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Family size, infection and atopy: the first decade of the "hygiene hypothesis"

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Introductory article

The magnitude of the effect of smaller family sizes on the increase in the prevalence of asthma and hay fever in the United Kingdom and New Zealand

K Wickens, J Crane, N Pearce, R Beasley

Background. *Declining family size is one factor that has been proposed to contribute to increasing asthma and hay fever prevalence, but its relative importance has not been quantified.* ***Objective.*** *Our purpose was to determine the change in asthma and hay fever prevalence that would be expected from the reduction in family size that has occurred in England/Wales and New Zealand over recent decades.* ***Methods****. The relative change in family size between 1961 and 1991 in England/Wales and New Zealand was determined from census data for these years. Summary weighted odds ratios were calculated for the associations among birth order, family size, and asthma and hay fever prevalence. The expected increase in the prevalence of asthma and hay fever between 1961 and 1991 resulting from changes in family size was then calculated.* ***Results****. The expected relative increase in the prevalence of asthma between 1961 and 1991 as a result of the smaller family size was 1% and 5% for England/Wales and New Zealand, respectively; smaller family size would be expected to increase the prevalence of hay fever prevalence in England/Wales by 4%.* ***Conclusions****. Changes in family size over the last 30 years do not appear to explain much of the reported increase in asthma or hay fever prevalence. The contribution that other risk factors have made to these increases could be assessed with use of a similar approach.* (*J Allergy Clin Immunol* 1999;**104**:554–8)

In 1989 I proposed a novel but speculative explanation for the principal epidemiological features of hay fever and the apparent rise in the prevalence of allergic diseases.[1] Colloquially named the "hygiene hypothesis", this stated, in summary:

"*These observations . . . could be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally . . . Over the past century declining family size, improved household amenities and higher standards of personal cleanliness have reduced opportunities for cross-infection in young families. This may have resulted in more widespread clinical expression of atopic disease.*"

At first this hypothesis was received with scepticism because the prevailing immunological thinking considered infection as a potential trigger of allergic sensitisation rather than as a protective influence.[2] However, during the early 1990s a plausible mechanism arose from the distinction of Th1 and Th2 lymphocyte populations in laboratory animals and the recognition that "natural immunity" to bacterial and viral infections induces a Th1 pattern of cytokine release, potentially suppressing the Th2 immune responses involved in IgE mediated allergy.[3 4] Although the Th1/Th2 paradigm may not be as clear in humans as it first appeared in rodents,[5] the "hygiene hypothesis" has remained of interest to both immunologists and epidemiologists throughout the 1990s and has been the subject of a number of editorials and review articles.[6–14]

This commentary summarises the epidemiological evidence which has emerged during the 1990s relating family size, infections, and immunisations to atopy and allergic diseases, and looks forward to the research agenda for the next decade.

Epidemiology of atopy and allergic diseases

HOUSEHOLD SIZE AND STRUCTURE

The introductory article by Wickens *et al*[15] concentrates on one of the most striking epidemiological features of allergy – the inverse association with family size, which has been consistently found in studies of hay fever, skin prick positivity, and specific IgE.[16] Many, but not all, studies find a stronger "protective" influence for older

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siblings than for younger siblings and, among the British and New Zealand studies summarised by Wickens *et al*,[15] this is the predominant pattern for hay fever, although not for asthma – a point to which we return shortly.

The "hygiene hypothesis"[1] was an attempt to offer a parsimonious and coherent explanation for the *current* observations of differential hay fever risk within families and the *historical* evidence suggesting that hay fever had emerged as a "post-industrial revolution epidemic" during the 19th century[17] and continued to increase in prevalence throughout the 20th century.[18–20]

In 1989 the influence of family size and structure was apparent only for parentally reported and self-reported hay fever and eczema in two national British birth cohorts,[1 21] and this raised the possibility of an artifactual association arising from differential awareness or labelling of allergic diseases by parents of firstborn and subsequent offspring. However, during the past decade this concern has been allayed by studies from several European countries showing the same inverse association of family size[22–28] or, more specifically, birth order[23 25 26] with objective measures of allergic sensitisation such as skin prick tests[22 23 25 26] or circulating levels of aeroallergen specific IgE.[24 27 28]

Large samples are required to distinguish reliably between the effects of older and younger siblings, but in two such studies[28 29] no statistically significant difference emerged, in contrast to the earlier observations on British populations.[1 21 30] It is intriguing that in both the former studies[28 29] a significantly stronger "protective" effect emerged for brothers than for sisters. Thus, while an inverse association between family size and allergic diseases remains a consistent feature, the possible modifying influences of household structure, including birth order, sibling gender, and parental ages[23 31 32] still require clarification.

