

Studies on Hypersensitivity to Bacterial Antigens in Intrinsic Asthma

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Twelve children, aged 4 to 14 years, with moderate to severe intrinsic asthma (IA) were studied. Symptom-score charts were used to confirm the relationship of acute respiratory tract infections to exacerbations of asthma. Hypersensitivity to eight commonly occurring bacteria from the normal flora of the upper respiratory tract was studied by skin test, by crossed immunoelectrophoresis, and by basophil histamine release *in vitro*, using ultrasonicates of the bacteria as antigens. Skin tests were all negative. All children contained low titers of precipitating antibodies against most of the bacteria, but in this respect they did not differ from normal children. In contrast, release of histamine was induced in leukocytes from the IA children by all, or most sonicates, while such reactions, were less frequent in control children. The pattern of responses indicated an element of specificity. There was no correlation to precipitating antibodies, or to the microbial flora of the children. Positive responses were characterized by low values of maximal histamine release, and by a tendency to fluctuations with time. Because of these fluctuations, and because the IA children and control children were tested on separate occasions, we cannot be certain as to the real difference between these two groups. Our studies do, however, demonstrate that water-soluble constituents of all the bacterial strains tested were capable of causing the release of histamine *in vitro*, but that this phenomenon is not restricted to IA. The clinical significance of these findings awaits further investigations on the mechanism(s) of release *in vitro* by such agents.

Key words: bacterial hypersensitivity; basophil histamine release; intrinsic asthma.

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The aetiology and pathogenesis of intrinsic asthma (IA) is largely unknown and the diagnosis is based upon exclusion criteria, i.e. the absence of demonstrable allergy. Children with IA show a close temporal relationship between exacerbations of symptoms and acute respiratory tract infections (ARI) (4, 11). This relationship, although not restricted to the intrinsic type, has repeatedly furnished speculations about hypersensitivity to microbial antigens being a pathogenic factor. Attempts to demonstrate type I hypersensitivity to killed bacteria, or extracts of bacteria, from the respiratory

tract by skin tests (4) or by bronchial provocation tests (BPT) (1) have generally failed. However, high concentrations may elicit delayed type skin reactions (4) and bronchoconstriction by BPT (1). These findings suggest that direct toxic effects and mixed types of immune reactions may prohibit the selective demonstration of, and quantitation of type I hypersensitivity to such complex compounds *in vivo*. We have therefore employed the technique of basophil histamine release *in vitro* to search for the presence of cell-bound specific antibodies to bacterial antigens in children with IA precipit-

able by ARI. At the same time we searched for precipitating antibodies to the same antigens, and for circulating immune complexes. Finally, we studied the microbial flora over prolonged periods.

SUBJECTS AND METHODS

Patients

Twelve children, aged 4 to 14 years were included in the study (Table 1). Criteria for entering were: a) a history of recurrent or chronic non-seasonal asthma of more than 1 year's duration, b) a physical diagnosis of reversible obstructive pulmonary disease, c) normal levels of serum-IgE, d) no evidence of extrinsic asthma from history, results of skin tests, radioallergosorbent (RAST) tests, and when doubtful, BPT, and e) absence of other known causes of obstructive pulmonary disease.

Controls

For basophil histamine release studies, eight normal non-asthmatic, non-atopic age-matched children, five males and three females, served as controls. For precipitating antibody studies, controls were age- and sex-matched normal healthy children with a negative history of pulmonary disease or atopic diseases.

Methods

Pulmonary function. Peak expiratory flow (PEF), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁) were examined repeatedly, using an electronic spirometer (Spirotron®, Dräger). Examination for exercise-induced asthma (EIA) was done by free running for 6 min or less, with pulse rates of 160 beats/min or more.

Scoring of symptoms of asthma and infection. As part of a separate study (to be published) the patients or their parents filled out symptom-score charts, where each day was fractionated into four pe-

riods, and the degree of asthma within each fraction recorded* (Fig. 1). Four symptoms of infection (mucous secretion from nose and throat, cough, expectoration, and general malaise) were graded from 0 to 3 (absent, slight, moderate, severe) and the degree of severity for each of these four symptoms for each day was recorded. The weekly sum total of these figures is defined as the "infection score" with a maximal value of 84 (Fig. 1).

