

# Dietary prevention of allergic diseases in infants and small children

## Part I: Immunologic background and criteria for hypoallergenicity\*

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The role of primary prevention of allergic diseases has been a matter of debate for the last 40 years. In order to shed some light into this issue, a group of experts of the Section of Pediatrics EAACI critically reviewed the existing literature on the subject. In this paper, the immunology of the fetus and newborn is reviewed as well as the post-natal development of the immune system. The influence of post-natal environment and breastfeeding on tolerance induction and sensitization are examined. Allergic diseases result from a strong relationship between genetic and environmental factors. Sensitization to food allergens occurs in the first year of life and cow's milk allergy is the first food allergy to appear in the susceptible infants. Hypoallergenicity of food formulas to be used is a critical issue both for treatment of cow's milk-allergic children and for prevention. Methods to document hypoallergenicity are discussed and evaluated in the preclinical and clinical steps.

**Antonella Muraro<sup>1</sup>, Sten Dreborg<sup>2</sup>, Susanne Halcken<sup>3</sup>, Arne Høst<sup>4</sup>, Bodo Niggemann<sup>5</sup>, Rob Aalberse<sup>6</sup>, Syed H. Arshad<sup>7</sup>, Andrea von Berg<sup>8</sup>, Kai-Håkon Carlsen<sup>9</sup>, Karel Duschén<sup>10</sup>, Philippe Eigenmann<sup>11</sup>, David Hill<sup>12</sup>, Catherine Jones<sup>13</sup>, Michael Mellon<sup>14</sup>, Göran Oldeus<sup>15</sup>, Arnold Oranje<sup>16</sup>, Cristina Pascual<sup>17</sup>, Susan Prescott<sup>18</sup>, Hugh Sampson<sup>19</sup>, Magnus Svartengren<sup>20</sup>, Yvan Vandenplas<sup>21</sup>, Ulrich Wahn<sup>21</sup>, Jill A. Warner<sup>13</sup>, John O. Warner<sup>13</sup>, Magnus Wickman<sup>22</sup> and Robert S. Zeiger<sup>14</sup>**

<sup>1</sup>Department of Pediatrics, University of Padua, Padua, Italy, <sup>2</sup>ESPACI Past President, Lerum, Sweden, <sup>3</sup>Department of Pediatrics, Sønderborg Hospital, Sønderborg, Denmark, <sup>4</sup>Department of Pediatrics, Odense University Hospital, Odense, Denmark, <sup>5</sup>Department of Pneumology and Immunology, University Children's Hospital Charité, Humboldt University, Berlin, Germany, <sup>6</sup>Department of Allergy CLB, Amsterdam, The Netherlands, <sup>7</sup>Clinical Allergy Research Unit, St Mary's Hospital, Newport, Isle of Wight, UK, <sup>8</sup>Marien-Hospital, Abt. für Kinderheilkunde, Wesel, Germany, <sup>9</sup>Voksentoppen National Centre of Asthma, Allergy and Chronic Lung Diseases in Children, Oslo, Norway, <sup>10</sup>Department of Paediatrics, University Hospital Linköping, Linköping, Sweden, <sup>11</sup>Allergologie/Pediatrie, University of Geneva, Geneva, Switzerland, <sup>12</sup>Department of Allergy, Royal Children's Hospital, North Melbourne, Vic., Australia, <sup>13</sup>Child Health, Level G (803) Centre Block, Southampton General Hospital, Southampton, UK, <sup>14</sup>Kaiser Permanente San Diego, San Diego, CA, USA, <sup>15</sup>Department of Paediatrics, County Hospital Ryhov, Jönköping, Sweden, <sup>16</sup>Department of Dermatology and Venerology, University Hospital (Sophia) Rotterdam, Rotterdam, The Netherlands, <sup>17</sup>Servicio de Alergia, Hospital Infantil Universitario La Paz, Madrid, Spain, <sup>18</sup>Department of Paediatrics, University of Western Australia, Subiaco, WA, Australia, <sup>19</sup>Department of Pediatrics, Division of Allergy and Immunology, Mount Sinai School of Medicine, NY, USA, <sup>20</sup>Department of Public Health Sciences, Division of Occupational Medicine, Karolinska Hospital, Stockholm, Sweden,

\*An extensive review by an expert group set up by the Section on Pediatrics, European Academy of Allergy and Clinical Immunology.

<sup>21</sup>A.Z.- Kinderen, Free University of Brussels, Brussels, Belgium, <sup>22</sup>Department of Environmental Health, Karolinska Hospital, Stockholm, Sweden

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Antonella Muraro MD PhD, Department of Pediatrics, University of Padua, Via Giustiniani 3, 35128 Padua, Italy

Tel.: +39 049 8213505-06, +39 049 8212538

Fax: +39 049 8213509

E-mail: muraro@pediatria.unipd.it

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This is the first of three reviews which will appear in successive issues of the Journal aimed at presenting the opinion of the Section on Pediatrics within the European Academy of Allergology and Clinical Immunology (SP-EAACI) on observational and interventional studies on the dietary prevention of allergic diseases, mainly of food allergy including cow's milk protein (CMP) allergy in early childhood. The topic was the main theme of a Section Meeting in Padua in February 2002.

