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The intrauterine and postnatal environments

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Pregnancy is associated with a strong skewing toward T_H2 cytokine pattern, which enables the survival of the fetus, including fetal allergen-specific immune responses. The postnatal maturation of the immune system which is characterized by the development of a balanced T_H1/T_H2 immunity is genetically determined and modified by the environment. The process seems to proceed at a slower rate in atopic than in nonatopic infants. There is a close immunologic interaction between the mother and her offspring through the breast milk. Individual variations in the composition of human milk may explain the controversy with regard to the possible allergy-preventive effects of breast-feeding. Recurrent respiratory infections have been suggested to enhance immune deviation. The microbial flora are a more likely source, however, because they are a major driving force in the maturation of the immune system. Changes in its composition, as a consequence of an altered lifestyle and diet, may play a role in the higher prevalence of allergy. So far, primary prevention of allergy has failed. Future studies should therefore focus on factors enhancing immune deviation (ie, "success" factors) rather than on "risk" factors. The intestinal microflora is one of these factors that deserves closer analysis. (*J Allergy Clin Immunol* 1999;104:1119-27.)

Key words: Allergy, immunology, maturation, gestation, infants

The reasons for the large increase in the prevalence of allergic diseases since World War are II largely unknown. Until recently, most of the interest was focused on identifying risk factors (ie, environmental factors that would increase the likelihood for sensitization). Thus excessive exposure to indoor allergens, particularly house dust mites, tobacco smoke, and poor indoor ventilation, have all been suggested as major risk factors. Because sensitization to ubiquitous environmental allergens usually occurs in early childhood and the increase in allergy prevalence has been particularly pronounced in children, much of the interest has been focused on this age group. Furthermore, the risk of sensitization is affected by the

Abbreviations used

BLG:	β -Lactoglobulin
DHA:	Docosahexenoic acid, C22:6n-3
DPA:	Docosapentaenoic acid, C22:5n-3
EPA:	Eicosapentaenoic acid, C20:5n-3
FTA:	Fecal tryptic activity
GI:	Gastrointestinal
LA:	Linoleic acid
MAC:	Microflora-associated characteristic
PMA:	Phorbol myristate acetate
PUFA:	Polyunsaturated fatty acid

time of exposure to allergens and the conditions under which exposure take place. There seems to be a period in early life during which an infant is particularly susceptible to sensitization.¹ The increasing prevalence and severity of atopic diseases seen in industrialized countries^{2,3} are in marked contrast to the low prevalence of allergy among children in the formerly socialist countries of Europe. In the latter countries, the lifestyle is similar to that prevailing in Western Europe 30 to 40 years ago.^{4,5} The increase may in part be caused by the major improvements in public health and personal hygiene practice, diet changes, reduction of average family size, and improvement of general living standards (ie, a changed overall exposure to microbial stimulation).⁶

The environment has undergone major changes in recent years. Rather than devoting all interest toward risk factors enhancing sensitization, a better way would be to search for factors that enhance the induction of tolerance. The discussion on the impact of environmental changes on the incidence of asthma and other allergies has mostly been limited to the possible effects of a deteriorating air quality, poorly ventilated houses, and an increased exposure to certain allergens, notably house dust mites. None of these factors can more than marginally explain the observed regional differences in the prevalence of allergic diseases. But "environment" is much more than emissions from traffic and exposure to mites and other allergens. The concept of "lifestyle" should therefore be expanded considerably (Table I) because an altered lifestyle also includes dietary changes, the microbial environment, extensive traveling to new environments, stress, and much more.

