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Study on cockroach antigen as a probable causative agent in bronchial asthma

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Bronchial provocative test (BPT) with cockroach (CR) antigen was performed in 22 asthmatic subjects. Sixteen had positive reaction (CR-positive) and 6 had negative reaction (CR-negative) to CR antigen by skin test. Immediate bronchoconstrictive response was noted following the antigen inhalation in 14 of 16 CR-positive asthmatics, while none of 6 CR-negative asthmatics showed bronchospasm. Late asthmatic responses also were noted in 13 of 16 CR-positive asthmatic individuals following BPT with CR antigen. The dual asthmatic reactions in CR-positive individuals were mostly inhibited by the prior administration of cromolyn sodium. Three-fold increases in peripheral eosinophil counts were noted 24 hr following BPT with CR antigen. Results indicate that CR-induced asthmatic responses are allergen-specific and CR plays a causative role in allergic asthma in the population studied.

Successful management of allergic bronchial asthma depends on proper identification of the causative allergens. In winter, as patients spend more time in a closed environment, house dust may become more important as a cause of bronchial asthma. However, the role of house dust as a cause of asthma has been controversial for a long time. One of the problems in assessing the importance of house dust is that its composition differs in different geographic locations. Animal hair and various domestic insects are known to alter the composition of house dust.

It is well known that cockroaches heavily infest urban dwellings, especially in

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TABLE I. Clinical information on 22 asthmatic subjects studied for BPT with CR antigen

Subjects	Age	Sex	Duration of BA (yr)	Worse season of year	Result of skin test	
					Positive reaction to cockroach	Positive allergy skin tests
1. A. Z.	18	F	10	W	+	Hd
2. M. O.	32	F	20	SP W	+	Ca, Tr, Hd
3. D. H.	20	F	18	P W	+	Gr, Do, Hd, Sm, Pm
4. A. W.	27	F	12	W	+	Hd
5. M. H.	16	F	6	W	+	Ca, Hd, Sm
6. L. T.	44	F	19	P W	+	Hd
7. S. A.	15	F	6	W	+	Hd
8. M. G.	40	F	10	P W	+	Ca, Hd, Do
9. P. W.	37	F	2	S	+	Tr, Ra, Ww, Ca, Hd
10. R. H.	21	F	20	P W	+	Tr, Gr, Ra, Ww, Do, Hd, Sm
11. L. B.	35	F	0.5	W	+	Do, Hd
12. V. W.	39	F	12	P W	+	Gr, Ra, Hd, Sm, Ca
13. C. H.	10	M	1	P S	+	Ca, Hd
14. M. S.	34	F	10	S F	+	Do, Ca, Ra, Hd
15. L. A.	9	F	5	W	+	Tr, Gr, Ca, Hd
16. L. P.	51	M	19	W	+	Hd
17. C. H.	22	F	3	P	0	None
18. T. G.	21	F	1	P	0	Sm, Hd
19. K. W.	38	M	7.5	P S	0	Tr, Gr, Ww, Do, Hd
20. E. W.	54	F	14	P	0	Ca, Hd, Mi
21. A. C.	43	F	4	P W	0	Hd
22. J. K.	46	M	6	P	0	None

W: winter Hd: house dust Pm: perennial molds SX: Symptom
SP: spring Ca: cat Do: dog DSCG: Disodium cromoglycate
P: perennial Gr: grass Tr: trees CR: Cockroach
F: fall Ra: ragweed Ww: western weeds ND: not done
S: summer Sm: seasonal molds BA: Bronchial asthma

*Degree of reaction expressed in scale of 0-4.

†Numbers in parentheses represent a number of inhalation of cockroach antigen (1:100).

lower socioeconomic communities.^{1, 2} Recently, a series of studies by Bernton and Brown³⁻⁵ clearly demonstrated that cockroach (CR) antigen has the capability of inducing skin-sensitizing antibodies, and they reported very high incidence of positive skin reaction to CR antigen in an atopic population (44%) as well as in a nonatopic population (7.5%). Even higher rate of positive skin reaction to CR antigen (58%) was detected in an atopic population of urban Chicago area recently.⁶ Such a high prevalence of reactivity to CR antigen cast a question on its significance in interpretation of allergy skin test.

Bronchial provocative test is well accepted as a specific test for detection of the causative antigen and a valuable procedure for an evaluation of allergic bronchial asthma.⁷⁻¹⁰

In this study, we performed the bronchial provocative test using CR antigen in asthmatic patients in order to evaluate the role of CR antigen in bronchial asthma.