A large study of specific IgE among adults from several European countries[28] has shown a statistically significant difference in the strength of the family size effect between subjects with a parental history of allergy and those without such a history. A protective effect of siblings on atopy was evident only in the group with no parental allergy, suggesting that environmental influences on allergic sensitisation may be "overwhelmed" by genetic predisposition to atopy. On the other hand, a study of German children suggested that the effect of sibship size was concentrated among the offspring of atopic fathers, although the statistical significance of the difference between paternal and maternal atopy was not assessed.[33]

The European study[28] found an additional protective effect of sharing a bedroom as a child, independent of family size. This would be consistent with the "hygiene hypothesis" which interprets the variation in allergy risk with family size as a reflection of differential exposure to infections acquired in childhood from contact with siblings. A similar effect might be expected from exposure to children outside the home, but inconsistent results have emerged from the few studies which have investigated this. One relatively small German study found a reduced risk of allergy among children who had entered day nursery at an early age,[34] but a larger Finnish study reported a significant association in the opposite direction.[35] An earlier Finnish study found no difference in allergic symptoms or skin test positivity with respect to day care attendance.[36] In the British 1958 birth cohort, attendance at a preschool nursery did not influence the prevalence of allergic sensitisation in early adult life[25] and, among European adults, day care attendance in childhood was associated with a small, non-significant excess of atopy.[28]

The balance of evidence does not therefore suggest a relationship between allergy and early child contacts outside the home, which is difficult to reconcile with the "hygiene hypothesis". In retrospect, it is interesting to note that Finnish investigators publishing in 1984[36] had obviously set out to test the hypothesis that early infection *increased*,[2] rather than *decreased* the risk of allergic symptoms. Finding no difference with respect to day care attendance, they concluded that "atopy probably cannot be prevented by protecting small children from infection". The direction of the causal hypothesis has certainly changed in the intervening years!

SOCIOECONOMIC VARIATIONS

Another feature of the epidemiology of allergic disease for which the "hygiene hypothesis" offers an explanation is the socioeconomic gradient, with a higher prevalence of hay fever[37 38] and eczema[39] among children and adults from more affluent families. The variation with parental socioeconomic status is independent of, and more powerful than, the effect of the offsprings own socioeconomic status as an adult,[31] and is also independent of family size and birth order.[26 31 38]

Theoretically, some of the socioeconomic gradient in hay fever prevalence could be due to differential reporting and labelling of symptoms,[40] but this cannot account for the similar trend in physician-diagnosed eczema[39] and positive skin prick tests.[25 26 41 42] Socioeconomic variations in the prevalence of allergy are evident not only in western countries such as Britain,[31 38 39] Italy,[26] and the USA,[37 41 42] but also in the German Democratic Republic before German unification[43] and in urban Ghana.[44]

Anecdotally, the association of allergy with affluence dates back to the 19th century.[17] Charles Blackley, who first demonstrated that hay fever was caused by allergy to grass pollen, described the disease as one of the urban educated classes and was puzzled to find that it was rare among farmers, despite their high exposure to pollen. Interestingly, several recent studies have reported a reduced prevalence of allergic rhinitis and/or objectively measured atopy among children of farmers compared with other children in rural communities.[35 45–47] The magnitude of these differences, a 2–3-fold variation, is similar to the comparison of extremes of parental social class or household size.[31]

A number of explanations can be offered for this reduced prevalence of childhood allergy in farming families.[48] Greater exposure to infection is likely in the farm environment, but other exposures including chemical pesticides and herbicides or bacterial endotoxins should be considered. There are also differences in lifestyle between farming and non-farming families, including diet, which may be influential, either directly or through their effects on intestinal microflora, as discussed below. The Alpine studies, at least, relate primarily to small scale livestock farming and frequency of animal contact accounts for most of the reduction in allergy risk among farming families.[46 47] This raises the possibility that zoonotic infections may offer important protection against allergic sensitisation.

In this context it may be relevant that, among European adults, exposure to a dog during childhood was associated with a reduced prevalence of atopy.[28] This finding may reflect pet avoidance by allergic families, although early exposure to cats was not independently associated with less atopy.[28] Studies of children generally

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show little variation in the prevalence of allergic disease with pet ownership,[49] although a recent Swedish study found a reduced prevalence of allergic rhinitis and asthma among children exposed to pets in the first year of life which persisted, albeit at reduced levels of statistical significance, after adjustment for pet avoidance.[50] While it would be premature to conclude that early pet *exposure* protects against allergic sensitisation, this possibility should be considered in the context of allergen avoidance strategies which aim to reduce the burden of allergic disease by pet *avoidance*.[51]

INTERNATIONAL VARIATIONS

Important insights into the epidemiology of atopic disease were offered with the opening of former socialist countries of eastern Europe to "western" investigators during the early 1990s. Comparisons between former West and East Germany[52–54] and across the Baltic[55 56] indicated a substantially lower prevalence of allergic symptoms and atopic sensitisation among children and adults from East Germany than from West Germany, and among children from Poland and Estonia compared with those from Sweden. These east–west differences were seen only in children and younger adults, and not among Germans born before the 1960s,[54] which suggests that a "cohort effect" may be operating with the lifetime allergy risk being influenced by living conditions in early childhood.[57] During the 1990s, however, the prevalence of hay fever and atopy in Leipzig appears to have increased, even among generations born before German reunification.[58] This suggests that the critical period for determination of these geographical variations in allergy risk is not confined to the perinatal period.

