

Allergens, IgE, mediators, inflammatory mechanisms

A dose-response relationship between exposure to cockroach allergens and induction of sensitization in an experimental asthma in Hartley guinea pigs

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Objective: An indoor allergen, cockroach allergen (CRa) has been partly implicated in the rising prevalence of inner-city asthma. This study investigates a dose-response relationship of exposure to CRa with the degree of sensitization and with airway inflammation through the use of our established guinea pig asthma model.

Methods: Four groups of Hartley guinea pigs were exposed to aerosolized saline (control) and three dose levels of CRa: low-dose, 0.5 mg (LD); medium-dose, 5 mg (MD); or high-dose, 25 mg (HD), nebulized twice a day, 5 days per week, for 4 weeks. The development of anaphylactic antibodies was measured by both allergy skin test and passive cutaneous anaphylaxis assay. Animals were challenged with CRa 5 days after the last sensitization, and specific airway resistance was measured continuously by a double-chamber plethysmograph while animals were in the conscious state. The inflammatory cells in bronchoalveolar lavage fluid (BALF) and the contractile responses of tracheal rings were analyzed in vitro at 24 hours after CRa challenge.

Results: The anaphylactic antibodies to CRa were detected in the CRa-sensitized animals by allergy skin test. Passive cutaneous anaphylaxis titers of IgG_{1a}-like antibody were zero in control, 1:2 in LD, 1:40 in MD, and 1:160 in HD. Total leukocytes in BALF were increased by CRa challenge in all three CRa-sensitized groups compared with control ($p < 0.0001$). The tracheal rings from CRa-sensitized guinea pigs constricted upon addition of incremental doses of CRa challenge in vitro in a dose-responsive manner ($p < 0.0001$). The leukocytosis in BALF and the anaphylactic contractile responses of the tracheal rings in CRa-sensitized groups were correlated to the levels of CRa to which the groups had been exposed during sensitization ($p < 0.001$). CRa-provoked increase in SRaw was noted in all three groups of the CRa-sensitized guinea pigs compared with control ($p < 0.01$). Yet,

among CRa-sensitized groups, a greater increase in specific airway resistance was noted in the LD group but not in the HD CRa group.

Conclusions: The development of anaphylactic cockroach sensitivity in guinea pigs was dependent on the levels of CRa exposure during sensitization, and the CRa-sensitized animals showed antigen-specific airway inflammation along with airway smooth muscle contractions. However, the severity of bronchospasm in conscious animals was not in agreement with the degree of CRa sensitivity. (*J Allergy Clin Immunol* 1998;101:653-9.)

Key words: Cockroach allergens, dose-response relationship, passive cutaneous anaphylaxis, bronchoalveolar lavage, airway resistance, airway, smooth muscle contraction, asthma, guinea pig

The rising mortality and morbidity of high-risk asthma have been partly attributed to the environmental exposure to indoor allergens at home.¹⁻³ Among indoor allergens, cockroach allergen (CRa) has long been recognized as a major indoor allergen, together with dust mites and domestic pets.⁴⁻⁶ Particularly, the significance of sensitivity to CRa lies in the fact that it is highly prevalent among inner-city asthmatic populations who require frequent emergency room and hospital visits, indicating extreme severity of asthma among the afflicted.^{7,8} The studies from our laboratory and others have indicated that exposure to CRa at home sensitizes atopic subjects and causes asthma.^{4-6,9,10} It has also been known that inhalation of CRa by cockroach-sensitive subjects with asthma produces asthmatic responses of variable types (immediate, late, dual, or persistent) and causes peripheral eosinophilia.^{11,12} More significantly, a recent National Cooperative Inner-City Asthma study has documented that the high levels of CRa in the bedroom dust of asthmatic children is directly linked to the increased morbidity of asthma and perhaps to the mortality of inner-city asthma.¹³

However, in humans it is unclear whether the exposure of CRa is dose dependent in induction of sensitization, asthma, or both. The prevalence of cockroach hypersensitivity has been known to loosely correlate with the number of cockroaches cited, the levels of CRa in the dwellings, or both of asthmatic subjects.^{4,5,10} Yet,

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Abbreviations used

ANOVA:	Analysis of variance
AST:	Allergy skin test
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
CRA:	Cockroach allergens
HD:	High dose
LD:	Low dose
MD:	Medium dose
PCA:	Passive cutaneous anaphylaxis
SRaw:	Specific airway resistance

the amount of allergen to which an individual is exposed is difficult to estimate because there are many other variables affecting the exposure. Therefore the measurement of allergens in the dust reservoir may not be an accurate index of exposure. Furthermore, the relationship between exposure to allergens such as CRA and induction of asthma in an individual subject has not been clearly understood other than within the context of clinical experience or by inference from epidemiologic studies.