Throughout, the papers we use the nomenclature proposed by the Task Force on Nomenclature within EAACI (Fig. 1) (1). Citation marks are used if the word 'atopic' has been used in previous publications not according to the definition in Ref. (1) (Appendix 1).

We discuss only studies fulfilling the criteria for statements of evidence, category I and II and grade of recommendations, A and B, as proposed by The World Health Organization (WHO) (2, 3). That means that we only focus on prospective studies of documented high quality.

## Immunologic background

Fetal and neonatal immunology and sensitization

At conception, the genotype of the individual is defined. During pregnancy the intra-uterine envi-

ronment and later the extra-uterine environment influence the growing individual. The ability of the fetus/newborn to respond to antigenic stimuli changes gradually with the maturation of the immune system. Based on present data the hypothesis is that in fetuses with an atopic genotype, the Th2 milieu of pregnancy (4) influences the immune response from the normal Th1 response [immunoglobulin G (IgG)] toward a Th2 response (IgE).

At 11 wk of age, the fetus starts to produce IgE (5). As evident at birth, T cells do react to antigens and allergens (6) as a sign of external influence. Children later developing atopic disease [eczema and skin prick test (SPT) positive] (1) show reduced soluble CD-14 levels in amniotic fluid (7). The mechanism is probably reduced down-regulation of IgE production by B cells within the lymphoid follicles of the gastrointestinal (GI) tract and reduced facilitation of switch from Th2 to balanced Th1/Th2 response in the gut.

At birth, total IgE is low, below 1 kU<sub>A</sub>/l (8), yet cord blood contains specific IgE at very low levels (9). Neonatal production of interleukin-4 (IL-4) and interferon- $\gamma$  (IFN- $\gamma$ ) levels are low (10, 11) and IFN- $\gamma$  production remains low during infancy in those developing atopy during infancy and early childhood (6). However, the production of IFN- $\gamma$  and IL-13 by peripheral blood mononuclear cells after allergen stimulation *in vitro* is reduced (10, 12, 13) in children later developing allergy.

Hereditary factors of importance are atopy (1) in the family (14), especially maternal atopy (15). Eczema or perennial rhinitis in the mother is associated with elevated cord blood IgE (16, 17), and maternal atopic eczema dermatitis syndrome (AEDS), asthma and sensitization are associated with AEDS and recurrent wheezing during the first 2 yr of life (18).

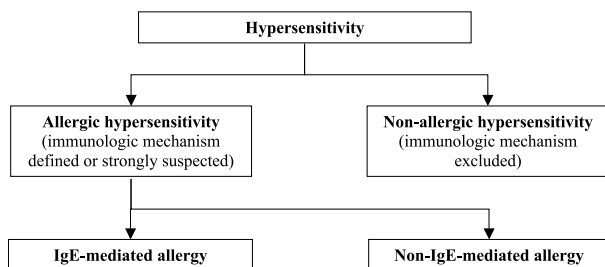


Fig. 1. EAACI nomenclature for allergy.

**Post-natal environment**

After birth GI bacteria are important antigenic stimulants. The gut is colonized from close contacts, especially the mother. In westernized cultures, the first and dominant allergen contact is that with CMP in gram quantities per day, mainly influencing the gut, but also the oral, nasal (and bronchial) mucosa by regurgitation. Later, pg to at the most ng/m<sup>3</sup> of indoor allergens (19) and still smaller amounts of outdoor allergens ( $\mu\text{g}/\text{season}$ )(20) mainly influence the mucosa of the airways.

**The influence of breastfeeding**

Human breast milk contains a number of immune-modifying substances, IgA antibodies toward bacteria, fungi, foods (21) and inhalants (22) and even inhalant allergens (22). Furthermore, breast milk contains pro-inflammatory cytokines [IL-1 $\beta$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6], anti-inflammatory [IL-10, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), TGF- $\beta$ 2], Th1 cytokines (IFN- $\gamma$ ), Th2 cytokines (IL-4, IL-5), growth factors (GM-CSF, G-CSF, M-CSF) and chemokines (IL8, GRO- $\alpha$ , RANTES, MCP-1, Eotaxin, IL-16) (23). Depending on the balance, components of breast milk can both enhance and suppress the immune response and participate in antigen exclusion.

The level of most of these factors, in breast milk does not influence the development of atopy in children (23). However, reduced content of soluble CD14 is found in breast milk from mothers of children who later develop atopic disease and/or eczema (7).

Both  $\omega$ -3 and  $\omega$ -6 levels are lower in the breast milk of atopic mothers than in that of non-atopic mothers (24). Low  $\omega$ -3 in breast milk and a high  $\omega$ -6/ $\omega$ -3 ratio favors the development of atopy in the offspring (24).