A total of 153 weekly observation periods were obtained, with a mean of 12.75 weeks per patient, and a range of 1–24 weeks.

Tests for extrinsic asthma. Skin tests were performed by conventional prick tests using a panel of 84 allergens (ALK, Copenhagen). Tests for allergen-specific IgE (AL-RAST) were performed with the aluminium-radioallergosorbent test (16) using standard mixtures of allergens from pollen, moulds, house dust, mite and feather, animal danders, milk, fish, meat, hen's egg, and rye, barley, and soya-bean (ALK, Copenhagen).

The total serum IgE levels were determined by paper radioimmunosorbent test (PRIST, Pharmacia, Sweden) (ALK, Copenhagen).

Bacterial antigens. Eight strains of bacteria were selected (Table 3). These strains belong to those most frequently encountered in the flora of the upper respiratory tract in normal Danish children (5), and constitute the "standard asthma vaccine" of Statens Seruminstitut Copenhagen (kindly provided by the late Dr. Elo Christensen). All bacterial strains were cultured on solid media and washed 3 times in 0.9% saline before preparation of antigens. The washed bacterial cells were sonicated and centrifuged (7), and the 0.22 µ Millipore®-filtered

* 0 = no asthma, 1 = slight asthma, i.e. slight wheezing, not affecting the general condition or daily activities, 2 = moderate asthma with obvious wheezing and difficulties in breathing out, but only slight affection of general activities, 3 = severe asthma with reduced level of activity inhibiting playing and school work, and 4 = indurable asthma with total inactivity and possibly cyanosis.

Table 1
Clinical and laboratory data in 12 patients with intrinsic asthma

Pt	Sex	Age	Family history		Age at onset	Attacks precipitated by			Pre-treatm. ^a severity	Usual ^b medication	Eos./μl ^c	Serum-IgE ^d U/ml
			Asthma	Atopy		Infection	Exercise	Unspec. agents				
1	M	14	pos	?	1	yes	yes	yes	very severe D	permanent C+S+B	180-405	24-82 (2-105)
2	F	14	pos	pos (?)	2	yes	yes	no	very severe D	permanent C+S+B+T+I	5-1605	59-75 (2-105)
3	F	11	pos	pos	4	yes	yes	yes	moderate W-M	permanent C+S	85-310	5-10 (2-105)
4	F	11	neg	neg	5	yes	yes	yes	moderate D-W	permanent C+S+B	85-410	8-14 (2-105)
5	F	10	pos	?	5	yes	yes	yes	severe D-W	permanent C+S	495-2250	86-191 (2-105)
6	F	8	pos	?	½	yes	yes	no	moderate D-W	permanent C+S+B+T	345-1560	62-93 (1-526)
7	F	7	pos	pos (?)	1	yes	yes	no	mild W	permanent C+S	275-650	114-158 (1-526)
8	F	8	pos	?	3	yes	yes	yes	moderate W	permanent S	545-1390	78-149 (1-526)
9	F	6	neg	neg	2	yes	yes	no	severe D(-W)	permanent S	130-1325	40-71 (1-526)
10	F	4	pos	?	1	yes	yes	yes	moderate W-M	permanent C+S	65-985	9-73 (1-526)
11	F	10	pos	?	1	yes	yes	yes	moderate D-W	permanent C+S+T	155-3240	25-176 (1-264)
12	F	4	neg	neg	½	yes	yes	no	mild W-M	permanent S	120-400	31-56 (1-264)

^a Attack rate: D = daily, W = weekly, M = monthly.

^b C = sodium cromoglycate, S = salbutamol, B = beclomethasone, T = theophylline, I = ipratropin.

^c Eosinophils: lowest and highest observed values.

^d Figures in brackets are normal values for age-matched control children.

supernatants constituted the antigen preparations used in all subsequent *in vivo* and *in vitro* studies. They were kept frozen at -20°C in small aliquots and used freshly thawed throughout. Table 3 also gives the protein contents.

Precipitating antibodies. Assays for the presence of precipitating antibodies in patient and control sera to the different bacterial strains were made by crossed immunoelectrophoresis (CIE),

as described in detail previously (6, 8), using the sonicates as antigens.

