

New visions for basic research and primary prevention of pediatric allergy: An iPAC summary and future trends

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Hydrolyzed formula feeding, delayed introduction of solid food, indoor allergen avoidance, smoke and pollutants avoidance have been applied for several decades as primary preventive measures for allergic diseases. Unfortunately, some of these strategies have had no or modest success. Therefore, resources need to be focused on better understanding of the early allergic events and on interventional studies to investigate new strategies of primary and secondary prevention. Accordingly, this review summarizes the state-of-the-art of genetic, immunological and clinical aspects of primary prevention of allergic diseases. Studies investigating gene-by-gene and gene-by-environment interactions suggest that prevention of allergic diseases must be tailored to the individual genetic susceptibilities ('gene profiling') and environmental exposures. The expanding knowledge on new T cell populations (Th17, TSLP (thymic stromal derived lymphopoietin)-dependent 'inflammatory Th2 cells') is also inspiring new concepts on the origins of allergic diseases. The old concept of 'blocking immunoglobulin G antibodies' has been re-appraised and it is likely to generate novel preventive and therapeutic strategies. The major task for future clinical research is to clearly define the timing of optimal exposure to potential allergens. In addition, the role of microbial products such as certain bacteria, or their components, and of helminths or their larvae at different times in early life, alone or with potential allergens, definitely need to be further investigated. The benefit of efficient allergy prevention, based on focusing resources on novel and promising research lines, will be of prime importance to both affluent countries and other parts of the world where allergy is only currently emerging.

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The optimal management of allergies is to prevent them from becoming clinically expressed by efficient primary prevention. Although a set of primary preventive measures, in large part without clear scientific validation, have been applied for several decades, the rate of allergic diseases has steadily increased over the years (1). Efficient primary prevention is largely hampered by our patchy understanding of immune events in early life. We need to link laboratory studies on the early maturation of the immune system with

clinical research projects focusing on the efficient primary prevention of allergy more closely. The aim of this review is to provide a state-of-the-art review linking early events influencing the immune system and clinical studies on primary prevention, as well as a prospective for future research.

The immune system with regard to allergy is relatively naïve in the newborn child. Although primary sensitization can occur during pregnancy or lactation, newborns and young infants

mostly lack detectable antibodies or memory cells to most environmental or food allergens (2). Elevated immunoglobulin E (IgE) levels in cord blood do not reliably predict the atopic status of the child (3) with allergen-specific IgE antibodies only arising later in life. Despite this, the long-lasting and often tedious natural progression of atopic diseases (often called the *allergic march* but better named *allergic marathon*) tends to start very early in life. The allergic marathon usually starts with atopic eczema and/or food allergy in young infants. Although one of the atopic diseases, atopic eczema is distinct as its primary causes are not always linked to common allergens, such as in allergic rhinitis or in allergic asthma, and are believed to be owing to an intrinsic defect of the skin barrier (4). According to revised definitions, 'eczema' has been therefore defined as IgE-associated (atopic eczema) or non-atopic eczema (5). In the progression of the allergic marathon, food-specific IgE antibodies might appear early in life, while environmental allergen-specific IgE antibodies mostly develop during the second year of life or later, often leading to allergic rhinitis and or allergic asthma in pre-school, or school-age children (6).

For decades, primary prevention has mostly focused on avoidance. Allergy-prone individuals have been fed early in life with hypoallergenic formulae and environmental control has emphasized house dust mite and animal dander control measures. On the other hand, over the last 10 yr, a new concept of primary prevention has progressively emerged, which proposes that early exposure to allergens is able to induce tolerance, at least in non-allergy prone individuals and in a subset of allergy-prone individuals. In addition, the concept of the hygiene hypothesis has also largely influenced our understanding of early-life

events (7). According to this hypothesis, early exposure to common bacterial triggers such as endotoxins or helminths might protect an allergy-prone individual from developing allergic diseases.

So far, primary prevention of allergic diseases has in large part failed to be shown to be efficient and safe. Many open questions including implication of specific genes and the interaction of the maturing immune system with the environment remain unanswered. The challenge of controlling the allergic marathon starts here.

Genetics

Already in the last century, clinical observations have linked the classic atopic diseases (atopic dermatitis, allergic asthma, allergic rhinitis and conjunctivitis) with familial inheritance patterns. Based on these observations, and powered by the rapid technical evolution of genetic studies, about 20 yr ago researchers started to look at gene linkage to clinical patterns of allergic diseases by different approaches (Table 1).

The first approach has been by *candidate gene analysis*, and to date, hundreds of reports have been published in the field of allergy. However, most of these studies provided orphan results as many of them could not be replicated. In an analysis of a large number of studies, Ober and Hoffjan (8) reviewed allergic diseases in association with the gene studies. They identified nearly 25 genes that could be associated with an asthma or atopy phenotype in six or more populations. In addition, 54 genes have been associated in two to five populations. However, atopy-associated phenotypes were rarely found within the same variance of the respective candidate gene. This specific approach has been hampered by the

Table 1. Strategies in genetics of allergic diseases: research perspectives

Strategy	Focus	Major outcomes	Research perspectives
Candidate gene analysis	Single genes	Nearly 500 genes associated with allergies, but only 25 in 6 or more study populations	Poor reproducibility of most observations; unfit to study complex disorders
Genome-wide linkage studies	Genome regions	Only a few regions significantly linked in more than two study populations	Strategy to expand mapping of areas to be further investigated with more focused approaches
Major polymorphic genes (single nucleotide polymorphism)	Single genes	Identification of relevant genes (e.g. filaggrin)	Strong reproducibility and great potential for future studies
Gene-by-gene interactions	Combination of gene variants	Synergistic effect of three genes increase asthma (27-fold) or high total immunoglobulin E (18-fold) risk	Promising strategy to identify complex genetic profiles linked to allergy sub-phenotypes
Gene-by-environment interaction	Mutual influence of polymorphic genes and environmental factors	Genes relevant only under environmental pressure (CARD vs. endotoxin, TIM1 vs. hepatitis A virus)	Useful approach to understand variability of gene impact among individuals and populations

variety of phenotypes of expression of allergic diseases, as there are as many 'allergy syndromes' as patients. Allergy as such or even on specific disease such as allergic asthma cannot be linked to a definite gene.

In a second type of approach, researchers have performed *genome-wide linkage studies*. To date, over 15 studies have been published for asthma and four for atopic dermatitis. However, few regions showed significant linkage in more than two study populations. In addition, there were no overlaps between atopic dermatitis and asthma. Again, this approach is linked to the phenotype of the disease and might be most promising in a peculiar allergic manifestation, such as in anaphylaxis owing to peanut allergy.

