



Cross-reacting carbohydrate determinants and hymenoptera venom allergy

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Purpose of review

Insect venom allergy is an important cause of anaphylaxis. Venom immunotherapy assume the clear identification of the culprit insect, but this is impeded by Immunoglobulin E (IgE) antibodies to cross reactive carbohydrate determinant (CCD) epitopes of common glycoproteins. Here we give an overview about inducers, importance, and relevance of anti-N-Glycan CCD IgE antibodies.

Recent findings

Pollen exposure and insect stings induce anti-CCD IgE antibodies interfering with in-vitro tests for allergy diagnosis due to extensive IgE cross-reactivity. Instead of being biologically active these antibodies are irrelevant for allergic reactions due to hymenoptera stings. The general response of the immune system to the ubiquitous exposure to N-glycan containing glycoproteins is still a matter of debate. CCD specific IgG antibodies in sera of bee keepers suggest tolerance induction due to high-dose exposure. Tolerance induction by pollen and food glycoproteins has not been proved.

Summary

Hymenoptera stings and pollen exposure induce anti-CCD IgE. In regard to anaphylaxis due to Hymenoptera stings these antibodies are not clinically relevant, but they are important for the specificity of in-vitro tests proving insect venom allergy. The introduction of component based diagnostic IgE testing improves the specificity of in-vitro tests if proteins devoid of CCD epitopes are used.

Keywords

cross reactive immunoglobulin E antibodies, glycoprotein, insect venom allergy

INTRODUCTION

Hymenoptera venom allergy comprises clinical symptoms from large local reactions to fatal anaphylactic shock. Up to 31% of all anaphylactic reactions treated in hospitals are caused by hymenoptera stings, and the incidence of fatal reactions ranges from 0.03 to 0.48 per million inhabitants per year. Venom immunotherapy (VIT) is highly effective and is indicated in patients with a history of a systemic insect sting reaction with a confirmed Immunoglobulin E (IgE)-mediated sensitization either by a skin test and/or by the determination of venom specific IgE antibodies.

Simultaneous IgE reactivity to bee and wasp venom of the sera from patients with hymenoptera allergy makes the identification of the culprit insect difficult. Apart from double sensitivity, cross reactivity due to IgE antibodies binding both bee and wasp allergens is more likely the reason for double serum reactivity. In the past inhibition tests have been used to distinguish between double sensitivity and cross reactivity. Nowadays component resolved

diagnostic (CRD) IgE testing seems to be more suitable.

Homology between the Hyaluronidase in bee venom (Api m 2) and wasp venom (Ves v 2) is the basis of cross reactivity due to protein epitopes, but the majority of cross reactivity is caused by clinically irrelevant IgE antibodies binding classical cross-reactive carbohydrate determinants (CCD) of N-linked glycans. Only the outnumbered patients with double sensitivity to both insect venoms require VIT with both venom extracts, others will be treated only with the venom that caused the reaction.

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KEY POINTS

- Hymenoptera venom allergy, a frequent cause of anaphylaxis, can be prevented by VIT in previously sensitized patients; the prerequisite is the clear identification of the eliciting venom.
- Frequently IgE antibodies to both bee and wasp venoms can be detected in serum samples of Hymenoptera venom allergic patients.
- Usually clinically nonrelevant IgE antibodies to CCD are responsible for serological double positivity.
- The introduction of nonglycosylated allergens and the ability to detect IgE to CCD epitopes are the solution for this problem.
- Serum samples with IgE to bee and wasp venom extracts should be analyzed for IgE antibodies to recombinant allergens and CCD.

The introduction of CRD in-vitro diagnostic IgE testing is a new strategy solving this problem if recombinant proteins devoid of CCD epitopes are used.

CLINICAL IMPACT OF INSECT VENOM ALLERGY

Hymenoptera stings are a major cause of anaphylaxis. A total of 1943 people died in the United States between 1991 and 2001 due to venomous and non-venomous animals. Venomous animals caused 39% of these fatalities, and hornets, bees, and wasps were responsible for 70.2% [1].

