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Identification of a German Cockroach-Specific Allergen by Human IgE and Rabbit IgG¹

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Abstract. To further study the immunology of insect hypersensitivity, we identified and partially characterized the principal allergens in whole body German cockroach (WBGCR) (*Blattella germanica*) and compared this extract to whole body antigens prepared from other insects. WBGCR extract was fractionated over a calibrated Sephadex G-200 column; peak allergenic activity was contained in fraction 3 (GCR3), containing components with apparent molecular weights ranging from 12,500 to 75,000 daltons. The antigenicity, allergenicity, and specificity of GCR3 components were tested by using rabbit antisera raised to GCR3 or true armyworm (*Pseudaletia unipuncta*). Radioimmunoassay, cross-inhibition and immunoblot studies revealed, particularly in the IgE system, that GCR3 contained an allergen with a pI of 6.7 and MW of 36,000 daltons that was unique to WBGCR extracts and not present in other insect species, including true armyworm, caddis fly, lakefly, yellow jacket, or honeybee. This GCR3 component may represent a specific marker for the diagnosis of cockroach hypersensitivity in an insect-sensitive population of individuals.

Introduction

Cockroach hypersensitivity has recently become of increasing importance in allergic diseases, especially asthma [1-5]. Almost half of economically disadvantaged patients in large urban areas of the United States give positive skin tests and bronchial provocation tests to cockroach extract [3, 6, 7]. One report indicated that cockroach hypersensitivity was even more prevalent than ragweed hypersensitivity [8]. Cockroaches, dead and/or alive, may infest houses for long periods, depending in part on the geographic area and on the hygienic status of the house. Richman et al. [1] found that most of the allergenic activity of the German cockroach was extractable from whole body and cast skins.

Using sera from cockroach-sensitive humans and hyperimmune sera from rabbits immunized with an allergenic fraction of German cockroach (GCR3), *Blattella germanica* (order: Dictyoptera), we have at-

tempted to identify and characterize the principal cockroach allergens and antigens. We further studied whether cockroach allergens and antigens were present in extracts of other insect orders. The German cockroach was specifically chosen for the study because it is one of the most widespread, not only in the United States, but in the world, and it is also highly resistant to several insecticides [9, 10]. Its immunogenic potential is greater than that of American or Oriental cockroach, as well [1, 10].

Materials and Methods

Insect Extracts

Frozen cockroaches were supplied by Hollister-Stier Laboratories (Spokane, Wash.). Whole insects were snap frozen in liquid nitrogen, lyophilized, defatted for 4 h with ether in a Soxhlet extractor, and dried at room temperature. Defatted cockroaches were weighed, crushed in a mortar and pestle, and extracted (1:10, w:v) in 200 mM ammonium bicarbonate. The whole body German cockroach (WBGCR) extract was clarified by centrifugation and dialyzed against 50 mM ammonium bicarbonate using nominal 3,500 MW cutoff casing (Spectrum Medical Industries, Inc., Los Angeles, Calif.). The dialyzed extract was again clarified by centrifugation and divided into two aliquots. To one was added an equal

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volume of glycerine, while the other was lyophilized. The extracts were then stored at -20°C .

An extract of true armyworm (TAW), *Pseudaletia unipuncta* (Haworth) (order: Lepidoptera), was prepared in a similar way, using insects kindly provided by Dr. William Showers (Research Entomologist, Agricultural Research Services, Ankeny, Iowa). 'Lake-flies' (LF) (mayflies; order: Ephemeroptera) were collected locally during a mid-summer swarm and an extract prepared from whole insects in a similar fashion to that for WBGCR. Powdered whole body caddis fly (CF) (order: Trichoptera), yellow jacket (YJ) and honeybee (HB) (order: Hymenoptera) were purchased from Hollister-Stier Laboratories and extracted in 200 mM ammonium bicarbonate in the following w:v ratios: HB (1:20), YJ (1:10), CF (1:10).

Fractionation of Cockroach Extract

Gel filtration was performed at 4°C on a calibrated 2.5×100 cm Sephadex G-200 column equilibrated and eluted with 200 mM ammonium bicarbonate at 15 ml/h. Eight separate applications (5–10 mg each) of lyophilized WBGCR extract were fractionated on the column. Five-milliliter aliquots were monitored for spectrophotometric absorbance at 280 nm and pooled into four separate fractions based on elution profiles. The protein concentration of each pooled cockroach fraction was determined by the method of Lowry et al. [11].

Rabbit Antibodies

Six rabbits each were immunized intradermally with 25 $\mu\text{g}/\text{kg}$ of either German cockroach fraction 3 (GCR3), the cockroach fraction with the highest allergen activity, or with crude TAW, with booster injections at 4, 6 and 10 weeks. Antigens were administered in complete Freund's adjuvant for the first injection, then incomplete Freund's adjuvant subsequently. The sera were harvested at the 12th week.