EPIDEMIOLOGICAL DISTINCTION BETWEEN ASTHMA AND ATOPY

It is important to note that neither the household size effect nor the socioeconomic or geographical variations apply so consistently or conspicuously to asthma symptoms. Although some large studies of asthma prevalence show a strong inverse relationship with birth order,[59] most show only a weak association.[15] Socioeconomic variations in asthma *prevalence* in Europe recently have been small, but asthma *severity* tends to be worse in the poorer groups.[60 61]

Some important epidemiological differences between allergy (indicated by hay fever) and wheezing illness (indicated by asthma or wheezy bronchitis) are summarised in figs 1 and 2 which present data from two national British birth cohorts studied at the age of 16, 12 years apart.[38 62] A possible explanation for these differences is that only a minority of asthmatic symptoms are attributable to atopy (about one third as estimated by a recent meta-analysis[63]). There is growing recognition of multiple wheezing syndromes, particularly in early childhood,[64] with "transient early wheezing", "recurrent wheezy bronchitis", and "allergic asthma" forming distinct phenotypes, each with characteristic epidemiology and natural history.[65–67]

If the "hygiene hypothesis" is correct, then childhood infection should protect against "allergic asthma". Other wheezing syndromes, however, are positively related to infection[64] and, even among allergic asthmatics, intercurrent viral illnesses are a common trigger for episodes of bronchospasm. Thus, we should not be surprised if the associations with household size and socioeconomic status which emerge clearly and consistently for hay fever (almost by definition, "allergic" rhinitis) and for direct measures of atopy are less evident or absent for wheezing illness. An important corollary is that we should not seek to explain the epidemiology of asthma solely on the basis of patterns of allergic sensitisation.[63]

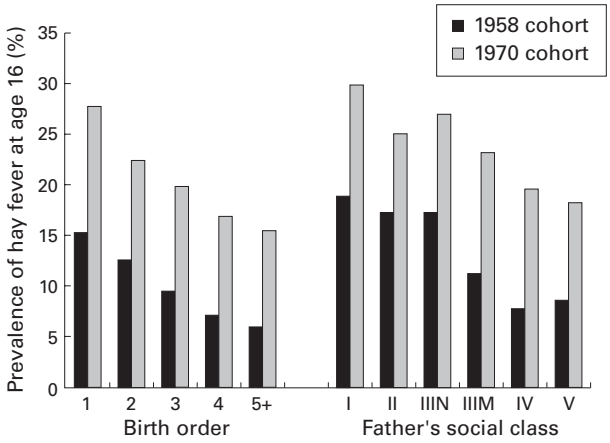

Figure 1 Prevalence of hay fever at the age of 16 in two national British birth cohorts born in 1958 and 1970, by birth order and father's social class.

Infection and risk of subsequent atopic disease

Epidemiological studies which have tested the hygiene hypothesis fall into two groups: those relating to specific infections or immunisations, and those assessing more generally the burden of infectious illness in early life.

GENERAL BURDEN OF CHILDHOOD INFECTIONS

Several large cross sectional studies of schoolchildren have obtained information retrospectively from parents on the nature or frequency of infections earlier in childhood and related this to the prevalence of atopic diseases[30 68] and skin prick test positivity.[26 68] The findings have been generally disappointing, in that no substantial inverse relationships have emerged. There are several important limitations to cross sectional data. Firstly, parental recall is likely to be incomplete, particularly for non-specific illnesses such as fever or respiratory infections, although it is arguably better for the "once off" viral infections (such as measles, mumps, and chickenpox). This would tend to dilute, but not to

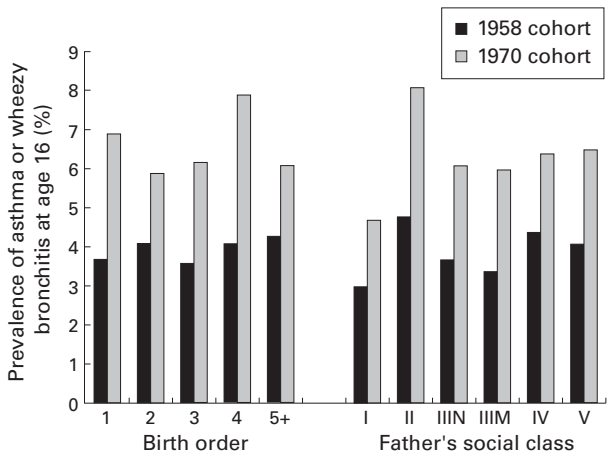

Figure 2 Prevalence of asthma or wheezy bronchitis at the age of 16 in two national British birth cohorts born in 1958 and 1970, by birth order and father's social class.

