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Editorial

Investigation of the hygiene hypothesis: current issues and future directions

A.-L. Ponsonby[1,2], A. Kemp[3,4]

[1]Murdoch Children's Research Institute, Melbourne, Vic.; [2]Menzies Research Institute, University of Tasmania, Hobart, Tas.; [3]Department Allergy and Immunology, The Children's Hospital, Westmead; [4]Discipline of Paediatrics and Child Health, The Children's Hospital at Westmead, University of Sydney, Sydney, NSW, Australia

Professor Anne-Louise Ponsonby
Head Environmental and Genetic Epidemiology Research Group
Infection, Immunity and Environment, Murdoch Children's Research Institute
Royal Children's Hospital
Flemington Road
Parkville
Vic. 3052
Australia

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The 'hygiene hypothesis' was first proposed in 1989 by Strachan et al. (1) who proposed that reduced opportunities for cross-infection in families may have resulted in the more widespread clinical expression of atopic disease. It was based on the observation of a striking inverse association between sibling number and hay fever in adulthood among those in the 1960 UK birth cohort study (1). It proposed that sibling number could be protective for allergic disease because siblings are the source of infection. The immunological mechanisms that might mediate the consequences of increased sibling exposure are unclear and the simplistic proposal that early life microbial deflects the immune system from a Th2 (allergic)- to a Th1(nonallergic)- response can be challenged (2).

In this journal, the extensive work by Bremner et al. utilizing two large general practice databases in the UK, reports that clinically apparent infections in the first year of life were not associated with subsequent child hay fever (3). Further, although older sibling number inversely associated with hay fever, sibling number did not predict clinically apparent infections in the first year of life.

The strengths of this study include the use of a large number of general practices as a population-based sampling frame and the large sample size providing adequate sample size to conclude that negative findings were not likely to be on account of type 2 error. Further, a careful evaluation of the potential spurious association between infection and hay fever on account of frequency of healthcare attendance was made, and control for socioeconomic status, previously linked to both infection and hay fever (4, 5), was possible.

In such studies, a significant issue is the scope and accuracy of the measurement of exposure. Presentation of an infection to a general practice may not accurately reflect the totality of microbial exposures occurring in early life (Fig. 1). In such cases, infections with minor symptoms that do not present to medical practitioners were not recorded. The likelihood of an infective illness presenting to a general practitioner may depend on many factors such as anxiety and childcare experience of the parent as well as the clinical symptomatology. That these issues of the measurement of an infection subset may be relevant to the present study is suggested by the low incidence of reported clinical infections among infants during the first year of life. For example, 48% of controls in the matched sample had not presented with an upper respiratory tract infection by one year (3). The proportion of infection-positive infants is lower than in the Oslo birth cohort, where

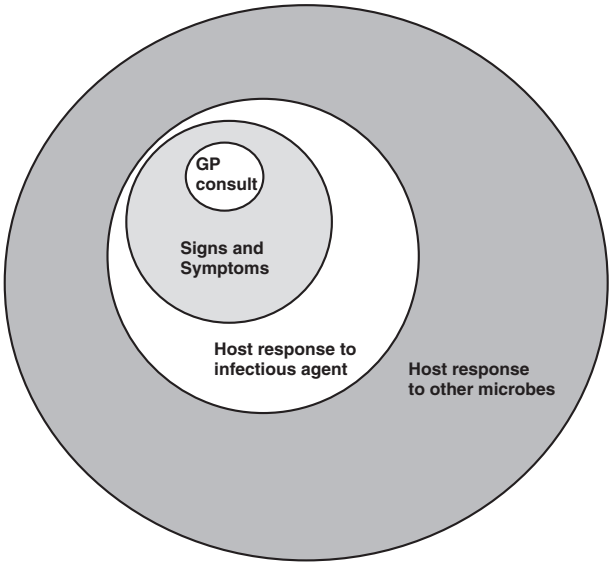

Figure 1. The scope of exposure assessment: investigating how infection and other microbial exposure influences the developing immune system in the context of the hygiene hypothesis.

69% of infants had a cold by 6 months and almost all by 12 months (6). Similarly, among the MAS cohort, and 86% were reported to have at least one viral infection, 67% of the children had had two or more runny noses by 1 year, the later associated with a reduced risk of atopic disease by age 5 (7). A prospective study of more than 800 18-month-old Swedish children over a 1-month period found that 93% of the children reported one or more symptom-days of infection; however, only one in five consulted a doctor (8). Thus the lower prevalence of any reported URI infection reported by Bremner is likely to reflect that only a subset of these minor infection episodes presented for medical care and were able to be studied in the record linkage study. Furthermore, even symptomatic infection, regardless of medical care presentation, is only a subset of microbial exposure (Fig. 1). Thus in a birth cohort of atopy prone infants, 40.2% were positive for viral PT-PCR by nasal probe by even 2 weeks of age (9), with a high proportion positive to even one virus (e.g. picornavirus, 68.5%) by 1 year of age (10).