IgG antibodies to GCR3 and TAW were isolated at pH 7.8 by precipitation in 35% saturated ammonium sulfate. The precipitated immunoglobulins were taken up to their original volume in phosphate-buffered saline (PBS) (pH 7.4), dialyzed against additional PBS, and clarified by centrifugation.

Human IgE Antibodies

A human serum pool containing elevated specific IgE for cockroach was prepared using sera from 12 urban patients who were cockroach-sensitive, as determined by both radioallergosorbent test (RAST) assay and skin testing (sera kindly supplied by Dr. Bann Kang, University of Kentucky, Lexington, Ky.). A similar pool was made with sera from 2 individuals having summer and fall rhinitis who were screened by RAST for elevated IgE to TAW and who had no history of known cockroach exposure. One of these patients experienced rhinitis on exposure to seasonal swarms of chironomids near Lake Winnebago, Wisc.

Preparation of Solid-Phase Allergens

Solid-phase allergens were prepared by adsorbing WBGCR, GCR3, or TAW extracts (0.1 ml) to Immulon-2 microtiter strips (Dynatech Laboratories, Inc., Alexandria, Va.). By checkerboard titration, optimal allergen adsorption was determined to be 2.6 μg of protein in 100 mM carbonate buffer, pH 9.2. The plates were incubated overnight at room temperature and washed three times with RAST buffer (200 mM phosphate buffer, 1% Tween 20, pH 7.4).

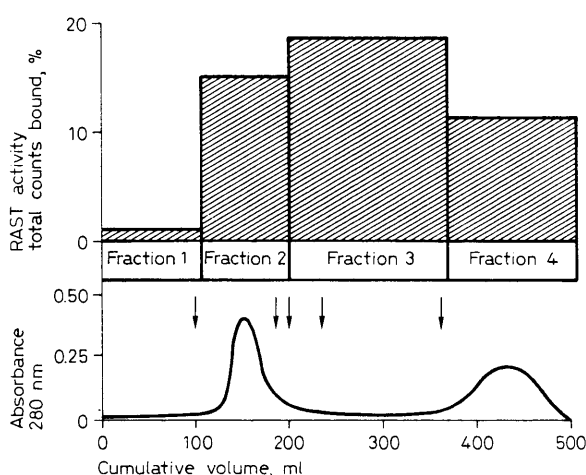


Fig. 1. Gel filtration of WBGCR extract on a calibrated Sephadex G-200 column. Shaded bars represent the percent counts bound in direct RAST assay using each pooled fraction (fractions 1–4) as a solid-phase and pooled sera from cockroach-sensitive persons. Serum from a nonsensitive control individual produced RAST binding of $<0.2\%$ with each fraction. The vertical arrows indicate elution volumes of protein markers of varying molecular weights, including from left to right, blue dextran (MW 2,000,000), ferritin (MW 460,000), catalase (MW 235,000), bovine serum albumin (BSA) (MW 66,000), and cytochrome C (MW 12,400).

Measurement of Allergenic Activity

Allergenic activity was measured by RAST inhibition assays in which various quantities of cockroach (WBGCR, GCR3) or TAW extracts were incubated in allergen-coated plates along with either human IgE antibody pool (undiluted) or rabbit IgG antibody preparation (1:100). The plates were incubated overnight in a humid chamber, washed thrice with RAST buffer, then incubated with 20 ng of either ^{125}I -antihuman IgE or ^{125}I -protein A. The plates were further incubated at room temperature overnight, washed as before, and counted in a gamma scintillation counter. Assay results were analyzed by regression analysis (percent inhibition vs. log inhibitor protein added) using a Hewlett-Packard 9845B computer (Hewlett-Packard Co., Palo Alto, Calif.).

Affinity Chromatography

To render the rabbit IgG anti-GCR3 specific for cockroach antigens, it was further purified by affinity chromatography. A mixture of 1.0 ml each of whole body insect extracts including TAW, CF, two separate preparations of LF (1:10, w:v) and HB (1:20, w:v) in 200 mM ammonium bicarbonate (total volume, 5.0 ml) was reacted with 5.0 ml CNBr-activated Sepharose 4B, which was then washed exhaustively with 200 mM ammonium bicarbonate to remove unbound antigens. The rabbit IgG anti-GCR3 antiserum was sequentially absorbed with three fresh aliquots of whole body insect-Sepharose, and the absorbed IgG antiserum in turn was coupled to activated Sepharose 4B (30 mg IgG/5 packed gel). WBGCR

