Tackling Bet v 1 and associated food allergies with a single hybrid protein

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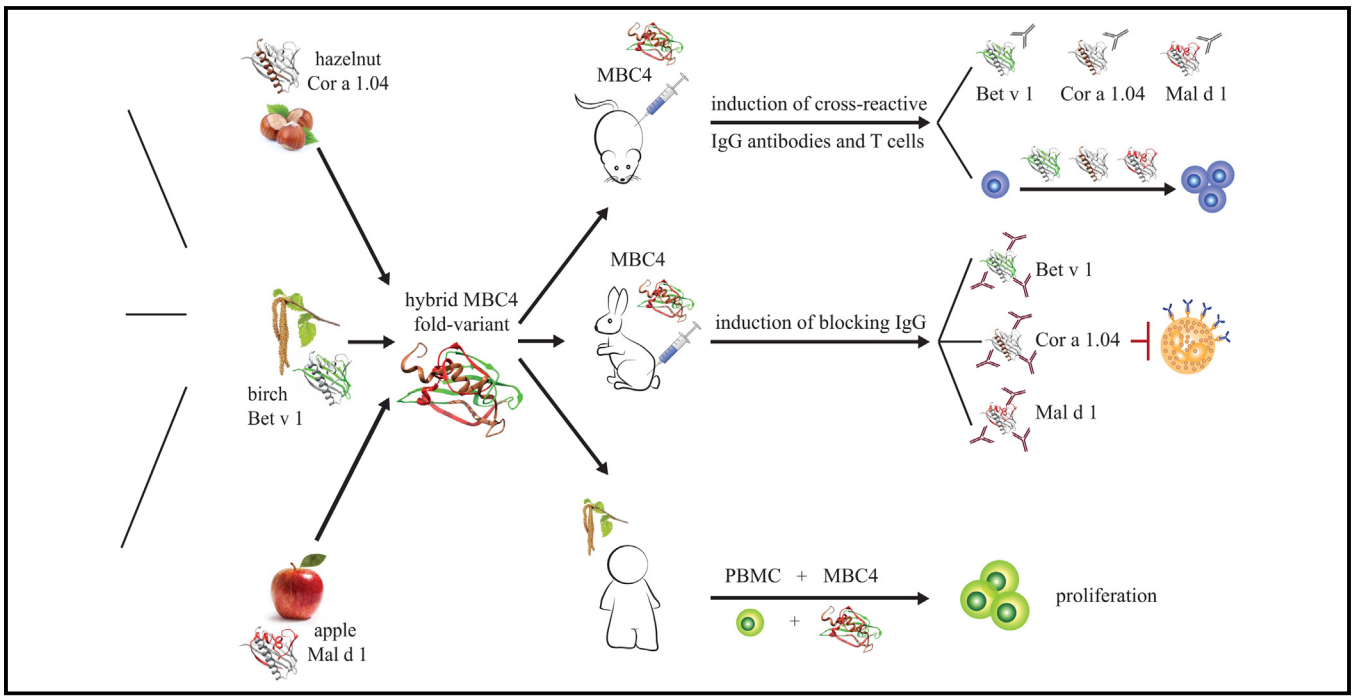
GRAPHICAL ABSTRACT



Background: Allergy vaccines should be easily applicable, safe, and efficacious. For Bet v 1–mediated birch pollen and associated food allergies, a single wild-type allergen does not provide a complete solution.

Objective: We aimed to combine immunologically relevant epitopes of Bet v 1 and the 2 clinically most important related food allergens from apple and hazelnut to a single hybrid protein, termed MBC4.

Methods: After identification of T cell epitope–containing parts on each of the 3 parental allergens, the hybrid molecule was designed to cover relevant epitopes and evaluated *in silico*. Thereby a mutation was introduced into the hybrid sequence, which should alter the secondary structure without compromising the immunogenic properties of the molecule.

Results: MBC4 and the parental allergens were purified to homogeneity. Analyses of secondary structure elements revealed substantial changes rendering the hybrid *de facto* nonreactive with patients' serum IgE. Nevertheless, the protein was monomeric in solution. MBC4 was able to activate T-cell lines from donors with birch pollen allergy and from mice

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Graphical abstract photos were purchased from Fotolia, and structural models were derived from the PBD entry 1bv1 (www.rcsb.org) and modified with UCSF Chimera 1.10.1 (www.cgl.ucsf.edu/chimera/).

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immunized with the parental allergens. Moreover, on immunization of mice and rabbits, MBC4 induced cross-reactive IgG antibodies, which were able to block the binding of human serum IgE.
Conclusion: Directed epitope rearrangements combined with a knowledge-based structural modification resulted in a protein unable to bind IgE from allergic patients. Still, properties to activate specific T cells or induce blocking antibodies were conserved. This suggests that MBC4 is a suitable vaccine candidate for the simultaneous treatment of Bet v 1 and associated food allergies. (J Allergy Clin Immunol 2017;140:525-33.)