We have attempted earlier to clarify how CRA sensitization occurs in guinea pigs and learned that an aerosolized exposure, which could be a likely route of exposure to CRA in human beings, is less likely to produce IgE-type reagenic antibody in the guinea pigs.^{14, 15} Yet, the guinea pigs sensitized with chronic aerosolized CRA developed asthma-like bronchial obstructions along with release of leukotrienes in association with production of IgG_{1a}-type reagenic antibody.¹⁵ In this study we further examined the dose-response relationship between exposure to aerosolized CRA and induction of "asthma" in this guinea pig asthma model.

METHODS

Animals

Male Hartley strain guinea pigs weighing 200 to 250 gm were purchased from Hilltop Laboratories (Scotsdale, Pa.). Upon arrival, two animals were housed in a plastic cage in a colony room with a 12-hour day-night cycle (lights on at 7 AM) through artificial illumination. They received food and water *ad libitum*. Before the start of the experiment, guinea pigs were quarantined and acclimatized to their housing for 1 week. The experiment was carried out with the approval of the Institutional Animal Care and Use Committee at the University of Kentucky.

Cockroach allergens

A lyophilized whole-body extract of German cockroaches was obtained from Greer Laboratories, Inc. (Lenior, N.C.) and used for allergen sensitization and challenge after appropriate dilution.

Allergen sensitization

CRA aerosol sensitization was done as previously described.^{14, 15} Briefly, guinea pigs were placed in a semiclosed chamber connected to an ultrasonic nebulizer (Devilbiss model 100, Somerset, Pa.) generating aerosol; droplet sizes ranged

from 0.5 to 30 μ m, with an output of 0.2 ml/min. Animals were exposed to three concentrations of CRA: 0.5 mg (low dose, LD), 5 mg (medium dose, MD), or 25 mg (high dose, HD) in 10 ml saline, for 45 minutes, twice a day, 5 days per week, for a total of 4 weeks. A group of guinea pigs (control) were sensitized with aerosolized saline (vehicle) in the same manner as CRA sensitization.

Allergy skin test

After 4 weeks of sensitization, guinea pigs were slightly sedated with sodium pentobarbital, 30 mg/kg, and their back skin was shaved for the allergy skin test (AST). With 27G skin test syringes, histamine (30 μ g) and CRA (0.5, 1, and 5 μ g) in 0.03 ml of saline was injected into the upper dermis intradermally. Two diameters, lengthwise and vertical of a wheal, were measured 20 minutes and 60 minutes after the injections, and a mean was used.¹⁵

Passive cutaneous anaphylaxis assay

Sera collected from the guinea pigs of each group (LD, MD, HD, and control) were pooled by group and used for passive cutaneous anaphylaxis (PCA) assays by the method described previously.^{14, 15} Briefly, 0.1 ml of a set of serially diluted sera with or without heat treatment (56°C, 30 minutes) was inoculated in duplicate intradermally into the shaved back skin of English short-hair guinea pigs. IgG_{1a} and IgE were measured after a latent period of 4 hours and 7 days, respectively. Size of "bluing" in the skin was measured 30 minutes after intravenous injection of 2 mg CRA in 1 ml phosphate-buffered saline mixed with 5 mg Evans blue dye.