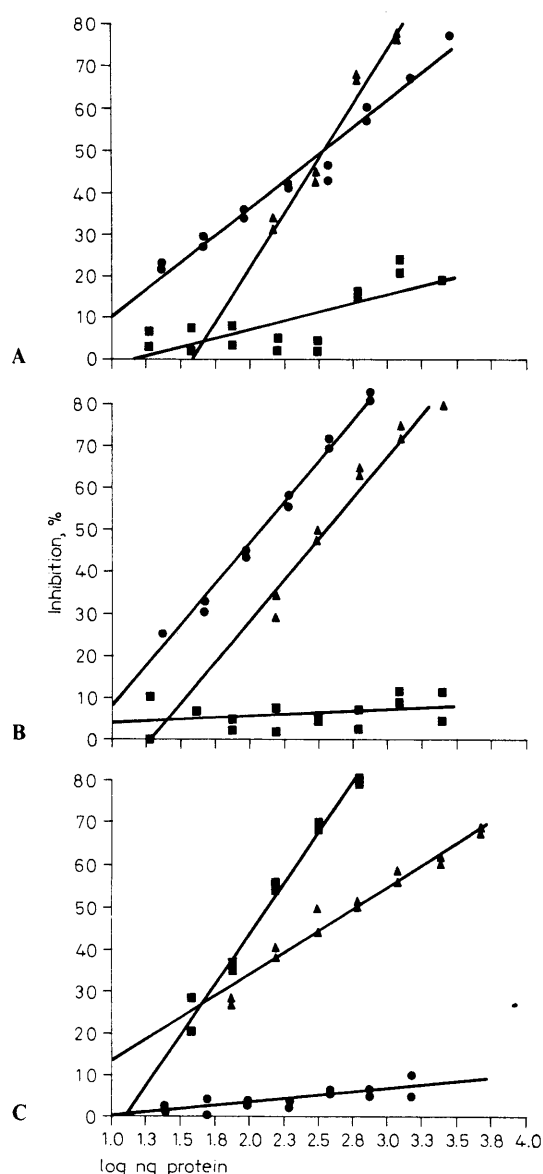


Fig. 2. RAST inhibition results using: (A) WBGCR; (B) GCR3, and (C) TAW as the solid-phase allergens, cockroach-sensitive human IgE serum pool and various fluid-phase inhibitors: ● = GCR3; ▲ = WBGCR; ■ = TAW. In the absence of inhibitors, this serum pool produced binding of 15.3% (A), 13.8% (B), and 3.9% (C) total counts, respectively. A normal serum used as control produced binding of 0.40% (A), 0.24% (B), and 0.22% (C) total counts, respectively.

extract (10 mg) was then applied to the latter column and allowed to incubate overnight. After the column was washed exhaustively with 200 mM NH_4HCO_3 (pH 7.4), affinity chromatography purified GCR3 antigens (ACP-3) were eluted from the column with glycine-HCl buffer (pH 2.8), collected into tubes containing an equal volume of 200 mM NH_4HCO_3 (pH 7.4), and concentrated by pressure filtration through an Amicon YM10 membrane.

SDS-PAGE/Immunoblotting

SDS-PAGE was carried out in 13 cm long 12.5% acrylamide gels using the discontinuous buffer system of Laemmli [12]. Replicate insect extracts, treated with mercaptoethanol, were applied in the stacking gel and electrophoresis was carried out at 20 mA until the tracking dye reached the end of the gel. After electrophoresis, gels were either stained with Coomassie brilliant blue (BioRad Laboratories) or protein bands were transferred to nitrocellulose [13]. Transfer was effected in a water-cooled (10°C) BioRad TRANS-blot apparatus at a constant voltage of 30 V for 18 h. After transfer, nitrocellulose membranes were washed in PBS for 30 min, followed by a 30 min wash in PBS containing 1% BSA to block unbound membrane sites. Sera were diluted 1:4 (human) or 1:100 (rabbit) in 10 mM Tris-HCl/0.1% Tween 20; individual nitrocellulose blotted membranes were incubated overnight on a shaker at room temperature with the human IgE antibody pool or rabbit antisera. Serum was then removed and each membrane was washed thrice with 10- to 15-ml aliquots of 10 mM Tris-HCl/0.1% Tween 20. Membranes incubated with rabbit sera were incubated with ^{125}I -protein A (20 ng/ml) in final volume of 10 ml for 16 h on a shaker at room temperature. Membranes incubated with human sera were incubated similarly with ^{125}I -antihuman IgE (20 ng/ml). Autoradiographs were prepared by incubating washed membranes with X-ray film (Kodak X-Omat) and exposing in a cassette equipped with a DuPont Cronex intensifying screen for 6 h at -70°C.

