

Review Article

Family and twin studies on methacholine hypersensitivity

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ABSTRACT

Essentially all asthmatics demonstrate a marked sensitivity to inhaled methacholine and histamine, termed non-specific bronchial (airway) hyperresponsiveness (BHR). Airway hyperresponsiveness is a characteristic not only of asthmatics, but can be found in many persons with allergic rhinitis as well as in members of asthmatics' families. The presence of BHR usually precedes the development of clinically identifiable asthma. In recent years there has been an emphasis on inflammation, inducing hyperresponsiveness. However, these factors increase airway hyperresponsiveness by a magnitude of only three-fold compared with normal subjects. The important question is not why asthmatics respond, but why normal subjects do not. The normal subjects are quite able to maintain normal airway function in the presence of high concentrations of methacholine or histamine *in vivo* but not *in vitro*, suggesting the presence of protective mechanisms *in vivo* that are either lacking in, or are less effective in, the asthmatic subjects. There is a strong correlation between the degree of airway hyperresponsiveness and the severity of asthma. In order to determine whether methacholine sensitivity could be used as a potential genetic marker, we studied 750 subjects from 53 asthma families and 26 control families. The best sensitivity and specificity is at 200 breath units. Only 6% of the allergic rhinitis subjects showed a high positive response, but 30% overlapped with asthmatics in that they reacted with 200 breath units or less. There was a group of non-atopic subjects from asthma families who responded by 200 breath units, but there was

a significantly lower percentage from normal families. Being from an asthma family is a risk factor in terms of subsequent development of asthma and increased airway reactivity. The parent data suggest that airway reactivity is transmitted to succeeding generations. Studies of twins have revealed that the concordance of asthma is higher in monozygotic than in dizygotic twins, but environmental factors are at least as important as genetic factors. Animal models of asthma comparing genetic strains can provide an important link between airway hyperresponsiveness and the allergic response. The inheritance of asthma fits a polygenetic pattern rather than a single-gene pattern.

Key words: adenylate cyclase, airway hyperresponsiveness, allergic rhinitis, asthma, asthmatics, bronchial (airway) hyperresponsiveness, chromosome, cytokines, eosinophils, families, genetic, histamine, IgE, methacholine, phosphodiesterase, twins.

INTRODUCTION AND HISTORICAL PERSPECTIVE

Asthma is a well-recognized and common condition. Nearly all asthmatics demonstrate recurrent episodes of shortness of breath, wheezing, and coughing. Asthmatics generally respond to bronchodilator medication. All asthmatics demonstrate a marked sensitivity to inhaled methacholine and histamine,¹ termed non-specific bronchial (airway) hyperresponsiveness (BHR). Airway hyperresponsiveness is a characteristic not only of asthmatics, but can be found in many persons with allergic rhinitis¹ and members of asthmatics' families.² A recent report suggested that the presence of BHR usually precedes the development of clinically identifiable asthma.³ We reported on 20 subjects who were studied before and after the onset of clinical asthma. Subjects were part of a larger ongoing study of the 'Natural History of Asthma'.

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The results showed that enhanced airway reactivity usually precedes the development of asthma, which could support the theory that asthma has a genetic basis.

Asthma is characterized by a constellation of biochemical, hematological, and pharmacological abnormalities. It is generally accepted that both genetic and environmental influences play a role in its development.

Figure 1 shows the characteristics of asthma that encompass its pathogenesis and need to be included in any theory regarding asthma.

BRONCHIAL HYPERRESPONSIVENESS

Viral infections, industrial pollutants, and exposure to allergens can all increase airway hyperresponsiveness and induce inflammation. In recent years there has been an emphasis on inflammation, inducing hyperresponsiveness. However, these factors increase airway hyperresponsiveness by a magnitude of only three-fold, whereas the hyperresponsiveness of the asthmatic is increased at least three log, or 1000-fold, compared with normal subjects.

The important question is not why asthmatics respond, but why normal subjects do not. Normal subjects have been challenged with up to 256 mg/mL of methacholine and have still failed to show a 20% fall in the forced expiratory volume in 1 s (FEV₁). However, if the airway is removed and put in a muscle bath, the normal subjects respond very effectively to histamine or methacholine at very low concentrations.^{4,5} A review of literature shows that there is essentially no difference *in vitro* between normal subjects and asthmatics in terms of methacholine or histamine sensitivity.^{5,6} The normal subjects are quite able to maintain normal airway function in the presence of high concentrations of methacholine or histamine *in vivo* but not *in vitro*. This suggests the presence of a protective mechanism *in vivo* that is either lacking in, or is less effective in, the asthmatic subjects.

There is a strong correlation between the degree of airway hyperresponsiveness and the severity of asthma;^{7,8} the more severe the asthma, the greater the airway hyperresponsiveness. The slope of the dose-response curve is steeper and the degree of response greater, with the maximum fall being obviously larger, in patients with severe asthma than in those with milder asthma (Fig. 2).

A plateau phenomenon is commonly seen in allergic rhinitis subjects as well as in many former asthmatics and indeed in current asthmatics following a therapeutic course of inhaled corticosteroids. Former asthmatics as a group are about one-tenth as sensitive as current asthmatics.⁷

This plateau phenomenon which may occur at different degrees of fall in FEV₁ and subsequent increasing concentrations of methacholine, does not result in a further decrease in the FEV₁. Subjects with current symptomatic asthma do not generally reach plateau.⁹ In asthmatic subjects, a significant direct correlation between airway sensitivity to methacholine or histamine and peak expiratory flow variability has been shown, but the relationship