Further genetic approaches included studies of *major polymorphic genes* (i.e. single nucleotide polymorphism, SNP). These studies showed a strong reproducibility. As an example, a Th2 type cytokine-encoding gene is significantly associated with elevated total serum IgE levels (9, 10). Furthermore, the gene encoding for filaggrin expressed in the epidermis is very strongly associated with atopic dermatitis (4). This approach of SNP polymorphism has a great potential to improve our understanding of genetic susceptibility in the future.

A further approach is characterized by *gene-by-gene interactions*. In this approach, a combination of single gene variants is linked to the expression of specific diseases. With this approach, a 27-fold increase of asthma risk could be shown by the interaction of three genes [coding for interleukin (IL)-4, IL-13 and STAT6 (Signal Transducers and Activators of Transcription)]. Similarly, an 18-fold increase in elevated specific IgE could be shown by a three-gene (coding for IL-4, the IL4 receptor and IL-13) interaction (11). This genetic approach takes into account the specificity of the atopic condition characterized by highly individual patterns as it acknowledges a combination of major features of allergy such the increased total IgE titers, personal pattern of specific IgE sensitizations as well as the expression of the disease. Taking the example of a child with a history of severe atopic dermatitis, highly increased levels of total IgE and allergic asthma to ragweed, one might target genes coding for IL-4 and IL-13, filaggrin as well as for specific major histocompatibility complex (MHC) class II overexpressed in ragweed allergic individuals.

Finally, the *gene-by-environment* approach has brought many insights into pathogenesis and the linkage of atopic diseases with specific polymorphic genes and the environment. This

approach might be best highlighted by expression of toll-like receptor 4 genes and CARD4 (Caspase Recruitment Domain) genes associated to atopic diseases expression specifically in the population of farmer's children (12). Similarly, the TIM1 (T cell immunoglobulin and mucin domain containing protein) gene has been associated with hepatitis A infections (13).

Immunology

The approach to elucidating the pathogenic mechanism(s) underlying atopic disease, which is proving to be most instructive, involves the use of birth cohorts. In principle, this approach [archetypal examples (14–18)] makes it possible to track the individual elements of this process from initial priming of Th cells, through to production of allergen-specific IgE antibodies and subsequent disease expression. The key research questions focus around why the immune system in some children follows this pathway, as opposed to development of protective immunological tolerance.

The specific question of when Th cell priming is initiated is central to this discussion, and remains controversial. A range of earlier observations, including the presence of putative allergen-specific T cells in cord blood (19–22) plus evidence supporting the transplacental transport of allergen to the fetus (23, 24), suggested that priming may in some cases be initiated antenatally. However, recent evidence suggesting that allergen-responsive Th memory cells in cord blood may be functionally immature naive thymocytes which interact 'non-specifically' with the allergen (25). Moreover, a recent prospective birth cohort study failed to detect any relationship between cord blood T-cell responses and subsequent sensitization (26) and instead demonstrated that specific Th memory responses were initiated beyond 6 months of age.

Accordingly, the main focus of this research is upon postnatal immune competence, especially CD4⁺ Th cell maturation which is delayed in subjects expressing the high-risk (HR) phenotype (27). However, as noted in the following, there are sound rationales for casting the net wider to encompass other T-cell subsets, as well as populations of antigen-presenting cells (APC). Additionally, it has become increasingly clear that in atopic diseases, exemplified by atopic asthma, the risk of ultimately expressing significant symptomatology requires environmental signal(s) beyond those triggered via activation of allergen-specific T cells. In particular, the maximal risk for persistent atopic asthma is observed

in children who experience concomitant early sensitization to inhalants with early lower respiratory tract (LRT) viral infections (28–30), suggesting synergistic interaction between these processes (i.e. between the inflammatory responses they trigger). Accordingly, postnatal maturation of the defence mechanism(s) protecting against the spread of viral infections to the LRT are becoming increasingly important elements in this debate.

The main cell populations of interest in this discussion, and some of the most pressing research issues related to the role of these cell types in atopy pathogenesis, are summarized next (Table 2).

T-cells

The original observation linking postnatal maturation of CD4+ Th function with genetic risk for atopy came from the study of isolated T-cell clones from HR and low-risk (LR) infants, which demonstrated that T cells from HR infants secreted less Th1 and Th2 cytokines than in their LR counterparts (27). The degree of reduction in the HR group was more profound with respect to Th1 cytokines, indicating an exaggeration of the normal Th2 skew present in the overall infant population. Similar findings have since been

reported by many groups but the emphasis has mainly been on the reduction in Th1 cytokines in HR subjects, and the concomitant (but less marked) Th2 attenuation has not been examined in detail. The mechanism(s) responsible for this attenuation in HR subjects remain unknown. A comparable cytokine phenotype (i.e. reduced Th1 and Th2) associated with HR has been reported in other contexts (31, 32), and hence it is likely that mechanisms other than those associated with 'immune deviation' phenomena underlie the overall HR phenotype. One possible example of such a 'pan T-cell' mechanism is the recent observation of reduced TcR-associated PKC ζ levels in CD4+ T cells from infants who develop atopy (33), and this and related mechanisms associated with T cell activation merit more detailed investigation.

An additional important research question relates to allergen-specific Th memory generation. Recent studies (26) in a prospective birth cohort has extended earlier findings (34) indicating that for variable periods during infancy allergen-specific responses to dietary and inhalant allergens fluctuate cyclically over time as the ultimate fate of each immune response (i.e. tolerance or sensitization) is finally decided. Thus, the results of single time-point assessments

Table 2. Immunity in allergic diseases: (re-)emerging paradigms and related research priorities