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reverse, associations. Secondly, parents who recall more accurately may also tend to report diseases more completely at the survey, generating a bias towards a positive association of early infection with allergic symptoms, but not with objective indicators of atopy such as skin prick tests. Thirdly, the early manifestations of a wheezing tendency may be recalled as episodes of chest infection, generating a non-causal positive association with later asthma and, indirectly, with associated atopic diseases.

Because of this third problem, an excess of early febrile illnesses and chest infections among children later suffering from asthma or wheeze[26 68] requires cautious interpretation. However, there was no association between the frequency of febrile episodes in the first year of life and subsequent hay fever among German children after those with asthma were excluded. There was a weak but non-significant inverse relationship between febrile episodes and skin test and RAST positivity, and a weak but significant inverse correlation of early fever with total IgE.[68]

The only childhood illness associated with a significantly lower prevalence of allergic disease among Aberdeen schoolchildren[30] was measles occurring after the age of three years in relation to asthma. However, this finding should be interpreted with caution because it arose from 30 comparisons (including one significant positive association between chickenpox and asthma), and eczema and hay fever were not similarly reduced among the children with later measles. When a composite index of exposure was generated by combining histories of illness due to measles, mumps, rubella, varicella and pertussis, the tendency was for a slightly higher risk of allergic disease in children with multiple infections.[30]

Longitudinal data are less prone to bias from parental recall, although they are equally prone to the problem of continuities in susceptibility to chest complaints. Investigators from the Tucson cohort study[69] interpreted an association between non-wheezing lower respiratory illness in early life and subsequent reduction in total IgE levels as possible evidence in favour of the "hygiene hypothesis". However, an alternative explanation is that the propensity to wheeze at times of viral infection in early childhood is in some children an early manifestation of "allergic asthma". Thus, the lower IgE levels among children with *non-wheezing* lower respiratory illnesses are probably due to selective removal from this group of the more atopic children who wheezed in response to early infection.

Longitudinal studies are preferable for exploring possible critical periods during which infection might influence allergic sensitisation. A large study of British adolescents[23] focused on infectious illnesses in the first month of life, ascertained by health visitors at a routine postnatal visit. No associations were found with hay fever or skin prick positivity in later childhood, but the spectrum of illness was relatively mild and records were restricted to the first month when passive immunity from maternal IgG is still influential.

A systematic review of the medical records of all children in a single general practice in Oxfordshire[70] has provided the only comprehensive insight to date of the relationship between common symptomatic illnesses throughout childhood and the occurrence of doctor-diagnosed asthma, hay fever, and eczema. The study was unusual in that social class, sibship size, and birth order were unrelated to the prevalence of allergy, and the only infectious illness examined which was related to birth order was croup. Others included in the analysis were otitis media, tonsillitis, upper and lower respiratory tract infections, urinary infections, whooping cough, measles, mumps, and chickenpox. No inverse associations were found between any of these infectious illnesses and the later development of hay fever, eczema or asthma, and there were significant positive relationships with croup, mumps, and chickenpox.[70] In addition, early antibiotic therapy was associated with a doubling in the risk of subsequent allergic disease, a point to which we will return later.

The totality of current evidence from cross sectional and longitudinal studies of common specific and non-specific infectious illnesses in infancy and childhood offers no support for the "hygiene hypothesis".

MEASLES INFECTION AND IMMUNISATION

Interest in the possibility that measles might be protective against allergy had previously arisen from the intriguing results of a longitudinal study of children in Guinea-Bissau.[71] The cohort was unusual because the area had been affected by an epidemic of wild measles when the children were young and before most had been immunised. Measles vaccination was offered after the epidemic to those who had not contracted wild measles infection. A substantially reduced prevalence of positive skin prick tests was found in the group who had been infected during the epidemic compared with those who received post-epidemic immunisation. The authors offered two interpretations – either that wild measles infection might protect against the development of atopy or that measles immunisation might promote allergic sensitisation. Correspondents[72 73] added a third possibility – namely, that children with impaired Th1 immune responses, who are normally at increased risk of IgE mediated allergy, were at a higher risk of dying during the measles epidemic and therefore the prevalence of atopy was artifactually lowered in the infected survivors.

Fortunately, evidence from the British 1970 birth cohort[21] was already available (and cited[71]) at this time to allay concerns about an increased risk of atopy from measles immunisation. About half of this cohort of over 13 000 children were vaccinated against measles and no substantial difference in the prevalence of hay fever or eczema was evident at the age of five when immunised and non-immunised children were compared; nor was there any evidence of a protective effect from wild measles infection.[21]

Subsequently, data on the prevalence of hay fever at the age of 16 in the same cohort were analysed in relation to measles infection, measles immunisation, and birth order.[74] Overall, the prevalence of hay fever was unrelated to wild measles or measles immunisation but, when stratified by birth order, a protective effect emerged in the children with many older siblings. However, we have examined the same statistical interaction of birth order and wild measles infection on hay fever at age 16 in the British 1958 cohort and found no evidence of effect modification (Butland BK, Strachan DP, unpublished analyses: available on request). Thus, it seems unlikely that there is a protective effect of measles selectively among larger families.

