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REVIEW ARTICLE

The High Affinity IgE Receptor (FcεRI) as a Target for Anti-allergic Agents

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ABSTRACT

Prevention of the effector cell activation via high affinity IgE receptor (FcεRI) is thought to be a straightforward strategy for suppressing the allergic reaction. Among the numerous methods to prevent the activation through FcεRI, three versions are described in this article. The first and second ideas involve inhibition of binding between FcεRI and IgE with a soluble form of the FcεRI α chain and a humanized antibody directed against the α chain, respectively. Both of these paths involve suppression the histamine release from human peripheral blood basophils *in vitro*. They also inhibited the allergic reaction *in vivo*. The soluble α inhibited the anaphylactic reaction in rodents and the Fab fragments of the humanized anti-FcεRI α chain antibody suppressed the dermal response in rhesus monkeys. The third idea involves repression of FcεRI expression by suppressing the transcription of the genes encoding the subunits of FcεRI. Although no plausible candidate molecule for actualizing this idea can be identified at present, further analyses of the transcriptional regulatory mechanisms in the human FcεRI α and β chain genes will lead to the discovery of novel targets for developing anti-allergic agents.

KEY WORDS

FcεRI, gene expression, humanized anti-FcεRI α chain antibody, IgE, soluble α

INTRODUCTION

The high affinity IgE receptor, FcεRI, plays a key role in triggering the allergic reaction. FcεRI is composed of three different subunits, α, β and γ, of which the α chain directly binds IgE, while the β and γ chains mediate intracellular signals. Crosslinking of IgE-bound FcεRI on the surface of effector cells such as mast cells and basophils by the multivalent antigen induces not only the release of chemical mediators in the early phase reaction but also the expression of cytokine genes leading to the late phase reaction. Therefore the allergic reaction is thought to be efficiently suppressed by intervention of initiation of activation signals from FcεRI. Many methods have been considered for preventing stimulation through FcεRI, for example, inhibition of binding between IgE and FcεRI by blocking IgE(Fig. 1-1) or FcεRI(Fig. 1-2), inhibition of IgE production (Fig. 1-3), inhibition of FcεRI expression (Fig. 1-4), etc. Here we will describe three models : (1) inhibition of IgE-FcεRI binding by trapping IgE with a soluble form of the FcεRI α chain, (2) inhibition of the IgE-FcεRI binding by blocking the receptor with a humanized antibody against the FcεRI α chain, (3) suppression of FcεRI expression by regulating α and β chain gene transcription.

RECOMBINANT SOLUBLE FORM OF THE FcεRI α CHAIN

The α chain of FcεRI, a membrane-anchoring glycosylated protein, binds IgE via its extracellular domain. A truncated form of the α chain containing 95% of its extracellular domain was successfully expressed in Chinese hamster ovary (CHO) cells as a secreted form, while retaining its binding activity for IgE.[1] The binding equilibration constant (K_A) of this recombinant soluble α chain and human IgE was 10^{10} /M in both surface plasmon resonance analyses and scattered plotting. This value was almost the same as the one between the cell surface FcεRI and IgE. Furthermore, the stoichiometry of IgE binding was confirmed to be 1 : 1 by gel filtration analyses of the soluble α-IgE mixture.[2]

