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REVIEW

Protective parasites and medicinal microbes? The case for the hygiene hypothesis

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Summary The incidence of allergic conditions continues to increase world wide. The underlying mechanisms and in particular the causes are however poorly understood. This article presents the evidence for the hygiene hypothesis which has been proposed in the debate on the causation of allergic disease.

Introduction

Since the coining of the term ''allergy'' by the Austrian Baron Clemens von Pirquet in 1903 the incidence of allergic conditions has risen dramatically [1,2]. In particular, the last four decades have seen a steep increase in atopy, hayfever, eczema and asthma [3]. With it has come an increased research effort and much is known, often in great detail about underlying physiological and immunological mechanisms. Knowledge about the underlying cause(s) however, remains elusive.

Genetic risk is without doubt important, but it alone cannot explain the rapid rise in the incidence of allergic disease. The attributable genetic risk has been estimated at between 35 and 80% for asthma and hayfever, and up to 72% for eczema [4,5]. Numerous genetic markers have been linked to asthma and atopy, and insight into the genetic and molecular mechanisms is helpful in the understanding what makes some individuals more susceptible to the development of allergic disease than others [6—8]. But, if some individuals are genetically more susceptible—what is it they are more susceptible to?

The answer must lie in our environment and associated lifestyle, both of which have undergone rapid and profound changes in the last century [9,10]. The so-called ''western and industrialised world'' tends to have smaller family size, cleaner living conditions, highly processed and sterilised food, with additives and altered nutrient content. Interestingly, the so-called ''developing world'' appears to be following suit now: parts of the population are moving to the cities to a ''better life'' (cleaner, treatment of infections) and it is becoming apparent that this is associated with a rise in allergic disease in these individuals [11—13]. Similarly, migrants from developing countries to developed countries show an increase in allergic disease [14].

It was not until Strachan published his observations on the effect of family size on the development of hayfever, that the ''hygiene hypothesis'' took shape [9]. He proposed that it might be the decrease of infections in childhood (a consequence of

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cleaner living conditions and less contact with other children) which could be seen as the loss of a protective factor, thus allowing the increasing development of atopic disease. When Strachan reviewed his original hypothesis a decade later with all the newly emerging immunological and epidemiological research findings, he stated in his conclusion:

> ''10 years have not changed my view that infections remain the most promising candidates for the underlying protective factor'' [15].

The hygiene hypothesis was quickly put to the test by scientists worldwide and a possible immunological explanation proposed, suggesting an imbalance between ''allergic'' and ''non-allergic'' inflammation, mediated by different types of T-helper (Th) cells and characterised by certain cytokines [16]. Type 1 T-helper cell (Th-1) inflammation was seen as the predominant response in non-allergic individuals, occurring also during acute infections. Type 2 T-helper cell (Th-2) inflammation was seen as the predominant response in allergic disease and also identified as the physiological immunological state at birth [17]. This ''Th2/Th1 Paradigm'' was linked with the hygiene hypothesis with the following reasoning:

At birth the overall physiological immune response is of the Th-2 type which is thought to be the foetal default response to protect the pregnancy [17]. The neonatal/infant immune system then requires a certain microbial pressure, such as childhood infections, to allow normal maturation of immune responses towards a Th-1 weighted response. If environmental microbial pressure is insufficient, the T-helper cell response remains skewed towards a Th-2-type rather than Th-1-type response, thus favouring the development of atopy. This fairly straightforward Th1/Th2 paradigm provided an initial immunological explanation of how decreased microbial pressure (clean living, small families, fewer infections) could delay maturation of the immune system, leaving it ''locked'' in the atopy-friendly Th2-state.

