REPORT

Model studies of transmembrane interaction of FcεRIα/FcRγ reveal novel strategies to inhibit allergic responses

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Abstract: The high-affinity IgE receptor complex plays an essential part in allergic responses and involved in downstream signaling, released inflammatory mediators that cause allergic responses. The transmembrane region of the high-affinity IgE has a conserved motif (LFAVDTGL) where a polar aspartate (D194) is important for the ligand binding. This modeling study proposes novel potential binding sites between high affinity immunoglobulin E receptor α subunit (FcεRIα) and FcRγ and as a consequence, we propose a new model of FcεRIα and FcRγ interaction (T194) which can mediate downstream signaling in allergic response. The docking of FcRγ with wild-type (D194) and mutant human high affinity immunoglobulin E receptor α subunit (D194T, D194I, D194L, D194A, D194V, D194E, D194S and D194R) has been performed on Autodock Vina. This modeling study is based on lab data obtained by carrying out site-directed mutagenesis done at residue D194 of FcεRIα to assess its functional importance for the mediation of intracellular signal cascade. HuFcεRIα D194 residue was replaced with threonine, leucine, serine, arginine, alanine, asparagine and glutamic acid. FcRγ docking on mutated huFcεRIα (D194T) indicated a new site of interaction and emphasizes the significance of the charge and size of an amino acid at position 194 in huFcεRIα subunit. Amino acids D & T at position 194 are important for cell surface localization, interactions, distribution and downstream signaling of IgE receptor subunit. These proposed models may herald in better therapeutic interventions to combat unfavorably allergic diseases.

Keywords: FcεRIα, FcRγ allergic responses, transmembrane, protein-docking.

INTRODUCTION

Epidemiological data shows globally rise in atopic diseases (Pawankar *et al.*, 2013). Immunoglobulin E and mast cells play vital role in the hypersensitive immune reactions. The newly synthesized and preformed mediators released by mast cells and basophils mediate immediate hypersensitivity in response to different stimuli. Sensitisation of basophils and mast cells expressing the high-affinity IgE receptors which firmly attached with antibody and activates in response to cognate allergen has well defined regulatory mechanism of secretion of dynamic active intermediately molecules mediates symptoms of hypersensitivity class I. Therapeutic intervention strategies are focused on developing rationally designed agents targeting the IgE receptor and its downstream signaling pathway (Liu *et al.*, 2011).

Conserved transmembrane (TM) motifs in immunoreceptors may have an important role in the assembly, function and expression of receptor. Several studies have shown the significance of chemical nature of amino acid inside the transmembrane (TM) in binding, stability and function of various cell surface receptors. TM domains of FcγRIIIα and FcεRIα subunits have invariant motif of eight amino acids (LFAV**D**TGL) which appeared to be conserved in humans, rats and mice. This motif that dominatingly made out of hydrophobic amino acids contains a hydrophilic aspartic acid residue (D194) which, in principle, is energetically not stable in the hydrophobic center of plasma membrane.

The detection of a transmembrane (TM) amino acid residues involved in downstream signaling would be useful to treat the IgE mediated hypersensitivity response through the incorporation of an amino acid in peptide sequence which may have the ability to inhibit the TM downstream signaling. The aim of our previous study was to analyse and understand the importance of conserved amino acid in the interaction between the subunits of high affinity immunoglobulin E receptor (FcεRI) and role in mediating TM signaling using site directed mutagenesis technique (Rashid *et al.*, 2010). In view of information got from the study, a model to express the interaction between alpha and gamma subunits in the TM region was proposed (Rashid *et al*., 2010). Further analysis of these

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results has now been carried out leading to the presentation of a novel proposal to predict productive interactions between alpha and gamma chains of IgE receptor in the propagation of downstream signal cascade.

MATERIALS AND METHODS

Rat basophilic leukemia (RBL)-2H3.1 were transfected with human high affinity immunoglobulin E receptor α subunit (huFcεRIα) to produce rat/human chimeric receptors and subsequently site-directed mutagenic huFcεRIα targeting the D194 to assess the effect of these mutations on subsequent receptor expression and signaling (Rashid *et al.*, 2012; Rashid *et al*., 2010). The FcεRIα and FcRγ protein interaction 3D model has been designed using I-TASSER algorithm tool (Zhang, 2009). The docking of FcRγ with wild-type (D194) and mutant FcεRIα (D194T, D194I, D194L, D194A, D194V, D194E, D194S and D194R) at D194 of FcεRIα and Thr 22 of FcRγ has been performed on Autodock Vina (Trott and Olson, 2010). The best model was selected on the basis of low energy minimization, RMDS at ±0.5 and C-score ±0.3.

