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Changes in bronchial responsiveness to histamine at intervals after allergen challenge

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ABSTRACT Bronchial responsiveness to inhaled histamine was measured two, seven, and 30 hours after allergen inhalation challenge in 19 atopic subjects. The provocative histamine concentrations causing a 20% fall in FEV_1 (PC_{20}) at these three times were compared with the baseline value, with values obtained two and seven hours after diluent inhalation, and with those obtained five to seven days after allergen challenge in the 12 late responders. Seven subjects had allergen induced isolated early asthmatic responses (ΔFEV_1 22·6% (SD 6·6%)) with less than a 5% late fall in FEV_1. There was no change in the six histamine PC_{20} values measured in these seven subjects; the geometric mean $PC_{20}$was 1·0–1·3 mg/ml on all six occasions. Twelve subjects had an allergen induced early asthmatic response (ΔFEV_1 26·3% (9·8%)) followed by a definite (>15% ΔFEV_1, n = 7) or equivocal (5–15% ΔFEV_1, n = 5) late asthmatic response. The geometric mean histamine PC_{20} was not significantly different two hours after allergen inhalation either from baseline (0·67 *v* 0·78 mg/ml) or from that seen two hours after diluent (0·67 *v* 0·95). It was significantly reduced at seven (0·24 mg/ml) and at 30 hours (0·44 mg/ml) but had returned to baseline when repeated five to seven days later (0·74 mg/ml). In 10 subjects with a dual response who had a repeat antigen challenge the mean early and late response and ΔPC_{20} at seven and 30 hours were similar. These data show that bronchial responsiveness to a non-allergic stimulus has not increased two hours after allergen inhalation following spontaneous recovery of the early asthmatic response but before the start of the late asthmatic response.

Non-allergic bronchial responsiveness (for example, to inhaled histamine or methacholine) may increase after exposure to allergen[1–3] or occupational sensitising agents[4 5] in a sensitised individual. There is a close relationship between allergen or occupational induced increase in non-allergic bronchial responsiveness and the late asthmatic response.[1–5] Non-allergic bronchial responsiveness does not appear to increase after isolated early asthmatic responses.[1 3] Bronchial responsiveness to histamine or methacholine is increased seven to eight hours after allergen challenge[1 2 4] towards the end of the late response and this may persist for days after the late response, at a time when all measurements of lung function have returned to baseline.[1 2 4] To our knowledge, change in bronchial responsiveness in the interval phase between the early and the late response to allergen has not been examined closely. In this study we measured bronchial responsiveness to histamine before and two, seven, and 30 hours after allergen inhalation in 19 subjects undergoing controlled allergen inhalation tests.

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Methods

SUBJECTS

Nineteen subjects were selected from volunteers and patients at the respiratory clinic, University Hospital, Saskatoon. All subjects had asthma, defined as wheezing dyspnoea on exposure to an allergen to which they had a positive prick skin test response. All subjects were atopic and had an FEV_1 greater than 70% of the predicted value and a histamine provocative concentration causing a 20% fall in FEV_1 (PC_{20}) of 10 mg/ml or less. They were using no medication other than an inhaled β_2 agonist occasionally as needed (n = 7). The investigations were approved