However, the Th2/Th1 paradigm could not be upheld in this relatively straightforward form. Since it was first proposed the following findings have confused the issue:

1. A strong Th2 response, as seen with intestinal parasite infection, can paradoxically be protective of atopy.
2. Th1 mediated autoimmune diseases (e.g. type 1 diabetes, Crohn's disease) are increasing at a similar rate to allergic diseases and share many of the epidemiological observations (small family size, sibship order, etc.) and thus have to be considered in light of the hygiene hypothesis as well.
3. Both, allergic and autoimmune disease can occur in the same patient thus making an isolated disturbed balance between Th1 versus Th2 less likely.
4. Th2 responses can be ''modified''—leading to tolerance instead of expected allergy (e.g. exposure to high levels of cat allergen can lead to tolerance instead of allergy) [18].

Although the precise immunological scenario is still incompletely understood, further work now indicates that within the complex web of immune-interactions it may be regulatory T-cells, which are affected by or susceptible to environmental stimuli [19—21]. The lack of a balanced T-regulatory response secondary to altered or reduced infectious/microbial stimuli, may be what allows exaggerated responses of the Th1 and/or the Th2-type. Fig. 1 summarises a proposed simplified mechanism; it is beyond the scope of this review to go in to the details of cytokine interactions, feedback mechanisms, etc.

How does the replacement of the simple Th2/Th1 paradigm with a more complex concept fit in with the hygiene hypothesis? The hypothesis simply encompasses a theory on how the environment, more specifically the exposure to microbes, interacts with the individual's genetic and immunological background. It is not affected by the changing theories on the underlying immunological mechanisms. Table 1 shows the main factors associated with atopic and autoimmune diseases. The evidence supporting the hygiene hypothesis with respect to the development of allergic disease (including allergic sensitisation, allergic asthma, hayfever, eczema, food allergies) will now be discussed in more detail.

Epidemiological

The increase of atopic conditions is most prevalent in societies with a westernised, industrialised lifestyle [15]. It has been associated specifically and consistently with decreasing family size, small sibling number, birth order and high socio-economic status—all of which reflect increased cleanliness, eradication of infections and a modern diet [22—24]. These factors are not likely to have a direct effect on the developing immune system, but should be considered as an indirect marker for an underlying change, which could for example reflect a change in the microbial exposure in infancy and childhood [15].

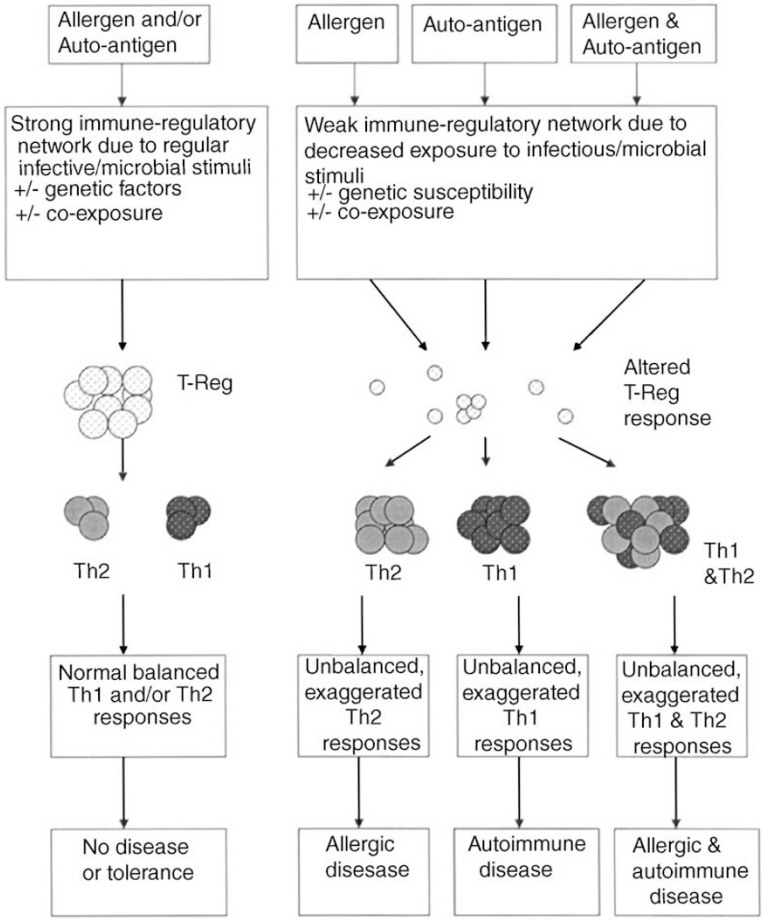

Figure 1 Simplified diagram of how absence of microbial stimuli could affect the immune response and facilitate the development of disease.