Basophil histamine release. The release of histamine was based on the radioactive assay of ^3H -histamine release (15). ^3H -histamine was incorporated in peripheral blood and the leukocytes were isolated by the Ficoll-Hypaque gradient method. The isolated fractions contained approximately 2% basophils. The cells were washed twice in Tris AMC buffer and resus-

pended in this medium. The cell suspensions, which did not contain serum, were incubated for 40 min at 37°C in the presence of the bacterial sonicates in final concentrations ranging from 10^{-2} to 10^{-6} of the undiluted sonicates. Release of labelled histamine was determined as the residual ^3H -histamine and expressed as percentage of the total labelled histamine of the sample. Since in numerous experiments the spontaneous release never exceeded 10%, a release of 10% or more was regarded as positive.

Circulating immune complexes. The sera of the patients were examined for the presence of circulating immune complexes by two independent methods; a polyethylene glycol precipitation complement consumption assay (PEG-CC) (2), and an automated rheumatoid factor dependent assay called PACIA (particle counting immunoassay) based on inhibitory activities of immune complexes towards agglutination of IgG-coated latex particles by rheumatoid factor (3).

Microbiological studies. During the investigation period, nasal and pharyngeal swabs and laryngeal secretions obtained by expectoration or endotracheal suction were examined twice with 1 week's interval. Distinction between secretions originating from the lower respiratory tract and from the pharynx was made by microscopy of Gram-stained smears, paying particular attention to the epithelial cells. Only bacteria associated with areas consisting of lower respiratory epithelial cells and pus cells, but without squamous epithelial cells, were considered of bronchopulmonary origin. Culturing, subculturing and identification of isolated bacteria and fungi were performed using standard microbiological methods as described previously (6, 9).

Identical studies had been done approximately 6 months earlier in all patients. In addition, since all patients were seen regularly in our clinic, a number of cultures were done during episodes of respiratory infections.

RESULTS

Clinical data.

The majority of patients were girls with early onset of moderate to severe asthma requiring permanent medication (Table 1). At the time of the present studies none of the children received systemic corticosteroids or theophylline. No medication was given 12 h prior to BPT. Several received oral sodium cromoglycate as part of a separate study (to be published).

All children had a clinical history of precipitation of exacerbations by ARI, and by exercise, and many gave a history of attacks on exposure to dust, humid air, and other unspecific environmental factors. A few patients gave a history suggesting offending allergens, but these could not be confirmed by tests. A family history of asthma was a frequent finding, and slightly more frequent than a definite positive family history of atopic disease (Table 1).

All patients were screened for other known causes of obstructive pulmonary disease, including cystic fibrosis, alpha-1-antitrypsin deficiency, and ciliary dyskinesia syndromes (13). Quantitative IgG-, -M, and -A were normal. *In vitro* and *in vivo* studies on the function of T-lymphocytes and phagocytes, done in most patients, revealed normal findings.

Test for extrinsic asthma

All but one child (No. 3) had blood eosinophilia (Table 1). In contrast, only one (No. 5) had marginally elevated serum IgE. Skin tests were negative with occasional small reactions to one or two allergens. Subsequent BPT and RAST could not confirm these scarce positive reactions. One patient (No. 11) developed allergy to dust mite 1 to 2 years after these studies, with a strongly positive skin test and RAST.

Most of the children were challenged orally with salicylate, tartrazine, and benzoate, often while on medication because of the severity of the disease. There were no positive reactions, but false negative reactions cannot be excluded in patients on medication.

Table 2

Lung function parameters in 12 children with intrinsic asthma

Pt	PEF %	FVC %	FEV _{1sec} %
1	86-128	73-103	43-75
2	53-93	77-108	33-73
3	98-112	81-107	69-85
4	39-120	60-135	58-95
5	40-69	47-60	24-52
6	50-90	58-116	35-82
7	90-135	67-111	50-94
8	104-129	82-127	70-95
9	63-127	46-92	33-92
10	69-100	54-92	58-67
11	55-90	40-70	30-50
12	72-96	58-100	55-82

Lowest and highest values over several months of observation – given as per cent of estimated normal values corrected for sex, age and height.