Table 1 *Anthropometric and clinical data on the subjects*

Patient No	Age	Sex	Height (cm)	Weight (kg)	FEV_1 (l)	FEV_1 (% predicted)	Histamine PC_{20} (mg/ml)	Treatment*	Allergen†	Allergic respon
1	19	M	188	90·9	5·2	109	8·0		Horse	E
2	18	F	160	59·0	3·1	96	6·8		Cat	E
3	22	M	173	80·5	4·7	108	5·4		Grass	D
4	19	F	175	86·4	4·1	110	3·8		Cat	D
5	17	F	160	64·5	3·6	110	3·8		Grass	D
6	23	F	165	52·7	3·5	106	2·5		Horse	E
7	27	F	160	84·1	3·0	101	2·1		Grass	D
8	28	F	165	74·5	3·7	117	1·2		Cat	D
9	22	M	180	81·8	4·2	92	1·2		Grass	E
10	26	F	160	60·9	3·2	100	0·80		Cat	E
11	23	M	178	73·6	4·8	110	0·58	*	Tree	D
12	20	F	157	73·2	3·2	104	0·40		Cat	D
13	22	F	165	63·2	3·4	102	0·40	*	Cat	D
14	21	F	157	58·9	3·1	100	0·36	*	Horse	D
15	20	F	165	56·8	3·4	102	0·32	*	Cat	D
16	23	F	170	70·9	3·1	89	0·20	*	Grass	D
17	22	M	165	78·2	3·7	92	0·20		Grass	E
18	58	F	163	72·3	1·6	71	0·14	*	Cat	E
19	36	M	170	63·2	2·8	76	0·12	*	Grass	D

*Inhaled β_2 agonist (fenoterol or salbutamol) as needed.
†Allergens used were cat 1:10 w:v, lot No 152072306; horse 20 000 PNU/ml, lot No J52115501; and mixed tree pollen 1:10 w:v, lot No 1498180 (purchased Hollister Stier Laboratories, Mississauga, Ontario); mixed grass pollen 1:20 w:v, lot No X30631 (purchased from Bencard Allergy Service, Western Ont
PC_{20}—provocative concentration causing a 20% fall in FEV_1; E—early; D—dual.

by the President's Ethics Committee of the University of Saskatchewan and signed informed consent was obtained. Data on the subjects are shown in table 1.

HISTAMINE INHALATION TEST

Bronchial responsiveness to inhaled histamine was measured as described previously.[6 7] Solutions were nebulised with a Wright nebuliser calibrated to give an output of 0·130 ml/min (airflow 8 l min^{-1}) of an aerosol whose particles had an aerodynamic mass median diameter 1–1·5 μm. Aerosols were inhaled by tidal breathing via a loose fitting facemask with the nose clipped. The FEV_1 was measured initially in triplicate. Phosphate buffered saline (PBS), the diluent for the histamine solution, was then inhaled for two minutes and the FEV_1 measurement repeated at 30 and 90 seconds. Doubling concentrations of histamine (0·03–8·0 mg/ml) were then inhaled for two minutes at five minute intervals until the FEV_1, measured again at 30 and 90 seconds, had fallen at least 20% or until the highest concentration had been given. The percentage reduction in FEV_1 was calculated from the lowest post-PBS value to the lowest post-histamine value and the histamine PC_{20} was calculated by interpolation of the last two data points on the concentration-response curve.[8]

ALLERGEN INHALATION TEST

Controlled allergen inhalation tests were carried out as previously described.[1 2] On day 1, the control day, subjects waited in the laboratory for at least 30 minutes before performing triplicate FEV_1 manoeuvres. Sterile isotonic buffered saline with 0·5% phenol, the diluent for the allergen solutions, was nebulised by a separate Wright nebuliser (output 0·130 ml/min, flow rate 8·5 l min^{-1}) and inhaled via a mouthpiece and Hans Rudolph valve with two filters (BB-50T, Pall Biomedical Inc, Fajardo, Puerto Rico 00648) in series on the exhaled line. Three two minute tidal breathing inhalations of diluent were done at 10 minute intervals, the FEV_1 being repeated in duplicate 10 minutes after each inhalation and then 20, 30, 40, 50, 60, 90 minutes and 2, 3, 4, 5, 6, and 7 hours after the final inhalation. The best FEV_1 at each time was retained for analysis. Histamine PC_{20} and the allergen concentration required to produce a 2 mm skin weal were determined during day 1 and these allowed prediction of the allergen PC_{20}.[9]

On day 2, usually the next morning, allergen inhalation was performed. In a fashion analogous to diluent inhalation, doubling amounts of the relevant aqueous allergen, beginning three dilutions below the predicted allergen PC_{20}, were inhaled for two minutes at 10 minute intervals until the FEV_1 had fallen at least 20%; the FEV_1 was then followed for seven hours as on day 1. The percentage fall in FEV_1 was calculated from the highest baseline to the highest post-allergen FEV_1.