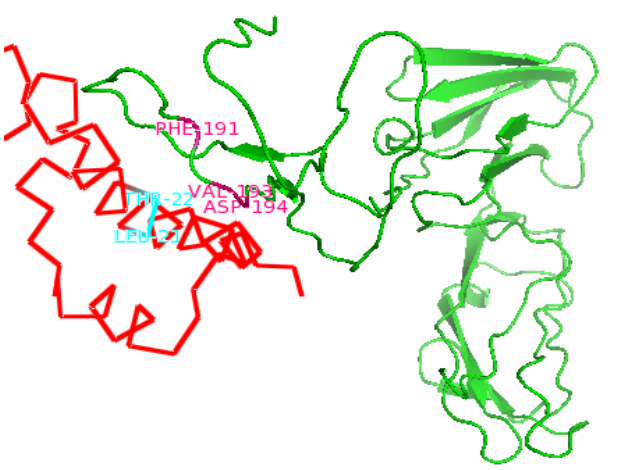

Fig. 1: Model of FcεRIα and FcRγ interaction involving D194 of FcεRIα and Thr 22 of FcRγ.

RESULTS

The D194 residue is important for the expression of cell surface FcεRIα and FcRγ (fig. 1). This interaction takes place at residue D194 of FcεRIα and at Thr22 of FcRγ Transition from D194 to T194 in FcεRIα created new binding sites with FcRγ (fig. 2). This model identified potential new binding interaction between FcεRIα and FcRγ. This was a functional receptor complex with cell surface expression as well as mediator release.

The replacement of aspartate with Serine did not support expression of the receptor complex (fig. 3). FcRγ residue Thr22, Gln-49, Val-20, Cys-26 have been computed to form binding with residue SER-194, PHE-181, PHE-101, Val-188 on FcεRIα.

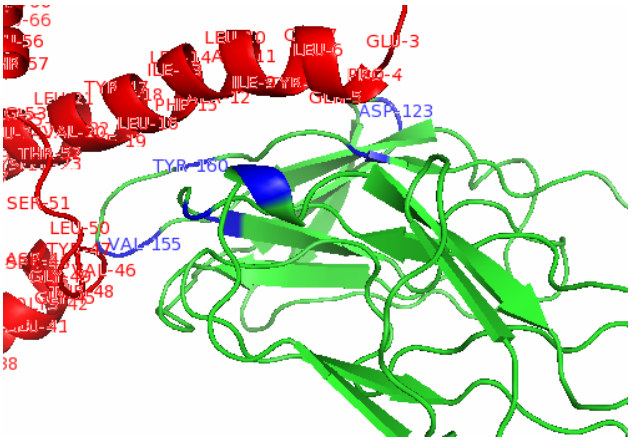

Fig. 2: D194T: Transition from D194 to T194 in FcεRIα

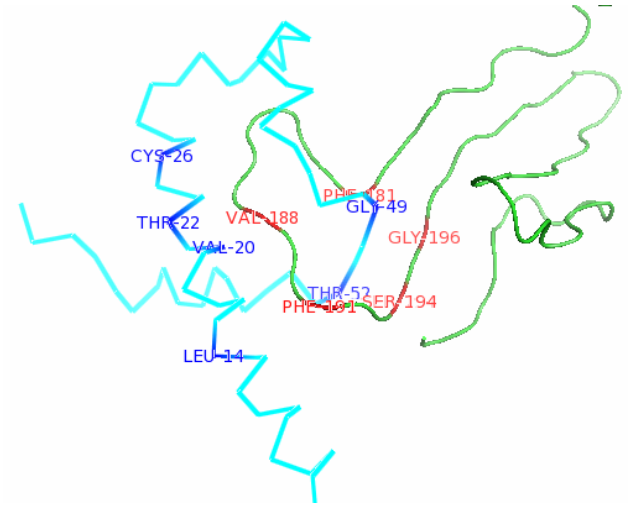

Fig. 3: The aspartate residue mutated to amino acid Serine

The substitution of aspartate with arginine was created new interaction of (fig. 4) residue Phe-15, lys-62, Thr-22, Tyr-25 in FcRγ with PHE-182, LEU-179, ARG-194 on FcεRIα. This ligand-receptor complex again failed to support receptor expression. The transmembrane of FcRγ involving residues Ile-13, Phe-15, Gly-18, Thr-48, Leu-16 with ILE-194, THR-195, VAL-197, ALA-192 in the TM of FcεRIα protein. This interaction failed to support receptor expression when aspartte was changed to isoleucine (fig. 5).

The figs. 6 and 7 show replacement of aspartate with alanine and glutamic acid in FcεRIα. As evidenced by the failure of such constructs to facilitate cell surface expression after binding with FcRγ. Transition D194L in FcεRIα is predicted to cause the change in binding sites of FcRγ. FcRγ residue Leu14, Leu16, TRP8 Leu10 Glu64, His 63 may form interactions at L194, SER200, HIS224 THR191 on FcεRIα protein (fig. 8).

