

German cockroach extract increases bronchial epithelial cell interleukin-8 expression

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Summary

Background Cockroach exposure has been recognized as a common trigger for asthma. While dust mite and *Aspergillus fumigatus* aeroallergens have been noted to have direct effects on airway epithelium, direct effects of cockroach proteins have not been determined.

Objective The purpose of this study was to investigate whether cockroach extract has a direct pro-inflammatory effect on airway epithelium.

Methods We examined the effect of crude German cockroach (*Blattella germanica*) extract on IL-8 expression in a human bronchial epithelial cell line (16HBE14o-cells) and primary human bronchial epithelial cells. Transcription from the IL-8 promoter and protein abundance were determined by reporter assay and enzyme-linked immunosorbent assay (ELISA), respectively. Endotoxin levels in the crude cockroach extracts were determined using the Limulus Amebocyte Lysate assay. Protease activity was assessed using Azocoll as a substrate.

Results We found that crude cockroach extract induced a synergistic increase in TNF- α -induced transcription from the IL-8 promoter. The synergistic effect was observed with as little as 0.3 $\mu\text{g/mL}$ of crude cockroach extract, while larger concentrations (30 $\mu\text{g/mL}$) approximately doubled TNF- α -induced IL-8 promoter activity. Similar effects of cockroach extract on IL-8 protein abundance were observed in both 16HBE14o- and primary human bronchial epithelial cells. Addition of endotoxin at concentrations found in the cockroach extract had no effect on TNF- α -mediated IL-8 expression. The serine protease inhibitors aprotinin and phenylmethylsulphonyl fluoride abolished cockroach-induced synergy, while the cysteine protease inhibitors E64 and leupeptin had little effect. Measurement of protease activity using Azocoll as a substrate confirmed the presence of protease activity in cockroach extracts. Addition of recombinant Bla g 2, Bla g 3 and Bla g 5 had no effect on TNF- α -induced IL-8 promoter activation. Finally, cockroach extract also increased TNF- α -induced transcription from the IL-6 promoter.

Conclusions German cockroach extract contains novel serine protease activity which has a direct pro-inflammatory effect on airway epithelial cells

Keywords *Blattella germanica*, human bronchial epithelial cells, serine protease, tumour necrosis factor- α

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Introduction

Cockroach sensitization has been recognized as an important cause of asthma for over 30 years. Cockroaches produce potent allergens which may elicit an IgE antibody response in predisposed individuals. IgE to cockroach is strongly associated with asthma, and exposure to cockroach is a major risk factor for hospital emergency room visits [1–3] and morbidity [4] among inner city children with asthma. At least three studies suggest that exposure to cockroach might be of particular importance

relative to other aeroallergens. Rosenstreich et al. [4] found that children who were both allergic to cockroach allergen and exposed to high levels of this allergen had greater hospitalization rates, unscheduled medical visits and more days of wheezing for asthma per year than other children, whereas similar patterns were not found for the combination of allergy to dust mites or cat dander and high levels of the allergen. In a prospective birth-cohort study of children of asthmatic/allergic parents from metropolitan Boston, the level of cockroach allergen, but not dust mite or cat allergen, was a significant predictor of recurrent wheeze [5]. Finally, children exposed in the first 3 months of life to cockroach, but not house dust mite or cat, develop allergen-specific T cell proliferative responses and repeated wheeze [6]. Taken together, these studies have led investigators to speculate that cockroach allergens may promote sensitization through direct pro-inflammatory effects on the airways [7].

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At present there is no evidence that cockroach allergens act on the respiratory tract other than by means of IgE-mediated sensitization. However, other allergens with enzymatic activity have been demonstrated to have a direct effect on airway epithelium. The house dust mite (*Dermatophagoides pteronyssinus*) cysteine protease Der p 1 causes disruption of intercellular tight junctions, detachment of lung epithelial cells and epithelial cell release of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-8 and IL-6 *in vitro* [8, 9]. Proteases from *Aspergillus fumigatus* induce IL-6 and IL-8 production in airway epithelial cell lines [10, 11].