CRA inhalation challenge and lung function measurement

Upon completion of sensitization, guinea pigs were allowed to rest for at least 5 days in their home cages. Animals then were tested for CRA provocation *in vivo*. The day before the CRA provocation, guinea pigs were acclimated to the double-chamber plethysmograph (Buxco Electronics, Inc., Troy, N.Y.) for at least 4 hours to minimize the influence of restraint stress. On the day of the CRA provocation experiment, guinea pigs were placed into the chamber and allowed to settle down while monitoring of specific airway resistance (SRaw) was continued with the guinea pigs in a conscious state. A segment of 10 minutes of data is used as baseline SRaw. The guinea pigs were then challenged with a nebulized CRA aerosol (1 mg/ml) at a flow rate of 3 L/min controlled by a bias flow machine for 10 minutes and then 30 minutes for the second challenge. The interval between the two challenges was 10 minutes. SRaw was recorded immediately after the first challenge and continuously monitored. Data were collected at a mean value of 10 minutes of every hour up to 8 hours. The guinea pigs were then released and returned to their home cages for resting and feeding overnight. SRaw then was measured again the next day at 24 hours after challenge, modified from the method described.¹⁶

Bronchoalveolar lavage

Immediately after 24-hour measurement of lung function in a conscious state, the guinea pigs were anesthetized by intraperitoneal injection of sodium pentobarbital. The trachea was exposed and cannulated. Bronchoalveolar lavage (BAL) was conducted by instillation of sterile saline (5 ml/time, two times) through the tracheal cannula; bronchoalveolar lavage fluid (BALF) was collected (approximately 70% volume of instilled solution) and analyzed for cell counts.¹⁶

TABLE I. Results of CRa skin test

Group	Saline	CRa 0.5 µg	CRa 1 µg	CRa 5 µg	Histamine
Control (<i>n</i> = 11)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	12.3 ± 0.4
LD (<i>n</i> = 6)	0.0 ± 0.0	7.6 ± 0.3	9.2 ± 0.7	12.5 ± 0.3	14.3 ± 0.3
MD (<i>n</i> = 6)	0.0 ± 0.0	7.5 ± 0.6	10.0 ± 0.6	12.4 ± 0.6	12.8 ± 0.3
HD (<i>n</i> = 6)	0.0 ± 0.0	7.4 ± 0.2	11.0 ± 0.2	13.1 ± 0.2	13.4 ± 0.1

Results are presented as means ± SEM size of wheals at 60 minutes after saline, histamine, or CRa injection on day 29.
Control, Saline-sensitized.

Inflammatory cell analysis in BALF

The collected BALF was centrifuged at 1500 rpm for 10 minutes. The pelleted cells were treated with Tris-buffered ammonium chloride solution (pH 7.2) to lyse red blood cells. The remaining cells were washed once with phosphate-buffered saline supplemented with 1% fetal calf serum and 0.1% antibiotics (from Gibco, Long Island, N.Y.). Total cell counts were determined with a hemocytometer. Differential leukocyte counts were undertaken on cytospin slides, which were prepared by centrifuge of BALF cells on a glass slide with a Cytospin centrifuge and stained with a Diff-Quick staining kit (Jorgensen Laboratories, Inc., Loveland, Colo.). A minimum of 200 leukocytes were counted with standard morphologic criteria.¹⁶

Measurement of airway smooth muscle contractility in vitro

After BAL, the guinea pigs were killed, and the trachea was isolated and soaked in ice-cold Krebs solution (NaCl 118 mmol/L, KCl 4.8 mmol/L, CaCl₂ 2.5 mmol/L, MgSO₄ 1.2 mmol/L, NaHCO₃ 24.9 mmol/L, KH₂PO₄ 1.15 mmol/L, glucose 5.6 mmol/L, and HEPEP 12.6 mmol/L, pH 7.4), with aeration by 95% O₂–5% CO₂. After clearing excess fat and connective tissues, the trachea was cut into 3 mm rings and mounted in organ baths containing Krebs solution (37°C). Airway smooth muscle contractility was measured as described previously¹⁷. The tracheal rings were initially stretched to a resting tension of 0.75 gm and equilibrated for 60 to 90 minutes with replacement of buffer every 20 minutes. Isometric tension development was measured with force-displacement transducers (model FT-03C, from Grass Instrument Co., Quincy, Mass.) and an EEG and polygraph data recording system (model 79D, Grass Instrument Co.).