Table 3

Strains of bacteria used for preparation of the antigens, and the protein concentrations in the final sonicates

Microorganism	Protein concentration mg/ml
<i>Hemophilus parainfluenza</i> – biotype III	9.6
<i>Corynebacterium</i> sp.	21.8
<i>Aerococcus</i> sp.	7.5
<i>Neisseria pharyngis</i>	3.7
<i>Streptococcus mitior</i>	6.4
<i>Streptococcus salivarius</i>	10.7
<i>Staphylococcus aureus</i> – phage type 29+	11.7
<i>Branhamella catarrhalis</i> ²	8.5

¹ Measured by refractometry with human IgG as standard.

² Previous name: *Neisseria catarrhalis*.

Pulmonary function test

Table 2 shows the highest and lowest values in lung function parameters over a 3 months' period. Most studies were done while the patients were on medication; however, reversible obstruction was readily demonstrable in all. All children had positive test for EIA with a greater than 20% fall in PEF and prompt response to inhalation of salbutamol (data not shown).

Relation of asthma to ARI

Fig. 1 shows the relation between the duration and the severity of asthma and the "infection score" for a total of 153 observation weeks. High infection score values are associated with asthma of longer duration, and of higher degrees of severity. There were 48 observation weeks without symptoms of infection (score value 0). In 32 of these, there was no asthma. Slight asthma, of limited duration covering only a few per cent of the total weekly period, was noted in 14. Conversely, in 19 weeks with a "score" of 28 or greater, none were free from asthma, and 15 were characterized by severe asthma lasting approximately one-tenth of the weekly period. Statistical analysis (Spearman's correlation coefficient r) revealed a significant correlation between "infection-score values" and duration of asthma for each of the four degrees of severity ($r = 0.4153, 0.5181, 0.6485$, and 0.3772 , respectively, all P -values below 0.001).

Skin tests with bacterial antigens

Prick tests with the undiluted sonicates (Table 3) regularly gave reactions characterized by erythema and itching, and sometimes slight infiltration. These reactions appeared within 10 to 20 min and gradually disappeared in 6 to 10 h. Identical reactions were seen as frequently in five normal non-atopic controls, as in the patients. There were no late reactions.

Used in dilutions 1:10, all sonicates gave completely negative reactions, when read immediately, at 30 min, and at 8 h, and further dilutions were not applied.

Precipitating antibodies

Table 4 shows the results of CIE. All patient sera contained precipitating antibodies to five or more of the bacterial strains. The antibody responses were not especially pronounced with only minor quantitative or qualitative variations between patients. On repeated examinations the same pattern and number of precipitation lines were found. Upon inspection of the plates, all patients showed an identical pattern

