

Humanized Anti-IgE mAb Hu-901 Prevents the Activation of Allergen-Specific T Cells

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Key Words

Allergen · T cell · Anti-IgE · Antigen presentation

Abstract

Background: As a result of the very efficient capture of allergens by IgE that focuses to CD23 on B cells or FcεRI on dendritic cells, allergen-specific T cells can be activated after exposure to very low levels of allergens. This IgE-mediated allergen presentation is 100- to 1,000-fold more efficient than fluid phase endocytosis. The aim of the present study was to determine whether humanized anti-IgE mAb Hu-901 can prevent the activation of allergen-specific T cells by inhibiting IgE-mediated allergen presentation. **Methods:** A house dust mite major allergen Der p 1-specific T cell line was generated from an allergic asthma patient, and a model was set up to show IgE-facilitated allergen presentation via CD23 on EBV-transformed B cells. In addition, experiments were performed by FACS analysis, detecting the presence of IgE-allergen complexes bound to EBV-B cells by polyclonal FITC-labeled anti-IgE antisera. **Results:** The anti-IgE mAb Hu-901 inhibited proliferation of allergen-specific T cells at low allergen concentrations. Inhibition was dose-dependent. This effect could be explained by Hu-901 inhibition of binding of allergen-IgE complexes to CD23 expressed on EBV-transformed B lymphocytes. **Conclu-**

sions: These data clearly indicate that anti-IgE antibodies for the treatment of allergy exert their effect not only by inhibiting mast cell/basophil degranulation, but also by preventing T cell activation, which possibly explains the effect of anti-IgE treatment on late-phase reactions noted in clinical studies.

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Introduction

Antigen-presenting cells (APC) of allergic patients exhibit increased expression of the high affinity IgE receptor, FcεRI, and of the low affinity IgE receptor, CD23. The expression of FcεRI and CD23 is controlled by serum IgE levels. Both IgE receptors have been shown to facilitate the presentation of allergens in the presence of allergen-specific serum IgE [1, 2]. This is a crucial step in the activation and persistence of the allergen-specific Th2 cells that are responsible for IgE class switching and eosinophilia.

Serum IgE-facilitated allergen presentation can be inhibited by polyclonal anti-IgE antibodies [1], and by allergen-specific IgG molecules induced by specific allergy vaccination [3]. Humanized anti-IgE mAb are currently being developed to prevent mast cell degranulation by inhibiting the interaction of IgE with its receptors [4, 5]. However, results from clinical trials with neutralizing

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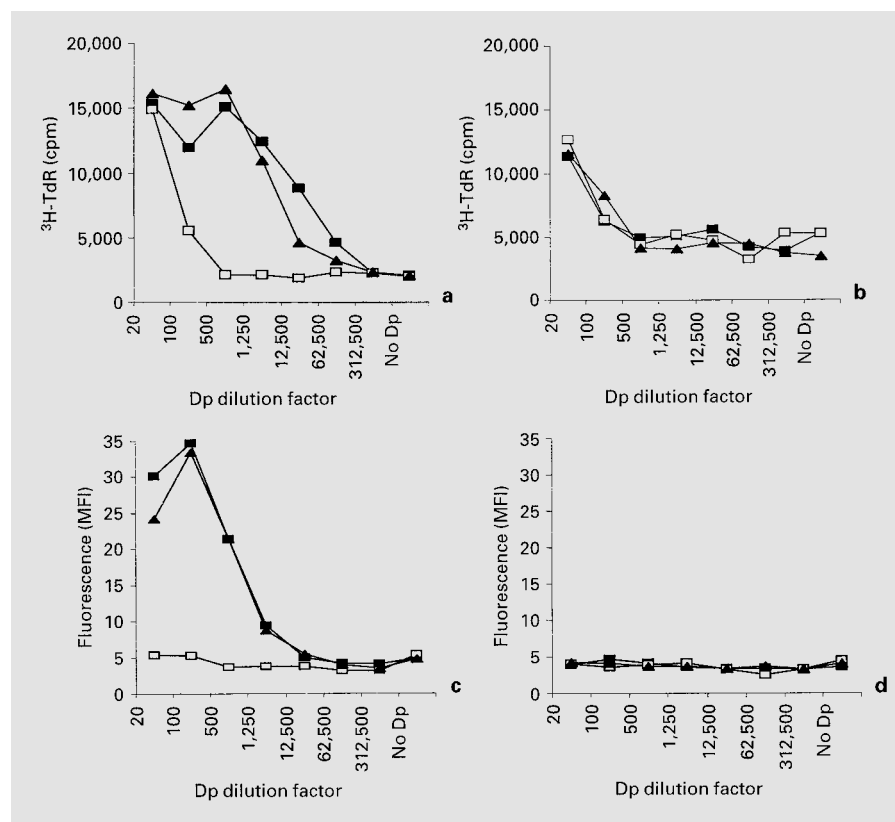
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Fig. 1. Serum IgE-facilitated allergen presentation to a Der p 1-specific T cell line was tested as described in Materials and Methods. Facilitation of antigen presentation is seen in the presence of serum from an allergic asthma patient (**a**, ■), but not in the presence of control serum (**b**, ■), and can be inhibited by the addition of Hu-901 mAb (□). The addition of an isotype control mAb has no inhibitory effect (**a**, b, ▲). The EBV-transformed B cells used as APC were stained with a polyclonal anti-IgE-FITC antibody. It is shown in **c** (asthma serum) and **d** (control serum) that IgE-allergen binding to the B cells is abrogated by the addition of Hu-901 (□), but not by control mAb (▲). MFI = Mean fluorescence intensity.



anti-IgE mAb rhuMAb-E25 have shown that anti-IgE mAb treatment not only has an effect on early-phase allergic responses, but also inhibits bronchial late-phase responses and the number of eosinophils in sputum of allergic asthma patients [4]. These data strongly suggest that IgE is crucial for the development of late-phase responses, and that inhibition of this mechanism results in decreased late-phase allergic reactions. As late-phase reactions are mainly T cell-dependent [6, 7], these data indicate that anti-IgE treatment may also inhibit T cell activation in vivo.

