Interaction of Human IgE with Soluble Forms of IgE High Affinity Receptors

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Purpose. Interaction of human IgE with its high affinity receptor (FcεRI) on mast cells and basophils is an important step for initiating IgE mediated immune responses. To characterize the IgE and FcεRI interaction, we investigated this interaction in terms of stoichiometry and binding affinity in solution. The binding of IgE and IgE FcεRI α chain, the extracellular portion of IgE high affinity receptor (sFcεRIα) was compared with the binding of IgE and IgE immunoadhesin (FcεRIα-IgG).

Methods. The interaction was characterized by analytical ultracentrifugation, size exclusion chromatography, light scattering and ELISA.

Results. We show that the sFcεRIα is only able to bind to one IgE, while the immunoadhesin can bind to two IgE. The interaction between IgE and FcεRI is very strong. Both forms of soluble receptors have similar intrinsic binding affinity with IgE.

Conclusions. Both soluble receptors (FcεRIα-IgG and sFcεRIα) can block the binding of IgE to its high affinity receptors on cell surface. The FcεRIα-IgG is a better IgE binding protein than sFcεRIα at physiological relevant conditions. A humanized anti-IgE monoclonal antibody, rhuMAb E25 that also can block the binding of IgE to its high affinity receptors appears to bind to IgE at slightly different regions or in a different manner as the soluble forms of IgE receptors.

KEY WORDS: analytical ultracentrifuge; anti-IgE monoclonal antibody; IgE Fc high affinity receptor; light scattering; complex.

INTRODUCTION

Human IgE is an important immunoglobulin that has been widely implicated in allergic responses. Two cell surface receptors, FcεRI (1), and FcεRII/CD23 (2) have been identified as the primary transducers of IgE activity. The high affinity IgE receptor, FcεRI, is a multi-subunit complex consisting of three polypeptides, an extracellular α-chain, a transmembrane β chain and two intracellular disulphide-linked γ chains. This receptor is primarily expressed on the surface of mast cells and basophils and is best known for its central role in type I hypersensitivity disorders (3). The extracellular portion of α-chain contains the binding sites for the Fc portion of human IgE. Therefore, the soluble form of the extracellular α-chain can be used as a specific IgE inhibitor that may be potentially useful as a therapy for IgE mediated allergic diseases.

Recently, Haak-Frendscho reported a novel immunoadhesin as a specific IgE inhibitor. This chimeric molecule, FcεRIα-IgG contains the extracellular portion of human FcεRI α-chain linked to a truncated form of IgG1 heavy chain. It has two functional binding sites to human IgE and a markedly longer plasma half-life than the single soluble extracellular binding domain, sFcεRIα (4). Therefore, potentially it may be more useful than simple sFcεRIα as a therapeutic agent to treat allergic diseases.

The ability of sFcεRIα and FcεRIα-IgG to inhibit the binding of IgE to its high affinity Fc receptor on mast cells and basophils, depends not only on their intrinsic binding affinity but also on the stoichiometry and stability of complex that was formed. In this study, we have explored in detail the formation of IgE—FcεRI-IgG and IgE—sFcεRIα complexes in solution using both analytical ultracentrifugation and light scattering approaches. Based on the stoichiometry of these complexes, the intrinsic binding affinity of both soluble receptors to IgE were determined and used to evaluate their potency at the physiological relevant condition.

MATERIALS AND METHODS

Materials

A humanized anti-IgE monoclonal antibody, rhuMAb E25 and human IgE were prepared essentially as described previously (5). The soluble extracellular domain of IgE high affinity receptor, sFcεRI-α was expressed in CHO cells and was kindly provided by Dr. Jarema Kochan from Hoffmann-La Roche (6). The human IgE receptor α-chain IgG chimera, FcεRI-IgG was constructed by transplanting the entire α-chain of the human FcεRI into the constant region of human IgG1 heavy chain as described previously by Haak-Frendscho (4). All samples for physical and chemical characterization were prepared in phosphate-buffered saline (PBS) consisting of 137 mM NaCl, 2.7 mM KCl, 7.9 mM Na_3PO_4, 1.14 mM K_3PO_4, pH 7.2.