**Post-natal immune development**

Infants who go on developing atopic disease show early consolidation of Th2 responses to allergens, often in the first year of life before disease is manifest (25). These responses are short-lived in infants without atopic heredity (25). The factors responsible are still unclear. After birth, maturation of Th1 immune function appears to have an important role in preventing persistent Th2 responses and subsequent allergic disease. Many studies have documented an association between neonatal Th1 immaturity and atopic risk (13, 26–29). Further delay in Th1

maturation in the post-natal period has also been associated with atopy (30). This leads to failure of normal post-natal ‘Th1-driven immune deviation’, and persistent allergen-specific Th2 responses (31).

There are age-related differences in allergen lymphoproliferation and allergen-specific IgE and IgG antibodies toward food and inhalant allergens. Most infants show early transient lymphoproliferation and IgG antibody responses to food allergens not associated with disease (32). A transient early IgE response is seen in many children not developing allergic diseases whereas a prolonged and higher response is seen in children later developing atopy (33, 34) and associated with Th2 cytokine production (34).

Responses to aeroallergens are present in some infants in the first year of life and increase in frequency and magnitude with age (35). T-cell responses to aeroallergens, such as cat or house dust mite, appear earlier in food allergic infants (34) who are more prone to develop persistent aeroallergen-related diseases. The presence of Th2 or Th0 cytokine responses and IgE antibodies to inhalants are the distinguishing immunologic features of children who develop inhalant allergies. The early life events leading to this immune dysregulation are still unclear.

Immaturity of antigen-presenting cell (APC) function (36) also contributes to the ‘Th2 skewed’ immune response of infants due to low pro-Th1 cytokine (IL-12) production that favors development of a default Th2 cytokine profile (37). Neonatal APC are also less efficient in providing T-cell co-stimulation that is likely to contribute to development of tolerance (38, 39). There is indirect evidence, but currently no direct proof, that APC co-stimulation is defective in neonates at high risk of atopy (39).

Influence by bacteria and bacterial products have been proposed to promote the switch from Th2 responses at birth to balanced Th1/Th0/Th2 responses in normal infants. In animals, there is evidence that bacterial endotoxin exposure can prevent allergic sensitization (40) if given before allergic responses are established. Animal models also suggest that bacterial colonization is essential for maturation of Th1 function and induction of oral tolerance (41). So far there are no human studies verifying these animal experiments.

**Tolerance**

While the immune system has principally evolved to protect the host from harmful stimuli, limiting

immune responses to harmless stimuli is equally important for health and survival.

The normal development of immunologic tolerance is poorly understood. Most children tolerate contact with common allergens but respond to exposure with production of IgG rather than IgE antibodies, i.e. the normal development in non-atopic children is, as mentioned above, a Th1-skewed immune response combined with clinical tolerance. According to animal experiments, immunologic tolerance can develop either during early T-cell development in the thymus (central tolerance) or later in the circulation (peripheral tolerance).

During early ontogeny, T cells, which respond to self-antigens, are deleted by 'negative selection'. This prevents most high-affinity auto-reactive T cells from reaching the circulation. In the periphery a number of processes also prevent inappropriate immune responses. The 'danger model' of peripheral tolerance (42) proposes that antigens do not evoke immune responses without inflammatory signals, which promote APC activation and expression of co-stimulatory molecules. In the absence of 'danger' and APC co-stimulation, potentially reactive T cells fail to respond and undergo clonal deletion by apoptosis.

However, the processes governing tolerance to food allergen or aeroallergen are still poorly understood, as neither class of allergens conform to this model. Lymphoproliferation to food allergens is present in most (all) infants (43), but is lost with age. Similarly, IgE responses to food allergens, often without symptoms, appear in a majority of children later developing atopic diseases (33, 44). However, food-specific cytokine and IgG antibody responses appear to persist (34), arguing against clonal deletion of food-responsive T cells and for immunomodulation, i.e. a switch from Th2/IgE to Th1/IgG. The normal IgG response toward many food allergens seen in children as well as adults is rather a sign of exposure than of sensitization and varies with degree of exposure (45).

The lymphoproliferative response to inhaled allergens normally increases with age regardless of allergic status (25, 34), despite their seemingly innocuous nature. Clinical tolerance to these allergens is mostly accompanied by IgG responses at exposure (43). These divergent patterns of response observed in the Th1/Th2 paradigm have provided the basis of the 'immune deviation' model of peripheral tolerance (30).

Once an allergic immune response is established, secondary tolerance may be induced, as seen with successful immunotherapy (46) or in children with CMA after months to a few years

(47). In both cases tolerance is associated with IgG production.

The natural course of food allergy

Food allergy is usually the first sign of allergy. Children producing IgE antibodies toward food allergens often later develop IgE antibodies against inhalant allergens (48) and may also develop inhalant allergies later in childhood (33, 44). Children with AEDS at 18 months of life and sensitization toward mite and grass develop asthma significantly more often than those who do not show these signs of atopic trait (49).

