Gene Expression and Tissue Distribution of the Major Human Allergen Bla g 1 in the German Cockroach, *Blattella germanica* L. (Dictyoptera: Blattellidae)

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ABSTRACT   Exposure and sensitization to cockroach allergens is an important risk factor for allergic disease in humans. Despite a recent burgeoning of clinical and socioeconomic studies regarding environmental pervasiveness and human exposure to cockroach allergens, little is known about the basic biology of these proteins. The purpose of this study was to ascertain gene expression patterns and the tissue distribution of *Blattella germanica* allergen 1 (Bla g 1), a perennial indoor environmental allergen, thought to be involved in digestion in cockroaches. We also investigated the relative potential contribution of different life stages of the German cockroach to environmental Bla g 1. Enzyme-linked immunosorbent assay was used to quantify the Bla g 1 contents of feces and various anatomical tissues, and Northern blot analysis was used to elucidate tissue-specific expression of Bla g 1. Results showed that the Bla g 1 protein is most prevalent in the midgut, and the Bla g 1 gene is exclusively expressed by midgut cells. Although Bla g 1 is produced by both sexes and all life stages of the German cockroach, adult females produce and excrete significantly more Bla g 1 in their feces than males or nymphs, even when corrected for body mass or mass of voided feces. Our results show that the concentration of Bla g 1 in feces of adult females is 6- to 7- and 30-fold higher than in adult males and nymphs, respectively, probably because females process more food than other life stages of the German cockroach.

KEY WORDS   German cockroach, *Blattella germanica* allergen 1, cockroach allergen, midgut, digestion

THE GERMAN COCKROACH, *Blattella germanica* L., is a synanthropic pest of considerable economic, veterinary, and medical importance (Schal and Hamilton 1990, Brenner 1995, Zurek and Schal 2004). Populations of this pest are intimately associated with human-built structures, such as homes, food service facilities, and various occupational environments. Since the mid-1970s, cockroaches have been recognized as a major source of indoor allergens, playing a causative role in allergic asthma (Kang 1976, Kang et al. 1979). Recent studies have confirmed that exposure and sensitization to German cockroach allergens can lead to the development and onset of allergic respiratory diseases, such as asthma, in genetically predisposed individuals, particularly in "inner-city," low-income homes, where German cockroach infestations are both frequent and severe (e.g., Rosenstreich et al. 1997).

Several German cockroach–produced human allergens have been identified and cloned (Schou et al. 1990, Stankus et al. 1990, Pollart et al. 1991, Arruda et

al. 1995a–c, Chapman et al. 1998, Pomes et al. 1998, Jeong et al. 2003). While analyses of their deduced amino acid sequences have led to some insight into the presumed function of these proteins, much remains unknown about their biological function, the tissues responsible for their production, and the developmental expression patterns of allergenic proteins.

Natural Bla g 1, originally identified through immunochemical techniques, has a variable molecular weight ranging from 33 to 37 kDa (Schou et al. 1990, Pollart et al. 1991). Bla g 1 isoallergens (cDNA clones Bla g 1.01 and Bla g 1.02) show 70–72% nucleotide sequence homology to the antigenically cross-reactive *Periplaneta americana* L. (American cockroach) allergen 1 (Per a 1), and together they comprise a family of structurally and antigenically related group 1 allergens (Pomes et al. 1998, Melen et al. 1999). A unique feature of this group of cockroach allergens, not previously found in allergen sequences, is that they consist of multiple tandem repeats of ≈100 amino acids (Pomes et al. 1998). Group 1 allergens are very important because they are the only cockroach allergens known to be produced by both *B. germanica* and *P. americana* and are likely common to all pest cockroaches. The nucleotide sequence of Bla g 1 also shows

94% identity to a previously described 4-kb sequence, Bla g Bd90K (Helm et al. 1996). Bla g 1 and Per a 1 share 32–42% deduced amino acid sequence identity with AEG12 of *Aedes aegypti* L. (accession no. AY038041) and ANG12 of *Anopheles gambiae* (Giles) (accession no. Q17040), both of which are induced in the midgut of the female mosquito after a blood meal. Furthermore, Bla g 1 sequences contain myristoylation and trypsin cleavage sites, suggesting that natural Bla g 1 is posttranslationally modified and secreted into the digestive tract, possibly serving a digestive function (Pomes et al. 1998, Smith et al. 2000). The AEG12 protein has also been described as a microvillar membrane protein (Shao and Jacobs-Lorena, accession no. AY050565).

Several tissues of the German cockroach have been shown to be allergenic to sensitized individuals by skin-prick and radioallergosorbent tests (RAST), including the digestive tract, Malpighian tubules, ovaries, oothecae, and exuvia, as well as whole body extracts and feces (Richman et al. 1984, Pollart et al. 1991, Zwick et al. 1991, Musmand et al. 1995). Pomes et al. (1998) showed that higher Bla g 1 titers were associated with the digestive organs, primarily the hindgut and proventriculus.

In this study, we quantify whole body and fecal Bla g 1 contents of nymphs and adult males and females and examine the anatomical distribution and expression patterns of Bla g 1 in various tissues of the German cockroach. Our results show that Bla g 1 is produced exclusively within the midgut and that, because adult females produce more Bla g 1 than males and nymphs, they contribute more to environmental residues of Bla g 1.

