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Induction of IgE Antibody Responses by Glutathione *S*-Transferase from the German Cockroach (*Blattella germanica*)*

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We report that a major 23-kDa allergen from German cockroach (*Blattella germanica*) is a glutathione *S*-transferase (EC 2.5.1.18; GST). Natural *B. germanica* GST, purified from cockroach body extracts by glutathione affinity chromatography, and recombinant protein expressed in *Escherichia coli* using the pET21a vector, showed excellent IgE antibody binding activity. *B. germanica* GST caused positive immediate skin tests in cockroach-allergic patients using as little as 3 pg of recombinant protein. The NH_2-terminal sequence of the natural protein and the deduced amino acid sequence from cDNA were identical except for one substitution ($Phe^9 \rightarrow Cys$). Assignment of this protein to the GST superfamily was based on binding to glutathione and sequence identity (42–51%) to the GST-2 subfamily from insects, including *Anopheles gambiae* and *Drosophila melanogaster*. *B. germanica* GST contained 18 of the 26 invariable residues identified in mammalian GST by x-ray crystallography and exhibited enzymic activity against a GST substrate. Our results show that cockroach GST causes IgE antibody responses and is associated with asthma. The data strongly support the view that the immune response to GST plays an important role in allergic diseases.

Cockroaches produce potent allergens, which give rise to IgE antibody (Ab)[1] responses in genetically predisposed individuals living in cockroach-infested housing (1–8). IgE Ab to cockroach are strongly associated with asthma, and sensitization to cockroach allergens is a major risk factor for hospital emergency room visits for asthma (5, 9, 10). Although cockroach may carry viral and bacterial pathogens, asthma is the only disease for which an unequivocal causal relationship with cockroach exposure has been established (9–11). Over the last few years, several allergens from the principal domiciliary cockroach species, *Blattella germanica* (German cockroach) and *Periplaneta americana* (American cockroach), have been cloned and insights into their biological function have been obtained (12–15). *B. germanica* allergen Bla g 2 shows sequence homology to aspartic proteases, whereas Bla g 4 is a ligand-binding protein or calycin (12, 13). In Taiwan, *P. americana* is an important cause of asthma, and a 72-kDa allergen, Per a 3, has recently been cloned, which shows homology to insect hemolymph proteins (arylphorins) (14).

We have identified an important *B. germanica* allergen with sequence homology to the glutathione *S*-transferase (GST) superfamily. These enzymes are involved in the detoxification of endogenous and xenobiotic toxic compounds and are widely distributed in most forms of life (16–18). In molecular biology, plasmid vectors have been constructed to express foreign proteins in *Escherichia coli,* as fusion proteins with the COOH terminus of GST from *Schistosoma japonicum.* These high level expression systems (pGEX vectors) have been widely used for purification of recombinant proteins by glutathione affinity chromatography (19).

Here, we report the sequence, purification, and recombinant expression of *B. germanica* GST, and demonstrate that this protein causes IgE Ab responses in ~70% of cockroach-allergic asthmatic patients. Homologous GST allergens were also identified in the house dust mite, *Dermatophagoides pteronyssinus*, and in the helminth parasite *Schistosoma mansoni* (20, 21), thus defining the importance of the GST superfamily in causing IgE antibody responses.

EXPERIMENTAL PROCEDURES

Molecular Cloning and Sequencing of B. germanica GST (or Bla g 5)—A *B. germanica* cDNA library was prepared in the UniZAP-XR phagemid expression vector as described previously (12, 13). Six cDNA clones were identified by screening the library with IgE Ab in a serum pool from eight cockroach-allergic patients (13). Further screening with individual sera from 20 cockroach-allergic patients and 4 non-allergic controls by plaque immunoassay revealed that the protein encoded by cDNA clone *bg*16 bound IgE Ab from 70% of the patients, but not from control sera. This protein was designated *B. germanica* allergen, 5 or Bla g 5, in keeping with the current allergen nomenclature (22). Double stranded sequencing of clone *bg*16 was carried out by dideoxynucleotide chain termination with a Sequenase kit (U. S. Biochemical Corp., Cleveland, OH) (23). The sequence was compared with the National Biomedical Research Foundation, Swiss-Prot, and GenBank data bases using FASTA, and the results revealed that the protein encoded by cDNA clone *bg*16 shared sequence homology with the GST. Sequence alignments were performed using the GCG program.