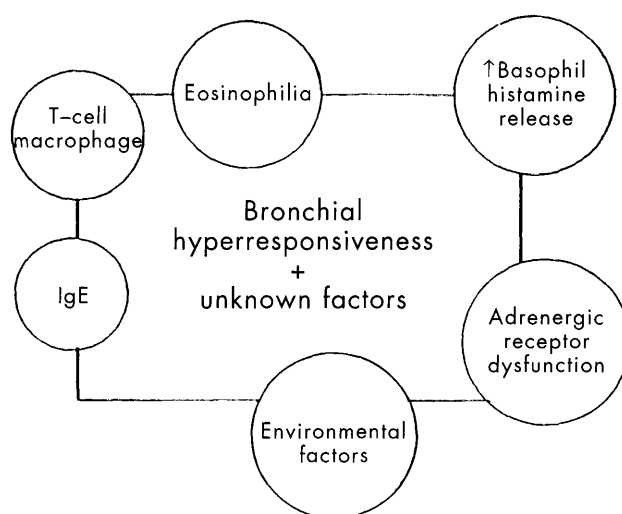


Fig. 1 These characteristics encompass the pathogenesis of asthma and need to be included in any theory regarding it.

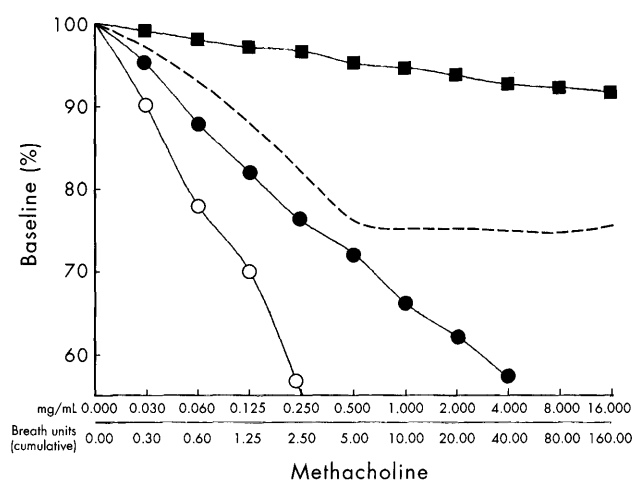


Fig. 2 Typical dose-response curves to methacholine in subjects with moderate (○) and mild (●) asthma, in subjects with past asthma (no wheezing for at least 12 months) or allergic rhinitis (---), and in normal subjects (■).

between peak expiratory flow variation and maximal response plateau to pharmacologic agents has not been investigated.^{10,11}

Recently, Cloosterman *et al.* reported on the relationship between the variability of peak expiratory flow rate in allergic rhinitis and mild asthma. These authors also reported on the variation among subjects with plateau response curves to methacholine and subjects without plateau.¹² They concluded that in both asthmatic patients and subjects with allergic rhinitis, the shape of the concentration–response curve to methacholine provided new information on the relationship between airway responsiveness and peak expiratory variability. Cloosterman *et al.* also concluded that some subjects with allergic rhinitis without evidence of plateau had degrees of diurnal peak expiratory flow variation similar to that found in patients with mild asthma. This could indicate that those subjects had subclinical inflammatory changes in the airways. It should be emphasized that most of the allergic rhinitis subjects develop a plateau at much lower falls in FEV₁ compared with asthmatic subjects. A maximal response plateau is a feature of normal persons and also of many subjects with allergic rhinitis.^{13–15} In contrast, in patients with current symptomatic asthma increasing doses of inhaled pharmacologic agents usually led to progressive airway narrowing without the achievement of a plateau.^{9,10}

Subjects with mild asthma had a lower prevalence (22%) of plateau on the concentration–response curves to methacholine than did subjects with allergic rhinitis (63%) or healthy subjects (94%). With regard to subjects for whom plateau was detected, the level of maximal response was significantly higher in subjects with asthma. These observations show that non-asthmatic subjects with allergic rhinitis without evidence of plateau have a degree of daily peak expiratory flow variation greater than do healthy subjects and similar to that found in patients with mild asthma. These individuals may be at greater risk of developing clinical asthma. These findings are consistent with the observations of Ryan,¹¹ who found that an increase in diurnal expiratory flow variation in some non-asthmatic subjects with allergic rhinitis was associated with increased histamine sensitivity.

On the basis of the above studies, it is reasonable to speculate that subjects with allergic rhinitis without evidence of plateau could be at increased risk of developing asthma, particularly if they also show increased expiratory variation and if they are exposed to sufficiently strong stimuli. Further prospective studies are needed to inves-

tigate this hypothesis. In this regard, Cloosterman *et al.* reported that house dust mite avoidance may improve peak flow and symptoms in patients with allergy but without asthma. They concluded that this results in a possible delay in the manifestation of clinical asthma.¹² These authors reported significant improvements in the treatment group in terms of symptom scores for disturbed sleep, wheeze and breathlessness following house dust mite avoidance compared with the placebo group. Larger studies with a long follow-up period are necessary to indicate protection against clinically manifest asthma in these subjects with house dust mite allergy.

Genetic studies using measurement of airway hyperresponsiveness

Family studies

In order to determine whether methacholine sensitivity could be used as a potential genetic marker, we studied 750 subjects from 53 asthma families and 26 control families.^{13,14} The asthma families were defined as having a proband with current bronchial asthma between the age of 6 and 25 years. Both parents had to participate in the testing.

The control families were usually neighbors of the asthma families, were of the same age group and had no atopic disease.

We measured the area under the dose–response curve using the best-fit parabolic curve.¹⁵ Subjects who had a highly positive response were very sensitive to methacholine and had a small area under the parabolic–response curve. We used a 35% fall in FEV₁ as an end point to take into consideration the plateau phenomenon. This allowed us to separate subjects who had a 20% fall in FEV₁ and then plateaued, from current asthmatics who, for the most part, did not show a plateau phenomenon. A negative study was defined as one having less than a 20% fall in FEV₁ after 800 cumulative breath units of methacholine.