Further evidence against a protective effect of wild measles infection has been published recently from a mass MMR vaccination programme of over half a million Finnish children conducted during the early 1980s.[75] This is by far the largest study of any infection in relation to the prevalence of allergy and shows a statistically significant *increase* in the prevalence of

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asthma, hay fever, and eczema, by one third or more in relative terms, among the 4% of children with a history of measles illness. Interpretation is not entirely straightforward because the incidence of wild measles had already been reduced by monovalent measles vaccination during the previous seven years, and it is possible that uptake of this vaccine differed between allergic and non-allergic families. Additionally, the clinical manifestation of measles in vaccinated individuals could vary according to the Th1/Th2 balance, just as case fatality might in the African context. However, it is reasonable to conclude that this latest study provides no support for a protective effect from wild measles infection.[76]

PERTUSSIS IMMUNISATION

The debate about a possible increase in allergy risk from measles immunisation resonated with concerns during the mid 1990s about the safety of whooping cough vaccine. These were raised in the correspondence columns of *JAMA* by a British general practitioner who reported an increase in the risk of asthma among children in his practice who had received whole cell pertussis vaccine.[77] These results were never published as a peer reviewed paper, but similar findings were subsequently reported from an Oxfordshire practice where pertussis immunisation was associated with a 75% relative increase in the risk of asthma, hay fever, or eczema.[70] A further study from New Zealand, based on extremely small numbers of unvaccinated infants, suggested an effect in the same direction.[78]

These results need careful interpretation because of the likelihood that, in countries with a high vaccine coverage (such as Britain and New Zealand), the families who choose not to immunise their children are unusual and possibly include fewer allergic parents. Furthermore, whooping cough illness is associated with an increased risk of subsequent asthma and wheezing illness,[67] although the direction of causality is not clearly understood; an early tendency to wheezing may exacerbate the symptoms of whooping cough, rather than pertussis damaging the airways to cause asthma.

Although in theory pertussis vaccine, or its associated adjuvant, may increase IgE production[79] and thereby the risk of allergy, the results of a small randomised controlled trial in Sweden exclude a major increase in the risk of allergy.[80] Among 669 children randomised to receive acellular pertussis vaccine, whole cell pertussis vaccine, or placebo (diphtheria and tetanus vaccines only), whole cell vaccine was associated with a relative reduction in the risk of atopic disease of 8% with an upper 95% confidence limit of 28% relative increase. The corresponding figures for acellular vaccine were a 10% relative increase with an upper 95% confidence limit of a 41% relative increase.[80]

Observations from a large British cohort born in the early 1990s[81] are also reassuring in relation to the risk of asthma or wheezing following pertussis vaccination. There were no significant differences in the incidence of early wheezing or later onset wheezing in relation to whole cell pertussis vaccination in the first six months of life, with upper 95% confidence limits of 23% relative increase and 5% relative increase, respectively.[81] No substantial reduction in the risk of asthma is evident for British cohorts born during the late 1970s[12] when pertussis vaccine coverage fell from over 75% to less than 40% for the 1974–7 birth cohorts as a result of concern about neurological side effects, with a consequent rise in notifications and fatalities from wild pertussis infection. It is important that a similar loss of confidence in an effective vaccine is not prompted by poorly founded concerns about its effect on the risk of allergic disease.[82]

MYCOBACTERIAL INFECTION AND BCG VACCINATION

A possible link between mycobacterial infection and reduced risk of allergy was suggested by a study of 867 Japanese children who underwent routine tuberculin tests prior to BCG vaccination at ages 6 and 12 years. An inverse relationship was observed between delayed hypersensitivity to tuberculin at the age of 12 and both total and allergen specific serum IgE levels at the same age.[83] The authors interpreted this as evidence that prior infection with tuberculosis or environmental mycobacteria might protect against the development of allergy.

However, the allergy data are presented in four groups by tuberculin positivity at ages six and 12, and the reduction in allergic symptoms and IgE levels was evident only in the children who were positive at both ages (n = 289) or positive at age 12 only (n = 213). This latter group would have received BCG at the age of six years, and their tuberculin positivity is likely to have reflected vaccination rather than exposure to natural mycobacteria. The comparison of particular interest is between 290 children who were tuberculin negative at both ages (who received BCG at the age of six but did not mount an effective cell mediated immune response) and 75 children who were tuberculin positive at the age of six (and therefore did not receive BCG) but negative at age 12. These two groups had similar prevalences of allergic disease, some three times higher than the tuberculin positive subjects, and similar prevalences of atopy as defined by allergen specific IgE. This would imply that BCG vaccination at the age of six did not affect allergic outcomes in this population.