Conclusions

Allergic diseases arise from complex interactions between genetic predisposition and environmental influences. Environmental factors may have a significant influence in pregnancy and early post-natal life when the immune system is first developing.

Initial immune responses to food allergens are evident in fetal life, although the significance of these responses is still unclear.

The mechanisms underlying the development of immunologic and clinical tolerance are not fully understood. Both primary and secondary tolerance seem to be accompanied by 'immune deviation' toward a Th1 pattern of immune response.

Food allergy is the first manifestation of allergy, and food-allergic children are at significant risk of persistent inhalant allergies.

### Hypoallergenicity

Foods intended for *treatment* of food allergy should have allergenicity much lower than the naturally occurring food as measured *in vivo* in allergics, i.e. be hypoallergenic. CM is the most common substitute for mother's milk in western countries and dominates the diet during the first year after weaning. Cow's milk allergy (CMA) is the first food allergy appearing in susceptible infants (50). Therefore, in this review much attention is paid to CMA. About 50% of infants with CMA have a non-IgE-mediated allergy (1) toward CM (51). Thus, roughly half of children reacting to CM in double-blind placebo-controlled food challenge (DBPCFC) have early reactions and produce IgE, whereas the other half reacts later (> 2 h), do not have an IgE-mediated disease, cannot be diagnosed by IgE tests and do not react to the same epitopes as the former ones. Children with IgE-mediated FA do

not, mostly, react to the present s.c. hypoallergenic formulae. In addition, many children with non-IgE-mediated allergy tolerate these formulae.

Food/formulae intended for *prevention* should have a very low if any allergenic activity until otherwise proved (52). In the future, it might be that other criteria can be set up for the development of food/formulae intended for prevention, based on the immunostimulatory effect of these products, but so far there are no criteria for the design of hypoallergenic foods for prevention available.

The documentation of hypoallergenic formulae includes the *in vitro* study of possible allergenicity, i.e. pre-clinically and for quality control, the *in vivo* documentation of reduced allergenicity in children with documented food allergy to the food in question, and the *in vivo* documentation of reduced induction of sensitization and symptoms in children at risk of development of allergy. The *in vivo* documentation of 'hypoallergenicity' of a food/formula for treatment and prevention should be carried out once and for all in a sample of sensitive patients and children at high risk, respectively.

The serum and cells from infants included in clinical trials documenting hypoallergenicity should be used for *in vitro* studies and their properties documented so that relevant epitopes are studied and sensitivity and reactivity can be documented and compared with that of other samples used in other centers.

Physicochemical, cellular and immunochemical methods are the most commonly used methods for the pre-clinical documentation of hypoallergenic foods/formulae and physicochemical and immunochemical methods for quality control. Physicochemical methods are primarily aimed at determining the size distribution of hydrolyzed molecules. Mass-spectroscopic procedures provide quantitative information on well-defined peptide fragments in hypoallergenic products, which will be of great interest in the future.

Human basophils have been used in two types of protocols: either basophiles from allergic subjects, which is practically difficult, or basophils from non-allergic subjects sensitized with IgE from a food-allergic child. The efficiency of the latter procedure has been improved considerably and should be validated.

Immunochemical procedures have been widely used for the study of hypoallergenic products. Because of its simplicity, most laboratories have been using the two-site enzyme-linked immunosorbent assay (ELISA). This

assay is prone to both specific and non-specific interference. Non-specific interference (matrix effects) may occur, for example, if the test sample contains fatty substances, emulsifiers or salts that may inhibit the antigen-antibody interaction. Specific interference may occur via peptides, large enough to bind to a single antibody, but too small to bind two antibodies (as required for detection in the ELISA). For these reasons it is critically important to test all samples with and without spiking, i.e. with and without addition of a known amount of the full allergen. Unless the spiked allergen can be fully recovered in the assay, the assay result is invalid, i.e. too low.

#### Pre-clinical documentation of hypoallergenicity

Before a product is launched for clinical trials, allergenicity should be documented by *in vitro* methods for grading of its allergenicity, including e.g. basophil histamine release studies and stimulation of T cells from several donors, specific for the food and patient age under study.

There is no good *in vitro* test for grading of the allergenicity of hypoallergenic products in infants with late reactions, i.e. non-IgE-mediated allergic reactions, often with AEDS (1).

#### Clinical documentation

*Clinical documentation of allergenicity for treatment.* For safety reasons, a phase II clinical study using skin testing should be the first step. Tests should be performed on a sample of patients clinically sensitive to the food to be avoided as documented by DBPCFC. To optimize the SPT, concentrated formula/food should be used and the technique be documented properly (53, 54). For safety reasons (55), in highly sensitive infants, skin prick testing should start with a concentration 100–1000 times lower (56–58), to reduce the risk of adverse reactions. In a second step, the concentrated product should be tested, as the flat dose response of allergen allows such an increase in concentration (54). Allergenicity should be expressed in times reduction of allergenicity using parallel line bioassay (54).