Cells, Ig	Emerging paradigm	Crucial new findings in the atopic child	New research priorities to identify targets of early intervention
CD4+ T cells	'Pan-T cell' activation theory	Children at high risk of allergy show overall reduced cytokine responses and low TCR-associated PKC ζ level	To investigate PKC ζ as a putative molecular target to induce normal T-cell responses in at risk children
CD4+ T and B cells	'Saw tooth' window period theory	Allergen-specific T and B memory fluctuate between activation and tolerization before atopy is fixed	To use microarrays to identify markers of T/B stable or unstable responses and molecular targets for intervention
Immature Tregs cells	'Naïve T cells' theory	Naïve recent thymic emigrants recognize allergens and produce large amounts of regulatory cytokines	To test if this T population prevents atopy and, if so, if it can be rescued and expanded in at-risk children
T-cell profiles	New T-cell populations and cytokines	iNK-T, Th17 and TSLP-dependent 'inflammatory Th2' cells contribute to symptom expression in asthma	To expand knowledge on the role of novel effector and regulatory T cells in asthma
CD8+ T cells	'Pro-atopic' CD8+ cells	High levels of CD8+ T-cell cytokine responses linked to atopy in early life	To investigate whether suppression of allergen-specific CD8+ prevents atopy
DC	'Delayed DC maturation' hypothesis	Reduced newborn's and postnatal antigen-presenting cell capacity of DC, perhaps due to low maternal exposure to PAMP	To test if transplacental exposure to microbial-derived TLR ligands affects postnatal maturation of DC and atopy
NK cells	'Reduced NK activity'	NK modulate DC functions, but NK activity is attenuated at birth	To test if a low NK activity is a risk factor for atopy development.
EOS	'Eosinophil precursor' hypothesis	Eosinophil precursors in CB and early childhood express IL-3R and GM-CSFR rather than IL-5R	To test if an anomalous shift of the EOS precursor compartment towards IL-5 dependence contributes to atopy
IgG	'Blocking IgG' theory	Cross-linking of Fc γ R and Fc ϵ R on MC and basophils inhibits cell activation and histamine release	To develop allergen-specific human IgG monoclonal antibodies with defined B epitope specificity to inhibit MC degranulation

NK, natural killer cells; DC, dendritic cells; Ig, immunoglobulin; IL, interleukin; EOS, eosinophils; TCR, T cell receptor; PKC, protein kinase C- ζ ; PAMP, pathogen-associated molecular pattern; TLR, toll-like receptors; CPB, cord blood; GM-CSFR, granulocyte-macrophage colony-stimulating factor receptor.

of allergen-specific T-cell reactivity performed on PBMC (peripheral blood mononuclear cells) from young children cannot be accurately interpreted in relation to risk for atopy, as we have no tools to discriminate between stable/unstable allergen-specific Th memory populations in these assays [the same may be said of early antibody responses; see Ref. (26)].

In comparison with the CD4+ T-cell compartment, the significance of CD8+ T-cell function in relation to risk for atopy remains relatively neglected. It is known that the epigenetic mechanisms which selectively downregulate the production of the Th1 cytokine interferon (IFN) γ in infant CD4+ Th cells are much less active in CD8+ T cells (35), and as a consequence CD8+ Th1 function is relatively 'mature' in early life. Moreover, high levels of CD8+ T-cell cytokine responses have been linked with risk for early sensitization (36) and airway symptomatology in older age groups (37). Accordingly, the potential role of this T-cell subset in atopy pathogenesis merits more detailed attention.

An increasingly important research focus in this area is T-regulatory cell (Treg) function. Two populations of Treg have been implicated in control of immunoinflammatory processes, antigen-non-specific Treg of direct thymic origin and allergen-specific Treg which arise at the periphery (38). Research from several groups have implicated these cells in the pathogenesis of allergic diseases (39–43), suggesting that Treg deficiencies may contribute to overall risk for atopy. In the pediatric context, evidence is accumulating to support a role for Treg in modulation of food allergy (43), and there is indirect evidence of Treg deficiency in HR children (44). This is a potentially important area for research in the future in relation to atopy development in childhood, and the prospective cohort approach appears particularly suited to this task. One issue of particular interest for the future is the generation of functionally active Treg from recent thymic emigrants in infants, given the demonstration that the latter appear to arise spontaneously in cultures of allergen-stimulated cord blood cells (25).

APC functions

There is clear evidence that innate immune capacity is significantly attenuated at birth (45), and this includes the functions of antigen-presenting dendritic cells (DC) which are the principal regulators of T-cell memory function. Given that DC play a central role in the development of protective tolerance to allergens at the gastrointestinal and respiratory mucosal

surfaces, including the generation of relevant Treg populations, it is likely that variations in the kinetics of maturation of DC competence will be important determinants of risk for atopy. This appears even more likely in light of evidence of DC abnormalities in atopic and/or asthmatic adults (46, 47), and some preliminary evidence already associates risk for atopic disease in early life with variations in DC populations in blood (48). This is an important area for future study, including the development of DC functions in the skin, which is a potentially important route for sensitization to both inhalant and dietary antigens during infancy.

The functional maturation of DC populations is controlled principally via interactions between surface Toll receptors and microbial-derived ligands. It is an intriguing possibility that the degree of functional competence of newborn DC may be influenced by antenatal exposure to transplacentally transported microbial-derived ligands (49), and this issue also merits more detailed study.

Granulocyte populations

The role of granulocytes in the etiology of atopy has been largely neglected. However, the growing realization that interactions between inflammation triggered by host responses to microbes and allergens can interact synergistically to drive atopic disease pathogenesis, provides the imperative to direct more research into this area. In relation to susceptibility to infection, the most relevant cell types to consider are the natural killer (NK) cells and neutrophils. The functional capacity of both these cell types is markedly attenuated at birth (50, 51), and the kinetics of functional maturation of these populations is not well understood. Given the heavy reliance of infants on these 'front-line defence' cell populations to defend against viral infections in the absence of T-cell memory, it is likely that they are important players in the pathogenesis of early childhood diseases such as asthma. NK cells have additionally been shown to be capable of modulating DC functions (52, 53), which may also be directly relevant in this context.

It is generally accepted that eosinophils play a central role in the pathogenesis of atopic diseases, and there is every reason to expect that this rule also applies to childhood atopy. Indeed, the exaggerated Th2 polarity of the immune system during early life may increase the contribution of eosinophils to immune responses in general, a prime example being responses to viral infections such as RSV (respiratory syncytial virus) (54, 55)

which has downstream implications in asthma pathogenesis (29). The functional capacity of eosinophils has not been studied in detail, but the high frequency of erythrum toxicum during infancy (51) suggests that dysregulation of this population may be common during this life phase. A recent study has addressed this issue in a cohort of HR infants and has demonstrated intriguing relationships between the numbers of eosinophil precursors in cord blood and subsequent susceptibility to wheezing respiratory infections (56).

Heterogeneity within T-cell response profiles among atopics: potential implications for treatment choice

It is enigmatic that while up to 50% of the children are sensitized to environmental allergens, significant clinical symptoms occur in only a subset of this population (57). While it is likely that genetically determined differences in end-organ sensitivity to inflammatory mediators is important in this context, it is equally likely that heterogeneity within the gene response profiles of ostensibly homogeneous allergen-specific Th2 clones contributes to this variation among atopics. An archetypal example is the recent report of heterogeneous Th1/Th2 response profiles among atopic 11-yr-olds, which suggested a significant role for Th1 cytokines in symptom expression (58). Additional examples which require more vigorous investigation in this context include potential contributions to symptom expression from iNK T cells (59), Th17 cells (60) and TSLP-dependent 'inflammatory Th2 cells' (61) which produce high levels of tumor necrosis factor (TNF) α as part of their effector response profile. The recent demonstration of a broad range of previously unrecognized effector and regulatory genes within the expression signature of activated primary human Th2 memory cells (62) indicates that this is a potentially rich territory for future exploration.