Over the past few years, however, novel discoveries

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TABLE I. Some examples of changes in "lifestyle" and environment after World War II in industrialized countries with market economy, which may possibly have affected incidence of sensitization to allergens

Urbanization	Major changes in habits, exposure to air pollution
Diet	Foods that are new to the area, wider range of foods, food additives, foods processed by industry, altered intestinal microbial flora
Buildings	New building materials, more efficient insulation, altered methods for construction, construction year round even in temperate climates leading to increased humidity
Homes	Larger dwellings, many new compounds at home (chemicals), increased indoor humidity in temperate and cold climates
Lifestyle	Most of time spent indoors, extensive traveling and exposure to new environments

related to the maturation of the immune system has resulted in an interest in identifying factors that protect against the development of manifest allergic disease rather than to continue to merely study risk factors. Such studies were prompted by the discovery that normal pregnancy in mammals, including man, is accompanied by an immune deviation toward T_H2 type immunity.⁷ As a consequence, the immune system of the newborn baby is also skewed toward T_H2 -type immunity. The postnatal maturation of the immune system is characterized by the development of a balanced T_H1/T_H2 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. In this review I will summarize recent data on the development of immune responses to inhalant allergens in the fetus and infant and discuss some possible environmental factors that may influence this process. Maternal immunity will be considered as a significant "environmental factor" during fetal life and early infancy. Particular interest will also be devoted to the possible role of the intestinal microflora in the development of a balanced immune system.

GESTATION PERIOD

Pregnancy is associated with a strong skewing toward the T_H2 cytokine pattern, which enables the survival of the fetus.⁷ If this does not occur in time, there is an increased risk for abortion. Studies suggest that T_H1 cytokines, especially IFN- γ , are harmful in pregnancy and can damage the placenta directly or by activation of cytotoxic cells. Because the immune responses of atopic individuals are characterized by a propensity toward T_H2 type responses to ubiquitous environmental allergens, it is possible that such individuals may be more likely to have a successful pregnancy. This notion was recently supported by the observation in an epidemiologic study that atopic compared with nonatopic mothers were more likely to have several children.⁸

TABLE II. Function of neonatal immune system in relation to that of adults

T cells	Increased proportion of naive cells Decreased cytotoxic capacity Decreased helper function Impaired cytokine production More easily $TH2$ skewed
B cells	Defective isotype switching Defective affinity maturation Decreased antibody secretion
Antigen-presenting cells	Defective $TH1$ -inducing capacity Decreased cytokine production
Granulocytes	Defective neutrophil function Eosinophilia common

There are several studies suggesting that premature infants are less likely than term babies to have allergic disease (but not wheezing). For example, in a large Swedish study comprising 148,000 conscripts for military service, the odds ratio for hay fever was only 0.8 for individuals who were born before the 36th week of gestation.⁹ Similarly, allergy was less common in conscripts who had a birth weight less than 2500 g. The reasons for the inverse relationship between premature birth and development of allergy are unknown. It is possible, however, that the observations could be explained by fewer pregnancy complications in atopic women because they may express a stronger or more rapid immune deviation toward T_H2 -type immunity during pregnancy. This possibility has not been explored.

There is now convincing evidence that the fetus may have low level, but specific, immune responses both to food and inhalant allergens. Thus studies from several groups have shown that cord blood T cells commonly proliferate when stimulated in vitro by ovalbumin, β -lactoglobulin (BLG), house dust mite, cat, and pollen allergens.¹⁰⁻¹² Furthermore, antigen-specific immune responses have been documented in T cells obtained from fetuses after 20 weeks of gestation. Recently it was confirmed by DNA analysis that the responding cord blood cells are of fetal and not maternal origin.¹³

POSTNATAL DEVELOPMENT OF IMMUNITY

The immune system at birth is not adapted for postnatal life in several respects, including poor cell-mediated immunity, inability to produce certain immunoglobulin isotypes, poor inflammatory responses, and impaired defenses against intracellular pathogens (Table II).^{6,14} For example, neonatal peripheral blood T cells functionally differ from adult cells in several respects (eg, high proliferative responses to polyclonal mitogens¹⁵ and direct activation by CD2 or CD3 is sluggish). Furthermore, neonatal T cells bear surface markers suggestive of an immature phenotype (eg, CD45RA), which is present in up to 90% of cord blood $CD4^+$ T cells and does not reach adult levels until after age 15 years.¹⁵ Similarly, CD45RO expression, which is associated with recall

antigen memory T cells, is seen in fewer than 5% of the cord blood T cells and then steadily increases throughout childhood toward adult levels (ie, about 50%).¹⁶

