

An in vitro Model for the Allergen-IgE-FcεRI Interaction

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Key Words

Type I allergy · Recombinant allergen · rBet v 1 · rBet v 1 specific chimeric IgE · Recombinant Fcε receptor alpha chain

Abstract

Background: The interaction of immune complexes consisting of allergens and allergen-specific IgE with the high-affinity Fcε receptor represents the key event in the induction of symptoms in type I allergic individuals. Immediate-type symptoms result from the release of biological mediators due to allergen-induced cross-linking of FcεRI receptors on mast cells and basophils, whereas FcεRI-mediated presentation of allergen-IgE complexes may contribute to late-phase symptoms through enhanced T cell activation. The interaction of allergens/allergen-specific IgE/FcεRI represents, therefore, an important target for therapeutic intervention strategies in type I allergy. **Methods and Results:** A molecular model of the allergen-IgE-FcεRI interaction was established. It consists of recombinant purified Bet v 1, the major birch pollen allergen, a chimeric Bet v 1 specific monoclonal IgE antibody, and the baculovirus-expressed purified human alpha chain of FcεRI. The chimeric Bet v 1-specific IgE antibody consists of the light chain and the heavy chain variable region of a

mouse monoclonal Bet v 1 specific antibody, Bip 1, and the constant region of human IgE. The interaction of rBet v 1, chimeric Bip 1, and human alpha chain was investigated by overlay experiments. Nitrocellulose-immobilized recombinant alpha chains was incubated with chimeric Bip 1 and, for control purposes, with mouse-derived Bip 1. Bound chimeric Bip 1 was detected with ¹²⁵I-labeled rBet v 1. The specific interaction of rBetv 1, chimeric Bip 1, and recombinant human alpha chain is demonstrated. We thus establish a molecular model of the allergen/IgE/alpha chain interaction. The usefulness of the described in vitro system is exemplified by the identification of a mouse monoclonal antihuman IgE antibody which blocked the IgE-alpha chain interaction. **Conclusions:** The module system consisting of rBet v 1, chimeric Bip 1, and recombinant alpha chain may be used for the identification of competitors of the allergic effector reaction by means of high throughput screening of compounds or by combinatorial chemistry.

Type I allergy represents a genetically determined hypersensitivity disease which results from the recognition of per se innocuous antigens by IgE antibodies [1]. As IgE antibodies represent the least abundant class of immunoglobulins in serum and other body fluids, IgE recognition of al-

