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Cross-reactivity of IgE antibodies to caddis fly with arthropoda and mollusca

V. L. Koshte, BSc, S. L. Kagen, MD,* and R. C. Aalberse, PhD

Amsterdam, The Netherlands, and Appleton, Wis.

We investigated the possibility that subjects with IgE antibodies to an inhalant insect allergen, such as caddis fly, might also have antibodies to cross-reacting carbohydrate determinants (CCDs). IgE antibodies to cross-reacting allergens in caddis flies, mussels, oysters, shrimps, crabs, honeybee, and yellow jacket venoms were determined by RAST, RAST inhibition, and immunoblot studies with sera from three different sources: (1) sera of patients with well-defined inhalant atopy to caddis fly, (2) sera with IgE anti-CCD antibodies from subjects without known exposure to caddis fly, and (3) hyperimmune antisera with IgG anti-CCD antibodies raised as a result of immunization of rabbits with grass-pollen extract, buckwheat glycoprotein, or with honeybee venom. Sera from groups 2 and 3 reacted with Sepharose-coupled caddis fly extract in a RAST-type assay and elicited virtually identical patterns on immunoblots of caddis fly extract separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, whereas the sera from group 1 atopic patients did not react with CCD-rich material. However, indications for other types of cross-reacting antibodies were detected. The IgE antibodies of one of the patients studied (who was allergic not only to caddis fly but also to shellfish) were found to detect a cross-reacting homologous protein in extracts of mussel, oyster, shrimp, crab, honeybee, and yellow jacket venom. Preliminary results suggest that this cross-reacting 13 kd protein, the most prominent caddis fly allergen, is an invertebrate hemoglobin (erythrocrucorin)-like molecule. These studies suggest the possibility that patients sensitized by exposure to caddis fly antigens could develop allergic reactions during their first exposure to shellfish or to their first bee sting. (J ALLERGY CLIN IMMUNOL 1989;84:174-83.)

Aquatic and terrestrial arthropods and mollusks are common causes of both inhalant and ingestant allergic disease. Inhalant allergy to caddis fly was one of the first well-documented causes of inflammatory airway

disease.¹ After initial studies by Parlato in 1929, subsequent clinical studies confirmed the prevalence of caddis fly atopy with symptoms varying from allergic conjunctivitis and rhinitis to life-threatening status asthmaticus. Allergy to various inhaled insect allergens has been known for several decades²⁻⁴ involving insects, such as caddis flies, cockroaches, moths, and chironomid larvae. A widespread IgE-mediated hypersensitivity of insect origin has been reported from Sudan,^{5, 6} Japan,^{7, 8} and the United States.⁹⁻¹¹

When we started our serologic investigations regarding the incidence of sensitization to nonstinging and nonbiting insects, we were surprised to find positive caddis fly RASTs with sera from patients without known exposure to caddis flies. We had found, previously,^{12, 13} that IgE antibodies in some human sera

From the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service and Laboratory for Clinical and Experimental Immunology, University of Amsterdam, Amsterdam, The Netherlands, and *Department of Medicine, College of Wisconsin, Appleton, Wis.

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Reprint requests: R. C. Aalberse, PhD, c/o Publication Secretariat, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, PO Box 9406, 1006 AK Amsterdam, The Netherlands.

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Abbreviations used

SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
CCD:	Cross-reacting carbohydrate determinant
MW:	Molecular weight
Con A:	Concanavalin A
PBS-T:	Phosphate-buffered saline with 0.05% Tween 20
kd:	Kilodalton
NC:	Nitrocellulose

reacted with a carbohydrate antigenic determinant on glycoproteins present in pollen, foods (e.g., grains, legumes, vegetables, and fruits), and in material of invertebrate origin (e.g., bee venom, vespid venom, shrimp, crab, or shellfish). This CCD was periodate sensitive, heat stable, and highly immunogenic in rabbits. In view of this unexpected cross-reactivity between foods and material of invertebrate origin, we decided to investigate the possibility of cross-reacting IgE antibodies in the caddis-fly system.

MATERIAL AND METHODS

Patients

Fi, Be, Br, Wi, Vi, Fr, and Gr are patients from the United States. They are all positive to caddis fly in skin tests as well as RAST. Three papermill workers, Fi, Be, and Br, were studied in detail.

Fi is a 25-year-old white man first evaluated in the intensive care unit during an episode of status asthmaticus at the time of a caddis fly hatch. On intradermal skin test at 10^{-3} (wt/vol) dilution of caddis fly extract, he reacted with a life-threatening anaphylactic reaction. Allergy investigation revealed a strongly positive scratch test to caddis fly extract as well as a positive reaction to ragweed and grass- and tree-pollen extracts. His intradermal skin tests were negative to midge and mold aeroallergens. Fi denied any history of shellfish-induced atopic symptoms.

Be is a 25-year-old white man with perennial symptoms of mild rhinitis and asthma. He experienced seasonal flares of asthma during caddis fly hatches. His intradermal skin test¹⁴ was 4+ (i.e., strongly positive) to caddis fly at 10^{-6} ; his skin tests for midge and mayfly were also positive; tests to ragweed, grass, and tree pollen were negative. He experienced pruritus of the mouth with shrimp exposure as his only food allergy.

