

Cockroach extract antigen increases bronchial airway epithelial permeability

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Background: The bronchial epithelial cells of airways are subject to recurrent environmental injury throughout the life of an individual. Recently, a high incidence of asthma has been reported in inner-city children. The increased incidence of asthma in inner-city children is thought to be caused, in part, by frequent exposure to allergens of the common household pest the cockroach. **Objective:** We sought to investigate whether cockroach extract antigen (CrAg) induces vascular permeability factor, also known as vascular endothelial growth factor (VEGF), and whether it increases permeability in bronchial airway epithelial cells (BAECs).

Methods: We estimated CrAg-induced VEGF release in BAECs by using an ELISA and VEGF mRNA expression by using an RT-PCR reaction. The influence of CrAg on BAEC barrier function was estimated by measuring electrical resistance with an electric cell substrate impedance-sensing system.

Results: Our results demonstrate that CrAg induces VEGF release in BAECs in a time-dependent manner. The VEGF induction was also confirmed by means of VEGF mRNA expression in CrAg-stimulated BAECs. CrAg decreased electrical resistance across BAEC monolayers. The maximum decrease in electrical resistance was noticed 6 hours after activation and reached a plateau thereafter. Neutralizing antibodies to VEGF significantly inhibited the decrease in BAEC electrical resistance caused by CrAg.

Conclusions: These data suggest that CrAg induces VEGF release in BAECs and alters bronchial airway permeability. (J Allergy Clin Immunol 2002;110:589-95.)

Key words: Bronchial airway epithelial cells, cockroach extract antigen, vascular endothelial growth factor, bronchial epithelial permeability

Allergen exposure causes edema and increased mucous secretion in bronchial airways. Previous studies have demonstrated that cockroaches are an important source of indoor allergens, and cockroach exposure has been implicated in the increasing prevalence of inner-city asthma. The phase I National Cooperative Inner-city Asthma Study found that cockroach allergen was the predominant allergen within inner-city homes,¹ and cockroach allergens were found in daycare centers in the United States.² Cockroach sensitivity has been shown to be associated with asthma for over 30 years.^{3,4}

Abbreviations used

BAEC: Bronchial airway epithelial cell

CrAg: Cockroach extract antigen

ECIS: Electrical cell substrate impedance-sensing system

GAPDH: Reduced glyceraldehyde-phosphate dehydrogenase

SFM: Serum-free medium

VEGF: Vascular endothelial growth factor

The prominent pathophysiologic features of asthma are airway edema and inflammation, with an increased microvascular permeability of bronchial circulation and airway epithelium and movement of inflammatory cells into the lumen of the airway.⁵ Allergen exposure in sensitive asthmatic patients causes acute increases in bronchovascular permeability.⁶ Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, causes permeability changes in peripheral vasculature.⁷⁻⁹ The VEGF family of growth factors includes *VEGF-A*, *VEGF-B*, *VEGF-C*, and *VEGF-D*, as well as placenta growth factor.¹⁰ There are 4 splice variants of *VEGF-A*: VEGF121, VEGF165, VEGF189, and VEGF206.¹¹ VEGF is widely expressed in normal and disease^{12,13} settings and is upregulated at sites of angiogenesis and inflammation.^{14,15} Recent studies demonstrate that activated bronchial airway epithelial cells (BAECs) release VEGF.¹⁶ However, it is not clear whether cockroach allergen causes VEGF release and induces bronchial epithelial permeability. In the current study we demonstrate that cockroach allergen induces bronchial airway epithelial barrier dysfunction through induction of VEGF.

METHODS

Reagents, bacteria, and cell cultures

Recombinant VEGF, VEGF antibody, and mouse isotype antibody were purchased from Peprotech Inc. FBS was obtained from Atlanta Biologicals, and Media 199 was obtained from Gibco BRL. The Human VEGF Quantikine ELISA kit was purchased from R&D Systems. Human bronchial epithelial cells (BEAS-2 cells, American Type Culture Collection no. CRL-9609) were purchased from the American Type Culture Collection.