Table 1. Characterization of methacholine inhalation response

Category	% Drop in FEV ₁	Cumulative dose	Area under the curve
High	≥ 20%	0–5	0–306
Medium	≥ 20%	51–200	306–1225
Low	≥ 20%	201–800	1225–4000
Negative	< 20%	> 800	4000–5600

FEV₁ = forced expired volume in 1 second.

Table 1 compares area and cumulative dose in breath units. A subject who had a 20% fall in FEV_1 with 50 breath units or less, would have had a maximum area of 306, whereas a subject with a medium response, with a 20% fall in FEV_1 at 200 breath units or less, would have had an area of up to 1225. The standard diagnostic method goes up to only 200 cumulative breath units.^{16,17}

Most children with asthma in our family study responded to 50 breath units or less. The few non-responders were former asthmatics who, by definition, had been free of all symptoms for at least 1 year. The majority of the normal children from the normal families failed to react, even at 800 breath units. If we analyze the pediatric population from these families, the best sensitivity and specificity was found at about 100 breath units.¹⁸ However, when the total population is analyzed, the best sensitivity and specificity is at 200 breath units.¹⁵ This illustrates that normal children have a greater bronchial reactivity than do adults.^{16,17,19}

Data for the whole population studied showed that the asthmatics, for the most part, reacted by 50 breath units or less, but that some asthmatics did not react until 200 breath units.¹⁵ Only 6% of the allergic rhinitis subjects showed a high positive response, but 30% overlapped with asthmatics in that they reacted with 200 breath units or less. There was a group of non-atopic subjects from asthma families who responded by 200 breath units, but there was a significantly lower percentage from normal families.¹⁵

Results for the normal non-atopic subjects from the asthma families versus the normal subjects from the non-atopic families are shown in Fig. 3. This shows a subgroup of subjects from asthma families who respond to methacholine and who are siblings of asthmatics.^{15,20} These responders are perhaps at greater risk for subsequent development of asthma. The normal subjects from normal families show a unimodal distribution. However, the normal subjects from the asthma families clearly show a bimodal response. When we compared the characteristics of the responders and the non-responders, there was no difference in the sex distribution, mean age, or serum IgE. There was a slight difference in the total intradermal skin test score, and although this was statistically significant, these scores are small compared with the scores of subjects with known atopic disease.

Data for the parents of normal children from the non-atopic families and the parents of the children with asthma have been reported.^{18,21} All asthmatic subjects were eliminated for the purpose of comparison. The parents of the

normal children from the normal families demonstrated a unimodal distribution. The results clearly show a bimodal distribution of the parents of the asthma families.

There was no difference in sex distribution, age or serum IgE when we compared these groups, but again there was a slight but significant difference in total skin test score.¹⁸ The parents of asthmatics who did respond to methacholine had a somewhat greater total intradermal skin test score.

This bimodal distribution of airway reactivity in the non-atopic siblings of asthmatics and in non-atopic parents of asthmatic children would suggest a single physiological, or perhaps a single genetic, abnormality. Consistent with these results is the report by Longo *et al.*, who also found bimodal distribution of airway reactivity in parents of asthmatic children but not in control parents.²² In our study, however, a simple genetic effect was not evident because segregation analysis of these families excluded a single-gene mechanism.^{14,20}

Clifford *et al.* studied 50 children who had one parent with asthma.²³ These children had a 50% increase in respiratory symptoms of cough, wheeze and shortness of breath when compared with control subjects. Over 90% of the asthmatic parents responded to methacholine with a 20% fall in FEV_1 by 6.4 μ mol, while 45% of the children had similar responses.

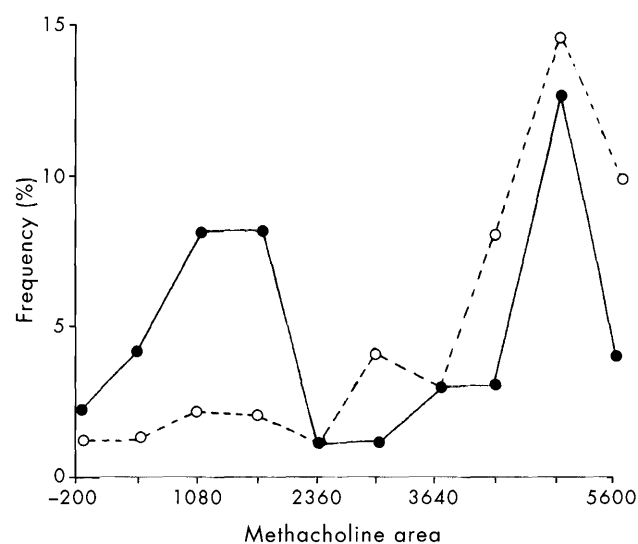


Fig. 3 Frequency plot of age-correlated area under dose-response curves for methacholine in normal subjects from both asthmatic (●) and control (○) families. Left-hand mode shows excess number of positive responders from asthmatic families.

We performed a segregation analysis on the bronchial response to a standardized methacholine inhalation challenge obtained from members of 83 families that were part of a Natural History of Asthma study population. Each bronchial response was expressed as the area under the best fitting parabolic dose-response curve. Standard methods of statistical analysis demonstrated that age, sex, and recent respiratory infection had a significant effect on the bronchial response to methacholine inhalation. Segregation analysis indicated that, although a familial component exists in the transmission of bronchial response to methacholine, the bimodal distribution of the bronchial response is not due to segregation at a single autosomal locus.¹⁴ Airway reactivity appears to be determined by both genetic and environmental factors. It is our conclusion that its inheritance is due to a polygenetic mechanism. There may be more than one major locus: polygenic inheritance, culturally transmitted environmental effect, or perhaps a mixture of these mechanisms.