Emergency physicians documented 333 cases of anaphylaxis in the Berlin area between 2008 and 2010 [2] of which 295 were severe. Among these, insect stings (19.3%) take the third place after food allergies (32.2%) and drug allergies (29.2%).

Population-based epidemiological studies demonstrated a prevalence of systemic reactions due to Hymenoptera stings in the range from 0.3 to 8.9%, with the lowest risk in children. The incidence of fatal anaphylactic sting reactions ranges from 0.03 to 0.48 per million inhabitants per year [3].

VIT with purified insect venom preparations became a standard therapy for patients with an elevated risk for subsequent insect sting anaphylaxis. VIT has a clear indication in patients with a history of systemic insect sting reactions ($\geq$ grade II according to the Ring and Messmer grading scale for anaphylactic reactions [4]) when an IgE-mediated sensitization can be confirmed by skin tests and/or the determination of venom specific IgE antibodies. So far VIT constitutes the only therapeutic treatment to induce an

active protection against severe allergic reactions due to insect stings.

INSECT VENOM PROTEINS

Several allergens in bee and wasp venom have been characterized; many of them are glycoproteins with an N-glycan structure (Table 1). Important major allergens of both venoms, like Api m1, Api m2 and Ves v2, are glycosylated and protein and CCD structures can perform as epitopes for IgE antibodies. Glycan epitopes of Phospholipase A2 of bee venom was already described in 1987 [5].

For diagnostic reasons it is important to note that major allergens of bee and wasp venom contain protein and CCD epitopes. The new approach of component resolved diagnostic (CRD) IgE testing using single proteins in in-vitro systems solve the problem of CCD antibody caused serological cross reactivity if recombinant proteins devoid of N-glycans are applied. The use of purified native proteins does not offer the option of differentiation between antibodies to protein and CCD epitopes and they will be less useful if IgE antibodies to CCD epitopes are not clinically relevant.

STRUCTURE OF GLYCOPROTEINS

The enzyme equipment of cells determines the post-translational modification of proteins (i.e. glycosylation) in the endoplasmatic reticulum (for details see [6]). Asparagine (N)-linked glycans carrying core α 1,3-fucose and β 1,2-xylose as main side chains form the essential parts of the two independent N-glycan epitopes of plant glycoproteins (pollen, vegetables, fruits and natural rubber latex) represented by the two typical complex-type N-glycan structures MMXF (horseradish peroxidase) and MUXF (bromelain) (Fig. 1). Insect glycoproteins contain core-linked fucose, and MMF3F6 represents a typical structure [7]. IgE antibodies directed to these glycan pan-epitopes provide the basis for in-vitro cross-reactivity of allergens from different sources. Bromelain, horseradish peroxidase and ascorbatoxidase are the glycoproteins routinely used to identify IgE reactivity to CCD determinants; additionally a test using the MUXF glycan from bromelain is commercially available.

INDUCTION OF CROSS REACTIVE CARBOHYDRATE DETERMINANTS SPECIFIC IMMUNOGLOBULIN E ANTIBODIES

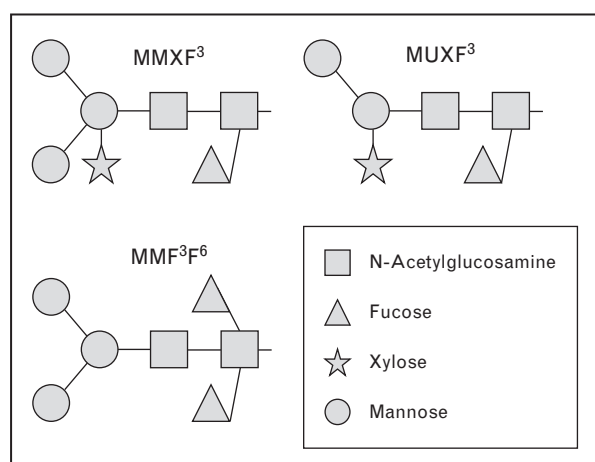
The most important inducers of specific IgE to CCD epitopes are pollen and insect stings. In up to 72% of