Materials and Methods

Insects. Insects were collected from a laboratory colony of insecticide-susceptible German cockroaches (American Cyanamid strain, Princeton, NJ) reared at 27°C, variable ambient relative humidity, and a photoperiod of L12:D12 h, and provided with water and rat chow (Purina no. 5012; Purina Mills, St. Louis, MO).

Bla g 1 Extraction and Quantitative Enzyme-Linked Immunosorbent Assay. Whole cockroaches, dissected tissues, or feces were homogenized in 1% bovine serum albumin (BSA)–phosphate-buffered saline (PBS)–Tween (1% BSA/PBS-T) containing protease inhibitors, using a hand-held pestle motor and sterile disposable pestles (Kimble/Kontes, Vineland, NJ). The homogenate was incubated with agitation on an orbital shaker at 4°C for ≈1 h and centrifuged at 10,000 rpm for 10 min, and the supernatants were collected and stored at −80°C until assay. Bla g 1 titers were measured using a monoclonal capture and polyclonal detector extraction and quantitative enzyme-linked immunosorbent assay (ELISA; INDOOR Biotechnologies, Charlottesville, VA) as described by Pollart et al. (1991). Bla g 1 content is expressed in arbitrary units because there are no national or international reference standards for cockroach allergens.

Whole Body and Fecal Bla g 1. The relationship between age of adult females (stage of the vitellogenic cycle) and amount of Bla g 1 they contained was examined. Females ($n = 10$ per age) were cold-anesthetized, briefly rinsed in PBS, and extracted; the Bla g 1 titers were measured by ELISA.

To examine relative sex or stage differences, feeding-stage cockroaches were assayed for whole body and fecal Bla g 1 titers. Individual 5-d-old adult males ($n = 5$) and females ($n = 5$) and groups of day 3 first instars (20 per replicate, $n = 5$) were cold-anesthetized on ice for 5 min, weighed, and extracted. To collect feces, same-sex groups of day 5 adult males (10 per replicate, $n = 5$) or females (10 per replicate, $n = 5$) were placed in 13 by 18-cm plastic cages with food and water. Twenty day 3 first instars per replicate ($n = 5$) were placed in 60 by 15-mm plastic petri plates and subjected to the same conditions as adults. Feces were collected after 24 h, weighed, and extracted, and the Bla g 1 titers were measured by ELISA.

Tissue Distribution of Bla g 1. Five-day-old adult females ($n = 5$) were cold-anesthetized and rinsed in PBS to remove any external debris. The head, thorax (including wings and legs), and abdomen were dissected and homogenized, and the Bla g 1 titers were measured by ELISA.

Another group of 5-d-old adult females ($n = 10$) was cold-anesthetized and briefly rinsed in PBS, and the following tissues dissected: alimentary tract, ovaries, fat body, abdominal sternites, and abdominal tergites. The anterior and posterior ends of the foregut, midgut, and hindgut were ligated with sterile cotton thread before separation to preserve lumen contents. Tissues were briefly rinsed in PBS after dissection. Hemolymph (1 μl) was collected from a severed cercus into a glass capillary tube before dissection and diluted into 1% BSA/PBS-T. The Bla g 1 titers were measured by ELISA. The total Bla g 1 content of hemolymph was derived by multiplying the measured Bla g 1 content of 1 μl of hemolymph by 21.9 ± 0.84 μl, the hemolymph volume of 5-d-old females (Sevala et al. 1999).

Northern Blot Analysis of Bla g 1 mRNA. Five-day-old adult females were cold-anesthetized and PBS rinsed, and various tissues dissected and immediately placed into TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) in 1.5 ml RNase-free microcentrifuge tubes. First, the entire alimentary tract, whole body without the alimentary tract, foregut, midgut, and hindgut were processed. To determine whether expression occurred in a distinct region of the midgut, it was further divided into a smaller anterior portion containing the gastric caeca (posterior to the proventriculus) and a much larger posterior portion comprised of the remaining midgut (ventriculus). Because preliminary Northern analysis of the gut sections showed that some expression might occur in the hindgut, it was further dissected into a smaller anterior portion (including the Malpighian tubules) and a much larger posterior region, including the colon and rectum. The Malpighian tubules were also dissected separately for analysis. For total RNA extraction, tissues were homogenized in 1 ml TRIzol Reagent, chlo-

roform extracted, precipitated with isopropyl alcohol, and washed with ethanol, and the RNA was resuspended in RNase-free TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.0). The RNA concentration was determined spectrophotometrically at 260 nm. Samples were aliquoted and stored at $-80°C$ until use.

Primer sequences were designed based on the known cDNA sequence of the clone Bla g 1.0101 (Pomes et al. 1998; accession no. AF072219). Forward (5′-CTTCCCGCAACCTCCAAG-3′) and reverse (5′-CTTATCAGTCTTGAGTTCTC-3′) primers were synthesized (GIBCO BRL, Carlsbad, CA) and stored at $-20°C$. First-strand cDNA was synthesized from 8 μg of total RNA isolated from the whole gut of day 5 females using StrataScript reverse transcriptase (Stratagene, La Jolla, CA) in the presence of oligo(dt) at $42°C$. The cDNA was used to amplify a Bla g 1 DNA fragment by polymerase chain reaction (PCR) in a PCRExpress thermal cycler (Thermo Hybaid, Franklin, MA) for 30 amplification cycles (45 s denaturation at $95°C$, 1 min annealing at $58°C$, and 2 min elongation at $72°C$), followed by a final extension at $72°C$ for 10 min. The resultant 617-bp PCR product was purified by gel extraction using a QIAquick kit (QIAGEN, Valencia, CA), according to the manufacturer's protocol, and eluted in 50 μl TE buffer. The gel-purified Bla g 1 DNA fragment was labeled for use as a probe for Northern blot analysis using the BrightStar Psoralen-biotin Nonisotopic Labeling Kit (Ambion, Austin, TX) according to the manufacturer's specifications.