The Japanese findings excited considerable interest and controversy, the more widely held explanation being that the degree of tuberculin reactivity was influenced by Th1 immunity and atopy by Th2 immunity. Their inverse relationship could therefore be a reflection of the hosts Th1/Th2 balance rather than a causal relationship between infection and atopy.[84] However, this would not be consistent with the observation that a family history of allergic disease was equally common in all four comparison groups described above.[83]

The results published in a recent issue of *Thorax* challenge this second interpretation. Among Norwegian adults who routinely received BCG vaccination as adolescents, the size of reaction to tuberculin testing was unrelated to total or specific IgE levels, or to skin prick responses to common aeroallergens.[85] Thus, in adults at least, it appears that individual variations in Th1/Th2 balance are insufficient to generate an epidemiological association between tuberculin positivity and measures of atopy.

On the other hand, if early exposure to mycobacteria does influence the risk of allergy, some protective effect might be expected from neonatal BCG vaccination. Two Scandinavian studies, one experimental[86] and the other observational,[87] suggest that the size of any such protective effect is probably small or non-existent, although a definitive large scale controlled trial has yet to be conducted.

The most definitive study to date of mycobacterial infection is a comparison of 456 Finnish children and 706 young adults (aged 17–20) who were diagnosed with active tuberculosis, and an age matched control group of similar size.[88] There was a slightly lower pre-

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valence of subsequent allergic disease among the tuberculosis cases (6.7% versus 9.0% overall), but a slightly higher prevalence of asthma (4.6% versus 3.6%). The authors focused in their interpretation on a marked reduction in allergic disease among females, but this is balanced by a marked excess of asthma among males and a more appropriate conclusion would be that clinical tuberculosis does not have a major impact on the incidence of subsequent asthma and allergy.

It has been argued that environmental mycobacterial exposure plays a major part in determining the risk of allergic sensitisation.[89] However, in the absence of more positive evidence of a protective effect from active tuberculosis or documented BCG vaccination, and one study showing a higher, rather than a lower, prevalence of cutaneous reactivity to atypical mycobacteria among allergic children,[87] this seems unlikely.

HEPATITIS A

Perhaps the most consistent evidence of an inverse relationship between infection and allergic sensitisation has emerged from studies of hepatitis A, an infection which is known to vary with family size and socioeconomic status in a direction opposite to the trends seen for allergy.

Among Italian military students, serological evidence of previous hepatitis A infection (in about one quarter of the sample) was associated with a halving in the prevalence of high aeroallergen specific IgE levels.[90] This effect was independent of age, sibship size, birth order, area of residence, and paternal education but, unusually, there was no strong relationship between paternal education and specific IgE, either before or after adjustment for hepatitis A seropositivity. This raises the possibility that unmeasured socioeconomic confounding may account for at least some of the association of hepatitis A seronegativity with allergic sensitisation in this sample.

In a further study based on the same study population the association was found to be fairly specific to hepatitis A; no significant association was found between high specific IgE concentration and seropositivity to measles, mumps, rubella, varicella, cytomegalovirus, herpes simplex, or *Helicobacter pylori*.[91] A significant inverse relationship was seen with seropositivity to *Toxoplasma gondii*, but whether this was statistically independent of the hepatitis A effect was not assessed. This second study does not provide additional evidence in favour of hepatitis A, but points towards foodborne and faecooral transmission, rather than respiratory infections, as the more likely determinants of allergy risk.

Only two other studies have addressed this relationship. A survey of a general population sample in San Marino generated similar findings to the Italian Air Force study – namely, a 40% relative reduction in atopy among those seropositive for hepatitis A.[92] In a case-control study of adult onset wheeze in Aberdeen a similar relationship was seen with a 37% relative reduction in atopy among seropositive individuals.[93]

INTESTINAL FLORA AND ANTIBIOTICS

The possibility that intestinal infection may be of particular relevance was first suggested on immunological grounds from observations on animals raised in a sterile environment.[94–96] Two pioneering studies comparing infants in Sweden and Estonia have shown marked differences in the types of faecal bacteria in unselected infants in the two countries[97 98] which broadly match the differences seen between atopic and non-atopic infants in each country.[98] So far these results have been based on small numbers of infants, but they raise the possibility that intestinal colonisation in early childhood may contribute to both the international variations and the individual risk of allergy within countries.

Lactobacilli are present in larger numbers in the faeces of Estonian and non-atopic infants.[97 98] It is therefore interesting that a comparison of children attending Steiner schools with other Swedish schoolchildren showed a lower prevalence of atopy in those in the Steiner schools where many families adopted an anthroposophic lifestyle.[99] The problem with such a comparison is that there are many aspects of the anthroposophic lifestyle which may be relevant, and it is impossible to disentangle them in an observational study.[100] These features include consumption of locally produced "organic" food, particularly fermented vegetables which are rich in lactobacilli; avoidance of immunisations; and restricted use of medications, including antibiotics.[99]

