Anti-IgE Monoclonal Antibodies that Inhibit Allergen-Specific Histamine Release

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100
LOOPEF
LOOPAB
LOOP CD
LOOPFG
60 80
FcERI-alpha Mae-11 Mae-13 Ξ Mae-17
T5
.5 601
w
i-1 40
20

IgE binding to its high affinity receptor (FcεRI) constitutes the initial biochemical event responsible for IgE mediated allergic reactions [1]. Identification of residues within the Cε3 domain of IgE responsible for receptor binding is necessary for the development of new therapeutic antagonists of IgE-mediated allergic disease. We identified anti-IgE monoclonal antibodies (mAbs) which recognize soluble but not FcεRI-receptor-bound IgE on human basophils. We compared the binding of anti-IgE mAbs and FcεRI to both native and recombinant mutants of IgE prepared by substituting Fcγ2 sequences into Fcε3 based on the homology between these two domains [2]. We demonstrate that anti-IgE mAbs recognize the same residues within the Fcε3 domain required for receptor binding (fig. 1). This observation was confirmed by demonstrating that anti-IgE mAbs could compete with the receptor on basophils for antigen-specific IgE binding by inhibiting basophil sensitization without affecting spontaneous histamine release (fig. 2).

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Cε3 SEQUENCES

Fig. 1. Binding of recombinant FcεRI α subunit on CHO 3D10 cells [3] and anti-IgE mAbs (Mae-11, -13 and -17) to recombinant Cε3 domain mutants. Mutants were prepared [4], transiently expressed and quantified by ELISA. Binding of CHO cells to 1 μg/ml IgE mutants was measured by immunofluorescence and analyzed by flow cytometry, and binding to anti-IgE mAbs was measured by ELISA. Values are the average of duplicate determinations from 1 of 3 experiments.

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Mae-11 Mae-13 Mae-17

Fig. 2. Effects of anti-IgE mAbs on spontaneous and ragweed-specific histamine release. Histamine release was performed as previously published [5]. Inhibition of ragweed-induced histamine release was measured by coincubating anti-IgE mAbs with 100 ng/ml ragweed-specific IgE for 2h at 37°C followed by a 30-min challenge with 100 ng/ml ragweed antigen. Data represents 1 of 3 experiments.

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