Northern blot analysis was carried out using the NorthernMax kit (Ambion) per manufacturer's protocol. Briefly, total RNA was fractionated on a denaturing formaldehyde-1% agarose gel and transferred to a positively charged nylon membrane. After UV crosslinking, the nylon membrane was prehybridized for >30 min at $42°C$ in hybridization solution and then overnight at $42°C$ in the same solution with 1 pM of biotin-labeled probe. Washing conditions consisted of two low stringency washes in $2\times$ SSC (sodium chloride, sodium citrate) containing 0.1% SDS (sodium dodecyl sulfate) for 5 min each at room temperature, followed by two high stringency washes in $0.1\times$ SSC containing 0.1% SDS for 15 min each at $42°C$. The hybridized probe was detected with streptavidin-alkaline phosphatase using the BrightStar Biodetect kit (Ambion) using the manufacturer's protocol.

Data Analysis. The titers of Bla g 1 in whole body, dissected tissues, and feces were log-transformed and subjected to analysis of variance (ANOVA; PROC GLM) in SAS 8.2 (SAS Institute 2001). In preplanned comparisons, mean separation was by Tukey's studentized range test ($\alpha = 0.05$).

Results

Whole Body and Fecal Bla g 1. Throughout, Bla g 1 content, as determined by ELISA, is expressed in arbitrary units, per manufacturer calibration, because there are no national or international reference standards for cockroach allergens. The Bla g 1 content of
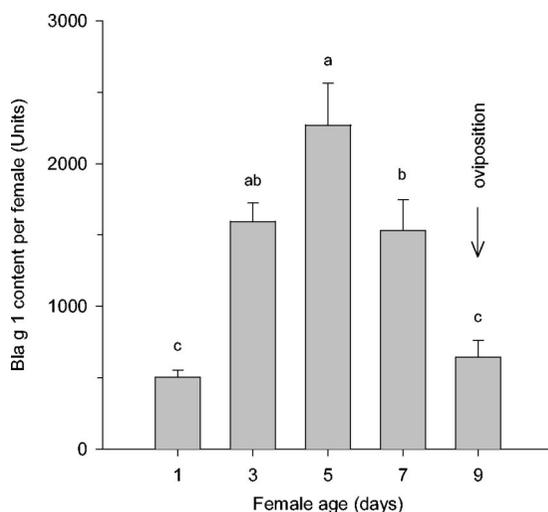

Fig. 1. Changes in the Bla g 1 content of female German cockroaches in relation to early stages of the reproductive cycle. Whole body Bla g 1 content was quantified by ELISA and expressed as units per female $\pm$ SEM ($n = 10$ females). Means with different letters are significantly different (Tukey's studentized range test, $P < 0.05$).

females increased in relation to oocyte maturation during the first 5 d after eclosion, but subsequently declined well before oviposition (Fig. 1). Oviposition normally occurs on days 8–9, followed by a protracted period of "pregnancy," during which the female carries an egg case. It thus seems that the Bla g 1 content is highest in feeding, vitellogenic females and lowest in previtellogenic females and in postoviposition "pregnant" females.

Comparative whole body Bla g 1 titers were determined in feeding-stage adult males (day 5), adult females (day 5), and first-instar nymphs (day 3). Adult females weighed more than twice as much as adult males and nearly 83 times as much as first instars (Table 1; $F_{2,14} = 1,117.45, P < 0.0001$). However, each female contained 3.2-fold more Bla g 1 than each male and 237-fold more than first-instar nymphs ($F_{2,14} = 1,534.87, P < 0.0001$). Corrected for body mass, therefore, females contained significantly more Bla g 1 than males, which in turn contained more than nymphs. Based on these results, females were used for subsequent tissue Bla g 1 assays.

The stage- and sex-specific differences in Bla g 1 were even more dramatic in feces collected over a 24-h period (Table 1). Females not only produced significantly more feces than other stages ($F_{2,14} = 618.47, P < 0.001$), but their fecal Bla g 1 titer was significantly greater when normalized for fecal mass ($F_{2,14} = 270.29, P < 0.0001$). Amazingly, females produced 6.8-fold more Bla g 1 per mg of feces than males and 30-fold more than nymphs. These results suggest that adult females might contribute a disproportionate amount of Bla g 1 to the allergen load in cockroach-infested structures.

Bla g 1 in Various Tissues. To identify the anatomical tissue(s) that contained the most Bla g 1, the head,

Table 1. Whole body and fecal Bla g 1 titers

Bla g 1 source	Stage/age	Mass per insect (mg)[a]	Amount of Bla g 1[a]	
			Units per insect	Units per mg
Whole body	Adult female day 5	$114.5 \pm 2.79a$	$3038.8 \pm 208.64a$	$26.5 \pm 1.33a$
	Adult male day 5	$47.8 \pm 0.94b$	$944.3 \pm 61.33b$	$19.8 \pm 1.57b$
	First instar day 3	$1.4 \pm 0.03c$	$12.8 \pm 1.16c$	$9.2 \pm 0.79c$
Feces (24 h)	Adult female day 5	$3.1 \pm 0.08a$	$1037.5 \pm 122.06a$	$333.1 \pm 42.60a$
	Adult male day 5	$0.2 \pm 0.02b$	$14.5 \pm 2.58b$	$49.0 \pm 6.06b$
	First instar day 3	$0.1 \pm 0.01c$	$0.9 \pm 0.08c$	$11.2 \pm 0.99c$