Statistical analysis

The data were analyzed by analysis of variance (ANOVA) and corrected with repeated measures when appropriate. In the event that ANOVA justified post hoc comparisons between group means (e.g., *p* < 0.05 for a main or an interaction effect), the Neuman-Keuls test was used for the multiple comparisons. This test contains important safeguards for reducing the risks of committing a type I error. In experiments in which there were only two groups and no repeated measures, statistical analysis was carried out with the unpaired Student's *t* test. In all analyses, main effects, interactions, and group differences were considered significant at *p* < 0.05.¹⁸

RESULTS

Anaphylactic sensitization

As shown in Table I, the saline-sensitized guinea pigs (control) showed negative reactions to the CRa skin test, whereas in the CRa-sensitized guinea pigs, LD, MD, or HD revealed positive reactions (*p* < 0.001). The size of

TABLE II. Results of PCA assay

Group	4-Hour PCA Titers (IgG _{1a})	7-Day PCA Titers (IgE)	
		Nonheat	Heat
Control	0 (0 × 0)	0 (0 × 0)	0 (0 × 0)
LD	1:2 (6 × 6)	0 (0 × 0)	0 (0 × 0)
MD	1:40 (9 × 8)	0 (0 × 0)	0 (0 × 0)
HD	1:160 (9 × 7)	1:5 (9 × 5)	1:5 (8 × 6)

Values in parentheses are the mean diameters of the blue area measured at 30 minutes after CRa challenge in duplicate.
Control, Saline-sensitized.

wheals in response to three concentrations of CRa showed dose-dependent responses. However, the average size of wheals of LD, MD, and HD groups were not significantly different from each other.

The anaphylactic antibody measured by PCA assay, however, showed a clear dose-response relationship between CRa exposure during the sensitization period and the titers of the antibody. As shown in Table II, all three groups of guinea pigs exposed to CRa aerosol produced IgG_{1a}-like antibodies, and only the HD group showed a questionable IgE antibody in low titer (1:5), whereas no antibody was detected in the control group. The PCA titers were higher in HD, medium in MD, and lower in LD groups.

Contractile responses of tracheal rings in vitro

The contractile responses of tracheal smooth muscles to CRa were measured from the tracheal rings prepared as described and that had been isolated from the guinea pigs 24 hours after CRa provocation in vivo. The results, expressed as percent of the maximal contraction of the tracheal ring to acetylcholine stimulation (3×10^{-5} mol/L), were not different between control guinea pigs and CRa-sensitized guinea pigs (data not shown).

The tracheal rings of the CRa-sensitized guinea pigs responded with contractions to the additions of CRa (3, 10, 30, and 100 µg/ml), whereas those of control-sensitized animals did not (Fig. 1). The contractile responses were also heightened to higher amounts of CRa added to the tissue bath in a dose-responsive manner (*p* < 0.001). There was no contraction upon the addition of ovalbumin, a nonspecific antigen (data not shown). Moreover, the magnitudes of the contractile responses of the tissues from the three groups of guinea

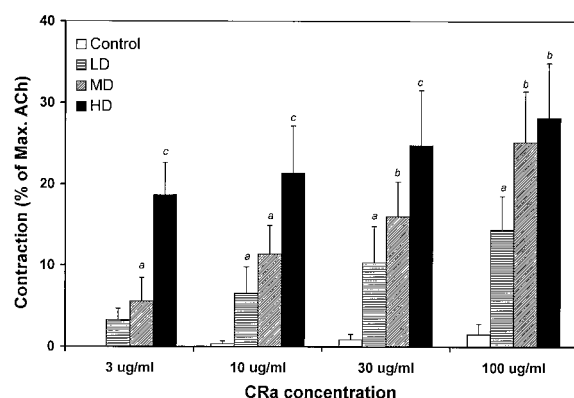


FIG. 1. Tracheal ring contractility to CRa challenge in vitro. Data are presented as mean $\pm$ SEM. Control, Saline sensitized; LD, low-dose CRa ($n = 6$); MD, medium-dose CRa ($n = 11$); HD, high-dose CRa ($n = 6$). a, significance between control and LD ($p < 0.05$); b, significantly different from both control and LD ($p < 0.05$); c, significantly different from control, LD, and MD ($p < 0.05$).

pigs sensitized with CRa in LD, MD, and HD were the strongest in HD, lower in MD, and lowest in LD to each respective dose of the CRa challenge ($p < 0.001$).