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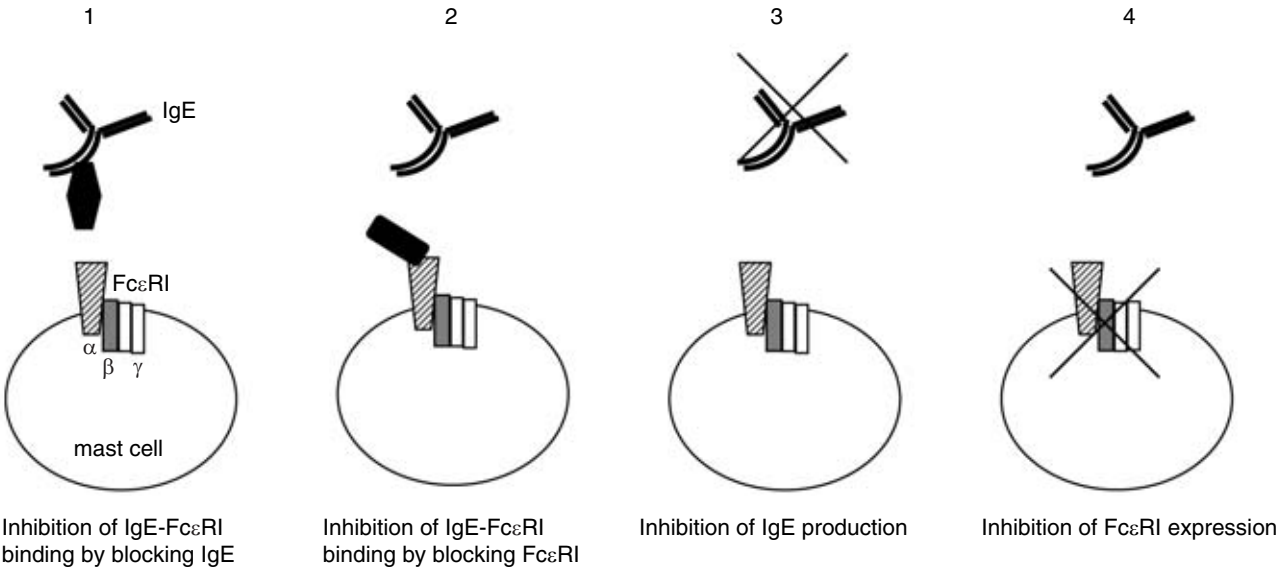

Fig. 1 Candidate methods for preventing activation through FcεRI.

