (B) Inhibition of binding of FITC-conjugated human IgE to CHO/ $\alpha\beta\gamma$ cells by the five mouse mAbs (bold line). Histograms for CHO/ $\alpha\beta\gamma$ cells only (dashed line) and for those incubated with FITC-conjugated human IgE only (solid line) are also shown as controls.

Expression of the FcεRIα fusion protein and its deletion mutants in *E. coli*

The extracellular portion of human FcεRIα and its deletion mutants were expressed as fusions with the maltose binding protein. Expression was confirmed by staining with Coomassie Brilliant Blue after SDS-PAGE and immunoblotting with the rabbit anti-human FcεRIα polyclonal Ab. The clones shown in Fig. 2 and Fig. 3 and the other clones shown in Fig. 4 produced the fusions as indicated by the main bands on SDS-PAGE, and the bands, except for the negative control (*malE-lacZ*), specifically reacted to the rabbit anti-human FcεRIα polyclonal Ab (Fig. 2).

Reactivities of the FcεRIα fusion protein and its deletion mutants to human IgE and five anti-human FcεRIα mAbs

To identify the regions recognized by human IgE or five mouse mAbs, reactivities of the FcεRIα fusion protein and its deletion mutants to human IgE and the mAbs were analyzed by immunoblotting. The results are shown in Fig. 3. Reactivities of other clones were analyzed by the same method and the results are summarized in Fig. 4. IgE and the five mAbs reacted to the fusion containing the entire extracellular portion of FcεRIα (1-172, D1D2). IgE reacted to the deletion mutants containing the whole D2 of FcεRIα (Fig. 4), but reactivities of two deletion mutants, 43-172 and 85-172 were significantly lower than that of 1-172 (D1D2) (Fig. 3A). Three inhibitory mAbs for IgE-binding to FcεRI, CRA2, CRA3, and CRA4, reacted to the deletion mutants containing the whole D2 (Fig. 3B and Fig. 4). The other two uninhibitory mAbs, CRA1, and CRA5, reacted to those containing the whole D1 (Fig. 3C and Fig. 4).

cDNA sequences of the V region of five mouse anti-human FcεRIα mAbs and the deduced amino acid sequences

The nucleotide sequences of the cloned V region cDNAs for five mouse mAbs are shown in Fig. 5 (The nucleotide sequences of V regions of the mouse anti-human FcεRIα mAbs have been submitted to the DDBJ, EMBL, and GenBank nucleotide sequence database with the following accession numbers: D88406 (CRA2VH), D88407 (CRA2VL), D88408 (CRA3VH), D88409 (CRA3VL), D88410 (CRA4VH), D88411 (CRA4VL), AB022778 (CRA1VH), AB022779 (CRA1VL), AB022780 (CRA5VH), and AB022781 (CRA5VL)). The use of VH and VL was decided by sequence similarity with mouse germline V and J genes. The VH and VL nucleotide sequences of CRA2 and CRA3 were highly similar. V genes that displayed 80% similarity with mouse germline V genes are shown in Table 2.³⁰⁾

The amino acid sequences of the variable regions of five mouse anti-human FcεRIα mAbs are shown in Fig. 6. The VH and VL amino acid sequences of CRA2 and CRA3 are highly similar consistent with the nucleotide sequences.

Table 2. VH and VL Family Usage by Anti-human FcεRIα Chain Monoclonal Antibodies

Hybridoma	VH	DH	JH	Vκ	Jκ
CRA2	7183	DFL16.le	4	21	2
CRA3	7183	DFL16.le	4	21	2
CRA4	J558	DFL16.2	4	12	1
CRA1	7183	DSP2.3, 4, 5	3	9	5
CRA5	7183	DSP2.3, 4, 6	4	5	5

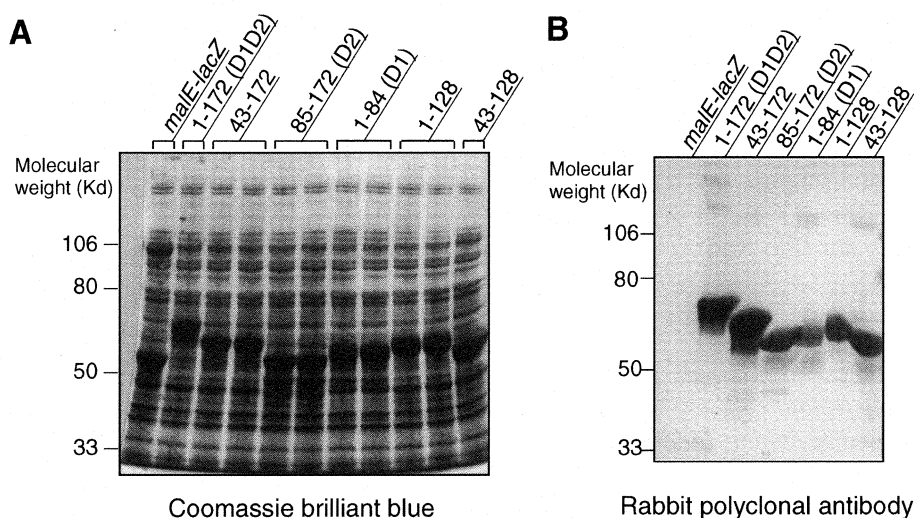


Fig. 2. Expression of the Human FcεRIα Fusion Protein and Its Deletion Mutants in *E. coli*.