Inflammatory cellular responses in the BALF

The effects of CRa provocation (by inhalation) in vivo on airway inflammation in the sensitized guinea pigs were examined by analyzing comparative cellular contents of BALF at 24 hours after CRa inhalation in four groups. As shown in Table III, three CRa-sensitized groups of guinea pigs (LD, MD, and HD) showed significant leukocytosis in BALF compared with that of control guinea pigs ($p < 0.001$). The leukocytosis in BALF was the highest in guinea pigs sensitized with HD CRa, and in decreasing order showed a dose-dependent relationship to the doses of CRa used to sensitize the guinea pigs. The increased BAL leukocytes were represented in all types of inflammatory cells: neutrophils, lymphocytes, monocytes, and eosinophils. Eosinophilia were also apparent in all three groups of the CRa-sensitized guinea pigs but in a moderate degree. The greatest increase in BAL eosinophils was observed in the HD group (Fig. 2). There was no leukocytosis in the BALF of the CRa-sensitized guinea pigs when the animals were challenged by saline aerosol. Control-sensitized guinea pigs showed a mild to negligible leukocytosis and no eosinophilia in response to CRa inhalation provocation. These results indicate that CRa-inhalation-provoked leukocytosis and eosinophilia are CRa specific and that the influx of the eosinophil-rich inflammatory cells in the sensitized animals is correlated to the dosages of the CRa used to sensitize the guinea pigs (Fig. 2).

Asthmatic responses to CRa inhalation provocation in conscious guinea pigs

The baseline lung function (measured before CRa challenge by SRaw) of four groups of guinea pigs were similar: 1.56 ± 0.12 for control, 1.03 ± 0.11 for LD-CRa,

TABLE III. Total leukocytes in BALF 24 hours after CRa provocation

Group	Total leukocytes ($\times 10^6$)
Control ($n = 11$)	4.91 ± 0.46
LD ($n = 6$)	7.03 ± 1.37
MD ($n = 6$)	$19.50 \pm 4.31^*$
HD ($n = 6$)	$30.83 \pm 5.08^\dagger$

Number of total leukocytes in BALF was expressed as mean $\pm$ SE.

Control, Saline-sensitized.

*Significantly different from control and LD ($p < 0.01$);

†significantly different from control, LD, and MD ($p < 0.01$).

1.54 ± 0.17 for MD-CRa, and 1.96 ± 0.40 for HD-CRa, respectively. The CRa provocation generally induced increases in SRaw in the CRa-sensitized guinea pigs compared with control-sensitized animals (Fig. 3). However, the degree of changes in SRaw from the baseline level was widely variable, from a negligible increase to close to 400% increase in the sensitized animals. Also, the changes in SRaw occurred at variable time points and showed early, late, dual, or persistent pattern, which did not significantly differ among the three groups of CRa-sensitized guinea pigs. In general, CRa provocation in conscious guinea pigs induced increases in SRaw immediately in all three groups of CRa-sensitized guinea pigs; then followed a trend of returning to close to baseline levels at 1 hour after CRa provocation before their increases became persistent and severe during the observed period ($p < 0.01$). There were greater variations of SRaw in the later phase in all three groups; the SRaw of LD CRa-sensitized guinea pigs was gradually elevated and reached a plateau between 4 to 8 hours after the challenge, whereas the increased SRaw in MD and HD CRa-sensitized guinea pigs was sustained for several hours, with the second peak appearing approximately 8 hours after the CRa provocation. The increased SRaw, however, returned almost to their baseline levels at 24 hours after CRa provocation in all three groups of CRa-sensitized guinea pigs.

Interestingly, however, the degree of increase in the SRaw on CRa provocation in the three dosage levels (LD, MD, and HD) of the CRa used to sensitize guinea pigs failed to show a dose dependency; in fact, the LD group showed the highest increase in SRaw. Yet, more animals in the LD group showed that SRaw returned to baseline value at 24 hours after challenge compared with animals in the MD or HD group.