Sedimentation Equilibrium

The average molecular weights of protein molecules and their complexes were determined by sedimentation equilibrium analysis. Sedimentation equilibrium experiments were performed at different rotor speeds at 10°C in a Beckman XLA ultracentrifuge using charcoal-filled Epon six-channel Yphantis cells essentially as described previously (5). The loading concentration of samples are from 0.05 mg/ml to 0.5 mg/ml. The partial specific volumes, $\bar{\nu}$, of sFcεRI-α (0.688 ml/g), FcεRI-IgG (0.700 ml/g) and IgE (0.716 ml/g) were calculated from their amino acid and average carbohydrate composition (7–9). The density of PBS (ρ = 1.006 g/ml) was measured directly with a digitized PAAR density meter (DMA 35, Anton Paar K. G.).

The data for sedimentation equilibrium were edited using a PC program, REEDIT. The edited data were analyzed as a single ideal species using the non-linear least square fitting program, NONLIN (10) to yield the weight average molecular weight.

Sedimentation Velocity

Sedimentation velocity experiments were also conducted in a Beckman XLA ultracentrifuge. Sedimentation velocity

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analyses were carried out at 60,000 rpm at 10°C using charcoal-filled Epon 12 mm double-sector centerpieces. The moving boundary was monitored by repetitive radial scanning at a constant time interval of one minute using the XLA UV absorption optical system.

Sedimentation velocity data from the acquisition software (XLA, Beckman, Inc.) were analyzed using the g(s) program of Stafford (11) to produce the differential sedimentation coefficient distribution. The apparent sedimentation coefficients ($S_{10,solvent}$) at 10°C in PBS were corrected to the standard condition in water at 20°C ($S_{20,w}$) using the standard method described previously (12).

Chromatography

All Size Exclusion Chromatography experiments were performed on a Hewlett-Packard 1090 HPLC system with a diode-array detector (DAD). TSK-Gel G4000SW$_{XL}$ and TSK-Gel G2000SW$_{XL}$ (TOSOHAAS) size exclusion columns were equilibrated with PBS at a flow rate of 0.5 ml/min. The BioRad Gel filtration standard, composed of thyroglobulin, γ-globulin, ovalbumin, myoglobin and cyanocobalamin, was used to monitor the performance of the column throughout the experiments. Separations of FcεRI-IgG, IgE and their complexes were achieved on a TSK-Gel G4000SW$_{XL}$ at room temperature.

Light Scattering

Light scattering for each fraction from the chromatography was measured using an on-line miniDAWN light scattering detector (Wyatt Technology) equipped with a laser light source at a wavelength of 690 nm and three detectors at different angles. The detector was connected directly after a size exclusion column on a Hewlett-Packard 1090 HPLC system installed with a 0.1 μm in-line filter. A Refractive index detector (HP 1047, Hewlett-Packard) was placed after the miniDAWN detector to measure the concentration distribution. The refractive index increment (dn/dc) was determined by injection of a small volume of sample with a known concentration into the RI detector. Data were acquired and processed using Wyatt ASTRA software version 4.0 for PC. This program calculated the weight average molecular weight of each slice of peak with a Debye plot using the Zimm equation (13).

Determination of Intrinsic Binding Affinity

The intrinsic binding affinity of IgE and receptor interaction was determined using an ELISA based measurement (14). Briefly, samples containing either sFcεRIα or FcεRIα-IgG immunoadhesin at various concentrations were mixed with 500 ng/ml IgE. After incubation, the free IgE at equilibrium was determined by a solid phase ELISA. The ELISA assay was performed in high binding flat bottom polystyrene plates (Costar, Cambridge, MA) coated overnight at 2–8°C with 100 ng of FcεRIα-IgG immunoadhesin in 100 μl of pH 9.6 carbonate buffer. The plates were washed with 0.05% Tween 20 in PBS, then incubated with 200 μl of assay diluent (0.5% bovine serum albumin, 0.05% Tween 20, 0.01% thimerosal in PBS) for 1 to 2 hours. FcεRIα-IgG captures the free IgE in solution. The plates were then incubated with a biotinylated anti-IgE monoclonal antibody and the color reaction was generated with avidin-horseradish peroxidase and o-phenylenediamine/H_2O_2. The concentration of free IgE was calculated from the standard curve and then applied into the mass association equation with the model determined by analytical ultracentrifuge and light scattering to calculate the intrinsic binding affinity.