Neonatal CD4 T cells have a deficient capacity to provide help for antibody secretion by B cells.^{17,18} The expression of CD40 ligand, which stimulates B cell differentiation and isotype switching,¹⁹ is reduced in neonatal relative to adult CD4⁺ T cells after phorbol myristate acetate (PMA) and ionomycin²⁰ after stimulation. The impaired neonatal T-cell secretion of cytokines that regulate B-cell activation and maturation also play a role.

The postnatal maturation of T-cell function may be particularly slow in atopic children.²¹ In 1978 Strannegård and Strannegård²² reported that children with atopic dermatitis have lower numbers of lymphocytes forming rosettes with sheep erythrocytes. This seems to be a primary defect rather than the result of disease manifestations because the abnormality is present in healthy infants with an atopic family history before any clinical symptoms have developed. With use of flow cytometry, we recently confirmed that the proportion of lymphocytes expressing CD2, which is the receptor for sheep erythrocytes, was lower in children with atopic eczema than in nonallergic children.²³ A reduced PHA responsiveness has also been observed in atopic children even before the onset of disease.²⁴ Furthermore, the postnatal accumulation of memory T cells responsive to anti-CD3 proceeds more slowly in children with a family history of atopic disease.²⁵ T cells from infants with no family history of allergy also tend to be less responsive than adult T cells but less significantly so.¹²

The capacity of T cells to secrete IFN- γ is markedly reduced in neonates.²⁶⁻²⁸ The kinetics of the postnatal development of this cellular function is heterogeneous, but in the majority of children adult levels are not attained until 3 to 5 years after birth.^{29,30} Reduced IL-3,^{15,31} IL-4,^{27,32} IL-5,³¹ IL-10,^{33,34} GM-CSF,^{31,35} and TNF- α ²⁷ production by mitogen-stimulated neonatal T cells has also been documented. Alloantigen-induced IL-5 and IL-10 responses are normal, however.³⁶ Mitogen-induced IL-4 production is reduced in neonates and in children less than 10 years old compared with adults and increases progressively with age.³²

The reduced neonatal IFN- γ -producing capacity is even more pronounced in atopic children. Several recent studies have shown that low mitogen-induced IFN- γ production at birth and early infancy is associated with a family history of allergy,^{10,37,38} development of eczema during the first year of life,^{11,39} and atopic sensitization up to 6 years.⁴⁰ The reduced IFN- γ production is at least partly intrinsic to the T cells because development of atopic symptoms during the first 2 years of life was associated with less IFN- γ secretion of cord blood cells.¹² The decreased IFN- γ production could possibly be related to the reduced function of the CD2 pathway because CD2 receptor stimulation enhances the responsiveness of activated T cells to IL-12^{41,42} and the monocyte-stimulated IFN- γ production by T cells is CD2⁻.^{42,43} There are no data on mitogen-stimulated IL-4 production in relation

to atopic disease because the levels were below the detection limits of the assays. Elevated IL-4 levels in serum has been reported, however, at ages 3, 6, 9, and 18 months in children in whom atopic disease subsequently developed.⁴⁴

The antibody producing capability is lower in neonates than in adults.⁴⁵⁻⁴⁷ IgM is the only isotype that is synthesized by the neonate to a significant degree,⁴⁵ whereas IgG, IgA, and IgE production is.^{46,47} Serum IgM levels increase rapidly after birth, reaching 75% of adult levels at age 1 year.⁴⁸ The IgG levels fall during the first 6 months of life because of the catabolism of maternal transplacentally derived antibodies and then the levels increase. The age when adult levels of the various IgG subclasses are reached vary, but it has been shown that IgG1 and IgG3 reach adult levels faster than IgG2 and IgG4 do.⁴⁹ Adult levels of IgA and IgE in serum are not attained until age 10 years.⁴⁸