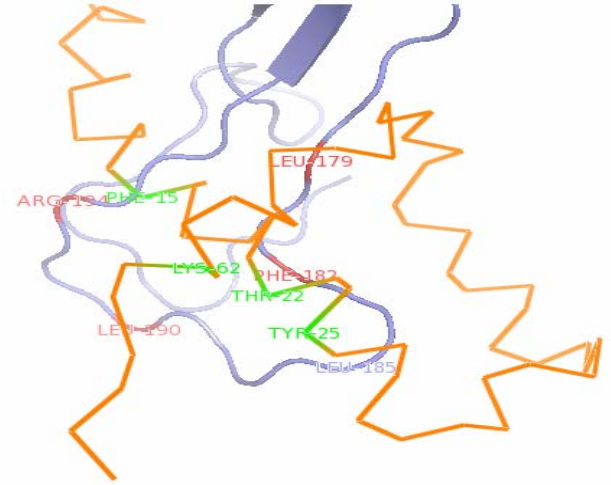

Fig. 4: Molecular model of FcεRIα after substitution of aspartate with arginine.

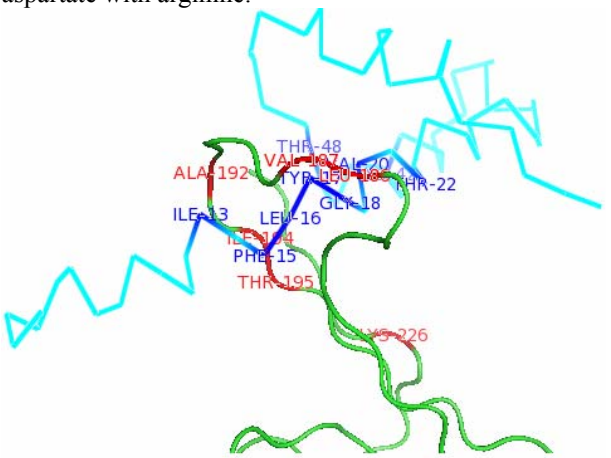

Fig. 5: Proposed computed model for interaction between FcεRIα and FcRγ chain. Transition of aspartate to isoluecine

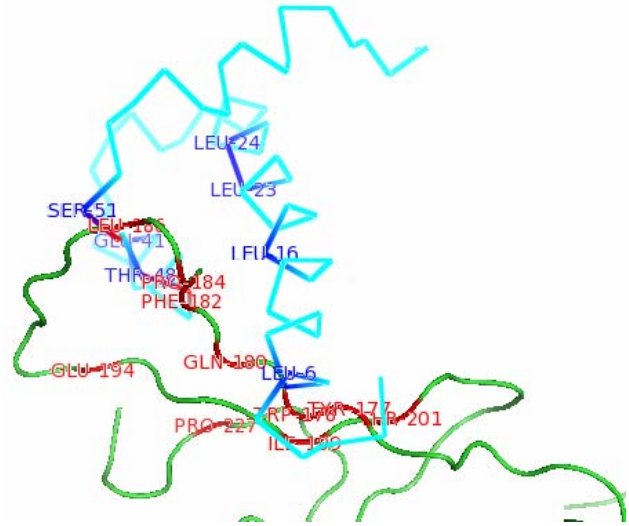

Fig. 6: Transition of aspartate to glutamic acid

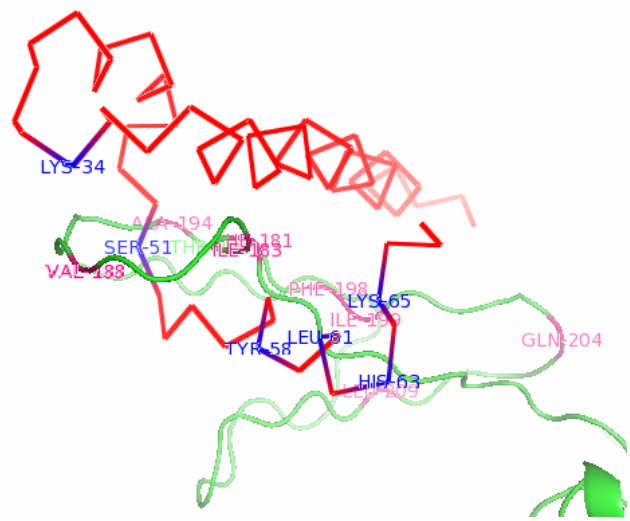

Fig. 7: Proposed computed model for interaction between FcεRIα and FcRγ chain after transition to alanine at position 194.