Purification of Natural B. germanica GST—*B. germanica* GST was purified from cockroach whole body extract by chromatography over glutathione-Sepharose. A *B. germanica* whole body extract containing 289 mg of protein/ml was prepared from 12 g of cockroach, as described previously (24). The extract (1.3 ml) was passed over a 0.5-ml glutathione-Sepharose column (Pharmacia, Piscataway, NJ), and *B. germanica* GST was eluted with 10 mM reduced glutathione. After dialysis, further purification was carried out using a Superdex 75 HR10/20 size-exclu-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U92412.

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[1] The abbreviations used are: Ab, antibody; GST, glutathione *S*-transferase; Bla g 5, *Blattella germanica* allergen 5; PAGE, polyacrylamide gel electrophoresis; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; CDNB, 1-chloro-2,4-dinitrobenzene.

```
   1 CT  TAT AAA CTG ACA TAC TGT CCC GTG AAG GCT CTG GGA GAG CCA     44
          Y   K   L   T   Y   C   P   V   K   A   L   G   E   P

  45     ATT CGC TTC CTT CTG TCT TAT GGA GAG AAA GAT TTT GAA GAT     86
          I   R   F   L   L   S   Y   G   E   K   D   F   E   D

  87     TAT CGT TTC CAG GAG GGA GAT TGG CCT AAT TTG AAA CCT TCC    128
          Y   R   F   Q   E   G   D   W   P   N   L   K   P   S

 129     ATG CCA TTT GGT AAA ACA CCA GTG TTG GAG ATT GAT GGG AAG    170
          M   P   F   G   K   T   P   V   L   E   I   D   G   K

 171     CAA ACA CAC CAG TCT GTT GCC ATT TCT CGC TAT CTT GGT AAG    212
          Q   T   H   Q   S   V   A   I   S   R   Y   L   G   K

 213     CAG TTT GGC CTC AGT GGT AAG GAT GAT TGG GAG AAC TTG GAG    254
          Q   F   G   L   S   G   K   D   D   W   E   N   L   E

 255     ATC GAC ATG ATC GTC GAC ACC ATC TCT GAC TTC AGG GCT GCC    296
          I   D   M   I   V   D   T   I   S   D   F   R   A   A

 297     ATT GCT AAT TAC CAT TAT GAT GCT GAT GAA AAT TCA AAG CAG    338
          I   A   N   Y   H   Y   D   A   D   E   N   S   K   Q

 339     AAG AAA TGG GAC CCT CTC AAG AAG GAA ACC ATT CCT TAC TAC    380
          K   K   W   D   P   L   K   K   E   T   I   P   Y   Y

 381     ACC AAA AAG TTT GAT GAA GTG GTG AAG GCT AAC GGA GGA TAC    422
          T   K   K   F   D   E   V   V   K   A   N   G   G   Y

 423     CTT GCT GCT GGA AAG CTG ACA TGG GCA GAC TTC TAC TTC GTT    464
          L   A   A   G   K   L   T   W   A   D   F   Y   F   V

 465     GCC ATT CTC GAC TAT TTG AAT CAC ATG GCT AAA GAA GAC CTG    506
          A   I   L   D   Y   L   N   H   M   A   K   E   D   L

 507     GTG GCC AAT CAA CCC AAT TTG AAG GCT TTG CGG GAG AAA GTA    548
          V   A   N   Q   P   N   L   K   A   L   R   E   K   V

 549     TTG GGT TTG CCT GCT ATC AAA GCA TGG GTC GCC AAG CGT CCT    590
          L   G   L   P   A   I   K   A   W   V   A   K   R   P

 591     CCT ACA GAT CTG TAA GAA AAA TGT GCC ATG GCA AAA AAA TTC    632
          P   T   D   L  ***

 633     ATG TTG CAT GTA ACA CTG AGA TCA TAA CGA TGT TCT AAA AGA    674
 675     AAT TTT GTT ACG CAT AAT GAT TTT ATG AAA GTA TTT TGT TAG    716
 717     CAG CTT TGC TCT ATA ATA ATC ACT AGA CCA TAT TTA AAA GGC    758
 759     AAA AAC GAA CAT TTT CTT CAT AAA AGG CAA AAA TAG CCA AAA    800
 801     AAT ACT TTT GTA TTA AAA TAT TCA TTG ACG CTG ATT CTT ACA    842
 843     TTT AAT TCT TCA CAA TTT AAG AAT TTT TTA ACA ATA GTA ATT    884
 885     ACG ATC AAC ATT TCA GAT CTG TTT AGA TAT GAT TGC AAA GCT    926
 927     TGT TTA TAA TCA GAA AAT GAC TTC CTA AAA TCA ACA GCA TAT    968
 969     GGC GCA AAA TTT TTC GTT CTA AAT TTC CAG TTT TTT AAA TGT   1010
1011     ATA ATT TTT TTG GTA AAC TTT TAT TTA CTA GAA ATT TGA TCC   1052
1053     AGA AGT AGA CTG ATA ATT TCC TTT ACT TAC TTT TTG GTA TTA   1094
1095     AAC AAA GTT GGA AAC AAA ATA ATT TTG A19           1140