Provided the product has been found probably suitable to be used in children allergic to the food in question, the product should be documented according to the American Academy on Pediatrics (AAP) criteria (59) as proposed modified in this paper by EAACI. Ninety percent of the infants with documented CMA should tolerate the formula with 95% confidence in DBPCFC. Trials should be performed in two independent

centers including consecutive children, and be divided into IgE-mediated and non-IgE-mediated cases before statistical treatment. The centers involved should be secondary centers, i.e. get referrals from general practice/health care centers. The distribution of threshold concentrations in the CM (other food) challenges, sex, age at onset of symptoms, CM/other allergen skin reactivity, CM/other allergen-specific IgE level, time of onset of reaction at challenge and present age should be documented. Children included with IgE-mediated and non-IgE-mediated allergy to CM/other allergens should be statistically treated separately and  $\geq 28$  of each type should be included in each trial. If one patient reacts, 46 subjects must be included and the remaining 45 must be without reactions. In order to ensure long-term tolerance a normal daily intake during a period of 3 months is recommendable. The child should be symptom-free or with minor constant symptoms for 2 wk prior to the challenge.

So far, only CM-based hypoallergenic formulae have passed testing according to AAP (60–62) and some have been tested without passing (63). When new formulae are tested, the procedure starts with a controlled challenge with CM (see food allergy, challenges), then the trial formula and at last, one formula known to fulfill the AAP criteria and placebo. Such extended challenging may not be performed more than necessary.

Elementary diets do not need any clinical testing provided the production is sufficiently controlled assuring no contamination, but the hypoallergenicity of elementary diets has been documented (64).

Furthermore, the nutritional value of the products should be documented (62, 64).

#### Documentation of individual tolerance

In children with less pronounced immediate symptoms to CM and especially those with late onset reactions, i.e. eczema or GI symptoms after 2 h or more, the formula can be given without initial SPT. All children with symptoms appearing within the next days should be inspected and evaluated by a trained pediatrician so that long-term symptoms from the formula are not overlooked or over-diagnosed (66).

In children with pronounced immediate type reactions the child should be tested by SPT using the concentrated formula before the formula is fed orally (53, 54), to assure absence of pronounced allergy to the formula and to avoid severe reactions to the formula at the introduction (60–62). Duplicate tests should be used due to the fact that the dose response of allergen in

SPT is flat (67), the precision of the SPT is bad (67) and there are no prospective, only one retrospective study (65), showing that duplicate tests can be avoided to reduce the risk of false-negative results. If the test is negative the first bottle of the chosen formula should be given in the office of a trained pediatrician or better pediatric allergist (66).

#### Clinical documentation of allergenicity of food/formulae for allergy prevention

What can be prevented is primarily sensitization followed by allergic symptoms due to the avoided food.

Newborns included in prevention studies should be from high-risk families (i.e. newborns with at least one degree relative [parent or sibling] with documented allergic disease). The infants should be randomized at birth and fed the formula when supplements are needed at least 4 months of age, and the results compared with infants fed a full CMP formula after weaning. Children should be investigated when symptoms appear and validated clinical criteria, including controlled food challenges, should be used for diagnosis. Scoring methods must be validated. Statistically significantly fewer allergies with the study formula should be demonstrated. Evaluation should be before introducing other foods. So far no product has been documented according to the criteria given here.

#### Quality control

The batch control of the allergenicity should preferably be by combining several methods.

Determination of molecular size can only be utilized for orientation, not for batch control.

Quality control methods designed for the control of the composition and consistency of hypoallergenic foods/formulae for treatment of non-IgE-mediated allergy and prevention have not been identified so far.

Elementary diets should be controlled regarding contamination during production.

#### Conclusions

- For research there are many *in vitro* methods, which can be used for determining the allergenicity of hypoallergenic foods/formulae.
- As there are drawbacks with the *in vitro* methods used for quality control, preferably several methods should be combined to assure batch to batch consistent allergenicity.

- *In vitro* methods used for research and quality control should be correlated with *in vivo* determination of allergenicity in samples of patients aimed to receive the product.
- Basic clinical documentation of the allergenicity of products for treatment and prevention of food allergy should follow the modified recommendations of the AAP.