Cockroach extract antigen and BAEC stimulation

Cockroach extract antigen (CrAg) purchased from Alk-Abello Inc was resuspended in serum-free medium (SFM). CrAg used in this study was from American cockroaches, and it resulted in negative Limulus amoebocyte assay results for endotoxin. Total protein in the extract was 1.6 mg/mL, as evaluated with BCA reagent (Pierce). Cockroaches produce several important allergens; we eval-

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uated Bla g 1 and Bla g 2 content in the extract by using a sandwich ELISA (Indoor Biotechnologies). The cockroach extract stock has 30 µg/mL Bla g 1 and 0.33 µg/mL Bla g 2. BAECs were incubated in the presence of varying concentrations of CrAg in SFM (ie, 8, 16, 32, 64, and 128 µg/mL) and incubated at 37°C in 5% CO₂. BAEC viability was checked by means of trypan blue dye exclusion testing at 24 hours. The cell viability was greater than 99% in all selected concentrations. On the basis of a concentration response curve of VEGF release, the optimal concentration of the extract (32 µg/mL) was selected. A 1:500 dilution of cockroach extract has 32 µg/mL total proteins. The Bla g 1 and Bla g 2 antigen contents in this concentration of cockroach extract were 0.06 and 0.007 µg/mL, respectively. BAECs were incubated in the presence of a 32 µg/mL concentration of CrAg in SFM to evaluate the response over time. The cultures were terminated at different time points (3, 6, 12, and 24 hours), and supernatants were collected and stored at -70°C until estimation of VEGF levels by means of ELISA. The cells were saved for total RNA extraction for the analysis of VEGF-specific mRNA.

Determination of enzyme activity in CrAg by using a milk-clotting microtiter plate assay

The enzyme activity in the CrAg preparation was evaluated by means of a milk-clotting assay, as described previously.¹⁷ Serial dilutions of CrAg samples (20 µL) were incubated in microtiter wells with 12% (wt/vol) dried milk in 0.2 mol/L sodium acetate (pH 5.4) and 10 mmol/L CaCl₂ (80 µL) and incubated for 1 hour at 37°C. The plate was inverted to evaluate the presence of milk-clotting activity. Milk clotting was indicated by curd formation at the bottom of the well. Pepsin and chymosin were used as standards for the enzyme activity, and pepstatin and phenylmethylsulfonyl fluoride were used as inhibitors of aspartic and serine proteinase activities, respectively.

Measurement of VEGF by means of ELISA

VEGF levels in CrAg-induced BAEC culture supernatants were measured by using a sandwich enzyme immunoassay (Quantikine ELISA, R&D System), as previously described.¹⁸ Briefly, the samples were added to 96-well microtiter plates, which were coated with a murine mAb to VEGF. The unbound protein was washed 3 times, and an enzyme-linked polyclonal antibody specific to VEGF was added. The plate was washed again 3 times, and substrate solution was added to the wells. After 30 minutes of incubation, stop solution was added to each well. The amount of VEGF was determined on the basis of OD of the samples by comparison with the standards at 450 nm with an ELISA reader. The detection limit of the kit was 2.0 pg/mL for cell culture supernatant and 3.0 pg/mL for serum or plasma samples.

Nonradioactive GEArray

VEGF gene expression was evaluated by using a nonradioactive GEArray (Super Array). Total RNA from resting and CrAg-activated BAECs was isolated by using the RNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. The RNA was converted to biotinylated cDNA probes by means of reverse transcription with a deoxyribonucleoside triphosphate mix containing biotin-16-2'-deoxyuridine-5'-triphosphate. Biotinylated cDNA probes were hybridized to gene-specific cDNA fragments spotted on the membranes. The GEArray membrane was then blocked with GEA-blocking solution and incubated with alkaline phosphatase-conjugated streptavidin. The relative expression levels of multiple genes then were detected on the basis of chemiluminescence signal by using the alkaline phosphatase substrate CDP-Star, as previously reported.¹⁹ The membrane was exposed to x-ray film, and the relative abundance of the transcript was estimated by comparing the signal intensity of the gene of interest with the signal derived from reduced glyceraldehyde-phosphate dehydrogenase (GAPDH) as a positive control.

RT-PCR

Total RNA from resting and CrAg-activated BAECs was isolated with the RNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. RT-PCR was performed as reported earlier.²⁰ One microgram of total RNA was reverse transcribed into cDNA. The first strand of cDNA was synthesized in a total volume of 20 µL in the presence of 5 mmol/L MgCl₂, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1 mmol/L deoxyribonucleoside triphosphates, 1 U/µL RNase inhibitor, 15 µmol/L primer, and 2.5 U/µL MuLV reverse transcriptase (Perkin Elmer Cetus). The reverse transcription was conducted at 42°C for 15 minutes, and the reaction was stopped by means of incubation at 99°C for 5 minutes.