Br is a 53-year-old white man experiencing seasonal asthma symptoms with caddis fly exposure. Intradermal skin test¹⁴ was 3+ at 10^{-4} (wt/vol) to caddis fly. Allergy investigation revealed positive scratch test to ragweed, tree pollens, *Dermatophagoides farinae*, and house dust. He also experienced angioedema of the lips and mouth with shrimp exposure.

Mu, Wo, Am, B1, B2, and B3 are sera of European origin. Mu, Wo, and Am were known to have a high titer

of IgE anti-CCD antibodies; for Wo, a relationship with a yellow jacket sting was probable; for Mu and Am, no such relationship could be established. A panel of 12 honeybee venom-positive sera was randomly selected from Hymenoptera-positive sera. When sera was tested in the caddis fly RAST, three of the 12 sera were positive. These sera were coded B1, B2, and B3.

Rabbit antisera

Rabbit antisera against grass pollen, buckwheat glycoproteins, and honeybee venom were prepared as described previously.¹²

Allergens and coupling to CNBr-activated Sepharose 4B

Caddis flies were collected in a light trap and killed with 70% alcohol in the vicinity of Appleton, Wis. A 10% (wt/vol) extract was prepared of these caddis flies in PBS. Five hundred microliters of this extract containing 200 μ g of protein was coupled to 100 mg of CNBr-activated Sepharose 4B. Mussels, oysters, shrimps, and crabs were obtained locally (Amsterdam), cleaned, and extracted uncooked at 10% (wt/vol) in water at pH 8, dialyzed, and lyophilized. Fifteen milligrams of the lyophilized extract was coupled to 300 mg of CNBr-activated Sepharose 4B. Four hundred milligrams of honeybee venom, grade IV, from Sigma Chemical Co., St. Louis, Mo., were filtered on a Sephadex G-75 column (2.4 by 45 cm); fractions were pooled until the first 10% of the mellitin peak was collected. Sixty milliliters of the pool was used for coupling to 4 gm of CNBr-activated Sepharose. The yellow jacket venom was prepared by extracting venom sacs. Fifty sacs were punctured in 0.1 mol/L of borate buffer at pH 8.3. This extract was then centrifuged, filtered, and coupled to 300 mg of CNBr-activated Sepharose. All allergen extracts were coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the manufacturers' instructions, apart from the inactivation step, which was performed with glycine rather than with ethanolamine.

RAST

Tests were performed by incubating 50 μ l of serum with 0.5 mg to 1.5 mg allergen-coupled Sepharose. After an overnight incubation, serum was washed and labeled with ¹²⁵I sheep antihuman IgE or sheep antirabbit in case of rabbit antisera. After this second incubation, the beads were washed again and measured for counts of bound radioactivity. The RAST scores are expressed after blank correction as percent bound of total radioactivity added.

RAST-inhibition assays were performed by first incubating the serum sample with an inhibitor allergen extract for 2½ hours followed by the RAST procedure mentioned earlier. The quantity of serum sample, inhibitor, and the allergosorbent were adjusted to elicit an uninhibited binding of 15% to 20% of the added radioactivity.

Periodate treatment

Periodate treatment of caddis fly extract was performed to inactivate by oxidation any possible presence of cross-

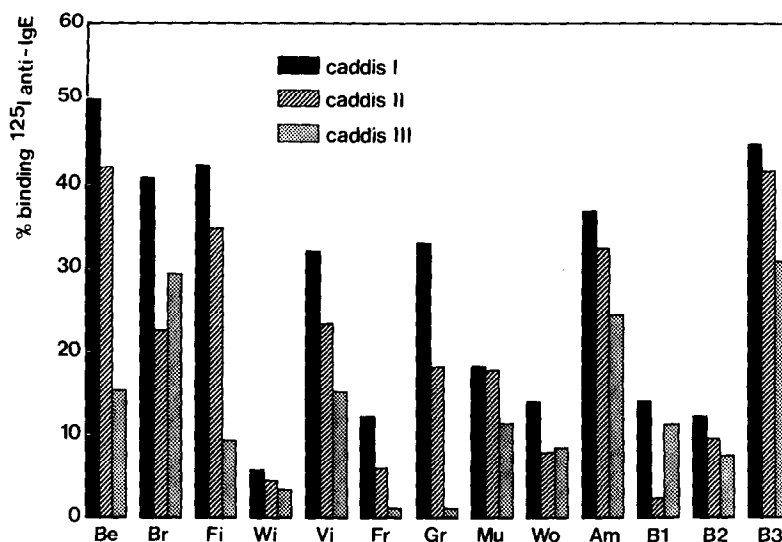


FIG. 1. RAST scores of 13 sera tested with three different caddis fly extracts. Results are expressed as percentage bound ^{125}I -labeled anti-IgE of the total added radioactivity.