$n = 5$. Values are means $\pm$ SEM.
[a] Unit is an arbitrary designation for Bla g 1 measurement. Different letters within columns and within Bla g 1 source indicate significant differences using Tukey's studentized range test ($P < 0.05$).

thorax, and abdomen of day 5 adult females were dissected, and the soluble protein fraction was extracted for assay by ELISA. Bla g 1 was present in all three body parts (Fig. 2). However, only trivial amounts were found in the head (0.23 ± 0.064 U Bla g 1), whereas the abdomen contained 8,208-fold more allergen than the head and 51-fold more than the thorax ($F_{2,14} = 70.78$, $P < 0.0001$).

To further determine the tissue distribution of Bla g 1, several tissues and 1 μl hemolymph were extracted from day 5 adult females and assayed by ELISA. While all tissues contained Bla g 1 (Fig. 3), the midgut titer ($2,574 \pm 308$ U) was significantly greater than all others ($F_{2,14} = 128.05$, $P < 0.0001$). The foregut, hindgut, and ovaries contained similar amounts, but ≈89-fold less Bla g 1 than the midgut. Although other tissues, including the abdominal sternites and tergites, fat body, and hemolymph, contained low amounts of Bla g 1, these values (from 1 U in fat body to 16 U in hemolymph) may nevertheless be significant clinically (see Discussion).

Northern Blot Analysis of Tissue Expression. Northern blot analysis was used to determine whether tissues that contained the Bla g 1 protein also expressed Bla g 1 mRNA. A 4-kb transcript hybridizing the Bla g 1 cDNA probe was found to occur exclusively in the midgut, both in the gastric caeca and in the ventriculus (Fig. 4). No expression was observed in the carcass, which excluded the alimentary tract. Because preliminary Northern analysis of the alimentary tract sections showed a faint banding associated with the hindgut, more comprehensive dissections were conducted. The hindgut was systematically separated into anterior (including the Malpighian tubules) and posterior regions (colon and rectum). Furthermore, the Malpighian tubules alone were dissected, to include or exclude them as a source of Bla g 1, and assayed independently of the hindgut. From these assays, we conclude that Bla g 1 expression is limited to the midgut.

Discussion

The German cockroach has a long history of intimate association with humans. It is most often regarded as an unpleasant pest of economic importance and occasionally implicated in transmission of enteric disease. Early observations several decades ago showed that 40–60% of asthmatic patients were sensitized to cockroaches (Bernton and Brown 1964, Kang et al. 1979). More recent studies, particularly those associated with the National Cooperative Inner-City Asthma Study (NCICAS), have established a causal relationship between cockroach infestations and the prevalence of allergic disease. The NCICAS found that ≈37% of asthmatic children were sensitized to German cockroach allergens (Rosenstreich et al. 1997). Among cockroach-allergic patients attending an allergy clinic, 47% were sensitive to purified Bla g 1 (Schou et al. 1990), underscoring the importance of this protein as a major allergen. A number of research and clinical trials have sought to develop approaches to mitigate the harmful effects of allergen exposure. For example, we recently showed that clinically relevant, sustained reductions of Bla g 1 below the sensitization (2 U/g of environmental dust) (Eggleston et al. 1998) and morbidity (8 U/g of dust) (Rosenstreich et al. 1997) thresholds for exposure could be achieved with pest control efforts, sanitation, and resident education (Arbes et al. 2003, 2004).

Despite the recent plethora of socioeconomic surveys, clinical reports, and environmental residue studies about the impact of cockroach allergens on cock-
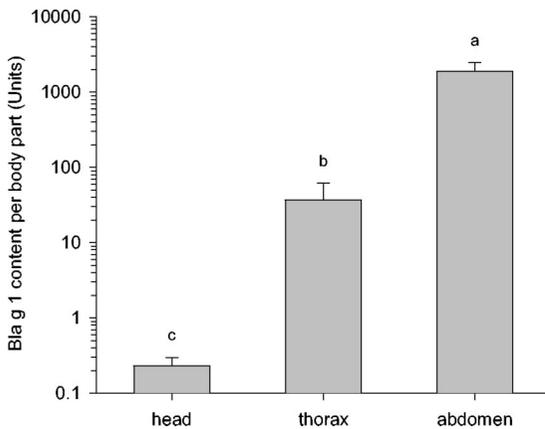