The cDNA was then amplified with specific primers for human GAPDH as a control. Primers for amplification were synthesized by using the phosphoramidite method with a Beckman 200A synthesizer. The primers used were 5'-GGTCGGAGTCAACGGATTGTG-GT-3' (sense) and 5'-TGTGGCCATGAGGTCCACCAC-3' (antisense) for GAPDH and 5'-AGGGCAGAATCATCACGAAG-3' (sense) and 5'-TTTAAGTCAAGCTGCCTCGC-3' (antisense) for VEGF (GeneBank accession no. X62568). PCR was performed with 5 µL of reverse transcription product in a reaction mixture containing 2 mmol/L MgCl₂, 50 mmol/L KCl, and 10 mmol/L Tris-HCl (pH 8.30), specific oligonucleotide primers (15 µmol/L), and 2.5 U of Taq DNA Polymerase (Perkin Elmer Cetus). The samples were amplified in a thermal cycler (GeneAmp PCR System 9600 Perkin Elmer Cetus) and preheated for 1.30 minutes at 95°C, followed by 30 cycles. Each cycle was composed of denaturation at 95°C for 15 seconds, primer annealing for VEGF of 55°C for 45 seconds, and extension at 72°C for 45 seconds. The amplification products were analyzed by means of agarose gel electrophoresis, and their identities were confirmed after sequence determination.

Measuring BAEC electrical resistance

BAEC electrical resistance was measured by using an electric cell substrate impedance-sensing system (ECIS; Applied Biophysics), as described previously.²⁰ Briefly, BAECs were cultured on the ECIS electrode array. (The ECIS electrode array has eight 1-cm² chambers. Each chamber contains 10 gold electrodes of 10⁻⁴ cm² each, and the culture media functions as the electrolyte.) The cockroach extract was added into the chambers on confluent cultures. In some of the wells, along with cockroach extract, either isotype antibody or neutralizing VEGF antibodies were included. The cockroach extract was dialyzed against PBS (pH 7.4) with a cut-off membrane of 12,000 to 14,000 d molecular weight to determine whether the extract retains its permeability-inducing ability after dialysis. The extract was boiled for 30 minutes and tested for permeability-inducing activity to evaluate its sensitivity to heat.

Total electrical resistance was measured dynamically across the BAEC monolayer and was determined on the basis of the combined resistance between the ventral surface of the cell and the electrode reflective to the resistance between the cells. The small gold electrode and the larger counter electrode (1 cm²) are connected to a phase-sensitive lock in amplifier (model 5301A, EG and G Instruments) with a built-in differential preamplifier (model 5316A, EG and G Instruments). A 1-V, 4000-Hz alternating-current signal was supplied through a 1-mΩ resistor to approximate a constant current source. Voltage and phase data were stored and processed with a Pentium 400-MHz computer that controlled the output of the amplifier and relay switches to different electrodes. Experiments were conducted on wells that achieved 5000 Ω of steady-state resistance. Resistance was expressed by the in-phase voltage (proportional to the resistance), which was normalized to the initial voltage and expressed as a fraction of the normalized value.

Statistical analysis

The significance of differences between the experimental and control groups was tested by using ANOVA with Sigma-Stat statistical software. The significance of difference between the 2 groups was tested by using the all-pairwise multiple comparison procedure (Student-Newman-Keuls method), and a *P* value of less than .05 was considered significant.

RESULTS

CrAg did not contain serine or aspartic proteinases

CrAg was tested for proteinases activity by using a milk-clotting assay. Cockroach extract did not cause milk clotting, even at 10-fold higher concentrations than those used for VEGF induction or for ECIS assay in this study. Inclusion of the serine protease inhibitor phenylmethylsulfonyl fluoride or the aspartic protease inhibitor pepstatin with the cockroach extract in the milk-clotting assay had no effect. Pepsin and chymosin were used as positive controls. Pepsin and chymosin showed milk clotting at 0.003 and 0.004 $\mu\text{g}/\text{well}$, respectively (data not shown).

CrAg induces VEGF release in BAECs

CrAg induced VEGF release in BAECs in a concentration- and time-dependent manner (Fig 1). A low concentration of cockroach extract was not effective in VEGF induction. Optimal response was noticed with 32 $\mu\text{g}/\text{mL}$ protein (1:500 dilution). At higher concentrations, no significant increase in VEGF release was noticed. Significant levels of VEGF were noticed as early as 3 hours. The VEGF release reached maximum levels by 12 hours, and thereafter, the response reached a plateau.