STUDY DESIGN

Subjects attended the laboratory on two days (which were usually consecutive) for control and allergen inhalation tests. All had a stable FEV_1 ($\leqslant$10% difference between the two days), none had suffered

Table 2 *Individual values (mg/ml) for histamine provocative concentration causing a 20% fall in FEV_1 (PC_{20})*

Patient No	Control	After diluent		After allergen				Second allergen challenge		
		2 h	7 h	2 h	7 h	30 h	5–7 d	18 h before	7 h after	30 h after
DUAL RESPONDERS										
3	5·4	7·9	5·1	2·8	1·7	3·9	7·9	9·3	3·7	4·2
4	3·8	3·4	7·2	4·2	1·8	4·1	3·1	2·8	1·5	1·8
5	3·8	9·0	8·8	2·6	0·9	1·4	3·1	6·5	0·22	1·9
7	2·1	1·9	3·1	2·3	0·71	2·7	2·9	3·4	0·70	1·6
8	1·2	1·3	1·3	1·3	0·30	0·50	1·0	1·4	0·23	0·41
11	0·58	0·62	0·49	0·46	0·29	0·22	0·37	0·37	0·17	0·24
12	0·40	0·59	0·34	0·30	0·37	0·15	0·80	0·48	0·19	0·13
13	0·40	0·33	0·30	0·29	0·045	0·26	0·22	0·37	0·14	0·25
14	0·36	0·23	0·21	0·17	0·10	0·13	0·18	0·25	0·12	0·19
15	0·32	0·34	0·39	0·37	0·15	0·35	0·41	—	—	—
16	0·20	0·80	0·20	0·18	0·052	0·096	—	—	—	—
19	0·12	0·12	0·15	0·19	0·024	0·048	0·13	0·15	0·030	0·078
Geometric mean PC_{20}	0·78	0·95	0·86	0·67	0·24**	0·44**	0·83	1·07	0·28	0·51
EARLY RESPONDERS										
1	8	8	3·5	3·8	2·7	4·8	—			
2	7·2	7·2	6·8	4·5	2·5	5·9	—			
6	2·5	1·0	2·6	1·6	3·8	1·8	—			
9	1·2	1·7	0·9	1·8	1·1	2·2	—			
10	0·8	1·1	1·3	4·0	1·3	1·4	—			
17	0·20	0·18	0·35	0·22	0·16	0·20	—			
18	0·17	0·10	0·09	0·06	0·19	0·19	—			
Geometric mean PC_{20}	1·24	1·10	1·12	1·15	1·02	1·29				
TOTAL GROUP										
log PC_{20}										
n	19	19	19	19	19	18				
Mean	−0·033	0·001	−0·023	−0·09	−0·38	−0·15				
SD	0·60	0·63	0·63	0·60	0·66	0·67				
Geometric mean	0·93	1·00	0·95	0·81	0·42**	0·70*				

*p < 0·01; **p < 0·001 compared with 7 h after saline.

allergen exposure or respiratory tract infection for at least four weeks, and all were able to withhold inhaled β_2 agonists for at least eight hours.

Histamine PC_{20} was measured six or seven times in all subjects. A baseline PC_{20} was obtained within two weeks of the study. Histamine inhalation was repeated, starting two and seven hours after completion of diluent inhalation on day 1 and two, seven, and 30 hours after allergen inhalation on day 2. In all cases the two hour histamine inhalation test was completed in less than 30 minutes and was therefore completed 2·25–2·5 hours after allergen inhalation. In subjects with a late asthmatic response and a significant reduction in histamine PC_{20} a seventh histamine inhalation test was done five to seven days later.

Ten subjects with equivocal or definite dual asthmatic responses underwent repeat allergen challenge within two months of the original study. The same dose of allergen was administered 10 minutes after inhalation of two puffs of freon propellant. Histamine PC_{20} was measured before and seven and 30 hours after allergen challenge but not at two hours.