Table 1 The main factors associated with atopic and autoimmune diseases

	Atopic disease	Auto-immune disease
Epidemiological findings		
Decreasing family size	⇑	⇑
Number of older siblings	⇑	⇑
High socio-economic status	⇑	⇑
Decreased day-care exposure	⇑	⇑
Evidence of cleaner houses		⇑
Evidence of previous oro-faecal infection (as a marker for poor hygiene)	⇓	?
Higher frequency of viral ''cold'' in early childhood (parentally reported)	⇓ ⇔	?
Environmental measurements		
High endotoxin exposure (e.g. on farms)	⇓	?
GI-flora		
Decreased Lactobacilli, Bifidobacteria	⇑	?
Supplementation with *Lactobacillus* GG	⇓	?
Increase in Clostridia (esp *C. difficile*)	⇑	?
GI-parasite infection		?
Active/chronic infection	⇓	
Treatment of parasite infection	⇑	

''And I didn't like it, and I didn't inhale'' (Bill Clinton, in Washington Post, 30.03.1992, p A1) [25].

Are we missing an inhaled protective factor?

Viral infections

Airborne viruses have been investigated in the context of allergy development, as they tend to elucidate Th1-type responses and were thought to provide a counterbalance to those of the Th2-type.

Several studies have indirectly shown a protective effect of frequent ''viral colds'', although the overall evidence is wholly inconclusive and the underlying immunological mechanisms not clear [15,22,26,27]. A protective effect of measles infection was suspected, but could not be conclusively proven [15,28—30].

Endotoxin

Endotoxin is a bacterial cell wall product (Lipopolysaccharin LPS), which has been implicated by several large studies as a potential protective factor. Braun—Fahrlander et al. showed in a large cross-sectional study of school children, that those who grew up on farms, spending time in the stables, had a significantly lower incidence of atopic sensitisation, hayfever and asthma [31]. The degree of protection correlated with the degree of endotoxin exposure measured in mattresses [32]. Other groups have found evidence supporting this observation, although findings are not always straightforward (e.g. Litonjua et al. found some protective effect of endotoxin on the development of wheeze in later childhood but observed increased wheeze in infants exposed to high levels) [33,34]. A German birth cohort designed to measure the effect of endotoxin from birth (LISA study) had initially shown that endotoxin decreased the risk for atopic dermatitis at age 6 months [35]. Recently the authors demonstrated, however, that children whose parents are atopic have an increased risk of sensitisation to inhalant allergens if exposed to high levels of mattress endotoxin [36]. Physiologically it is not obvious either, how inhaled endotoxin should be producing the protective effect. It is known to cause inflammation in the lung after inhalation, associated with increased mucosal permeability, thus theoretically making it easier for allergens to come into contact with the immune system [37]. It can exacerbate existing asthma as well as cause increased wheezing in infancy [34]. Could it be that endotoxin just reflects another factor or marks the presence of a co-factor, which is either inhaled or ingested?

