Mouse/Human Chimeric IgE Antibodies Directed to the House Dust Mite Allergen Der p 2

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Key Words

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To study functional aspects concerning the cross-linking of IgE molecules bound to IgE receptors on basophilic granulocytes, mast cells and antigen-presenting cells, we are developing mouse/human chimeric antibodies directed to the house dust mite allergen Der p 2. These antibodies consist of a mouse variable heavy-chain domain (VH) coupled to human constant domains of the IgE isotype. The complete light chain is of mouse origin.

From a panel of 22 mouse monoclonal antibodies directed to Derp2, we selected two antibodies, α -DpX [1] and 2B12B3 [2] known to be directed to non-overlapping epi-topes of Derp2.

To obtain mouse/human chimeric antibodies, we followed a three-step protocol, combining the methods as described by Orlandi et al. [3] and Parren et al. [4]. (1) Heavy-chain plasmids were constructed with the VH domain cloned from hybridoma cells coupled to the human ϵ heavy-chain constant region. These coding sequences were under control of the mouse heavy-chain promoter and enhancer. (2) Spontaneously arising 'heavy-chain-loss variants' (producing light chains only) were isolated from the original hybridomas. (3) Transfection of the heavy-chain constructs in-

to their respective 'heavy-chain-loss variants' gave rise to the expression of whole chimeric antibodies.

With this method, we obtained expression of chimeric IgE antibodies of monoclonal 2B12B3. This anti-Derp2 IgE antibody showed the expected reactivity with mite extract, anti-IgE and anti-light chain antibodies coupled to Se-pharose (fig. 1). The chimeric α -DpX IgE antibody showed no reactivity to mite extract whereas reactivity to anti-light chain and anti-IgE antibodies was

observed (data not shown). The cause for this unexpected loss of mite reactivity is now being studied.

The chimeric IgE antibodies prepared in this study were able to sensitize the IgE receptor as present on human basophilic granulocytes. Cross-linking of this IgE with anti-IgE antibodies caused the release of histamine (data not shown).

In conclusion, a chimeric IgE antibody was obtained and shown to have expected allergen-binding and receptor-binding properties. Such chimeric antibodies will be used for studying aspects of IgE cross-linking on human mast cells, basophils and antigen-presenting cells.

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80

40-

← 20

k:

0.1 1 10 100 1,000 IgE Concentration (IU/test)

Fig. 1. Reactivity of chimeric 2B12B3 IgE antibodies with mite extract (O), anti-human IgE (•) and anti-mouse κ light-chain (◻) antibodies. Different concentrations of chimeric 2B12B3 IgE (1IU = 2.4 ng) were incubated with mite extract, anti-(human) IgE or anti-(mouse) κ light-chain antibodies coupled to Sepharose. The bound chimeric antibodies were detected by 251 I-labelled anti-IgE antibodies.

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