Methacholine and histamine challenges are the most sensitive indicators of non-specific bronchial reactivity. Other tests such as exercise and osmotic challenges have been used to determine the degree of bronchial sensitivity in asthmatics and allergic rhinitis subjects. Exercise challenges have been used to determine the degree of bronchial lability in relatives of asthmatics. Konig and Godfrey reported exercise challenge and skin test results in 65 first degree relatives of 12 children with asthma.²⁴ Positive index for enhanced bronchial lability was obtained in 10 of the 12 asthmatic children. Overall, 28 of the 65 family members responded. It is impressive that many non-asthmatic subjects responded to a challenge that generally is not considered to be as sensitive as methacholine.

The use of non-isotonic aerosols for evaluating bronchial hyperresponsiveness has been studied extensively by Anderson *et al.*²⁵ Challenges with non-isotonic aerosols, that is osmotic challenges, including those using hypertonic saline (4.5%), require little patient cooperation and the equipment required is portable, relatively inexpensive, and readily available. Bronchial challenge by inhaling hyperosmolar saline has the advantage of being a physiologic challenge rather than a pharmacologic one. It can also be used in children. These osmotic challenges cause bronchoconstriction indirectly by causing the endogenous release of mediators to which the subject is sensitive. Although these osmotic challenges are less sensitive in detecting bronchial hyperreactivity than challenges with methacholine and histamine, they have a high positive predictive value for identifying bronchial hyperreactivity associated with moder-

ate to severe asthma.^{26,27} Anderson *et al.* reported no false-positive tests in osmotic challenges in healthy subjects.²⁵ This is in contrast to histamine and methacholine challenges which, as mentioned, have relatively low specificity but a high negative predictive value. In the population studies and family studies discussed, approximately 30% of persons with hyperresponsiveness to methacholine and histamine have no symptoms of asthma.

The Bronchoprovocation Committee of the American Academy of Allergy, Asthma and Immunology has conducted a multicenter study of the effect of hypertonic saline challenges in asthmatics, allergic rhinitis subjects and control non-atopic subjects. In this study of 80 subjects, all asthmatics responded to methacholine whereas only 75% responded to hypertonic saline with a 20% fall in FEV₁. Two of the 20 subjects with allergic rhinitis had a positive response to methacholine and these subjects also responded to hypertonic saline. No normal subject responded to methacholine or hypertonic saline. Overall, it can be said that the osmotic challenges are more specific but less sensitive than the direct pharmacologic challenges using histamine and methacholine. Anderson *et al.* have compared the airway responses to methacholine and histamine with 4.5% saline in 26 subjects.²⁵ The results demonstrate that a PD₂₀ to 4.5% saline of less than 15 mL is associated with a PD₂₀ to histamine of less than 2 μ mol (PC₂₀ of 4 mg/mL). A PD₂₀ for methacholine was also less than 2 μ mol. In patients with a PD₂₀ to 4.5% saline less than 15 mL, the authors always observed responsiveness to methacholine or histamine.

Twin studies

Although there are limitations in using the twin model to examine the influence of heredity, genetic analysis of twins remains an accessible and practical method of estimating the weight of genetic influence on clinically measurable parameters. This is true because members of a monozygous pair share a genotype and a relatively similar environment. By comparing clinical or laboratory parameters of a disease condition in monozygous (MZ) twins and dizygous (DZ) twins, it is possible to provide evidence for the genetic determination of those parameters, under reasonable assumptions.²²

The comparison of intrapair correlation coefficients (*r*) provides an indicator of the role of heredity as a clinical marker of atopic disease or bronchial hyperresponsiveness. An estimate of heritability ((H)², *r*MZ-*r*DZ) has been used.²⁸ This heritability index provides an estimation of the

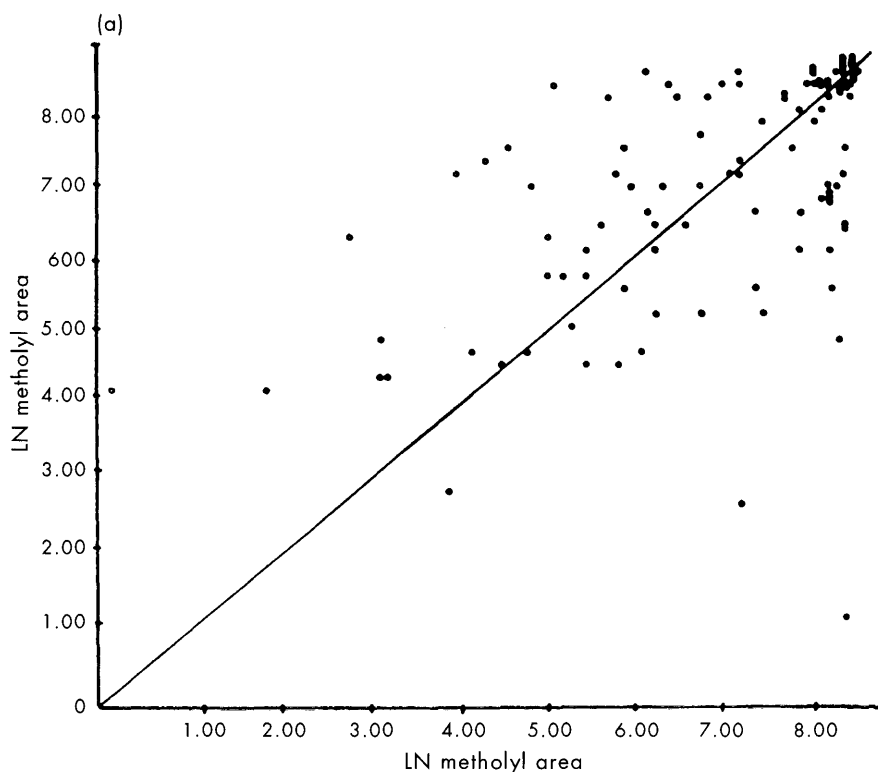


Fig. 4 (a) Intrapair correlation of methacholine (metholyl) sensitivity as determined by the area under the dose-response curve. Methacholine area expressed as the natural log shows a correlation coefficient of 0.67 in monozygous (MZ) twins (○; $r = 0.67$) and 0.34 in dizygous (DZ) twins (●; $r = 0.34$).

contribution of heredity to the variants of the measured trait or marker. When $H = 1$, the variance of the measurement is entirely due to heredity. As H approaches 0, the variance of the measurement is presumed to be determined by environmental factors. Pair and casewise (proband) concordance rates of a trait are also commonly used to express the results of twin data.