The different kinetics in the development of IgE antibodies in atopic and nonatopic infants is well known because excessive IgE antibody formation is a hallmark of the atopic individual. IgE antibodies often appear before any clinical symptoms have developed and the demonstration of such antibodies in an apparently healthy infant is therefore highly predictive of subsequent allergic disease.^{50,51} It is less well established that atopy may also be associated with a transient deficiency of IgA in serum^{52,53} and secretions.^{54,55} The capacity to produce IgA2 matures more slowly than IgA1-producing ability does.^{49,56,57} The reduced neonatal B-cell activity is partly the result of the immaturity of the T-helper cells, but the B cells themselves are also functionally immature.⁴⁶

In conclusion, several aspects of neonatal immunity are immature, including T-cell function such as diminished cytotoxic and helper activity, antibody production, antigen presentation, and innate immunity. This is at least partly the result of reduced cytokine production and a high proportion of naive cells. The maturation, at least functional T-cell maturation, seems to proceed at a slower rate in atopic than in nonatopic infants. This seems to be particularly true for the maturation of T_H1 function.

DEVELOPMENT OF IMMUNE RESPONSES TO ALLERGENS

The first encounters with ubiquitous allergens occur during the first year of life or even before birth. Thus allergen-specific immune responses may be detected in fetal blood and they are common in T cells from cord blood.^{12,58}

The cord blood levels of IgG antibodies to foods and inhalant allergens, including cat, house dust mite, and birch, are higher in newborn infants with an atopic, compared with nonatopic, mothers.⁵⁹ These findings may reflect quantitatively different immune responses in atopic than in nonatopic individuals with a bias toward the production of type-2 cytokines in the former. The significance of transplacentally transferred IgG antibodies

to allergens has been studied in a few prospective studies. In a Belgian study⁶⁰ high levels of IgG antibodies to food antigens were associated with the development of atopic symptoms in the infants, whereas in a Japanese study⁶¹ the opposite was the case. In 3 larger, carefully controlled prospective Swedish studies,⁶²⁻⁶⁴ however, there was no association between cord blood levels of IgG or IgG subclass antibodies to cow's milk or egg protein on one hand and the development of allergic disease or sensitization on the other.

In contrast to food antibodies, high cord blood levels of IgG antibodies to inhalants seem to be associated with less development of allergy in the infants. Thus children in whom allergy did not develop over the first 8 years of life despite a positive family history more often had high levels of IgG antibodies to birch and cat in the cord blood compared with children who had allergic disease or became sensitized to inhalants.^{59,65} The reasons for the different effects of maternal antibodies to foods and inhalant allergens are unknown. Possibly, the much higher doses of food allergens compared with doses of inhalants to which the infant is exposed stimulate the immune system to such extent as to decrease the importance of any maternally derived antibodies.

The effect of maternal IgG antibodies on immune responses in the offspring could possibly be explained by antigen neutralization. Alternatively, maternal allergen-specific antibodies could also actively suppress humoral immune responses to allergens in the children. Furthermore, it may be speculated that maternal IgG antibodies could affect cytokine production in the child, as suggested by the observation that intravenous Ig therapy increases the plasma levels of IFN- γ .⁶⁶