A second study of Steiner schools in New Zealand made comparisons within the schools but did not compare with non-anthroposophic children.[101] The lifetime prevalence of asthma was increased fourfold in the one third of children who had received antibiotics in the first year of life, with a dose-response relationship to the number of antibiotic courses. In this context, antibiotic use might simply be a marker for non-adherence to the anthroposophic lifestyle, and in the Swedish study a composite "Steiner index" showed evidence of a graded inverse relationship to atopy.[99]

More generally, however, antibiotics are commonly prescribed for early episodes of chest illness and this may generate a spurious association with later asthma.[102] Associations with hay fever and eczema may be a better indicator of a true relationship with allergic sensitisation, and a large German study showed an excess of hay fever and eczema among children receiving six or more courses of antibiotics in the first year of life, even after asthmatics were excluded. However, there was no increase in the prevalence of atopy, as determined by skin prick and RAST tests.[68] A retrospective review of records in an Oxfordshire general practice found a doubling of the risk of hay fever and eczema among children who had received any antibiotics by the age of two years which was independent of the clinical indication for antibiotic treatment. Cephalosporins and macrolides were associated with a higher risk than penicillins.[70]

These observations could be linked to effects of early antibiotic treatment on bacterial colonisation of the infant gut,[103] thus offering a paradoxical link between an increased risk of allergic disease associated with early non-intestinal infection (often treated with antibiotics) and a decreased risk of allergic disease associated with a "protective" pattern of bowel colonisation, which is disturbed by the non-specific effects of antimicrobial therapy.[96]

Explaining the time trends in atopic disease

The "hygiene hypothesis" was developed as a putative explanation for time trends in allergic disease, and Wickens *et al*[15] have examined whether changes in family size could account for the rising prevalence of asthma and hay fever among British and New Zealand children. The approach they use is indirect in that the relationship between family size or birth order and the disease outcomes is determined by literature review and meta-

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analysis and then applied to documented changes in family structure in each country over time. A more direct approach is to compare successive birth cohorts and to examine risk factors within each cohort and the between-cohort differences in a single statistical analysis. This integrated approach was adopted for the 1958 and 1970 British birth cohorts (figs 1 and 2), and a range of perinatal and socioeconomic factors were considered in addition to birth order.[38 62] None of these factors, individually or in combination, accounted for a substantial portion of the doubling in prevalence of hay fever[38] or asthma and wheezy bronchitis[62] over a 12 year interval. Given that these British cohorts are both included in the analysis by Wickens *et al*[15] and are the largest studies in their meta-analysis, it is not surprising that the conclusions of the two types of analysis are similar.

It would be simplistic to dismiss the family size effect just because relatively small changes in family composition do not explain recent time trends in allergic diseases. It is extremely implausible that family size, birth order, and socioeconomic status are the *direct* determinants of allergy prevalence. Rather, they should be considered as *indirect* and therefore imprecise measures of some other biologically relevant factor, which must increase with household size and relative poverty in many cultures, and which protects against allergic sensitisation more directly. Whether or not this protective factor is indeed some form of infection, as suggested by the "hygiene hypothesis", it must meet these two fundamental criteria in order to generate what are now highly consistent epidemiological observations of a reduced prevalence of allergic sensitisation in larger families and less privileged households.

When analysing time trends it may be useful to consider table 1 which shows the arithmetic association between the degree of protection offered by a putative protective factor, the prevalence of exposure to this factor in any study group (for instance, firstborn children), and the prevalence of disease which may be expected in the study group, expressed as a percentage of the prevalence in a totally unexposed population. It is not often appreciated that, in order to generate a twofold difference in disease prevalence – for instance, the difference in the prevalence of hay fever between firstborn children and those with three or more older siblings (fig 3 in Wickens *et al*[15]) – there needs to be a large difference in exposure to quite a powerful protective factor. For instance, a twofold difference could result from comparison of groups with 10% and 60% exposure to a "biological" factor which reduces the prevalence of disease tenfold (relative risk 0.1); or from comparison of groups with exposure prevalences of 50% and 90% to a protective factor with a relative risk of 0.2, and so on (table 1). The situation becomes more extreme if we attempt to explain threefold or larger differences in the observed prevalence of allergy such as occur with combinations of birth order and social class.[31]

The proper interpretation of the consistent variations in atopy and allergic rhinitis with family size and socioeconomic status (fig 1) is that these are important clues to the presence of a powerful underlying environmental determinant of allergic sensitisation, and it is likely that the rising prevalence of hay fever, and probably part of the rising prevalence of asthma,[104] is attributable to changes in the degree of exposure to this underlying cause. It is also pertinent to note that this interpretation places some constraints on the exposure prevalences which must apply in groups defined, for instance, by birth order, at the start and end of the follow up period. Since the prevalence of hay fever has increased in all birth order groups and all social classes (fig 1), the exposure prevalence in any of these groups cannot have been zero in 1974. This suggests that smaller changes in the proportion exposed to a *very* strong protective factor (relative risk of 0.05 or less) has been the underlying cause of the rise in the prevalence of allergy and its current distribution by family size and birth order, assuming that both are influenced by the same factor.