It is not unreasonable to suggest that more detailed knowledge of the T-cell response profiles of individual atopic children will eventually provide blueprints for more informed treatment choice [e.g. selection of appropriate blocking agent(s); potential susceptibility to immunotherapy, etc.], and for more accurate identification of candidates for early prophylactic immunointerventions.

The re-emerging concept of IgG 'blocking antibodies': therapeutic implications

The idea that allergen-specific IgG antibodies may inhibit allergic reactions, a popular concept

in the seventies (63), re-emerged recently after the observations that IgG antibodies can inhibit several phenomena, such as the allergen-induced boost of memory IgE production, IgE-facilitated antigen presentation to T-cells, allergen binding to cell-bound IgE antibodies and the IgE-mediated degranulation [by cross-linking Fc ϵ and Fc γ receptors on the membrane of MC (mast cells) and basophils; reviewed in Ref. (64)]. Interestingly, technologies to produce large amounts of monoclonal human IgG antibodies (Hu-IgG MoAb) of the desired specificity and for therapeutic purposes have been recently described (65). Passive administration of properly selected, allergen-specific Hu-IgG MoAb is therefore a plausible therapeutic strategy of IgE-mediated allergic diseases and a novel research priority.

Prevention

The concept of primary prevention has been - until recently - mostly guided by the dogma that allergen avoidance is the most effective measure to prevent allergic sensitization and its pathological consequences. Based on this, primary prevention measures have specifically targeted nutrition in the newborn and the young child as well as on environmental control (Table 3). The goal of these measures was dual, preventing the disease by preventing IgE-type sensitization. In addition, the notion of target populations became soon relevant, resulting in prevention studies mostly focusing on HR families for atopic diseases.

It is clear now in the light of our current knowledge of immunology as well as in the light of the results gathered over these last decades that these concepts need to be revised. We will here shortly point out relevant studies on primary prevention addressing the nutritional modification as well as environmental control.

Breast-feeding is highly recommended for all infants irrespective of atopic heredity. Interventional studies for allergy prevention focused on two aspects, supplementation by hydrolyzed formula as well as the late introduction of solid foods. For ethical reasons, primary prevention studies based on modified formula feeding were performed in children in whom mothers could not or did not want to breast-feed. Recently, the section on pediatrics of EAACI (European Academy of Allergology and Clinical Immunology) has reviewed observational non-interventional studies on cow's milk allergy and trials with hydrolyzed formula feeding for primary prevention of allergy (66). Interventional studies with partially or extensively hydrolyzed formula

Table 3. Old primary prevention strategies of allergic diseases and their outcomes

Strategy	Proposed rationale	Major outcomes of intervention in children
Prolonged breast feeding	Breast milk protects against allergy	Prolonged breast feeding is useful for the child's health and may prevent allergic sensitization in early childhood without clear benefit for the development of inhalant allergic diseases later in childhood
Hydrolyzed formula feeding	Formula feeding prevents allergy to cow's milk	A diet with specific hydrolyzed formula in at-risk children reduces the cumulative incidence of food allergy and atopic eczema up to 3–5 yr but has no benefit beyond the sixth month of life
Delayed introduction of solid foods	Early introduction of solid foods facilitates food allergy	No evidence that delayed introduction of solid food after 6–8 months of life is useful to prevent food allergy
Indoor allergen avoidance or exposure reduction	Exposure to indoor allergens causes sensitization and asthma	Reduction of exposure to indoor allergens reduces the risk of sensitization but may not prevent asthma in childhood
Smoke and pollutants avoidance	Smoke and pollutants induce allergic sensitization in infants	Smoke and pollutant avoidance is mandatory to maintain respiratory health, and may be effective in reducing the risk of asthma and allergy

have been analyzed by considering various outcomes such as the occurrence of food allergy, atopic dermatitis or respiratory symptoms. The greatest possible impact of preventive measures has been found on food allergy as well as on atopic eczema. However, a large variation of potential efficacy has been found, mostly in relation with various specificities of the formula (i.e. the degree of hydrolysis and the protein source), with extensively hydrolyzed, casein protein-based formula being the most effective in one study (67). The efficacy of hypoallergenic formulae should always be evaluated per brand and not per source of protein (68). However, more recent studies tend to show that either a delayed introduction shows no benefits or even could be detrimental with regard to early sensitization to foods such as egg (69). In addition, an intriguing observation has been made following the advice in the United Kingdom for mothers to avoid peanuts during pregnancy and lactation. During the time period of the advice, the prevalence of peanut allergy had raised from 1.3% to 3.3% suggesting either no effect of these avoidance measures or even a potential detrimental effect (70). On the other hand, evidence suggests also that advice may not have been followed properly (71).

Similarly, regarding development of cow's milk protein allergy, serial birth cohort studies in Denmark suggested that daily exposure to minute amounts via breast milk may be beneficial and facilitate tolerance induction (72, 73). In the same studies, however, the administration of supplements of cow's milk-based formula during the first 5 days in the newborn nursery increased the risk of specific sensitization. Thus, the outcome (tolerance vs. sensitization) of the administration of cow's milk in early life may

be dose-dependent. Our current knowledge is lacking and more studies are urgently needed.

A growing interest has been raised by the hypothesis that allergic asthma is linked to a deficit of fat-soluble vitamins, like vitamins D and E (74). Cross-sectional studies in adults have suggested that antioxidants are inversely associated with the prevalence of established asthma and allergies (74). Prospective studies suggested that a higher intake of antioxidants by pregnant mothers may influence the fetal immune system and reduce the risk of asthma and allergies (75, 76). These preliminary evidences may inspire intervention trials of vitamin D or vitamin E supplementation in pregnant women and infants in an attempt to prevent asthma in children (74).

House dust mite is a typical environmental antigen on which primary prevention has focused in several prospective cohort studies. The rationale for specifically targeting house dust mite sensitization and related allergies comes from the world-wide distribution of this allergen. House dust mites can be found in large parts of Europe, North America, Australia and New Zealand, South America as well as Asia. In most of these areas, they play a key role in the pathogenesis of respiratory allergic diseases and more specifically of asthma.