The principal domestic cockroach species are the German cockroach (*Blattella germanica*) and the American cockroach (*Periplaneta americana*). *B. germanica* is a small cockroach that commonly infests houses in the US, while *P. americana* is larger and commonly infests larger buildings such as schools and hospitals [7]. Several allergens have already been cloned from *B. germanica*. Bla g 2 shows sequence homology to aspartic proteases and was found in high concentrations in cockroach digestive organs [12]. However, there are critical substitutions in the enzymatic active site and is thought to be an inactive protease [7, 13]. Bla g 4 is a ligand-binding protein or calycin (lipocalin) which has been implicated in eliciting an IgE response [14]. Bla g 5 has sequence homology to glutathione S-transferase superfamily proteins, which are important for detoxification of endogenous and xenobiotic toxic compounds [15]. Bla g 6 is a troponin C [7, 16]. Bla g 1 contains multiple tandem amino acid repeats and shares sequence homology to the mosquito protein precursor ANG 12 [17].

Although none of the characterized cockroach allergens is thought to be an active protease, protease activity has been noted in extracts of *B. germanica* [13] and *P. americana* [18–20]. Many other allergens have sequence homology with known enzymes or have functional enzymatic activity. The house dust mite (*Dermatophagoides pteronyssinus*) contains allergens which include cysteine proteases, trypsins, chymotrypsins and serine proteases [8]. Domestic cats contain an allergen, *Felis domesticus* (Fel d 1), which degrades gelatin and fibronectin [21].

In the present study, we demonstrate that, in cultured human bronchial epithelial cells, *B. germanica* extracts synergistically increase TNF- α -induced transcription from the IL-8 promoter, and that the observed IL-8 expression is dependent on serine protease activity. Together, these data suggest that German cockroach extract contains at least one protein with serine protease activity that may have direct pro-inflammatory effects on airway epithelial cells.

Methods

Cell culture

A derivative of 16HBE14o-human bronchial epithelial cells, provided by S. White (University of Chicago), was studied. Cell lines were originally established from bronchial epithelial tissue by transfection with pSVori-, which contains the origin-defective SV40 genome [22]. Unlike the parental line, these cells do not grow in distinct clusters and demonstrate improved transfection efficiency. Cultures show specific immunostaining with pan-cytokeratin c11 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), bind galactose or galactosamine-specific lectins particular to basal epithelial cells [23] and express

β 1-, α 2-, α 3- and α 6-integrin subunits on their cell surface [24]. Cells were grown on coated plates (fibronectin, 10 μ g/mL; collagen, 30 μ g/mL; bovine serum albumin, 100 μ g/mL) in minimum essential medium with 10% fetal bovine serum, 1% penicillin-streptomycin and 200 mM of L-glutamine.

Normal human bronchial epithelial cells (Clonetics, Walkersville, MD, USA) were grown in basal medium supplemented with bovine pituitary extract (65 ng/mL), hydrocortisone (0.5 μ g/mL); epidermal growth factor (0.5 ng/mL), epinephrine (0.5 μ g/mL), transferrin (10 μ g/mL), insulin (5 μ g/mL), retinoic acid (0.1 μ g/mL), triiodothyronine (6.5 ng/mL); gentamicin (50 μ g/mL) and amphotericin B (50 μ g/mL).

Preparation of whole body cockroach extracts

Lyophilized whole body German cockroach (*Blattella germanica*) extracts were purchased from Greer Laboratories (Lenoir, NC, USA). Four separate lots (X8, R15, R14 and R13) were studied. Extracts were suspended in endotoxin-free double-distilled water and centrifuged to remove debris (13 000 g for 2 min at 4 °C).

Transient transfection of human airway epithelial cells

The –162/+44 fragment of the human IL-8 promoter subcloned into a luciferase reporter was obtained from A. Brasier (University of Texas, Galveston) [25, 26]. The –158/+11 fragment of the human IL-6 promoter subcloned into a luciferase reporter was obtained from S. Barik (University of South Alabama, Mobile) [27]. pCMV-LacZ was provided by M. Rosner (University of Chicago). Cells were grown to 70% confluence and transfected with Lipofectamine (Gibco Invitrogen, Carlsbad, CA, USA), as described [28]. After 8 h serum starvation, selected cultures were treated with TNF- α (3 ng/mL, R & D Systems, Minneapolis, MN, USA), cockroach extract (30 μ g/mL) or both. In selected experiments, cockroach extract was incubated with aprotinin (10 μ g/mL), phenylmethylsulphonylfluoride (PMSF, 100 μ M), E64 (10 ng/mL), or leupeptin (100 μ M; each from Sigma Chemical, St Louis, MO, USA) for 15 min prior to addition to the cells. In other experiments, cells were incubated with *Escherichia coli* endotoxin (BioWhittaker, Walkersville, MD, USA) or recombinant Bla g 2, Bla g 4 or Bla g 5 (3 μ g/mL; Indoor Technologies, Charlottesville, VA, USA). Sixteen hours after treatment, cells were harvested and analysed for luciferase and β -galactosidase activities [28, 29].