A standardized exercise challenge test has been used to compare eight identical and seven-non-identical twin pairs.²⁹ These authors found that six pairs of MZ twins were concordant for exercise lability, while only one of the DZ pairs was concordant.

In our twin studies (Fig. 4a,b), the correlation of methacholine area in 61 pairs of MZ and 47 pairs of DZ twins was determined.

We observed a significantly greater correlation coefficient of methacholine sensitivity in the MZ twins (0.67) than in the DZ twins (0.34).³⁰

We also compared serum IgE levels in the MZ and DZ twins and found a higher correlation coefficient in the MZ twins. The heritability estimate for the IgE was 0.61, and for the methacholine sensitivity it was 0.66.³⁰

Insight into the genetic contribution to pulmonary function as reflected in twin studies was reported by Ackerman *et al.*, who investigated 39 twin pairs at a mean age of 11.4 months.³¹ This study included 25 MZ and 14 DZ pairs. Thirty per cent of the twin pairs had a positive family history of asthma. Pulmonary function in the infants was determined in a sleep state and the measurement of maximal expiratory functional residual capacity was performed with rapid chest compression. A greater intrapair variation in pulmonary measurements was observed between DZ twins than between MZ twins. Similar findings had previously been reported for a large population of adult twins in a study using standard pulmonary function testing.³²

We concluded that methacholine can determine the

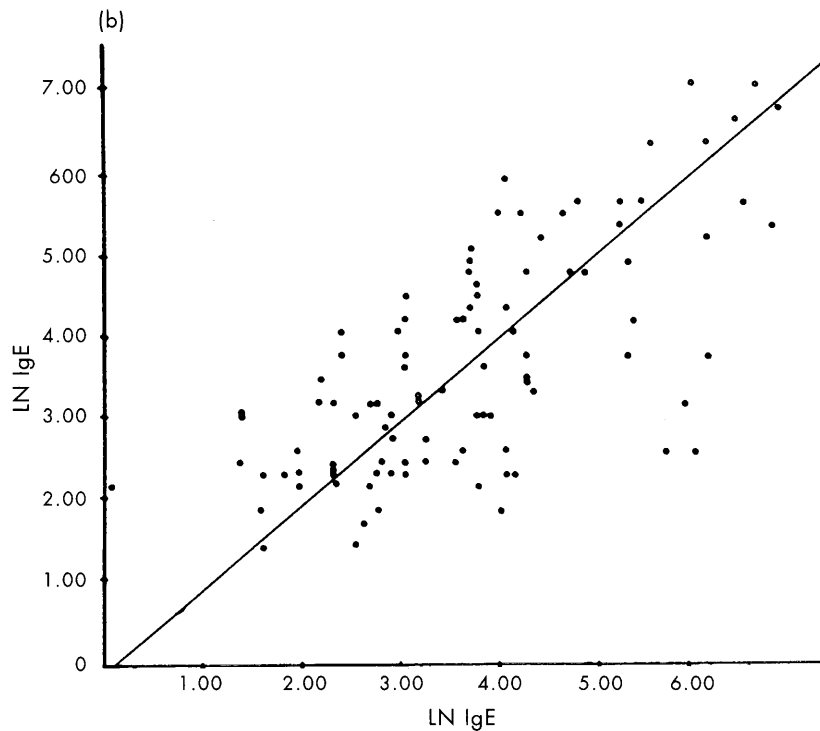


Fig. 4 (b) Intrapair correlation of serum immunoglobulin E (IgE) expressed as the natural log. Serum IgE shows a correlation coefficient of 0.825 in the MZ twins ($\circ$; $r = 0.825$) and 0.52 in the DZ twins ($\bullet$; $r = 0.52$).

degree of bronchial reactivity, which is certainly a hallmark of asthma. Just coming from an asthma family is a risk factor in terms of subsequent development of asthma and increased airway reactivity. The parent data suggest that airway reactivity is transmitted to succeeding generations. Studies of twins have revealed that the concordance of asthma airway reactivity and IgE is higher in monozygotic than in dizygotic twins, but that environmental factors are at least as important as genetic factors. Animal models of asthma comparing genetic strains can provide an important link between airway hyperresponsiveness and the allergic response. The inheritance of asthma fits a poly-genetic pattern rather than a single-gene pattern.

Effect of age on bronchial hyperresponsiveness

We have investigated the effect of age on methacholine sensitivity in a population study of over 750 subjects.¹⁹ When methacholine responsiveness is plotted against age (Fig. 5) for subjects who have no history of asthma,

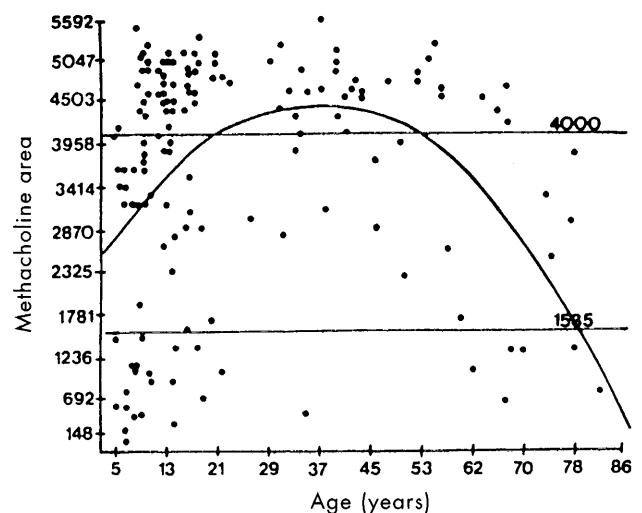


Fig. 5 Methacholine responses of subjects studied. Results ≥ 4000 indicate normal responses. Values ≤ 1535 indicate values that are ≤ 200 breath units of methacholine. The best-fitting curve is superimposed. $r = 0.42$; $P < 0.00001$.

it can be very easily appreciated that the younger and older subjects have significantly increased bronchial responsiveness. If this factor is not taken into account in clinical practice, there could be a tendency to suggest falsely the presence of hyperresponsive airways in these populations.