PATIENTS AND METHODS

Patients

During a period of over one year (November, 1973, to February, 1975), 102 patients with bronchial asthma with or without rhinitis underwent skin test with CR antigen as well as 25 to 30 other common inhalant allergens (Holister-Stier Co.). Among these patients, 22 active

Bronchial provocative test			Immediate asthmatic reaction		Late SX	Eosinophils (count/mm ³)	
Cockroach	Saline	DSCG + CR	Early SX*	Early sign*		Before challenge	After 24 hr
++ (5)	—	—	4	4	4	233	305
+ (6)	—	ND	3	3	3	971	1,220
+ (5)	—	—	2	2	4	290	527
+ (5)	—	—	4	4	3	289	444
+ (5)	—	—	4	2	4	500	1,066
+ (5)	±	+	2	4	4	61	83
+ (5)	—	—	3	2	1	538	449
— (10)	—	—	0	0	0	438	499
+ (13)	—	+	2	1	0	200	ND
+ (5)	—	+	4	4	3	ND	ND
+ (10)	—	—	3	3	2	222	442
+ (5)	—	—	4	4	2	200	1,465
+ (10)	—	—	4	3	1	55	5,772
— (10)	—	—	0	0	0	582	760
+ (5)	—	—	3	3	2	500	1,100
+ (10)	ND	ND	4	4	3	ND	ND
— (10)	—	ND	0	0	0	2,070	2,542
— (10)	—	ND	0	0	0	377	438
— (10)	—	ND	0	0	0	278	183
— (10)	—	ND	0	0	0	170	894
— (10)	ND	ND	0	0	0	111	83
— (10)	ND	ND	0	0	0	205	205

asthmatics were selected for bronchial provocative test using CR antigen. All of these patients had moderately severe bronchial asthma, and their last asthmatic attack was 1 wk to 6 mo prior to the test. They were on one or more bronchodilators "round the clock" except Patient T. G. (No. 18), and 3 of them (2 CR-positive asthmatics and 1 CR-negative asthmatic) were also on alternate-day steroid at the time of this study. Of these 22 patients, 16 had positive skin reaction to CR antigen (CR-positive) and 6 had negative skin reaction to the antigen (CR-negative). Average age of CR-positive asthmatics was 29 yr, ranging from 9 to 51, while that of CR-negative asthmatics was 37 yr, ranging from 21 to 54 yr. Mean durations of bronchial asthma in CR-positive asthmatics and CR-negative asthmatics were 6.9 yr and 5.9 yr, respectively.

Sixteen CR-positive asthmatics had extrinsic type of bronchial asthma with seasonal variation in severity of their asthma (Table I). The number of significantly positive skin reactions to most common inhalants was 1 to 6 allergens when 30 inhalants were tested in addition to the CR antigen. Among 6 CR-negative asthmatics, 4 were intrinsic type (Subjects 17, 18, 20, and 22) and 2 were extrinsic type (Subjects 19 and 21) of bronchial asthma (Table I).

Allergy skin test

Routine allergy survey and allergy skin tests were carried out at the outpatient department. The skin tests included the prick test and intracutaneous test with 30 common inhalant allergens (1:20 w/v for the prick test and 500 PNU/ml for the intracutaneous test). Results were read in 15 min by measuring diameter of wheals. Interpretation of positive result indicates that the size of the wheal was over 13 mm in diameter and/or 3-fold larger than the diameter of the control wheal.

Bronchial provocative test (BPT)

The detailed procedure for the BPT and possible adverse reactions were explained to the volunteer subjects before the procedure was carried out. BPT was undertaken while the patients were hospitalized, and all bronchodilators were withheld for 4 to 10 hr prior to the test. An

TABLE II. Bronchial response of asthmatic subjects to bronchial provocative test with CR antigen

	FVC ₁ (%) [*]	FEV ₁ (%) [*]	PFR	Immediate symptoms	Signs	Late symptoms
CR-positive	57.6 ± 5.7	64.4 ± 4.8	57.2 ± 5.9	14/16	12/16	13/16
CR-negative	97.4 ± 2.0	95.8 ± 2.9	92.8 ± 5.9	0/6	0/6	0/6

Data expressed as mean ± standard error.

^{*}% of baseline values at point of maximal response to antigen.