Total cellular proteins of *E. coli* TB1 transformed with the expression vector of the extracellular portion of FcεRIα fused with the maltose binding protein (1-172), its deletion mutants (43-172, 85-172, 1-84, 1-128, and 43-128) or the control vector: pMAL-c encoding a fusion of the maltose binding protein and the β-galactosidase (*malE-lacZ*) were put on SDS-PAGE and electroblotted under non-reduced conditions. The membranes were stained with Coomassie Brilliant Blue (A) or probed with combination of the rabbit anti-FcεRIα polyclonal Ab and an HRP-conjugated anti-rabbit IgG Ab (B).

Purification of two mouse/human chimeric Abs, chimeric CRA2 and chimeric CRA4, secreted into the culture supernatant of COS7 cells

Mutations were introduced into the amino acid sequence in the N-terminal region in the VL sequences of chimeric CRA2 and chimeric CRA4 (QVQL was substituted for DIQM) and in the VH sequences of chimeric CRA4 (QVQL was substituted for QVKL). This was a result of the use of genomic DNA on the pMHgpt vector encoding not only the H chain signal sequence but also the N-terminal region of the H chain of the mouse anti-human cALLA mAb.³³⁾ The mouse/human chimeric Abs with the V regions of CRA2 or CRA4 and C regions of the κ light chain and the IgG1 heavy chain were expressed in COS7 cells. The transiently expressed chimeric CRA2 and chimeric CRA4 were secreted into the culture supernatant of the cells. Each of the secreted chimeric Abs was detected as a band of approximately 150 kDa in molecular mass on SDS-PAGE under non-reduced conditions (Fig. 7A). On SDS-PAGE of purified chimeric Abs, there was a band of approximately 150 kDa under non-reduced conditions and two bands of approximately 50 kDa for the H chain and approximately 20–30 kDa for the L chain under reduced conditions (Fig. 7B).

Activities of chimeric CRA2 and chimeric CRA4 to bind to CHO/ $\alpha\beta\gamma$ cells and to inhibit binding of human IgE to the cells

Chimeric CRA2 and chimeric CRA4 bound to CHO/ $\alpha\beta\gamma$ cells (Fig. 8A) and significantly inhibited the binding of FITC-conjugated human IgE (Fig. 8B). However, the inhibitory effect by chimeric CRA2 was weaker than that by mouse CRA2 (Fig. 1B and Fig. 8B).

Discussion

The glycosylated extracellular portion of human Fc ϵ RI α produced by recombinant CHO cells²²⁾ was used for immunization of mice to establish hybridoma cells secreting the five anti-human Fc ϵ RI α mAbs used in this study. Three mAbs, CRA2, CRA3, and CRA4, were inhibitory for IgE binding to Fc ϵ RI and the other two, CRA1 and CRA5, were not (Fig. 1B). Human IgE and all of the five mAbs bound to the Fc ϵ RI α fusion protein expressed in *E. coli* (Fig. 3). Human Fc ϵ RI α contains seven potential N-glycosylation motifs (Fig. 4), and the native Fc ϵ RI α and the recombinants expressed in mammalian cells are actually highly glycosylated.^{37,38,8)} The protein expressed in *E. coli* is not glycosylated. Therefore,

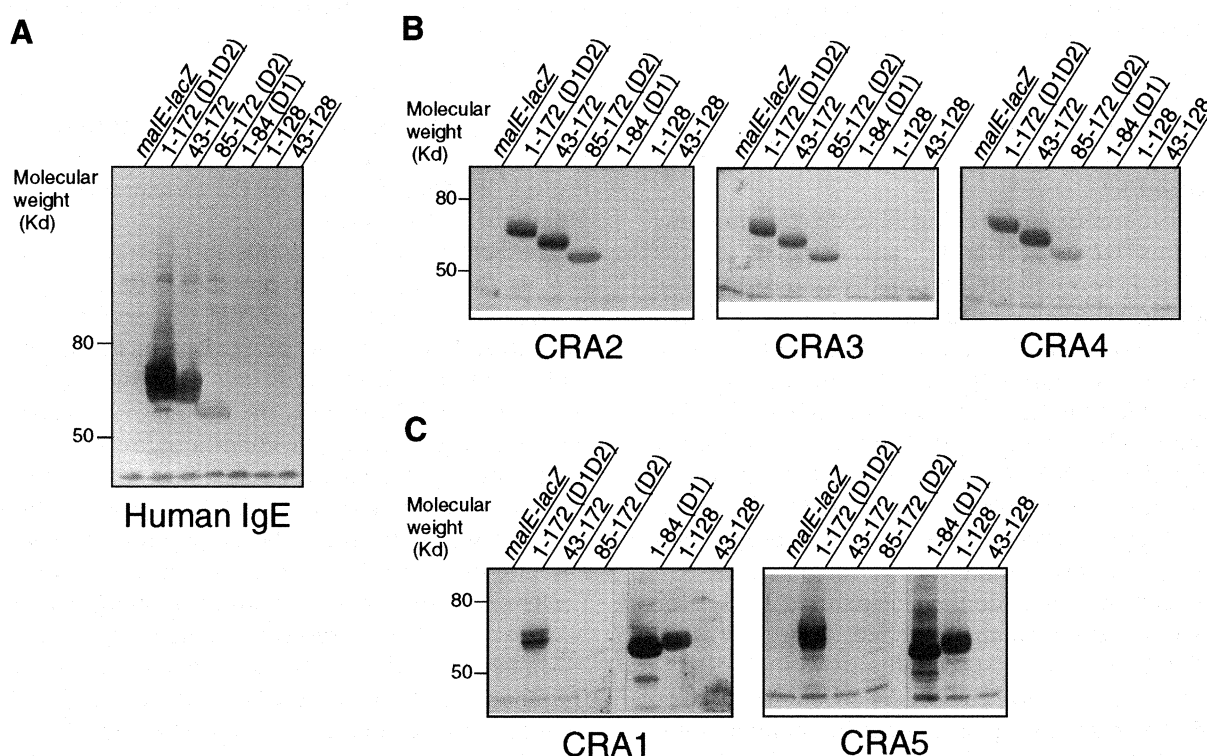


Fig. 3. Reactivities of the Human Fc ϵ RI α Fusion Protein and Its Deletion Mutants Expressed in *E. coli* to Human IgE and Five Mouse Anti-human Fc ϵ RI α mAbs.