ANALYSIS

Analysis was performed by means of logarithmic transformation of PC_{20} values and paired t tests.[10]

Results

There was less than 5% change in FEV_1 during the control day in 17 of the 19 subjects. In the remaining two (Nos 16 and 18) there was a gradual fall in FEV_1 of 15–20%. A correction was made for the presence and magnitude of the late response in these two subjects in the allergen study.

After allergen challenge in the 19 subjects the mean early fall in FEV_1 was 24·5% (SD 8·86%). A definite early asthmatic response (>15% fall in FEV_1) was seen in 18 and an equivocal (5–15%) response in one

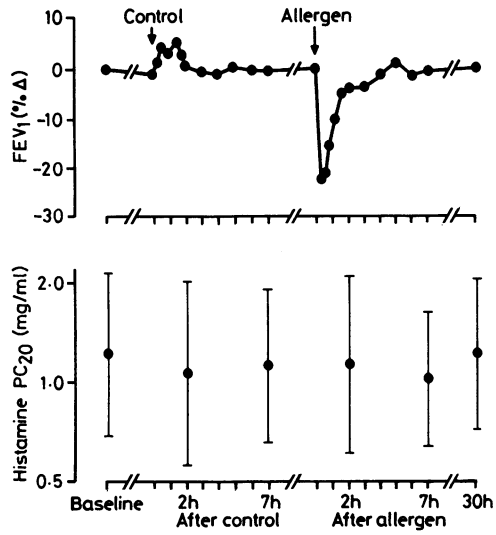

Fig 1 *Changes in FEV_1 and histamine PC_{20} (provocative concentration causing a 20% fall in FEV_1) in seven subjects with an isolated early asthmatic response. The mean FEV_1 (% change) in the upper graph and the geometric mean (with SEM) histamine PC_{20} in the lower graph are plotted against time (variable scale) on the horizontal axis.*

subject. This subject was included because he had a reproducible late response with an associated fall in histamine PC_{20}. The mean late fall in FEV_1 was 11·8% (10·9%) ($p < 0·001$). There was a definite late response (> 15%) in seven subjects, an equivocal late response (5–15%) in five, and no late response (< 5%) in seven subjects.

CHANGES IN HISTAMINE PC_{20} AFTER ALLERGEN CHALLENGE

Changes in histamine PC_{20} seven and 30 hours after allergen challenge were compared with those occurring seven hours after saline and with prior "baseline" measurements in all 19 subjects (table 2). Geometric mean histamine PC_{20} values were 0·93 mg/ml at baseline, 0·95 mg/ml seven hours after saline, 0·42 mg/ml seven hours after allergen, and 0·70 mg/ml (n = 18) 30 hours after allergen. Mean and SD log histamine PC_{20} values are shown in table 2. The values seven and 30 hours after allergen were significantly lower than those seven hours after saline ($p < 0·001$ and $p = 0·01$ respectively). In six of the seven subjects with an isolated early response there was less than a twofold reduction in PC_{20} at both seven and 30 hours after allergen compared with seven hours after saline. On the other hand, all 12 subjects with either a definite or an equivocal late response showed a greater than twofold reduction in PC_{20} at either seven hours (n = 10) or 30 hours (n = 6) following allergen. Despite a trend for larger falls in histamine PC_{20} with larger late responses to allergen there was no significant correlation in this study, nor was there any difference between mean Δ log PC_{20} in the seven definite and five equivocal late responders. The 12 definite and equivocal late responders were therefore compared with the seven early responders; the late responders had an early response of 26·3% (SD 9·8%) fall in FEV_1 and a late response of 17·4% (10·5%) compared with 22·3% (6·6%) and 2·5% (2·0%) for the early responders.