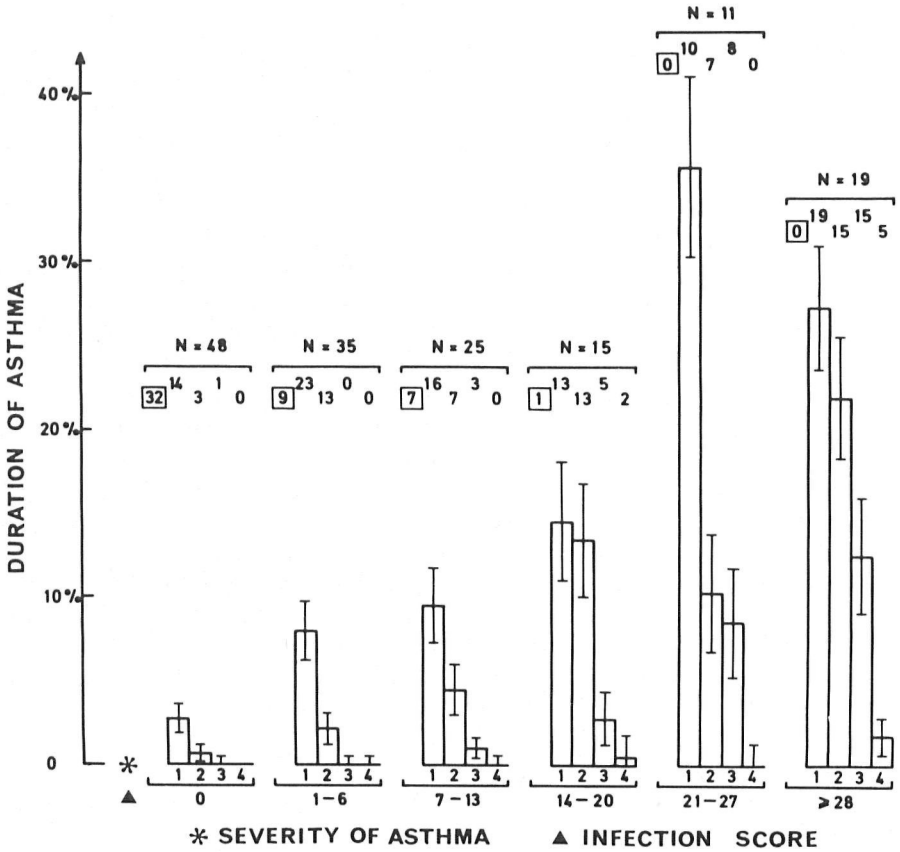


Fig. 1. Temporal relationship between exacerbations of asthma and acute respiratory tract infection in 12 children with intrinsic asthma. The data represent a total of 153 observation weeks. The infection score gives severity and duration of symptoms of respiratory tract infection, and is calculated as described in Methods. Maximal score value: 84. Individual observation weeks are grouped according to infection score, as indicated on the abscissa. *N*, denotes the number of observation weeks within each group of score values. The duration of asthma is given as per cent of the total weekly period for each of the four degrees of severity, as described in Methods. Note that a particular patient may have experienced asthma of different degree of severity during the same observation week. Therefore, the sum of weeks with asthma (below line in figure) can exceed the total number of weeks (*N*) above the line.

of precipitation lines for each sonicate, indicating that the antibodies in all patients were directed against the same antigenic determinants.

An identical pattern and number of precipitation lines were found in the sera of the control children (Table 4).

Basophil histamine release

Incubation of peripheral leukocytes from the patients with the bacterial sonicates *in vitro* often caused release of histamine. Fig. 2 shows

some representative dose-response curves. Most frequently, was a low response, consisting of release of 10 to 30%. The magnitude of responses showed a rather marked tendency to fluctuation with time on repeat studies (data not shown). Half of the patients responded to all eight sonicates, and the rest responded to between four and seven sonicates (mean 6.8) (Table 5).

These results were obtained in August and September. In the same months, but 1 year later, similar experiments were done in eight

Table 4

Precipitating antibodies against bacterial antigens in 12 children with intrinsic asthma, and in age-matched healthy children.
Number of precipitation lines by CIE

Pt	<i>H. parainfluenzae</i>	<i>Corynebacterium</i> sp.	<i>Aerococcus</i> sp.	<i>N. pharyngis</i>	<i>S. mitior</i>	<i>S. salivarius</i>	<i>S. aureus</i>	<i>Branhamella catarrhalis</i>
1	2	3	2	0	0	2	2	1
2	2	3	2	0	1	2	1	1
3	1	3	2	0	0	2	2	1
4	2	2	1	0	0	1	2	1
5	2	2	0	0	0	2	1	1
7 (6 ^a)	2	3	1	0	0	2	1	1
8	2	3	2	0	0	2	2	1
9	2	3	2	0	0	2	3	1
10	1	3	2	0	0	3	1	1
11	1	3	2	0	0	2	1	1
12	2	2	1	0	0	2	1	1

Controls								
1	1	2	2	0	1	2	1	1
2	1	2	1	0	0	2	2	1
3	0	2	2	0	0	2	1	1
4	2	3	2	0	1	2	3	1
5	1	3	2	0	0	2	2	0
6	2	2	1	0	0	3	3	0
7	1	2	2	1	0	2	3	1
8	1	2	2	0	0	2	2	0
9	1	2	2	0	1	3	1	0
10	1	3	2	0	0	2	1	1
11	2	3	2	0	0	1	3	1
12	2	2	1	0	0	1	2	0

^a not done.

