A Fully Human Monoclonal Antibody Isolated From a Beekeeper Targets the Immunodominant IgE Epitope of Api m 10

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Honeybee venom (HBV) allergy is a severe, potentially life-threatening condition presenting as anaphylaxis upon a honeybee sting [1]. The dominant major allergen in HBV is Api m 1. Other major allergens are Api m 2, 3, 5, 6, and 10; the minor allergens include the high-abundance peptidic component melittin, Api m 4 [2,3]. Predominant sensitization to Api m 10 may represent a risk factor for failure of venom immunotherapy (VIT) [4]. Underrepresentation of Api m 10 in venom extracts is thought to result from molecular instability, and the absence of stable secondary structures in the allergen hampers structural analysis [5]. The specificity of IgE (sIgE) to Api m 10 in sensitized individuals has been deconvoluted using an Api m 10 peptide library spanning the entire sequence of Api m 10 variant 1 [6]. One immunodominant IgE epitope, P54, was recognized by 100% of the tested Api m 10–positive sera from HBV-allergic patients. Furthermore, IgE reactivity to P54 represented, on average, 67% of the total Api m 10 peptide sIgE. Epitope P54 is thus highly relevant for diagnostics and for making decisions on therapy in Api m 10–sensitized individuals.

In order to precisely determine the residues involved in sIgE binding to Api m 10 within the immunodominant epitope P54, an alanine scan peptide library for P54 was generated and assessed for immunoreactivity of 8 sera with high levels of P54 sIgE (Figure, Supplementary data figure 1). A clear decrease in sIgE reactivity was observed for alanine substitutions in P54-7, P54-8, P54-9, and P54-13 (P<.001) and in P54-1 and P54-10 (P<.05). Data on individual patients' sIgE reactivity to alanine scan peptides and Api m 10 can be found in Supplementary data figure 2.

Single-cell sequencing of memory B cells from a beekeeper exhibiting no sIgE reactivity but exhibiting specific IgG and IgG4 reactivity to Api m 10 (Supplementary data figure 3), yielded a single Api m 10–specific IgG1 monoclonal antibody (mAb). In order to compare the reactivity profile with patient

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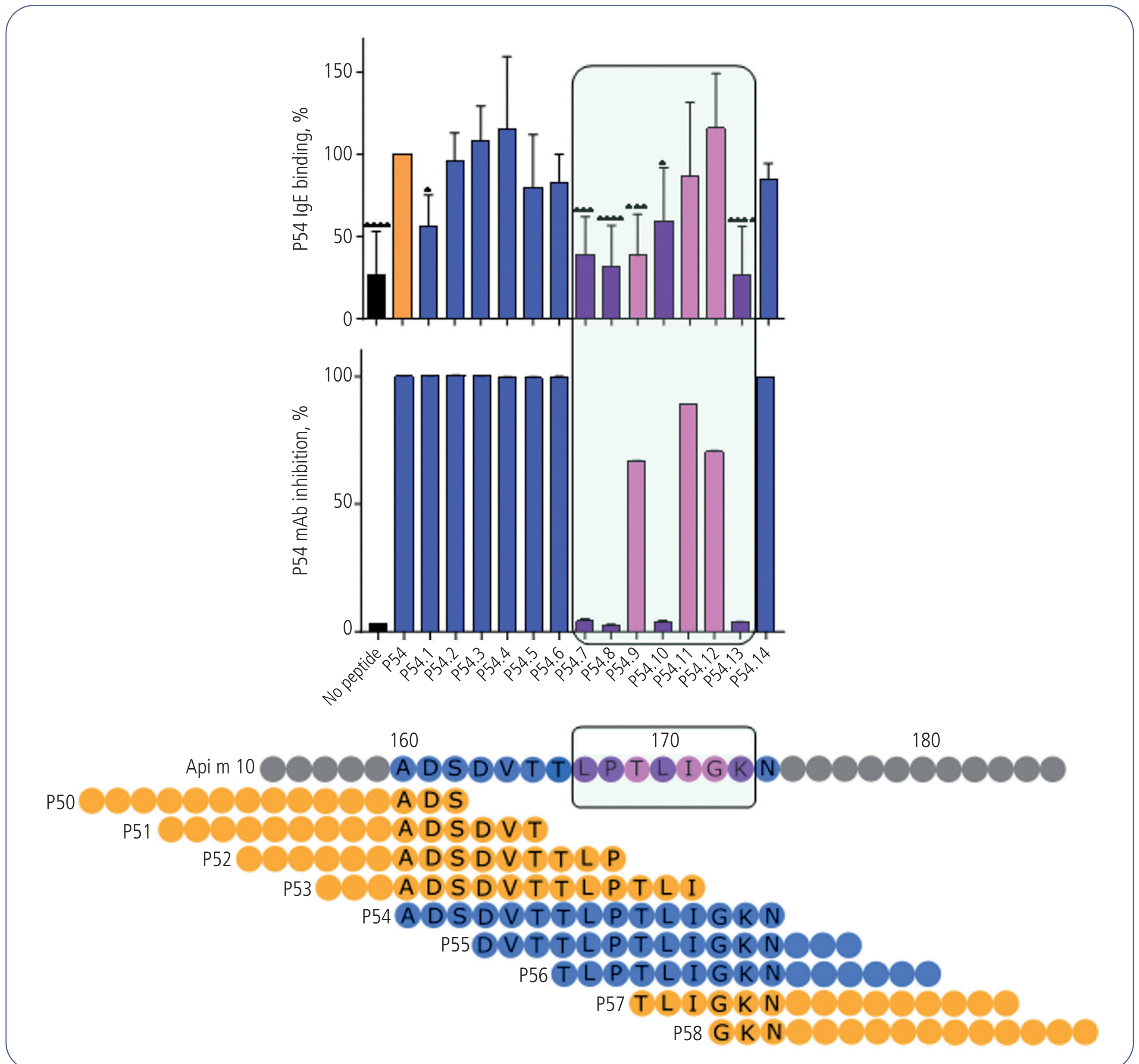