Total cellular proteins of *E. coli* TB1 transformed with the expression vector of the extracellular portion of Fc ϵ RI α fused with the maltose binding protein (1–172), its deletion mutants (43–172, 85–172, 1–84, 1–128, and 43–128) or the control vector: pMAL-c encoding a fusion of the maltose binding protein and the β -galactosidase (*malE-lacZ*) were put on SDS-PAGE and electroblotted under non-reduced conditions. The membranes were probed with a combination of human myeloma IgE and an HRP-conjugated anti-human IgE Ab (A), with that of inhibitory mAbs (CRA2, CRA3, and CRA4) and an HRP-conjugated anti-mouse IgG Ab (B), or with that of non-inhibitory mAbs (CRA1 and CRA5) and an HRP-conjugated anti-mouse IgG Ab (C).

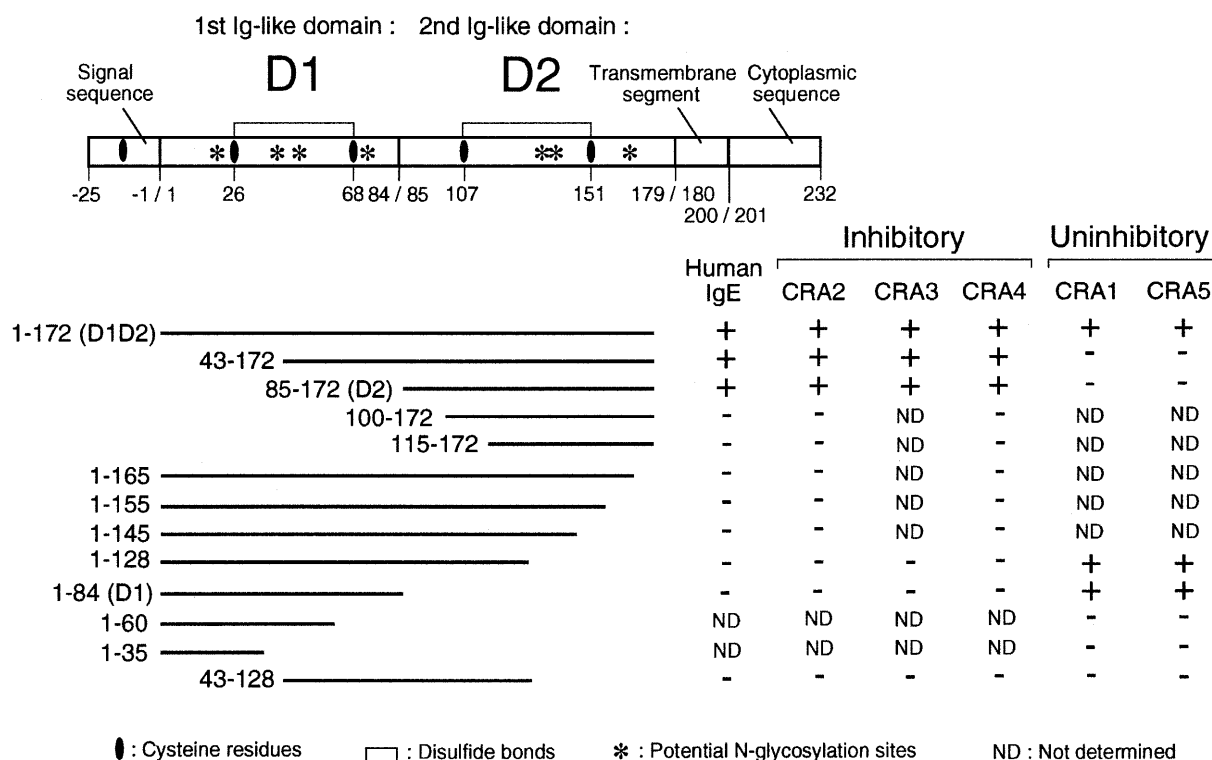


Fig. 4. Schematic Representation of Deletion Mutants of Human FcεRIα Expressed in *E. coli* and Their Reactivities to Human IgE and Five mAbs.

(+) denotes positive reaction. (-) denotes no reaction. The reactivities were measured by immunoblotting. A schematic view of the whole FcεRIα protein encoded by the cDNA is also displayed.²⁵⁾

glycosylation of FcεRIα was concluded not to be essential for the binding of the five mAbs as well as for that of IgE.

