Assessment of component-resolved *in vitro* diagnosis of celeriac allergy

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Background: Previous studies have demonstrated insufficient sensitivity of commercially available celeriac extract reagents in the diagnosis of celeriac allergy.
Objective: We sought to assess the diagnostic performance of specific IgE determination based on recombinant and purified natural celeriac allergens in comparison with an extract-based assay and to investigate interference by IgE to cross-reactive carbohydrate determinants and its biologic activity.
Methods: Twenty-four subjects with a positive double-blind, placebo-controlled food challenge result to celeriac; 20 atopic control subjects with birch pollen allergy who tolerated celeriac; and 20 nonatopic subjects were enrolled. IgE binding was investigated for celeriac allergens (rApi g 1.01, rApi g 4, and nApi g 5), extract reagents (celeriac, birch, mugwort, and timothy grass pollen), birch pollen allergens (rBet v 1 and rBet v 2), and cross-reactive carbohydrate determinants by means of ImmunoCAP analysis. Biologic activity of allergens was determined based on basophil mediator release.
Results: Component-resolved ImmunoCAP analysis considerably increased the sensitivity to detect celeriac-specific IgE by 20%. Sensitization to carbohydrate structures was detected in 38% of patients with celeriac allergy, and there was an excellent correlation between sensitization to the glycoprotein Api g 5 and isolated glycan. Positive results among atopic control subjects were mainly caused by protein allergens, whereas the effect of carbohydrate epitopes was marginal. The ability of allergens to induce mediator release decreased in the order Bet v 1 > Api g 1 > Api g 5, confirming the low biologic activity of IgE to carbohydrate epitopes.
Conclusion: Component-resolved diagnosis allowed an increase in diagnostic sensitivity from 67% to 88% compared with extract-based diagnosis. Sensitization to Api g 5 was attributable to its glycan moieties but did not interfere with diagnostic specificity.
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Key words: *Component-resolved diagnosis, celeriac allergy, ImmunoCAP, recombinant allergens, cross-reactive carbohydrate determinants, pollen-related food allergy, cross-reactivity*

Allergy to celery tuber (celeriac) is one of the most important food allergies in central Europe and is particularly prevalent in Switzerland, Germany, and France.[1,2] In these countries celeriac is consumed raw or cooked in soups and sauces and in dry powdered form in spice mixes. Celeriac allergy is frequently associated with birch pollinosis, mugwort pollinosis, or both,[2-5] a pattern that is referred to as the birch-mugwort-celeriac syndrome.

Symptoms of celeriac allergy range from mild oral allergy syndrome (OAS) to severe and even life-threatening reactions.[1,6,7] According to European Directive 2007/68/EC (amending Directive 2000/13/EC), the presence of celeriac must be indicated on the label of prepackaged foods in the European Union. To date, 3 celeriac allergens have been identified, the Bet v 1 homologous protein Api g 1, the profilin Api g 4, and the glycoprotein Api g 5.

The major celeriac allergen Api g 1 belongs to the so-called PR-10 family of pathogenesis-related proteins and has a molecular weight of approximately 16 kd. Thus far, 2 different isoforms have been detected.[8-10]

The celeriac profilin Api g 4 is a 14 kd homologue to the birch pollen protein Bet v 2.[11,12] It is a minor allergen, showing a heat stability greater than that of Api g 1 but less than that of carbohydrate structures.[13,14]

Api g 5 has been described as a mixture of 2 polypeptides with molecular weights of 53 and 57 kd. The 2 polypeptides belong to the family of flavoproteins.[15,16] According to mass spectrometric analysis, the protein core carries at least 3 N-glycans of the Manα1-6(Manα1-3)(Xylβ1-2)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc and Manα1-6(Xylβ1-2)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc ($MUXF^3$) types attached at different amino acid positions. Removal of the carbohydrate structures results in complete

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Abbreviations used
CCD: Cross-reactive carbohydrate determinant
CRD: Component-resolved diagnosis
DBPCFC: Double-blind, placebo-controlled food challenge
MUXF^3: Glycan structure Manα1-6(Xylβ1-2)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc
OAS: Oral allergy syndrome
PR-10: Pathogenesis-related class 10 protein
RBL: Rat basophilic leukemia

loss of the IgE-binding capacity of the glycoprotein, indicating that the IgE binding of Api g 5 is attributable to the carbohydrate moiety.[15] The effect of cross-reactive carbohydrate determinant (CCD) sensitization on the outcome of *in vitro* diagnostic procedures is subject to debate.[17]

Our previous studies on celeriac allergy have shown a diagnostic sensitivity of the celeriac extract ImmunoCAP (Phadia, Uppsala, Sweden) of 73%,[18] indicating a need for further improvement of the diagnostic reagents. To evaluate whether purified allergen components from celeriac would enable improved clinical diagnosis, we designed a component-resolved diagnosis (CRD) study using the ImmunoCAP system to analyze the sensitization pattern of a patient group with confirmed celeriac allergy. Furthermore, we wanted to investigate whether specific serum IgE levels to single celeriac allergens or allergen combinations would correlate (or predict) the type or severity of symptoms. Finally, the effect of glycan-specific IgE on the *in vitro* diagnosis of allergy to celeriac and the biologic activity of the glycoprotein Api g 5 in comparison with the protein allergens rBet v 1 and rApi g 1 were investigated.