Fig. 2. Distribution of Bla g 1 in the head, thorax, and abdomen of day 5 adult female German cockroaches. Bla g 1 content was quantified by ELISA and expressed as units per body part $\pm$ SEM ($n = 5$ females). Means with different letters are significantly different (Tukey's studentized range test, $P < 0.05$).
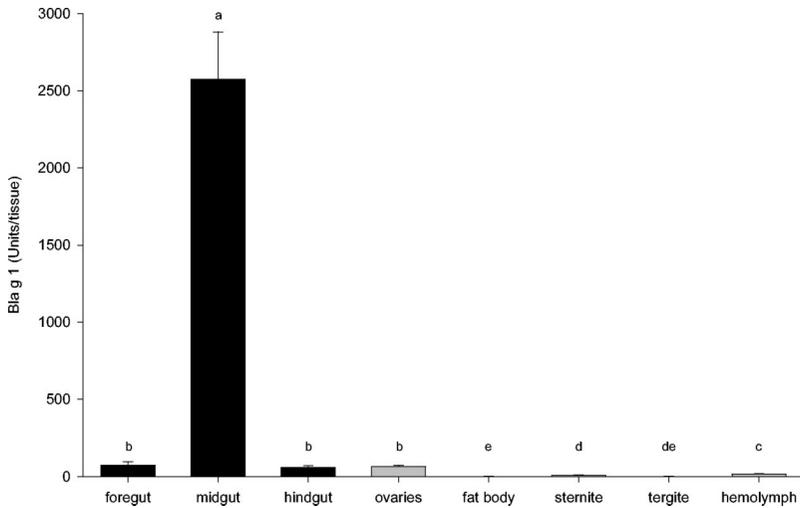
Fig. 3. Anatomical tissue distribution of Bla g 1 in day 5 adult female German cockroaches. Bla g 1 content was quantified by ELISA and expressed as units per tissue $\pm$ SEM ($n = 10$). Means with different letters are significantly different (Tukey's studentized range test, $P < 0.05$).

roach-sensitized individuals and the prevalence of allergens in the indoor environment, little is known about the basic biology of these allergenic proteins. We aimed to elucidate the anatomical tissue that produces Bla g 1 and the relationships between sex, stage, and age of cockroaches and Bla g 1 production.

Midgut Produces Bla g 1. Several cockroach tissues have been considered as sources of allergens (Richman et al. 1984, Zwick et al. 1991), but the anatomical tissue(s) responsible for allergen production has not been clearly defined for any of the six *B. germanica* allergens. Bla g 1 sequence analysis, ELISA (Pomes et al. 1998), immunohistochemistry (Zwick et al. 1991), and its presence in feces suggested that this allergen might be secreted into the alimentary canal. Using a highly specific ELISA, we first showed that the abdomen contained 51 times more Bla g 1 than the thorax, whereas Bla g 1 levels in the head were barely detectable (Fig. 2). Next, we systematically dissected, extracted, and quantified Bla g 1 levels in various abdominal tissues. While previous research implicated the hindgut and proventriculus (Pomes et al. 1998), our results showed that the midgut contained 44 times more Bla g 1 than the hindgut and nearly 36 times more than the foregut, which contains the proventriculus (Fig. 3). Results from RNA hybridization of various regions of the gut showed that Bla g 1 was not only secreted into the midgut, but also produced by midgut cells (Fig. 4). Our 617-bp probe hybridized to a single 4-kb mRNA, which showed 93% identity (BLAST; Altschul et al. 1997) to Bla g Bd90K (Helm et al. 1996), now considered to be Bla g 1, and 99% identity to previously reported Bla g 1 clones (Pomes et al. 1998). Careful dissections of the gastric caeca and the remaining midgut (ventriculus) and the Malphigian tubules, which are associated with the anterior hindgut, further confirmed that Bla g 1 expression was exclusively within the midgut.

Other important arthropod allergens are associated with the alimentary canal. Bla g 2, an allergenic aspartic protease, was found in highest titers in the gut of German cockroaches (Arruda et al. 1995c). Immunohistochemical studies have also shown that Der p I, a major allergen produced by the house-dust mite *Dermatophagoides pteronyssinus* (Trouessart) and thought to be a digestive enzyme, is found in highest concentrations just anterior to the hindgut (presumably the midgut) (Thomas et al. 1991).

The digestive system of the cockroach is relatively simple, with food processing, digestion, and absorption occurring in one or more of the morphologically distinct regions. Most digestion occurs in the foregut, primarily by enzymes passing forward from the midgut that act on the slow-moving food bolus (Dow 1986, Terra et al. 1996). The midgut epithelium contains absorptive and secretory cells, the latter producing digestive enzymes, and a dense array of apical microvilli. Although the function of Bla g 1 is currently unknown, amino acid sequence analysis suggests that Bla g 1 follows a secretory pathway into the digestive tract (Pomes et al. 1998). Based on the data presented here and our unpublished data on the relationship between Bla g 1 and food intake, we speculate that Bla g 1 is secreted by the midgut epithelium and serves a digestive function. This is in keeping with a 40% deduced amino acid identity to AEG12 of *Aedes aegypti* (accession no. AY038041) and ANG12 of *Anopheles gambiae* (accession no. Q17040). Both proteins are expressed in the midgut of female mosquitoes and undergo changes in expression relative to blood feeding (Müeller and Crisanti, accession mo. Q17040; Morlais et al. 2003). Expression of AEG12 is female-specific; it is low in the female gut before blood feeding, significantly up-regulated 12 h after feeding, and thereafter declines to prefeeding levels (Morlais et al. 2003). It is worth noting that the mosquito proteins
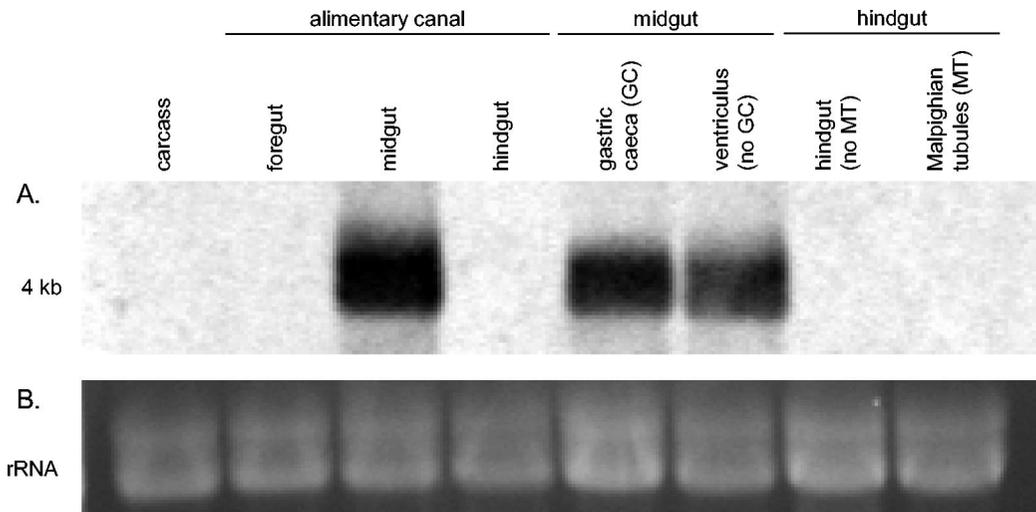
Fig. 4. (A) Northern blot of Bla g 1 expression in alimentary tissues of day 5 adult females. Total RNA (6 μg) was fractionated by 1% agarose-formaldehyde gel electrophoresis. The blot was hybridized with a Bla g 1 cDNA probe. The estimated size of the Bla g 1 mRNA is displayed at the left. (B) Ethidium bromide–stained rRNA, indicating relative equal loading of RNA in each lane.