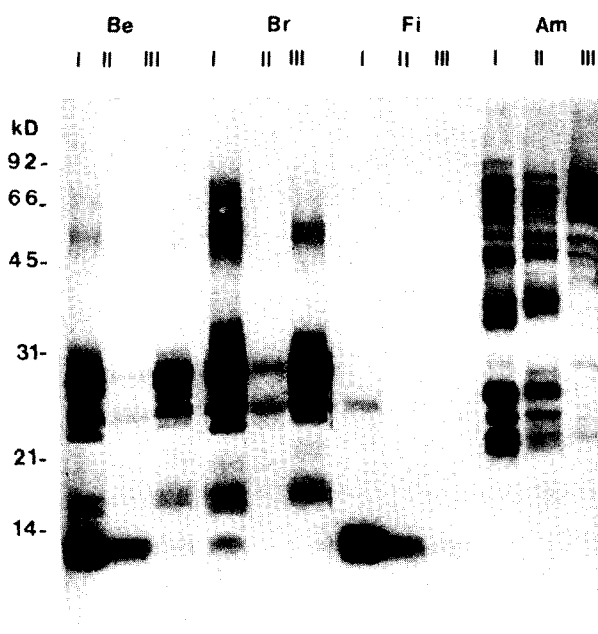


FIG. 2. Immunoblot of three caddis fly extracts: autoradiography of IgE-binding components as recognized by caddis fly-sensitized sera Be, Br, Fi, and anti-CCD IgE antibody containing serum Am. Position of the MW markers is denoted on the left.

reacting carbohydrate antigens, as described previously for other allergens.¹²

Affinity chromatography

Two absorbents were used to remove glycoprotein and carbohydrate antigens. The first absorbent, Con A Sepharose, was obtained from Pharmacia. The second absorbent, anti-CCD Sepharose, was prepared as follows: rabbit antiserum to buckwheat glycoproteins was absorbed onto

Sepharose-coupled potato extract, the antibodies bound to the potato Sepharose (anti-CCD antibodies) were eluted at pH 2.5 and neutralized, and 1.5 mg of anti-CCD antibody was coupled to 100 mg of CNBr-activated Sepharose. For depletion experiments, caddis fly extract (175 μl) was absorbed on 100 mg of Con A Sepharose or on anti-CCD Sepharose for 4 hours, after which the supernatants, that is, the depleted caddis fly extracts, were coupled to CNBr-activated Sepharose 4B.

Immunoblots

Immunoblots were prepared by electrophoresis of the allergen extracts in a 12.5% SDS/PAGE gel.¹⁵ After the electrophoresis, the proteins were electroblotted onto an NC membrane¹⁶ for at least 6 hours to ensure complete transfer of proteins to the NC membrane, and the membranes were washed in distilled water. The allergen-bound NC paper was then cut into strips of 5 mm, blocked two times by PBS-T for 20 minutes each step, followed by incubation overnight with 200 μl of serum sample, and 3.5 ml of PBS-T containing 0.1% bovine serum albumin. The strips were then washed three times with PBS-T for 10 minutes each and incubated again in the above-mentioned incubating buffer containing 4.5% bovine serum, 0.5% sheep serum, and 50,000 counts of ^{125}I -labeled sheep antihuman IgE or ^{125}I -labeled sheep antirabbit in case of rabbit antiserum. After an overnight incubation, the strips were washed three times with PBS-T for 10 minutes followed by a last rinse for 1 minute in distilled water and air-dried. The dry strips were then exposed to a Kodak X-ray film (Eastman Kodak Co., Rochester, N.Y.) in a cassette with an intensifying screen for 48 hours at -70°C .

RESULTS

Variability of different caddis fly extracts

The three different extracts of caddis flies were tested. Extract I was from a mixture of unidentified

TABLE I. Specificity of caddis fly extract in the RAST

Caddis fly (inhibitor)	A		B		C	
	Allergosorbent caddis fly		Allergosorbent <i>D. pteronyssinus</i>		Allergosorbent cat dander	
	-	+	-	+	-	+
Be	25.9	0.3				
Br	9.5	0.3				
Fi	13.9	0.2				
Wi	4.7	0.3				
Vi	8.0	0.7				
Fr	12.2	0.2				
Gr	12.3	0.1				
Mu	11.6	0.8				
Wo	5.7	1.0				
Am	16.7	0.6				
BV1	12.9	0.6				
BV2	13.1	1.8				
BV3	20.1	1.2				
Hr			36.1	34.8		
Wl			45.3	43.8		
Hs			35.0	34.1		
Ti					14.6	15.4
De					11.5	11.8

A, Caddis fly allergosorbent tested with caddis fly-positive sera; the RAST score against RAST score after inhibition by caddis fly extract. B, *D. pteronyssinus* allergosorbent tested with three *D. pteronyssinus*-positive sera; the RAST score against RAST score after inhibition by caddis fly extract. C, Cat-dander allergosorbent tested with two cat-dander-positive sera; the RAST score against RAST score after inhibition by caddis fly extract.

RAST scores are expressed as percent bound ^{125}I -labeled anti-IgE of the total added radioactivity. Concentration of the allergosorbent and the serum adjusted to make the assay sensitive to inhibition. All inhibitions performed with a quantity of extract containing 10 μg of protein.

caddis fly species, whereas the other two extracts were prepared from distinct, identified species. Marked quantitative as well as qualitative differences were found between these extracts when tested in RAST (Fig. 1) or in immunoblot analysis (Fig. 2). It is unclear as yet if these differences were indeed caused by species differences. All other tests were performed with extract I.