Expirometer (Warren E. Collins, Inc.) was used to measure forced expiratory volume in one second, (FEV₁), forced vital capacity (FVC), and peak flow rate (PFR). Either antigen extract or saline was aerosolized by a glass nebulizer (No. 40, DeVilbiss Co.) using compressed air. Paired tests (saline and antigen challenge) were carried out at approximately the same time on two consecutive days in a randomized sequence, so that each patient served as his/her own control for antigen challenge. Each patient inhaled 5 to 10 breaths of the antigen extract (1:100 w/v), corresponding to a total volume of 0.1 to 0.2 ml of the antigen extract (containing 400 to 800 protein nitrogen units [PNU] of CR antigen).

Pulmonary functions were measured at control state (baseline) and at 5, 10, and 15 min following inhalation of the allergen or saline. The result was considered as positive when a reduction of 15% or more from the baseline value was obtained. Subjective symptoms and objective signs were recorded in detail during the procedure and for the following 24 hr. Subjects were given 5 breaths of 1:200 isoproterenol following the BPT with antigen or saline to reverse the induced bronchospasm.

In 14 subjects who showed a definite bronchoconstriction following the antigen challenge, the provocative test was repeated using the same dose of antigen a few days later in the same manner 30 min after an administration of 40 mg of disodium cromoglycate (DSOG) by inhalation.

Total eosinophil counts were obtained from peripheral blood taken before the challenge and at 12, 24, 48, and 72 hr after the BPT with CR antigen.

RESULTS

Summarized clinical data on 22 asthmatics studied are shown in Table I. Extrinsic type of asthmatics who showed positive skin reaction to CR antigen are the first 16 subjects listed in the table. They were allergic also to other inhalants such as ragweed and/or house dust except Subject 1, who was allergic only to CR antigen. It is interesting to observe that 12 of 16 CR-positive asthmatics had more severe and frequent attacks of bronchial asthma during the winter, as shown in Table I. Furthermore, 7 of them were relatively free of symptoms during warmer months of the year, and they were allergic to cockroach and house dust by skin test, while the tests with other 30 common inhalants were negative. CR-negative asthmatics were listed as the latter 6 subjects in the table, and skin test to CR antigen in these patients was negative. However, 2 of 6 CR-negative asthmatics (Nos. 19 and 21) were allergic to other allergens, i.e., grass and ragweed in Subject 19 and house dust in Subject 21.

CR antigen inhalation caused a significant decrease in pulmonary function as well as inducing symptoms and signs of asthmatic reaction in 14 of 16 CR-positive asthmatics, while saline inhalation failed to induce bronchospasm in the same population. None of CR-negative asthmatics showed significant decrease in their pulmonary functions following BPT with 10 inhalations of CR antigen.

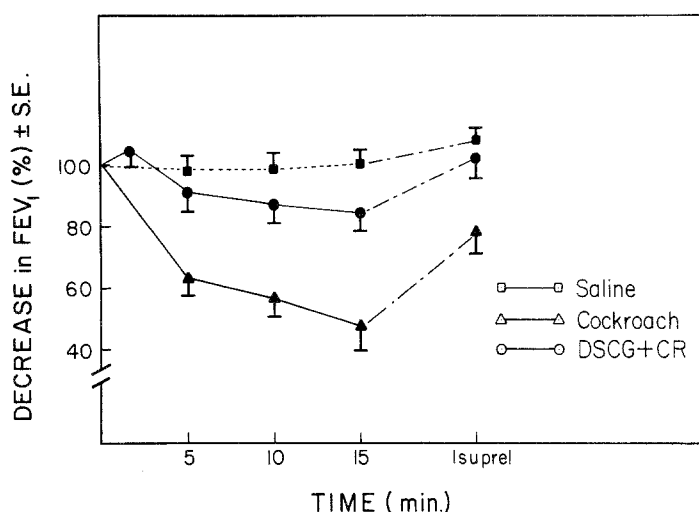


FIG. 1. Changes in FEV₁ following BPT using CR antigen in 14 asthmatic subjects with positive skin reaction to the antigen.