Three inhibitory mAbs reacted to deletion mutants containing D2 as well as IgE did (Fig. 3). On the other hand, two uninhibitory mAbs reacted to those containing D1. These results are consistent with the report by Robertson¹⁰⁾ that the binding site for IgE on FcεRIα was localized to the D2 domain, using a phage display method. The binding sites on FcεRIα for the inhibitory mAbs that recognize D2 might overlap that for IgE and could be targets of therapeutic reagents that inhibit the complementary interaction between IgE and FcεRI. For more precise epitope mapping, more mutants such that deletions were introduced into each domain recognized by IgE or mAbs were generated and analyzed. However, binding of IgE and mAbs to such mutants was not detectable (data not shown). The results are summarized in Fig. 4. The results suggest that the immunoglobulin-like fold of the whole D2 is essential for the binding to IgE and the inhibitory mAbs at least in immunoblot analysis, and that of the whole D1 for the binding to the uninhibitory mAbs. The reactivities of the two deletion mutants, 43-172 and 85-172, which contained the whole D2 but lost D1 partially or completely, to IgE were significantly lower than that of the fusion containing the whole D1D2, 1-172

(Fig. 3A). This suggests that some residues in D1 contribute to the binding to IgE or/and the folding of the IgE-binding structure of D2 to express the high affinity of IgE for FcεRIα.

The FcεRIα fusion was efficiently expressed in *E. coli* as the densest band on SDS-PAGE (Fig. 2). Robertson¹⁰⁾ previously reported the production of the extracellular portion of FcεRIα in the medium and periplasmic space of *E. coli*. But judging from the results of SDS-PAGE, the productivity seems to be not so efficient and the conditions for expression are critical. FcεRIα might be stabilized when fused to the maltose binding protein. The high productivity in our case would facilitate the preparation of a large amount of sample of the extracellular portion of FcεRIα and/or the IgE binding domain, D2, for NMR and/or X-ray crystallographic analysis.

Mallamaci *et al.*³⁹⁾ analyzed binding of IgE and anti-human FcεRIα mAbs to COS cells transfected with vectors coding chimeric receptors. They reported that transfectants with genes for single-domain α chains could not bind IgE and mAbs and that diversity exists in cell-surface expression levels among different chimeric receptors. Cook *et al.*⁴⁰⁾ used mutants of the extracellular portion of FcεRIα secreted from mammalian transfectant cells for the analysis of interaction with IgE. The efficiency of translocation to the cell surface and/or secretion of the variants of FcεR-

A

(7183)	10	20	30	40	50	60	70	80	90
CRA2VH	CAG GTG CAG CTG CAG GAG TCA GGG GGA GGT TTA GTG CAG CCT GGA GGG TCC CTG AAA CTC TCC TGT ACA GCC TCT GGA TTC ACT TTC AGC								
CRA3VH	 A.								
CRA1VH	 A.								
CRA5VH	 A.								
(J558)	 A.								
CRA4VH	 A.A								
CDR1					CDR2				
	100	110	120	130	140	150	160	170	180
CRA2VH	ACC TAT CCC ATG TCT TGG GTT CGC CAG ACT CCA GAG AAG AGG CTG GAG TGG GTC GCA TTC ATT AGT AAT CGT GGT GGT AGC ACC TAC TAT								
CRA3VH	C								
CRA1VH	.AT ... GG.								
CRA5VH	GA. ... TA. ... T.								
CRA4VH	.G. .C TAT .A CAC G AAG GG .T .GA C. .G.A .T A.T .G. .GG ... TA. CC. AAA AA. .T. .AT .T A.G ...								
	190	200	210	220	230	240	250	260	270
CRA2VH	CCA GAC ACT GTA AAG GGC CGA TTC ACC GTC TCC AGA GAC AAT GCC AAG AAT ATC CTG TAT CTG CAA ATG ACC AGT CTG AAG TCT GAG GAC								
CRA3VH	 A. .T.								
CRA1VH	G. .G.								
CRA5VH	G. .G.								
CRA4VH	AAT .G .GG T.C AAG GC. .A C.G A.T .C. A T. .TCC .GC .CA GCC .C A. .G C.C .G. .C CC ...								
JH									
CDR3									
	280	290	300	310	320	330	340	350	360
CRA2VH	ACG GCC ATG TAT TAC TGT GCA AGA CAT AAT TAT GGA GGA --- --- --- ATG GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA								
CRA3VH	C								
CRA1VH	 T.								
CRA5VH	..A GGA .C ... A.G TAC GGC TAT GCT G. ...								
CRA4VH	T.T .G G.C T. G CTT ACA GC. CGG .CT ACG --- --- GCT ...								

. : Identity with CRA2. - : Gap.

B

(VK21)	10	20	30	40	50	60	70	80	90
CRA2VL	GAC ATC CAG ATG CCC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG CAG AGG GCC ACC ATA TCC TGC AGA GCC AGT GAA AGT GTT GAT								
CRA3VL	 A.G								
(VK9)	xxx xxx xxx ... A.G ...								
CRA1VL	xxx xxx xxx ... A.G ...								
(VK5)	 A.								
CRA5VL	 A.								
(VK12)	 A.								
CRA4VL	 A.T ...								
CDR1					CDR2				
	100	110	120	130	140	150	160	170	180
CRA2VL	AGT TAT GGC AAC AGT TTT ATG CAC TGG TAC CAG CAG AAA CCA GGA CAG TCA CCC AAA CTC CTC ATG TAT CTT GCA TCC AAC CTA GAA TCT								
CRA3VL	T ...								
CRA1VL	--- G.C ATT .T .C .A .T.A AGT T. .C ...								
CRA5VL	--- ATA A.T TC. .A. .AC T. .T.T T ...								
CRA4VL	--- A. ATT T. ... AA. T.A GCA T ...								
	190	200	210	220	230	240	250	260	270
CRA2VL	GGG GTC CCT GCC AGG TTC ACT GGC AGT GGG TCT AGG ACA GAC TTC ACC CTC ACC ATT GAT CCT GTG GAG GCT GAT GAT GCT GCA ACC TAT								
CRA3VL	G.								
CRA1VL	A T.A ...								
CRA5VL	..A A .T C.C G.								
CRA4VL	..T .G .A T.A G.								
JK									
CDR3									
	280	290	300	310	320	330			
CRA2VL	TAC TGT CAG CAA AAT AAT GAG GAT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATC AAA CGG								
CRA3VL	T ...								
CRA1VL	..T TA .G T. .G. .A TT. ... CT. ...								
CRA5VL	C G GG. .G. AGT ATA .A CT. ...								
CRA4VL	A .T TT. TGG .GT AC. GG ...								