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FIG. 1. **Nucleotide and deduced amino acid sequence of *B. germanica* cDNA clone *bg*16, encoding *B. germanica* GST.** The initiation methionine is lacking from the deduced sequence. No potential *N*-linked glycosylation sites were identified, and the stop codon TAA is shown (*).

sion column (Pharmacia). To assess purity, *B. germanica* GST was analyzed by silver-stained SDS-PAGE, using a PhastSystem (Pharmacia). The amino acid sequence of 35 NH_2-terminal residues (spanning residues 1–38) was determined by Edman degradation.

Expression of Recombinant B. germanica GST in E. coli—Recombinant *B. germanica* GST was expressed as a non-fusion protein using the pET21a expression vector (Novagen, Madison, WI). A 973-base pair DNA fragment containing the coding sequence for *B. germanica* GST was generated by polymerase chain reaction. Ten nucleotides were added at the 5′-end, encoding the initiation Met and the first three NH_2-terminal amino acid residues (Ala-Pro-Ser) lacking in the original cDNA. Primers for polymerase chain reaction were designed as follows: ′- GGAATTC**CATATG**GCACCGTCTTATAAACTGACATAC-3′ (sense), containing an *Nde*I restriction site, and 5′-CTGTTGATTTTAGGAAGTCAT-3′ (antisense), downstream from a *Hin*dIII site in the 3′-untranslated region (nucleotides 922–927). The 973-base pair polymerase chain reaction-amplified DNA was ligated into *Nde*I-*Hin*dIII digested pET-21(a) vector. Expression of the 23-kDa *B. germanica* GST was induced in *E. coli* strain BL21(DE3) with 1 mM isopropyl-1-thio-β-D-galactopyranoside at 30 °C, and recombinant protein was purified from cell lysates using glutathione-Sepharose (yield typically 3–4 mg/liter of culture). Recombinant *B. germanica* GST was analyzed by silver-stained SDS-PAGE and size exclusion chromatography. The amino acid sequence of the five NH_2-terminal residues was confirmed by Edman degradation.

Immunoassays for Recombinant and Natural B. germanica GST—IgE Ab to natural and recombinant *B. germanica* GST were compared using an antigen-binding RIA (25). Briefly, 10 and 20 μg of natural and recombinant *B. germanica* GST were labeled with 0.5 mCi of ^{125}I, using the chloramine T technique (specific activity, 35 and 23 μCi/μg), respectively. Serum samples diluted 1:2 and 1:10 were incubated with either ^{125}I-labeled allergen (~100,000 cpm added) for 4 h at room temperature. Immune complexes were precipitated overnight at 4 °C with 50 μl of sheep anti-human IgE (The Binding Site, San Diego, CA), using IgE myeloma serum (patient P. S.) diluted 1:200 as a carrier. Precipitates were counted in a γ-counter following washing with BBS. Quantitation of both assays was carried out using a control curve, constructed with serum from patient I. H., assigned to contain 1,000 units/ml IgE Ab.

A direct binding ELISA was also developed for measuring IgE Ab to recombinant *B. germanica* GST. Antigen was coated to a microtiter plate (0.5 μg/well) in carbonate-bicarbonate buffer, pH 9.6, overnight at 4 °C. Plates were washed with PBS-Tween and serum samples diluted 1:2 and 1:10 in horse serum were added for 1–2 h, after blocking with 1% bovine serum albumin, phosphate-buffered saline-Tween for 1 h at room temperature. Antibody binding was detected using peroxidase-conjugated goat anti-human IgE and the ABTS substrate system (26).

```
                     1        10        20        30
Natural Bla g 5:     APSYKLTYFPVKALGEPIRFLLS_GE__FEDYRFQEGD
                        ::::: :::::::::::::: ::  ::::::::::
Deduced sequence:       YKLTYCPVKALGEPIRFLLSYGEKDFEDYRFQEGD
 (from cDNA)
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FIG. 2. **Sequence alignment between the NH_2-terminal amino acid sequence of natural *B. germanica* GST (or Bla g 5) and the deduced amino acid sequence from cDNA.** The *solid lines* represent undetermined residues where the amino acids could not be identified without ambiguity. There was only one amino acid substitution, at position 9 (Phe → Cys), compared with the deduced sequence from cDNA.