''His mouth had been used as a latrine. . . ''—is it protection via the gut? (Kingsley Amis, in Lucky Jim 1953, Chapter 6)

In contrast to the respiratory route, the oral route of exposure to microbes appears more promising. Matricardi et al have used serological evidence of previous infection with the hepatitis A virus (HAV) and/or *Toxoplasma gondii* (both transmitted via the oro-faecal route) as a marker for poorer levels of hygiene [38]. They found that previous infection with HAV and/or *T. gondii* was protective for atopy and hayfever [39]. They also showed that family size and birth order (the more older sibs the better) decreased the risk for atopy and hayfever [40]. This protective effect has also been shown using data from the United States NHANES III Survey involving >30,000 individuals age 1—90 years [41]. In this study they could for the first time demonstrate, that the protective effect extended to a food allergen (peanut) strengthening the theory that HAV and *T. gondii* have an effect on gastrointestinal (food) allergy development, be it direct or indirect. Linneberg et al. found the same protective effect of HAV and *T. gondii* in the Danish Copenhagen Allergy Study [42]. They showed in addition, that it was the serological evidence of previous systemic, but relatively asymptomatic oro-faecal infections which was associated with a protective effect, whereas food-borne pathological gastrointestinal bacteria such as *Campylobacter jejuni*, *Yersinia enterocolitica* and *Clostridium difficile* were associated with a higher prevalence of atopy. We have previously shown that wheezy, atopic infants had significantly higher serum IgG levels for *C. difficile* compared to age-matched non-atopic, non-wheezy infants. These findings further emphasise that it may be differences in the intestinal microflora, which are involved in the development of atopic disease [43].

Gastrointestinal bacteria—further evidence from the Micro(be)-Cosmos

The gastrointestinal tract has come under immunological scrutiny at a surprisingly late stage, considering the size of its mucosal surface allowing potential interaction between the immune system

and the ingested and commensal environment. Its vital role in the maturation of the immune system is now well documented and it is certainly a place where the hygiene hypothesis can be tested (and supported) further.

Colonisation of the gastrointestinal tract by bacteria is a pre-requisite for the normal development of systemic as well as local mucosal immune responses [44,45]. Absence of bacteria is associated with impaired immune responses and a predominance of Th2 type responses. The latter has been shown to allow allergy rather than tolerance to develop. Introduction of a GI microflora on the other hand has been shown to have a protective effect and to allow tolerance to develop [44]. These experiments have been carried out in rodents, however, the role of the GI-microflora in the development of allergic disease in humans has been studied to some extent now, with the following key findings:

1. Different GI-flora between Estonian (living conditions similar to Sweden in 1960s) and Swedish children. More Lactobacilli in Estonian children, less atopy in Estonian children and vice versa in Swedish children [46,47].
2. Different GI-flora between allergic and non-allergic children (allergic: less Lactobacilli, less Bifidobacteria, more Clostridia) [47–49].
3. Different GI-flora between neonates who went on to develop allergy versus those who didn't [50].
4. Fewer Lactobacilli and/or Bifidobacteria associated with manifest allergic disease [51].
5. Evidence that *C. difficile* increased in individuals with allergic disease [42,43].
6. Supplementation with Lactobacilli can improve atopic dermatitis and food allergy and even prevent development of atopic dermatitis in some children [52–54].

Overall, these findings present convincing evidence that the colonisation of the GI tract with bacteria is crucial to immune development and that the presence or absence of certain bacteria is linked to allergic disease. The latter fits in nicely with the hygiene hypothesis, as previously (in a more unhygienic life) Lactobacilli used to be consumed more in fermented foods and then were eradicated form our diet. They also respond poorly to antibiotic treatment and can be replaced by *Clostridia* species, which are associated with increased allergy.

How exactly the GI-flora influences the systemic and local immune response is however still poorly understood.

Parasites—is it a case of ''the worms have turned''?