Measurement of protein abundance

Cells were growth factor deprived for 8 h and treated overnight with TNF- α , cockroach extract or both. Supernatants were collected and centrifuged to remove debris (13 000 g, 3 min). IL-8 and IL-6 protein abundance was measured by enzyme-linked immunosorbent assay (ELISA, Amersham Life Sciences, Arlington Heights, IL, USA and Pierce Endogen, Rockford, IL, USA, respectively).

Measurement of protease activity

Cockroach extract protease activity was measured by the Azocoll method [30]. Azocoll is an insoluble protein-dye conjugate hydrolysed by a variety of proteinases to yield soluble peptides. A standard curve was generated by digestion of Azocoll with known concentrations of subtilisin (Type VII protease, Sigma Chemical).

Statistical analysis

When applicable, statistical significance was assessed by one-way repeated measures analysis of variance (ANOVA). Differences identified by ANOVA were pinpointed by Student–Newman–Keuls' multiple range test.

Results

German cockroach synergistically increases TNF- α -induced IL-8 expression

We hypothesized that exposure to cockroach proteins may have direct pro-inflammatory effects on human airway epithelial cells. To test this, we transiently transfected SV40-transformed human bronchial epithelial cells (16HBE14o-cells) with a cDNA encoding the –162/+44 fragment of the IL-8 promoter. Treatment with crude cockroach extract alone elicited minor effects on transcription from the IL-8 promoter. However, addition of crude cockroach extract (0.3–30 $\mu\text{g/mL}$) significantly increased TNF- α (3 ng/mL)-induced transcription from the IL-8 promoter (Fig. 1a). A range of TNF- α concentrations from 0.1 to 10 ng/mL was originally tested. A concentration of 3 ng/mL TNF- α was chosen as we found the largest level of synergy with cockroach extract with this concentration. However, it should be noted that we found a synergistic, albeit smaller, effect with concentrations as low as 0.1 ng/mL TNF- α (40% increase in IL-8 promoter activity). Increased IL-8 promoter activity was associated with an increase in IL-8 protein abundance in both 16HBE14o-cells (Fig. 1b) and primary human bronchial epithelial cells (Fig. 1c). Together, these data demonstrate that German cockroach extract increases IL-8 mRNA and protein expression in human airway epithelial cells.

We tested whether crude cockroach extract elicited cell damage (trypan blue exclusion, lactase dehydrogenase assays) or induced cell proliferation (cell counting, [^3H]-thymidine incorporation assay). At concentrations at or below 100 $\mu\text{g/mL}$, there were no significant effects on any parameters studied (data not shown).

Crude cockroach synergistically increases TNF- α -induced IL-6 expression

We hypothesized that other cytokines would also be affected by treatment with cockroach extract. We tested the effect of crude cockroach extract on TNF- α -induced transcription from the IL-6 promoter. 16HBE14o-cells were transiently transfected with α -galactosidase and a cDNA encoding the –1158/+11 fragment of the IL-6 promoter [27]. Addition of crude cockroach extract (30 $\mu\text{g/mL}$) synergistically increased TNF- α -induced IL-6 promoter activity (Fig. 2a) and protein expression (Fig. 2b).

Endotoxin does not play a role in the synergistic effect

Endotoxin in concentrations of 10–100 $\mu\text{g/mL}$ has been reported to increase IL-8 expression in airway epithelial cells [31, 32]. Two individual lots of German cockroach extract were tested by Greer Laboratories for endotoxin by Limulus Amebocyte Lysate assay (BioWhittaker). The two cockroach preparations varied slightly in the amount of endotoxin present (162–340 ng/mg protein). Incubation of the cells with 30 $\mu\text{g/mL}$

