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PRO AND ANTI: THE BIOTICS OF ALLERGIC DISEASE

J Crane

Introductory article

Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial

M Kalliomäki, S Salminen, H Arvilommi, P Kero, P Koskinen, E Isolauri

*Background: Reversal of the progressive increase in frequency of atopic disease would be an important breakthrough for health care and wellbeing in western societies. In the hygiene hypothesis this increase is attributed to reduced microbial exposure in early life. Probiotics are cultures of potentially beneficial bacteria of the healthy gut microflora. We assessed the effect on atopic disease of Lactobacillus GG (which is safe at an early age and effective in treatment of allergic inflammation and food allergy). Methods: In a double-blind, randomised placebo-controlled trial we gave Lactobacillus GG prenatally to mothers who had at least one first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma, and postnatally for 6 months to their infants. Chronic recurring atopic eczema, which is the main sign of atopic disease in the first years of life, was the primary endpoint. Findings: Atopic eczema was diagnosed in 46 of 132 (35%) children aged 2 years. Asthma was diagnosed in six of these children and allergic rhinitis in one. The frequency of atopic eczema in the probiotic group was half that of the placebo group (15/64 (23%)) vs 31/68 (46%); relative risk 0.51 (95% CI 0.32–0.84)). The number needed to treat was 4.5 (95% CI 2.6–15.6). Interpretations: Lactobacillus GG was effective in prevention of early atopic disease in children at high risk. Thus, gut microflora might be a hitherto unexplored source of natural immunomodulators and probiotics for prevention of atopic disease. (**Lancet** 2001;357:1076–9)*

In the last decade there has been a growing interest in the relationships between infection, hygiene, and allergic disease and the hypothesis that infections and allergy are inversely related. Kalliomäki and colleagues, in publishing their randomised controlled trial of probiotics in the prevention of atopic eczema in the first 2 years of life, have placed probiotics in the forefront of the minds of allergists and paediatricians, and provided the first tangible and apparently effective intervention to stem from the "hygiene hypothesis".[1] At first sight it seems somewhat surprising that administering a rather innocuous Gram positive bacterium (*Lactobacillus* GG) in infancy could influence the natural history of eczema. However, the biological plausibility behind this study lies in the potential of probiotics to stimulate the infant's immune system and to reduce oral allergen exposure through effects on the gut.

HYGIENE AND ALLERGY

Atopic disease and infections

The concept of an inverse relationship between infection and allergic disease was first suggested by Gerrard in 1976. A number of previous studies described communities with a high prevalence of helminth infections and raised total IgE but little atopic disease.[2] Gerrard first suggested that bacterial and viral infections might also protect against allergic disease.[3] In a study in Saskatchewan he observed the low prevalence of asthma and allergic disease among Metis Indian children compared with white children living in the same region and concluded:

"*It is suggested that atopic disease is the price paid by some members of the white community for their relative freedom from diseases due to viruses, bacteria and helminths*."

J Crane
Wellington Asthma Research Group, School of Medicine and Health Sciences, Wellington, New Zealand;
crane@wnmeds.ac.nz

Atopic disease and household size

This idea seems to have been forgotten for a decade or so. Its re-emergence and epidemiological delineation by David Strachan, together with plausible immunological support, has come to be known as the "hygiene hypothesis". The first associations from a large cohort showed that the prevalence of hay fever and eczema were inversely related to household size. It was proposed that in large households, with close contact with older siblings, there would be increased infection

which promotes a Th1 profile and reduces subsequent allergic disease in younger siblings.[4] The initial hypothesis was based on self-reported atopic disease but was soon confirmed for atopy, measured by skin prick testing.[5] Matricardi *et al*[6] found a clear inverse association between family size and atopic sensitisation to common allergens with a 3% decrease in prevalence for each additional sibling in 11 000 Italian army recruits. In a study of pregnant women Strachan *et al*[7] showed that self-reported inhalant allergy was inversely associated with the number of brothers, but not sisters. Many studies have subsequently confirmed a relationship between family size and allergic disease.[8 9] However, it has been suggested that postnatal infection may not explain this association. For example, Sunyer *et al*[10] noted an independent inverse relationship between maternal atopy and parity, suggesting that increasing parity may have some direct effect on maternal atopy and, in turn, on the atopic status of the offspring. Karmaus *et al*[11] have shown that cord IgE decreases with increasing parity, and suggested that parity may influence fetal programming. Presumably the same mechanisms could be invoked to explain both of these results.