References

1. JOHANSSON SG, HOURIHANE JO, BOUSQUET J, BRUIJNZEEL-KOOMEN C, DREBORG S, HAAHTELA T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001; 56: 813–24.
2. US Department of Health and Human Services AfHCPaR. Clinical Practice Guidelines Development. 95-0045, 1993.
3. ECCLES M, FREEMANTLE N, MASON J. North of England evidence based guidelines development project: methods of developing guidelines for efficient drug use in primary care. *BMJ* 1998; 316: 1232–5.
4. LIN H, MOSMANN TR, GUILBERT L, TUNTIPOPIPAT S, WEGMANN TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J Immunol* 1993; 151: 4562–73.
5. PUNNONEN J, AVERSA GG, VANDEKERCKHOVE B, RONCAROLO MG, DE VRIES JE. Induction of isotype switching and Ig production by CD5+ and CD10+ human fetal B cells. *J Immunol* 1992; 148: 3398–404.
6. PRESCOTT SL, MACAUBAS C, YABUHARA A, et al. Developing patterns of T cell memory to environmental allergens in the first two years of life. *Int Arch Allergy Immunol* 1997; 113: 75–9.
7. JONES CA, HOLLOWAY JA, POPPLEWELL EJ, et al. Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. *J Allergy Clin Immunol* 2002; 109: 858–66.
8. KJELLMAN NI, CRONER S. Cord blood IgE determination for allergy prediction – a follow-up to seven years of age in 1,651 children. *Ann Allergy* 1984; 53: 167–71.
9. HOST A, HUSBY S, GJESING B, LARSEN JN, LOWENSTEIN H. Prospective estimation of IgG, IgG subclass and IgE antibodies to dietary proteins in infants with cow milk allergy. Levels of antibodies to whole milk protein, BLG and ovalbumin in relation to repeated milk challenge and clinical course of cow milk allergy. *Allergy* 1992; 47: 218–29.
10. 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.
11. KONDO N, KOBAYASHI Y, SHINODA S, et al. Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders–6-year follow-up study. *Clin Exp Allergy* 1998; 28: 1340–4.
12. WARNER JA, MILES EA, JONES AC, QUINT DJ, COLWELL BM, WARNER JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a

- predictor of atopic eczema? *Clin Exp Allergy* 1994; 24: 423–30.
13. PRESCOTT SL, MACAUBAS C, SMALLACOMBE T, et al. Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. *Clin Exp Allergy* 1998; 28(Suppl. 5): 39–44.
14. KJELLMAN NI, JOHANSSON SG. IgE and atopic allergy in newborns and infants with a family history of atopic disease. *Acta Paediatr Scand* 1976; 65: 601–7.
15. RUIZ RG, KEMENY DM, PRICE JF. Higher risk of infantile atopic dermatitis from maternal atopy than from paternal atopy. *Clin Exp Allergy* 1992; 22: 762–6.
16. BJERKE T, HEDEGAARD M, HENRIKSEN TB, NIELSEN BW, SCHIOTZ PO. Histamine release from cord blood basophils is influenced by plasma IgE concentration, osmolarity, gestational age at birth and atopic disposition. *Pediatr Allergy Immunol* 1994; 5: 193–201.
17. JOHNSON CC, OWNBY DR, PETERSON EL. Parental history of atopic disease and concentration of cord blood IgE. *Clin Exp Allergy* 1996; 26: 624–9.
18. 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.
19. SAKAGUCHI M, INOUE S, IRIE T, et al. Airborne cat (Fel d 1), dog (Can f 1), and mite (Der I and Der II) allergen levels in the homes of Japan. *J Allergy Clin Immunol* 1993; 92: 797–802.
20. MARSCH DG. Allergens and the genetics of allergy. In: SELA M, ed. *The Antigens*. New York: Academic Press 1975: 271–359.
21. DUCHEN K, CASAS R, FAGERAS-BOTTCHER M, YU G, BJORKSTEN B. Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin A antibodies and early childhood allergy. *Pediatr Allergy Immunol* 2000; 11: 29–39.
22. CASAS R, BJORKSTEN B. Cat-specific IgA antibodies in breast milk from atopic and non-atopic mothers: detection of Fel d 1-IgG immune complexes in cord blood and sera. *Int Arch Allergy Immunol* 1999; 118: 317–8.
23. FAGERAS BM, JENMALM MC, GAROFALO RP, BJORKSTEN B. Cytokines in breast milk from allergic and nonallergic mothers. *Int Arch Allergy Immunol* 1999; 118: 319–20.
24. DUCHEN K, YU G, BJORKSTEN B. Polyunsaturated fatty acids in breast milk in relation to atopy in the mother and her child. *Int Arch Allergy Immunol* 1999; 118: 321–3.
25. PRESCOTT SL, MACAUBAS C, SMALLACOMBE T, HOLT BJ, SLY PD, HOLT PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353: 196–200.
26. TANG ML, KEMP AS, THORBURN J, HILL DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994; 344: 983–5.
27. RINAS U, HORNE G, WAHN V. Interferon-gamma production by cord-blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr Allergy Immunol* 1993; 4: 60–4.
28. MARTINEZ FD, STERN DA, WRIGHT AL, HOLBERG CJ, TAUSSIG LM, HALONEN M. Association of interleukin-2 and interferon-gamma production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J Allergy Clin Immunol* 1995; 96(Pt 1): 652–60.