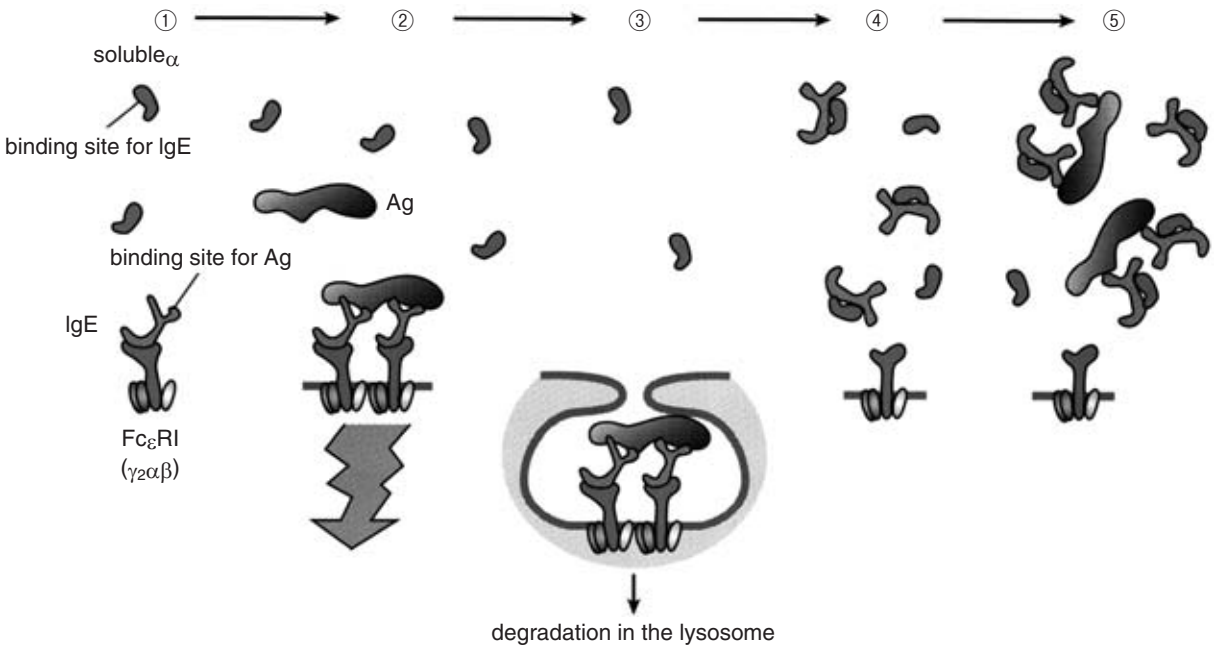

Fig. 2 Prevention of the allergic reaction by the soluble α chain.
① IgE is bound to FcεRI on mast cells. ② The first antigen entry after the administration of the soluble α chain causes crosslinking of FcεRI through IgE and activation of mast cells. ③ The crosslinked FcεRI is endocytosed and degraded in the lysosome. ④ Newly synthesized FcεRI is expresed on the surface of mast cells, but it is unable to bind IgE which is trapped by the soluble α chain. ⑤ Repeated antigen invasion does not activate mast cells.[4]

The physiological activity of the soluble α chain was examined both *in vitro* and *in vivo*. The soluble α chain inhibited the release of histamine from human peripheral blood basophils in a dose dependent manner *in vitro*.[1] *In vivo*, it suppressed PCA reactions in the rat even if the soluble α chain treatment was performed after IgE sensitization, although a larger amount of the recombinant protein was required than in pre-treatment before IgE sensitization.[1] The soluble α chain also prevented death from anaphylactic shock in a murine model when administered after IgE sensitization. In addition, the short interval time

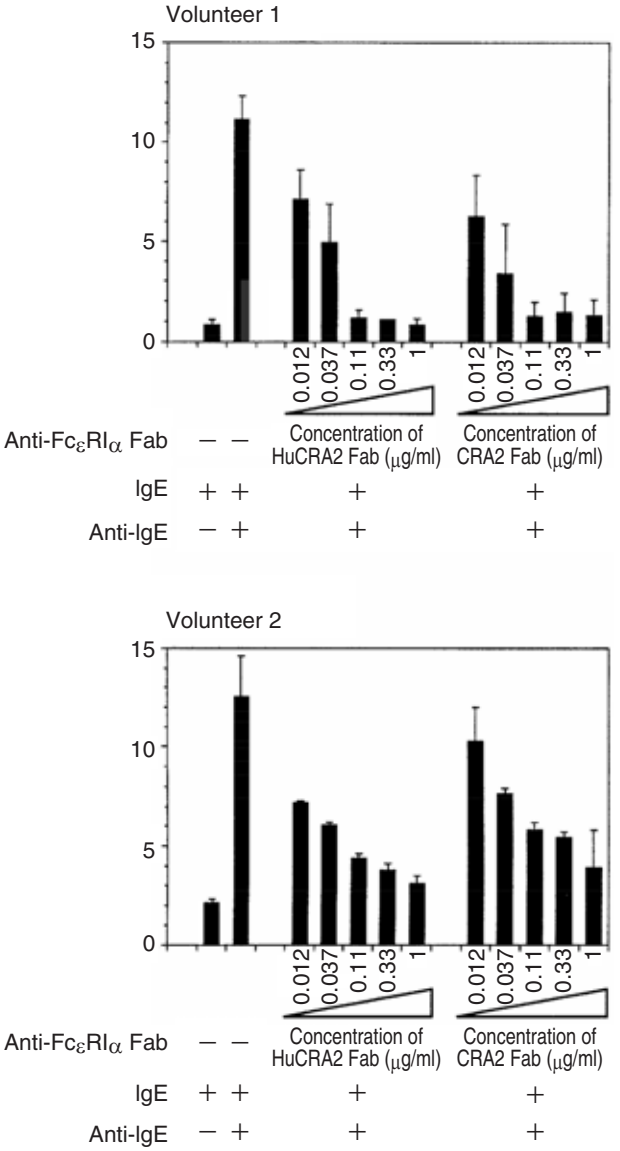

Fig. 3 Inhibition of histamine release from human peripheral blood basophils by the Fab fragment of humanized anti-human FcεRI α mAb.