It thus remains to be determined to what extent the protection from allergic disease afforded by large households is related to hygiene. Regardless of the mechanism, the inverse association between household size and atopic disease is the most consistent feature of the hypothesis. A recent literature review of 53 studies showed a protective effect of household size in all 17 studies of hay fever, nine of 11 studies of eczema, 14 of 16 studies of atopic sensitisation, but only 21 of 31 studies of asthma.[12]

Atopic disease and specific infections

Inverse associations between atopy or atopic disease and specific infections have been noted. These include measles in West Africa,[13] *Mycobacterium tuberculosis* exposure in Japan,[14] and hepatitis A and other orofaecal infections in Italy.[15] A protective effect of malaria has also recently been suggested.[16] For all of these associations alternative arguments have been constructed around increased susceptibility to infection or greater severity of infection, consequent upon being atopic.[17 18] One logical extension of these studies was to examine the effects of antibiotics in early childhood. If infections in early life protect against atopy, then antibiotics in early life might increase the risk, assuming that bacterial infections are protective. As most antibiotics are given for viral infections, this seems unlikely. A more plausible explanation is that antibiotics alter bowel flora and might reduce critical Th1 promoting mechanisms. A number of studies have examined this relationship and have shown positive associations between exposure to antibiotics and the development of asthma, atopy, or atopic disease.

Atopic disease and antibiotics

Farooqi *et al*[19] showed, in a large general practice birth cohort, that children who had received antibiotics in the first two years of life were at increased risk of doctor diagnosed asthma, hay fever, and eczema. They were also able to show that this effect was independent of the number of GP visits, was greater for broad spectrum antibiotics, and was evident regardless of the indication for the antibiotic treatment. The effect was, however, strongest for antibiotics prescribed for respiratory conditions. Among children at a Rudolf Steiner school—a population less likely to engage in the full panoply of orthodox medicine—those exposed to antibiotics in early life were significantly more likely to report the symptoms of asthma in later childhood.[20] The risk was greater for exposure in the first year of life, and showed a dose response relationship for the number of courses of antibiotic treatment. Alm *et al*,[21] in a cross sectional study of Rudolf Steiner families in Sweden, showed that children adopting an anthroposophic lifestyle—which included less antibiotic use, less childhood immunisations, and a diet much higher in naturally fermented vegetables—had significantly less atopy, as measured by skin prick testing or RAST, than children living in families in the same region who did not adopt this lifestyle. They were not, however, able to tease out the separate effects of individual aspects of the anthroposophic lifestyle. Droste *et al*[22] found a positive association between antibiotics in the first year of life and reported asthma, hay fever, eczema and atopic sensitisation, which was confined to children with a family history of atopic disease. A recent historical birth cohort study has also identified antibiotic use, but not infections, as a risk factor for subsequent allergic disease, although after adjustment for consulting behaviour this remained significant only for a diagnosis of asthma.[23] Mechanistic support for these studies comes from Oyama and colleagues who have recently shown in mice that kanamycin raised serum levels of IgE and IgG1 and, in vitro, enhanced stimulated spleen cell secretion of interleukin (IL)-4 and reduced interferon (IFN)γ secretion, thus demonstrating a perturbation of Th1/Th2 cell maturation.[24]

The authors of these studies have pointed out that such retrospective associations cannot entirely exclude reverse causation as an explanation. A recently published prospective study in children with a family history of asthma or allergy found no association between antibiotic treatment in the first year of life and a diagnosis of asthma, hay fever or eczema, or a reported history of wheezing in children by 5 years of age. An association between antibiotics and transient wheezing (which may not reflect allergic respiratory disease) disappeared when wheezing associated antibiotic prescription was removed from the analysis, suggesting that response bias and reverse causation are more likely reasons for previous associations.[25] Subsequent associations between early antibiotic use and atopy when these children are older will be of interest. In contrast, Farooqi *et al*[19] had noted that, when individuals who had atopic disease before antibiotic administration were removed from their analysis, antibiotic treatment still predicted subsequent atopic disease. They defined wheezing before the age of 2 years as wheezy bronchitis and wheezing after 2 years as asthma, and found that the association between antibiotics and wheezing after 2 years of age was stronger than between antibiotics and wheezing before 2 years. On balance, however, the positive association between early antibiotic exposure and subsequent allergic disease is probably explained by reverse causation.