Thin-Layer Isoelectrofocusing (TLIEF)

TLIEF (LKB-PAG plate, pH 3.5-10) was performed using the LKB Multiphor system. Samples of WBGCR, GCR2, GCR3, and ACP-3 extracts were applied at the cathode margin and run under constant current for 45 min at 10°C; wicks containing the allergen were then removed and electrofocusing was continued at 900 V for 60 min. After electrofocusing, the gel was removed and stained by the silver nitrate method (BioRad).

Results

Fractionation of Cockroach Antigens

The Sephadex G-200 chromatogram is shown in figure 1. Although two major 280-nm absorbance peaks were observed, the fractions with the highest specific allergenic activity (measured by direct RAST) were those with apparent MW between 12,500 and 75,000 daltons, eluting between the two major absorbance peaks. These allergen-rich fractions were pooled and designated GCR3.

RAST Inhibition

To assess whether the same or similar allergens were present in WBGCR, GCR3 and TAW extract preparations, RAST inhibition experiments were performed using WBGCR, GCR3 and TAW as solid-phase allergens and pooled IgE from cockroach-sen-

sitive patients (fig. 2). Each of the extracts was tested for its ability to inhibit binding of IgE to each support. Using either the WBGCR (fig. 2A) or GCR3 (fig. 2B) as solid supports and the cockroach-reactive human IgE serum pool and the three fluid-phase inhibitors, extracts of WBGCR and the GCR3 preparation showed RAST inhibitory activity, whereas no RAST inhibitory activity was seen with TAW extract. However, when TAW was used as the solid phase (fig. 2C) with cockroach-reactive human IgE serum pool and fluid-phase inhibitors, TAW and WBGCR extracts showed RAST inhibitory activity, whereas no RAST inhibitory activity was seen with GCR3 preparation. Although the IgE serum pool from German cockroach-sensitive patients had antibodies which recognized both TAW and WBGCR, no RAST inhibitory activity was seen with GCR3. These findings collectively suggested that the GCR3 preparation contained allergens that may be specific for cockroach, but that there also appeared to be allergens shared between WBGCR and TAW.

To further address this question, we repeated the same experiments using the IgE serum pool from 2 individuals with summer and fall rhinitis, high RAST binding to TAW, and no known history of cockroach exposure (fig. 3). Both WBGCR and TAW extracts showed good RAST inhibitory activity, whereas limited allergenic reactivity (<40% inhibition) was seen with GCR3 for both solid-phase reagents, further suggesting that allergens present in GCR3 may be specific to allergens present in cockroach and not in TAW extracts and that these donors had small amounts of antibody to these common allergen(s). With either WBGCR or GCR3 as solid-phase supports rabbit anti-GCR3 serum and fluid-phase inhibitors only TAW failed to cross-react in RAST inhibition experiments (data not shown). Thus, both human IgE from individuals sensitive to cockroach and rabbit IgG antiserum to GCR3 appeared to recognize unique antigens in cockroach extract that did not cross-react with TAW antigens. However, rabbits immunized with crude TAW antigens produced antibody against high molecular weight components also contained in GCR3 and to a greater degree in whole body cockroach.

SDS-PAGE/Immunoblot

Between 20- and 600- μ g amounts of various insect extracts or fractions thereof were applied to a 12.5% SDS-PAGE gel (fig. 4). Although considerable differ-

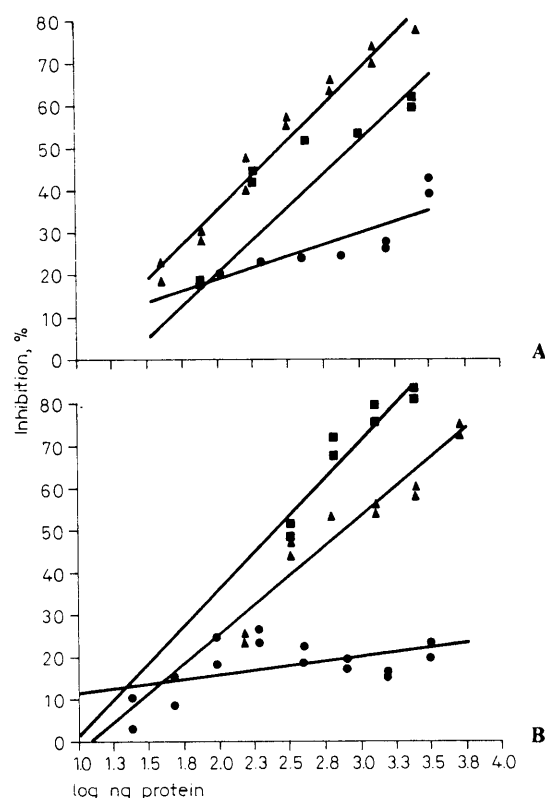


Fig. 3. RAST inhibition results using: (A) WBGCR and (B) TAW as the solid-phase allergens, and TAW-sensitive human IgE serum pool and various fluid-phase inhibitors: ● = GCR3; ▲ = WBGCR; ■ = TAW. In the absence of inhibitors, this serum pool produced binding of 4.9% (A), and 3.9% (B) total radioactive counts, respectively. Normal serum used as control produced binding of 0.24% (A), and 0.26% (B) total counts, respectively.