The assay was quantitated using a control curve constructed with serum from patient I. H., assigned with 1,000 units/ml IgE ab.

Immediate Skin Testing—Quantitative intradermal skin testing was carried out using serial 10-fold dilutions of *B. germanica* extract (1:20, w/v, Allergy Laboratories of Ohio, Columbus, OH) or purified recombinant *B. germanica* GST from 10^{-5}-10^{0} μg/ml, as described previously (12, 13, 24). Skin testing of human subjects using recombinant allergens was approved by the University of Virginia Human Investigation Committee.

Human Sera—Sera from 40 cockroach-allergic asthmatic patients had been obtained from patients who participated in previous studies on emergency room asthma (5, 9, 10, 12, 13). All patients had IgE Ab to cockroach by RAST (82-6, 400 units/ml, 1 unit~ of 0.1 ng IgE). Four non-allergic control subjects were also studied.

Enzymatic Activity of B. germanica GST—The enzymatic activity of *B. germanica* GST was assessed using the 1-chloro-2,4-dinitrobenzene (CDNB) substrate and compared with the activity of rat liver glutathione *S*-transferase (Sigma). The absorbance was monitored at 340 nm and activity was calculated using the extinction coefficient of 9.6 mM^{-1} (27).

RESULTS

Identification of a Major B. germanica Allergen Bla g 5 as a Member of the GST Family—The nucleotide and deduced amino acid sequence of cDNA clone *bg*16 is shown in Fig. 1. The 1,140-base pair sequence contains a 600-base pair open reading frame, encoding a 200-residue polypeptide. A stop codon (TAA) was found at position 603. NH_2-terminal sequencing of the natural protein revealed that the sequence encoding the initiation methionine and the first three amino-terminal residues (Ala-Pro-Ser) were lacking from the original cDNA clone (Fig. 2). The estimated molecular mass of the 203-amino acid residue protein was 23,176 Da and no potential *N*-linked glycosylation sites were found.

Sequence similarity searches revealed that the protein encoded by cDNA clone *bg*16 was a GST. These enzymes catalyze the nucleophilic addition of the thiol of reduced glutathione to organic compounds, and their active site contains two binding sites, one for glutathione (G-site) and one substrate binding (H-site). Five different gene families encode GST, including four classes of cytosolic enzymes referred to as alpha, mu, pi, and theta, and a class of microsomal enzymes. The highest degree of homology was found with the GST-2 subfamily of insect GST from *Anopheles gambiae* (51.5%), *Musca domestica* (housefly) (47.8%), *Drosophila melanogaster* (46.8%), and *Manduca sexta* (tobacco hornworm) (42.9%) (28–30). Significant homology was also found with other GST including human, rat, and mite GST (Fig. 3). In particular, 18 of the 26 invariable residues identified in mammalian GST by x-ray crystallography were present in *B. germanica* GST, including Tyr-8, Gln-63, and Asp-97, which are involved in glutathione binding at the G-site of the molecule (17, 18, 31–35).