Allergic non-asthmatic children have increased non-specific bronchial responsiveness and this increased bronchial responsiveness persists over time.³³ We have postulated that persons with allergies and family members of asthmatic subjects retain this enhanced bronchial responsiveness, thus increasing their likelihood of subsequently developing asthma. These findings, along with others, would require that increased bronchial responsiveness precede the development of asthma symptoms. In our asthma family population we evaluated bronchial reactivity over several years and were able to show that 17 of 20 children who years later developed asthma had pre-existing bronchial reactivity.³ Increased airway responsiveness pre-existing in children who later became clinically asthmatic has also been reported by Sears *et al.*³⁴ These studies suggest, but do not prove, that airway hyper-responsiveness may be genetically determined.

The natural history and significance of asymptomatic airway hyperresponsiveness (AHR) are still to be defined. Over a 3 year period, Laprise and Boulet compared clinical, immunologic, and physiologic features of 30 subjects who had asymptomatic AHR with those of 30 symptomatic asthmatic subjects and 30 normal non-responder subjects (age, 31.9 ± 1.4 years; mean $\pm$ SEM; $n = 90$).³⁵ These tests were repeated annually, at the same period of the year, for 3 years. Subjects with asymptomatic AHR had greater bronchodilator responses ($P = 0.001$), variability of peak expiratory flow rate ($P = 0.002$), and prevalence of atopy ($P = 0.02$) than did the normoreactive subjects. After 3 years, the concentration of methacholine provoking a 20% decrease in FEV₁ (PC₂₀) had decreased significantly in the asymptomatic AHR subjects ($P < 0.0001$) as compared with the other two groups, and of the 28 subjects studied at this time, four (14.3%) had developed asthma symptoms. These last four subjects were atopic and had been exposed to animals when they developed asthma. They also had a familial history of asthma and an increased baseline AHR compared with the subjects who did not develop symptoms. In conclusion, this study showed that over a 3 year period, subjects with asymptomatic AHR had a greater increase in airway responsiveness

and frequency of development of asthma symptoms than did normoresponsive subjects. Allergen exposure in sensitized subjects at the time of the study, and genetic predisposition, seemed the main risk factors for the development of symptomatic asthma in this population.³⁵

Studies in animals: Dogs, guinea pigs and mice

Animal models of asthma have demonstrated increased non-specific bronchial responsiveness. Basenji-greyhound (BG) crosses have proved a valuable animal model. Each breed (i.e. Basenji and greyhound) has minimal or no BHR inherently, but the BG dog does have inherent BHR.³⁶ The Basenji dog and the BG dog often have atopic dermatitis, although non-atopic BG dogs can have BHR.

Thirty years ago we reported that sensitization to anaphylaxis and some of its pharmacologic mediators could be induced by a blockade of the beta adrenergic receptors in certain specific strains of mice and guinea pigs. In Hartley strain guinea pigs time of onset of severe dyspnea (Fig. 6) after inhalation of serotonin, histamine or methacholine was significantly decreased when the strain was pretreated with a beta adrenergic blocking agent. This is not unlike the effect of beta adrenergic blocking agents seen in asthmatics.³⁷ In contrast, the beta adrenergic blocking agent did not induce significant increased reactivity to serotonin, histamine or to methacholine in the Trapani-Campbell strain.³⁸

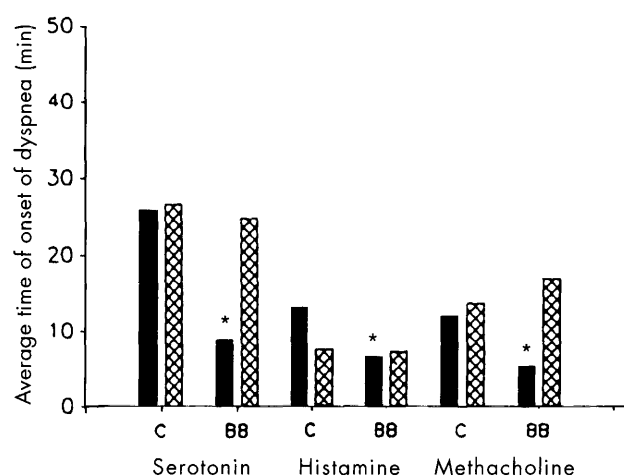


Fig. 6 Bronchial sensitivity to serotonin, histamine, and methacholine in β -adrenergically blocked guinea pigs. (■), Hartley; (▨), Trapani-Campbell; C, control; BB, Beta Blockade. * $P < 0.01$.