To fully investigate the hygiene hypothesis, we need to move towards deepening our understanding of how host immune responses to infectious or other microbial agents influence the developing immune system. Further support for the idea that microbial exposure may underlie the inverse association between sibling number and atopic disease has been sought from examination of immunoglobulin seropositivity, which can provide evidence concerning past microbial exposure. As reviewed by Bremner et al. (3), hepatitis A virus, *H. pylori*, herpes simplex virus 1 and cytomegalovirus seropositivity have all been inversely associated with atopic disease. Further, a link between higher sibling number and seropositivity has also been reported (11, 12) although, unfortunately, not many studies have evaluated the interplay between sibling number and seropositivity .

The study in this journal is to be commended for not only examining 30 clinically apparent infections but doing so within the context of also considering older sibling-hay fever associations. This two-pronged approach of assessing not only the putative 'hygiene' factor (day-care, infection, antibiotic use, IgG seropositivity, gut flora) in relation to disease but also in relation to sibling number is often missing in much of the current and past investigations of the hygiene hypothesis. Rather, there has been a tendency to examine either sibling-exposure patterns or an alternative putative exposure, one that siblings may be acting through, but not examine them both, and the interrelationships between the two in the same study. Here, the report by Bremner et al. indicates that clinically apparent infections did not explain the inverse association between siblings and hay fever (3). This is similar to findings from the Danish cohort, where the inverse association between siblings and atopic dermatitis was not explained by early clinically apparent infection (13).

In addition to the exposure by infectious agents, evidence continues to accumulate that general microbiological exposures may also be of importance with several studies now indicating a possible protective role for farm animals (14) and even suggesting that previous innocuous exposures, such the bacterial composition of drinking water may influence the developing immune system. For example, pollen sensitization, often linked to hay fever, is more common in Finland than North Karelia in Russia (15). The total numbers of microbial cells in drinking water were many-fold higher in Russia than in Finland. Further, high and intermediate levels of water contamination were associated with reduced risk of atopy (odds ratio 0.34, 95% CI 0.20–0.57 and 0.39, 95%CI 0.23–0.69, respectively), independently from other factors (15). Findings such as these indicate that we need better markers of microbial exposure in early life. Relevant biomarkers that have been used include tuberculin sensitivity (16) and IgG responses to agents but these markers reflect not only the microbial exposure of interest, but also the specific host response. The influence of microbial exposures on intestinal microflora has been examined in microbiological studies and efforts are under way to now utilize the new field of metabolomics to carefully document gut colonization. In a parallel development, viral DNA load can now be also studied in blood to indicate latent infection. These initiatives may provide new information on the role of early life microbial exposure and the development of atopic diseases such as hay fever. However, it will be important for such future work to also investigate the interplay with sibling exposure.

There are several important new directions for investigation of the hygiene hypothesis. We need to move

beyond a consideration of whether the infection or a microbial exposure occurred in a binary sense (no/yes). Our increased knowledge of immune mechanisms over time now indicates an important role for early microbial antigenic exposure to drive immune system training through sequential events. Thus, a consideration of repeated exposures and the sequence of exposures may also be required. We have previously reported that the sibling effect appears to be stronger for rye grass and house dust mite sensitization when using mutually exclusive categories (17). We have postulated that this may reflect a difference in the timing of allergen exposure (17). Ideally, studies of infection exposure in early life would also consider whether allergen exposure preceded, was concomitant or followed such infection because the results from animal studies suggest the effect of infection may differ according to the timing of allergen exposure (18, 19). Further, a comparative disease approach has been proposed to allow the concomitant evaluation of sibship, the hygiene hypothesis and related issues across a range of immune disorders (20). This reflects recent immunological findings that that early life microbial exposures can also have important effects on the development of innate and adaptive immunity by pathways beyond those involved in allergic disease only.

So, where does this report by Bremner et al. (3) leave the hygiene hypothesis? It leaves it intact but remaining unexplained in that a robust inverse association between older siblings and hay fever was again observed, but not explained by the exposure measure of infections presenting for clinical care in the first year of life. We agree with Bremner et al.'s conclusion that supporters of the hygiene hypothesis need to look beyond clinical infectious illness for an explanation of the increase in atopic diseases in developed countries.

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