The aim of this study was to investigate whether Hu-901, a humanized anti-IgE mAb that was originally selected for its ability to inhibit the binding of IgE to CD23 as well as FcεRI [8], can inhibit serum IgE-facilitated allergen presentation. If so, this may explain the clinical effects on late-phase reactions reported after anti-IgE treatment.

Materials and Methods

FACS Analysis of EBV-Transformed B Cells

Sera from an allergic asthma patient and from a nonallergic control donor were incubated for 30 min at 37°C with serial dilutions of extract made from house dust mite *Dermatophagoides pteronyssinus*-

spont culture medium (Dp) in IMDM (BioWhittaker). CD23+ EBV-B cells were added at a concentration of 5×10^5 /ml to the allergen-complexed serum and incubated for 30 min at 37°C. The EBV-B cells were washed twice in PBS with 1% BSA and 0.01% NaN₃ and labeled on ice for 30 min with FITC-labeled goat anti-human IgE antiserum (Kirkegaard and Perry Laboratories, Gaithersburg, Md.). Cells were washed again and fluorescence was analyzed on a FACSCalibur flowcytometer (Becton Dickinson, San Jose, Calif.). In blocking experiments with anti-IgE mAb Hu-901 (human IgG1) or AD439 (isotype control) the sera were incubated with these mAbs and Dp simultaneously for 1 h at 37°C before adding the EBV-B cells.

Proliferative Responses of Der p 1-Specific T Cells

A Der p 1-specific T cell line was prepared from the peripheral blood of a house dust mite-allergic asthma patient as described elsewhere [1]. Der p 1-specific T cells were washed twice in HBSS and antigen-specific proliferation was assessed by stimulation of the T cells, $2-4 \times 10^4$ cells/well, by antigen using 3,000 rad-irradiated autologous EBV-transformed B cells as APC ($1-2 \times 10^4$ cells/well). The cells were cultured for 24 h in the presence of 5% allergic donor serum or control serum, and in the presence of serial dilutions of Dp extract and Hu-901 or isotype control mAb AD439. This incubation was followed by a 16-hour pulse in the presence of tritiated methyl thymidine (³H-TdR; NEN DuPont, Wilmington, Del.), 0.3 μCi/well. Proliferation was expressed as mean cpm of ³H-TdR incorporation of triplicate cultures.

Results and Discussion

Here we show that the activation of a T cell line reacting to Der p 1, the major allergen of *D. pteronyssinus*, is 100-fold more efficient in the presence of house dust mite-specific IgE. Preincubation of the mite-specific IgE with anti-IgE mAb Hu-901, but not with isotype control mAb, completely inhibited IgE-facilitated antigen presentation to Der p 1-specific T cells (fig. 1a,b). This inhibition by Hu-901 is dose-dependent (data not shown). These data are consistent with the hypothesis that Hu-901 prevents the binding of IgE-allergen complexes to CD23 on the membrane of the EBV-B used as antigen-presenting cells. To visualize this binding, FACS experiments were performed using FITC-labeled goat-anti-human IgE to detect IgE binding to CD23, the low affinity IgE receptor, present on the B cells. Binding of allergen-IgE complexes to CD23 expressing EBV-B could be measured in the presence of Dp and patient serum, but not when B cells were treated with control serum or with patient serum without allergen. This binding of IgE to CD23-bearing EBV-B cells was inhibited by treating the patient serum with Hu-901 (fig. 1c, d), demonstrating that anti-IgE prevents IgE-allergen complexes from interacting with CD23.

This means that anti-IgE treatment has an effect at several levels. Anti-IgE treatment decreases free serum IgE levels, as a result of which the expression of FcεRI is downregulated [9], further preventing mast cell and baso-

phil degranulation. Serum IgE levels and FcεRI expression increase upon discontinuation of anti-IgE treatment, but do not reach pretreatment levels [10], suggesting that IgE production by B cells may be partially decreased. This may be explained by our finding that anti-IgE prevents the activation of allergen-specific T cells at low allergen doses, and may therefore interfere in IgE production and allergic inflammation, both of which are dependent on the activation of Th2 cells.

Such a prolonged prevention of T cell activation in vivo should lead to a decreased frequency of allergen-specific Th2 cells, and as a result possibly even shift the production of Th2 to Th0 cytokines because newly formed allergen-specific T cells will not mature in a Th2-biasing environment. If this is the case, chronic treatment of patients with anti-IgE may give even better clinical efficacy and may be a causal treatment of the disease with long-term efficacy. In addition, because inhibition of serum IgE-facilitated allergen presentation is one of the effects noted after specific allergy vaccination [3], and because IgE-mediated side effects may occur as a result of this treatment, anti-IgE in combination with specific allergy vaccination may enhance the safety profile and efficacy of allergy vaccination with allergenic extracts.

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