ences in the intensity of Coomassie blue staining proteins were demonstrated, multiple stained bands were demonstrated with extracts of TAW, WBGCR and GCR2; fewer bands were noted with GCR3, GCR4, YJ and HB extracts. The LF and CF extracts showed staining material only at low MW (12,400 daltons). GCR3 showed a predominant band at approximately 36,000 daltons with fainter bands at approximately 55,000, 25,000 and 15,000 daltons. The ACP-3 preparation isolated from WBGCR extract showed a Coomassie-stained pattern similar to that of GCR3, suggesting that either: (a) cross-reacting antibodies were still present in the absorbed rabbit IgG anti-GCR3 antiserum used for affinity purification, or (b) that the washing was incomplete before elution of ACP-3 (results not shown).

The autoradiograph from immunoblot analysis

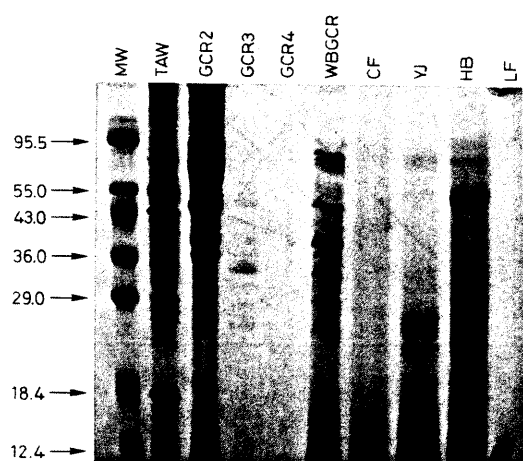


Fig. 4. Coomassie blue stain of SDS-PAGE (Laemmli) gel of German cockroach and various insect extracts. MW = Molecular weight markers; TAW (203 μ g); GCR2 (480 μ g), GCR3 (252 μ g), GCR4 (384 μ g) = Sephadex G-200 pooled fractions of German cockroach; WBGCR = whole body German cockroach (400 μ g); CF = caddis fly (192 μ g); YJ = yellow jacket (272 μ g); HB = honeybee (562 μ g); and LF = lake fly (22 μ g).

using cockroach-reactive human IgE pool and radio-labeled anti-IgE (fig. 5A) demonstrated two distinct radiostained bands in WBGCR extract corresponding to MW of 36,000 and 55,000 daltons; similar radiostained bands were identified in GCR3. Many radiostained bands were demonstrated in GCR2 and none in GCR4. The presence of radiostained bands in GCR2 corresponding with bands in GCR3, TAW, HB, and LF extracts indicated that IgE from German cockroach-sensitive patients may recognize proteins in these other insects.

The autoradiograph of immunoblots prepared using TAW-reactive human IgE (fig. 5B) showed several radiostained bands in the GCR2 fraction. However, no radiostained bands appeared in GCR3 and very faint bands were seen in the WBGCR extract. Dense radiostaining was identified in the CF and LF extracts. Thus, these TAW-sensitive individuals appear to have IgE that recognizes proteins in various insect extracts including GCR2 but not in GCR3 or GCR4.

Immunoblot autoradiography using rabbit anti-GCR3 IgG and radiolabeled protein A showed a faint band in GCR3 at approximately 36,000 daltons corresponding to a similar band in WBGCR extract; however, there was radiostaining in the 55,000-dalton region of GCR2, TAW, WBGCR, YJ, CF, and HB ex-