In addition, it has been found that the best single predictor for childhood asthma are both a positive mother's history for asthma as well as allergic sensitization to house dust mites (77, 78). House dust mite sensitization is also often found early in childhood and is related to the level of domestic exposure (79). Thus, there is a strong rationale for reducing house dust mite exposure in the environment of newborn and young children. Most of the studies investigating the efficacy of house dust mite avoidance measures

chose sensitization to house dust mites cough and/or wheeze as clinical end points. One of the earliest trials was performed in the Isle of Wight (80–82). Early interventions to reduce exposure to house dust mites have resulted in significantly decreased house dust mite sensitization in the intervention group combined with significantly less wheeze at the age of 8 yr, i.e. 13.8% vs. 27.4% in the control group. However, these positive results could not be reproduced in other studies. As an example, the SPACE (Study of Prevention of Allergy in Children in Europe) study has used mattress covers without showing any beneficial effect in the intervention group (83). In the PIAMA (Prevention and Incidence of Asthma and mite Allergy) study, intervention measures had a significant effect on measured levels of dust mite allergens, however, without a significant clinical benefit up to 4 yr of age (84). In this study, only the prevalence of the night cough in the second year of life was lower in the intervention group. In addition, house dust mite avoidance measures had no effect on levels of house dust mite-specific IgE titers. The MAAS (Manchester Allergy and Asthma Study), performed in Manchester (UK), specifically targeted HR infants with both parents suffering from allergic diseases (14). Environment control measures were multiple in order to provide a low allergen environment including reduction of house dust mite allergen levels. Interestingly, an inverse relation was found at 3 yr of age between sensitization rates to house dust mites, and wheeze and lower airway resistance (85).

Overall, prevention studies addressing the environment with drastic avoidance measures show rather disappointing results. If any positive outcome of avoidance was shown, they were mostly short-lasting with various effects on sensitization as well as on the respiratory outcome. It must also be emphasized that differences in study design largely hamper thorough comparison of dust mite avoidance studies.

One of the main benefits of these studies was the emerging notion of target groups for preventive measures and a specific response according to the inherent risk of allergy of an individual. Clearly, further interventional studies on house dust mite avoidance for primary prevention of allergic respiratory disease could be mostly beneficial in HR children. However, the selection criteria to assign individuals to such a group are not well defined yet.

It needs to be briefly stressed that an indirect effect of environmental tobacco smoke has been shown to enhance the risk of allergic diseases. A meta-analysis failed to show a consistent risk of

environmental tobacco smoke for childhood allergic sensitization (86) but this result is probably linked to reverse causation (smoke avoidance in families with a history of asthma). On the other hand, in the German MAS study, smoking during pregnancy was found to favor later sensitization to inhalant allergens (87). Indoor pollution is also a primary risk factor of asthma. In poor areas of many developing countries, indoor pollutants (e.g. solid fuels used for cooking or heating) cause asthma, other chronic respiratory diseases and lung cancer; therefore, WHO considers prevention of indoor pollution as a public health priority and a primary prevention measure of asthma, especially in developing countries (88).

Future strategies

This summary of our knowledge leading to the allergic march has made apparent large black boxes hampering the search for efficient primary prevention. We suggest that a number of experimental approaches should be adopted to address this problem; these are summarized in Table 4.

In the last decades, Th2-type mechanisms and IgE have been the hallmark of our understanding of immune events in allergic diseases. However, missing pieces in the puzzle of early event leading to the allergic march have made it apparent that both the adaptive and innate immune systems strongly influence the development of allergic diseases. Clearly mechanistic studies will have to be linked to clinical studies, including interventional studies providing 'beneficial infectious signals' early in life. In addition, genetic studies should be integrated and mostly focus on gene-by-gene interactions as well as on gene-by-environment interactions. These are the approaches that have so far shown the best value for a better understanding of allergic diseases.

A major task of basic research in collaboration with clinical research is to identify the best markers for the allergic risk. Current observational and interventional studies have made apparent that the concept of risk populations and target groups for intervention is essential for better allergy prevention at all levels. Specific gene profiles might help to identify risk groups, but future research should also address other markers such as antibody profiles or other measurable mediators of the immune system such as cytokines or other soluble markers.

The major task for future clinical research is clearly to improve the quality of the methodology in both prospective non-interventional birth cohort studies and prospective randomized con-

Table 4. New prevention strategies of allergic diseases and their expected outcomes

Research topic	Open questions	How to address it	Expected outcome
Define early events leading to atopy	Which molecular mechanisms link innate immunity to adaptive immunity at the initial stages of atopy development?	Animal models designed to investigate how maternal exposure to environmental factors influences the newborn's innate immunity and its allergen-specific responses	Elucidation of the mechanism of maternally derived atopy protection to improve prevention and treatment of atopic diseases
Identify markers for the atopic risk	Can gene profiling help to identify the genetic risk?[]Can antibody tests help to identify the genetic risk?	Perform genetic studies with various approaches (gene by gene interaction, gene-environment interaction) in atopic populations with various phenotypes[] Define antibody response patterns to various antigens in atopic populations with various phenotypes	Reduce the prevalence of allergic diseases
Unspecific immune modulation	Can microbial or parasitic compounds modulate the immune towards protection from allergy?	Prospective birth cohort studies in families with a defined risk of allergy by using interventional groups with either microbial compounds (PAPS), probiotics, prebiotics or helminths	Reduction of the severity and the rate of expression of allergic diseases
Primary prevention of allergy	Can antigen-specific tolerance be induced early in life by high dose exposure?	Prospective studies in selected allergy risk infants by early introduction of specific foods (e.g. peanuts, egg)[] Prospective studies in selected allergy risk infants by early exposure to other types of allergens (e.g. pollens and/or environmental) by SLIT or other mucosal routes	Effective prevention of specific disease expression, i.e. food allergy[] Effective prevention of specific disease expression, i.e. respiratory allergy
Secondary prevention of allergy	How can the progression to clinical manifestations of allergy in already sensitized children be stopped?	Prospective studies in children already sensitized to common allergens (e.g. pollens and/or environmental) by preventive immunotherapy (e.g. SLIT, SOTI or SIT)	Prevention of progression to respiratory allergy
Prevent disease progression by non-antigen-specific measures	Can the pathogenesis of the allergic march be interrupted by anti-inflammatory treatments (e.g. with anti-immunoglobulin E or Th2 antagonists)?	Interventional studies in infants with early manifestations of the allergic march (e.g. atopic eczema)	Non-specific prevention of atopic disease expression

SLIT, sublingual immune therapy; SOTI, specific oral tolerance induction; SIT, specific immune therapy.