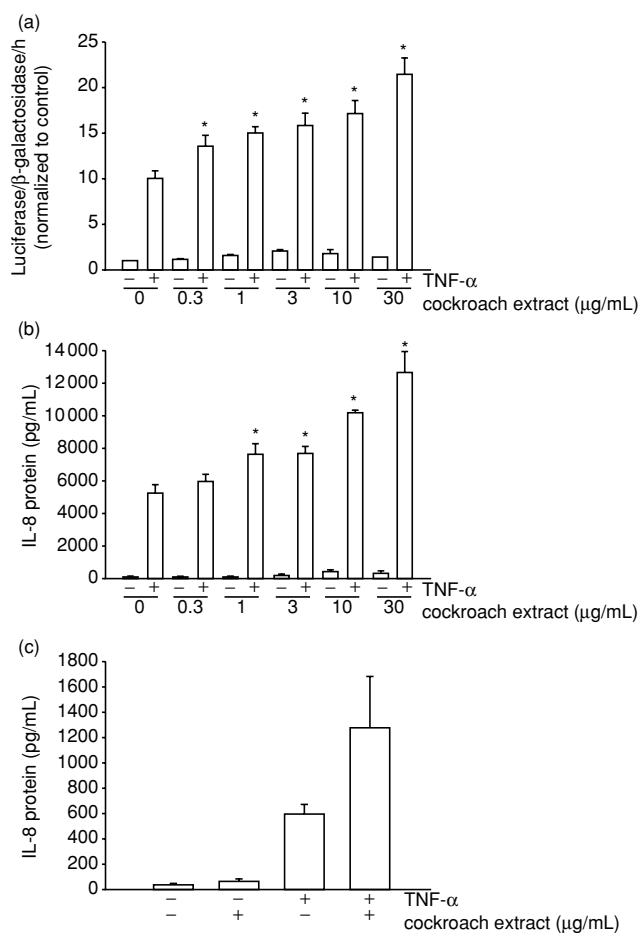


Fig. 1. Cockroach extract synergistically increases TNF- α -induced IL-8 expression in airway epithelial cells. (a) 16HBE14o-cells transiently cotransfected with cDNAs encoding the IL-8 promoter and β -galactosidase were treated with cockroach extract (0.3–30 $\mu\text{g/mL}$), human TNF- α (3 ng/mL) or both for 16 h. Data are expressed as luciferase/ β -galactosidase/h normalized to the control vector (-fold increase). Addition of cockroach extract to TNF- α significantly increased transcription from the IL-8 promoter compared to TNF- α alone. Data represent means $\pm$ SEM for 3–4 experiments (*different from TNF- α alone, $P < 0.05$). (b) 16HBE14o-cells were treated with cockroach extract (0.3–30 $\mu\text{g/mL}$), human TNF- α (3 ng/mL) or both overnight. Supernatant IL-8 protein levels were measured by ELISA. The addition of cockroach extract to TNF- α significantly increased IL-8 protein abundance compared to TNF- α alone. Data represent means $\pm$ SEM for 3–4 experiments (*different from TNF- α alone, $P < 0.05$). (c) Primary human airway epithelial cells were treated with cockroach extract (30 $\mu\text{g/mL}$), human TNF- α (3 ng) or both overnight (*different from TNF- α alone, $P < 0.05$, data represent means $\pm$ SEM for 3 experiments).

of cockroach extract therefore resulted in a final concentration of 4.8–10.2 ng/mL of endotoxin.

To test whether such endotoxin concentrations could induce IL-8 expression in our system, we incubated 16HBE14o-cells with endotoxin in the absence and presence of TNF- α (Fig. 3). Addition of endotoxin (10 ng/mL–10 $\mu\text{g/mL}$) had minimal effects on transcription from the IL-8 promoter, suggesting that endotoxin is not responsible for cockroach-mediated increments in IL-8 expression.

Serine protease activity is present in German cockroach extract and is responsible for the observed increase in transcription from the IL-8 promoter. Protease activity in the various cockroach preparations was measured by colourimetric assay [30]. This assay employs Azocoll, an insoluble protein-dye