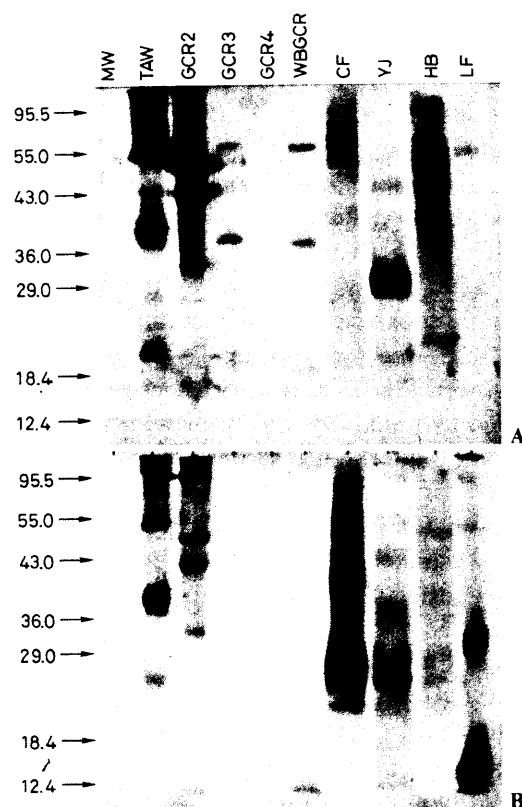


Fig. 5. Autoradiograph of SDS-PAGE/immunoblot of German cockroach, Sephadex G-200 cockroach fractions and other whole insect extracts using: (A) cockroach-sensitive human IgE antibody pool and radiolabeled anti-IgE, and (B) TAW-sensitive human IgE antibody pool and radiolabeled anti-IgE. Abbreviations as in figure 4.

tracts (fig. 6A). As in figure 5A with human IgE antibodies, multiple radiostained antigens appeared in various insect extracts capable of binding to rabbit anti-GCR3 IgG. The binding in the 55,000-dalton region in these other extracts was somewhat surprising; however, it was apparent that GCR3 contained a Coomassie-stained protein in this 55,000-dalton region that appeared to be highly immunogenic and demonstrated significant radiostaining with proteins in other insect extracts.

In the autoradiograph pattern using rabbit IgG to TAW (fig. 6B), the prominence of the 55,000-dalton component was evident in TAW, GCR2, GCR3, WBGCR, CF, YJ, and HB. The lack of a discrete band at 36,000 daltons in GCR3 supported our suggestion that the 36,000-dalton component was specific for WBGCR while the 55,000-dalton component appeared to be common in several insect extracts.

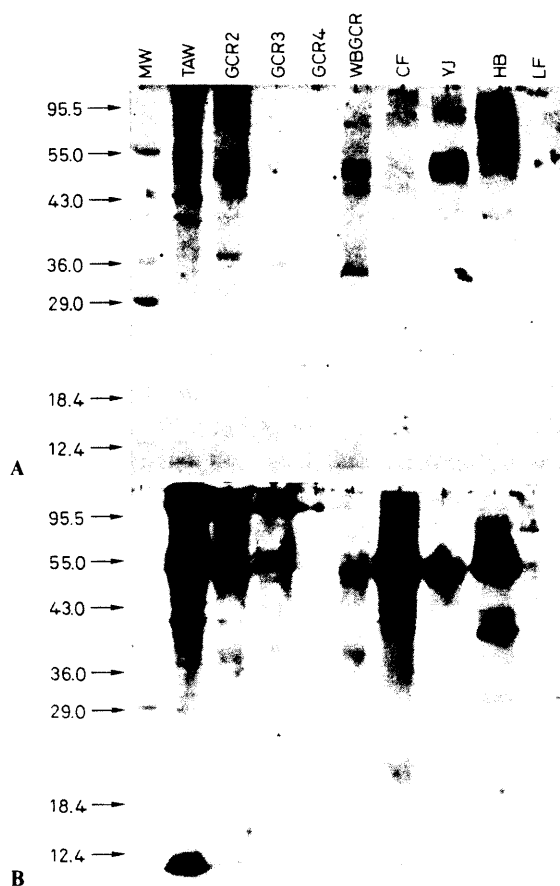


Fig. 6. Autoradiograph of SDS-PAGE/immunoblot of German cockroach, Sephadex G-200 cockroach fractions, and other whole insect extracts using: **(A)** rabbit IgG to GCR3 and radiolabeled protein A, and **(B)** rabbit IgG to TAW and radiolabeled protein A. Abbreviations as in figure 4.

TLIEF

Several silver-stained bands with pIs from 7.0 to 3.5 were present in WBGCR, GCR2 and GCR3 (fig. 7). Although there were several bands in GCR3, a band at approximately pI 6.7 predominates. When WBGCR was passed over an affinity column of rabbit anti-GCR3 IgG and the acid eluate (ACP-3) analyzed by TLIEF, a band was demonstrated at the same relative pI (6.7) suggesting this antigen can be purified from WBGCR by affinity chromatography.