CrAg induced VEGF expression in BAECs

The VEGF family of growth factors consists of several closely related members, including *VEGF-A*, *VEGF-B*, *VEGF-C*, and *VEGF-D*.¹⁰ Each of these members displays different biologic functions. Among these members, *VEGF-A* has, predominantly, a permeability-enhancing ability on epithelial and endothelial cells. A nonradioactive GEMArray precoated with *VEGF-A*, *VEGF-B*, *VEGF-C*, and *VEGF-D* genes was used to understand the effect of cockroach extract on the differential response of these VEGF members. A significant increase in *VEGF-A* and *VEGF-D* expression was noticed in CrAg-induced BAECs when compared with that seen in resting cells (Fig 2). CrAg did not induce *VEGF-B* and *VEGF-C* genes in BAECs. A relatively higher expression of *VEGF-A* gene was noticed when compared with that of *VEGF-D* in CrAg-induced cultures. There was a 2.5-fold increase in *VEGF-A* in CrAg-induced BAECs when compared with that seen in resting cells. *VEGF-D* gene expression increased by 1.3-fold in CrAg-induced cultures when compared with that seen in control resting cells.

CrAg-stimulated BAECs express VEGF mRNA

Alternative splicing of *VEG-F* mRNA (or *VEGF-A*) results in 4 main isoforms: VEGF121, VEGF165,

VEGF189, and VEGF206.¹¹ Among these, the VEGF165 splice variant is a potent permeability-inducing factor. To delineate the effect of cockroach extract, we analyzed the various isoforms of *VEGF-A* semiquantitatively by means of RT-PCR. We did not test the *VEGF-B*, *VEGF-C*, and *VEGF-D* expression by means of RT-PCR. The primer sequence selected was known to amplify all 4 isoforms of *VEGF-A* (ie, VEGF121, VEGF165, VEGF189, and VEGF206). *VEG-F* mRNA expression in resting BAECs was minimal (Fig 3, A). Cockroach antigen induced *VEG-F* mRNA expression in a time-dependent manner in BAECs (Fig 3, B). The peak expression was noticed at 12 hours. In the CrAg-induced BAECs, the VEGF165 (444 bp), VEGF189 (516 bp), and VEGF206 (567 bp) isoforms were amplified, whereas in resting BAECs these isoforms were not expressed. In CrAg-induced BAECs, the VEGF165 (444 bp) isoform was predominantly expressed. VEGF165 (444 bp) increases permeability in several types of cells. The VEGF165 (444 bp) isoform was highly expressed in CrAg-stimulated BAECs, whereas in resting cells it was marginally expressed.

Cockroach antigen causes a decrease in resistance of epithelial cell monolayers

Confluent BAEC monolayers exhibited a mean electrical resistance of 5000 Ω/cm^2 . A significant decrease in electrical resistance was noticed across BAEC monolayers that were incubated with CrAg (Fig 4). CrAg decreased electrical resistance across the confluent BAEC monolayer in a time-dependent manner. CrAg induced a significant drop in BAEC barrier function as early as 3 hours. Inclusion of neutralizing VEGF antibody inhibited CrAg-mediated BAEC barrier dysfunction. However, inclusion of an isotype antibody to irrelevant antigen did not show any effect on CrAg-induced BAEC barrier dysfunction. The cockroach extract did not lose its permeability-inducing ability on dialysis, but the activity was decreased on heat inactivation (data not shown).

DISCUSSION

The bronchial epithelial cells of airways are subject to recurrent environmental injury throughout the life of an individual. A number of agents, including infectious organisms, environmental allergens, and inhaled toxic substances, affect the bronchial airways. Although cockroach allergen exposure has been associated with asthma, little is known about its effect on bronchial epithelial permeability. In the current study we evaluated the BAEC responses to CrAg in vitro. Our results demonstrated that CrAg induced VEGF release in BAECs in a time-dependent manner. RT-PCR studies also confirmed the antigenic VEGF release. CrAg significantly decreased the electrical resistance across the BAEC monolayer. VEGF production was directly correlated with the decreased electrical resistance in CrAg-stimulated BAECs. Addition of neutralizing VEGF antibodies restored the CrAg-mediated decrease in electrical resistance.