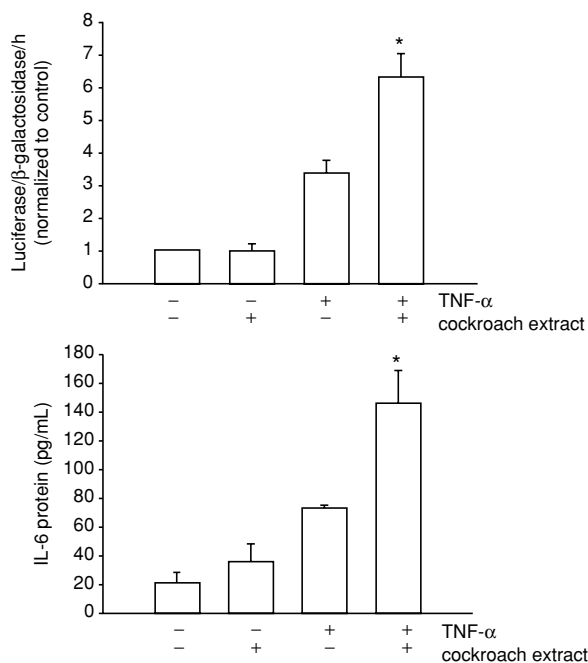


Fig. 2. Cockroach extract synergistically increases TNF- α -induced IL-6 expression in airway epithelial cells. (a) 16HBE14o-cells were transiently cotransfected with cDNAs encoding the IL-6 promoter and β -galactosidase. Selected cells were treated with cockroach extract (30 μ g/mL), human TNF- α (3 ng/mL) or both for 16 h. Cells were lysed and assayed for luciferase and β -galactosidase activity. Data are expressed as luciferase/ β -galactosidase/h normalized to the control vector (-fold increase). Addition of cockroach extract to TNF- α significantly increased transcription from the IL-6 promoter compared to TNF- α alone. Data represent means $\pm$ SEM for 3 experiments (*different from TNF- α alone, $P < 0.05$). (b) Cockroach extract significantly increased TNF- α -induced IL-6 protein expression compared to TNF- α alone, as determined by ELISA. Data represent means $\pm$ SEM for 3–4 experiments (*different from TNF- α alone, $P < 0.05$).

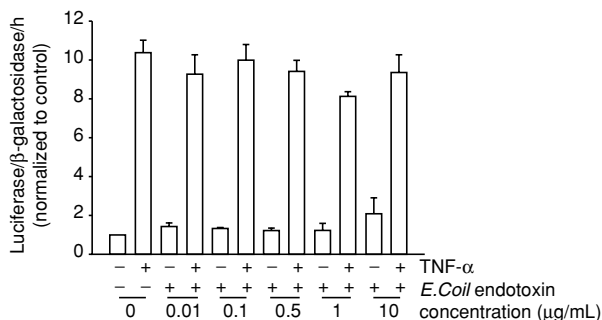


Fig. 3. Endotoxin is not responsible for the synergistic effect on transcription from the IL-8 promoter. 16HBE14o-cells were transiently transfected with the IL-8-luciferase promoter and β -galactosidase. Selected cells were treated with purified endotoxin from *E. coli* at concentrations from 10 ng/mL–10 μ g/mL in the absence or presence of TNF- α (3 ng/mL). Data are expressed as luciferase/ β -galactosidase/h normalized to the control vector (-fold increase). Data are representative of two separate experiments.

conjugate which is hydrolysed by a variety of proteinases, as substrate. Samples were incubated with Azocoll suspension at 37 °C for 2 h. Protease activity was measured at room temperature using a spectrophotometer at 520 nm absorbance. Subtilisin, an active serine protease, was used as a standard to quantify protease activity. The average protease activity in the four lots

of cockroach extract was 21.2 ± 3.9 μ g/mL (mean $\pm$ SEM) of extract (which is a specific activity of 0.029 ± 0.005 U/mg). Incubation of the cells with 30 μ g/mL of cockroach extract would therefore have resulted in a final average protease concentration of 89.8 ± 15.7 ng/mL of protease activity.

To test whether protease activity was responsible for the observed IL-8 expression, aliquots of cockroach extract were pre-incubated with either the serine protease inhibitors aprotinin or PMSF or the cysteine protease inhibitors E64 or leupeptin for 15 min at 37 °C. Pre-treatment with the serine protease inhibitors significantly inhibited cockroach protease activity (aprotinin inhibited 90% and PMSF about 60%), while pre-treatment with the cysteine proteases had little effect (15% inhibition or less; data not shown). Pre-treatment with aprotinin and PMSF each attenuated cockroach-mediated increases in TNF- α -induced IL-8 promoter activity (Fig. 4a, b). Pretreatment with cysteine protease inhibitors did not attenuate the response (Fig. 4c). Together, these data suggest that the cockroach extracts contain an active serine protease which is responsible for the observed synergistic effect on TNF- α -induced IL-8 expression.