More recently, studies using mice have shown that specific strains have an increased response whereas others are totally unresponsive. Breeding between strains has shown a genetic susceptibility to bronchial hyperreactivity in mice that is not associated with atopy.^{38,39} A marked strain difference in sensitivity to serotonin and methacholine exists in mice.^{38,40,41}

We investigated the sensitivity of different mouse strains to histamine and methacholine. Pertussis toxin or low dose of the beta adrenergic blocking agent sotalol followed by histamine challenge resulted in the death of all CFW strain mice.^{38,41} In CF1 mice, however, the same low dose of beta blocker was not lethal and this strain was also resistant to the sensitizing effect of pertussis toxin. However, in the CF1 mice, this resistance to histamine was overcome when a higher dose of beta adrenergic blocker was used and resulted in a mortality rate of 80%. The obese hyperglycemic mouse, a genetic strain that has been used to study Type II diabetes, is resistant to sensitivity to histamine induced even by high doses of beta adrenergic blocking agents. However, the normal litter mates of these obese hyperglycemic mice are sensitive to histamine following a high dose of beta adrenergic blocker. Because of the recent advances in measuring airway sensitivity in awake, unanesthetized mice, the fact that pure inbred strains of mice are readily available and that environmental factors can be easily controlled and manipulated, it is now possible to design experiments to study the various components involved in the pathogenesis of asthma in this mouse model (Fig. 1). Animal models and human studies support the hypothesis of genetic control of bronchial hyperreactivity. Intense efforts to relate this bronchial hyperreactivity by linkage analysis are currently under way in a number of laboratories around the world.

GENETICS OF IMMUNOGLOBULIN E, T CELLS AND CYTOKINES

The presence of atopic diseases, such as allergic rhinitis, has a strong genetic determination.^{42,43} The ability to generate IgE response to a specific antigen has been conclusively shown to be associated with specific histocompatibility antigen loci.⁴³ Cookson *et al.* have recently analyzed maternally derived alleles from 155 sibling pairs affected by atopy to markers on chromosome 11q13.⁴⁴ They found that the β subunit was a candidate for the chromosome 11 atopy locus. These investigators also found that paternal transmission of atopy does not

occur at the chromosome 11q13 locus. At present, the exact localization of gene(s) for the predilection to be atopic (IgE) is in question. The original observations linking atopy with chromosomes 11 and 5 need further investigation. An intensive effort to identify the genes associated with asthma and airway hyperresponsiveness is currently in progress in Europe, North America and Asia.

Mouse strains have been shown to have a T-helper population (Th2) preferentially producing cytokines (IL-4 and IL-5) that induce a response to parasites.⁴⁵ Several laboratories have recently reported an induction of airway hyperresponsiveness, increased IgE and eosinophilia in a mouse model of asthma after ovalbumin inhalation in sensitized mice.^{46,47} In sensitized animals, repeated inhalation of ovalbumin induces airway hyperresponsiveness *in vivo*, infiltration of eosinophils and CD4⁺ cells in lavage and airway tissue with Th2-type cytokines IL-4 and IL-5. The role of IL-16 in upregulation of IgE, eosinophil infiltration and airway hyperresponsiveness has been demonstrated by these authors.⁴³ This response was significantly inhibited after the addition of antibodies to IL-16, a T lymphocyte chemo-attractant factor that utilizes CD4 molecules as its receptor. Interleukin-16 is more potent than platelet activating factor as an eosinophil chemo-attractant. As reviewed by Hessel *et al.*,⁴⁷ IL-16 may play an important role in airway inflammation in allergic asthma. The recent finding that IL-16 is present in bronchial lavage fluid of allergic asthmatics 6 h after allergen bronchial provocation, but not in allergen challenged normal subjects or allergen challenged allergic rhinitis subjects, supports this hypothesis. Furthermore, airway epithelial cells of asthmatics, but not normals or atopic non-asthmatics, contain both IL-16 mRNA and protein. Given that histamine has been reported to induce release of IL-16 and because it is a potent chemo-attractant for CD4⁺ cells and eosinophils and induces airway hyperresponsiveness, it is reasonable to speculate that IL-16 provides a link between the early and late allergen response. Furthermore, IL-16 could be an important factor in target organ specificity because it is a CD4⁺ chemo-attractant and is present in the bronchial lavage of allergic asthmatics but not in allergic non-asthmatics after allergen challenge. Could IL-16 acting by the above mechanism determine why some atopic individuals have bronchial asthma whereas other subjects who are equally sensitive to an allergen develop allergic rhinitis?

A study in human atopic asthmatics showed significant increases in Th2-like broncho-alveolar lavage fluid cytokines, in comparison with normal subjects including IL-4, IL-5, and granulocyte-macrophage colony-stimulating

factor.⁴⁸ The presence of specific phenotypically different human CD4⁺ T cells has not been clearly established to date, although T cell clones from mite-sensitive subjects produce IL-4 and IL-5 on exposure to mite allergen.⁴⁹ In humans, the genetic drive to produce a Th2-like cytokine pool (IL-4, IL-5, IL-10 and IL-13) in response to allergen exposure is highly probable.

In atopic subjects there is an increased percentage of monocytes and macrophages that have surface IgE receptor positivity. Macrophages reside at the critical air-surface interface, and the presence of IgE on macrophage surfaces may be critical in asthma. Macrophages have the ability to release LTB₄ and platelet activating factor, both eosinophil chemo-attractants. Interferon- γ alters macrophage function. This lymphokine, as well as IL-4, may regulate IgE receptor density on lung macrophages.⁵⁰

ADRENERGIC RECEPTOR DYSFUNCTION AND PHOSPHODIESTERASE

Recent studies using bronchial alveolar macrophages have shown that both salbutamol (homologous) and prostaglandin E (heterologous) stimulation of cAMP production in asthmatics is deficient, suggesting a non- β 2-receptor defect.⁵¹ Since β -receptor hyporesponsiveness is probably not directly caused by intrinsic defect(s) in the β -receptor itself, it would suggest potential defects in the signal transduction pathway involving guanine nucleotide regulatory protein, adenylate cyclase enzyme, or even phosphodiesterase (PDE). The Basenji-greyhound dog model demonstrates a role for PDE levels in the pathogenesis of the atopic phenotype of this animal.⁵² Phosphodiesterase levels and bronchial responsiveness to methacholine are increased in the cross-breed, intermediate in the Basenji, and low in the greyhound. Increased PDE activity has been demonstrated in atopic dermatitis and allergic rhinitis subjects.⁵³

Phosphodiesterase inhibitors are under clinical investigation in asthma.⁵⁴ Five isoenzyme types of PDE are recognized, with PDE4 having the highest affinity for cAMP. Studies of PDE4 levels in basal U-937 cells showed that exogenous β -agonists increased PDE4 levels in a time- and dose-dependent manner.⁵⁵ This suggests an important functional link between β -agonists and the PDE4 level. The five isoenzymes are coded by distinct but probably related genes. The genetic profile for PDE in asthmatics or allergic subjects is yet unknown.