The "hygiene hypothesis" in the 21st century

This review of the first decade of the "hygiene hypothesis" presents a number of paradoxes. An idea which was introduced on the basis of epidemiological data and contrary to immunological orthodoxy now survives as much on the grounds of biological plausibility as confirmatory epidemiological observations. What the past 10 years has confirmed beyond reasonable doubt is that the original findings of variation in hay fever and allergic sensitisation with birth order[1] and socioeconomic status[31] are real (objectively confirmed), consistent, and statistically independent. There is undoubtedly something to explain, but the results of studies which have more directly addressed infection as the explanatory factor have been disappointing and often difficult to interpret.

If the argument in the previous section applies, then we are seeking to detect quite powerful protective effects from an exposure which varies substantially across groups defined by birth order and/or socioeconomic status. Whereas large surveys have been necessary to confirm these "surrogate" risk markers, theoretically even small studies which measure the "true" protective factor should offer sufficient statistical power, for instance, to detect relative risks of 0.1 or below. Yet even the most promising evidence for an infective agent, in relation to hepatitis A, involves relative risks in the region of 0.5–0.7.[90–93] This suggests that none of the specific agents studied hitherto are the "true" protective exposure.

Table 1 Prevalence of disease, expressed as a percentage of the prevalence in a totally unexposed population, by prevalence of exposure to a putative protective factor and the degree of protection offered

Relative risk*	Percentage exposed to the protective factor										
	0	10	20	30	40	50	60	70	80	90	100
0.5	100	95	90	85	80	75	70	65	60	55	50
0.3	100	93	86	79	72	65	58	51	44	37	30
0.2	100	92	84	76	68	60	52	44	36	28	20
0.1	100	91	82	73	64	55	46	37	28	19	10
0.05	100	90	81	72	62	53	43	34	24	15	5
0.01	100	90	80	70	60	51	41	31	21	11	1

* Prevalence of disease in exposed individuals compared with unexposed individuals.
The figures in the body of the table may be derived by the formula $100 \times (1-(1-R)e)$ where R = relative risk and e = proportion exposed.

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LEARNING POINTS

- **A higher prevalence of hay fever, eczema, skin prick positivity, and allergen specific IgE in individuals brought up in smaller and more affluent families are consistent features of the epidemiological literature during the 1990s**
- **Asthma shows a different epidemiological pattern to these indicators of atopy**
- **The "hygiene hypothesis", which postulates that infection protects against atopy, is considered immunologically plausible and is consistent with the epidemiological features of atopy**
- **However, an inverse association between infection and atopy has not been confirmed directly by epidemiological studies. The available data are either inconsistent or inconclusive**
- **Recently identified areas for research include the influence of intestinal bowel flora on immunological maturation, and the modulating effects of antibiotic therapy and diet, acting through gut flora or other mechanisms**
- **The inverse association of family size with allergic sensitisation remains an enigmatic but potentially informative lead in the search for underlying causes of the rising prevalence of atopic disease in Western societies**

Where should we look for these stronger effects? One possibility is that infection is not the influential exposure, and we should be seeking other developmental, lifestyle, or environmental influences which vary *strongly* with birth order, family size, or social class. Paradoxically, despite the unpromising results relating indices of infection to allergy, no alternative explanation has emerged for the variations of hay fever and atopy within populations and families over the past 10 years. It is with those well established associations that investigation should start.

Few of the infections studied so far show very strong variations in relation to birth order or family size, never mind with allergic disease. This may be because the wrong infections have been studied, or because the timing, rather than the nature, of the infection is particularly important. Further work is needed relating early infection to later atopic outcomes, but this is a demanding research agenda requiring longitudinal studies with follow up over several years. More rapid progress might be made by examining the epidemiology of a wide range of infections by age at onset, in relation to sibship size, and socioeconomic status. Only those showing large variations in relation to these variables would be good candidate exposures under the "hygiene hypothesis". For instance, the recent findings in relation to infant bowel flora are intriguing but little is known about the association of intestinal colonisation with demographic or social factors.

Future research needs to consider carefully whether the "critical period" or "window of opportunity" relates more closely to immunological development in general, as proposed for intestinal bacteria,[96] or to the first exposure to each allergen, which might explain why some allergic individuals become sensitised to one ubiquitous allergen (house dust mites in the UK) but not to another (grass pollen). In this respect, hay fever provides a particularly useful epidemiological model because the timing of early allergen exposure can be imputed from the local pollen season.

Conclusions

At the start of the 21st century the "hygiene hypothesis" retains its appeal because it offers a parsimonious, coherent, and biologically plausible explanation for the variations in allergy over time, between countries, between more and less affluent households, larger and smaller families, and by position within the family. For these reasons, 10 years have not changed my view that infections remain the most promising candidates for the underlying protective factor. The task for the next decade is to identify the nature of the protective agent, the timing of its effect on atopic sensitisation, and the opportunities this offers for prevention of allergic disease.

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