. : Identity with CRA2. - : Gap. x : Not determined.

Fig. 5. cDNA Sequences Coding for V Regions of Five Mouse Anti-human FcεRIα mAbs.

Nucleotide sequences of VH cDNAs (A) and VL cDNAs (B) of five mAbs. Dots (.) indicate the residues identical to those of CRA2. Bars (-) indicate gaps. Lines above the nucleotide sequences indicate the definition of CDRs by Kabat *et al.*³⁰ (CDR1, CDR2, and CDR3). The letter x indicates the residue was not identified.

A

		10	20	30	40	50	60
(7183)							
CRA2VH	QVQLQESGGGLVQPGGSLKLSCTASGFTFSTYPMSSWRQTPEKRLQWAFISNRGGSTYY						
CRA3VH	..K.....V.....Y.....						
CRA1VH	..K.....K.....V..E...N.G.....T..GD.SY.F.						
CRA5VH	..K..Q.....AT.....D.Y.F.....K.....Y..DGD.....						
(J558)							
CRA4VH	..K..Q..PE..K..A.VRI..K...Y..TS.YIH..K.R.GQG...IGW.YPKNVN.K.						

		70	80	90	100	110	120
CRA2VH	PDTVKGRTVSRDNKNILYLQMTSLKSEDTAMYYCARHNYGG---MDYWGQGTITVTVSS						
CRA3VH	...IM...I.....T.....N.....						
CRA1VH	..S.....I.....N.....S..R....L.F.ISLF.RSS--FPF.....						
CRA5VH	I.....T.....SR.....G..RYGYAV.....						
CRA4VH	NERF..KA.LTT.KSSSTA.M.LS..T...S.V.F..LTARAT--A.....						

B

		10	20	30	40	50	60
(Vk21)							
CRA2VL	DIQMPQSPASLAVSLGQRATISCRASESVDSYGNFHWYQQKPGQSPKLLMYLASNLES						
CRA3VL	...T.....P.....						
(Vk9)							
CRA1VL	xxx.T...S.MYA...E.V..T.K..Q---DI..YLS.FH...K...T.I.R.KR.VD						
(Vk5)							
CRA5VL	...T...TTM.A.P.EKI..T.S..S---ISSNYL.....F.....I.RT...A.						
(Vk12)							
CRA4VL	...T.....S..V.ETV..T.....NII.NLA.....Q.K..Q..V.A.T..AD						

		70	80	90	100	110
CRA2VL	GVPARFTGSGSRTDFTLTIDPVEADDAATYYCQNNEDPYTFGGGKLEIKR					
CRA3VL	S.....H.....					
CRA1VL	...S...S...GQ.YS...SSL.YE.MGI...L.YD.F.L...A.....x					
CRA5VL	S...G.SYS...GTM..E.V.....GSSI.L...A.....L..					
CRA4VL	...S...S...G.QYS.K.NSLQSE.FGS...HFWGT.W.....					

.. : Identity with CRA2. - : Gap. x : Not determined.

Fig. 6. Amino Acid Sequences of V regions of Five Mouse Anti-human Fc ϵ RI α mAbs Deduced from the cDNA Sequences.

Amino acid sequences of VH (A) and VL (B) of five mAbs were deduced from the cDNA sequences shown in Fig. 5. Dots (.) indicate the residues identical to those of CRA2. Dashes (-) indicate gaps. Lines above the sequences indicate the definition of CDRs by Kabat *et al.*³⁰⁾ (CDR1, CDR2, and CDR3). An x indicates the residue was not identified.

I α or the extracellular portion in the mammalian host cells is important in such experiments and preparation of the protein samples. In this study, the Fc ϵ RI α fusion protein and its deletion mutants are expressed in *E. coli* with high efficiency and with approximate equivalence in quantity among the variants. Therefore, the expression system would be convenient to analyze reactivities of numbers of mutants of the extracellular portion of Fc ϵ RI α and/or D2 to IgE and mAbs.

Primary and tertiary structures of antigen binding sites of the inhibitory mAbs would be candidates for therapeutic inhibitors for the interaction of Fc ϵ RI with IgE.^{41,14)} The primary structures of the inhibitory anti-Fc ϵ RI α mAbs (Fig. 6) that recognized D2 would provide useful information for designing such inhibitors. CRA2 and CRA3 were highly similar in the entire sequences of both VL and VH cDNAs (Fig. 5) and we concluded that they had differentiated

in the affinity maturation process from the same B cell clone. On the basis of differences in sequence, CRA2, CRA4, CRA1, and CRA5 were concluded to have differentiated from different origins (Fig. 5 and Table 2). Purified mouse/human chimeric Abs for the two inhibitory mAbs were confirmed as fully assembled by SDS-PAGE under reduced or non-reduced conditions (Fig. 7). The chimeric Abs bound to CHO/ $\alpha\beta\gamma$ cells and significantly inhibited the binding of FITC-conjugated human IgE (Fig. 8). The weaker inhibitory effect by chimeric CRA2 than by mouse CRA2 (Fig. 1B and Fig. 8B) might be attributable to the N-terminal region in the VL sequences of chimeric CRA2, where QVQL was substituted for DIQM, on the basis of further analysis for humanization of the mAb (Takai *et al.*, manuscript in preparation). Wright and Lim¹⁸⁾ predicted the binding site and amino acid residues on IgE that are critical for an inhibitory anti-IgE mAb, the primary structure of