Figure. Immunoreactivity and epitope mapping of serum IgE and mAb immunoreactivity to Api m 10 peptides. The similarity of the epitope is shown by the reactivity of patient IgE (n=8) and mAb binding to rApi m 10 towards alanine scan peptides. Inhibiting peptides are marked in blue, noninhibiting peptides in violet, and partially inhibiting peptides in pink. Peptides P54 (amino acids 160-174) (nongray), P54-7, 8, 10, and 13 (purple) and P54-9, 11, and 12 (pink) in the context of the Api m 10.0101 amino acid sequence.

sIgE, the antibody was converted and recombinantly expressed as fully human IgE in human HEK293 cells. The resulting IgE mAb displayed pronounced and specific immunoreactivity to recombinant Api m 10 and HBV (Supplementary data figures 4 and 5). The binding affinity (kD) to rApi m 10 was determined to be 137 pM (Supplementary data figure 6), suggesting efficient in vivo affinity maturation.

The epitope of the Api m 10–specific mAb was mapped using the overlapping Api m 10 peptide library, as described elsewhere [6]. Pronounced inhibition of rApi m 10 binding was obtained for peptides P54, P55, and P56 in a dose-dependent manner (Supplementary data figure 7), suggesting specificity for the same linear epitope as that being predominantly recognized by the allergic patients' IgE.

Subsequent fine mapping of the epitope was performed using the alanine scan P54 peptide library's ability to inhibit binding of the mAb to rApi m 10 (Figure). Amino acid replacements in P54-7, P54-8, P54-10, and P54-13 completely abolished the inhibitory capacity of the peptides, whereas replacements in P54-9, P54-11, and P54-12 diminished the inhibition to a lesser extent.

The complementary data obtained for sIgE from sensitized patients' sera and the beekeeper-derived monoclonal IgG antibody corroborate the dominant nature of the epitope and

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further reveal a shared recognition mode driven by highly defined residues within P54. The principles underlying this predominant recognition have not been studied. However, the identification of a linear IgE epitope in an HBV allergen lacking a stable fold extends our insights into the crucial allergen–antibody interaction.

The results of our study reveal an IgE epitope that is not only overlapping but also equivalent to an IgG epitope. To our knowledge, such epitopes shared on the level of individual residues are scarce, with a notable exception being cross-reactive carbohydrate determinants [7]. Xenobiotic carbohydrate linkages induce both IgG and IgE reactivity to the same structure, as seen for the α-Gal epitope or the fucose-modified core glycan epitope [8].

The biochemical peculiarities of P54 include an unusually low isoelectric point and the asparagine residue at P54 position 15, which represents one of the glycosylation sites of Api m 10. While the absence of glycosylation in the synthetic peptides and the Api m 10 used in the ImmunoCAP apparently does not affect sIgE reactivity, a relevant role for glycosylation in the immunogenicity of the natural Api m 10 peptide cannot be excluded. In general, the role of glycosylation of Api m 10 and other HBV allergens in recognition by IgE and IgG should be addressed in the future.

The high-affinity mAb that binds the immunodominant residues of Api m 10 was isolated as an Api m 10–specific IgG1 from a memory B cell from a beekeeper extensively exposed to HBV. Hence, an authentic Api m 10–specific blocking IgG could have the potential for establishing selective protection in patients predominantly sensitized to Api m 10. Furthermore, the mAb is an excellent tool for quantifying and assessing the presence of Api m 10 for diagnosis and in therapeutic preparations.

In summary, we demonstrated recognition of defined residues in a dominant, linear epitope in Api m 10 by both patient IgE and beekeeper-derived IgG. These data support the concept of identical IgE and IgG epitopes recognized in HBV and could pave the way for advanced tools for diagnostics, functional analysis, and interventional approaches.

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Conflicts of Interest

Anders Lund, Lars Harder Christensen, and Frederic Jabs are employees at ALK Abelló A/S. The remaining authors declare that they have no conflicts of interest.

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