

The gastrointestinal flora and the skin – is there a link?

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Introduction

The reasons for the large increase in the prevalence of allergic diseases since the Second World War are unknown. The environment has undergone major changes during these years. None of the numerous suggested risk factors, e.g. air pollution, poor indoor climate and exposure to allergens can more than marginally explain the variations in allergy prevalence over time and in space. Interest has therefore been devoted to the possibility that protective factors have been lost in industrialised countries with a market economy.

For many years it was thought that infections are potent risk factors for the development of sensitisation and the induction of allergies. In recent years, the general validity of this relationship has been questioned in the light of novel research. Thus, there is now more or less consensus that the maturation of the immune system after birth is largely dependent on microbial stimulation. As a consequence, infections would under certain conditions actually protect against allergic diseases.

Immunological background

Normal pregnancy in mammals, including man, is accompanied by an immune deviation towards Th2 type immunity (1). As a consequence, the immune system of the newborn baby is also skewed towards Th2-type immunity. The post-

natal maturation of the immune system is characterised by the development of a balanced Th1/Th2 immunity. The efficiency and kinetics of this process is to some extent genetically determined, but it seems also to be modified to a considerable degree by the environment (2).

The postnatal maturation of T cell function may be particularly slow in atopic children. As early as 1978, Strannegård had already reported that children with atopic dermatitis have lower numbers of lymphocytes forming rosettes with sheep erythrocytes (3). Since then, numerous studies employing different methods have confirmed differences in T cell function between atopic and non-atopic infants (4).

It is also known that Th2 skewed immune responses to allergens are already common at birth. Over the first few years of life an immune deviation takes place and the neonatal Th2 skewed immune responses are modified into low level immunity predominantly expressing Th1 cytokines and low levels of IgG antibodies, particularly of the IgG1 subclass (4). This immune deviation appears to occur more readily for food antigens than for inhalants and more readily in non-atopic than in atopic infants.

The role of microbes

It is well established that infections and other microbial pressure may drive the immune system towards a Th1-like response. Consequently, it has been suggested that recurrent infections may

enhance skewing towards Th1 type responses by exerting a pressure on the immune system (5). It would seem unlikely, however, that a stimulus that is potentially harmful to the host should be necessary for the postnatal maturation of a balanced immune system. The suggestions were based on epidemiological studies and the evidence for a relationship between respiratory tract infections and protection against allergy is still largely circumstantial. The observed relationships may therefore be spurious. For example, a suggested relationship between the exposure to tuberculosis and a low prevalence of allergy was based on the observation that allergic individuals had strongly positive tuberculin reactions less often, compared to non-allergic individuals (6). However, this observation is more likely due to the fact that atopic individuals have depressed cell-mediated immune responses, including delayed-type hypersensitivity (7). When smaller tuberculin reactions were included in the comparisons, the atopic and non-atopic individuals had a similar prevalence of tuberculin positivity. Weak or absent tuberculin reactivity would therefore merely be an indication of a Th2-skewed immunity and should not be regarded as an indication of less exposure to mycobacteria. Similarly, reported associations between various respiratory infections, including measles, have been refuted in carefully controlled studies (4, 8).

As the immune responses to environmental allergens seem to develop over a period of several years, occasional respiratory infections would not seem to be adequate to induce immune deviation efficiently in an infant. Chronic infections as hepatitis or tuberculosis would theoretically be long-lasting and potent enough to induce immune deviation similar to that shown in animal experiments. It would seem unlikely, however, that a stimulus that is potentially harmful to the host should be necessary for the postnatal maturation of a balanced immune system. Also, the decline in exposure to these agents took place long before there was a major increase in the prevalence of allergic diseases, e.g. in Northern Europe. Furthermore, the suggestions were based on epidemiological studies and the evidence for a relationship between respiratory tract infections and protection against allergy is circumstantial.

The intestinal microflora

The biological and medical communities now embrace the still-developing realisation that the microflora of the large gut may have important roles in both human health and disease. The perspective of the human colon in health and

disease is by no means a new concept, as Metchnikoff, a century ago indicated the clinical importance of the host colonic microflora. Rapid advancements have been made, particularly over the past 10 years, and the bacterial microflora of the human gut is now accepted as an integral component of the host defence system and has generated considerable interest in the functional food/nutraceutical industry.