After dissociating cell-bound immunoglobulins, leukocytes from human peripheral blood were preincubated with Fab fragments of humanized CRA2 or mouse CRA2. Cells were sensitized with human IgE followed by stimulation with anti-human IgE Ab.[7]

between the administration of the soluble α chain and the antigen challenge demonstrated no preventing effect, indicating that the soluble α chain could facilitate the dissociation of IgE from FcεRI on the cell surface in addition to trapping free IgE.[2] The soluble α chain may have no effect on the activated mast cells which have surface FcεRI already occupied by IgE and crosslinked by the antigen, however it will act effectively when the crosslinked receptors are endocytosed and empty receptors come up on the cell surface because the presenting receptors are unable to bind IgE in serum which has already been trapped by the soluble α chain. In fact, this was proven by using a novel murine model of type I allergy which was established by transplantion of the hybridomas secreting antigen-specific IgE in syngeneic mice.[3] In this model, administration of the soluble α chain before the first antigen challenge and between the first and second challenge suppressed the ear swelling response in the second challenge but not in the first challenge, which suggests that repeated administration of the soluble α chain may ultimately prevent the allergic reaction in an IgE-sensitized individual exposed to the repeated antigen invasion (Fig. 2).[4]

Additionally, the soluble α chain was shown to bind to membranous IgE on the surface of B cells and to specifically repress IgE production in B cells by inhibiting the mRNA expression of the mature ε chain.[5] Although the negative signals elicited by the binding of the soluble α to the surface IgE on B cells have not been precisely characterized, the production of IL-6 from B cells was shown to be suppressed.[6] It is therefore expected that the soluble α chain efficiently suppresses the allergic reaction not only by preventing IgE-FcεRI binding on mast cells and basophils but also inhibiting IgE production in B cells.

HUMANIZED ANTIBODY AGAINST THE FcεRI α CHAIN

There is an another strategy for preventing IgE-FcεRI binding by using a humanized anti-FcεRI α chain antibody. A murine monoclonal antibody against the human FcεRI α chain, CRA2, was successfully humanized by CDR grafting to reduce the antigenicity in humans.[7] The humanized CRA2 had almost the same binding activity for the α chain as original CRA2 and showed reduced antigenicity in Japanese monkeys.[8] To prevent crosslinking of FcεRI by antibodies which compete with IgE and cause activation of cells, Fab fragments of the humanized CRA2 were prepared in place of the whole antibody. The Fab fragments of the humanized CRA2 suppressed histamine release from the human peripheral blood basophils *in vitro* (Fig. 3).[7] Furthermore, the Fab fragments of the humanized CRA2 suppressed the dermal reaction in rhesus monkeys through the local intradermal administration.[8]

Although precise analyses of stability *in vivo* and development of optimal administration methods are required before initiation of clinical trials, the soluble α chain and the humanized anti-FcεRI α chain antibody are expected to have suppressive activities against allergy in humans, based on favorable results in clinical trials using omalizumab (E25), a humanized anti-IgE antibody developed by Genentech/Novartis/Tanox which also inhibits the IgE-FcεRI binding.[9,10]