DISCUSSION

This study further demonstrates that aerosolized CRa exposure alone sensitizes animals to CRa and that the sensitized animals develop an antigen-specific "asthma-like" airway obstruction on CRa inhalation. This confirms previous studies from this laboratory.^{14,15} Moreover, the anaphylactic sensitization appears to be correlated to the levels of CRa exposures of the guinea

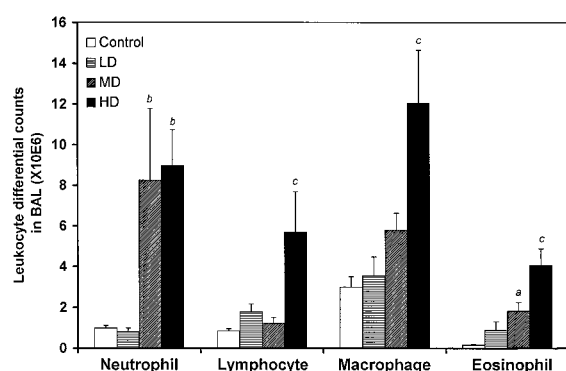


FIG. 2. Leukocytes in BALF 24 hours after CRa provocation. The number of leukocytes was absolute number and expressed as mean $\pm$ SEM. Control, Saline sensitized ($n = 11$); LD, low-dose CRa ($n = 6$); MD, medium-dose CRa ($n = 6$); HD, high-dose CRa ($n = 6$). a, significantly different from control ($p < 0.05$); b, significantly different from both control and LD ($p < 0.05$); c, significantly different from control, LD, and MD ($p < 0.05$).

pigs during the sensitization period (Fig. 1 and Tables II and III). However, the airway obstruction as manifested by the increased SRaw in conscious guinea pigs did not show a parallel dose-responsive relationship to the levels of CRa exposures during the sensitization period, even though the sensitized animals had shown a dose-responsive increase to the CRa challenge in vitro, that is, tracheal contractility and cellularity in BALF.

The CRa-provoked airway obstruction, defined as increased SRaw greater than 150% of the baseline value, was noted in 26 of the 27 CRa-sensitized guinea pigs, whereas it was increased in nine of the 20 control-sensitized guinea pigs (data not shown). Such an antigen-specific airway obstruction noted in the sensitive guinea pigs is quite similar to the asthmatic responses noted in cockroach-sensitive, asthmatic subjects.^{7, 11, 12} The CRa-sensitive human beings show elevated IgE antibody, whereas the CRa-sensitized guinea pigs produced mostly increased IgG_{1a} antibody. However, the fact that CRa-sensitized guinea pigs responded to CRa provocation with airway obstruction in vivo (together with the evidence in vitro, tracheal ring contractions, and so forth) suggests that the IgG_{1a} antibody appears to be a reaginic antibody in guinea pigs, as has been established.^{19, 20} The presence of anaphylactic antibody was apparent by AST and PCA. PCA showed a clear dose-response relationship; however, AST made it unclear. Yet, this probably is due to the inherent differences in the sensitivities of these two assays. It is true that skin testing in human beings also has a limitation unless one uses an end-point titration skin test to detect the reaginic sensitivity. Therefore although AST is a simple and sensitive assay for the determination of the status of allergic sensitization, it might be less sensitive in detecting quantity differences.

Inflammatory cellular influx in response to CRa provocation in the airways of the CRa-sensitized animals was apparent in the study (Table III and Fig. 2). The

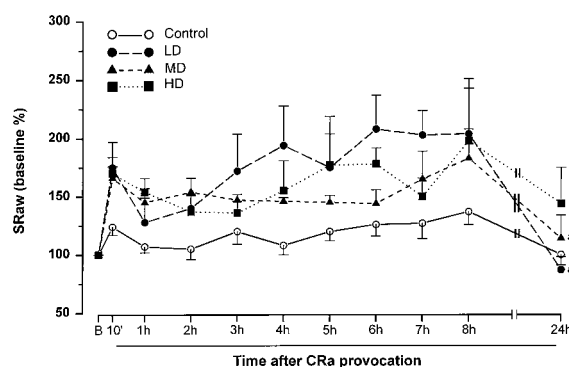


FIG. 3. Changes in SRaw after CRa provocation. The SRaw data were expressed as percent of baseline (B), mean $\pm$ SEM. Control, saline sensitized ($n = 11$); LD, low-dose CRa ($n = 6$); MD, medium-dose CRa ($n = 6$); HD, high-dose CRa ($n = 6$). a, significantly different from control ($p < 0.0001$).

