



Inhibition of Cross-Reactive Carbohydrate Determinants in Allergy Diagnostics

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Abstract

Despite being clinically largely irrelevant, antibodies against cross-reactive carbohydrate determinants (CCD) are an important issue in the in vitro diagnostics, as they may produce false positive or falsely elevated results of the immunoglobulin E class (asIgE) in relation to the actually present level of asIgE. The present chapter demonstrates an effective resolution of this diagnostic issue by the use of a CCD inhibitor in in vitro tests. A synthetic CCD inhibitor, Polychex® CCD inhibitor, was used in the laboratory diagnostics of 24 children diagnosed with allergic diseases. The anti-CCD antibody content was measured in the serum using a Polychex® Atopic 30-I panel (Biocheck GmbH; Münster, Germany), a screening assay for the quantitative determination of multiple allergen-specific IgE. We found that the baseline anti-CCD antibody content, without the CCD inhibitor, ranged from 0.7 to 3.5 kU/L in the sera of the majority of 16 out of 24 children. When the CCD

inhibitor was applied, the anti-CCD antibody content decreased in 16, remained unchanged in 3, and increased in 5 samples. In samples positive for plant allergens, the asIgE content dropped by an average of 72% when the CCD inhibitor was used in the assay, except the antibodies to tree and grass pollen allergens, for which the asIgE content remained above 100 kU/L. We conclude that the use of a CCD inhibitor in in vitro assays is a viable option to mitigate the influence of anti-CCD antibodies on the measured level of asIgE immunoglobulin, which increase the reliability of testing particularly in cases displaying multiple allergies.

Keywords

Allergy · Anti-CCD antibodies · CCD inhibitor · Cross-reactive carbohydrate determinants (CCD) · IgE immunoglobulin

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1 Introduction

Cross-reactive carbohydrate determinants (CCD) are structurally simple carbohydrate residues which become attached to amino acid protein chains during glycosylation as part of post-translational processing in eukaryotic cells. This process, which leads to glycoprotein production, is common in both plants and animals. What

makes CCD different from other carbohydrate residues is their antigenicity and ability to stimulate the production of allergen specific immunoglobulin E (asIgE). The term “cross-reactive” determinants was first used more than 30 years ago (Aalberse et al. 1981). Subsequent research has demonstrated that anti-CCD antibodies are of no clinical relevance, since their presence is not associated with any symptoms (Mertens et al. 2010; Muller et al. 2009; Mari et al. 2008; Ebo et al. 2004; Mari 2002). While basophil activation tests have demonstrated bioactivity of anti-CCD asIgE, no connection has been substantiated to clinical symptoms in patients (Mertens et al. 2010; Foetisch et al. 2003).

Despite the seeming clinical irrelevance, anti-CCD antibodies pose a serious problem in case of the in vitro asIgE diagnostics with respect to the allergens in which they appear, making it harder to correctly diagnose the allergy source. That is caused by the structural heterogeneity of CDD and their resemblance in various allergen sources. The most important type of CCD, having a sequence of N-glycan with alpha(1,3)-fructose attached to the polypeptide chain through nitrogen atom in the asparagine radical, is present in both plant allergens, including inhalable allergens and various food allergens, hymenoptera venom, and latex (Altmann 2016). Therefore, when an examined person produces anti-CCD antibodies, they can bind with allergens which contain CDDs during the test, giving a false positive or falsely elevated result in relation to the actual asIgE content. Taking into account that the prevalence of anti-CCD antibodies in people subjected to the in vitro asIgE testing can reach 25%, they pose a serious diagnostic problem in the allergy testing (Muller et al. 2009; Ebo et al. 2004; Mari et al. 2008). The prevalence of anti-CCD antibodies is even higher among young people, amounting to 35%.

One way to circumvent or eliminate the problem posed by anti-CCD antibodies in the in vitro asIgE diagnostics is to perform the component-resolved diagnostics, based on the antigens free from CCD or utilizing a CCD inhibitor. The premise behind the present study was to evaluate the influence of a CCD inhibitor on the asIgE content in individuals previously diagnosed with

positive levels of anti-CCD antibodies. To this end, we compared the asIgE content in relation to 30 allergens using multiparametric Polycheck® tests with and without a CCD inhibitor.