TABLE III. Changes in pulmonary function following BPT with CR antigen, in 16 asthmatics who showed positive skin reaction to the antigen

	FEV ₁ (L)				FVC (L)				Peak flow rate (L/sec)			
	Base-line	5 min	10 min	15 min	Base-line	5 min	10 min	15 min	Base-line	5 min	10 min	15 min
Saline	1.93 ±0.1	1.90 ±0.1	1.88 ±0.14	1.88 ±0.16	2.47 ±0.15	2.45 ±0.16	2.29 ±0.18	2.38 ±0.20	3.9 ±0.27	4.1 ±0.30	3.9 ±0.29	3.9 ±0.38
Cock- roach	1.95 ±0.13	1.36 ±0.15	1.27 ±0.15	1.22 ±0.21	2.42 ±0.16	1.89 ±0.19	1.75 ±0.18	1.65 ±0.21	3.9 ±0.36	2.5 ±0.3	2.42 ±0.32	2.5 ±0.44
DSCG + CR	1.90 ±0.11	1.70 ±0.12	1.68 ±0.17	1.62 ±0.17	2.29 ±0.14	2.42 ±0.14	2.19 ±0.19	2.12 ±0.19	3.9 ±0.35	3.9 ±0.33	3.5 ±0.37	3.3 ±0.30

Data expressed as mean $\pm$ standard error.

The decrease in pulmonary functions following CR antigen challenge in CR-positive asthmatics was statistically significant ($p < 0.005$) as compared to their baseline values or to the decrease observed in CR-negative asthmatics. The FEV₁ values appeared to be more sensitive to the allergen challenge, and its measurement of the change was more consistent than other parameters measured (Table II).

When 3 of the 16 CR-positive subjects were challenged with unrelated antigen, grass antigen, or ragweed antigen (1:50 w/v), to which they are nonreactive by skin test, none of the subjects showed a detectable bronchoconstriction. On the other hand, a CR-negative subject (Subject 19) developed a significant bronchoconstriction (34% fall in FEV₁) and other signs of asthmatic response when he was challenged with ragweed antigen to which his skin test showed positive response.

Baseline values of various pulmonary functions obtained in two different test days showed no significant difference (Table III).

Average changes in FEV₁ on CR-responsive asthmatics are illustrated in Fig. 1. Inhalation of CR antigen in CR-positive asthmatics induced an average 49%

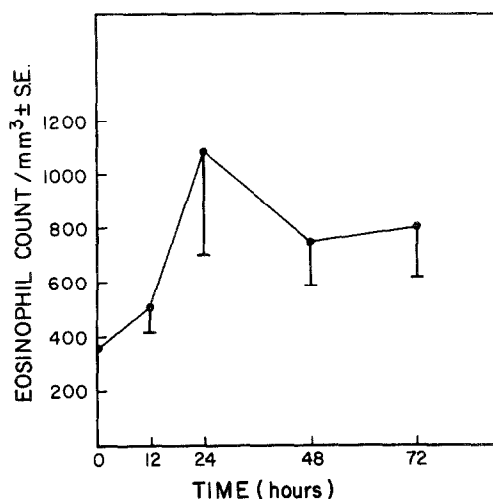


FIG. 2. Changes in peripheral eosinophil count following BPT with CR antigen in 16 asthmatic subjects with positive skin reaction to the antigen.

reduction in FEV_1 , while inhalation of saline in the same population showed no effect on the FEV_1 . This difference between antigen and saline in their effect on FEV_1 was statistically significant ($p < 0.0005$). The values for FVC and PFR were in accord with the result of FEV_1 .

The reduction in FEV_1 by the CR challenge was mostly prevented by a prior medication with DSCG, as illustrated in Fig. 1 and Table III.

The immediate symptoms following antigen inhalation were coughing, tightness of the chest, itching throat, and a sense of "air hunger." These symptoms appeared in a few minutes to 15 min following BPT with CR-antigen. Usually these symptoms worsened gradually until the responses were interrupted by inhalation of isoproterenol. Three subjects responded with generalized weakness and profuse sweating in addition to the symptoms listed above. Objective signs were prolonged expiratory phase, inspiratory and expiratory wheezing, tachycardia, and tachypnea. In 6 of 16 CR-positive subjects, immediate reactions were so severe that isoproterenol had to be administered before concluding the usual 15-min observation period.

Late asthmatic responses were noted in 13 of 16 CR-positive subjects, while none of 6 CR-negative subjects showed noticeable reactions (Tables I and II). Patients with the late asthmatic responses were gradually developing cough and shortness of breath accompanied by bilateral wheezing. These appeared usually 6 to 10 hr after the BPT with CR allergen in patients who had severe, immediate asthmatic reactions to the CR inhalation challenge. The late asthmatic responses developed in CR-positive asthmatics in spite of their usual "round-the-clock" bronchodilator medication. In some cases, the late asthmatic response was so severe that it was necessary to administer parenteral medications such as Susphrine and/or aminophylline in addition to the patient's regular oral medications. However, there were no febrile responses nor changes in chest x-rays during this period.