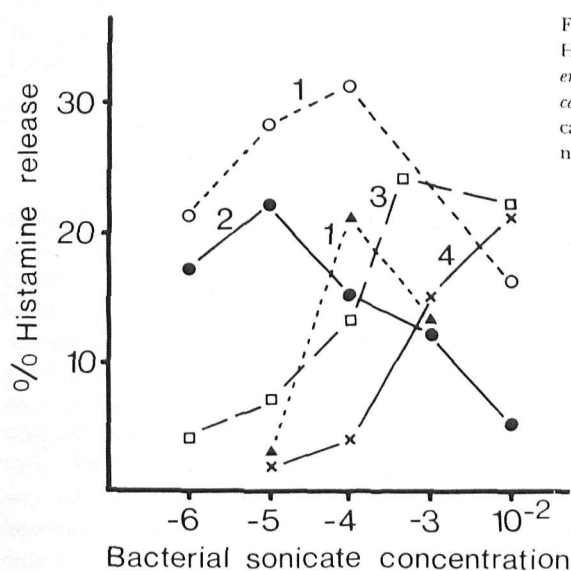


Fig. 2. Bacterial histamine release in intrinsic asthma patients. Histamine release was induced by sonicates of *H. parainfluenzae* (1), *S. salivarius* (2), *Corynebacterium* sp. (3), and *B. catarrhalis* (4) in final concentrations of the sonicates as indicated. Each point represents the mean of duplicate determinations.

Table 5

Basophil histamine release from 12 patients with intrinsic asthma, and from eight normal non-atopic, age-matched children, in presence of bacterial antigens

Pt	<i>H. parainfluenzae</i>	<i>Corynebacterium</i> sp.	<i>Aerococcus</i> sp.	<i>N. pharyngis</i>	<i>S. mitior</i>	<i>S. salivarius</i>	<i>S. aureus</i>	<i>Branhamella catarrhalis</i>
1	+	+			+	+	+	+
2		+	+	+	+	+		+
3	+	+	+	+	+	+	+	+
4		+	+	+	+	+		+
5			+		+	+		+
6		+	+	+	+			+
7	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+
10		+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+
Controls								
1					+			
2				+			+	+
3		+	+	+				+
4								
5								
6		+		+				+
7								
8								

normal children (Table 5). The number of positive reactions was clearly smaller, and in four of the controls none of the sonicates caused release. The pattern of distribution of positive reactions between the strains in the control children was clearly reminiscent of that seen in the patients. A similar pattern was seen in normal adults, where positive release was found more frequently than in normal children (to be published).

Circulating immune complexes

In the PEG-CC assay all sera but one were negative, and this single positive result was not detectable in the PACIA-system, where all sera were negative.

Microbiological studies

Table 6 shows the results of the microbiological studies. All patients harboured a normal flora in

the upper respiratory tract. More important, only one of the children (No. 7) showed consistent heavy growth of any single organism over the observation period. The pathogenic bacteria, isolated at one or several occasions over approximately 1 year, were those usually isolated from the respiratory tract in this age group.

DISCUSSION

Leukocytes from the patients released histamine when exposed *in vitro* to most, or all of the sonicates. The maximal release was usually low, and the responses fluctuated with time on repeat studies (data not shown). More consistent reactions and higher values of maximal release are seen in pollen-allergic patients with the appropriate allergen (14). Because of the fluctuations with time, and because the control children were studied on another occasion, we unfortunately cannot be certain as to the existence of a real difference between IA children and