Studies in rodents⁶⁷⁻⁶⁹ suggest that maternal IgG antibodies to allergen can suppress the development of IgE antibodies in the offspring in an antigen-specific way. There are studies in humans supporting this possibility (eg, fewer positive skin prick tests to grass in children whose mothers underwent rye grass immunotherapy during pregnancy and consequently had high IgG antibody levels compared with untreated mothers).⁷⁰ If maternal IgG antibodies with specificity against inhalant allergens are indeed protective, this may be an alternative explanation to numerous observations in epidemiologic studies showing an increased risk for allergy to seasonal allergens in children born before the relevant pollen season.^{71,72} The findings have been interpreted as indicating an increased risk for allergy when exposure takes place early in life and they formed the background for numerous, largely futile efforts to prevent allergy by reducing exposure to indoor allergens during infancy. It seems more reasonable to suggest that the higher incidence of pollen allergy in children born shortly before the pollen season could be the result of low maternal immunity to seasonal allergens at the time of birth.⁵⁹

After birth, antibody responses to food allergens peak in early childhood, whereas responses to inhalants such as rye grass, house dust mite, birch, and cat increase and then decline with age for several years.^{63,73-75} The IgG

responses during infancy are largely restricted to the IgG1 subclass, whereas IgG4 antibodies mostly appear after the first year of life.^{65,75}

Exposure to cow's milk early in life may have a long-lasting effect on IgG subclass antibody responses. Thus feeding with cow's milk during the first 3 months of life is associated with high levels of IgG subclass antibodies at least up to age 8 years.⁷⁵ This may suggest that the tolerance-inducing mechanisms of the intestine are less effective during the first months of life. In another study⁷⁶ the effects of early antigen exposure was studied in even more detail. Newborn babies were either exclusively breast-fed or fed with either a cow's milk infant formula or with a protein hydrolysate for the first 3 days of life. They were then exclusively breast-fed for at least 2 months. The short-lasting exposure to cow's milk protein was associated with high levels of IgG antibodies to cow's milk proteins but not with more allergy at least up to age 2 years. Children with atopic symptoms and sensitivity to allergens often fail to down-regulate IgG4 antibodies to cow's milk protein.⁷⁵

In conclusion, T_H2 skewed immune responses to allergens are common already at birth. Over the first few years of life an immune deviation takes place and the neonatal T_H2 skewed immune responses are modified into low-level immunity predominantly expressing T_H1 cytokines and IgG antibodies, particularly of the IgG1 subclass. This immune deviation appears to occur more readily for food antigens than for inhalants and more readily in nonatopic than in atopic infants.

THE ROLE OF BREAST MILK

Breast-feeding is undoubtedly the best food early in life and there is ample evidence for its role in nutrition and host defense against infections. However, the role of even exclusive breast-feeding in allergy prevention is highly controversial. During early infancy there is a close immunologic interaction between the mother and her offspring through the breast milk, but relatively little is known about the exchange of immunologic information. Besides numerous components that help in the protection against infection, human milk contains components that enhance the maturation of the immune system of the newborn infant.⁷⁷ Observations include an early stimulation of IgA antibody synthesis in breast-fed infants⁷⁸ and transfer of cell-mediated immunity and cytokines.⁷⁹ Thus human milk would not only provide passive protection against infections but also actively stimulate infant immunity. There are considerable individual variations in the composition of human milk, however. This may explain the controversy with regard to the possible allergy-preventive effects of breast-feeding. If individual variations in the milk modulate the development of immunity in the neonate, then maternal immunity may represent an environmental factor, which would influence the risk for allergic manifestations in the child, possibly even several years later.