29. LIAO SY, LIAO TN, CHIANG BL, et al. Decreased production of IFN gamma and increased production of IL-6 by cord blood mononuclear cells of newborns with a high risk of allergy. *Clin Exp Allergy* 1996; 26: 397–405.
30. HOLT PG, CLOUGH JB, HOLT BJ, BARON-HAY MJ, ROSE AH, ROBINSON BW, et al. Genetic 'risk' for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992; 22: 1093–9.
31. HOLT PG. Development of sensitization versus tolerance to inhaled allergens during early life. *Pediatr Pulmonol Suppl* 1997; 16: 6–7.
32. PRESCOTT SL, HOLT PG, JENMALM M, BJORKSTEN B. Effects of maternal allergen-specific IgG in cord blood on early postnatal development of allergen-specific T-cell immunity. *Allergy* 2000; 55: 470–5.
33. HATTEVIG G, KJELLMAN B, JOHANSSON SG, BJORKSTEN B. Clinical symptoms and IgE responses to common food proteins in atopic and healthy children. *Clin Allergy* 1984; 14: 551–9.
34. NG TW, HOLT PG, PRESCOTT SL. Cellular immune responses to ovalbumin and house dust mite in egg-allergic children. *Allergy* 2002; 57: 207–14.
35. WAHN U, LAU S, BERGMANN R, et al. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol* 1997; 99(Pt 1): 763–9.
36. TAYLOR S, BRYSON YJ. Impaired production of gamma-interferon by newborn cells in vitro is due to a functionally immature macrophage. *J Immunol* 1985; 134: 1493–7.
37. JANKOVIC D, KULLBERG MC, HIENY S, CASPAR P, COLLAZO CM, SHER A. In the absence of IL-12, CD4(+) T cell responses to intracellular pathogens fail to default to a Th2 pattern and are host protective in an IL-10(-/-) setting. *Immunity* 2002; 16: 429–39.
38. RIDGE JP, FUCHS EJ, MATZINGER P. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science* 1996; 271: 1723–6.
39. POHL D, BOCKELMANN C, FORSTER K, RIEGER CH, SCHAUER U. Neonates at risk of atopy show impaired production of interferon-gamma after stimulation with bacterial products (LPS and SEE). *Allergy* 1997; 52: 732–8.
40. TULIC MK, KNIGHT DA, HOLT PG, SLY PD. Lipopolysaccharide inhibits the late-phase response to allergen by altering nitric oxide synthase activity and interleukin-10. *Am J Respir Cell Mol Biol* 2001; 24: 640–6.
41. SUDO N, SAWAMURA S, TANAKA K, AIBA Y, KUBO C, KOGA Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; 159: 1739–45.
42. MATZINGER P. Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994; 12: 991–1045.
43. JENMALM MC, BJORKSTEN B. Development of immunoglobulin G subclass antibodies to ovalbumin, birch and cat during the first eight years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1999; 10: 112–21.
44. KULIG M, BERGMANN R, TACKE U, WAHN U, GUGGENMOOS-HOLZMANN I. Long-lasting sensitization to food during the first two years precedes allergic airway disease. The MAS Study Group, Germany. *Pediatr Allergy Immunol* 1998; 9: 61–67.
45. FALTH-MAGNUSSON K, OMAN H, KJELLMAN NI. Maternal abstention from cow milk and egg in allergy risk pregnancies. Effect on antibody production in the mother and the newborn. *Allergy* 1987; 42: 64–73.
46. BOUSQUET J, LOCKEY R, MALLING HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; 102(Pt 1): 558–62.
47. DANNAEUS A, INGANAS M. A follow-up study of children with food allergy. Clinical course in relation to serum IgE- and IgG-antibody levels to milk, egg and fish. *Clin Allergy* 1981; 11: 533–9.
48. KJELLMAN NI, NILSSON L. From food allergy and atopic dermatitis to respiratory allergy. *Pediatr Allergy Immunol* 1998; 9(Suppl. 11): 13–17.
49. WARNER JO. A double-blinded, randomized, placebo-controlled trial of cetirizine in preventing the onset of asthma in children with atopic dermatitis: 18 months' treatment and 18 months' posttreatment follow-up. *J Allergy Clin Immunol* 2001; 108: 929–37.
50. HOST A, JACOBSEN HP, HALKEN S, HOLMENLUND D. The natural history of cow's milk protein allergy/intolerance. *Eur J Clin Nutr* 1995; 49(Suppl. 1): S13–8.
51. HOST A, HALKEN S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy* 1990; 45: 587–96.
52. HOST A, KOLETZKO B, DREBORG S, et al. Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. *Arch Dis Child* 1999; 81: 80–84.
53. DREBORG S. Diagnosis of food allergy: tests in vivo and in vitro. *Pediatr Allergy Immunol* 2001; 12(Suppl. 14): 24–30.
54. DREBORG S. Skin testing in allergen standardization and research. DOLEN WK, ed. *Immunol Allergy Clinics N Am*. Skin testing, 2001: 329–354.
55. DEVENNEY I, FALTH-MAGNUSSON K. Skin prick tests may give generalized allergic reactions in infants. *Ann Allergy Asthma Immunol* 2000; 85(Pt 1): 457–60.
56. DREBORG S, BASOMBA A, BELIN L, et al. Biological equilibration of allergen preparations: methodological aspects and reproducibility. *Clin Allergy* 1987; 17: 537–50.
57. DREBORG S, HOLGERSSON M, NILSSON G, ZETTERSTROM O. Dose response relationship of allergen, histamine, and histamine releasers in skin prick test and precision of the skin prick test method. *Allergy* 1987; 42: 117–25.
58. DREBORG S. Skin testing. The safety of skin tests and the information obtained from using different methods and concentrations of allergen. *Allergy* 1993; 48: 473–5.
59. AAP Committee on Nutrition. Clinical testing of hypoallergenic formulas. *Pediatrics* 2000; 106: 346–9.
60. GIAMPIETRO PG, KJELLMAN NI, OLDAEUS G, WOUTERS-WESSELING W, BUSINCO L. Hypoallergenicity of an extensively hydrolyzed whey formula. *Pediatr Allergy Immunol* 2001; 12: 83–86.
61. HALKEN S, HOST A, HANSEN LG, OSTERBALLE O. Safety of a new, ultrafiltrated whey hydrolysate formula in