Table 1. Bee and wasp venom proteins

Venom	Biological function	Name	Positive up to	Glycosylated
	Phospholipase A2	Api m 1	<100%	+
	Hyaluronidase	Api m 2	<100%	+
	Acid phosphatase	Api m 3	37.5%	+
	Mellitin	Api m 4	<18.2%	–
	Dipeptidylpeptidase IV	Api m 5	40.0%	+
	Unknown	Api m 6	41.9%	–
	CUB Serineprotease	Api m 7	?	+
	Carboxylesterase	Api m 8	?	+
	Serinecarboxypeptidase	Api m 9	?	?
	Icarapin Variant 2, carbohydrate -rich protein	Api m 10	51.47%	+
	Major Royal Jelly Protein	Api m 11	34.04%	+
	Vitellogenin	Api m 12	?	?
wasp	Phospholipase A1	Ves v 1	<81.8%	–
	Hyaluronidase 2	Ves v 2b	<76.5%	+
	Dipeptidylpeptidase IV	Ves v 3	50.0%	+
	Antigen 5	Ves v 5	<100%	–
	Vitellogenin	Ves v 6	?	?

Major allergens are bold.

the serum samples of insect venom allergic patients IgE to CCD was detected. It is interesting that 62% of the venom allergic patients with CCD-specific IgE are not atopic [8]. The natural exposure by a sting can lead to a temporary increase in CCD-specific IgE, whereas a comparable increase of CCD-specific IgE is not induced by seasonal exposure to CCD containing pollen allergens [9]. This indicates a stronger CCD sensitization potential for hymenoptera venoms compared with pollen.

**FIGURE 1.** Examples of plant and insect venom N-glycans.

INCIDENCE AND CLINICAL RELEVANCE OF CROSS REACTIVE CARBOHYDRATE DETERMINANTS SPECIFIC IMMUNOGLOBULIN E ANTIBODIES

The importance of CCD epitopes for cross reactions between food, pollen and Hymenoptera venom was first described by Aalberse in 1981 [10].

In 23% of 1831 serum samples of patients with a suspected allergic respiratory disease, IgE antibodies to bromelain were detected [11]. The prevalence was 5% in nonallergic individuals and 71% in patients with prick test reactivity to five or more different pollen extracts. Prick tests with several CCD epitope bearing molecules confirmed a low biological activity of anti-CCD IgE antibodies.

Up to 59% of the patients with Hymenoptera venom allergy reveal positive results to more than one venom. More than the half of them (56%) had specific IgE antibodies to CCDs, with a higher prevalence seen in bee rather than in wasp venom allergic patients [12].

Although plant glycoproteins carry fucose and xylose as main side-chains, insect venom carries only fucose. The prevalence of positive specific IgE determination to pollen allergens due to anti-CCD IgE antibodies induced by insect stings was analyzed in 259 hymenoptera venom allergic patients. Only 16% had anti-CCD antibodies cross reacting with different types of pollen [9].

The prevalence of CCD sensitization is associated with alcohol consumption [13–15]. In sera of 11 of 20 heavy drinkers wine protein binding IgE antibodies were detected. By immunoblot analyses and inhibition tests it was shown that these antibodies are directed to CCD epitopes. Skin prick tests (SPT) to bromelain were negative in all patients, whereas only one had a positive SPT to horseradish peroxidase. In basophil activation test (BAT) basophils of the patients could be activated by CCD bearing allergens, but none of these patients reported symptoms of hypersensitivity to insect stings, food or wine [15].