Discussion

Patients with inhalant insect allergy appear to produce IgE to several individual insect allergens. Be-

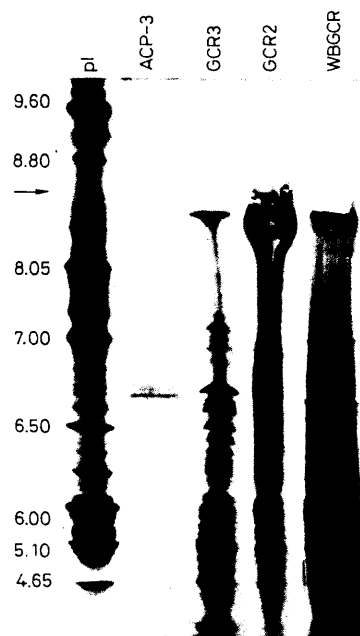


Fig. 7. A silver stain (BioRad) of thin layer isoelectric focusing gel (pH 3.5–10, LKB Produkter) of cockroach extracts. Lanes from left to right: BioRad pI markers; ACP-3; GCR3, GCR2; WBGCR. Fifty microliters of each preparation were added irrespective of protein concentration. Application site is indicated by the arrow.

cause an individual may be exposed to a large number of different insects in dwellings, schools, workplaces, or outdoors, it is difficult to determine whether a patient has multiple sensitivities because of multiple insect exposures or because of widespread insect cross-reactivity.

Several studies have been used to document IgE-mediated sensitization to cockroach-derived materials. In a study of 35 patients in the Boston area, there was concordance of skin test reactivity to commercial extracts of either German or American cockroach [2]. In another study, cockroach-specific IgE was measured in the sera of 13 cockroach-sensitive patients, and it was suggested that the cockroach cross-reactivity may have been due to exposure to multiple species and that most of the allergenic activity of the German cockroach could be extracted from whole bodies and cast skins [1]. Other studies have documented the high prevalence of cutaneous reactivity to cockroach allergens among urban patients with asthma and the presence of both immediate- and dual-phase bronchoconstrictive responses in patients with extrinsic asthma following bronchial challenge with cockroach

allergens [3, 4, 8]. Recently, a clinical and immunologic study on cockroach-laboratory workers demonstrated that American cockroach hemolymph possessed a broad range of cross-reactivity not only with American cockroach fecal antigens but with a whole range of whole body allergens from different cockroach species, as well as with a beetle larval antigen [14]. These data supported the findings of Baur et al. [15], who suggested that increases in asthmatic disease reported in the Sudan and other areas were predominantly caused by sensitization to hemoglobin molecules of chironomids. Although chironomid hemoglobins showed a high degree of polymorphism, primary structures were identified as soluble components of body fluids either in forms of monomers (MW approximately 16,000) or homodimers (MW approximately 32,000).

Few studies have attempted to isolate and purify the major antigens/allergens involved in immediate hypersensitivity to insect-derived materials. Twarog et al. [2] identified three antigens in German and American cockroach extracts localizing them by sequential purification on Sephadex G-75, diethylaminoethyl (DEAE) cellulose and agarose gel electrophoresis. CRI (MW 65,000) and CRII (MW 25,000) were the two major fractions eliciting skin test reactions in 70% of cockroach-sensitive subjects. Chang et al. [16] reported similar allergenic fractions by Sephadex G-75 filtration; however, in the analysis of their CrPI (equivalent to CRI of Twarog), they found at least three subfractions of with MW of 378,000, 440,000 and 651,000 by polyacrylamide gel electrophoresis.

The similarities between allergens identified in various insect-derived materials studied suggested that common properties may have contributed to their allergenicity. To further elucidate the immunologic profile of cockroach-derived allergens, we combined the high resolving power of electrophoresis in polyacrylamide gels with the sensitivity of immunoblot analysis to determine the immunologic reactivity of various insect extracts using sera from cockroach-sensitive individuals. We first identified a fraction isolated from Sephadex G-200 (GCR3, apparent MW 12,500–75,000 daltons) which contained the major allergenic activity as measured by direct RAST using pooled sera from cockroach-sensitive individuals. Although this heterogeneous range of MW proteins contained the equivalent of both CRI and CRII of Twarog et al. [2], we further analyzed the specificity of this fraction by determining the immunologic reactiv-

ity of GCR3 to extracts from different orders of insects utilizing RAST inhibition, SDS-PAGE/immunoblot and TLIEF analysis. Interestingly, several radiostained bands in SDS-PAGE/immunoblot analysis using either human IgE reactive to WBGCR or TAW suggested that cross-reacting allergens may be present in a variety of insect extracts. In RAST inhibition experiments and SDS-PAGE/immunoblot analysis, GCR3 partially purified from WBGCR extracts demonstrated specificity for WBGCR when compared to extracts of insects from several other orders. Moreover, in our preliminary affinity chromatography purification scheme we were able to identify, by TLIEF, a component (pI 6.7) that can be isolated from WBGCR extracts. At present, it is not clear from these data whether sensitization to unique or common insect allergens exists in species, genera, and order because of the limited number of insects and patients sera analyzed.