lack the multiple tandem amino acid repeats found in Bla g 1.

However, the AEG12 protein has also been described as a microvillar membrane protein (Shao and Jacobs-Lorena, accession no. AY050565), and it is possible that Bla g 1 might serve a structural rather than an enzymatic function. This might be consistent with the large amounts of Bla g 1 found in cockroach feces.

Time-Course of Bla g 1 in Females: Relation to Food Intake? We showed that adult females modulate production of Bla g 1 in relation to stages of their reproductive cycle. The female's Bla g 1 content rises through day 5 (Fig. 1), a pattern that parallels early stages of vitellogenesis, or events associated with it, such as an escalating juvenile hormone titer (reviewed in Schal et al. 1997). However, Bla g 1 declines considerably after day 5, whereas the juvenile hormone titer continues to rise through day 7 (Sevala et al. 1999). It thus seems that modulation of Bla g 1 content is less related to the changes in the gonadotropic hormone and more linked to patterns of food intake. Food intake in female *B. germanica* peaks around days 2–4 and dramatically declines as the female converts nutrients to yolk proteins, provisions the eggs, oviposits, and incubates her developing embryos for 20 d, during which only low and sporadic bouts of feeding occur (Hamilton and Schal 1988, Schal et al. 1994, DeMark and Bennett 1995, Osorio et al. 1998). A link between Bla g 1 and food intake is consistent with our observations that Bla g 1 is expressed exclusively in the midgut, and it has a high sequence identity to mosquito midgut proteins that are induced by a blood meal and involved in digestion. However, the hypothesis of a link between feeding and Bla g 1 production will need to await quantification of Bla g 1 levels throughout the reproductive cycle and results from starvation and feeding experiments, which are in progress.

Comparative Bla g 1 Production in Adults and Nymphs. A comparison of whole body and fecal Bla g 1 of feeding-stage adults and first instars showed some remarkable disparities among them. Not surprisingly, more or less consistent with differences in body mass, the Bla g 1 content of day 5 females was three times higher than in adult males and 237 times as much as in nymphs (Table 1). However, sex and stage differences persisted after Bla g 1 levels were normalized for fresh body mass of the assayed cockroaches, indicating that adult females have higher Bla g 1 titers, probably because of their enormous capacity to eat and digest more food during a short vitellogenic burst.

German cockroach feces are widely known as a source of allergens (Richman et al. 1984, Zwick et al. 1991), and adult females also produced significantly more feces over a 24-h period than either males or first-instar nymphs. While adult females modulate food intake in relation to reproduction, adult males feed comparatively little, without any apparent cycles (Hamilton and Schal 1988, DeMark and Bennett 1995). Nymphs of all instars feed extensively during the early one-half of each intermolt period and little or not at all in preparation for the molt (Valles et al. 1996, Young and Schal 1997). Presumably, feces production would follow a similar pattern, although with a slight delay related to the passage of food through the gut.

Therefore it is not surprising that the larger females defecated 71-fold more Bla g 1 than males and a striking 1,179-fold more than nymphs. However, even when corrected for fecal mass, the concentration of Bla g 1 in female feces was 6.8-fold more than in male feces and 30-fold more than in the feces of nymphs. In 24 h, a single female can produce ≈520 times the proposed human sensitization threshold (2 U/g of environmental dust) and ≈130 times the morbidity threshold (8 U/g of dust) for exposure to Bla g 1.

Richman et al. (1984) suggested, based on direct RAST analysis of patient sera, that fecal extracts contain less allergenically relevant proteins than extracts from whole bodies or exuvia. Our results confirm higher Bla g 1 content in whole body extracts than in feces. However, the concentration of Bla g 1 in feces (units per milligram) is ≈12-fold higher than in whole body extracts of females and ≈1.2- to 2.5-fold higher in nymphs and males. Nevertheless, it is difficult to extrapolate our results with Bla g 1 to studies using blends of allergens because the differential contribution of various allergens to allergic disease has not been resolved.