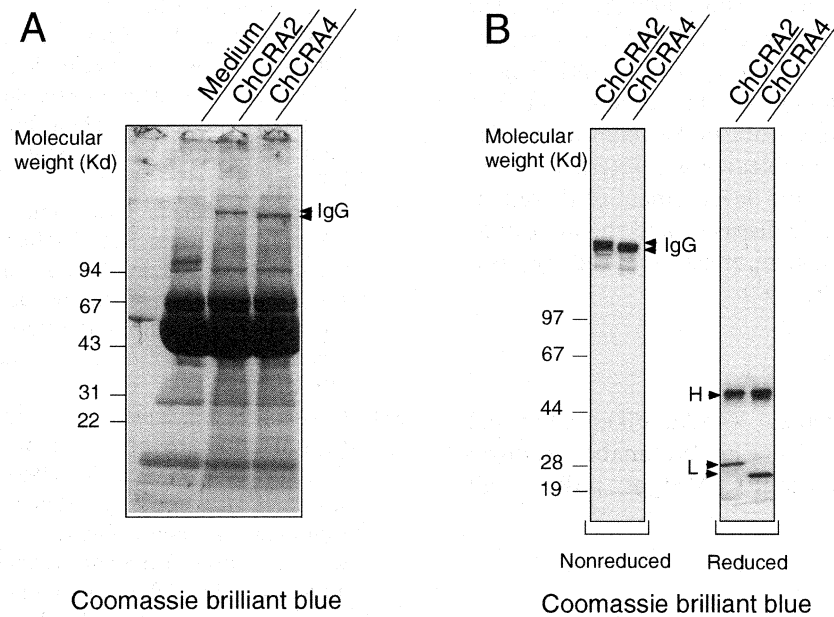


Fig. 7. Expression of Two Mouse/Human Chimeric Abs, Chimeric CRA2 and Chimeric CRA4, in COS7 Cells.

(A) Secretion of the chimeric Abs, into the culture supernatants of COS7 cells. The bands for the chimeric Abs are indicated as IgG. SDS-PAGE (4–20%) was done under non-reducing conditions. (B) Purified chimeric Abs. The bands for the chimeric Abs, their H chains, and their L chains are indicated as IgG, H, and L, respectively. SDS-PAGE (4–20%) was done under non-reducing or reducing conditions.

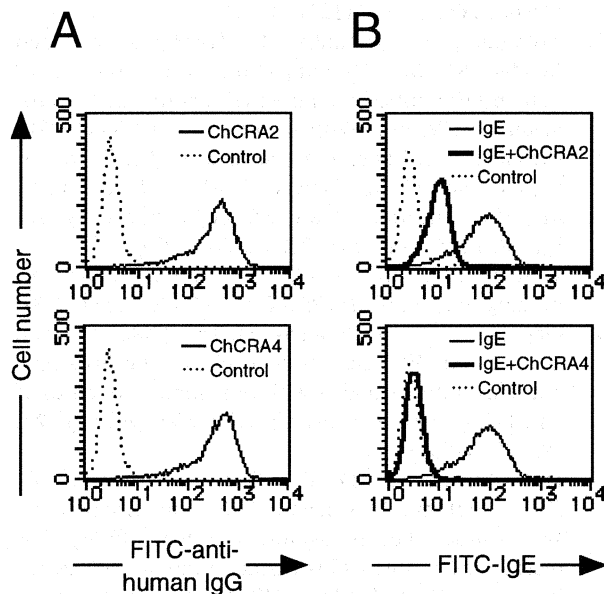


Fig. 8. Activities of Purified Chimeric CRA2 and Chimeric CRA4 to Bind to CHO/αβγ Cells and to Inhibit Binding of Human IgE to the Cells.

(A) Binding of the two mouse/human chimeric Abs, chimeric CRA2 (ChCRA2) and chimeric CRA4 (ChCRA4), to CHO/αβγ cells (solid line). Histograms for CHO/αβγ cells only are also shown as negative controls (dashed line). (B) Inhibition of binding of FITC-conjugated human IgE to CHO/αβγ cells by the chimeric Abs (bold line). Histograms for CHO/αβγ cells only (dashed line) and for those incubated with FITC-conjugated human IgE only (solid line) are also presented as controls.

which had been analyzed, by simulations of docking of a molecular model of the anti-IgE mAb to that of IgE. Prediction of binding sites for the anti-FcεRIα mAbs in this study might be possible by a similar simulation of docking of the molecular models of the anti-FcεRIα mAbs to the recently reported crystal structure of the extracellular portion of FcεRIα.¹³⁾ The inhibitory chimeric Abs are also useful as prototype Abs in antibody engineering to humanize the Abs for therapy.

Chronic urticaria (CU) is characterized by the recurrent and/or continuous degranulation of skin mast cells, through anti-IgE and/or anti-FcεRIα autoAbs.²¹⁾ IgG against FcεRIα is considered a major factor in the pathogenesis of CU. In humans, besides mast cells and basophils, Langerhans cells, peripheral blood dendritic cells, monocytes, and eosinophils have been demonstrated to express FcεRI. These FcεRI-expressing antigen-presenting cells (APCs) may act as tuners of specific immune responses. The APCs represent further cellular targets of anti-FcεRIα autoAbs. Anti-FcεRIα autoAbs might modulate the antigen presentation and migratory properties of the APCs.³⁾ Therefore, the inhibitory and uninhibitory recombinant Abs with human C regions, which could be generated by the use of cDNAs of V regions cloned in this study, might be also useful for analysis of the functional modulation of FcεRI-expressing APCs by anti-FcεRIα autoAbs. Inhibitory chimeric Abs were expressed in this study.