The total mucosal surface area of the adult human gastrointestinal tract is up to 300 m², making it the largest body area interacting with the environment. It is colonised with over 10¹⁴ microorganisms, weighing over 1 kg and corresponding to more than 10 times the total number of cells in the body (9).

The gastrointestinal tract of the newborn baby is sterile. Soon after birth, however, it is colonised by numerous types of microorganisms. Colonisation is complete after approximately 1 week but the numbers and species of bacteria fluctuate markedly during the first 3 months of life. When the microflora have been established they are surprisingly stable over time and environmental changes, e.g. a treatment period with antibiotics only temporarily changes the composition of the microflora.

The gut flora is thus the quantitatively most important source of microbial stimulation and may provide a primary signal for driving the postnatal maturation of the immune system, thus inducing Th1-like immunity. Rook and Stanford (10) recently suggested two major syndromes that could be the result of inadequate microbial stimulation early in life, i.e. inadequate priming of T helper cells, leading to an incorrect cytokine balance and a failure to fine-tune the T cell repertoire in relation to epitopes that are cross-reactive between self and microorganisms. The authors coined the expressions "input deprivation syndrome" and "uneducated T-cell regulation syndrome".

The gut-associated lymphoid tissue is continuously confronted with a vast array of antigens, ranging from food antigens to pathogenic microorganisms. The gut immune system has the capacity to distinguish between potentially harmful antigens, e.g. microbes and harmless antigens, e.g. foods. The former induce strong mucosal and systemic immunity and the latter immunological (oral) tolerance. The difference between the two types of antigens is probably related to how they are presented by antigen-presenting cells. Thus, microbial antigens are presented mainly by macrophages capable of phagocytosing whole bacteria. Phagocytosis stimulates the cells to express of surface bound T cell

co-stimulatory molecules and the secretion of cytokines capable of activating T cells. Animals lacking a normal intestinal microflora display enhanced immunity to fed proteins, compared with conventional animals (11).

We have reported recently considerable differences in the composition of the gut flora of healthy 1 year-old infants in Estonia, with a low prevalence of allergy, and Sweden. For example, *Lactobacilli* and *Eubacteria* were more common and the counts were higher in Estonian than in Swedish infants, whereas the reverse was true for *Clostridium difficile* (12). Postnatal colonisation seems to be more intense in Estonian than in Swedish babies as the counts of aerobes, particularly staphylococci, enterococci and enterobacteria are much higher during the first week of life in the former (13). At 1 month, the bacterial counts in the Swedish babies have reached the same level as in the Estonian infants. At that age, however, 80% of Estonian infants, compared to only 30% of Swedish babies, are colonised with lactobacilli. Differences in the composition of the intestinal microflora have also been demonstrated between allergic and non-allergic 2-year-old children, both in Estonia and Sweden (14). Thus, lactobacilli and bifidobacteria were more prevalent in the latter, while the counts of coliform bacteria were higher in the atopic children.

An alternative approach to study the microbial flora is to investigate the functional status of the flora, i.e. "what have the microbes done?" A microflora-associated characteristic (MAC) has been defined as any anatomical, physiological, immunological or biochemical function in a macroorganism which has been acted on by the microflora. Examples of MACs include the formation of short chain fatty acids (SCFAs) by anaerobic microbes in the human colon, microbial transformation of cholesterol to coprostanol, and the microbial inactivation of intestinal trypsin which can be measured by the remained faecal tryptic activity (FTA) in faeces. This approach to study the gut flora has several advantages over more traditional analyses based on isolation and enumeration of the microorganisms, as the latter are expensive, time-consuming and associated with problems in obtaining samples from parts of the GI-tract that are normally inaccessible.

Employing this approach, an analysis of short chain fatty acids was performed in stool samples from 25 allergical and 47 non-allergic Swedish infants (15). Iso-caproic acid was detected almost exclusively in allergic infants. This compound is associated with the presence of *C. difficile*. In

contrast, the levels of several fatty acids associated with *Lactobacillus* flora were higher in the non-atopic infants.