Selected recombinant cockroach proteins do not increase airway epithelial cell IL-8 promoter activity

We tested whether known German cockroach proteins Bla g 2, Bla g 4 or Bla g 5 increase transcription from the IL-8 promoter in human bronchial epithelial cells. Cells were treated with recombinant Bla g 2, Bla g 4, or Bla g 5, either alone (3 μ g/mL each) or in combination (total Bla g protein, 3 μ g/mL), in the absence or presence of TNF- α . No increase in IL-8 promoter activity was observed (Fig. 5).

Discussion

In this study, we show that German cockroach extract synergistically increases TNF- α -induced transcription from the IL-8 promoter, as well as protein expression, in cultured human bronchial epithelial cells. Increased protein expression was demonstrated in both a human bronchial epithelial cell line (16HBE14o-cells) and primary cells. While the responses of primary cells were less vigorous than the cell line, these data demonstrate that German cockroach extracts may have direct pro-inflammatory effects on human airway epithelium in the absence of allergic mechanisms. Because many allergens have sequence homology with known enzymes and have functional enzymatic activity, we tested whether cockroach extracts held protease activity. We found that cockroach extracts contain significant protease activity, and that the observed effect of cockroach extract on TNF- α -induced IL-8 expression was serine protease inhibitor-sensitive, consistent with the notion that a serine protease was responsible for the pro-inflammatory effect. Incubation of airway epithelial cells with recombinant Bla g 2, 4 and 5 had no effect on transcription from the IL-8 promoter. Although endotoxin at low concentrations was found in the cockroach extracts, treatment of epithelial cells with endotoxin did not increase TNF- α -induced IL-8 promoter activity. Taken together, these data suggest that cockroach extract contains serine protease activity that has a pro-inflammatory effect on human bronchial epithelial cells.