The association between early antibiotic exposure and atopic sensitisation or atopic disease illustrates the problems and complexity of trying to establish causal relationships in this area. Case ascertainment relies on reported symptoms, and a diagnosis often relies on the same reported symptoms. In early childhood, recurrent wheezing is often unrelated to atopy.[26] Antibiotics are likely to be given for some of these symptoms and are also likely to be prescribed for viral conditions that may themselves protect against the subsequent development of atopy.[27] Prospective studies from birth to adolescence are required or, alternatively, a randomised controlled trial of minimal antibiotic use during infancy in a general population sample.

Atopic disease and farm exposures

Another facet of the hygiene hypothesis relates to farming. The recent demonstration of the protective effect of a farm environment in early childhood has centred on animal and

stable exposures. This protection might be conferred by increased microbial exposure,[28] possibly mediated by inhalation of microbial products such as endotoxin from Gram negative organisms and peptidoglycans from Gram positive organisms, both of which are found in high concentrations in animal sheds.[29] These studies have also shown protection from early exposure to farm milk, an effect that we have recently confirmed in children living in rural New Zealand who were afforded protection against eczema, current allergic rhinitis, and wheezing by the consumption of unpasteurised milk and regular yoghurt in early life.[30] Thus, microbial exposure via the gastrointestinal tract may also explain part of the protection observed in farm exposed children. In the European studies the principal protection appears to be against hay fever and sensitisation to seasonal allergens rather than house dust mite or cat allergens. The exposures do not appear to protect against eczema and protect to a lesser extent against asthma.[31] The protection afforded for asthma appears independent of atopic status.[28] Exposure to farm milk appears to be as protective as exposure to farm animals, although the greatest protection appears to be when animals and farm milk exposures are combined.

Back to Blackley: hygiene or tolerance?

Protection from hay fever in farming communities was first described by Charles Blackley 130 years ago[32]:

"*One very curious circumstance in connection with hay fever is that persons who are most subjected to the action of pollen belong to a class which furnishes the fewest cases of the disorder, namely, the farming class. This remarkable fact may be accounted for in two different ways: it may on the one hand be due to the absence of the predisposition which mental culture generates; or, on the other hand, it may be that in this disease there is a possibility of a patient being rendered insusceptible to the action of pollen by continued exposure to its influence. If this latter hypothesis be correct it shows that, in one case at least, the enjoyment of health does not merely depend upon the presence of a high state of vitality, but also, to some extent, upon the acquisition of a certain degree of insusceptibility to the action of the exciting cause*."

A key question remains the one posed originally by Blackley. Does the lower prevalence of hay fever in farmers reflect a form of tolerance to allergens or some other protective effect? Blackley suggested a "mental" predisposition to hay fever among the educated classes in whom it was much more common. This would now be interpreted as a socioeconomic effect, which has been observed in a number of studies[33 34] and can now be re-cast in terms of the hygiene hypothesis—that is, decreased exposure to microbes or infection explaining the higher prevalence of hay fever among higher socioeconomic groups.[35 36] The alternative explanation is that the increased exposure to seasonal allergens induces tolerance. It was Blackley's observation that led Noon[37] to examine inoculation against hay fever with pollen in 1911, a therapeutic intervention still in practice today. Further evidence for such natural tolerance (surprisingly taking 128 years to surface) has recently been found in association with high levels of exposure to cats. Platts-Mills and colleagues[38] suggest that this state of tolerance may occur in the form of a modified Th2 response involving allergen specific IgG4, with no specific cat IgE and no allergic symptoms to cats. A similar finding in the occupational setting has been observed for exposure to pigs and laboratory animals.[39] There is also some evidence to suggest that allergen immunotherapy in monosensitised subjects can reduce further new sensitisation,[40] raising the possibility that tolerance to one or more seasonal allergens might reduce subsequent sensitisation to other allergens. Perhaps the protection afforded by early farm exposures is associated with both mechanisms, involving a complex interaction between allergen and microbial exposures with dose, timing and underlying genetic factors all being important.