Phosphodiesterase-4 inhibitors have modulatory effects on peripheral blood mononuclear cell (PBMC) cytokine

secretion. Reports of their effect on production of the immunosuppressive cytokine IL-10 differ, but there is overall agreement that they inhibit tumor necrosis factor (TNF)- α secretion. The effect of two PDE4 inhibitors on IL-10 and TNF- α production by PBMC from non-atopic versus atopic individuals has been compared.⁵⁶ The PDE4 inhibitors caused a concentration-dependent inhibition in the secretion of both TNF- α and IL-10. These drugs were more potent in suppressing cytokine secretion by PBMC from atopic than from non-atopic donors. They were also more potent in preventing TNF- α than IL-10 secretion.

Since the PDE4 inhibitors are selectively more potent in inhibiting TNF- α secretion by cells from atopic than from non-atopic donors, but not as effective in the suppression of IL-10, they may have a potentially important role in the treatment of allergic disease.

ENVIRONMENTAL FACTORS

Two factors commonly associated with the onset of asthma include viral illness and exposure to tobacco smoke. Bronchial asthma, which is always debilitating and occasionally fatal, is one of the most common chronic diseases in the world. In the USA, asthma is the number one chronic disease causing school absences and its prevalence in industrialized nations has doubled in the last 20 years. Furthermore, in spite of recent advances in treatment and in the prescribing of therapy, deaths from asthma have continued to rise to epidemic levels. Although the reason for the onset of this asthma epidemic is unknown, the localization of the problem to industrialized nations suggests causative agents such as poorly ventilated, allergen-friendly modern homes, air pollution, smoking or, as we suspect, the decline in incidence of childhood infections.

It is now recognized that allergies are the result of an imbalance in the immune system (Fig. 7). This skews the inflammatory response to common, harmless antigens so that high levels of IgE are made and eosinophils are activated. Childhood infections frequently cause the other arm of the immune system to be activated, so that IgG is formed and there is a non-allergic immune or delayed type of hypersensitivity response, as exemplified by the tuberculin skin test reaction. Studies have revealed that these two arms of the immune system provide a system of checks and balances for each other. For example, IL-4, one of the immune proteins secreted by cells (Th2) involved in allergy, prevents delayed type hypersensitivity reactions. In comparison, the immune proteins IL-12 and

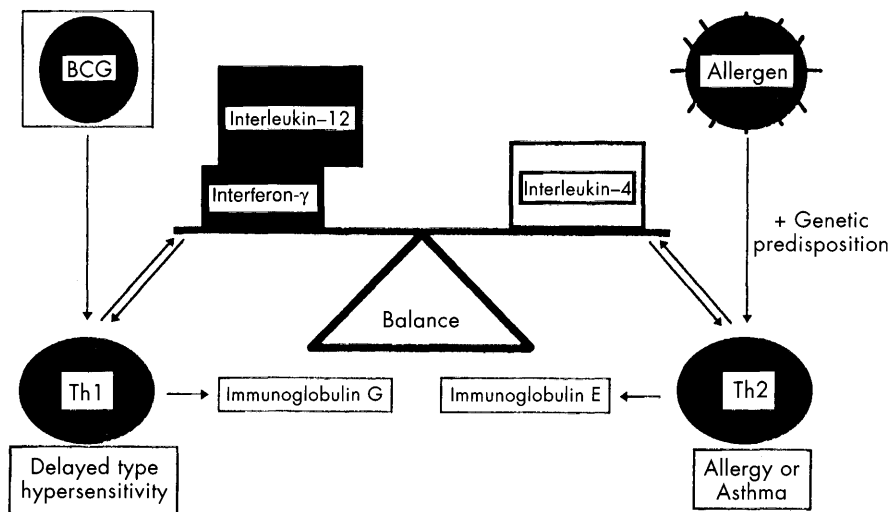


Fig. 7 Immune balance.

interferon- γ , which are secreted by cells (Th1) involved in delayed type hypersensitivity, inhibit the Th2 cells, which mediate allergic responses. Because the main risk factor for the development of asthma is allergy, it is logical that a decrease in childhood infections may increase the incidence of allergy by allowing the immune balance to shift to the right (Fig. 7). Indeed, the literature provides some evidence that this is the case.^{57,58} Conversely, we predict that if one can drive the immune balance towards the delayed type hypersensitivity reaction (left), allergy symptoms will decline.

CONCLUSION

The findings of this study were as follows:

1. Methacholine can determine the degree of bronchial reactivity, which is certainly a hallmark of asthma.
2. Being from an asthma family is a risk factor in terms of the subsequent development of asthma and increased airway reactivity.
3. The parent data suggest that airway reactivity is transmitted to succeeding generations.
4. Studies of twins have revealed that the concordance of asthma is higher in monozygotic than in dizygotic twins, but that environmental factors are at least as important as genetic factors.
5. Animal models of asthma comparing genetic strains can provide an important link between airway hyperresponsiveness and the allergic response.
6. The inheritance of asthma fits a polygenetic pattern rather than a single-gene pattern.

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