trolled clinical trials (66). Importantly studies on development of allergic diseases should follow accepted recommendations regarding study design, methodology, predefinition of outcome measures, well-defined diagnostic criteria, investigation at onset of symptoms/disease, control for confounding, sample size, power and adequate statistical analyses (89). Many pitfalls should be avoided when interpreting both observational and interventional studies on development of allergic diseases, e.g. disease-related modification of exposure, selection bias and reverse causation and avoidance behavior in families with allergic predisposition. More good studies are needed in particular to define the timing and dose of optimal exposure of potential allergens (89). For example, it should be investigated whether exposure to large amounts of cow's milk particularly in HR infants may induce sensitization, whereas daily exposure to minute amounts via breast milk may be beneficial and facilitate tolerance induction, as suggested by

observational studies (72, 73). In addition, the role of microbial products such as certain bacteria, or their components and of helminths or their larvae given at different times in early life, alone or with potential allergens, definitely need additional investigations. In this regard, mouse models of asthma have been treated with different kinds of microbial or parasite products to prevent the development of allergic sensitization or suppress allergic inflammation. A central, common feature of all these studies is the attempt of properly stimulating Toll-like receptors on cells of the innate immune system (e.g. DC) to produce cytokines directing naïve T cells into desired T-cell subtypes (e.g. Th1, Th3, Tr1, etc.). Some of these approaches have been already paralleled by attempts to prevent allergic diseases in humans (90). Most of the recent clinical data in humans concern the use of mycobacteria (BCG) and lactobacilli, but the results have been unfortunately either totally negative (91) or, when positive (92), the trial protocol was affected

by astonishing methodological flaws (93). Thus, not only trials of higher quality, but also new strategies for immunomodulation studies by early immunotherapy intervention are strongly needed. Again, the notion of well-identified target groups will be essential in these studies. Finally, primary prevention will never be fully effective, and efficient strategies for secondary prevention will need to be well documented. Children already in early phases of the allergic marathon (e.g. with atopic eczema) need to have efficient treatment options to stop disease progression. Future studies will need to address inflammation, development of new allergens sensitizations and progression from sensitization to disease. Besides efficient immunotherapy protocols, new pharmacological agents are urgently needed.

In conclusion, our current knowledge clearly shows that allergy is a pediatric disease and that efficient preventive measures need to be addressed early in life. However, interventional studies have so far failed to provide an efficient primary prevention, as the most efficient measures have only halved the number of allergic manifestations, and in most cases, only for a limited time. The iPAC judges that resources will need to be focused in a better understanding of early allergic events, as well as on interventional studies aimed to identify efficient primary and secondary prevention. The benefit of efficient allergy prevention will be of prime importance to affluent countries, but also in large part of the world where allergy is only currently emerging.

References

- GRUNDY J, MATTHEWS S, BATEMAN B, DEAN T, ARSHAD SH. Rising prevalence of allergy to peanut in children: data from 2 sequential cohorts. *J Allergy Clin Immunol* 2002; 110: 784–9.
- PRESCOTT SL. Early origins of allergic disease: a review of processes and influences during early immune development. *Curr Opin Allergy Clin Immunol* 2003; 3: 125–32.
- BERGMANN RL, EDENHARTER G, BERGMANN KE, et al. Predictability of early atopy by cord blood-IgE and parental history. *Clin Exp Allergy* 1997; 27: 752–60.
- PALMER CN, IRVINE AD, TERRON-KWIATKOWSKI A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; 38: 441–6.
- JOHANSSON SG, HOURIHANE JO, BOUSQUET J, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI Nomenclature Task Force. *Allergy* 2001; 56: 813–24.
- KULIG M, BERGMANN R, KLETTKE U, WAHN V, TACKER U, WAHN U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 1999; 103: 1173–9.
- STRACHAN DP, TAYLOR EM, CARPENTER RG. Family structure, neonatal infection, and hay fever in adolescence. *Arch Dis Child* 1996; 74: 422–6.
- OBER C, HOFFMAN S. Asthma Genetics 2006: the long and winding road to gene discovery. *Genes Immun* 2006; 7: 95–100.
- GRAVES PE, KABESCH M, HALONEN M, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000; 105: 506–13.
- LIU X, BEATY TH, DEINDL P, et al. Associations between total serum IgE levels and the 6 potentially functional variants within the genes IL4, IL13, and IL4RA in German children: the German multicenter atopy study. *J Allergy Clin Immunol* 2003; 112: 382–8.
- KABESCH M, SCHEDEL M, CARR D, et al. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. *J Allergy Clin Immunol* 2006; 117: 269–74.
- EDER W, KLIMECKI W, YU L, et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004; 113: 482–8.
- MATRICARDI PM, ROSMINI F, FERRIGNO L, et al. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. *BMJ* 1997; 314: 999–1003.
- CUSTOVIC A, SIMPSON BM, SIMPSON A, KISSEN P, WOODCOCK A. Effect of environmental manipulation in pregnancy and early life on respiratory symptoms and atopy during first year of life: a randomised trial. *Lancet* 2001; 358: 188–93.
- KULIG M, KLETTKE U, WAHN V, FORSTER J, BAUER CP, WAHN U. Development of seasonal allergic rhinitis during the first 7 years of life. *J Allergy Clin Immunol* 2000; 106: 832–9.
- KUSEL MM, DE KLERK NH, HOLT PG, KEBADZE T, JOHNSTON SL, SLY PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006; 25: 680–6.
- MARTINEZ FD, WRIGHT AL, TAUSSIG LM, HOLBERG CJ, HALONEN M, MORGAN WJ. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995; 332: 133–8.
- ODDY WH, HOLT PG, SLY PD, et al. Association between breast feeding and asthma in 6 year old children: findings of a prospective birth cohort study. *Br Med J* 1999; 319: 815–19.
- KONDO N, KOBAYASHI Y, SHINODA S, et al. Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992; 67: 1003–7.
- PIASTRA M, STABILE A, FIORAVANTI G, CASTAGNOLA M, PANI G, RIA F. Cord blood mononuclear cell responsiveness to beta-lactoglobulin: T-cell activity in 'atopy-prone' and 'non-atopy-prone' newborns. *Int Arch Allergy Immunol* 1994; 104: 358–65.
- PICCINI MP, MECACCI F, SAMPOGNARO S, et al. Aero-allergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993; 102: 301–3.
- PRESCOTT SL, MACAUBAS C, HOLT BJ, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; 160: 4730–7.