A highly efficient expression system for FcεRIα in *E. coli* was developed for rapid epitope analysis and

the inhibitory mAbs used in this study are useful for analyzing the IgE binding site on Fc ϵ RI α . The primary structures of the V regions of the anti-Fc ϵ RI α mAbs and the availability of the recombinant Abs with human C regions would make them useful for the analysis of the complementary interaction between IgE and Fc ϵ RI α , the development of therapeutic reagents for allergic diseases, and the molecular dissection of the biological effects induced by anti-Fc ϵ RI α autoAbs in CU.

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References

- Ishizaka, T. and Ishizaka, K., Activation of mast cells for mediator release through IgE. *Prog. Allergy*, **34**, 188–235 (1984).
- Metzger, H., Alcaraz, G., Hohman, R., Kinet, J.-P., Pribluda, V., and Quarto, R., The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.*, **4**, 419–470 (1986).
- Bieber, T., Fc ϵ RI-expressing antigen-presenting cells: new players in the atopic game. *Immunol. Today*, **18**, 311–313 (1997).
- Hasegawa, S., Pawankar, R., Suzuki, K., Nakahata, T., Furukawa, S., Okumura, K., and Ra, C., Functional expression of the high affinity receptor for IgE (Fc ϵ RI) in human platelets and its intracellular expression in human megakaryocytes. *Blood*, **93**, 2543–2551 (1999).
- Ra, C., Jouvin, M. H., and Kinet, J. P., Complete structure of the mouse mast cell receptor for IgE (Fc ϵ RI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. *J. Biol. Chem.*, **264**, 15323–15327 (1989).
- Blank, U., Ra, C., Miller, L., White, K., Metzger, H., and Kinet, J. P., Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature*, **337**, 187–189 (1989).
- Hakimi, J., Seals, C., Kondas, J. A., Pettine, L., Danho, W., and Kochan, J., The α subunit of the human IgE receptor (Fc ϵ RI) is sufficient for high affinity IgE binding. *J. Biol. Chem.*, **265**, 22079–22081 (1990).
- Blank, U., Ra, C., and Kinet, J. P., Characterization of truncated α chain products from human, rat and mouse high affinity receptor for immunoglobulin E. *J. Biol. Chem.*, **266**, 2639–2646 (1991).
- Sayers, I. and Helm, B. A., Structural basis of human IgE-Fc receptor interactions. *Clin. Exp. Allergy*, **29**, 585–594 (1999).
- Robertson, M. W., Phage and *Escherichia coli* expression of the human high affinity immunoglobulin E receptor α -subunit ectodomain: domain localization of the IgE-binding site. *J. Biol. Chem.*, **268**, 12736–12743 (1993).
- McDonnell, J. M., Beavil, A. J., Mackay, G. A., Jameson, B. A., Korngold, R., Gould, H. J., and Sutton, B. J., Structure based design and characterization of peptides that inhibit IgE binding to its high-affinity receptor. *Nature Struct. Biol.*, **3**, 419–426 (1996).
- Yagi, S., Yanagida, M., Tanida, I., Hasegawa, A., Okumura, K., and Ra, C. High-level expression of the truncated α chain of human high-affinity receptor for IgE as a soluble form by baculovirus-infected insect cells: biochemical characterization of the recombinant product. *Eur. J. Biochem.*, **220**, 593–598 (1994).
- Garman, S. C., Kinet, J. P., and Jardetzky, T. S., Crystal structure of the human high-affinity IgE receptor. *Cell*, **95**, 951–961 (1998).
- Rodríguez-Romero, A., Almog, O., Tordova, M., Randhawa, Z., and Gilliland, G. L., Primary and tertiary structures of the Fab fragment of a monoclonal anti-E-selectin 7A9 antibody that inhibits neutrophil attachment to endothelial cells. *J. Biol. Chem.*, **273**, 11770–11775 (1998).
- Riske, F., Hakimi, J., Mallamaci, M., Griffin, M., Pilson, B., Tobkes, N., Lin, P., Danho, W., Kochan, J., and Chizzonite, R., High affinity human IgE receptor (Fc ϵ RI): analysis of functional domains of the α -subunit with monoclonal antibodies. *J. Biol. Chem.*, **266**, 11245–11251 (1991).
- Kolbinger, F., Saldanha, J., Hardman, N., and Bendig, M. M., Humanization of a mouse anti-human IgE antibody: a potential therapeutic for IgE-mediated allergies. *Protein Eng.*, **6**, 971–980 (1993).
- Presta, L. G., Lahr, S. J., Shields, R. L., Porter, J. P., Gorman, C. M., Fendly, B. M., and Jardieu, P. M., Humanization of an antibody directed against IgE. *J. Immunol.*, **151**, 2623–2632 (1993).
- Wright, J. D. and Lim, C., Prediction of an anti-IgE binding site on IgE. *Protein Eng.*, **11**, 421–427 (1998).
- Grattan, C. E. H., Francis, D. M., Hide, M., and Greaves, M. W., Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin. Exp. Allergy*, **21**, 695–704 (1991).
- Hide, M., Francis, D. M., Clive, E. H., Grattan, C. E. H., Hakimi, J., Kochan, J. P., and Greaves, M. W., Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N. Engl. J. Med.*, **328**, 1599–1604 (1993).
- Fiebiger, E., Stingl, G., and Mauer D., Anti-IgE and anti-Fc ϵ RI autoantibodies in clinical allergy. *Curr. Opin. Immunol.*, **8**, 784–789 (1996).
- Ra, C., Kuromitsu, S., Hirose, T., Yasuda, S., Furuichi, K., and Okumura, K., Soluble human high-affinity receptor for IgE abrogates the IgE-mediated allergic reaction. *Int. Immunol.*, **5**, 47–54 (1993).
- Suzuki, K., Hirose, T., Matsuta, H., Hasegawa, S., Okumura, K., and Ra, C., The Fc receptor (FcR) γ subunit is essential for IgE-binding activity of cell-surface expressed chimeric receptor molecules constructed from human high-affinity IgE receptor (Fc ϵ RI) α and FcR γ subunits. *Mol. Immunol.*, **35**, 259–270 (1998).