RAST and inhibition assays

We analyzed in the RAST three sera with IgE antibodies to caddis fly in some more detail, sera from patients Fi, Br, and Be, all three with an allergic reaction to caddis fly. None of these sera had IgE antibodies to CCD-rich material, such as grains, legumes, vegetables, and fruits. Fi had no IgE antibodies to shellfishes, crustacea, bee venom, and yellow jacket venom. Br had IgE antibodies to oyster, shrimp, crab, yellow jacket venom, and *D. pteronyssinus*. Be had all the previously mentioned IgE antibodies and was also positive to bee venom and to mussel. For comparison, we analyzed the CCD-

positive sera Mu, Wo, Am, and the venom-positive sera B1, B2, B3; these six sera were from patients with no known exposure to caddis fly and were found to be positive in caddis fly RAST.

We performed RAST inhibition assays with serum Be and Br by testing the inhibition of the caddis fly RAST with shrimp, oyster, mussel, crab, bee venom, yellow jacket venom, *D. pteronyssinus*, and vice versa, using caddis fly extract as the inhibitor. The caddis fly RAST of all the caddis fly-positive sera was completely inhibited by caddis fly extract, whereas neither the cat-dander RAST of two cat-allergic patients nor the *D. pteronyssinus* RAST for three mite-allergic patients was inhibited by the caddis fly extract (Table I). The caddis fly RAST of serum Be was not inhibited by other allergens. Most other RASTs for Be were significantly inhibited by caddis fly extract except bee venom RAST, and *D. pteronyssinus* RAST was inhibited only 12% and 27%, respectively. In case of serum Br, the caddis fly RAST was inhibited 15% by oyster and yellow jacket venom and 30% by shrimp and *D. pteronyssinus* extract; cad-

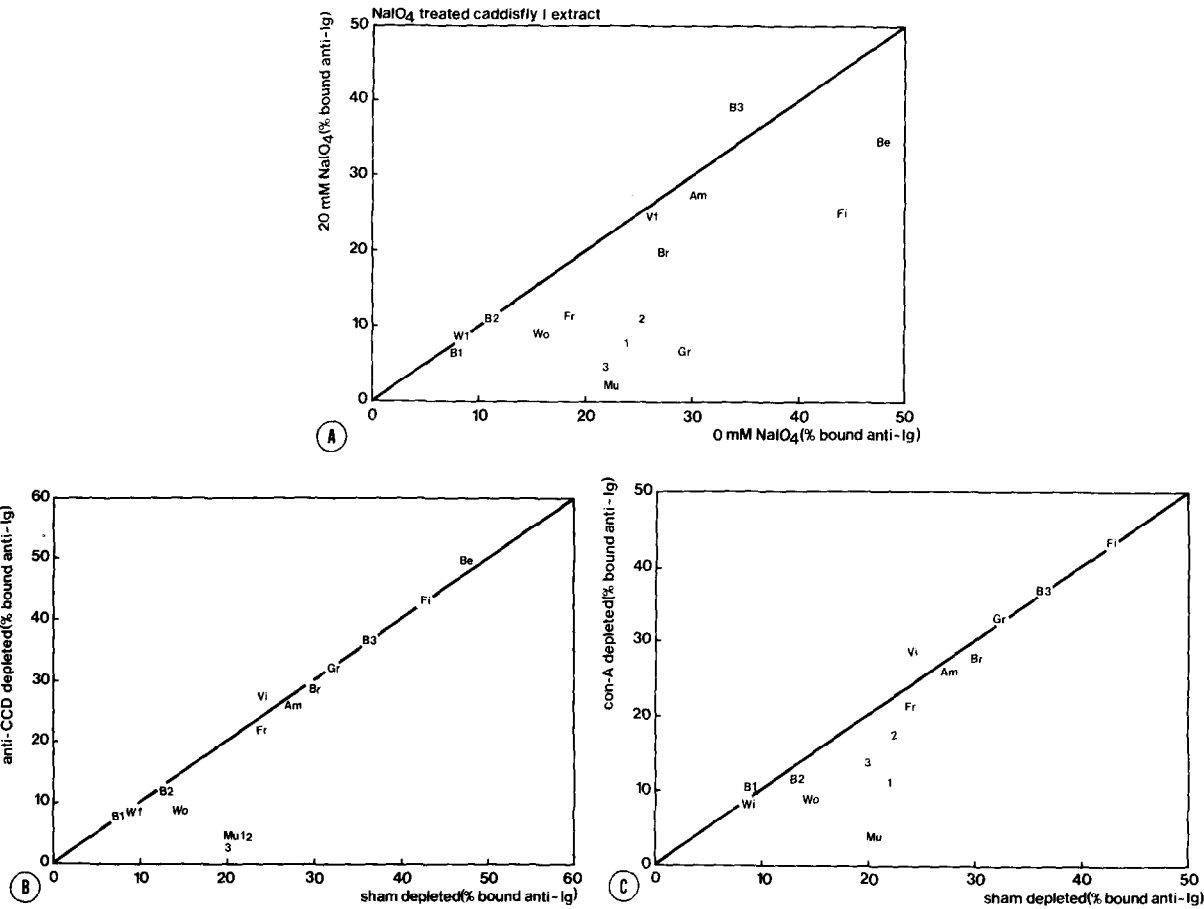


FIG. 3. Effect of CCD depletion. Correlation between RASTs with Sepharose-coupled treated or depleted caddis fly extract. **A.** Effect of periodate treatment. **B.** Effect of treatment with anti-CCD absorbent. **C.** Effect of treatment with Con A absorbent. Human sera (coded with letters) tested with ¹²⁵I-labeled anti-IgE; rabbit sera (coded with numbers: 1, antigrass pollen; 2, antibuckwheat glycoprotein; 3, antihoneybee venom) tested with ¹²⁵I-labeled antirabbit IgG. RAST results are expressed as percentage binding of total added radioactivity.