RESULTS AND DISCUSSION

Characterization of IgE, sFcεRIα and FcεRIα-IgG

The molecular weights of IgE, sFcεRIα and FcεRIα-IgG were determined by sedimentation equilibrium and light scattering (Table I). The experimentally determined molecular weights of FcεRIα-IgG and sFcεRIα are much larger than their core proteins calculated from their amino acid sequences. This result is consistent with previous observation that both FcεRIα-IgG and sFcεRIα are highly glycosylated (3). Each protein was monitored by SDS-PAGE and sedimentation velocity (Data not shown) to ensure its stability throughout the experiments.

Stoichiometry of Complex Formed by IgE and sFcεRIα

Both sedimentation equilibrium (Figure 1) and sedimentation velocity (Data not shown) experiments showed that IgE and sFcεRIα are able to form complexes of limited size. The highest average molecular weight, approximately 215,000 Da, acquired at equal molar ratio gives a very similar molecular weight to a hetero-dimer (Table I). This suggests that the complex formed by IgE and sFcεRIα contains the same amount of both components.

The hetero-dimer appears to have very similar hydrodynamic properties as IgE (Table I). Both sedimentation velocity and size exclusion chromatography show no significant separation between IgE and complex, indicating that they may have similar shape.

To further characterize its stoichiometry, size exclusion chromatography was utilized to isolate the complex. Since the complex formed by IgE and sFcεRIα elutes almost at the same position as IgE on a TSK G2000SW$_{XL}$ column, the isolation of complex was conducted under conditions where there is a large excess of sFcεRIα to ensure that most of the IgE was converted to the complex. The molecular weight of isolated complex determined from sedimentation equilibrium again suggests a stoichiometry of a hetero-dimer with one IgE and one sFcεRIα (Table I). This result confirms the previous observation that each IgE is only able to bind one IgE high affinity receptor (15).

Stoichiometry of Complexes Formed by IgE and sFcεRIα-IgG

Both size exclusion chromatography and sedimentation analyses showed that IgE and FcεRIα-IgG are able to form two types of complexes (Figure 1, 2, 3). At two to one molar ratio (IgE:sFcεRIα-IgG = 2:1), IgE and FcεRIα-IgG form the most of the largest complex with sedimentation coefficient around 13 s. The distribution of complex formation not only depends on the concentration of the two interacting components but also on their molar ratios. Under conditions where there is a large excess of IgE, the predominant complex formed by IgE and FcεRIα-IgG appears to have the stoichiometry of the largest complex, while under conditions where there is a large excess of FcεRIα-IgG, an intermediate complex with a sedimentation

Table I. Characterization of IgE, sFcεRIα, FcεRIα-IgG and Isolated Complexes

	MW(SE)[a]	MW(LS)[b]	Calculated MW[c]	$S_{20,w}$[d]
IgE	193,000 ± 10,000	187,000 ± 20,000	190,000	8.2 ± 0.1
sFcεRIα	34,500 ± 750	35,000 ± 3,000	36,000	2.7 ± 0.1
FcεRIα-IgG	136,000 ± 3,000	136,000 ± 30,000	140,000	6.3 ± 0.1
IgE:sFcεRIα Dimer	229,000 ± 10,000	220,000 ± 10,000	226,000	8.3 ± 0.2
IgE:FcεRIα-IgG Dimer	320,000 ± 20,000	343,000 ± 30,000	330,000	10.3 ± 0.2
IgE_2:FcεRIα-IgG Trimer	520,000 ± 30,000	533,000 ± 40,000	520,000	13.5 ± 0.2

[a] Weight average molecular weights of IgE, soluble receptors and purified complexes, determined by sedimentation equilibrium.
[b] Weight average molecular weight determined by light scattering.
[c] Molecular weight from amino acid and carbohydrate composition.
[d] Sedimentation coefficient at standard condition of water at 20°C.

coefficient approximately of 10 s accounts for the majority of the major complexes.