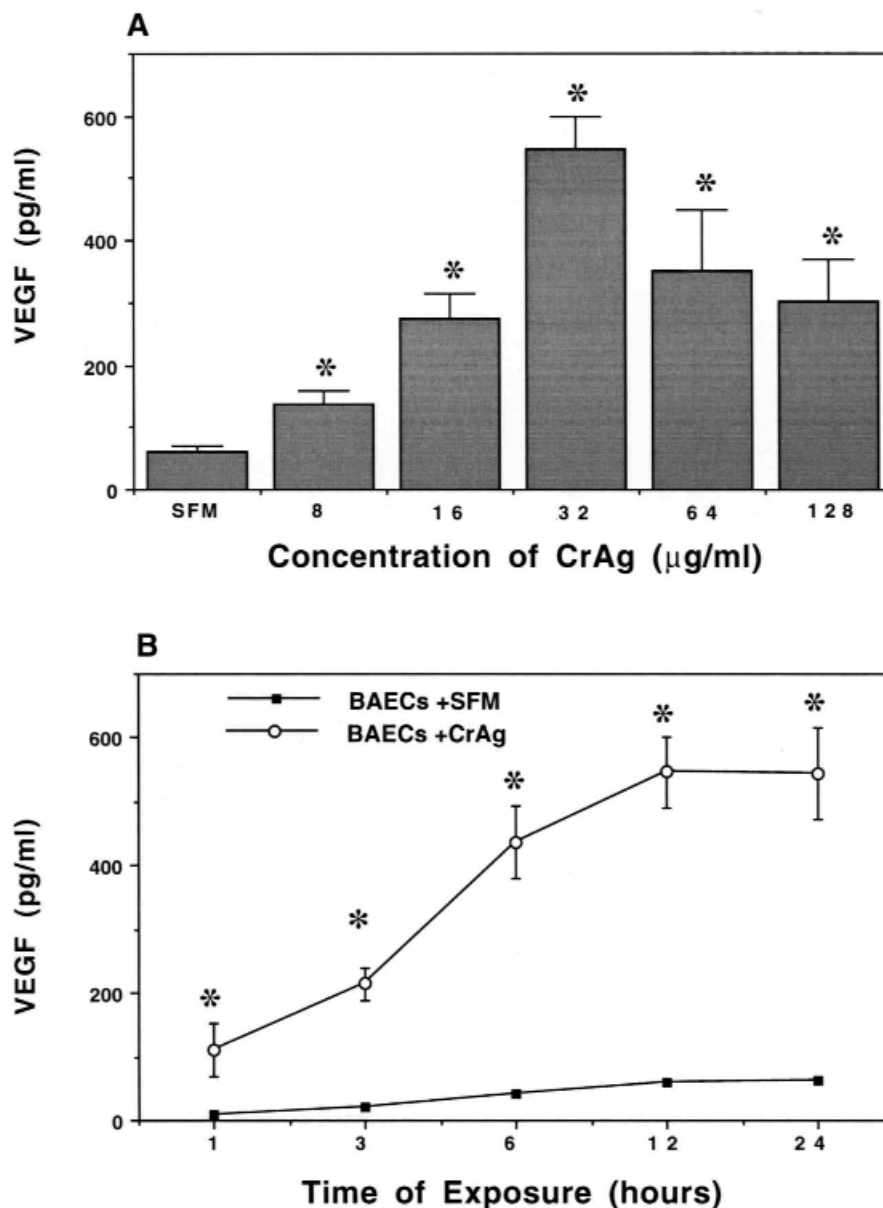


FIG 1. CrAg-induced VEGF release in BAECs: **A**, concentration response curve; **B**, time-dependent response to 32 µg/mL CrAg. Data represent mean $\pm$ SE of 3 separate experiments. Asterisk denotes statistical significance ($P < .001$) in comparison with control cultures.

Traditionally, asthma has been described as an allergy-mediated phenomenon. In certain individuals nonspecific irritation of the airway mucosa might cause bronchoconstriction. The clinical features of asthma include bronchial obstruction, cough, and mucus hypersecretion. Histologic changes that occur in bronchial asthma include thickening of the subepithelial layer, edema and cellular infiltration in the lower mucosa, and dilation of the arterial vessels.²¹ The mechanisms causing an increase in the permeability of the airway epithelium and of the arterial endothelium in asthma are not yet completely understood. However, plasma exudation in the airway interstitium and lumen caused by an increase in the endothelial and epithelial permeability might be due

to the release of inflammatory mediators, which in turn causes microvascular and epithelial leakage.²² Cockroach allergen is recognized as important in the pathogenesis of asthma, at least among children living in the inner cities of the United States.^{4,23} Several studies demonstrate that cockroach allergen sensitivity has been associated with asthma.^{3,4} Our findings demonstrating VEGF production in BAECs suggest that direct exposure of CrAg causes VEGF release in BAECs. VEGF causes endothelial barrier abnormality and increases microvascular permeability.⁹ VEGF is expressed in diseases, such as Graves disease¹² and Castleman disease,¹³ and it is upregulated at the site of inflammation.^{14,15} VEGF induces permeability changes in the endothelial cell²⁴