Purification of Natural B. germanica GST and Production of Recombinant B. germanica GST in E. coli—Natural *B. germanica* GST was purified from whole body cockroach extract by glutathione affinity chromatography and size exclusion. The protein gave a single homogeneous band on silver-stained SDS-PAGE, with a molecular mass of 23 kDa (Fig. 4*A*). NH_2-terminal sequence analysis demonstrated that the natural and deduced amino acid sequences were identical except for one amino acid substitution, Phe to Cys, at position 9 (Fig. 2). This substitution occurs in a hydrophobic region of the molecule next to one of the glutathione binding residues (Tyr-8). The mosquito and tobacco hornworm sequences also have Phe at position 9. The amino acid change from Cys (TGT) to Phe is a conservative substitution. The results imply that there are other isoform(s) of *B. germanica* GST with the Phe substitution at position 9. The yields of natural allergen from cockroach extracts were very low (0.002% of the protein in the cockroach extract). Recombinant *B. germanica* GST was produced as a non-fusion protein in *E. coli*, using the pET21a vector system, and purified from bacterial lysates by glutathione affinity chromatography. The pure protein migrated as a single 23-kDa band on SDS-PAGE (Fig. 4*B*). Following size exclusion chromatography, recombinant *B. germanica* GST eluted as a single peak with an estimated molecular mass of 46 kDa, suggesting that the recombinant protein may form a homodimer. The enzymatic activity of recombinant *B. germanica* GST was evaluated using the CDNB substrate. The CDNB conjugation activity of recombinant cockroach-GST was 0.05 μM/min/mg (mean of two experiments), which was very low, compared with the rat liver GST control (13.65 μM/min/mg, 273 times higher).

```
                  1                                  •                       50
Cockroach         .......... .....APSYK LTYCPVKALG EPIRFLLSYG EKDFEDYRFQ
Anopheles         LSSSSISRSS LKCNIMPDYK VYYFNVKALG EPLRFLLSYG NLPFDDVRIT
Tobacco hornworm  .......... .....MPKVV FHYFGAKGWA RPT.MLLAYG GQEFEDHRVE
Human             .......... ....MAEKPK LHYSNIRGRM ESIRWLLAAA GVEFEEKFIK
Mite              .......... .....MSQPI LGYWDIRGYA QPIRLLLTYS GVDFVDKRYQ

                  51                                           •        100
Cockroach         .......E.G DWPNLKPS.. MPFGKTPVLE IDGKQTHQSV AISRYLGKQF
Anopheles         .......R.E EWPALKPT.. MPMRQMPVLE VDGKRVHQSL AMCRYVAKQI
Tobacco hornworm  .......Y.E QWPEFKPN.. TPFGQMPVLE IDGKKYAQSL AISRYLGRKY
Human             .......SAE DLDKLRNDGY LMFQQVPMVE IDGMKLVQTR AILNYIASKY
Mite              IGPAPDFDRS EWLNEKFNLG LDFPNLPYYI DGDMKMTQTF AILRYLGRKY

                  101                       •                           150
Cockroach         GLSGKDDWEN LEIDMIVDTI SDFRAAIANY HYDADENSKQ KKWDPLKKET
Anopheles         NLAGDNPLEA LQIDAIVDTI NDFRLKIAIV AYEPDDMVKE KKMVTLNNEV
Tobacco hornworm  GLAGNDIEED FEIDQIVDFV NDIRASAASV EYEQDAANKE VKHEENMKNK
Human             NLYGKDIKEK ALIDMYIEGI ADLGEMILLL PFSQPEEQDA KLALIQEKTK
Mite              KLNGSNDHEE IRISMAEQQT EDMMAAMIRV CYDANCDKLK PDYLKSLPDC

                  151                                                   200
Cockroach         IPYYTKKFDE VVKANGGYLA AGKLTWADFY FVAILDYLNH MAK.EDLVAN
Anopheles         IPFYLTKLNV IAKENNGHLV LGKPTWADVY FAGILDYLNY LTK.TNLLEN
Tobacco hornworm  YPFQLNKLSE IITKNNGFLA LGRLTWADFV FVGMFDYLKK MLRMPDLEEQ
Human             NRYFPAFEKV LKSHGQDYLV GNKLSRADIH LVELLYYVEE LD..SSLISS
Mite              LKLMSKFVGE .....HAFIA GANISYVDFN LYEYLCHVKV MV..PEVFGQ

                  201                                             245
Cockroach         QPNLKALREK VLGLPAIKAW VAKRPPTDL. .......... .....
Anopheles         FPNLQEVVQK VLDNENVKAY IAKRPITEV. .......... .....
Tobacco hornworm  YPIFKKPIET VLSNPKLKAY LDSAPKKEF. .......... .....
Human             FPLLKALKTR ISNLPTVKKF LQPGSPRKPP MDAKSLEESR KIFRF
Mite              FENLKRYVER MESLPRVSDY IKKQQPKTFN APTSKWNASY A....
```

FIG. 3. **Sequence alignment of cockroach GST and GST enzymes.** *B. germanica* GST showed 42–51% sequence identity with other GST-2 insect enzymes, from mosquito (*Anopheles gambiae,* GenBank L07880), housefly (*M. domestica,* GenBank U02616), fruit fly (*D. melanogaster*, GenBank M95198), and tobacco hornworm (*M. sexta*, GenBank L32092). Alignments of the cockroach-GST sequence with anopheles, tobacco hornworm, human (alpha class, Genbank S27110), and mite GST sequences (O'Neill *et al.*, Ref. 20), obtained using the GCG program, are shown. *B. germanica* GST contains 18 of the 26 invariable residues (marked in *bold*), which have been identified by x-ray crystallography (31). The residues involved in glutathione binding (Tyr^8, Gln^{63}, and Asp^{97} in cockroach-GST) are indicated (·).