Table 6
Microbiological studies in 12 patients with intrinsic asthma

Pt	Isolated pathogenic bacteria from nose ^N , throat ^T or laryngeal secretions ^L		Heavy growth of other bacteria (besides a normal flora)	
	At time of study	Within last year	At time of study	6 Months earlier
1	<i>S. pneumoniae</i> ^L <i>H. influenzae</i> ^L <i>S. aureus</i> ^{NT}	<i>S. haemolyticus</i> ^T <i>S. aureus</i> ^L <i>H. influenzae</i> ^L <i>S. pneumoniae</i> ^L	none	<i>S. non-haemolyticus</i> ^T
2	<i>H. influenzae</i> ^L <i>S. aureus</i> ^N	<i>H. influenzae</i> ^L <i>S. aureus</i> ^L	none	<i>S. non-haemolyticus</i> ^T <i>Neisseria</i> sp. <i>H. haemolyticus</i> ^T
3	none	<i>S. pneumoniae</i> ^L	none	<i>S. non-haemolyticus</i> ^T <i>Neisseria</i> sp.
4	none	<i>S. pneumoniae</i> ^L	<i>S. non-haemolyticus</i> ^{NT} <i>Corynebacterium</i> sp. ^N	none
5	none	<i>H. influenzae</i> ^L	none	none
6	<i>S. aureus</i> ^L	<i>H. influenzae</i> ^L <i>S. aureus</i> ^L <i>Neisseria</i> sp. ^L	<i>S. non-haemolyticus</i> ^T <i>Neisseria</i> sp. ^{TL} <i>H. haemolyticus</i> ^T	none
7	none	<i>S. aureus</i> ^L	<i>S. non-haemolyticus</i> ^T <i>H. haemolyticus</i> ^T	<i>S. non-haemolyticus</i> ^T <i>Neisseria</i> sp.
8	<i>S. pneumoniae</i> ^{NT} <i>S. aureus</i> ^{NT}	none	<i>H. haemolyticus</i> ^{NT}	<i>S. non-haemolyticus</i> ^T <i>Neisseria</i> sp.
9	<i>S. aureus</i> ^N	<i>S. aureus</i> ^L <i>H. influenzae</i> ^L	<i>S. non-haemolyticus</i> ^T	none
10	<i>H. influenzae</i> ^L	<i>S. pneumoniae</i> ^L <i>H. influenzae</i> ^L	<i>S. non-haemolyticus</i> ^T	none
11	<i>H. influenzae</i> ^L	<i>S. pneumoniae</i> ^L <i>H. influenzae</i> ^L	none	none
12	<i>S. pneumoniae</i> ^L <i>S. aureus</i> ^L	<i>S. pneumoniae</i> ^L <i>S. aureus</i> ^L	none	<i>S. pneumoniae</i> ^N

normal children. In a separate report we describe the fluctuating pattern in relation to season of the year (in preparation).

The mechanism of *in vitro* histamine release with these sonicates is unknown. The restriction of positive reactions within patients and controls, and within the different sonicates indicates some kind of specificity. Specificity could be ascribed to the involvement of cell-bound antibodies with antibacterial specificities. These could in theory belong to both the IgG and IgE

class. The lack of correlation between precipitating antibodies and histamine release (Tables 4 and 5) and the lack of difference in precipitating antibodies between IA children and normal children (Table 4) are indirect evidence against involvement of IgG. Circulating immune complexes were not detectable in the patients.

If IgE is involved in the mechanism of release *in vitro*, our studies suggest the normal occurrence of low titers of IgE antibodies with

antibody specificities against a wide range of common bacteria from the respiratory tract. As mentioned above, we do not know whether such antibodies are quantitatively increased in IA children. In theory, the patient basophils may have reacted more strongly to the same stimulus than normal basophils.

The relevance of *in vitro* histamine release to the bacterial sonicates for the pathogenesis of IA is speculative at the present moment. We confirmed the relationship of ARI to exacerbations of asthma, but it should be recalled that the majority of ARI causing exacerbations of symptoms in IA as well as in EA (extrinsic asthma) are caused by viral agents (11). On the other hand, release of histamine does seem to take place *in vivo* during attacks in IA (10) and bacterial constituents could conceivably gain access to mast cells locally in the respiratory tract during inflammatory disease states. We were concerned with the composition of the bacterial flora – not with the aetiology of individual episodes of ARI. We found that IA children harboured a normal flora, with no particular dominance in quantity, and with no particular dominating organism over prolonged time, and the isolated pathogens were inconspicuous for the age. More important, there was no relationship between the flora and the histamine release or the precipitating antibodies in individual patients.

Release of histamine *in vitro* may in theory also be due to unspecific "non-immunological" interaction of the sonicates with the cell membrane causing stimulation and degranulation. In fact, our preliminary studies on the effect of removal of surface-bound immunoglobulin, indicate that Ig-mediated, as well as unspecific mechanisms are involved in release *in vitro* (12). Further investigations are in progress to clarify the role of immunoglobulin and of direct cell interaction in order to throw light on the significance of the release in IA.

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