Mouse/Human Chimeric Antibodies Directed to Two Nonoverlapping Epitopes of the House Dust Mite Allergen, Der p 2

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Key Words

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The interaction of free allergen with two (or more) IgE molecules bound to the high-affinity receptor for IgE (FcεRI) on mast cells and basophilic granulocytes leads to the release of inflammatory mediators. To study cross-linking of IgE bound to IgE receptors on basophils, we have developed mouse/human chimeric antibodies directed to two non-overlapping epitopes of the house dust mite allergen, Der p 2. These chimeric antibodies were constructed from the heavy-chain variable region and light chain of mu-rine origin, and of the heavy-chain constant region of human origin. We produced chimeric IgE, IgG1 and IgG4 antibodies originating from two hybridoma cell lines: 2B12 [1] and αDpX [2]. The chimeric antibodies derived from 2B12, named Dp2A have been described previously [3, 4]. hIgG1 and hIgG4-Dp2A antibodies were prepared as well [manuscript in preparation]. For studying cross-linking of IgE bound to IgE receptors present on mast cells, basophils and antigen-presenting cells we also generated a panel of chimeric antibodies directed to another nonoverlapping epi-tope of Der p 2, αDpX. The chimeric αDpX-derived monoclonal antibodies named hIgE-Dp2B, hIgG1-Dp2B and hIgG4-Dp2B showed binding to mite extract coupled to CNBr-activated Sepharose (data not shown). The expression level of these chimeric antibodies was about 20, 2,000 and 400 ng/ml, respectively. This shows that the hIgE-Dp2B cell line is by far the lowest antibody-producing cell line. Several attempts to obtain a more productive cell line failed. The nucleotide and deduced amino acid sequences of the V_H domains of 2B12 (Dp2A) and αDpX (Dp2B) are shown in figure 1.

This panel of chimeric IgE antibodies enables studying allergen-induced cross-linking. The chimeric IgE antibodies were able to bind to FcεRI present on basophilic granulocytes. Cross-linking of basophil-bound sensitized chimeric IgE by anti-light chain or anti-IgE antibodies resulted in the release of histamine. Only sensitization with a mixture of chimeric Dp2A-IgE and

Dp2B-IgE antibodies induced a response upon incubation with the allergen Der p 2. The modulating effect of chimeric IgG1 and IgG4 antibodies on this IgE-mediated activation of basophils is another major point of interest.

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Fig. 1. Nucleotide and deduced amino acid sequence of rearranged VH-domains of monoclonal antibody 2B12 [1] and α DpX [2]. By using primers previously described [4] the VH domains of α DpX and 2B12 were amplified and cloned in the vector pUC19 for sequence analysis. The assignment of the amino acid numbering, the framework regions (FR) and the complementarity-determining regions (CDR) was performed according to Kabat et al. [5]. The heavy chain expression construct (described previously for 2B12) [4] consisted of the mouse IgH enhancer, mouse IgH promoter and mouse IgH leader sequence, the antigen-binding part of the heavy chain V μ α DpX or VH2B12, intron fragment and the human genomic C ϵ -gene or C γ 1 or C γ 4-gene [6]. Restriction sites for PstI, Bsu36I and HindW λ are underlined. The expression vector used for these chimeric antibodies was pSV2-gpt [7, 8].

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