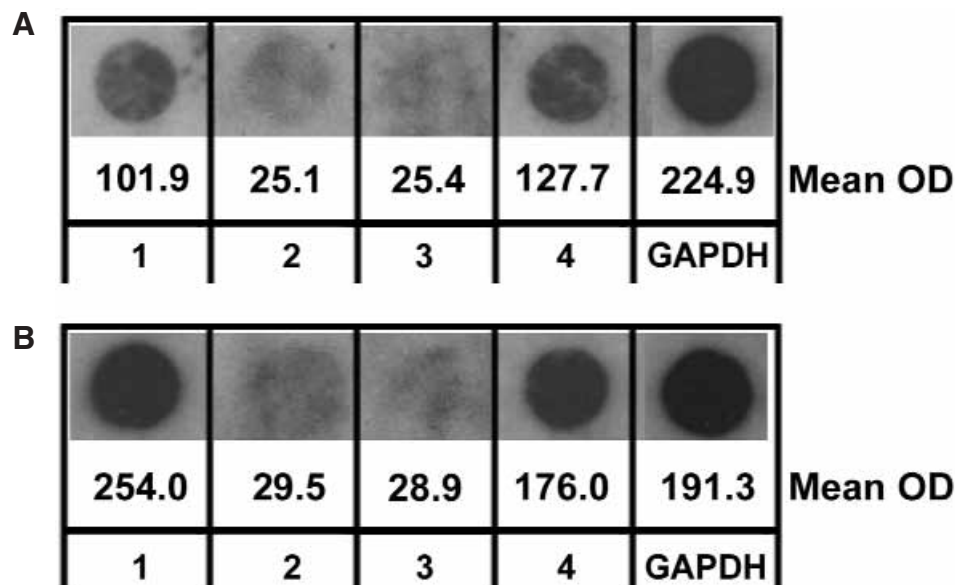


FIG 2. CrAg-induced expression of VEGF family members in BAECs: **A**, resting BAECs; **B**, CrAg-induced BAECs. Lanes 1, 2, 3, and 4 represent *VEGFA*-, *VEGFB*-, *VEGFC*-, and *VEGFD*-specific genes, respectively. *GAPDH* served as a positive control gene. Results are representative of 3 independent experiments.

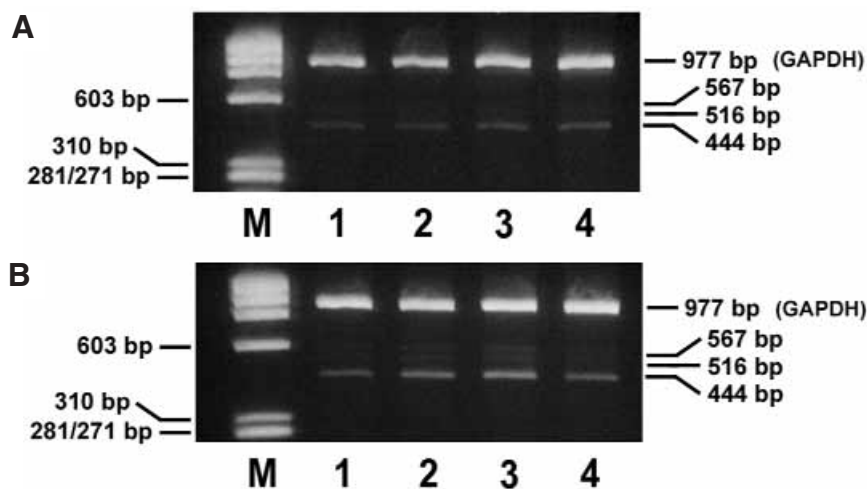


FIG 3. CrAg-induced *VEGFA* mRNA expression in BAECs: **A**, resting BAECs; **B**, CrAg-stimulated BAECs. Lane *M* indicates molecular weight marker (*Hae*-III), and lanes 1-4 indicate 3, 6, 12, and 24 hours, respectively. *GAPDH* served as a positive housekeeping gene. Results are representative of 3 independent experiments.

and mesothelial cell monolayers.²⁰ *VEGF-D* is mitogenic to endothelial cells.¹⁰ Cockroach extract predominantly enhanced *VEGF-A* rather than *VEGF-D* in BAECs. RT-PCR analysis demonstrated an increased *VEGF165* isoform expression, which is a permeability-inducing factor. The *VEGF-B* and *VEGF-C* expression was low. These findings together demonstrate that cockroach extract predominantly induces permeability, enhancing VEGF isoforms in BAECs. In a similar study with recombinant cockroach allergen, Alp et al²⁵ reported an increased cytokine release in neutrophils.

The mediators released during inflammation influence both the epithelial and vascular compartments. The airway inflammation involves the vascular bed and determines hyperplasia and dilation of blood vessels.²⁶ The VEGF expression in nasal polyp stroma cells was involved in the development of edema in chronic inflammation.²⁷ VEGF antagonism reduced the edema formation and damage after ischemia-reperfusion injury.²⁸ We noticed that CrAg significantly ($P < .001$) decreased the electrical resistance across BAEC monolayers, indicating that CrAg exposure results in BAEC barrier dysfunction.