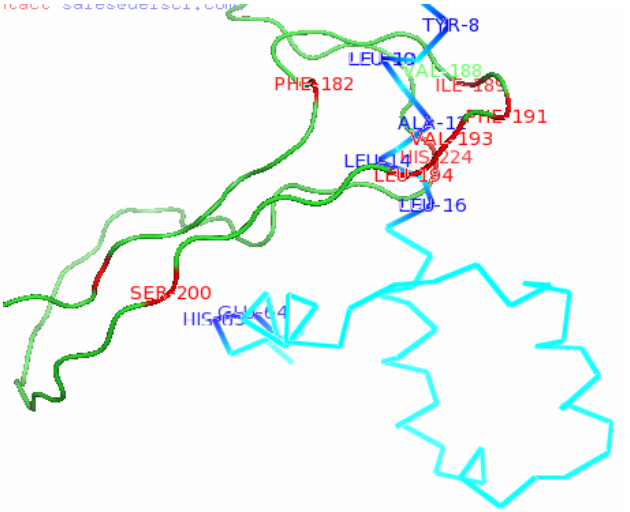

Fig. 8: Transition of aspartate to leucine in FcεRIα is predicted to cause the change in binding sites of FcRγ.

DISCUSSION

Immunoglobulin E is a key player in producing the allergic phenomena (Ishizaka and Ishizaka, 1967) that is responsible for causing multiple debilitating allergic diseases which have been labelled as an epidemic in the absence of an infection and scourge of the 21st century (Isolauri *et al.*, 2004). FcεRI is composed of ligand binding α-chain (Hakimi *et al.*, 1990; Blank *et al.*, 1991; Ra *et al.*, 1993), signal-amplifying β-chain (Scharenberg and Kinet, 1997; Kinet, 1999) and signal-transducing γ subunits (Kinet, 1999). A model for the interaction between various subunits of IgE receptor was first described by Faber and Sears but rejected by our group on the basis of data obtained in our study (Rashid *et al.*, 2010).

D194 in huFcεRIα is the main interacting site with Thr 22 of FcRγ chain (Fig. 1). This chimeric receptor complex exhibited cell surface expression and supports mediator release and intracellular tyrosine phosphorylation of γ-chain and calcium mobilization. The mutation of the polar aspartic acid at position 194 of the transmembrane domain of the huFcεRIα, to a slightly smaller polar threonine residue and subsequent transfection into parental RBL-2H3.1 cell line (D194T) resulted in a functional rat/human chimeric receptor which mediated intracellular, tyrosine phosphorylation of γ-chain, calcium mobilization and Syk kinase when activated via huIgE and antigen. Transition from D194 to T194 in FcεRIα caused the change in binding sites to FcRγ chain. FcRγ is computed to bind to a new site at FcεRIα at residue SER85, TRP 156, TRP 160, VAL 155. The predicted binding residues of FcRγ were Leu50, Gln 5, TRP47. The transition in FcεRIα D194T gave expression although interaction may form at new sites. Collectively, this modeling study has led to the identification of novel potential binding sites between FcεRIα and FcRγ. As a consequence, we propose a new model of FcεRIα and FcRγ interaction (T194) which can mediate downstream signaling in allergic response (Fig. 2). This model may serve a significant role to develop new anti-allergic with more effective responses other than natural mode of action.

In contrast, the mutations to serine (D194S), isoleucine (D194I), arginine (D194R), glutamic acid (D194E) alanine (D194A) and leucine (D194L) were failed to create the assembly of a functional chimeric receptor complex (Fig 3-8) as evidenced by previous study of our group suggesting the importance of a polar residue at the 194 position (Rashid *et al*., 2010). These interactions inhibited receptor complex expression in cells transfected with construct coding this mutant. These models suggested that specific arrangement is required for interaction between FcεRIα. and FcRγ to mediate intracellular allergic response. Lacking of these residues might intercept the signaling cascade. Our results propose the future perspective of cell surface expression deficient receptor complex in the therapeutic intervention. These alternative interactions deficient in expression could be effective approaches to inhibit the release of mediators important for downstream allergic signaling cascade.

CONCLUSION

Our protein docking analysis proposal gives rise to two new potential model structures consistent with the outcome of mutagenesis studies targeting residues in the TM of FcεRIα and FcεRIγ in supporting expression of receptor complex. D194 and T194 in FcεRIα are the important interactive residues which can support expression of the FcRγ. Results obtained following transfections with various mutant receptor subunit constructs confirmed this analysis where FcεRIα- FcRγ complex only expressed with either D194 or T194 present in TM of FcεRIα. Similarly, Thr22 in FcRγ is important for its interaction with the TM of the ligand binding domain of the receptor to support significant extracellular expression of the receptor complex. Our models predict that FcRγ can interact with FcεRIα on alternative sites in the TM domain but such complexes are non-productive and will not facilitate receptor expression.

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