Using sedimentation equilibrium to analyze the average molecular weight of isolated complex, the stoichiometry of the largest complex was identified as a hetero-trimer with one FcεRIα-IgG and two IgE. The intermediate complex formed by IgE and FcεRIα-IgG is a hetero-dimer with one IgE and one FcεRIα-IgG. Similar results were also obtained from light scattering experiments. The average molecular weights determined from chromatography and light scattering data are very consistent with the model of a hetero-trimer for the largest complex and a hetero-dimer for the intermediate complex (Table I). These results further confirmed that the FcεRIα-IgG is a bivalent molecule and the two IgE high affinity α chains are fully functional and capable of interacting with two IgE. In contrast, the IgE appears to be monovalent and only binds to one FcεRIα-IgG. As a result, IgE and FcεRIα-IgG are only able to form two types of complexes.

Binding Affinity of IgE with IgE High Affinity Receptor

The binding affinity of IgE with either FcεRIα-IgG or sFcεRIα was initially studied using sedimentation equilibrium. To avoid the interference from incompetent IgE or receptor monomer, the largest complex that was formed by IgE and receptor was first isolated using size exclusion chromatography. For IgE and sFcεRIα interaction, the complex that was formed by IgE and sFcεRIα is extremely stable. No apparent dissociation was observed during size exclusion chromatography. In addition, the average molecular weight of this complex obtained from sedimentation equilibrium at the lowest concentration (~0.1 mg/ml) that we tested gives almost exactly the same molecular weight of a hetero-dimer with one IgE and one sFcεRIα (Table I). The sedimentation equilibrium data were fitted using a non-linear least square fitting program, NONLIN.

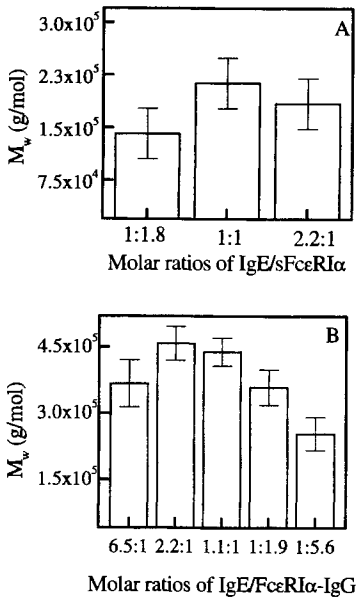

Fig. 1. Sedimentation equilibrium analysis of IgE and receptors complex formation in PBS at 10°C. The weight average molecular weights of IgE with sFcεRIα (A) and IgE with FcεRIα-IgG (B) at different molar ratios were obtained by analyzing the data from different rotor speeds as a single ideal species simultaneously. The error bars correspond to a 95% confidence interval.

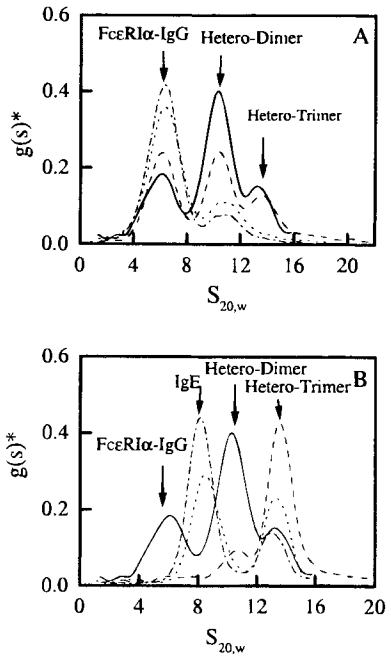

Fig. 2. Sedimentation velocity analysis of IgE and FcεRIα-IgG complex formation at (A) 1:1 (—), 1:2 (---), 1:6 (····) and 1:10 (-·-·-) molar ratios; (B) 1:1 (—), 2:1 (---), 6:1 (····) and 10:1 (-·-·-) molar ratios. The sedimentation coefficients have been corrected to the standard condition of water at 20°C. No faster moving species were observed at early scanning.