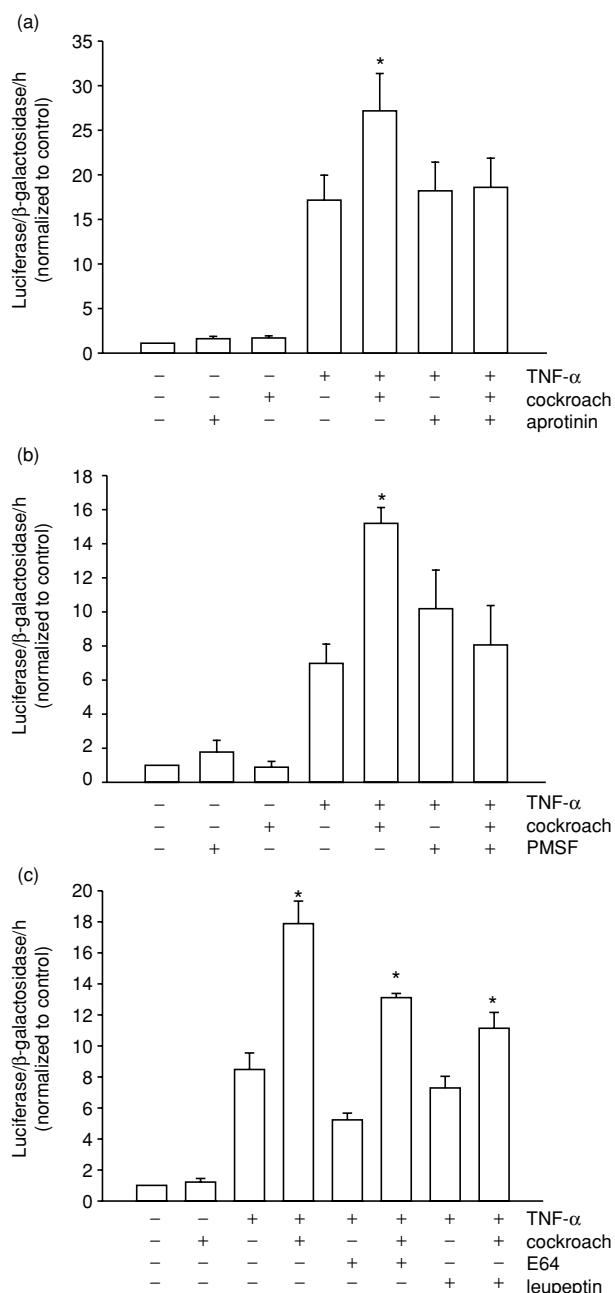


Fig. 4. Effect of protease inhibitors on TNF- α -induced transcription from the IL-8 promoter. (a) Effect of the serine protease inhibitor aprotinin. Data are expressed as luciferase/ β -galactosidase/h normalized to the control vector (-fold increase). Data represent means $\pm$ SEM for 6 experiments (*different from TNF- α alone, $P < 0.05$, ANOVA/Student–Newman–Keuls' multiple range test). (b) Effect of the serine protease inhibitor PMSF. Data represent means $\pm$ SEM for 3 experiments (different from TNF- α alone, $P < 0.05$). (c) Effects of the cysteine protease inhibitors E64 and leupeptin. Data represent means $\pm$ SEM for 3 experiments (different from TNF- α alone, $P < 0.05$).

These data extend previous studies demonstrating that house dust mite (*D. pteronyssinus*) and *A. fumigatus* proteases may increase lung epithelial cell cytokine release [8–11]. Owing to the apparent importance of cockroach exposure in the pathogenesis of inner-city asthma, a direct pro-inflammatory effect of cockroach proteins on the airways has been postulated [7]. The present study, performed in a cultured cell system, suggests the possibility that German cockroach proteins, when

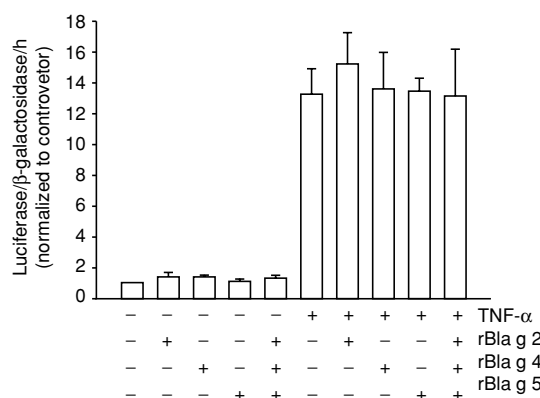


Fig. 5. Recombinant Bla g proteins do not increase TNF- α -induced transcription from the IL-8 promoter. 16HBE14o-cells were transiently transfected with the IL-8-luciferase promoter and β -galactosidase. Selected cells were treated with recombinant Bla g 2, Bla g 4, or Bla g 5 proteins (3 μ g/mL each) either alone or in combination (1 μ g/mL each). Data are expressed as luciferase/ β -galactosidase/h normalized to the control vector (-fold increase). Data represent means $\pm$ SEM for 5 experiments.

combined with cytokines already present in inflamed airways, may indeed have pro-inflammatory effects, independent of their ability to generate an allergic response.

Expression of IL-8, a potent neutrophil chemoattractant and activator, is increased in the airway epithelium of patients with asthma [33], and patients with status asthmaticus exhibit dramatic increases of this cytokine in their airways [34]. TNF- α is also increased in the airways of patients with asthma [35]. These data are consistent with the notion that interactions between cockroach proteins and TNF- α may be relevant to human asthma. Nevertheless, we did not test whether cockroach induces the expression of airway epithelial cell cytokines other than IL-8 and IL-6, nor did we test for synergistic effects with other cytokines besides TNF- α . Further, it is unclear whether the concentrations we employed in this study (30 μ g/mL) could be encountered by children and adults in cockroach-infested homes. Bla g 1 concentrations as high as 14306 U/g and Bla g 2 concentrations as high as 1946 U/g (78 μ g/g) have been found in the settled kitchen dust of low-cost public housing [36]; the total concentration of cockroach proteins would be significantly higher. After disturbance, cockroach-carrying particles greater than 10 μ m in diameter may be deposited in the airways [37]. Once in the airways, proteins elute from these particles and may reach high local concentrations. It is therefore conceivable that cockroach-carrying particles could contain sufficient allergen to induce chronic inflammation on inhalation. Finally, preliminary studies show that lower concentrations of cockroach extract (1–10 μ g/mL) may also have synergistic, albeit smaller, effects on TNF- α -induced IL-8 expression (K. Page and M. Hershenson, unpublished data).