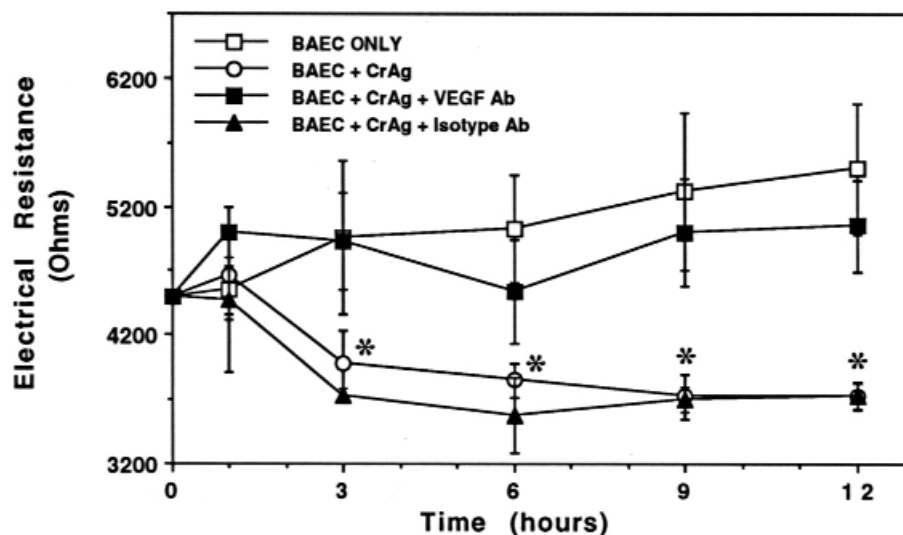


FIG 4. Real-time electrical resistance across BAEC monolayer. Values represent mean $\pm$ SE of 3 separate experiments. Asterisks denote statistical significance ($P < .001$) in comparison with control (unstimulated) cultures.

Neutralizing antibodies to VEGF restored the CrAg-induced decrease in BAEC electrical resistance, suggesting that the permeability changes noticed were due to VEGF release in BAECs. Earlier studies demonstrated that allergen exposure induces an acute increase in bronchovascular permeability.⁶ The bronchial mucus plugs from the asthmatic patients show large amounts of albumin, suggesting that an inflammatory exudate enters the airway lumen.²⁹ Taken together, these findings suggest that the exudate secretion in the atopic allergic patient's bronchial airway lumen might be due to the allergen-induced epithelial permeability. There have been no specific reports on the airway levels of cockroach antigen in patients with positive skin test responses. The amount of Bla g 1 (0.06 μ g/mL) present in the cockroach extract concentration used in our study is comparable with the median Bla g 2 range of exposure (0.08-0.15 μ g/mL) noticed in schoolchildren.^{17,30} Thus it is conceivable that exposure of the airway epithelium to a range of Bla g protein, such as that used in our experiments, for a short period of time would cause changes in airway permeability. A more permeable airway epithelium would be more susceptible to further insult from repeated allergen exposure.

Our study demonstrated that cockroach allergen exposure increases bronchial epithelial monolayer permeability and results in barrier dysfunction of the bronchial airway epithelium. Direct exposure of CrAg causes VEGF release in BAECs. Thus released VEGF might in turn facilitate allergen entry into the bronchial airways, causing sensitization of airways and triggering an allergenic response. Sensitization to cockroach allergen and continuous exposure to the same allergen might be related to the development of the characteristic pathologic feature of asthma, airway edema, and increases in luminal secretions and cells.