- children with cow milk allergy: a clinical investigation. *Pediatr Allergy Immunol* 1993; 4: 53–59.
62. SAMPSON HA, BERNHISEL-BROADBENT J, YANG E, SCANLON SM. Safety of casein hydrolysate formula in children with cow milk allergy. *J Pediatr* 1991; 118(Pt 1): 520–5.
63. RAGNO V, GIAMPIETRO PG, BRUNO G, BUSINCO L. Allergenicity of milk protein hydrolysate formulae in children with cow's milk allergy. *Eur J Pediatr* 1993; 152: 760–2.
64. SAMPSON HA, JAMES JM, BERNHISEL-BROADBENT J. Safety of an amino acid-derived infant formula in children allergic to cow milk. *Pediatrics* 1992; 90: 463–5.
65. DEVENNEY I, FALTH-MAGNUSSON K. Skin prick test in duplicate: is it necessary? *Ann Allergy Asthma Immunol* 2001; 87: 386–9.
66. BUSINCO L, DREBORG S, EINARSSON R, et al. Hydrolysed cow's milk formulae. Allergenicity and use in treatment and prevention. An ESPACI position paper. European Society of Pediatric Allergy and Clinical Immunology. *Pediatr Allergy Immunol* 1993; 4: 101–11.
67. ISOLAURI E, SUTAS Y, MAKINEN-KILJUNEN S, OJA SS, ISOSOMPI R, TURJANMAA K. Efficacy and safety of hydrolyzed cow milk and amino acid-derived formulas in infants with cow milk allergy. *J Pediatr* 1995; 127: 550–7.

**Appendix 1**

Full name	Definition
Atopy	A personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis or eczema/dermatitis
Allergic diseases	The clinical manifestation of allergy
Allergy	A hypersensitivity reaction initiated by immunologic mechanisms
Allergenicity	The ability to stimulate an IgE response and induce IgE-mediated reactions
Antigenicity	Ability to stimulate an immune response
Cow's milk allergy	An immunologically mediated hypersensitivity reaction to cow's milk, including IgE-mediated and/or non-IgE-mediated allergic reactions
Food allergy	An immunologically mediated hypersensitivity reaction to any food, including IgE-mediated and/or non-IgE-mediated allergic reactions
Partly hydrolyzed formula	A formula with moderately reduced allergenicity*
Extensively hydrolyzed formula	A formula with extensively reduced allergenicity*
Hypersensitivity	Objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus that is tolerated by normal subjects
Hypoallergenic	With reduced allergenicity both IgE and non-IgE-mediated allergenicity
Hypoallergenic formula for treatment	A formula with low allergenicity tolerated with 95% confidence by 90% of CMA patients
IgE-mediated allergy	An allergy with proven IgE-mediated reaction
Non-IgE-mediated allergy	An allergy (immunologically mediated hypersensitivity reaction) without involvement of IgE. In food allergy probably T-cell mediated
Sensitization, immunologic	Any immune response to a foreign antigen
Sensitization, IgE-mediated	An IgE response to foreign antigen (allergen), as measured by <i>in vitro</i> IgE determination or SPT
Sensitization, non-IgE-mediated allergy	A reactivity toward allergen as measured by cell stimulation or APT

\*Commission of the European Communities. Commission directive 96/4 EC of February 16, 1996 amending directive 91/321/EEC on infant formulae and follow-on formulae. Official Journal of the European Commission 1996; 39:12–16: 'Reduced allergenicity' defined as content of immunoreactive protein of <1% of nitrogen-containing substances.