We found using a colourimetric assay that German cockroach extracts contain small (0.029 U/mg) but significant level of protease activity, the presence of which is required for cockroach extract-induced responses. This relatively low activity may explain why extracts alone had no effect on IL-8 expression, while augmenting TNF- α -induced responses. Several German cockroach allergens have been characterized including Bla g 2 (inactive aspartic proteinase), Bla g 4 (calycin), Bla g 5 (glutathione-S-transferase), the group 1 cross-reactive allergens

Bla g 1 and Per a 1, and tropomyosin [12, 14, 15, 17]. However, none of these proteins have active protease activity. Further, we found that incubation of airway epithelial cells with recombinant Bla g 2, Bla g 4 and Bla g 5, either alone or in combination, failed to increase IL-8 expression. It is therefore likely that the pro-inflammatory effect of crude cockroach extract depends on a novel serine protease. Protease activity has recently been found in German cockroach extracts [13], and has also been found in crude extracts of American cockroach. Wongtim et al. [18] found that 1 mM PMSF reduced the proteolytic activity of American whole-body cockroach extract by 80%, suggesting the presence of a serine protease. Iraneta and colleagues [19] found that American cockroach extracts contained a complex collection of gelatinolytic proteins, including both cysteine and serine proteases. Finally, Fujimoto et al. [20] identified a 29-kDa member of the papain family in *Sarcophaga peregrina* (flesh fly) extracts, the cDNA of which was also isolated in *P. americana*.

Proteases have previously been demonstrated to modulate airway epithelial cell responses. As noted above, *D. pteronyssinus* and *A. fumigatus* proteases induce cytokine expression [8–11]. Proteases from other origins, particularly neutrophils, mast cells and alveolar macrophages, have also been shown to elicit the release of pro-inflammatory cytokines from epithelial cells [38–41]. The potential importance of serine proteases is underlined by the expression of serine proteinase inhibitors by airway epithelial cells [42–44]. Cockroach and other proteases may induce airway epithelial cell responses via G protein-coupled proteinase-activated receptors (PARs). Stimulation of PAR2 has recently been demonstrated to induce airway epithelial cell release of GM-CSF, a neutrophil and eosinophil chemoattractant and survival factor [45], and up-regulation of PAR2 has recently been noted in the respiratory epithelium from patients with asthma [46]. Alternatively, it is conceivable that protease-induced airway epithelial cell structural changes, including deformation of the cytoskeleton, especially microtubules, may contribute to IL-8 expression [47]. Finally, it should be noted that allergens may elicit airway epithelial cell cytokine responses via protease-independent mechanisms. For example, pollen extract devoid protease activity increased expression of IL-8, IL-6 and monocyte chemoattractant protein-1 in A549 cells [48].

We considered the possibility that endotoxin in cockroach extracts was responsible for the observed pro-inflammatory effects. While the endotoxin concentration of cockroach extracts was significant, concentrations of endotoxin similar to that present in cockroach-treated cultures were insufficient to increase airway epithelial cell transcription from the IL-8 promoter, either alone or in combination with TNF- α . We did, however, observe increased IL-8 expression following treatment with high concentrations (10 μ g/mL) of endotoxin, consistent with previous studies [31, 32]. Together, these data suggest that endotoxin was not responsible for cockroach extract-induced IL-8 expression.

While cockroach extract alone increased transcription from the IL-8 promoter in some experiments, more significant responses were observed in combination with TNF- α . On this basis, we speculate that human cockroach exposure may have more significant pro-inflammatory effects in susceptible individuals, or when combined with other airway insults such as air pollution, cigarette smoke, and the like. For example, Der p 1

and cigarette smoke have been previously noted to have additive effects on airway epithelial cell monolayer permeability [49], and epidemiologic studies have shown similar additive effects of tobacco use and ozone on asthma morbidity [50].

In conclusion, we have shown that *B. germanica* extracts synergistically increase TNF- α -induced transcription from the IL-8 promoter in human airway epithelial cells, and that the observed IL-8 expression is dependent on serine protease activity. These data suggest that German cockroach proteins may have direct pro-inflammatory effects on the airway, and raise the possibility that cockroach sensitization may not be required or sufficient for cockroach-associated asthma. Additional studies characterizing the precise protein or proteins responsible for this effect, as well as underlying mechanisms, may clarify the roles of cockroach exposure and sensitization in asthma, and lead to new therapeutic interventions.

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