REFERENCES

- Gergen PJ, Mortimer KM, Eggleston PA, et al. Results of the National Cooperative Inner-City Asthma Study (NCICAS) environmental intervention to reduce cockroach allergen exposure in inner-city homes. *J Allergy Clin Immunol* 1999;103:501-6.
- Fernandez-Caldas E, Codina R, Ledford DK, Trudeau WL, Lockett RF. House dust mite, cat, and cockroach allergen concentrations in daycare centers in Tampa, Florida. *Ann Allergy Asthma Immunol* 2001;87:196-200.
- Mendoza J, Snyder RD. Cockroach sensitivity in children with bronchial asthma. *Ann Allergy* 1970;28:159-63.
- Kang B. Study on cockroach antigen as a probable causative agent in bronchial asthma. *J Allergy Clin Immunol* 1976;58:357-65.
- Chung KF, Rogers DF, Barnes PJ, Evans TW. The role of increased airway microvascular permeability and plasma exudation in asthma. *Eur Respir J* 1990;3:329-37.
- Fick RB Jr, Metzger WJ, Richerson HB, et al. Increased bronchovascular permeability after allergen exposure in sensitive asthmatics. *J Appl Physiol* 1987;63:1147-55.
- Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989;246:1306-9.
- Lichtenbeld HC, Ferrara N, Jain RK, Munn LL. Effect of local anti-VEGF antibody treatment on tumor microvessel permeability. *Microvasc Res* 1999;57:357-62.
- Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. *J Cell Sci* 1995;108(suppl):2369-79.
- Li X, Eriksson U. Novel VEGF family members: VEGF-B, VEGF-C and VEGF-D. *Int J Biochem Cell Biol* 2001;33:421-6.
- Nicosia RF. What is the role of vascular endothelial growth factor-related molecules in tumor angiogenesis? *Am J Pathol* 1998;153:11-6.
- Nagura S, Katoh R, Miyagi E, Shibuya M, Kawaoi A. Expression of vascular endothelial growth factor (VEGF) and VEGF receptor-1 (Flt-1) in Graves disease possibly correlated with increased vascular density. *Hum Pathol* 2001;32:10-7.
- Nishi J, Maruyama I. Increased expression of vascular endothelial growth factor (VEGF) in Castleman's disease: proposed pathomechanism of vascular proliferation in the affected lymph node. *Leuk Lymphoma* 2000;38:387-94.
- Ferrara N, Keyt B. Vascular endothelial growth factor: basic biology and clinical implications. *EXS* 1997;79:209-32.
- Brown LF, Detmar M, Claffey K, et al. Vascular permeability factor/vas-

- cular endothelial growth factor: a multifunctional angiogenic cytokine. *EXS* 1997;79:233-69.
16. Lee CG, Yoon HJ, Zhu Z, et al. Respiratory syncytial virus stimulation of vascular endothelial cell growth Factor/Vascular permeability factor. *Am J Respir Cell Mol Biol* 2000;23:662-9.
 17. Pomes A, Chapman MD, Vailes LD, Blundell TL, Dhanaraj V. Cockroach allergen Bla g 2: structure, function, and implications for allergic sensitization. *Am J Respir Crit Care Med* 2002;165:391-7.
 18. Mohammed KA, Nasreen N, Ward MJ, Antony VB. Induction of acute pleural inflammation by *Staphylococcus aureus*. I. CD4+ T cells play a critical role in experimental empyema. *J Infect Dis* 2000;181:1693-9.
 19. Chapoval AI, Ni J, Lau JS, et al. B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. *Nat Immunol* 2001;2:269-74.
 20. Mohammed KA, Nasreen N, Hardwick J, Logie CS, Patterson CE, Antony VB. Bacterial induction of pleural mesothelial monolayer barrier dysfunction. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L119-25.
 21. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985;131:599-606.
 22. Regnard J, Lockhart A. Vascular factors in bronchial hyperresponsiveness and asthma. *Respir Med* 1992;86:179-81.
 23. Rosenstreich DL, Eggleston P, Kattan M, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med* 1997;336:1356-63.
 24. Cohen AW, Carbajal JM, Schaeffer RC Jr. VEGF stimulates tyrosine phosphorylation of beta-catenin and small-pore endothelial barrier dysfunction. *Am J Physiol* 1999;277(suppl):H2038-49.
 25. Alp H, Chapman M, Brees A, Moy J. Cytokine release by recombinant cockroach allergen-stimulated neutrophils (PMN) [abstract]. *J Allergy Clin Immunol* 1999;103(suppl):S106.
 26. Laitinen A, Laitinen LA. Vascular beds in the airways of normal subjects and asthmatics. *Eur Respir J Suppl* 1990;12:658s-62s.
 27. Ito A, Hirota S, Mizuno H, et al. Expression of vascular permeability factor (VPF/VEGF) messenger RNA by plasma cells: possible involvement in the development of edema in chronic inflammation. *Pathol Int* 1995;45:715-20.
 28. van Bruggen N, Thibodeaux H, Palmer JT, et al. VEGF antagonism reduces edema formation and tissue damage after ischemia/reperfusion injury in the mouse brain. *J Clin Invest* 1999;104:1613-20.
 29. Hogg JC. The pathophysiology of asthma. *Chest* 1982;82(suppl 1):8S-12S.
 30. Sporik R, Squillace SP, Ingram JM, Rakes G, Honsinger RW, Platts-Mills TA. Mite, cat, and cockroach exposure, allergen sensitisation, and asthma in children: a case-control study of three schools. *Thorax* 1999;54:675-80.

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