While the hygiene hypothesis has spawned many tantalising associations, aspects of it do appear to involve the bowel. It is through this organ that the first exposures to foreign proteins occur and eczema is often the first manifestation of an allergic response to them. It is with this in mind that Kalliomäki and colleagues in Finland progressed to their probiotic intervention. Probiotics have a long history in food preparation and are normal commensals of the human bowel, particularly in the first few weeks of life.

Fermented foods

The introduction of fermented milk products to Western Europe in the form of yoghurt, and scientific interest in their health giving properties, were largely due to the interest of Ilya Mechnikov in the early 1900s. Following his studies on phagocytosis at the Pasteur Institute, for which he shared the Nobel prize in 1908, Mechnikov became interested in the subject of longevity. In his book[41] he argued that the reduction in human lifespan was the result of putrefaction in the bowel. He reasoned that the consumption of putrefied foods, and further putrefaction in the bowel, could cause acute disease and death. Milk products, on the other hand, naturally produce a self-preserving acid environment through the action of lactic acid producing bacteria. Soured milk is mentioned in the bible, had been used in Egypt since antiquity, and was consumed frequently in parts of Eastern Europe where it was known as "yahourth". Mechnikov associated the large number of centenarians found in parts of Eastern Europe with their diet which commonly comprised large amounts of fermented milk. His thesis rested on replacing the putrefying organisms in the bowel with lactic acid producers, noting as he had that they were able to overgrow and keep in check many pathogenic organisms. By this means he proposed that lifespan might be increased by consuming yoghurt. Mechnikov was also aware that the newborn infant's meconium filled and sterile gastrointestinal tract rapidly becomes colonised almost exclusively by lactic acid producing bifidobacteria, first discovered by Tissier in 1900,[42] under the influence of breast milk. While his bacterial theories of longevity and the bowel flora of centenarians have not progressed since, fermented milk products have progressively entered the western diet and there has been continuing interest in colonisation of the infant bowel.

Bowel flora

A number of studies have confirmed the predominance of bifidobacteria in the bowels of healthy infants in the first weeks of life.[43 44] In 1976 Bullen *et al*[45] confirmed high counts of bifidobacteria associated with exclusive breast feeding, but much lower counts in bottle fed infants. In bottle fed infants, or when supplementary cow's milk was given, *E coli* and *S faecium* were commonly found together with clostridia, *Proteus* species, and *Pseudomonas aeruginosa*.[45] These authors confirmed the high pH (8–9) of bottle fed infant faeces compared with the acidic fermenting faeces (pH 5–6) of breast fed infants. This predominance of bifidobacteria associated with exclusive breast feeding is thought to be at least one reason why breast fed infants are protected against gastroenteritis.[46]

Interestingly, subsequent studies of infant bowel flora have failed to show this exuberant colonisation with bifidobacteria in exclusively breast fed infants.[47 48] Hall *et al* speculated that

this change in early gut colonisation with bifidobacteria might be related to changes in maternal diet or changing obstetric practices. In a group of preterm infants these authors showed that treatment with antibiotics or being nursed in an incubator significantly reduced early colonisation with lactobacilli and bifidobacteria. In both term and preterm infants birth by caesarean section was also associated with much lower counts of these organisms.[48] The clinical significance of these findings is unclear, though one recent study has suggested an increased risk of asthma and a non-significant trend for increased atopic sensitisation in children born by caesarean section compared with vaginal delivery.[49] Possible associations between bowel flora and atopy or eczema have been suggested by Björkstén and colleagues in one small study of Swedish and Estonian infants during the first year of life.[50] Allergic infants had fewer enterococci and bifidobacteria and more clostridia than non-allergic infants. Quantitative studies comparing bowel flora in 1 year old Swedish and Estonian infants showed more clostridia in Swedish infants and more lactobacilli in Estonian infants; bifidobacteria were found equally in both groups.[51] The authors suggested that these differences might reflect more generalised changes in bowel flora in Western industrialised countries which, in turn, might be associated with an increased prevalence of atopy and atopic disease.

While such associations are extremely tentative, they are broadly consistent with the studies cited previously which suggest that early bowel colonisation with lactose fermenting organisms in exclusively breast fed infants may have decreased over time, possibly as a result of changes in maternal diet, obstetric hygiene, methods of delivery, and the use of antibiotics in early life. In essence, changes in bowel flora in infancy might represent another facet of the hygiene hypothesis, and restoring these probiotic organisms to the early bowel might exert a protective effect.