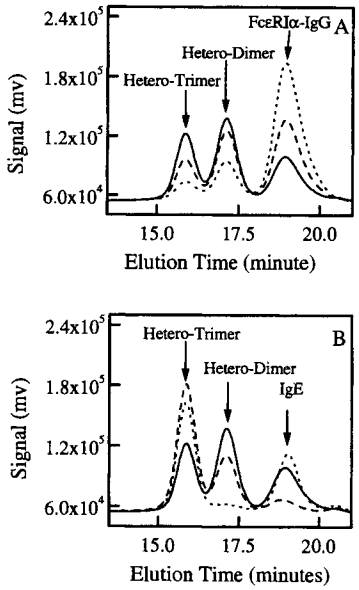

Fig. 3. Size exclusion chromatography analysis of IgE and FcεRIα-IgG complexes at (A) 1:1 (—), 1:2 (---) and 1:6 (····) molar ratios; (B) 1:1 (—), 2:1 (---) and 6:1 (-·-·-) molar ratios. A refractive index detector was applied to measure the concentration distribution. Separation of complexes was achieved in TOSOHAAS TSK-Gel G4000 SW_{XL} size exclusion column with a flow rate of 0.5 ml/min.

A single ideal species model gives the best fit (Figure 4), as seen by the random distribution of the residuals and low root mean square. Taken together, these results confirm the previous observation that IgE strongly interacts with sFcεRIα.

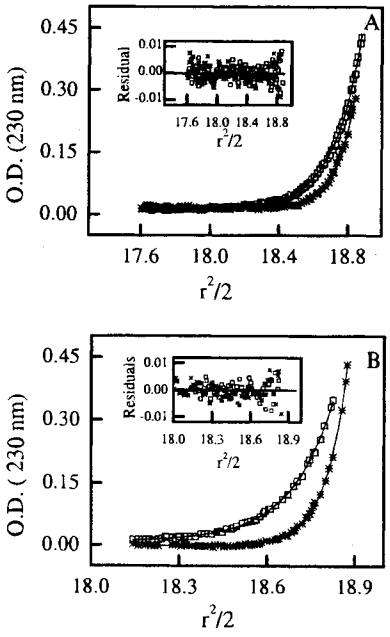

Fig. 4. Non-linear least square analysis of sedimentation equilibrium experiments of the purified largest complex. The data were collected at (A): 10,000 rpm (□) and 14,000 rpm (*) for the largest complex formed by IgE and FcεRIα-IgG; (B): 14,000 rpm (□) and 17,000 rpm (*) for the largest complex formed by IgE and sFcεRIα. These data were globally fitted to a model of a single ideal species to obtain the weight average molecular weight for each complex. The insert figure shows the residuals of the fit.

Similar results have also been obtained for IgE and FcεRIα-IgG interaction. The purified largest complex is very stable during the chromatography and the average molecular weight at the concentrations (0.05 mg/ml to 0.5 mg/ml) that we tested is almost the same as that of a hetero-trimer with two IgE and one FcεRIα-IgG (Table I and Figure 4).

To further investigate the binding affinity of IgE and receptor, an ELISA based free IgE assay was developed. Previous studies with the α subunit of the human IgE receptor showed that the dissociation rate of IgE and receptor complex was extremely slow, while the association rate was very fast (6). Moreover, a control study with this ELISA based free IgE assay showed that the equilibrium has not been disturbed during the time of measurements (less than 60 minutes, data not shown). Therefore, it is possible to determine the concentration of free IgE in solution at equilibrium by using an ELISA with a relatively short period of incubation time. Based on the initial concentration of interacting components and stoichiometry of complex determined by sedimentation and light scattering methods, we calculated the average intrinsic binding affinity of IgE with both FcεRIα-IgG ($7.1 \times 10^9 \pm 4.4 \times 10^9\ M^{-1}$) and sFcεRIα ($7.4 \times 10^9 \pm 4.0 \times 10^9\ M^{-1}$). Both soluble receptors were shown to strongly interact with IgE with similar intrinsic binding affinity. The result further confirmed the previous observation (6) that the IgE high affinity receptor on cell surface interacts very strongly with IgE.