Total eosinophil counts in peripheral blood following the BPT with CR antigen revealed quite variable results (Table I). The average eosinophil counts in the peripheral blood before antigen challenge were similar in both asthmatic groups. Following the antigen challenge, the average total eosinophil counts showed slow increase until 24 hr, when it became 300% of the baseline value in CR-positive asthmatics and 135% of the baseline value in CR-negative asthmatics. The average eosinophil count measured 72 hr after the challenge was still higher than the baseline but lower than the 24 hr value in CR-positive asthmatics (Fig. 2).

DISCUSSION

An immediate asthmatic response following the CR inhalation in asthmatic individuals has been first reported by Bernton, McMahon and Brown.¹¹ Six of their 10 asthmatic subjects showed reduction in FEV₁, 15% or more from the baseline value, following the inhalation of 36 to 2,480 PNU of the CR antigen. However, development of a late asthmatic response was not mentioned in their report. Uy and co-workers¹² reported that CR antigen challenge on asthmatic children showed neither immediate asthmatic response nor late asthmatic response. They used, however, a minute amount of antigen (5 PNU/ml) which was far less than the concentration used in this study or in other similar studies.^{7, 11, 13, 14}

Late asthmatic reaction following house dust inhalation in asthmatic individuals has been described by several other investigators,^{7, 13, 15, 16, 17} and the rate of its occurrence varied from 15% to 47%, depending on the investigators. In asthmatic subjects with positive skin reaction to the ragweed antigen, late asthmatic response developed following ragweed allergen inhalation in as high as 60% of the subjects studied¹⁴—but late asthmatic response to CR antigen inhalation has never, to my knowledge, been reported.

The findings from this study clearly demonstrate that inhalation of CR antigen induces the immediate and late asthmatic responses in asthmatic individuals who have positive reaction to the antigen by skin test. Though the mechanism of the dual asthmatic responses induced by the CR allergen challenge was not the scope of this study, the following observations indicate that immunologic reaction, particularly reagin-allergen reaction, is likely the basic mechanism of these asthmatic responses: (1) BPT with CR antigen, even with a higher amount than that used in CR-positive asthmatic subjects, failed to induce the asthmatic response in CR-negative asthmatics; (2) the asthmatic responses were mostly in accord with the skin test reactivity, and immediate asthmatic response was allergen-specific, as shown in Table I and Fig. 1; (3) dual asthmatic response induced by the ragweed antigen challenge is well known to be an IgE allergen reaction,^{14, 18} and the CR antigen challenge induced similar dual asthmatic response; (4) the development of dual asthmatic response following the CR challenge was mostly prevented by a prior medication with DSCG, which is well known to exert an inhibitory effect on reagin-mediated reactions^{16, 19, 20}; (5) a significant peripheral eosinophilia was induced only in the CR-positive asthmatic group by the CR challenge.

Baseline values of pulmonary functions obtained in two different test days were not significantly different ($p > 0.9$); thus, the reliability of pulmonary function data measured after the BPT is secured. BPT with the diluent buffer solution did not induce any detectable response in the population studied, and CR antigen failed to induce the asthmatic response in CR-negative asthmatic subjects, thus ruling out the possibility of a nonspecific, irritant effect of the antigen to be the cause of the change in pulmonary functions.

Significance of the peripheral eosinophilia following the BPT with CR antigen remains to be determined by a further study. It is, however, interesting to note an observation by Booij-Noord and co-workers¹³ on the eosinophilic response to house dust challenge. They noted 191% increase in peripheral eosinophil count following the house dust challenge in subjects who showed dual asthmatic response, while the increase in eosinophil count was 101% in subjects who manifested only an immediate response to the challenge. It appears that a probable role of eosinophils in asthmatic response to allergen challenge, particularly in late asthmatic manifestation, warrants a further study.

Entomologic study on cockroaches by Sheffield²¹ suggests that dead cockroaches and/or excreta of the insects may remain in the house for long periods, depending on the degree of the infestation by cockroaches and the hygienic status of the house. Consequently, it is not unreasonable to presume that components of cockroaches become a part of house dust while they disintegrate slowly in their domain.

In an evaluation of asthmatics from urban residence particularly, who have more frequent exacerbations in the winter, the CR antigen should be considered as one of the probable causative agents for their allergic bronchial asthma.

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