2 Methods

The study was approved by the institutional Ethics Committee and was performed according to the guidelines of the Helsinki Declaration for Human Research. There were 24 children of either genders (12 boys and 12 girls) aged 3–10 (average 5 years) enrolled into the study. They were hospitalized with chronic upper and lower respiratory tract symptoms and skin symptoms in the Regional Branch of Warsaw Institute of Tuberculosis and Lung Diseases in the mountainous city of Rabka-Zdrój from July 1 to August 2017. The goal of hospitalization was to conduct the diagnostics procedures and to determine therapy. Seventeen children were diagnosed with asthma, associated with allergic rhinitis in 8 of them, and 7 children were diagnosed with moderately severe atopic dermatitis. Anti-CCD antibodies were found in the sera of all children in a range of 0.43–25 kU/l (median 1.5 kU/l).

The Polycheck® Atopic 30-I (Biocheck GmbH, Münster, Germany), an allergy test routinely used in allergy screening, was the diagnostic method applied. This test is a multiparametric, quantitative test with a lower limit of detection of 0.15 kU/l. As recommended by the producer, it was assumed that positive results start from the level of 0.35 kU/L. The allergens included in the assay were the following: cow's milk, alpha-lactalbumin, beta-lactoglobulin, casein, egg white, egg yolk, codfish, peanut, cacao, soybean, apple, carrot, tomato, flour mix, chicken meat, citrus mix, rice, grass mix, rye, dog epithelia, cat epithelia, *Cladosporium herbarum*, *Alternaria alternata*, *Aspergillus fumigatus*, *Dermatophagoides pteronyssinus*, *D. farinae*, hazel pollen, birch pollen, and mugwort pollen. The test also includes a CCD marker, pineapple bromelain.

A Polycheck® CCD inhibitor was used according to the producer's recommendations, added at a ratio of 1:50 to each tested serum. This synthetic inhibitor is composed of polyvalent CCD structures of bromelain, with up to four amino acids left after the proteolysis and purification processes, which guarantees the lack of peptide epitopes. The glycopeptide obtained in such a way is attached to a protein carrier, which is highly purified human serum albumin.

Data were presented in the tabular form as two nominal variables (sample number and the absence or presence of CDD inhibitor) and one measurement variable (content of anti-CCD antibody). Differences in the content of anti-CCD antibody assayed without and with the addition of a CCD inhibitor, expressed as the percentage of the former, were statistically analyzed using the Wilcoxon signed-rank test. A p -value <0.05 defined a statistically significant difference. Statistical analysis was performed using a commercial GraphPad Prism v7 package (San Diego, CA).

3 Results

We found that the anti-CCD antibody content was significantly lower when the samples were incubated in the serum containing the CCD inhibitor than in those without the inhibitor ($p < 0.05$). The results for individual samples are presented in Table 1. The anti-CCD content decreased in 16, remained unchanged in 3, and increased in 5 samples. In most of the samples (16 out of the 24), the initial anti-CCD antibody content, without the inhibitor, was in a range 0.7–3.5 kU/L, i.e., in radioallergosorbent test (RAST) class 2, which is in line with the studies presented in the literature (Holzweber et al. 2013).

From among the samples analyzed, special attention should be paid to those displaying a positive result in relation to plant allergens. Table 2 presents an example of the presence of a multitude of plant allergens in a patient's serum and their changes after the addition of the CDD inhibitor. After application of the inhibitor, there was an across-the-board decrease in the anti-CCD

Table 1 Anti-CCD antibody content assayed without and with the CCD inhibitor (kU/L)

Sample	CCD inhibitor		Difference
	Without	With	%
1	25.00	6.30	−75
2	0.47	0.22	−53
3	0.78	0.41	−47
4	1.00	0.56	−44
5	2.40	1.40	−42
6	1.90	1.20	−37
7	8.90	6.10	−31
8	0.89	0.62	−30
9	0.80	0.59	−26
10	0.82	0.63	−23
11	0.76	0.60	−21
12	2.70	2.30	−15
13	0.56	0.49	−13
14	2.80	2.50	−11
15	0.87	0.80	−8
16	3.40	3.30	−3
17	1.50	1.50	0
18	2.00	2.00	0
19	2.20	2.20	0
20	1.50	1.60	+7
21	1.00	1.10	+10
22	0.43	0.48	+12
23	2.40	2.90	+21
24	6.30	7.90	+25