These studies indicate an imbalance in the gut flora of allergic infants. As the microbial flora is driving the maturation of the immune system, changes in its composition, as a consequence of an altered lifestyle and diet in industrialised societies, may conceivably play a role for the higher prevalence of allergy. All these findings could suggest that differences in the indigenous intestinal flora might affect the development and priming of the immune system in early childhood and that the observed differences between Estonian and Swedish infants and between allergic and non-allergic children are not secondary phenomena.

Probiotics as a potential for prevention and treatment

Probiotics are living organisms that exert beneficial or health-enhancing effects by improving the characteristics of the intestinal microflora (9). The most important are species of lactic acid bacteria that are able to survive through the stomach and small intestine.

Over the past few years there has been an increasing interest in the possible role of certain microbes in promoting and maintaining health. Several probiotic bacteria have been marketed with various claims to enhance human wellbeing. These claims are mainly poorly documented. It is known, however, that many probiotic strains can colonise the human GI tract transiently and this colonisation may result in augmented immune response. Probiotic lactic acid bacteria have been linked to many effects, including improving rates of recovery from gastroenteritis and diarrhoea of viral and bacterial origin. They have also been suggested to modulate immunity, both local and systemic (16).

It has been suggested that certain strains of *Lactobacilli*, e.g. *L. casei*, can inhibit allergen induced IgE production by murine splenocytes, possibly through induction of IL-12 secretion by macrophages (17). Thus, the intestinal flora may play a crucial role in generating Th2 cell populations fully susceptible to oral tolerance induction (12). Furthermore, the severity of atopic eczema improved in infants treated with extensively hydrolysed whey formula fortified with *L. GG* (16).

Interestingly, products from *Lactobacilli* degraded casein proteins from cow's milk have been shown to stimulate or inhibit lymphocyte proliferation and IL-4 production, and enhance IFN- γ production (18). In mice fed *Lactobacilli*,

the spleen cells were predominantly Th1 cytokines such as IFN- γ and IL-2, while the production of Th2-associated cytokines such as IL-4, IL-5, IL-6 and IL-10 were lower than in the control mice (17). As *Lactobacilli* are more common in Estonian than in Swedish children (13) it is tempting to speculate that the gut flora of the Estonian children may be able to downregulate Th2-like responses to cow's milk. In Estonian children, atopic symptoms were associated with a trend towards high levels of IgG₄ antibodies to birch at 12 months, whereas the reverse was true at 5 years (Jenmalm and Björkstén, unpublished). This could be explained by a transient increased Th2-like response early in life in atopic, compared to non-atopic children, which later disappears.

Very recently, Martinez and co-workers demonstrated a gene polymorphism in the locus for controlling the CD14 region in humans (19). This region is the receptor for lipopolysaccharide (LPS), which is a component of Gram-negative bacteria. A low sensitivity of the receptor was associated with high levels of IgE in serum. This could indicate that at least some atopic individuals, having a low sensitivity of the LPS receptor, require a stronger stimulus than individuals with low S-IgE to trigger the immune response. If this were indeed the case, then such individuals would be particularly susceptible to reduced microbial exposure from the environment.

Concluding remarks

The major lifestyle and environmental factors triggering sensitisation probably exert much of their influence during the first few years of life. The conclusion from a broad compilation of what is known regarding triggers of allergy is that we cannot identify which environmental factors that are the major reasons for the large increase in the prevalence in recent years. Thus, prospective intervention studies aiming at reducing exposure to allergen have been largely disappointing as a mean for primary prevention and the effects are, at best, marginal. The future search for significant environmental factors should be directed towards other areas that have not yet been explored. Future research should be truly interdisciplinary and "lifestyle" should be given a broad interpretation. In particular, factors enhancing immune deviation should be looked for, i.e. "success" factors, rather than "risk" factors. Epidemiological observations and experimental studies over the past years suggest that the exposure to microbial products due to increasing hygiene may be associated with the rising trend in allergies. In this respect, the

possible role of the intestinal microflora in modifying systemic and local immune responses deserves closer analysis.

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