Although adult females might contribute a disproportionate amount of Bla g 1 to the allergen load in infested structures, several features of males and small nymphs would suggest that they nevertheless might be highly clinically relevant. Adult males tend to forage over larger ranges than females and therefore might distribute their feces over larger spatial spans than other stages. Small nymphs are not only more numerous than adults, but they also produce much smaller fecal pellets that are probably more likely to adhere to clinically relevant dust that can become airborne and thus available for contact with the pulmonary mucosa of allergic individuals. Moreover, all stages of the German cockroach can engage in necrophagous behavior (Gahlhoff et al. 1999), which could concentrate allergenic proteins in feces. However, small nymphs facultatively ingest feces (coprophagy) more than other life stages (Kopanic and Schal 1999, Kopanic et al. 2001), and this behavior, coupled with the tendency of small nymphs to remain near their birth site, would result in the coprophagous ingestion of the more concentrated adult Bla g 1 and its processing into smaller, more clinically relevant particles.

Acknowledgments

References Cited

Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389–3402.

Arbes, S. J., Jr., M. Sever, J. Archer, E. H. Long, J. C. Gore, C. Schal, M. Walter, B. Neubler, B. Vaughn, H. Mitchell, E. Liu, N. Collette, P. Adler, M. Sandel, and D. C. Zeldin. 2003. Abatement of cockroach allergen (Bla g 1) in low-income, urban housing: a randomized controlled trial. J. Allergy Clin. Immunol. 112: 339–345.

Arbes, S. J., Jr., M. Sever, J. Mehta, J. C. Gore, C. Schal, B. Vaughn, H. Mitchell, and D. C. Zeldin. 2004. Abatement of cockroach allergens (Bla g 1 and Bla g 2) in low-income, urban housing: month 12 continuation results. J. Allergy Clin. Immunol. 113: 109–114.

Arruda, L. K., L. D. Vailes, D. C. Benjamin, and M. D. Chapman. 1995a. Molecular cloning of German cockroach (Blattella germanica) allergens. Int. Arch. Allergy Immunol. 107: 295–297.

Arruda, L. K., L. D. Vailes, M. L. Hayden, D. C. Benjamin, and M. D. Chapman. 1995b. Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycins) as a cause of IgE antibody responses. J. Biol. Chem. 270: 31196–31201.

Arruda, L. K., L. D. Vailes, B. J. Mann, J. Shannon, J. W. Fox, T. S. Vedvick, M. L. Hayden, and M. D. Chapman. 1995c. Molecular cloning of a major cockroach (Blattella germanica) allergen, Bla g 2. J. Biol. Chem. 270: 19563–19568.

Bernton, H. S., and H. Brown. 1964. Insect allergy, preliminary studies of the cockroach. J. Allergy. 35: 506–513.

Brenner, R. J. 1995. Medical and economic significance, pp. 77–92. In M. K. Rust, J. M. Owens, and D. A. Reierson (eds.), Understanding and controlling the German cockroach. Oxford University Press, New York.

Chapman, M. D., L. D. Vailes, E.M.L.K. Arruda, and A. Pomes. 1998. Source characterization and molecular structure of cockroach allergens. Rev. Francaise D'Allergologie. 38: 842–845.

DeMark, J. J., and G. W. Bennett. 1995. Adult German cockroach (Dictyoptera, Blattellidae) movement patterns and resource consumption in a laboratory arena. J. Med. Entomol. 32: 241–248.

Dow, J.A.T. 1986. Insect midgut function, pp. 187–328. In P. D. Evans and V. B. Wigglesworth (eds.), Advances in insect physiology. Academic, Orlando, FL.

Eggleston, P. A., D. Rosenstreich, H. Lynn, P. Gergen, D. Baker, M. Kattan, K. M. Mortimer, H. Mitchell, D. Ownby, R. Slavin, and F. Malveaux. 1998. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. J. Allergy Clin. Immunol. 102: 563–570.

Gahlhoff, J. E., Jr., D. M. Miller, and P. G. Koehler. 1999. Secondary kill of adult male German cockroaches (Dictyoptera: Blattellidae) via cannibalism of nymphs fed toxic baits. J. Econ. Entomol. 92: 1133–1137.

Hamilton, R. L., and C. Schal. 1988. Effects of dietary protein levels on reproduction and food consumption in the German cockroach (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. 81: 969–976.

Helm, R., G. Cockrell, J. S. Stanley, R. J. Brenner, W. Burks, and G. A. Bannon. 1996. Isolation and characterization of a clone encoding a major allergen (Bla g Bd90K) involved in IgE-mediated cockroach hypersensitivity. J. Allergy Clin. Immunol. 98: 172–180.

Jeong, K. Y., J. Lee, I.-Y. Lee, H.-I. Ree, C.-S. Hong and T.-S. Yong. 2003. Allergenicity of recombinant Bla g 7, German cockroach tropomyosin. Allergy. 58: 1059–1063.

Kang, B. 1976. Study on cockroach antigen as a probable causative agent in bronchial asthma. J. Allergy Clin. Immunol. 58: 357–365.

Kang, B., D. Vellody, H. Homburger, and J. W. Yunginger. 1979. Cockroach cause of allergic asthma. Its specificity and immunologic profile. J. Allergy Clin. Immunol. 63: 80–86.

Kopanic, R. J., and C. Schal. 1999. Coprophagy facilitates horizontal transmission of bait among cockroaches (Dictyoptera: Blattellidae). Environ. Entomol. 28: 431–438.