magnitudes of leukocytosis and eosinophilia in BALF showed a trend of dose-response relationship to the degree of CRa sensitization. The greater leukocytosis noted in the HD CRa-sensitized group, together with the evidence in the contractile responses of the tracheal rings (Fig. 1), than that, noted in the LD- and MD-sensitized groups could be interpreted as a representation of more severe pulmonary inflammation. This could be an indication for stronger anaphylactic sensitization creating severe inflammation. However, the individual leukocyte population of the inflammatory cells in BALF was rather diffusely increased, with a modest dose-dependent increase in eosinophils. This tendency is similar to the reports of others.^{21, 22} Therefore the data could be interpreted to mean that there may be a dose-response relationship between levels of CRa exposure in sensitized animals and severity of the antigen-induced airway inflammation in these guinea pigs, as BALF leukocytosis, with neutrophilia and eosinophilia, have been used as indicators of pulmonary inflammation particularly of the late phase in human asthma.^{23, 24}

However, a most intriguing point is that despite the abovementioned evidence shown in vitro, there was no clear-cut dose-response relationship in the degree of airway obstructions noted in the animals in vivo in response to CRa inhalation provocation. Although all CRa-sensitized animals showed antigen-specific SRaw increases, the magnitude of increase in the SRaw of the HD group was smaller than that of the LD group guinea pigs (Fig. 3). There is a disassociation in the anaphylactic parameters measured in vitro and anaphylactic bronchospasm measured in conscious animals in vivo. The reason or reasons for the dissociation between bronchospasm and airway inflammation is not clear at present. One possible explanation could be that animals immunized with HD CRa had an ongoing severe degree of chronic airway inflammation at the time of CRa provocation. Consequently, the HD group of animals might have had less CRa to penetrate the airways to induce more severe asthmatic responses than those of LD or

MD animals. This explanation, however, does not explain the cellularity of BALF of the HD animals, which is greater than those of LD or MD animals (see Table III and Fig. 2). However, in an earlier study from this laboratory there was a dose-responsive bronchospasm noted in the anesthetized guinea pigs.¹⁵ A similar discrepancy between bronchospasm in vivo and quantity of anaphylactic antibodies produced against complex allergen, such as dust mites (*Dermatophagoide farinae*), has been reported.^{25, 26} However, in these studies animals were immunized differently from those in our study. A similar dissociation between the degree of inflammatory cellular responses and the degree of bronchospasm was observed in human asthma.^{27, 28} It seems that there might be a corrective mechanism or an inherent protective mechanism that is perhaps activated in asthmatic responses in conscious animals, even in fully anaphylactically sensitized status. It could be possible that maintenance of normal lung function is so vital to the body that bronchospasm resulted from inhalation of the allergen-induced airway constriction, or the induced inflammation might be counteracted by a compensation mechanism of the body. It could be mediated by secretion of glucocorticoids by the adrenal gland,^{29, 30} through activation of nonadrenergic and noncholinergic inhibitory mechanisms, or both.³¹ Therefore even if there are changes in the contractilities of airway smooth muscles in vivo, inflammatory cellular changes induced by allergic inflammation, or both, their expression may not necessarily produce proportional changes in the lung function in conscious animals. The antigen-induced reduction of lung function in conscious animals may require a profound alteration in lung pathophysiology, such as extreme cholinergic hyperreactivity, airway epithelial damage, or both to show as asthmatic responses in a conscious state. Nevertheless, it appears that the impact of the CRa, an indoor allergen, depends on its ability to induce allergic sensitization and airway inflammation. The allergic sensitization appears to be related to the amount of CRa exposed during sensitization, perhaps along with the duration of the CRa exposure. Yet the severity of asthma manifested in conscious guinea pigs is not quite dose dependent to the amount of CRa exposure. Further study to investigate the impact of the duration of CRa exposure and the interaction between the dosages and duration of CRa exposure on the severity of asthma is warranted.

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