23. CASAS R, BJORKSTEN B. Detection of Fel d 1-immunoglobulin G immune complexes in cord blood and sera from allergic and non-allergic mothers. *Pediatr Allergy Immunol* 2001; 12: 59–64.
24. SZEPPFALUSI Z, LOIBICHLER C, PICHLER J, REISENBERGER K, EBNER C, URBANEK R. Direct evidence for trans-placental allergen transfer. *Pediatr Res* 2000; 48: 404–7.
25. THORNTON CA, UPHAM JW, WIKSTROM ME, et al. Functional maturation of CD4+CD25+CTLA4+CD45RA+ T regulatory cells in human neonatal T cell responses to environmental antigens/allergens. *J Immunol* 2004; 173: 3084–92.
26. ROWE J, KUSEL M, HOLT BJ, et al. Prenatal versus postnatal sensitization to environmental allergens in a high-risk birth cohort. *J Allergy Clin Immunol* 2007; 119: 1164–73.
27. HOLT PG, CLOUGH JB, HOLT BJ, et al. Genetic 'risk' for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992; 22: 1093–9.
28. HOLT PG, SLY PD. Interactions between respiratory tract infections and atopy in the aetiology of asthma. *Eur Respir J* 2002; 19: 538–45.
29. KUSEL MM, DE KLERK NH, KEBADZE T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007; 119: 1105–10.
30. ODDY WH, DE KLERK NH, SLY PD, HOLT PG. The effects of respiratory infections, atopy, and breastfeeding on childhood asthma. *Eur Respir J* 2002; 19: 899–905.
31. MACAUBAS C, DE KLERK NH, HOLT BJ, et al. Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. *Lancet* 2003; 362: 1192–7.
32. WILLIAMS TJ, JONES CA, MILES EA, WARNER JO, WARNER JA. Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. *J Allergy Clin Immunol* 2000; 105: 951–9.
33. PRESCOTT SL, IRVINE J, DUNSTAN JA, HUI C, FERRANTE A. Protein kinase ζ : a novel protective neonatal T-cell marker that can be upregulated by allergy prevention strategies. *J Allergy Clin Immunol* 2007; 120: 200–6.
34. HATTEVIG G, KJELLMAN B, BJORKSTEN B. Appearance of IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1993; 4: 182–6.
35. WHITE GP, WATT PM, HOLT BJ, HOLT PG. Differential patterns of methylation of the IFN- γ promoter at CpG and non-CpG sites underlie differences in IFN- γ gene expression between human neonatal and adult CD. *J Immunol* 2002; 168: 2820–7.
36. ROWE J, HEATON T, KUSEL M, et al. High IFN- γ production by CD8+ T cells and early sensitization among infants at high risk of atopy. *J Allergy Clin Immunol* 2004; 113: 710–16.
37. MAGNAN AO, MELLY LG, CAMILLA CA, et al. Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma. Increased IFN- γ -producing CD8(+) T cells in asthma. *Am J Respir Crit Care Med* 2000; 161: 1790–6.
38. HAWRYLOWICZ CM, O'GARRA A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol* 2005; 5: 271–83.
39. AKBARI O, FREEMAN GJ, MEYER EH, et al. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med* 2002; 8: 1024–32.
40. AKDIS M, VERHAGEN J, TAYLOR A, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199: 1567–75.
41. CARDONA ID, GOLEVA E, OU LS, LEUNG DY. Staphylococcal enterotoxin B inhibits regulatory T cells by inducing glucocorticoid-induced TNF receptor-related protein ligand on monocytes. *J Allergy Clin Immunol* 2006; 117: 688–95.
42. KARLSSON MR, RUGTVEIT J, BRANDTZAEG P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004; 199: 1679–88.
43. SLETTEN GB, HALVORSEN R, EGAAS E, HALSTENSEN TS. Memory T cell proliferation in cow's milk allergy after CD25+ regulatory T cell removal suggests a role for casein-specific cellular immunity in IgE-mediated but not in non-IgE-mediated cow's milk allergy. *Int Arch Allergy Immunol* 2007; 142: 190–8.
44. HADDELAND U, KARSTENSEN AB, FARKAS L, et al. Putative regulatory T cells are impaired in cord blood from neonates with hereditary allergy risk. *Pediatr Allergy Immunol* 2005; 16: 104–12.
45. LEVY O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat Rev Immunol* 2007; 7: 379–90.
46. MATSUDA H, SUDA T, HASHIZUME H, et al. Alteration of balance between myeloid dendritic cells and plasmacytoid dendritic cells in peripheral blood of patients with asthma. *Am J Respir Crit Care Med* 2002; 166: 1050–4.
47. UCHIDA Y, KURASAWA K, NAKAJIMA H, et al. Increase of dendritic cells of type 2 (DC2) by altered response to IL-4 in atopic patients. *J Allergy Clin Immunol* 2001; 108: 1005–11.
48. HAGENDORENS MM, EBO DG, SCHUERWEGH AJ, et al. Differences in circulating dendritic cell subtypes in cord blood and peripheral blood of healthy and allergic children. *Clin Exp Allergy* 2003; 33: 633–9.
49. EGE MJ, BIELI C, FREI R, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J Allergy Clin Immunol* 2006; 117: 817–23.
50. FEHNIGER TA, CALIGIURI MA. Ontogeny and expansion of human natural killer cells: clinical implications. *Int Rev Immunol* 2001; 20: 503–34.
51. HOLT PG. Postnatal maturation of immune competence during infancy and childhood. *Pediatr Allergy Immunol* 1995; 6: 59–70.
52. EBATA K, SHIMIZU Y, NAKAYAMA Y, et al. Immature NK cells suppress dendritic cell functions during the development of leukemia in a mouse model. *J Immunol* 2006; 176: 4113–24.
53. MARCENARO E, FERRANTI B, MORETTA A. NK-DC interaction: on the usefulness of auto-aggression. *Autoimmun Rev* 2005; 4: 520–5.
54. CULLEY FJ, POLLOTT J, OPENSHAW PJ. Age at first viral infection determines the pattern of T cell-mediated disease during reinfection in adulthood. *J Exp Med* 2002; 196: 1381–6.