- 24) Mania, C. V., Riggs, P. D., Grandea III, A. G., Slatko, B. E., Moran, L. S., Tagliamonte, J. A., and McReynolds, L. A., An *Escherichia coli* vector to express and purify foreign proteins by fusion to and separation from maltose-binding protein. *Gene*, **74**, 365–373 (1988).
- 25) Kochan, J., Pettine, L. F., Hakimi, J., Kishi, K., and Kinet, J. P., Isolation of the gene coding for the α subunit of the human high affinity IgE receptor. *Nucleic Acids Res.*, **16**, 3584 (1988).
- 26) Takai, T., Yuuki, T., Mori, A., Okumura, Y., Mori, A., and Okudaira, H., Determination of the N- and C-terminal sequences required to bind human IgE of the major house dust mite allergen Der f 2 and epitope mapping for monoclonal antibodies. *Mol. Immunol.*, **34**, 255–261 (1997).
- 27) Laemmli, U. K., Cleavage of structural proteins during assembly of the head of the bacteriophage T4. *Nature*, **227**, 680–685 (1970).
- 28) Tovey, E. R. and Baldo, B. A., Standardization of Allergens. Qualitative definition of house dust mite extracts following electroblotting and detection of components with antibody and lectin probes. *Int. Arch. Allergy Appl. Immunol.*, **75**, 322–329 (1984).
- 29) Badley, J. E., Bishop, G. A., St. John, T., and Frelinger, J. A., A simple rapid method for the purification of poly A⁺ RNA. *BioTechniques*, **6**, 114–116 (1988).
- 30) Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S., and Foeller, C., Sequences of Proteins of Immunological Interest. 5th Edit. U.S. Department of Health and Human Services, Public Health Service National Institutes of Health (1991).
- 31) Kanda, H., Mori, K., Koga, H., Taniguchi, K., Kobayashi, H., Sakahara, H., Konishi, J., Endo, K., and Watanabe, T., Construction and expression of chimeric antibodies by a simple replacement of heavy and light chain V genes into a single cassette vector. *Hybridoma*, **13**, 359–366 (1994).
- 32) Takebe, Y., Seiki, M., Fujisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M., and Arai, N., SRα promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell. Biol.*, **8**, 466–472 (1988).
- 33) Kudo, A., Nishimura, Y., and Watanabe, T., The antibody molecule to common acute lymphocytic leukemia (cALL) antigen used the identical or closely related VH gene segment as that of MOPC-21 immunoglobulin heavy chain. *J. Immunol.*, **135**, 642–645 (1985).
- 34) Sharp, P. A., Splicing of messenger RNA precursors. *Science*, **235**, 766–771 (1987).
- 35) Hieter, P. A., Max, E. E., Seidman, J. G., Maizel, J. V., and Leder, P., Cloned human and mouse kappa-immunoglobulin constant and J region genes conserve homology in functional segment. *Cell*, **22**, 197–207 (1980).
- 36) Takahashi, N., Ueda, S., Obata, M., Nikaido, T., Nakai, S., and Honjo, T., Structure of human immunoglobulin gamma genes: implications for evolution of a gene family. *Cell*, **29**, 671–679 (1982).
- 37) Kanellopoulos, J. M., Liu, T. Y., Poy, G., and Metzger, H., Composition and subunit structure of the cell receptor for immunoglobulin E. *J. Biol. Chem.*, **255**, 9060–9066 (1980).
- 38) LaCrix, E. L., and Froese, A., The N-linked oligosaccharides of the Fcε receptors of rat basophilic leukemia cells. *Mol. Immunol.*, **30**, 321–330 (1993).
- 39) Mallamaci, M. A., Chizzonite, R., Griffin, M., Nettleton, M., Hakimi, J., Tsien, W. H., and Kochan, J. P., Identification of sites on the human FcεRI α subunit which are involved in binding human and rat IgE. *J. Biol. Chem.*, **268**, 22076–22083 (1993).
- 40) Cook, J. P. D., Henry, A. J., McDonnell, J. M., Owens, R. J., Sutton, B. J., and Gould, H. J., Identification of contact residues in the IgE binding sites of human FcεRIα. *Biochemistry*, **36**, 15579–15588 (1997).
- 41) Monfardini, C., Kieber-Emmons, T., VonFeldt, J. M., O'Malley, B., Rosenbaum, H., Godillot, A. P., Kaushansky, K., Brown, C. B., Voet, D., McCallus, D. E., Weiner, D. B., and Williams, W. V., Recombinant antibodies in bioactive peptide design. *J. Biol. Chem.*, **270**, 6628–6638 (1996).