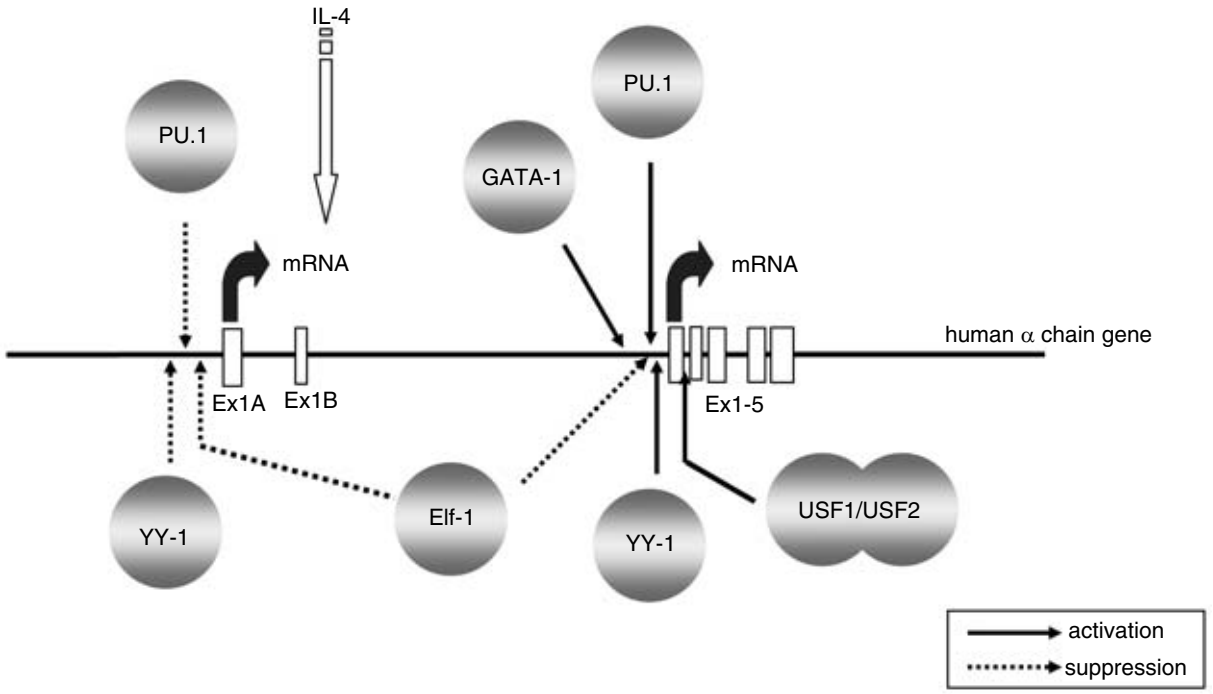

Fig. 4 Structure of the human FcεRI α chain gene and transcription factors regulating gene expression.

REGULATION OF THE FcεRI EXPRESSION AT THE TRANSCRIPTIONAL LEVEL

In addition to preventing FcεRI-IgE binding, suppression of FcεRI expression can also inhibit the FcεRI mediated allergic reaction. Due to the fact that the α and β chains but not the γ chain of FcεRI are expressed specifically in the FcεRI expressing cells, the expression of FcεRI would be specifically suppressed by repressing the transcription of the α or β chain gene. The α chain knockout mouse was reported to have a significantly reduced anaphylactic response,[11] indicating that the allergic reaction can be suppressed by repression of α chain expression. Furthermore, intracellular signals mediated by the γ chain, as well as the cell surface expression of the receptors, are amplified in the presence of the β chain,[12-14] although functional receptors are expressed both as tetrameric ($\alpha\beta\gamma_2$) and trimeric ($\alpha\gamma_2$) forms in humans.[15] These facts add further support to the argument that repression of the β chain expression raises the threshold of the induction of the allergic reaction.

Tanscriptional regulatory mechanisms of the human FcεRI α and β chain genes have recently been analyzed. As for the α chain (Fig. 4), both the regulatory elements close to the promoter region and the associated transcription factors binding to them have been identified.[16,17] GATA-1 cooperatively activated the α chain promoter with PU.1 and YY-1 transcription factors, while Elf-1 suppressed it by competing for the recognition site with PU.1 and YY-1. This was due to the fact that the binding site of Elf-1 partially overlaps with the PU.1 and YY-1 sites.[17] On the other hand, an element in the first intron was identified as an enhancer which was bound by a complex of USF 1/USF 2. Moreover, USF 2 antisense actually repressed the α chain expression.[18] In addition, two novel exons were found at 18.4 kb and 12.6 kb upstream of the reported first exon. These exons were shown to be transcribed, which indicates the presence of an additional distal promoter.[19] Although the activity of the distal promoter seemed to be weaker than the major promoter located upstream of the previously reported first exon, upregulation of α chain expression by IL-4 is possibly mediated by this distal promoter as evidenced by the fact that mRNA containing the novel two exons was increased by IL-4 stimulation.[20]

On the other hand, the analyses of the human FcεRI β chain gene region around the transciption initiation site revealed that two Oct-1 recognition sites were required for cell-type specific transcriptional activation.[21] And recently, the transcription factor MZF-1 was found to suppress β chain gene expression via an element in the fourth intron.[22]

Finally, although there is currently no concrete evidence of a candidate molecule which specifically inhibits the expression of the genes encoding FcεRI at present, it is hoped that the progress of the above mentioned studies will lead the way to the discovery of specific novel targets for agents against allergy.

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