Probiotics

The term "probiotic" was originally introduced to describe factors produced by protozoa that promote the growth of other protozoa.[52] By 1989 the term had been refined to refer more specifically to "a live feed supplement which beneficially affects the host animal by improving its microbial balance".[53] Among the first examples of a therapeutic application of such a supplement were faecal enemas given from healthy individuals to patients with pseudomembranous colitis.[54] A similar approach was used in chicks to prevent *Salmonella* infection.[55] In terms of readily available live organisms for human consumption, they have been largely confined to organisms that are naturally found in the healthy human gastrointestinal tract or that have been used historically in food preparation. These are predominantly the lactose fermenting organisms (LABs) that occur naturally in milk and on vegetables and are used to ferment and thus preserve a variety of human foods. The term "probiotics" has thus come to refer to these organisms in particular.

Until very recently advocacy for the use of probiotics in human health and disease, with one or two exceptions, was largely confined to complementary rather than conventional practice. An obvious application, and one with a reasonable evidence base, is in the management of acute bacterial, viral, and antibiotic associated diarrhoea. In viral diarrhoea lactobacilli have been shown to reduce duration of symptoms and viral shedding,[56] and a recent meta-analysis of nine studies showed an overall benefit of lactobacilli in the treatment of childhood diarrhoea.[57] There has also been some interest in the use of probiotics as an adjunct to eradication of *Helicobacter pylori*.[58] Anti-cancer properties of probiotics have also been suggested from animal and epidemiological studies. Enhanced uptake and metabolism of nitrites in the gastrointestinal tract (thereby reducing their availability for conversion to nitrosamines) and enhancement of immunological effects are postulated mechanisms by which such properties might be mediated.[59]

Probiotics and immune effects

Probiotics appear to have effects on both innate and acquired immunity. In vivo immunological effects of orally administered LABs show enhancement of murine macrophage phagocytic activity.[60] Immunological interest in LABs has focused on the cell wall and its two principal components, glycans and teichoic acid, the latter being unique to Gram positive organisms. Glycans consist of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid units. These sugars, which are linked to peptides (peptidoglycans), are polymerised at the cell surface to form relatively thick osmotically resistant envelopes.[61] It is this greater thickness that retains the Gram stain. Lysozyme breaks the linkage between the sugar building blocks, thus destroying the wall structure and lysing the cell. Further breakdown of peptidoglycans by lysozyme yields a muramic acid dipeptide fragment, muramyl dipeptide (MDP), which has independent immunological activity. LABs are particularly sensitive to lysozyme digestion.[59] Both MDP and teichoic acid have demonstrated Th1 immune stimulating properties, with increased production of IL-1, IL-6, and tumour necrosis factor (TNF)-α from a variety of cells.[62] Stimulation of human peripheral blood mononuclear cells by LABs leads to increased Th1 cytokine production and gene upregulation.[63] The pattern of cytokine upregulation is not, however, quantitatively or qualitatively uniform among LAB strains, and some strains have even demonstrated inhibition of Th1 cytokines. Such evidence suggests that the complex milieu of bowel flora can lead to competing driving forces on T helper systems.[64]

While there is evidence of in vitro immunological effects of LABs and their cell wall products, there is less evidence that this translates into distinct immunological benefits in vivo. The few studies that have been reported have usually involved live LABs in fermented milk products in the form of yoghurt. Yoghurt can increase specific IgA to attenuated *Salmonella typhi* and increase total serum IgA.[65] Large quantities of yoghurt daily (450 g) increased IFNγ from T cells in young adults,[66] but 200 g daily did not.[67] A randomised crossover study of 20 adults with a history of atopic disease showed no effect of 450 g yoghurt daily (made from *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) on cellular, humoral or phagocytic function.[68] A similar dose containing *Lactobacillus acidophilus* given to 15 adults with moderate asthma reduced eosinophilia and increased IFNγ from stimulated lymphocytes, but had no effect on IL-2, IL-4, IgE, clinical parameters, peak expiratory flow, or spirometric measurements.[69]