TABLE II. Inhibition assay of cross-reacting IgE antibodies of sera Be and Br

	Uninhibited RAST % bound anti-IgE	% Inhibition by caddis fly extract	% Inhibition of caddis fly RAST
Serum Be			
Shrimp	20	65	5
Oyster	6	83	2
Mussel	9	55	3
Crab	15	87	3
Bee venom	9	12	5
YJ venom	19	90	2
<i>D. pteronyssinus</i>	15	27	0
Serum Br			
Shrimp	26	27	30
Oyster	27	89	15
Crab	13	62	0
YJ venom	23	70	14
<i>D. pteronyssinus</i>	24	78	30

YJ, Yellow jacket.

Allergosorbent or extract (column 1) for Be and Br. The RAST score is expressed as percent bound ¹²⁵I-labeled I-anti-IgE of the total radioactivity added (column 2); percent inhibition of the RAST after incubation with caddis fly extract containing 10 µg of protein (column 3) and percent inhibition obtained of the caddis fly RAST after incubation with 100 µg of lyophilized extract (column 4). Concentration of the allergosorbent and the serum is adjusted to make the assay sensitive to inhibition.

dis fly was an efficient inhibitor in all RASTs, except the shrimp RAST in which only 27% inhibition was obtained (Table II).

Rabbit antisera against grass pollen, buckwheat glycoproteins, and bee venom also elicited binding in RAST-type assays. In these tests, caddis fly extract also proved to be as effective an inhibitor as grass-pollen extract. In the bee venom RAST of rabbit anti-grass pollen, 50% inhibition was obtained with 47 μ l of caddis fly extract per test; in the grass-pollen RAST of rabbit antivee venom, 50% inhibition was obtained with 6.9 μ l of caddis fly extract per test (data not presented). When we compared the inhibitory activity of caddis fly extract with that of dialyzed and lyophilized grass-pollen extract, the caddis fly extract was equivalent to 365 or 334 μ g/ml of grass-pollen extract in the bee venom RAST and in the grass-pollen RAST, respectively. This would indicate that on a raw material basis, the 10% (wt/vol) caddis fly extract is approximately equipotent in these assays with a 1% (wt/vol) grass-pollen extract.

Periodate treatment and affinity chromatography

The caddis fly-sensitized patients Fi, Be, Br, Wi, Vi, and Fr retained >70% of its RAST activity on periodate-treated caddis fly extract (Fig. 3, A); an exception was serum Gr that lost >70% of its RAST activity. Mu and Wo (two anti-CCD sera) lost 80% and 50% respectively, of the binding activities. Unexpectedly, the activity of serum Am was unaffected by periodate treatment of the antigen. The bee-venom, venom-positive sera B1, B2, and B3 behaved more like sera from caddis fly-sensitive patients than sera of CCD-positive patients because it remained reactive with periodate-treated caddis fly extract. Rabbit antisera to bee venom, buckwheat glycoprotein, and grass pollen lost approximately 75% of the binding reactivity when these were tested on periodate-treated caddis fly extract (Fig. 3, A). The results with anti-CCD-depleted (Fig. 3, B) and Con A-depleted (Fig. 3, C) caddis fly extract were largely similar to the results obtained with periodate treatment of the extract. For removal of CCD activity, periodate proved to be less specific than depletion with anti-CCD antibodies (compare serum Fi and Gr in Fig. 3, A and B), whereas depletion with Con A Sepharose was less efficient (compare the rabbit antisera in Fig. 3, B and C).

Profiles on the immunoblot

We found that the three sera from patients with known caddis fly allergy elicited different profiles when sera were tested on immunoblots for IgE antibodies (Fig. 4). Serum Fi was found to have specific antibodies to caddis fly reacting with a 13 kd com-