It should be noted that the binding affinity determination performed here has not considered the presence of incompetent or less competent monomers of either IgE or receptors. Such micro-heterogeneity of biological molecules can be very common since most of the purification scheme is based on their physical chemical properties, rather than their biological function. Indeed, our binding data from both sedimentation and size-exclusion chromatography suggest that a small amount of IgE or FcεRIα-IgG may have less binding affinity. These molecules tend to form the weakly associated trimer at the concentration we tested even when the IgE is in excess. As a result, the intrinsic binding affinity from ELISA may only reflect a lower limit of the real binding affinity.

FcεRIα-IgG Is a Better Inhibitor than sFcεRIα

We showed here that both FcεRIα-IgG and sFcεRIα can strongly bind to IgE in solution. The binding affinities of these soluble receptors to IgE are similar to those described for the high affinity FcεRI receptors on cultured cord blood basophils, COS and CHO cells transfected with the either α and γ subunits, or chimeric α subunit (6,16–17). Since the initial IgE/receptor binding is a reversible process, the use of these high affinity soluble receptors can then specifically prevent the binding of IgE to its high affinity receptor on mast cell and basophils. Therefore, they can be potentially used as a therapeutic agent to treat allergic disease.

The FcεRIα-IgG and sFcεRIα have similar intrinsic binding affinity with IgE. However, the overall binding ability (or avidity) of FcεRIα-IgG with IgE is very different from the sFcεRIα with IgE (Figure 5). The magnitude of such a difference not only depends on the intrinsic binding affinity of these molecules, but also relies on the stoichiometry of complexes

that were formed, since FcεRIα-IgG is capable of forming both hetero-dimer and hetero-trimer with IgE, while the sFcεRIα only forms a hetero-dimer. When there is a large excess of FcεRIα-IgG, the concentration of hetero-dimer is much higher than that of hetero-trimer. As a result, the FcεRIα-IgG is only about twice as potent as the sFcεRIα. In contrast, if the IgE is in large excess, such a difference can be significantly increased, since the FcεRIα-IgG and IgE can form much more hetero-trimer than the hetero-dimer. Therefore, the bivalent FcεRIα-IgG is a much more effective antagonist than the monovalent sFcεRIα under such conditions.

In addition, an immunoadhesin molecule, such as FcεRIα-IgG contains the portion of human IgG1 heavy chain. The addition of an immunoglobulin Fc domain has been demonstrated to extend the plasma half life of the soluble extracellular binding domain (18–19). Such a feature can largely increase the efficiency of those competitive inhibitors. The application of such inhibitors to block the binding of IgE to its high affinity receptor has recently been demonstrated by Haak-Frendscho. They showed that the FcεRIα-IgG is a potent inhibitor that can block the IgE binding to intracutaneous mast cells *in vivo*. Therefore, it may be potentially useful, as an alternative approach for the treatment of IgE-mediated disease.

Comparison with IgE and Anti-IgE Interaction

It has been shown that the binding site of human IgE for its high affinity receptor is localized in three loops, which form a putative ridge on the most exposed side of the Fcε3 domain of IgE and include Arg-408, Ser-411, Lys-415, Glu-452, Arg-465, and Met-469 (20). One approach to block the binding of IgE to FcεRI is to use an anti-IgE monoclonal antibody that specifically targets the region of IgE that is involved in its interaction with FcεRI. A humanized anti-IgE monoclonal antibody, rhuMAb E25 has been recently developed by Genentech to treat IgE-mediated allergic disease. Both *in vivo* and *in vitro* studies have shown that rhuMAb E25 can effectively block the binding of IgE to its receptor (21). Like the FcεRIα-IgG, the rhuMAb E25 is a bivalent molecule. It was specifically designed to target the FcεRI binding region of IgE, however the interaction of rhuMAb E25 with IgE appears to be very different from the interaction of FcεRIα-IgG with IgE (Figure 6). First, the size of the complexes formed by rhuMAb E25 and IgE are much larger than the complexes formed by IgE and FcεRIα-IgG (5). Second, each IgE is capable of interacting with two rhuMAb E25, but only with one FcεRIα-IgG. These results suggest that the rhuMAb E25 may interact with IgE in a different manner than its receptor.