Similar to the anti-infective properties, an antiallergic

effect by S-IgA antibodies in human milk was proposed by Machtinger and Moss,⁸⁰ who observed low levels of total IgA and IgA antibodies against cow's milk in milk from mothers of food-allergic infants. This could not be confirmed in a larger prospective study.⁶³ A disturbed composition of polyunsaturated fatty acids (PUFA) has been reported in milk from mothers of atopic infants. For example, the levels of the essential fatty acid linoleic acid (LA), as well as its metabolites, were lower in early mature milk from atopic compared with nonatopic mothers and the ratio between n-6 and n-3 fatty acids was higher.⁸¹ Differences in the fatty acid composition of human milk seems to have a relevance for the breast-fed infants. Thus lower levels of the n-3 fatty acids, eicosapentaenoic acid, C20:5 n-3 (EPA), docosapentaenoic acid, C22:5 n-3 (DPA), and docosahexaenoic acid, C22:6 n-3 (DHA) have been observed in mothers of infants who had allergic disease during the first year of life compared with mothers of babies who did not have any allergic manifestations.⁸² These differences were independent of maternal allergy. Similarly low levels of certain polyamines have been observed in milk from atopic mothers.⁷⁷ Because these compounds are required for optimal DNA synthesis, variations in the levels could affect immune responses to foreign antigens in breast-fed infants.

THE ROLE OF MICROBES

It is well established that infections and other microbial pressure may drive the immune system toward a T_H1 -like response. Furthermore, it has been suggested that recurrent infections may enhance skewing toward T_H1 -type responses by exerting pressure on the immune system.⁸³ 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 the results of epidemiologic studies, and the evidence for a relationship between respiratory tract infections and protection against allergy is 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 less often had strongly positive tuberculin reactions compared with nonallergic persons.⁸⁴ However, this observation is more likely due to the fact that atopic individuals have depressed cell-mediated immune responses, including delayed-type hypersensitivity.⁸⁵ When smaller tuberculin reactions were included in the comparisons, the atopic and nonatopic individuals had a similar prevalence of tuberculin positivity. Weak or absent tuberculin reactivity would therefore merely be an indication of a T_H2 skewed immunity and should not be regarded as an indication of less exposure to mycobacteria.

Infections can have long-lasting nonspecific systemic effects on the nature of the immune response to unrelated antigens. For example, natural measles have been suggested to reduce the incidence of atopy in contrast to vac-

cination against measles,⁸⁶ although this suggestion was not confirmed in a British study.⁸⁷ Other childhood infections may have a similar effect, as suggested by several studies reporting an inverse relationship between the incidence of atopy and family size and particularly the number of older siblings.^{88,89} It has also been suggested that vaccination against pertussis may be increasing the incidence of T_H2 -mediated disease, but this was refuted in a recent large prospective study in which this issue was specifically addressed.⁹⁰

THE INTESTINAL MICROFLORA

The biological and medical communities now embrace the still-developing realization 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 because Metchnikoff⁹¹ 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 defense system and has generated considerable interest in the functional food/nutraceutical industry.

The gastrointestinal tract of the newborn baby is sterile. Soon after birth, however, it is colonized by numerous types of micro-organisms. Colonization 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 has been established, it is surprisingly stable over time and environmental changes (eg, a treatment period with antibiotics only temporarily changes the composition of the microflora).

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 colonized with more than 10¹⁴ micro-organisms, weighs more than 1 kg, and corresponds to more than 10 times the total number of cells in the body.⁹²

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 T_H1 -like immunity. Rook and Stanford⁹³ recently suggested 2 major syndromes that could be the result of inadequate microbial stimulation early in life: 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 micro-organisms. 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 micro-organisms. The gut immune system has the capacity to distinguish between potentially harmful antigens (eg, microbes) and harmless antigens (eg, foods). The former induces strong mucosal and systemic immunity and the latter immunologic (oral)

tolerance. The difference between the 2 types of antigens is probably related to how they are presented by antigen-presenting cells. Thus microbial antigens are mainly presented by macrophages capable of phagocytosing whole bacteria. Phagocytosis stimulates the cells to express of surface bound T-cell costimulatory 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.⁹⁴