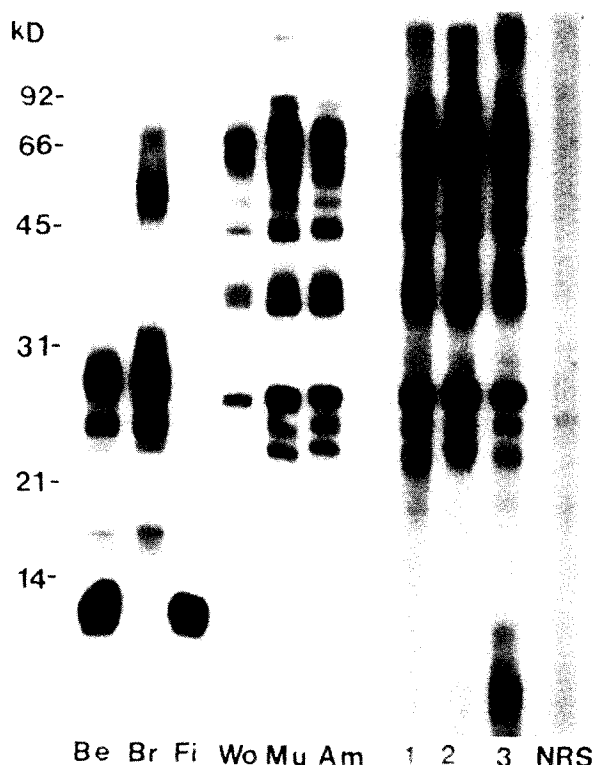


FIG. 4. Immunoblot of caddis fly extract I. Autoradiography demonstrating IgE binding components in three caddis fly-sensitive patients, Be, Br, and Fi, and in three sera, Wo, Mu, and Am, with IgE antibodies to CCD. For comparison, three rabbit antisera with IgG antibodies to CCD are presented: 1, antigress pollen; 2, antibuckwheat glycoprotein; 3, antivee venom, and *NRS*, normal rabbit serum. The position of the MW markers is denoted on the left.

ponent. The second serum, Be, had antibodies reactive at the same location of 13 kd, but also had distinct antibodies in the 25 to 35 kd range. This serum was found to contain IgE antibodies that reacted with a 13 kd range polypeptide, also found in other extracts of invertebrate origin, that is, in Hymenoptera, crabs, shrimps, mussels, and oysters (Fig. 5). On a bee venom immunoblot, only Be bound IgE antibodies to 13 kd molecules, whereas none of 14 sera from bee venom-positive subjects had binding at this location (Fig. 6). The third serum, Br, had almost no antibody reactive at the 13 kd location. It was also reactive in the intermediate MW range from 25 to 40 kd. In addition, we found distinct IgE binding in the 60 to 75 kd area. This serum also had IgE antibodies to other invertebrate allergens comparable to serum Be, but not to bee venom or mussel. When the serum was tested on immunoblots, the predominant reactivity with these other invertebrates was found not in the 13.5 kd range but in the higher MW range. When serum Be and serum Br were preincubated with caddis fly extract followed by testing on the previously men-

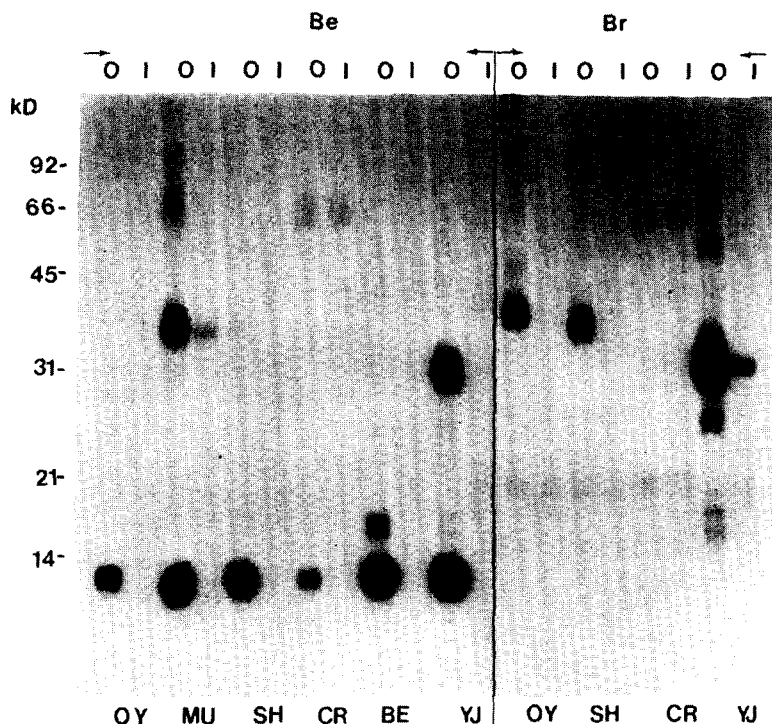


FIG. 5. Inhibition assay of IgE antibodies by caddis fly extract I on immunoblots; oyster (OY), mussel (MU), shrimp (SH), crab (CR), honeybee venom (BE), and yellow jacket venom (YJ) for serum Be and Br. O, uninhibited; I, inhibited. MW markers are indicated on the left.

tioned allergen immunoblots, almost all IgE binding was abolished (Fig. 5).

When the IgG antibodies of rabbit antisera were tested on immunoblots, the antibodies mimicked the binding pattern of the three human sera Wo, Mu, and Am with anti-CCD IgE antibodies; these six sera elicited identical patterns (Fig. 4). In three other caddis fly RAST-positive human sera with IgE antibodies to bee-wasp venoms, B1 bound IgE in the 20 to 23 kD and 30 to 40 kD area, B2 bound IgE to the whole of the NC strip without any distinct band, and B3 bound IgE antibodies in the 50 to 60 kD area very vaguely (not presented). The important point is that the immunoblot pattern of these sera was distinctly different from that of Fi, Be, and Br. Particularly noteworthy is that none of these patients had IgE antibodies reactive in the 13.5 kD range.

DISCUSSION

The problem of cross-reactions between taxonomically unrelated species appears to be more diverse than we originally assumed. When we described the periodate-susceptible CCD structures on a wide variety of glycoproteins of vegetable or invertebrate origin,^{12,13} it appeared to us at the time that this was the main, if this were not the only, structure respon-

sible for unexpected cross-reactivities demonstrable with human IgE antibodies. Based on data at that time, we suggested that IgE antibodies to these periodate-susceptible determinants were likely to be of no clinical relevance.