METHODS

Determination of specific IgE levels

Specific IgE antibodies to extracts of celeriac, birch, mugwort, and timothy grass pollen, as well as to the single allergens rApi g 1.01 (termed rApi g 1 in the following), rApi g 4, and nApi g 5 from celeriac; rBet v 1, rBet v 2, and rBet v 4 from birch; and CCDs (MUXF^3 carbohydrate epitope purified from bromelain) were measured by using the CAP FEIA system (ImmunoCAP 250 assay instrument, Phadia), according to the manufacturer's instructions. Specific IgE concentrations of 0.35 kU_A/L or greater were considered positive.

Purified celeriac allergens were produced as described previously, and their identity, structural integrity, and IgE reactivity were confirmed in several physicochemical and immunochemical tests.[19] Proteins were coupled to the ImmunoCAP solid phase, as previously described.[20,21] For all other antigens, commercial ImmunoCAP tests were used. IgE binding to the bromelain-derived glycan MUXF^3 was used as an indicator of anti-CCD reactivity.

Patients

Twenty-four patients with allergy to celeriac, as demonstrated by a positive double-blind, placebo-controlled food challenge (DBPCFC) result, were included in the study.[6] All patients with celeriac allergy had a positive skin prick test response to celeriac extract. The median age was 27 years (range, 12-49 years), and the female/male ratio was 15/9. Celeriac-induced symptoms, according to history and under DBPCFC, are summarized in Table I.

Twenty atopic subjects with a median age of 29 years (range, 20-53 years) and with a history of birch pollen allergy but without celeriac allergy, as well as 20 nonatopic subjects with a median age of 37 years (range, 20-43 years), were included as control subjects. All subjects were from Switzerland, except for 4 German nonatopic control subjects.

Skins prick tests and DBPCFCs

Skin prick tests and DBPCFCs were performed as previously described.[6] Control subjects were subjected to an open food provocation consisting of ingestion of 20 g of raw celeriac, with which no reaction to celeriac was confirmed. Written consent was provided by all study participants, and ethical approval was obtained from the local ethics committee.

To compare our results from *in vitro* determination of specific IgE levels to celeriac allergens, we mainly relied on symptoms reported by the patients (Table I) because challenges were usually interrupted before the most severe symptoms occurred[6] for ethical reasons. Ten (42%) patients reported OAS as the only symptom after ingestion of celeriac (symptom group 1, OAS only), 8 (33%) patients reported urticaria or flush (symptom group 2, skin reactions), 3 (13%) patients reported gastrointestinal symptoms (eg, emesis; symptom group 3, gastrointestinal reactions), 2 (8%) patients reported respiratory symptoms (eg, rhinoconjunctivitis; symptom group 4, symptoms of the upper respiratory tract), and 1 (4%) patient experienced a decrease in blood pressure during the DBPCFC (symptom group 5, severe systemic reactions).

Mediator release from humanized rat basophilic leukemia cells

The rat basophilic leukemia (RBL) mediator release assay was performed as described previously.[22] Briefly, RBL-30/25 cells were plated in 96-well, flat-bottom culture plates (Nunc [ordered through VWR International], Darmstadt, Germany) at a density of 1.5×10^6 cells/mL. The cells were passively sensitized by means of overnight incubation with human sera diluted 1:10. After washing of the cells, allergen was added at serial dilutions, ranging from 10^{-5} to 10^0 μg/mL for Bet v 1 and 10^{-4} to 10^1 μg/mL for Api g 1 and Api g 5. Specific mediator release was quantified by determining the β-hexosaminidase activity and expressed as a percentage of the total amount of β-hexosaminidase activity measured after cell lysis with Triton-X-100 (Sigma-Aldrich, Taufkirchen, Germany). Spontaneous release and potential nonspecific effects were measured by incubating naive cells with cell culture medium, allergen, or the patients' sera, respectively. Nonspecific release was not higher than 5% of the total β-hexosaminidase content. As a positive control, cells were sensitized with polyclonal human myeloma IgE and cross-linked with an anti-human IgE antibody.

Statistical analysis

Statistical analysis of the results was performed by means of a multivariate ANOVA with the Wilk λ exact test to test for an overall difference between symptom groups (according to the severity of reactions) for different sensitization profile groups. For analysis of the results obtained for patients with OAS versus patients with more severe or systemic reactions, the Wilcoxon rank sum test was applied. Statistical significance was accepted for P values of less than .05.

RESULTS

Sensitization patterns in patients with celeriac allergy are complex

The results of specific IgE testing are shown in Table I, and the prevalence of detectable specific IgE antibodies among the patients with celeriac allergy and atopic control subjects is shown in Fig 1, *A*. Sixteen (67%) of 24 patients with celeriac allergy showed specific IgE to celeriac extract, and 21 (88%) showed specific IgE to at least 1 of the celeriac allergen components tested.

Ten (42%) of the patients with celeriac allergy were monosensitized to 1 celeriac allergen, 8 to rApi g 1, and 2 to rApi g 4. Monosensitization to nApi g 5 or CCDs did not occur.

There was a good correlation between the reactivity to celeriac extract and specific reactivities to celeriac allergen components, which was expressed as the sum of specific IgE values of all single celeriac allergens (see Fig E1 in this article's Online Repository at www.jacionline.org), indicating that the used allergen components were represented in the celeriac extract.

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