Table 2 Anti-CCD antibody content assayed without and with the CCD inhibitor in a patient positive for plant-based allergens (kU/L)

Polycheck® Atopic 30-I	CCD inhibitor		%
	Without	With	
Peanut	3.50	2.00	−43
Cacao	0.34	0.25	−26
Soybean	0.78	0.38	−51
Apple	6.20	0.42	−93
Carrot	56.00	16.00	−71
Tomato	4.20	0.15	−96
Flour mix	4.00	0.58	−86
Citrus mix	9.20	0.72	−92
Rice	9.00	0.65	−93
Grass mix	>100	>100	−
Rye	>100	>100	−
Hazel pollen	>100	>100	−
Birch pollen	>100	>100	−
Mugwort pollen	>100	>100	−
CCD	0.82	0.63	−23

antibodies to plant allergens, except for the antibodies related to tree and grass pollens which remained at a level exceeding 100 kU/L.

4 Discussion

A CCD inhibitor is used in the asIgE detection test to limit false positive reactions, by competitively binding anti-CCD antibodies in the sample assayed before they become bound with allergens containing a CCD residue. In general, CCD inhibitor, as opposed to allergen-extract molecules, is composed of a mix of molecules that are unassociated with the solid phase of the test, which is why the CCD inhibitor-anti-CCD IgE complexes can be washed out along with the other unbound elements during the procedure, and thus do not influence the test result. The CCD inhibitor used in this study has been tested before in a group of more than 1,300 CCD-positive patients, effectively reducing the number of false positives (Holzweber et al. 2013).

The present study shows that the use of a CCD inhibitor lowers the content of anti-CCD antibodies directly analyzed in the test, which complies with the previous observations (Altmann 2016). The absence of an inhibitory effect in some samples we analyzed remains unexplained. That could stem from the lack of duplicate measurements. Also, a small quantity of the material tested or unique patient's antibody characteristics may constitute some limitation. A low content of asIgE found in the present sample of patients (median 1.5 kU/L, with no inhibitor) can be yet another factor making the statistical evaluation difficult, based on the CCD marker alone. Be it as it may, other authors have also noticed the lack of a uniformly inhibitory effect on the anti-CCD antibody content of a CCD inhibitor, although there was a marked reduction in false positives (Holzweber et al. 2013).

As exemplified by a sample showing, in *in vitro* testing, different plant-based allergies, the action of the CCD inhibitor was clearly visible in case of reduced specific asIgE (Table 2). In case of the tomato allergen, asIgE dropped below

the limit of detection, completely mitigating the false positive. On average, the level of the entire anti-CCD antibody content was lowered by 72%, despite a 23% decrease in the CCD marker. That clearly illustrates an advantage of the use of a CCD inhibitor, instead of testing different levels of a CCD marker. Unfortunately, in the patients investigated in this study, despite positive values for CCD, only one sample displayed numerous positive results for asIgE. It is worth emphasizing that the use of multiparametric tests makes it much easier for a physician to identify the presence of anti-CCD IgE antibodies than when interpreting individual results (Altmann 2016; Holzweber et al. 2013).

The CCD inhibitor is a tool for *in vitro* asIgE testing, which helps reduce the effect of anti-CCD antibodies on the test results, and thereby increases the specificity of the method. The application of this reagent can contribute to the better and faster diagnostics in cases of multiple allergies tested *in vitro*. The present findings lead to a conclusion that the use of CCD inhibition is a viable option in a clinical setting. Multiparametric tests in the diagnostics of IgE-dependent allergies facilitate the identification of an influence of anti-CCD antibodies on the results for other allergens. However, due to additional costs stemming from the need to retest with CCD inhibitor, the use of a CCD inhibitor should be considered on a case-by-case basis.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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