Comparison of IgE Antibody Binding to Natural and Recombinant B. germanica GST—Results of antigen binding RIA, using ^{125}I-labeled natural or recombinant *B. germanica* GST, demonstrated that both proteins had excellent IgE Ab reactivity *in vitro*. IgE Ab binding to natural and recombinant *B. germanica* GST was found in 27/40 (67.5%) and 29/40 (72.5%) of patients' sera, respectively. There was a good correlation between IgE Ab binding to natural and recombinant GST (r = 0.67, $p < 0.05$, Fig. 5*A*), and between the RIA and ELISA for

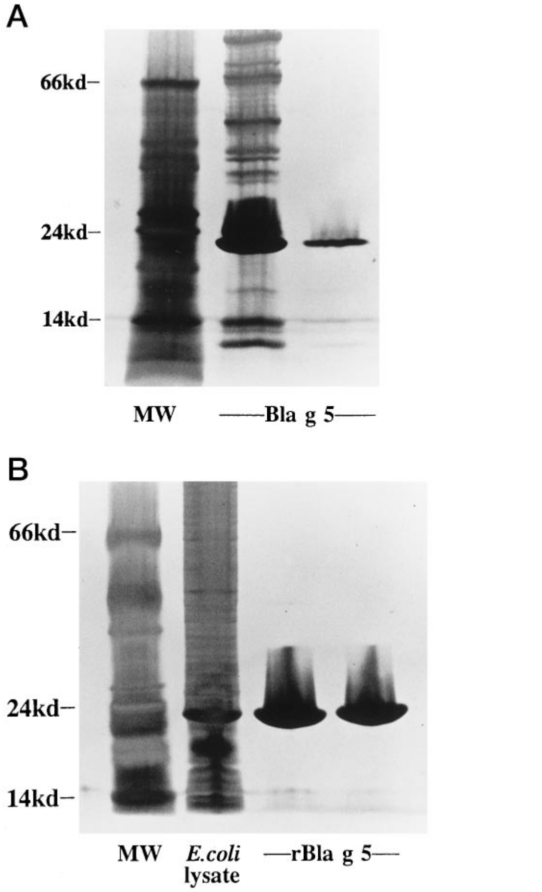

FIG. 4. **SDS-PAGE analysis of natural and recombinant *B. germanica* GST.** *A*, natural *B. germanica* GST was purified from whole body cockroach extract using glutathione affinity chromatography followed by size exclusion. *B*, recombinant *B. germanica* GST was purified from *E. coli* lysates over glutathione-Sepharose. The recombinant allergen migrated as a single 23-kDa band on silver-stained SDS-PAGE gel, which co-migrated with the same molecular mass natural *B. germanica* GST (*A*).