Kopanic R. J. Jr., G. L. Holbrook, V. Sevala, and C. Schal. 2001. An adaptive benefit of facultative coprophagy in the German cockroach *Blattella germanica* (L.). Ecol. Entomol. 26: 154–162.

Melen, E., A. Pomes, L. D. Vailes, L. K. Arruda, and M. D. Chapman. 1999. Molecular cloning of Per a 1 and definition of the cross-reactive Group 1 cockroach allergens. J. Allergy Clin. Immunol. 103: 859–864.

Morlais, I., A. Mori, J. R. Schneider, and D. W. Severson. 2003. A targeted approach to the identification of candidate genes determining susceptibility to *Plasmodium gallinaceum* in *Aedes aegypti.* Mol. Genet. Genom. 269: 753–764.

Musmand, J. J., W. E. Horner, M. Lopez, and S. B. Lehrer. 1995. Identification of important allergens in German cockroach extracts by sodium dodecylsulfate-polyacrylamide gel electrophoresis and Western blot analysis. J. Allergy Clin. Immunol. 95: 877–885.

Osorio, S., M. D. Piulachs, and X. Bellés. 1998. Feeding and activation of corpora allata in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae). J. Insect Physiol. 44: 31–38.

Pollart, S. M., D. E. Mullins, L. D. Vailes, M. L. Hayden, T.A.E. Platts-Mills, W. M. Sutherland, and M. D. Chapman. 1991. Identification, quantitation, and purification of cockroach allergens using monoclonal antibodies. J. Allergy Clin. Immunol. 87: 511–521.

Pomes, A., E. Melen, L. D. Vailes, J. D. Retief, L. K. Arruda, and M. D. Chapman. 1998. Novel allergen structures and tandem amino acid repeats derived from German and American cockroach. J. Biol. Chem. 273: 30801–30807.

Richman, P. G., H. A. Khan, P. C. Turkeltaub, F. J. Malveaux, and H. Baer. 1984. The important sources of German cockroach allergens as determined by RAST analyses. J. Allergy Clin. Immunol. 73: 590–595.

Rosenstreich, D. L., P. Eggleston, M. Kattan, D. Baker, R. G. Slavin, P. Gergen, H. Mitchell, K. McNiff-Mortimer, H. Lynn, D. Ownloy, and F. Malveaux. 1997. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N. Engl. J. Med. 336: 1356–1363.

SAS Institute. 2001. SAS/STAT user's guide, version 8.2. SAS Institute, Cary, NC.

Schal, C., and R. L. Hamilton. 1990. Integrated suppression of synanthropic cockroaches. Annu. Rev. Entomol. 35: 521–551.

Schal, C., X. Gu, E. L. Burns, and G. J. Blomquist. 1994. Patterns of biosynthesis and accumulation of hydrocarbons and contact sex-pheromone in the female German cockroach, *Blattella germanica.* Arch. Insect Biochem. Physiol. 25: 375–391.

Schal, C., G. L. Holbrook, J.A.S. Bachmann, and V. L. Sevala. 1997. Reproductive biology of the German cockroach: juvenile hormone as a pleiotropic master regulator. Arch. Insect Biochem. Physiol. 35: 405–426.

Schou, C., P. Lind, E. Fernandez-Caldas, R. F. Lockey, and H. Lowenstein. 1990. Identification and purification of an important cross-reactive allergen from American (*Periplaneta americana*) and German (*Blattella germanica*) cockroach. J. Allergy Clin. Immunol. 86(935–946).

Sevala, V., S. Shu, S. B. Ramaswamy, and C. Schal. 1999. Lipophorin of female *Blattella germanica:* characterization and relation to hemolymph titers of juvenile hormone and hydrocarbons. J. Insect Physiol. 45: 431–441.

Smith, A. M., A. Pomes, and M. D. Chapman. 2000. Molecular biology of indoor allergens. Clin. Rev. Allergy Immunol. 18: 265–283.

Stankus, R. P., W. E. Horner, and S. B. Lehrer. 1990. Identification and characterization of important cockroach allergens. J. Allergy Clin. Immunol. 86: 781–786.

Terra, W. R., C. Ferreira, and J. E. Baker. 1996. Compartmentalization of digestion, pp. 206–235. *In* M. J. Lehane and P. F. Billingsley (eds.), Biology of the insect midgut. Chapman & Hall, London, United Kingdom.

Thomas, B., P. Heap, and F. Carswell. 1991. Ultrastructural localization of the allergen *Der p* I in the gut of the house dust mite *Dermatophagoides pteronyssinus.* Int. Arch. Allergy Immunol. 94: 365–367.

Valles, S. M., C. A. Strong, and P. G. Koehler. 1996. Inter- and intra-instar food consumption in the German cockroach, *Blattella germanica.* Entomol. Exp. Appl. 79: 171–178.

Young, H. P., and C. Schal. 1997. Cuticular hydrocarbon synthesis in relation to feeding and developmental stage in nymphs of *Blattella germanica* (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. 90: 655–663.

Zurek, L., and C. Schal. 2004. Evaluation of the German cockroach (*Blattella germanica*) as a vector for verotoxigenic *Escherichia coli* F18 in confined swine production. Vet. Microbiol. 101: 263–267.

Zwick, H., W. Popp, K. Sertl, H. Rauscher, and T. Wanke. 1991. Allergenic structures in cockroach hypersensitivity. J. Allergy Clin. Immunol. 87: 626–630.