Observations that parasite infections appear to suppress atopy (i.e. sensitisation) were first made 20 years ago [55]. Some researchers then investigated whether it could work the other way, i.e. atopy be a protective factor with regard to intensity of parasitic disease [56,57]. Recently, the influence of parasite infections on the incidence of allergic disease has been receiving more attention again, with studies in Ethiopia and Le Gambia as well as Taiwan showing, that parasite infestation is associated with decreased atopic sensitisation and in some cases decreased incidence of clinical allergic disease [13,19,58,59]. Similarly, these observations are now being reported in some autoimmune diseases such as Crohn's disease, ulcerative colitis and in a mouse diabetes-model [60–62]. In the case of Crohn's disease and ulcerative colitis helminth ova (Trichuris) have actually been used in a small pilot study to treat the condition with some success [63]. The overall proposed mechanism of protection is thought to be immune stimulation of the regulatory T cells by the parasite as shown in the highly simplified diagram in Fig. 1 (for recent review on how helminths do it—see Nature Immunology Reviews 2003 article) [21]. These studies present some intriguing evidence, in support of the hygiene hypothesis: exposure to worms may be protective of allergic and some autoimmune conditions.

Conclusion

There is ample evidence to support the hygiene hypothesis. Our immune system appears to require the interaction with microbes that we have traditionally labelled with the ''YUK-factor'' and tried to avoid as best as we could. Of course we cannot return to the times of poor hygiene with high infant and child mortality due to severe infections, but we certainly need to continue to aim for a better understanding of the underlying mechanism. We need to elucidate not only which microbial stimuli are best, but when they are required, at what dose and for which duration(Fig. 2). The role of genetics and co-exposures needs to be clarified. In particular the gastrointestinal tract deserves more detailed and larger scale research attention. To achieve this objective, we require large, well-designed, longitudinal birth cohort studies with adequate collection of biological and environmental samples.

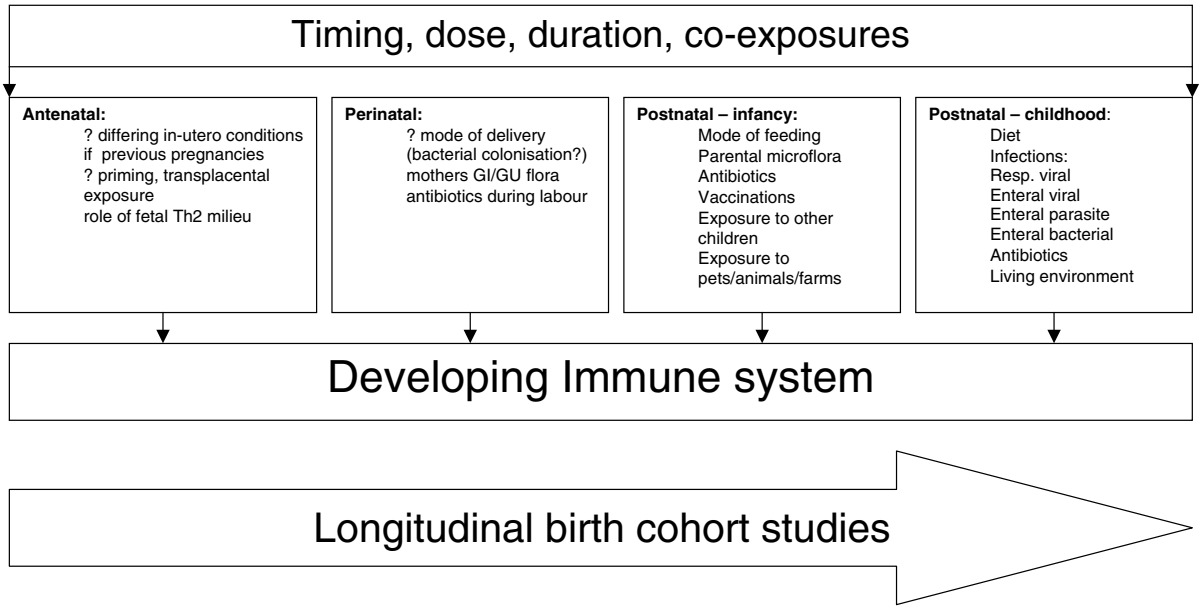

Figure 2 Outline of factors which affect the developing immune system at certain timepoints.

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