We have recently reported considerable differences in the composition of the gut flora of healthy 1-year-old infants in Estonia, with a low prevalence of allergy,⁵ and Sweden.⁹⁵ For example, *Lactobacilli* and *Eubacteria* were more common and the counts are higher in Estonian than in Swedish infants, whereas the reverse was true for *Clostridium difficile*. The postnatal colonization seems to be more intense in Estonian than in Swedish babies because the counts of aerobes, particularly staphylococci, enterococci, and enterobacteria are much higher in the former.⁹⁶ The bacterial counts in the Swedish babies reached the same level as in the Estonian infants at age 1 month. At that age, however, 80% of Estonian infants, compared with only 30% of Swedish babies, are colonized with lactobacilli. Very recently, differences in the composition of the intestinal microflora were demonstrated between allergic and nonallergic 2-year-old children, both in Estonia and Sweden.⁹⁷ Thus lactobacilli and bifidobacteria were more prevalent in the latter, whereas 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 (ie, "what have the microbes done?") A microflora-associated characteristic (MAC) has been defined as any anatomic, physiologic, immunologic, or biochemical function in a macro-organism that has been acted on by the microflora.⁹² Examples of MACs include the formation of short-chain fatty acids 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 fecal tryptic activity (FTA) in feces. This approach to study the gut flora has several advantages over more traditional analyses that are based on the isolation and enumeration of the micro-organisms because the latter are expensive, time consuming, and associated with problems in obtaining samples from parts of the gastrointestinal (GI) tract that are normally inaccessible.

With use of this approach, an analysis of short-chain fatty acids was performed in stool samples from 25 allergic and 47 nonallergic Swedish infants.⁹⁸ Isocaproic 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 a *Lactobacillus* flora were higher in the nonatopic infants.

These studies indicate an imbalance in the gut flora of allergic infants. Because the microbial flora is driving the maturation of the immune system, changes in its compo-

sition, as a consequence of an altered lifestyle and diet in industrialized societies, may 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 nonallergic children are not secondary phenomena.

Probiotics are living organisms that exert beneficial or health-enhancing effects by improving the characteristics of the intestinal microflora.⁹⁹ 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 well-being. Mostly these claims are poorly documented. It is known, however, that many probiotic strains can transiently colonize the human GI tract and this colonization may result in augmented immune responses.¹⁰⁰ Probiotic lactic acid bacteria have been linked to many effects, including improving rates of recovery from gastroenteritis and diarrhea of viral and bacterial origin. They have also been suggested to modulate immunity, both local and systemic.

It has been reported that certain strains of lactobacilli (eg, *Lactobacillus casei*) can inhibit allergen-induced IgE production by murine splenocytes, possibly through induction of IL-12 secretion by macrophages.¹⁰¹ Thus the intestinal flora may play a crucial role in generating T_H2 cell populations fully susceptible to oral tolerance induction.⁹⁴ Furthermore, the severity of atopic eczema improved in infants treated with extensively hydrolyzed whey formula fortified with *Lactobacillus* GG.¹⁰²

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.¹⁰¹ In mice fed lactobacilli, the spleen cells were predominantly T_H1 cytokines such as IFN- γ and IL-2, whereas the production of T_H2-associated cytokines such as IL-4, IL-5, IL-6, and IL-10 were lower than in the control mice.¹⁰³ Because *Lactobacilli* are more common in Estonian than in Swedish children,⁹⁵ it is tempting to speculate that the gut flora of the Estonian children may be able to down-regulate T_H2-like responses to cow's milk. In Estonian children atopic symptoms were associated with a trend toward high levels of IgG₄ antibodies to birch at 12 months, whereas the reverse was true at 5 years (Jenmalm and Björkstén, unpublished data). This could be explained by a transient increased T_H2-like response early in life in atopic compared with nonatopic children that later disappears.

CONCLUSION

The major lifestyle and environmental factors triggering sensitization 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 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 toward other areas that have not yet been explored. In particular, factors enhancing immune deviation should be sought (ie, "success" factors rather than "risk" factors). The intestinal microflora is one of these factors that deserve a closer analysis. Future research should be truly interdisciplinary and "lifestyle" should be given a broad interpretation.

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