

565 Recombinant Human IgE Antibodies to Analyze Antigenic Determinants in Group 1 Mite Allergens for the Design of Immunotherapy



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RATIONALE: Little is known about the molecular features of IgE antibody binding epitopes and their distribution on allergenic molecules. The goal is to analyze IgE antigenic determinants of group 1 dust mite allergens in order to, through a knowledge-based approach, design modified allergens with reduced IgE reactivity and retained immunogenicity, as candidates for immunotherapy, given their potential to decrease side-effects due to IgE cross-linking.

METHODS: Der p 1-specific single-chain variable fragments (scFv) were isolated from combinatorial libraries constructed from the IgE repertoire of mite allergic patients and displayed on filamentous phage. They were produced fused to immunoglobulin Fc domains and analyzed for allergen binding by direct and inhibition immunoassays. A scFv was expressed in yeast *Pichia pastoris* and purified by affinity chromatography.

RESULTS: Two isolated scFv bound strongly to natural Der p 1 by ELISA with different epitope specificity, by comparing with Der p 1-specific mAb of known specificity. A soluble Der p 1-specific scFv did not bind the homolog Der f 1, despite interfering with the binding of mAb-4C1, which recognizes a cross-reacting epitope in both allergens. The overlap of both epitopes was proven by: a) its lack of recognition of the allergen captured by mAb-4C1 and b) the inhibition of the scFv binding to nDer p 1 by mAb-4C1. The scFv was expressed in yeast *Pichia pastoris* in mg amounts suitable for crystallographic analysis.

CONCLUSIONS: Recombinant IgE from combinatorial libraries provide tools for analysis of antigenic determinants in the context of the human IgE antibody repertoire and for the design of immunotherapy.

566 The Measure of Specific IgE to Whole-Allergen Extracts May Not be Useful for Primary Sensitization Diagnosis in Children with Atopic Dermatitis and Asthma



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RATIONALE: Children with atopic dermatitis and asthma are mainly polysensitized thus use of specific IgE to allergen extracts in sensitization diagnosis may lead to misdiagnosis. In this cross-sectional, prospective study the degree of cross-reactivity using component resolved diagnostics (CRD) was analyzed.

METHODS: Serum specific IgE to 112 allergen components were measured by using multiplex microarray (ISAC) in 297 sensitized children (aged 1-18 years) with atopic dermatitis (mAD-moderate, sAD-severe, objective SCORAD index 15-40 and >40 respectively), asthma (A1-episodic, A2-mild persistent or A3-moderate) or both. Sensitization to 7 main cross-reactive allergen families (C-RAF) and specific IgE to cross-reactive carbohydrate determinants (CCD) was identified and evaluated depending on clinical diagnosis and age.

RESULTS: IgE reactivity to C-RAF and CCD was found in 81,1% of children (52,9%, 17,5%, 14,8%, 4,7%, 52,2%, 21,6%, 14,5% and 9,4% in pathogenesis-related class 10 proteins, non-specific transfer lipid proteins, profilins, polcalcins, lipokalcins, serum albumins, tropomyosins and CCD respectively). Depending on clinical diagnosis IgE to C-RAF was found in 75,3% children with asthma (57,9%, 78,9% and 85,0% in A1, A2 and A3 respectively), in 76,0% with atopic dermatitis (61,1% in mAD and 87,3% in sAD) and in 92,6% children with both atopic dermatitis and asthma. IgE

reactivity to C-RAF was different depending on age: in 60,8%, 84,1% and 92,8% of children at age 1-2, 3-6 and 7-18 years respectively.

CONCLUSIONS: In children with atopic dermatitis and/or asthma CRD is essential to due to widespread sensitization to C-RAF and high cross-reactivity to components within each family.

567 Production of Human Monoclonal IgE from Patients with Allergic Bronchopulmonary Mycosis



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RATIONALE: Allergy to mold is associated with significant morbidity, including increased rates of hospitalization and ICU admission for asthmatics. A subset of patients with asthma will go on to develop exuberant Th2 inflammation directed against mold colonizing their airway (Allergic Bronchopulmonary Mycosis). Understanding the development of the IgE response to mold has been limited by the lack of human monoclonal IgE antibodies directed against mold.

METHODS: Patients within the Vanderbilt system with diagnosis of allergic bronchopulmonary mycosis were identified and recruited to the study. Peripheral blood mononuclear cells were isolated and placed under conditions to enhance B-cell growth in cell culture. B-cell cultures were screened for IgE secretion by ELISA and fused with human derived myeloma partners using electrical cytofusion methods. Human hybridomas were identified by placing in selective media (hypoxanthine, aminopterin, and thymidine) and subsequently assaying for IgE secretion.

RESULTS: We report the production of IgE secreting human hybridomas from patients with allergic bronchopulmonary mycosis. Additional characterization of these antibodies will be presented.

CONCLUSIONS: Production of monoclonal IgE represents a novel tool to understand the evolution of the B-cell response in allergic patients.

568 IgE Anti-Haemophilus Influenzae Type b (Hib) Antibodies Detected in Serum of Hib Vaccinated Asthmatic and Non-Asthmatic Pediatric Patients



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RATIONALE: Haemophilus influenzae type b (Hib) is a bacterium that causes severe illness in infants and children and has not been linked to atopy and asthma. While the effects of certain vaccinations on atopic disease have been well-studied, little is known about the relationship between Hib vaccination and diseases of altered IgE regulation (asthma and atopy).

METHODS: Total serum IgE and IgE-and IgG-anti-Hib antibody responses were studied in Hib vaccinated asthmatic (N=14) and non-asthmatic children (N=26) (VaccZyme™ Human Anti Hib Enzyme Immunoassay Kit). Data are reported as mean optical density (OD) values.

RESULTS: We found that (1) total serum IgE levels were higher in asthmatic compared with non-asthmatic subjects ($P<0.001$), and (2) IgE- and IgG- anti Hib antibody responses were similar in both asthmatic and non-asthmatic subjects (0.722 ± 0.279 , 0.681 ± 0.28 , respectively, $P=0.65$; 0.450 ± 0.505 , 0.573 ± 0.779 , respectively, $P=0.584$).

CONCLUSIONS: The universal Hib vaccine antigen does not result in either increased IgE- or IgG anti-Hib antibody responses in asthmatic or non-asthmatic subjects. Thus, in this cohort, we did not find an association between Hib vaccination and asthma status.