Evidence available now indicates that these two assumptions may have to be reassessed. CCD structures prove to be only one of several possible sources of unexpected cross-reactions. Moreover, anti-CCD antibodies do not all have an identical specificity. With respect to the clinical relevance of periodate-susceptible determinants, the investigations in the sea squirt system are of interest.¹⁷ From these studies, it is evident that at least some periodate-susceptible determinants are active in the skin test. We do not know as yet whether the periodate-susceptible determinants in the sea squirt system are reactive with anti-CCD antibodies.

From the data presented in this article, it is evident that caddis fly extract itself contains various allergenic components, including CCD structures (Fig. 2) and that these CCDs may elicit positive reactions in the RAST. To distinguish antibodies to the caddis fly CCDs from antibodies to other caddis fly epitopes, immunoblots were found to be very helpful. The pattern obtained with human IgE anti-CCD antibodies

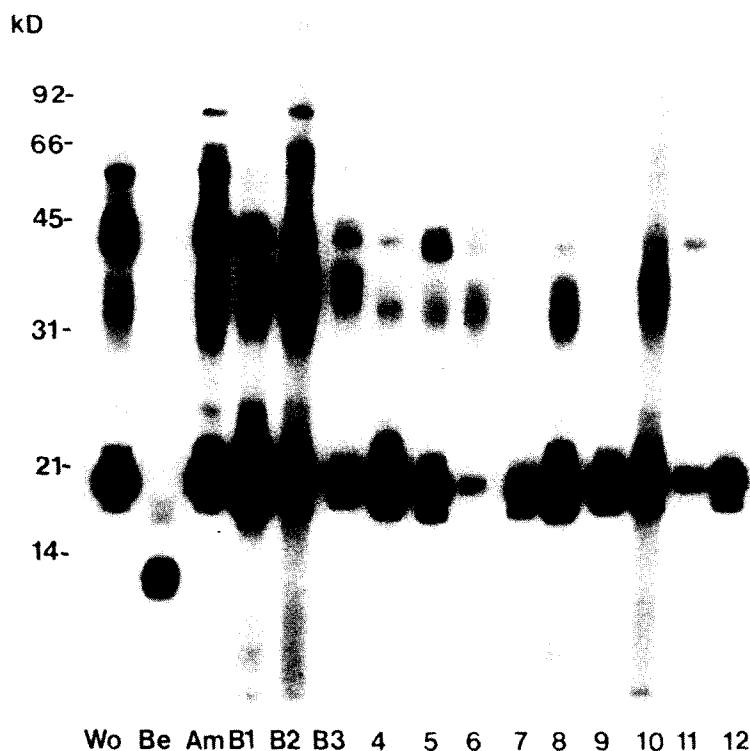


FIG. 6. Immunoblot of honeybee venom. Autoradiography demonstrating IgE binding components. Wo and Am are sera with IgE antibodies to CCD. Be is the caddis fly-positive serum with the cross-reacting IgE antibodies. B1, B2, B3, and 4 to 12 are the bee venom-positive patients. Note the exceptional pattern of serum Be.

was strikingly similar to the pattern obtained with rabbit IgG anti-CCD antibodies found in serum from rabbits immunized with diverse antigen sources (Fig. 4).

With respect to the other criteria for anti-CCD antibodies, we encountered problems with some sera, notably with serum Am. When we compare the reaction pattern of this serum with a "well-behaving" serum like Mu, we found marked differences both with respect to the effect of periodate treatment of the antigen (Fig. 3, A) and to the effect of depletion of the extract with Sepharose-coupled anti-CCD antibodies (Fig. 3, B) or with Sepharose-coupled ConA (Fig. 3, C). The conclusion is inescapable that serum Am contains different antibodies to caddis fly, presumably in addition to anti-CCD antibodies, and yet the blot patterns of these two sera are virtually identical (Fig. 4). One possible explanation for this discrepancy is that the additional antibodies in serum Am are directed against a component that does not react well in the SDS-immunoblot system used.

We now have clear evidence that the unexpected cross-reactions of IgE antibodies to invertebrate or vegetable antigens are not all due to anti-CCD antibodies. Three cross-reactive structures were found

in birch pollen.¹⁸ Apart from the CCD-type cross-reactions, two other types of unexpected cross-reactions were identified. One of these structures is responsible for the cross-reactivity between birch pollen, apple, and stony fruits; this cross-reacting epitope was located on a 20 kD protein. The other cross-reacting structure, responsible for the cross-reactions between grass pollen, potato, carrots, and other vegetables, was located on an 18 kD molecule.

The specificity of caddis fly extract in the RAST was established in three ways:

1. Most sera with IgE titer of >1000 IU/ml were negative in the caddis fly RAST. A serum from a patient with microfilaria with an IgE level of 50,000 IU/ml was tested at a dilution of 1:10 and was found to be negative.
2. Fi, Wi, Fr, and Gr did not have IgE antibodies to grains, legumes, vegetables, fruits, or invertebrates other than insects. This indicates that the caddis fly RAST of these sera is not due to IgE antibodies to CCD-type structures.
3. The caddis fly RAST of all the caddis fly positive sera was completely inhibited by caddis fly extract. This inhibition was specific because caddis fly ex-

tract did not inhibit the cat-dander RAST of sera from cat-allergic patients or the *D. pteronyssinus* RAST of sera from mite-allergic patients (Table I).