Low binding affinity was felt to be the reason for the lack of clinical significance for these antibodies but affinity binding analysis showed this to be incorrect [16]. Instead high affinity IgG antibodies seem to act as blocking antibodies and impede the clinical activity of anti-CCD IgE. The affinity is more important than the relative amount of these CCD-specific antibodies. A suggested mechanism explaining the clinical insignificance of CCDs is a comparably high IgG affinity and a not too high titer of CCD-IgE.

The theory of tolerance to CCD was confirmed by studying serum samples from bee keepers; here CCD specific IgE antibodies were present in only 7.7%. It was discussed that the high venom exposure due to repeated bee stings induces tolerance to CCD indicated by anti-CCD IgG antibodies in most of these serum samples [17].

BAT with bee/wasp venom in monosensitized hymenoptera venom patients were positive with both venoms in 67% of those patients having anti-CCD IgE antibodies. Using deglycosylated venoms the BAT of these patients was normally positive with only one of the venoms [18]. Therefore, a biological activity of anti-CCD IgE antibodies was obvious, but the study suggested that these antibodies are clinically irrelevant.

The clinical irrelevance of IgE-antibodies to plant glycans was proved by double blind oral provocation tests with lactoferrin (glycan chains of this glycoprotein are similar to those found in horse radish peroxidase) [19]. All five patients with IgE to glycans tolerated up to 1 g of the glycoprotein without any subjective or objective adverse reaction. Currently, it is thought that antibodies directed to protein epitopes are usually clinically relevant, whereas IgE to CCD epitopes of N-glycans have no clinical relevance for insect venom sensitized patients.

Altmann proposed the model of 'natural glyco-immune therapy'. This means that everybody is exposed to plant glycoproteins by ingestion and inhalation probably every day inducing a common tolerance to CCDs [7].

IMPACT OF IMMUNOGLOBULIN E AND CROSS REACTIVE CARBOHYDRATE DETERMINANT ANTIBODIES FOR THE IN-VITRO DIAGNOSIS OF INSECT VENOM ALLERGY

IgE antibodies reacting with bee and wasp venom could be detected in about 30–50% of patients with insect venom allergy. The serologic double sensitivity could be due to the following:

- (1) double sensitivity to both venoms as a result of bee and wasp stings
- (2) cross-reactive IgE antibodies against Hyaluronidase or dipeptidylpeptidase [20,21]
- (3) cross-reactive IgE antibodies against CCD [22,23].

Although double sensitized patients may need VIT with both insect venoms, in the case of cross reactivity, patients will be treated only with the venom of the insect, which elicited the clinical reaction. In 76 of 121 serum samples from patients with systemic insect sting reaction, IgE antibodies to bee and wasp venom extracts were detected [24²²]. Double reactivity was caused by anti-CCD IgE antibodies (MUXF3) in 54%; the rest were double sensitized.

These results are in accordance with other clinical trials demonstrating that IgE antibodies to CCD are most frequently responsible for serologically double positivity. CCD specific IgE antibodies are not biologically inactive; the activity of these antibodies was demonstrated by BATs. Compared with proteins the CCD-induced activation of basophils was low. This can indicate a correlation between the stimulatory potency of allergens in the BAT with their biological activity *in vivo* [18,25²³].

CONCLUSION

IgE to CCD causes the majority of serological double positivity, which hampers an unambiguous interpretation of in-vitro test results based on the determination of specific IgE antibodies. Nevertheless, for a clinical decision it is mandatory to distinguish between double sensitivity and cross reactivity. The introduction of CRD solves this problem if proteins devoid of CCD epitopes are used for in-vitro tests.

It should be kept in mind that clinical irrelevance anti-CCD IgE is still a matter of debate; it cannot be excluded that in some individuals CCDs induce clinical symptoms. Interestingly, the responsibility of another carbohydrate moiety (galactose- α -1,3-galactose) for severe anaphylactic reactions was recently demonstrated [26].

Although this is a completely different type of CCD, it emphasizes the necessity to explore the importance of carbohydrate epitopes in allergy.

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Conflicts of interest

There are no conflicts of interest for this work.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 453–454).

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