Although WBGCR significantly inhibited the TAW RAST (fig. 2C), the inhibitory activity is believed to reside in common components shared by whole body insect extracts. We failed to demonstrate significant inhibition with GCR3 in either WBGCR or TAW solid-phase polymers using human IgE-reactive to TAW whereas both TAW and WBGCR showed comparable inhibitory activity. This suggests that whole body insect extracts of TAW and GCR share common allergenic components and that GCR3 may contain a GCR allergen not present in TAW extracts.

Alternatively, a variation in the qualitative/quantitative display of antigens/allergens on the solid-phase discs of the WBGCR and TAW, respectively, would account for the reduced inhibitory capacity of one extract with respect to the other extract. For example, if the WBGCR solid phase had a greater number and/or a more complex array of antigens/allergens bound relative to the shared TAW solid-phase bound antigens/allergens then the WBGCR extract would inhibit well in the TAW RAST, but the TAW extract would not inhibit well in the WBGCR RAST. Our SDS-PAGE/Western blots demonstrated that with either human IgE or rabbit IgG, considerable high molecular weight radiolabelled components are shared among various insect extracts; however, our partially purified GCR3 component at 36,000 daltons appeared to be CR specific. However, by utilizing a more refined affinity chromatographic scheme to purify the GCR3 component, the identification and

characterization of the major allergen in WBGCR extracts will contribute significantly to the correlation between history and the various immunologic tests used to determine immediate hypersensitivity to WBGCR.

Furthermore, a detailed immunologic characterization of various other insect allergens utilizing similar purification schemes may reveal homologous structural sites (e.g. hemoglobin) essential for triggering the allergic response.

Our studies focused on the characterization of WBGCR and the partially purified GCR3 fraction as allergens which cross-react with other insect extracts. Subjects chosen for our IgE serum pool were originally defined by skin test reactivity and RAST to a commercial WBGCR. We opted to further characterize the allergens in GCR3 because it had the highest allergen activity per unit protein absorbed to microtiter wells. By SDS-PAGE/Western blot analysis we clearly demonstrated that GCR2 had very similar components to those found in GCR3; however, there was some degree of separation which allowed us to use the various fractions for further study by RAST inhibition and SDS-PAGE/Western blot. In separate studies we have documented the variability of individual sera to WBGCR extracts by SDS-PAGE/Western blot analysis (data not shown); however, for our purposes in this study we were primarily interested in the cross-reactivity of cockroach-sensitive patients' sera with various other insect extracts. Thus, we assembled a serum pool containing a maximal array of IgE antibody responses to cockroach extracts and so as not to identify minor allergen(s) that evoke IgE antibody responses in a minority of CR-sensitive persons. Because our patient cockroach IgE pool was collected from the Chicago-based area we were unable to skin test these patients with the various cockroach fractions. Furthermore, we presently have only a partially purified component that cross-reacts with TAW allergens and would not be clinically significant as a 'major' allergen for purposes of epidemiologic studies, diagnostic testing, and immunotherapy. Since our findings strongly suggest the presence of a 'major' cockroach allergen within GCR3, we believe it essential to further purify this allergen before attempting clinical trials to determine that this allergen represents a species-specific and/or shared interspecies allergen preparation among insect-sensitive individuals.

An interesting concept to consider is the manifesta-

tion of insect sensitivity following outdoor or indoor exposures [17-19]. Insect sensitization following outdoor exposure might be attributed to be a common cross-reacting insect antigen 'mosaic'. It is conceivable that these common insect antigens may contribute to the aeroallergenic burden in geographic regions highly infested with insects. This concept may well explain the cross-reactivity shown by many insects [20]. On the other hand, with indoor insect exposure or exposure to heavy localized (regional) single insect species outdoors, the individual is more likely to be sensitized to specific allergenic components unique to the infesting insect.

Although we have identified extensive cross-reactivity to insect allergens using IgE from clinically sensitive individuals, we have also demonstrated an allergen with a pI of 6.7 and a MW of 36,000 daltons that can be isolated from WBGCR. It must be kept in mind that GCR3 is only partially purified and contains minor components of WBGCR that cross-react with TAW allergens (see SDS-PAGE/immunoblot results). The clinical relevance of sensitization to this allergen vis a vis cross-reacting cockroach allergens is at present unknown. This allergen may or may not play a central role in cockroach-related symptoms, but regardless, it would seem useful as a specific marker for cockroach sensitivity.

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