EARLY VERSUS DUAL RESPONDERS

In the seven early responders the geometric mean histamine PC_{20} was 1·0–1·3 mg/ml on all six occasions (fig 1). In the 12 subjects with a dual response the geometric mean histamine PC_{20} values showed little fluctuation between baseline, two and seven hours after the control challenge, and five to seven days after allergen, ranging from 0·74 to 0·95 mg/ml. Two hours after allergen, when the FEV_1 was reduced by 5·2% (SD 5·7%), there was a small reduction in geometric mean PC_{20} to 0·67 mg/ml, which was non-significant when compared with two hours after diluent ($p > 0·05$). The small reduction was due largely to three subjects in whom PC_{20} fell slightly more than twofold; in the remaining nine subjects the change was less than twofold. At seven hours the

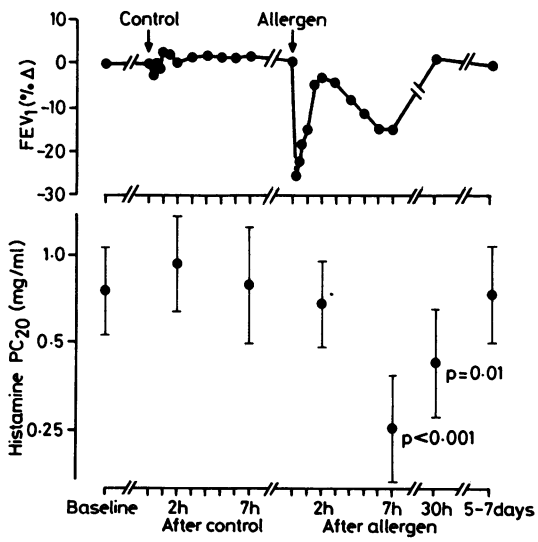

Fig 2 *Changes in FEV_1 and histamine PC_{20} (see fig 1) in 12 subjects with a dual asthmatic response. The mean FEV_1 (% change) in the upper graph and the geometric mean (with SEM) histamine PC_{20} in the lower graph are plotted against time (variable scale) on the horizontal axis.*

FEV_1 was reduced by 13·7% (SD 10%) ($p < 0·001$) and the histamine PC_{20} was reduced almost fourfold to 0·24 mg/ml ($p < 0·001$). At 30 hours the FEV_1 was back to baseline but the PC_{20} was still reduced almost twofold to 0·44 mg/ml ($p < 0·01$). Changes in FEV_1 and histamine PC_{20} in subjects with a dual response are shown in figure 2.

REPEAT ANTIGEN STUDIES

In the 10 dual responders undergoing a repeat antigen challenge the repeat values for the early response (30·2% (SD 15·8%) *v* 26·3% (9·8%)), late response (21·8% (13·6%) *v* 17·4% (10·5%)), Δlog PC_{20} at seven hours (0·58 (0·36) *v* 0·55 (0·31)), and Δlog PC_{20} at 30 hours (0·32 (0·16) *v* 0·31 (0·15)) were reproducible. This suggests that the performance of a histamine test two hours after allergen challenge did not affect the subsequent development (or magnitude) of the late response, or change histamine responsiveness.

Discussion

These data show that non-allergic bronchial responsiveness to histamine had not increased when measured 2–2·5 hours after allergen exposure in nine of 12 subjects with subsequent allergen induced late responses. The three subjects who had a fall in histamine PC_{20} slightly more than twofold did not have unusually large (12%, 22%, and 24%) or unusually early late responses.