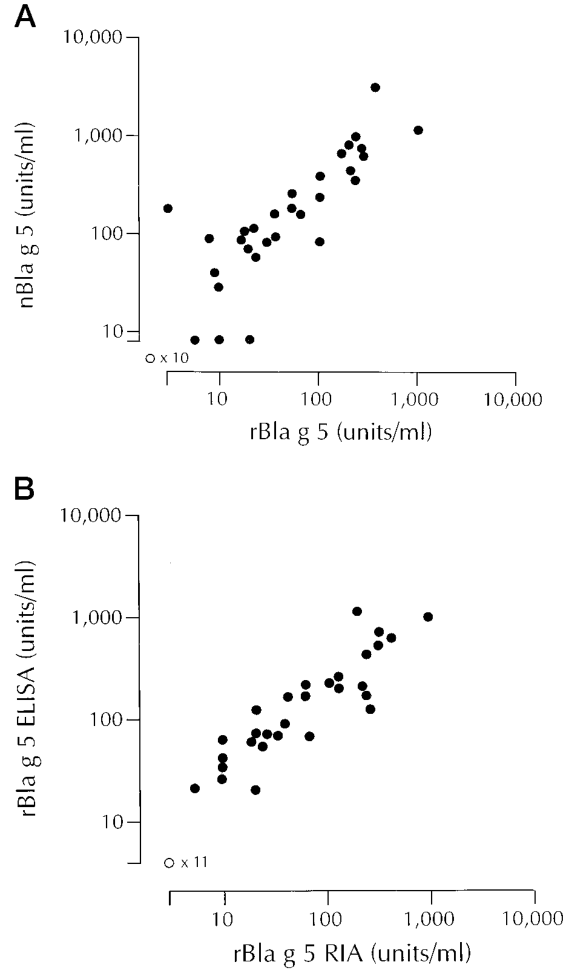

FIG. 5. **Comparison of IgE Ab binding to natural and recombinant Bla g 5.** Sera from 40 cockroach-allergic patients were analyzed for IgE Ab by antigen binding RIA using ^{125}I-labeled allergens (*A*). The prevalence of IgE Ab to the natural or recombinant Bla g 5 was 67.5 and 72.5%, respectively. There was a significant quantitative correlation between the levels of IgE Ab to nBla g 5 and rBla g 5 ($r = 0.67$; $p < 0.05$) and between RIA and ELISA for measuring IgE Ab to rBla g 5 (Fig. 5B; $r = 0.79$, $p < 0.001$).

measuring IgE Ab ($r = 0.79$, $p < 0.001$, Fig. 5*B*). Most patients who had IgE Ab to *B. germanica* GST also had IgG Ab to this allergen, as assessed by RIA. Only 3/40 patients had IgE Ab with no IgG Ab, and conversely only 1/40 had IgG Ab with no detectable IgE antibody. There was no significant correlation between levels of specific IgE and IgG antibodies ($r = 0.41$, $p = 0.09$, data not shown).

Demonstration of Biologic Activity of Recombinant B. germanica GST in Vivo—Quantitative intradermal skin testing was used to evaluate the reactivity of recombinant *B. germanica* GST *in vivo*. Five of the seven cockroach-allergic asthmatic patients who underwent skin testing showed a positive reaction ($>8 \times 8$-mm wheel diameter) 15 min following the injection of 0.03 ml of *B. germanica* GST, at concentrations of 10^{-4}-10^{-1} μg/ml (Table I). All patients with IgE Ab to *B. germanica* GST also had IgE Ab to whole cockroach extract, and there was a good correlation between the levels of serum IgE Ab to *B. germanica* GST and the concentration of allergen giving a positive skin test reaction. Three non-allergic control subjects gave negative skin tests using *B. germanica* GST at a concentration of 1 μg/ml.

DISCUSSION

We have reported the identification, purification, molecular cloning, and sequencing of a glutathione *S*-transferase from the cockroach *Blattella germanica*. Although it was previously known that cockroach extracts contained GST activity (36, 37), this is the first time that cockroach-GST has been purified and the structure of the molecule has been defined. Three pieces of evidence characterize the *B. germanica* protein as an important allergen and GST. First, the high prevalence of IgE Ab binding and potent biologic activity of the allergen *in vivo* (positive immediate skin tests to as little as 3 pg injected allergen). Second, the sequence homology to other GST, particularly those of insect origin (up to 51% sequence identity); and finally, the ability to bind glutathione, allowing purification of both natural and recombinant cockroach-GST.

Sequence analysis has shown that insect GST can be classified into two distinct groups, GST-1 and GST-2 (28–30, 38–42). There is 44–97% sequence homology within the GST-1 and GST-2 subgroups, but only 15–24% homology between the two groups. The GST-1 enzymes have higher identity to cytosolic mammalian theta and alpha GST classes, whereas GST-2 are more similar to the pi and mu classes, and are antigenically distinct from GST-1. Based on the degree of sequence homology, *B. germanica* GST has been assigned to the GST-2 subfamily. An intriguing aspect of our results was that, contrary to other insect GST, the *B. germanica* GST showed low activity against the CDNB substrate. This could imply that *B. germanica* GST has higher affinity against substrates other than CDNB, or may have unusual substrate specificity. *B. ger-*

TABLE I
Skin tests and serum IgE antibodies to recombinant B. germanica allergen Bla g 5

Patient	Skin test to *B. germanica*[a]	Bla g 5		
		Cockroach RAST	Skin test	Serum IgE
		units/ml	*μg/ml*	*cpm bound*[b]
J. S.	8 × 10 mm	1,395	10^{-4}	38,934
S. E.	7 × 15 mm	1,290	10^{-3}	49,554
T. B.	14 × 15 mm	450	10^{-3}	24,384
B. S.	8 × 9 mm	270	10^{-2}	17,097
R. M.	10^{-5}	1,065	10^{-1}	3,704
S. T.	10^{-5}	1,080	>1	243
J. H.	7 × 7 mm	<10	>1	ND[c]
Controls (n = 3)	Neg	<10	>1	382 ± 162[d]

[a] Wheal diameter on prick test or dilution of extract inducing an 8 × 8-mm wheal on intradermal test.
[b] Measured by antigen-binding RIA.
[c] ND, not done.
[d] Mean ± 2 S.D.

manica may also produce other GST isoenzymes with higher CDNB activity than the Bla g 5 allergen.