Probiotics and allergic disease

Against this rather unpromising clinical background for allergic disease come the studies from Kalliomäki and colleagues. The provenance of their studies lies in their interest in childhood infectious diarrhoeal disease, food allergy, and eczema.[70] They noted, as others had, that LABs can shorten the duration of diarrhoea[71] and increase rotavirus specific IgA,[72] and that this effect was dependent on live bacteria.[73] Experimental rotavirus infection of normal and germ free mice increased intestinal permeability, and the extent and timing of this increased permeability was altered by intestinal microflora.[74]

Cow's milk protein is one of the first foreign proteins encountered in infancy. Infants who become allergic to cow's

Learning points

- Alterations in the composition of bowel flora in early life may increase or decrease the risk of developing atopic disease
- Antibiotic exposure in early life may alter bowel flora, but the positive association with subsequent atopic disease probably reflects reverse causation
- Exposure to organisms in unpasteurised milk may partly explain the protective effect of a farming environment in early life on the risk of atopic disease
- Probiotics are lactose fermenting bacteria which occur naturally in the human gastrointestinal tract, in milk, and on vegetables and may have beneficial effects on infant immune function
- Changes in maternal diet and obstetric practice and reductions in exclusive breast feeding may have contributed to the rise in atopy by reducing beneficial probiotic colonisation of the infant bowel
- Recent studies suggest that the administration of probiotics may have a useful role in the prevention and treatment of infant atopic eczema, but probably not atopy or other atopic diseases

milk develop eosinophilic intestinal inflammation and increased gut permeability.[75] This inflammation and increased permeability can be reversed by the addition of LABs to their diet.[76] LABs have also been shown to enhance gut IgA responses to milk proteins in animal models.[77] Recently, Isolauri *et al* showed a significant improvement in atopic eczema in infants receiving two probiotic strains of bifidobacteria and lactobacilli.[78] These studies in animal models and in infants, showing beneficial effects of LABs on viral gut infections and on allergic dermatitis in food allergic infants, set the scene for a primary allergy prevention study using probiotics. By concentrating on allergic eczema in infancy (a condition in which the bowel and its flora are likely to be very important), and by administering the organisms directly rather than as a fermented food (allowing much greater numbers of organisms to be reliably administered), they maximised their chances of demonstrating an effect. As Murch[79] has noted, the results were remarkable with a halving of the risk of developing eczema in the first 2 years of life among a group of infants at increased risk of developing allergic disease. However, there was no reduction in positive skin prick tests or specific IgE by 2 years and, surprisingly, there was no reduction in severity of disease in those children who developed atopic eczema.[1]

There is a need to replicate these studies to confirm if probiotics can prevent and modify allergic eczema. Similarly, trials of probiotic supplementation in established allergic rhinitis and allergic asthma are worth undertaking, although it appears less likely that they will have a beneficial effect on these conditions. The benefits may be confined to allergic eczema in infancy and have very little to do with the longer term development of atopy or other atopic diseases, reflecting local gastrointestinal effects rather than any significant systemic immune effects. Administration of probiotics is straightforward, does not appear to be associated with any obvious adverse effects in otherwise healthy individuals, and is easily studied. Should the benefits be confirmed, then it would be important to determine which organisms provide the best response; the optimum dose, timing and duration of treatment; and whether maternal exposure is important. These questions are of practical importance given that probiotics are readily available over the counter and are likely to be administered to infants by parents with a history of atopic diseases. A much greater challenge will be to try and unravel the in vivo effect of probiotics on immunity, and the relationships between the developing immune system, the gut, and its flora.

In a wider sense, the various streams of the hygiene hypothesis and its most recent extension to the gut and possible interventions with probiotics emphasise the importance of carefully defining disease outcomes and their relationship with atopy. In the study by Kalliomäki *et al*[1] probiotics appeared to reduce the incidence of eczema at 2 years but not atopic sensitisation or the severity of new eczema. Yet, paradoxically, probiotics appeared to reduce the severity of established atopic eczema in other studies. In the European studies of children brought up in a farm environment, exposure to farm milk in the first year of life protected against grass pollen sensitisation and hay fever, but not eczema. For asthma, farm exposure appears to protect against both allergic and non-allergic asthma, and for all of these conditions prenatal exposure may be more important than postnatal exposure. While it is premature to encourage the widespread consumption of probiotics by mother and child, or to encourage frequent farm holidays in central Europe during pregnancy and early childhood, this fascinating corner of the hygiene hypothesis is clearly worthy of further exploration.

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