55. HOLT PG, SLY PD. Interactions between RSV infection, asthma, and atopy: unraveling the complexities. *J Exp Med* 2002; 196: 1271–5.
56. FERNANDES R, KUSEL M, CYR M, et al. Cord blood hemopoietic progenitor profiles predict acute respiratory symptoms in infancy. *Pediatr Allergy Immunol* 2008; 19: 239–47.
57. WOOLCOCK AJ, PEAT JK, TREVILLION LM. Is the increase in asthma prevalence linked to increase in allergen load? *Allergy* 1995; 50: 935–40.
58. HEATON T, ROWE J, TURNER S, et al. An immunoepidemiological approach to asthma: identification of in vitro T-cell response patterns associated with different wheezing phenotypes in children. *Lancet* 2005; 365: 142–9.
59. AKBARI O, FAUL JL, HOYTE EG, et al. CD4+ invariant T-cell-receptor+ natural killer T cells in bronchial asthma. *N Engl J Med* 2006; 354: 1117–29.
60. STEINMAN L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 2007; 13: 139–45.
61. LIU YJ, SOUMELIS V, WATANABE N, et al. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annu Rev Immunol* 2007; 25: 193–219.
62. BOSCO A, MCKENNA KL, DEVITT CJ, FIRTH MJ, SLY PD, HOLT PG. Identification of novel Th2-associated genes in T memory responses to allergens. *J Immunol* 2006; 176: 4766–77.
63. LEVY DA, LICHTENSTEIN LM, GOLDSTEIN EO, ISHIZAKA K. Immunologic and cellular changes accompanying the therapy of pollen allergy. *J Clin Invest* 1971; 50: 360–9.
64. FLICKER S, VALENTA R. Renaissance of the blocking antibody concept in type I allergy. *Int Arch Allergy Immunol* 2003; 132: 13–24.
65. LANZAVECCHIA A, CORTI D, SALLUSTO F. Human monoclonal antibodies by immortalization of memory B cells. *Curr Opin Biotechnol* 2007; 18: 523–8.
66. HOST A, HALKEN S, MURARO A, et al. Dietary prevention of allergic diseases in infants and small children. *Pediatr Allergy Immunol* 2008; 19: 1–4.
67. VON BERG A, KOLETZKO S, GRUBL A, et al. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol* 2003; 111: 533–40.
68. MURARO A, DREBORG S, HALKEN S, et al. Dietary prevention of allergic diseases in infants and small children. Part I: immunologic background and criteria for hypoallergenicity. *Pediatr Allergy Immunol* 2004; 15: 103–11.
69. ZUTAVERN A, BROCKOW I, SCHAAF B, et al. Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. *Pediatrics* 2006; 117: 401–11.
70. HOURIHANE JO, AIKEN R, BRIGGS R, et al. The impact of government advice to pregnant mothers regarding peanut avoidance on the prevalence of peanut allergy in United Kingdom children at school entry. *J Allergy Clin Immunol* 2007; 119: 1197–202.
71. DEAN T, VENTER C, PEREIRA B, GRUNDY J, CLAYTON CB, HIGGINS B. Government advice on peanut avoidance during pregnancy – is it followed correctly and what is the impact on sensitization? *J Hum Nutr Diet* 2007; 20: 95–9.
72. HOST A, HALKEN S, JACOBSEN HP, CHRISTENSEN AE, HERSKIND AM, PLESNER K. Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. *Pediatr Allergy Immunol* 2002; 13 (Suppl. 15): 23–8.
73. KJAER HF, ELLER E, HOST A, ANDERSEN KE, BINDSLEV-JENSEN C. The prevalence of allergic diseases in an unselected group of 6-year-old children. The DARC Birth Cohort Study. *Pediatr Allergy Immunol* 2008. [Epub ahead of print].
74. WEISS ST, LITONJUA AA. Childhood asthma is a fat-soluble vitamin deficiency disease. *Clin Exp Allergy* 2008; 38: 385–7.
75. MARTINDALE S, MCNEILL G, DEVEREUX G, CAMPBELL D, RUSSELL G, SEATON A. Antioxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life. *Am J Respir Crit Care Med* 2005; 171: 121–8.
76. LITONJUA AA, RIFAS-SHIMAN SL, LY NP, et al. Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 years of age. *Am J Clin Nutr* 2006; 84: 903–11.
77. LAU S, ILLI S, SOMMERFELD C, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. *Lancet* 2000; 356: 1392–7.
78. SEARS MR, HERBISON GP, HOLDAWAY MD, HEWITT CJ, FLANNERY EM, SILVA PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy* 1989; 19: 419–24.
79. KUEHR J, FRISCHER T, MEINERT R, et al. Mite allergen exposure is a risk for the incidence of specific sensitization. *J Allergy Clin Immunol* 1994; 94: 44–52.
80. ARSHAD SH, MATTHEWS S, GANT C, HIDE DW. Effect of allergen avoidance on development of allergic disorders in infancy. *Lancet* 1992; 339: 1493–7.
81. HIDE DW, MATTHEWS S, MATTHEWS L, et al. Effect of allergen avoidance in infancy on allergic manifestations at age two years. *J Allergy Clin Immunol* 1994; 93: 842–6.
82. ARSHAD SH, BATEMAN B, MATTHEWS SM. Primary prevention of asthma and atopy during childhood by allergen avoidance in infancy: a randomised controlled study. *Thorax* 2003; 58: 489–93.
83. HALMERBAUER G, GARTNER C, SCHIERL M, et al. Study on the prevention of allergy in children in Europe (SPACE): allergic sensitization at 1 year of age in a controlled trial of allergen avoidance from birth. *Pediatr Allergy Immunol* 2003; 14: 10–17.
84. CORVER K, KERKHOF M, BRUSSEE JE, et al. House dust mite allergen reduction and allergy at 4 yr: follow up of the PIAMA-study. *Pediatr Allergy Immunol* 2006; 17: 329–36.
85. LOWE LA, WOODCOCK A, MURRAY CS, MORRIS J, SIMPSON A, CUSTOVIC A. Lung function at age 3 years: effect of pet ownership and exposure to indoor allergens. *Arch Pediatr Adolesc Med* 2004; 158: 996–1001.
86. STRACHAN DP, COOK DG. Health effects of passive smoking. 5. Parental smoking and allergic sensitisation in children. *Thorax* 1998; 53: 117–23.
87. MURARO AM, DREBORG SW, HALKENLAU S, et al. Dietary prevention effect of allergic diseases in infants pre- and postnatal tobacco smoke exposure on specific sensitization to food and small children. Part III: critical review of inhalant allergens during the first 3 years of published peer-reviewed observational and interventional studies and final recommendations for life. Mul-

- ticenter Allergy Study Group, Germany. *Pediatr Allergy Immunol* 2004; 1554: 291220–307228.
88. WHO REPORT. Prevention of Allergy and Allergic Asthma. WHO, Geneva, 2003. Report WHO/NMH/MNC/CRA 03.2.
 89. MURARO A, DREBORG S, HALKEN S, et al. Dietary prevention of allergic diseases in infants and small children. Part II. Evaluation of methods in allergy prevention studies and sensitization markers. Definitions and diagnostic criteria of allergic diseases. *Pediatr Allergy Immunol* 2004; 15: 196–205.
 90. MATRICARDI PM, BJORKSTEN B, BONINI S, et al. Microbial products in allergy prevention and therapy. *Allergy* 2003; 58: 461–71.
 91. TAYLOR AL, DUNSTAN JA, PRESCOTT SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007; 119: 184–91.
 92. KALLIOMAKI M, SALMINEN S, ARVILOMMI H, KERO P, KOSKINEN P, ISOLAURI E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357: 1076–9.
 93. MATRICARDI PM. Learning from doubts when the evidence is confusing. *Allergy* 2002; 57: 1078.