It might appear surprising that the RAST inhibition between caddis fly and, for example, oyster, is not fully reciprocal. However, we assume that in serum Be or Br, only a fraction of the antibodies to caddis fly is cross-reactive with oyster, and the positive RAST to oyster is due to cross-reacting IgE antibodies. In this situation, one would expect caddis-fly extract to inhibit the oyster RAST, whereas the caddis-fly RAST would be inhibited only slightly by oyster extract. As is indicated by the results presented in Table I, caddis fly extract does not elicit nonspecific inhibition.

The most prominent antigenic structure in caddis fly extract is a molecule migrating in SDS-PAGE at 13 kd. It exhibits both specific epitopes (serum Fi, with no reactions to other invertebrate) but also cross-reactive epitopes (serum Be). It is likely that this molecule is to some extent susceptible to treatment with periodate. This is illustrated by the results with serum Fi, which has a low but significant loss of reactivity in the RAST on periodate treatment of the extract. When serum Be was tested on a honeybee-venom immunoblot, along with Wo, Am, and 12 venom-positive sera (Fig. 6), only with serum Be did we find IgE antibodies in the 13 kd range. Similar results were found with immunoblots of yellow jacket venom, shrimp, crab, mussel, and oyster.

One of the many proteins in this MW range that might be involved is the invertebrate hemoglobin (erythrocrurin) molecule. From the work of Baur et al.,^{19, 20} it is known that this is a potent allergen involved in sensitization to chironomid larval material. It is generally assumed that among the insects only chironomid larvae contain appreciable quantities of erythrocrurins. The gene involved in expressing erythrocrurins in chironimids is present in *phylum arthropoda* and *mollusca*, but it does not appear to be expressed to any extent.²¹ However, preliminary measurements of the differential spectrum of oxygenated and reduced caddis fly extract with a diode-array spectrophotometer indicated the presence of an oxygen-binding component, spectroscopically similar to hemoglobin.

It is not yet completely clear to what extent these cross-reactive determinants cause cross sensitization, although the diagnostic implications are obvious. From the data presented here, we conclude that, as with other allergens, the results of IgE antibody assays with caddis fly extract should be interpreted with care. A positive result of such a test does not necessarily indicate that the patient is exposed to caddis fly and

even less that the patient's symptoms are caused by these insects. Symptoms caused by inhalation of caddis fly allergens do occur, but the diagnosis of inhalant insect allergy requires more than an IgE-antibody assay.

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Asthma from childhood to adulthood—A follow-up study of 20 subjects with special reference to work capacity and pulmonary gas exchange

S. Friberg, MD,* S. Bevegård, MD,** V. Graff-Lonnevig, MD,* and
I. Hallbäck, technician** Stockholm, Sweden

*Twenty men with a mean age of 24.9 years, who had moderate to severe asthma during childhood, underwent a follow-up examination of their physical fitness, working capacity, maximal oxygen uptake, and pulmonary function during and after physical work. Comparison was made with similar data obtained at the age of 10.9 years. The young men had a normal physical working capacity with a normal oxygen uptake of 3.65 ± 0.56 L/min. The respiratory parameters and the arterial blood gases during work demonstrated values as in healthy individuals, and no differences between groups with mild and severe asthma during childhood were observed. The alveolar-arterial oxygen difference was normalized, compared to that at 10.9 years. Only a few subjects still had exercise-induced asthma with a postexercise fall of $>20\%$ in FEV₁ compared to childhood. The mean FEV₁ values for the whole group after exercise were lowered and had not returned to the baseline level even after 20 minutes. Oxygen pressure (tension), arterial, measured during 20 minutes after work, demonstrated continuously decreasing values. This might reflect a persisting disturbance in the pulmonary ventilation perfusion relationship caused by the underlying asthma disease. (*J ALLERGY CLIN IMMUNOL* 1989;84:183-90.)*

The natural course of bronchial asthma from childhood to adulthood has been the subject of several prospective studies.¹⁻⁷ These studies deal mainly with the clinical course of asthma; only a few studies include data on pulmonary function and exercise tests.^{6,7}

The relation between physical activity and EIA has

Abbreviations used

EIA:	Exercise-induced asthma
$\dot{V}O_2$:	Volume of oxygen used
PaO ₂ :	Partial pressure of oxygen, arterial
PaCO ₂ :	Partial pressure of carbon dioxide, arterial
HR:	Heart rate
A-a:	Alveolar-arterial

From *Sachs' Children's Hospital and the **Department of Clinical Physiology, Karolinska Institute, Söder Hospital, Stockholm, Sweden.

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Reprint requests: Sten Friberg, MD, Sachs' Children's Hospital, S-116 69 Stockholm, Sweden.

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been thoroughly investigated during childhood, but there is no information on how children with asthma develop up to adult age in this respect. A few authors have reported retrospective studies on the frequency of EIA in adults who had asthma during childhood⁸⁻¹⁰ but there are no data on changes in the working capacity, $\dot{V}O_{2max}$ and EIA from childhood to adulthood.