Key words: *Birch pollen allergy, Bet v 1, birch pollen–associated food allergy, allergy vaccine candidate, molecular allergology*

Abbreviations used
AIT: Allergen-specific immunotherapy
ANS: 1-Anilino-8-naphthalene sulfonate
AP: Alkaline phosphatase
APC: Antigen-presenting cell
CD: Circular dichroism
PDB: Protein Data Bank
PFS: Pollen-food syndrome
RBL: Rat basophil leukemia
TCL: T-cell line
WT: Wild-type

Birch pollen allergy is dominated by a single disease-eliciting allergen, namely Bet v 1, with reactivity rates of greater than 90% among patients with birch pollen allergy. Moreover, it has been reported that more than 70% of patients with birch pollen allergy react to at least 1 Bet v 1–associated allergenic food source, including pomaceous and stone fruits, vegetables, nuts, and legumes. With 80% reactivity among patients with food allergy, apple was most frequently recognized, followed by hazelnut, which triggered allergic symptoms in 59% of patients with pollen-food syndrome (PFS).[1]

For the sustainable treatment of birch pollen allergy, it has been shown that extract-based therapeutics can be successfully substituted by purified rBet v 1 applied either by means of subcutaneous or sublingual immunotherapy.[2,3] However, successful birch pollen allergen-specific immunotherapy (AIT) does not necessarily correlate with the amelioration of concomitant food allergies. In a study by Kinaciyan et al,[4] SLIT performed with birch pollen extract induced protective Bet v 1–specific IgG_4 antibodies in test subjects, which were not cross-reactive with Mal d 1 from apple. Moreover, the treatment reduced exclusively the responsiveness of Bet v 1–specific T cells, and the T-cell response to Mal d 1 remained practically unaltered.[4]

In a follow-up the presence of specific blocking IgG_4 antibodies, as well as the IgG_4/IgE ratio, was linked to food tolerance. Such blocking antibodies can be either innately present in food-tolerant patients or can be induced through AIT.[1,5] The majority of AIT-induced IgG_4 is thought to recognize IgE epitopes on the allergens; however, also allergen-specific unique IgG_4 specificities, which do not overlap with the IgE epitope, will eventually develop during therapy.[5]

In light of the above, it is necessary to tackle Bet v 1–associated food allergies at both the B- and T-cell levels, and AIT based on wild-type (WT) Bet v 1 seems insufficient for this task. Therefore the structure of Bet v 1, which is immunologically distinct from the structures of the related food allergens from apple and hazelnut, might be the cause of this observation.[6]

Thus we aimed to design a hybrid molecule by combining immunologically relevant epitopes of Mal d 1 from apple and Cor a 1.04 from hazelnut with the major birch pollen allergen. Recently, we successfully developed a strategy to produce a vaccine candidate for patients who are multisensitized to Bet v 1–like Fagales pollen allergens by generating a hybrid molecule. Sequence regions of the 5 most important allergens from birch, hazel, alder, oak, and hornbeam were combined into a single protein that showed reduced binding of patients' IgE but preserved immunogenicity.[7] In analogy to this study, we identified T-cell epitopes on Mal d 1, Bet v 1, and Cor a 1.04 and combined T cell–reactive stretches to a hybrid protein (MBC) showing the same overall length as the parental allergens. To reduce IgE binding of the protein, we generated a fold variant of our hybrid (MBC4) by introducing a mutation previously identified as important for Bet v 1 to adopt its native fold.[8,9] This particular mutation was shown to conserve sufficient potential IgG-reactive secondary structure elements on the surface of Bet v 1 to efficiently induce blocking antibodies while preexisting IgE epitopes would be eliminated. In addition, the structural modification should not affect the immunogenic properties of the fold variant.

METHODS

Patients and sera

Patients with birch pollen allergy and concomitant PFS were selected based on case history, positive skin prick test responses, and/or *in vitro* IgE detection (CAP System; Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden; see Table E1 in this article's Online Repository at www.jacionline.org). Inclusion criteria were a CAP class of greater than 3 to birch and greater than 1 to apple and hazelnut. Experiments with patients' sera were approved by the Ethics Committee of the University of Vienna (EK028/2006) and Salzburg (415-E/1398/4-2011). Written informed consent was obtained from all subjects included in the study.

Design, *in silico* evaluation, and cloning of MBC and MBC4

Hybrid proteins were designed and evaluated *in silico*, as described in the Methods section in this article's Online Repository at www.jacionline.org. Thereafter, the hybrid allergen MBC was cloned by means of PCR recombination of 3 overlapping fragments from the genes Mal d 1.0108 (AF126402), Bet v 1.0102 (X77266), and Cor a 1.0401 (AF136945), respectively. Subsequently, the gene was cloned into a pET28b vector (Novagen, Merck Millipore, Billerica, Mass). By using MBC as a template, a mutation was introduced in the Bet v 1.0102 part of the molecule to generate the MBC4. Moreover, in MBC4 the C-terminal cysteine of Cor a 1.0401 was mutated to a serine to abrogate dimerization.[6] A detailed description is provided in the Methods section in this article's Online Repository.

Expression and purification of recombinant proteins

The parental allergens Bet v 1.0101, Mal d 1.0108, and Cor a 1.0401 were produced as nonfusion proteins, as described, and are referred to as Bet v 1, Mal d 1, and Cor a 1.04, respectively, in the following.[6,8] Recombinant MBC was expressed in *Escherichia coli* and produced from the soluble fraction,