

Nonapeptides Selected by Phage Display Mimic the Binding Sites of Monoclonal Antibodies BIP1 and BIP4 on Bet v 1, the Major Birch Pollen Allergen

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Key Words

Phage · Bet v 1 · Mimotopes · Monoclonal

In birch pollen allergy the major allergen Bet v 1 is recognized by IgE from more than 90% of allergic patients. Moreover, 60% of the patients show exclusive IgE binding to Bet v 1 [1]. For these patients, interference of IgE binding with this allergen represents an intriguing therapeutical concept. Synthetic B cell epitopes could be used for peptide therapy, or for the precise induction of blocking IgG by immunotherapy. From studies with monoclonal antibodies, it is known that IgG specific for this allergen affects the IgE-allergen interaction [2]. We defined B cell epitopes of monoclonal anti-Bet v 1 antibodies BIP1 and BIP4 [3] by using random nonapeptide phage display peptide libraries [4, 5] and compared the selected mimotopes with the sequence and 3D structure of Bet v 1 [6, 7].

Our analyses showed that specific ligands were selected from the constrained library. As expected, alignments with the sequence of Bet v 1 showed no homology of the BIP1 mimotope CFPYCYPSESA and the BIP4 mimotope CRQTRTMPGC. The highest rate of similarities for the BIP1 mimotope was found between Phe⁵⁸ and Tyr⁶⁷ of Bet v 1. Alignment with the 3D structure showed that this part of the sequence composes an exposed loop between beta strands 3 and 4 (fig. 1). The majority of amino acids of this

hypothetical epitope are less accessible, we speculate that they may participate to binding by induced fit.

As the phage ligands were shown to be capable of inducing anti-Bet v 1 IgG in mice [8], our data point towards a possible use of phage-displayed peptides for the precise induction of a blocking antibody for (longlasting) treatment of allergic disease.

Materials and Methods

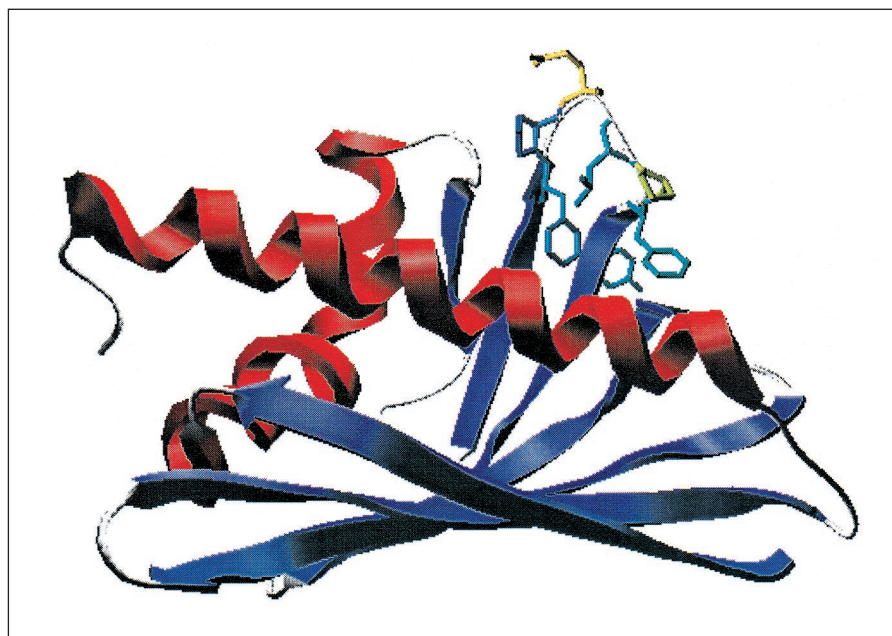
Phage Libraries and Biopannings

Biopanning was performed with decreasing amounts of monoclonal antibodies BIP1 and BIP4 for selections [8]. Ligands for BIPs were selected from random phage libraries expressing linear (pVIII 9aa) [4] or circular (pVIII 9aa.Cys) [5] nonapeptides fused to pVIII of the filamentous bacteriophage fd. These libraries were kindly provided by IRBM (Istituto di Ricerche di Biologia Molecolare P. Angeletti SPA, Rome, Italy).

Sequencing and Alignment with the Bet v 1 Sequence

DNA sequencing was performed by the Sanger dideoxy method and analyzed by a LI-COR DNA sequencer 4000L (LI-COR Inc., Lincoln, Nebr., USA). The sequences of mimotopes were compared with the 3D structure of Bet v 1, Brookhaven protein database, accession number 1BV1, by using the Swiss Pdb Viewer software program (Geneva Biomedical Research Institute, Glaxo Wellcome Research and Development SA, Geneva, Switzerland).

Fig. 1. The putative binding site of the BIP1 antibody on the loop between beta strands 3 and 4 of the three-dimensional structure of the major birch pollen allergen Bet v 1 [5, 6]. This illustration is based on sequence alignments with the mimotope of monoclonal BIP1 characterized by means of a phage display peptide library. Yellow and green = accessible residues; blue = residues which are less accessible.



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