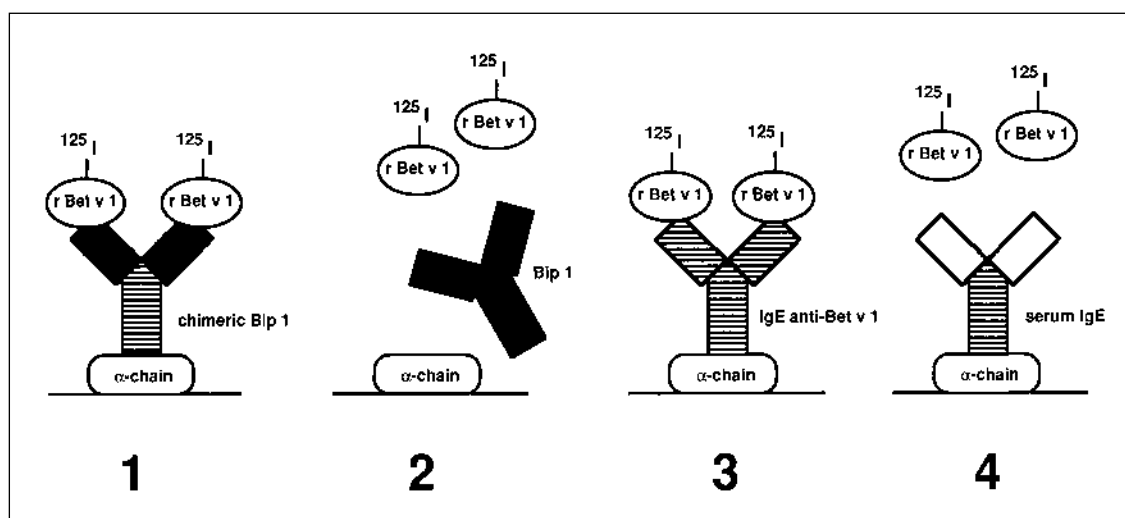


Fig. 1. Molecular model of the allergen-specific IgE-FcεRI interaction. Recombinant nitrocellulose-immobilized alpha chain was incubated with chimeric Bip 1 (1), with serum containing Bet v 1 specific IgE (3), and for control purposes with mouse-derived Bip 1 (2) or serum IgE without reactivity to Bet v 1 (4). Bound Bet v 1-specific IgE antibodies were detected with ^{125}I -labeled recombinant Bet v 1.

lergens would likely remain unnoticed, if it was not amplified by receptor-mediated cellular activation. Immediate symptoms of type I allergy are caused by cross-linking of FcεRI on allergic effector cells (mast cells, basophils) by allergen-IgE complexes and the subsequent release of biological mediators (e.g., histamine, leukotrienes) [2]. Support for the assumption that the more chronic manifestations of atopy may be triggered as well by IgE-allergen complexes that bind to FcεRI on antigen-presenting cells (e.g., monocytes, dendritic cells) comes from experiments demonstrating that IgE-FcεRI-mediated presentation of allergens leads to a greatly increased activation of allergen-specific Th2 cells [3–6].

As the IgE-FcεRI interaction represents one of the key mechanisms leading to atopy, considerable effort has been spent in the molecular analysis of the contact sites on IgE and FcεRI [7–9]. Despite extensive research efforts, information regarding the interaction domains is controversial, but a number of attempts have been made to develop competitors of the IgE-FcεRI interaction. It has been shown that passive cutaneous sensitization or IgE-FcεRI interaction can be inhibited by IgE-derived peptides [10, 11]. It was further demonstrated that active immunization with IgE domains can lead to the induction of autoantibodies that prevent allergen-induced mast cell degranulation [12]. A humanized monoclonal antihuman IgE antibody which has been developed in order to remove IgE from the circulation

as well as from effector cells is currently in clinical evaluation [13, 14].

Screening of compound libraries in order to identify other and, perhaps, better suited competitors would benefit from the availability of a simple in vitro system for the allergen-IgE-FcεRI interaction.

In this work we describe an allergen-specific in vitro model of the allergen-IgE-FcεRI interaction consisting of purified recombinant proteins. The interacting components comprise (1) recombinant Bet v 1, the major birch pollen allergen [15]; (2) a Bet v 1 specific mouse monoclonal antibody, Bip 1, which was expressed as a chimeric antibody with a human IgE constant region and Bip 1 derived variable regions [16, 17], and (3) baculovirus-expressed alpha chain of the human FcεRI [18]. All three components were purified and tested for interaction in an overlay assay.

Recombinant alpha chain was immobilized to nitrocellulose by dot blotting (fig. 1). It was then incubated with chimeric Bip 1 (fig. 1.1), serum IgE from a Bet v 1 allergic patient (fig. 1.3), and, for control purposes, with mouse-derived Bip 1 (fig. 1.2) or serum IgE without specificity for Bet v 1 (fig. 1.4). Bound Bet v 1 specific IgE was then detected with ^{125}I -labeled recombinant Bet v 1. The results obtained showed that alpha chain-bound Bet v 1-specific IgE could be detected with ^{125}I -labeled Bet v 1. The usefulness of the described in vitro system for identifying competitors of the IgE-FcεRI interaction was exemplified by the selec-

tion of a mouse monoclonal antihuman IgE antibody which prevented IgE binding to nitrocellulose-bound alpha chain. We suggest to use the molecular model to search for high-affinity competitors of the IgE-FcεRI interaction by means of throughput screening of compounds (antibodies, proteins, peptides, nucleic acids, and chemical compounds).

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