Non-allergic bronchial responsiveness to histamine and cholinergic agonists has been shown to increase after both natural[3 11–14] and artificial (laboratory)[1 2 4 5] exposure to both allergens and occupational sensitising chemicals. This is sometimes maximal seven to eight hours after exposure, when reduced airway calibre may play a role in increased responsiveness.[1–2] Non-allergic responsiveness, however, may continue to increase for several days[1 2] and may remain increased after sensitive tests of airway function have returned to baseline.[2 4] To our knowledge there are no fully published data regarding changes in bronchial responsiveness preceding the late asthmatic response. Milillo stated, during a conference discussion, that his group had observed increased non-specific responsiveness three hours after allergen challenge.[15] Recently Durham *et al* have reported in abstract form increased responsiveness to histamine after two to three hours in six subjects with late asthmatic responses to occupational challenges.[16] These observations are not incompatible with our own. The current data, combined with those from previous studies,[1 2] lead to the conclusion that bronchial responsiveness in late responders must develop between two and seven hours after allergen exposure. It is therefore not surprising that significant changes may have occurred by three hours. In fact, the observation of probably significant falls in histamine PC_{20} in three of our 12 subjects at two hours supports the idea that increased responsiveness may develop between two and three to four hours after exposure, which is before the late response occurs. The studies in occupational asthma[16] may not, however, be entirely analogous. The immunopathology of many of these remains somewhat obscure and the late or non-immediate asthmatic responses are more common, may begin earlier, and may last longer than those that follow allergen exposure.[17 18]

Both the late responses and the changes in bronchial responsiveness to histamine were substantially smaller in this study than in previous studies.[1 2] Previous observations suggest that more severe late responses start earlier and last longer. Possibly therefore more severe late asthmatic responses are associated with earlier changes in histamine responsiveness. Such a trend was not observed in our study. The most severe late responses, 40% and 32% falls in FEV_1, were not associated with an early change in PC_{20}, while the three subjects with a greater than twofold fall in PC_{20} two hours after allergen did not have particularly large late responses (12%, 22%, and 24%). In two of these three subjects the greater than twofold PC_{20} difference from the value two hours after diluent was due to an unexpected high PC_{20} value after diluent; neither showed a twofold change when the two hour value was compared with the baseline determination.

The precise pathophysiology of the late asthmatic response and associated transient increases in airways responsiveness remains uncertain. Early speculation that a type III immune response played a part[17] was not supported by clinical and laboratory data.[19] Late cutaneous allergic responses, which are likely to be of similar immune pathogenesis, appear to be IgE dependent.[20 21] While the exact mediator or mediators and cells that are concerned in the late responses are unknown, there is presumptive evidence to suggest that inflammation and oedema[22 23] in addition to bronchoconstriction play a part in its pathogenesis. Inflammation is also likely to be the cause of non-allergic airway responsiveness.[24] We propose the hypothesis that the allergen induced late asthmatic response and allergen induced increase in bronchial responsiveness to histamine are both manifestations of underlying airway inflammation. There are some preliminary data to suggest that changes in bronchial responsiveness may occur earlier than the late response. Significant changes in histamine PC_{20} have occurred, in this study and others,[1 2] in subjects with a very small (5–15% FEV_1 reduction) late response

that would have been ignored previously. In an occupational setting reductions in PC_{20} have been documented in two patients in the absence of any change in FEV_1,[25 26] although one of these had a late response to a larger occupational exposure.[26] These data, in addition to the three hour data[15 16] and the observations that changes in non-allergic responsiveness persist after any measurable alteration in lung function has resolved,[2 4] suggest that changes in airway responsiveness may be a more subtle or more sensitive reflection of "allergen induced late airways inflammation" than the late asthmatic response.

In summary, we have confirmed that allergen induced isolated early asthmatic responses, with less than a 5% late asthmatic response, are not associated with change in non-allergic bronchial responsiveness to inhaled histamine two, seven, or 30 hours after exposure. Twelve subjects with allergen induced dual asthmatic responses were significantly more responsive to inhaled histamine seven and 30 hours but not two hours or five to seven days after allergen inhalation. The increase in non-allergic bronchial responsiveness appears to develop with, or perhaps slightly before, the late asthmatic response, persists beyond any measurable late response, and may appear after or during subclinical late asthmatic responses. It is suggested that allergen induced late asthmatic responses and allergen induced increases in non-allergic bronchial responsiveness are both manifestations of allergen induced inflammatory changes occurring in the airways, and that the changes in responsiveness may be more sensitive—that is, may appear without and persist beyond the late response.

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