At least two possibilities might account for such a difference. The first is that the binding area of IgE to FcεRI may be larger and may require the involvement of both heavy chains, while rhuMAb E25 may interact with only one heavy chain. This hypothesis is supported by the observation of relatively higher intrinsic binding affinity between IgE and FcεRI than that of IgE and rhuMAb E25. In addition, each extracellular portion of FcεRI α chain has two similar domains that may well cover any potential binding sites on IgE for another receptor. The hydrodynamic modeling study of IgE-Fc and sFcεRI by Keown *et al.*, has further suggested that the binding region of IgE and its high affinity IgE-Fc receptor is very likely to involve both IgE heavy chains (22). The second possibility is that the rhuMAb E25 may have a more flexible structure than the FcεRIα-IgG. Therefore, it can avoid the interference that is caused by the effect of steric hindrance. However, this is unlikely since the sFcεRIα, that is much smaller and more compact in size than rhuMAb E25, demonstrates almost the same behavior as FcεRIα-IgG. Further studies with X-ray crystallography and two-dimensional NMR certainly will provide valuable structural information to determine the cause of such a difference.

CONCLUSIONS

In conclusion, we determined the stoichiometry and intrinsic binding affinity of IgE and soluble forms of IgE high affinity receptor using several different biochemical and biophysical techniques. Both FcεRIα-IgG and sFcεRIα have similar intrin-

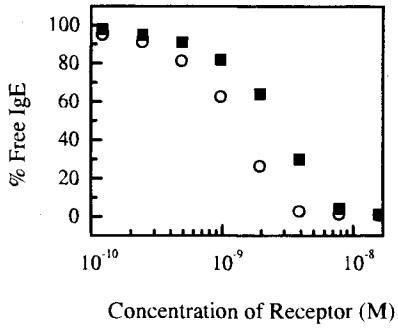

Fig. 5. Comparison of complexes formation by IgE-sFcεRIα (■) and IgE-FcεRIα-IgG (○). Human IgE that has a initial concentration of 1 μg/ml (5.1×10^{-9} M) was titrated with increasing amount of soluble receptors. The amount of free IgE at equilibrium was calculated using the model determined by sedimentation and light scattering experiments.

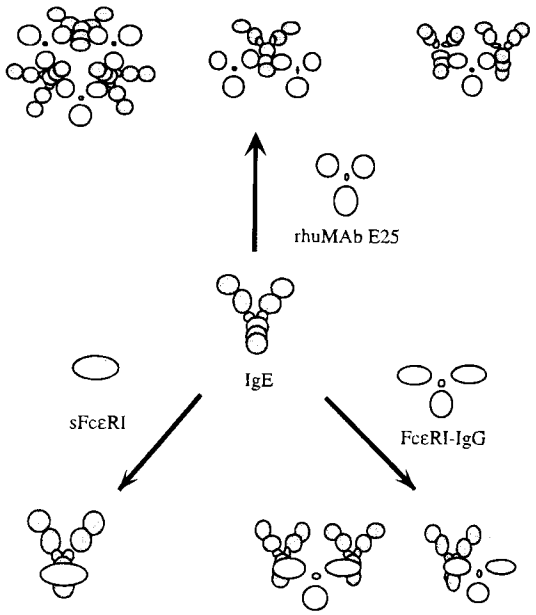

Fig. 6. Schematic diagram of complex formation by IgE and an anti-IgE monoclonal antibody, rhuMAb E25, IgE and FcεRIα-IgG, and IgE and sFcεRIα.

sic binding affinity with IgE as the receptor on the surface of mast cells and basophils. The FcεRIα-IgG overall is more efficient than sFcεRIα in blocking the binding of IgE to its high affinity Fc receptor on cell surface. It was also shown that the humanized anti-IgE monoclonal antibody, rhuMAb E25 and soluble form of IgE receptor may bind to slightly different regions of human IgE.

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