Insect GST are thought to play an important physiologic role in the detoxification of foods and other substances ingested by cockroach. Up-regulation of GST production in insects is associated with resistance to insecticides (particularly organophosphorates) (38, 43–45). Our findings that *B. germanica* GST is a potent allergen suggest that attempts to control cockroach populations in the homes of allergic patients could, paradoxically, lead to increased GST expression and environmental allergen exposure. Infestation of housing with cockroach can be difficult to control and often requires prolonged treatments with insecticides. The effect of these treatments on cockroach allergen levels are not known. The implication of our results is that insecticide treatments could increase exposure to the GST allergen which in turn could lead to increased risk of allergic symptoms. This hypothesis could be investigated by monitoring *B. germanica* GST levels in the environment.

It has been reported that a house dust mite (*D. pteronyssinus*) allergen, Der p 8, which reacts with IgE Ab in sera from ~40% mite allergic patients, is a GST (20). In addition, *S. mansoni* GST, a promising candidate for a vaccine against schistosomiasis, induces IgE and IgA Ab, which are thought to be beneficial in reducing parasitic infection (21, 46–48). IgE Ab responses to schistosomes may protect against human infection and vaccination with *S. mansoni* GST has been associated with reduced egg production and resistance to infection (in animal models). The degree of sequence homology of *D. pteronyssinus* GST and *S. mansoni* GST with *B. germanica* GST is low (27 and 26.2%, respectively). However, the three-dimensional structures of the alpha, mu, and pi mammalian GST classes (sharing only ~30% sequence identity) have been recently determined by x-ray crystallography and these proteins have similar folding topology (17, 18, 31, 32).

Diagnosis of cockroach allergy is carried out by immediate skin testing or serologic assays for IgE Ab, using extracts of ground cockroach bodies that are not standardized. Production of highly purified recombinant allergens will enable these reagents to be used for diagnostic purposes. A mixture of recombinant cockroach allergens will be required, based on the fact that multiple allergens cause IgE Ab responses. There is a high prevalence of IgE Ab to Bla g 2 (60%), Bla g 4 (40–60%), and Bla g 5 (GST, 70%) among cockroach-allergic patients (12, 13, 24). Each of these allergens is an important cause of sensitization to cockroach and preliminary comparisons show that the *in vivo* purified allergens give positive skin test reactions at 10^{-3}-10^{-5} μg/ml (49). Serologic analyses of ~40 sera suggest that measuring IgE Ab to Bla g 1, Bla g 2, Bla g 4, and Bla g 5 will demonstrate sensitization in 95% of *B. germanica* allergic patients.[2] Bla g 2 and Bla g 4 are not expressed in *P. americana* and recent results showed that *P. americana* extracts did not inhibit IgE Ab binding to Bla g 5 in RIA (12, 13). Thus if *P. americana* produces a GST allergen, it is a different isoform or GST class to *B. germanica* GST. We have recently cloned the cross-reactive Bla g 1 homologue from *P. americana* (Per a 1) and this allergen, in addition to Per a 3, could be used for diagnosis of sensitization to *Periplaneta* species (14).

Over the last few years, the primary structure of most major allergens has been determined and there do no appear to be particular structural features in these molecules that selectively induce IgE Ab responses (50). There is evidence that the biochemical activities of some allergens (*e.g.* Der p 1, bee venom phospholipase A_2) could contribute to allergenicity (50–54). It has also recently been reported that the enzyme leukotriene C4 synthase is a GST (54, 55). However, this enzyme appears to be a unique GST, with no significant sequence homology to other members of the GST family. The possibility that cockroach-GST may potentiate IgE Ab responses in the airways through its enzymatic activity could be investigated using site-directed mutagenesis to modify cockroach-GST at conserved residues involved in binding glutathione.

In summary, we have reported the identification and cloning of a major *B. germanica* allergen Bla g 5, which is a member of the GST superfamily. Recombinant cockroach GST has been produced in *E. coli*, with comparable immunologic activity to the natural protein. Results of these studies will lead to better approaches to control environmental exposure to this ubiquitous insect, including the development of GST inhibitors. In addition, cockroach-GST will provide a model for studying both the cellular and IgE Ab responses to GST and for establishing the role of these proteins in the development of asthma and other allergic diseases.

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