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REVIEW ARTICLE

Dissecting the Causes of Atopic Dermatitis in Children: Less Foods, More Mites

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ABSTRACT

Atopic dermatitis (AD) is a common, chronic or chronically relapsing, multifactorial skin disease that mainly occurs in children but affects also adults. AD usually begins early in life and often concerns people with a personal or family history of asthma and allergic rhinitis. AD is characterized by eczematous changes in the epidermis and originates from a late, T-cell mediated reaction associated to the formation and production of memory T-cell of TH2 type, occurrence of homing receptor at skin level and cutaneous lymphocyte-associated (CLA) antigens. Extrinsic or allergic AD, but not intrinsic AD, shows high total serum IgE levels and the presence of specific IgE for environmental and food allergens. A pivotal role in the pathogenesis of AD is played by filaggrin, a protein contained in the granular layer of the epidermis regulating the aggregation of keratin filaments. Mutation in the filaggrin gene causes decreased barrier function of the corny layers of the epidermis. This favours the enter through the skin of environmental allergens, especially the house dust mite, that further facilitates such entering by the proteolytic activity of its major allergen Der p 1. In fact, recent advances suggest that the dust mite, more than foods, is the major cause of allergic AD. As far as the causal diagnosis of AD is concerned, there is notable evidence supporting the capacity of the atopy patch test (APT) to reproduce the pathophysiologic events of AD. This makes APT a valuable diagnostic tool for AD.

KEY WORDS

atopic dermatitis, atopy patch test, dust mite allergy, filaggrin, food allergy

INTRODUCTION

"Everyone knows how eczema looks like, yet no one knows what eczema is."

Heinrich Adolf Gottron (1890-1974)

Atopic diseases, including rhinitis, asthma, and atopic dermatitis are a major health problem worldwide and their prevalence is particularly high in children.[1] In 2004, the World Allergy Organization (WAO) introduced a revised nomenclature for atopy and atopic diseases, defining atopy only in association with IgE-sensitization. Hence, the term atopy should be used in combination with documented specific IgE antibodies in serum or with a positive skin prick test.[2]

Thus, the non-IgE-associated (formerly defined as intrinsic or atopiform dermatitis) form has to be distinguished from the IgE-associated (formerly defined as extrinsic) form. Concerning atopic dermatitis (AD), even if some authors propagate the concept of 2 distinct forms, i.e. atopiform dermatitis versus atopic dermatitis, the non-IgE associated form may represent a transitional phase of the IgE-associated form, at least in infancy.[3]

AD is a common, chronic or chronically relapsing, severely pruritic, multifactorial skin disease that mainly occurs in children.[4] AD is characterized by eczematous changes in the epidermidis. It normally begins early in life and often occurs in people with a personal or family history of asthma and allergic rhinitis.[5] The lifetime prevalence of AD is estimated to 15-30% in children and 2-10% in adults, with a mean prevalence of 17%, while the incidence of AD has increased 2-3 fold during the past 3 decades in industrialized countries. The highest prevalence was typically found in Northern Europe.[1] The prevalence of AD in

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rural areas and in non affluent countries is significantly lower, emphasizing the importance of life-style and environment in the mechanisms of atopic diseases, which could be explained by the "hygiene hypothesis",[6] however this is a concept still subject to debate.[7] Theories to explain the rise in AD include a decrease in the number of children who are exclusively breast-fed, an overall increased awareness of AD, an increased exposure to air pollution, and an increased exposure to allergens.[8,9] Extensive research has demonstrated that a combination of food allergy, defects in the gut mucosal barrier, and increased intestinal permeability concur to the pathogenesis of AD.[10]

In children, the onset of AD occurs in 45% during the first 6 months of life, in 60% during the first year, and in 85% before the age of 5.[11] Up to 70% of these children have a spontaneous remission before adolescence. The disease can also start in adults (the so-called late-onset AD), and in a substantial number of these patients there is no sign of IgE-mediated sensitization.[12]

FACTORS UNDERLYING AD

The role of genetic factors in AD is clearly demonstrated by twin studies: the concordance rate for AD is higher among monozygotic twins (77%) than among dizygotic twins (15%).[13] The importance of genetic factors in AD is further underlined by the finding that a positive parental history is the strongest risk factor for AD; the incidence rate is doubled if AD is present in one parent, and tripled if both parents are affected.

Whereas, allergic asthma or allergic rhinitis in a parent appears to be a minor factor in the development of AD in the offspring, suggesting AD-specific genes.[14] Genome-wide scans[15] have highlighted several possible AD-related loci on chromosomes 3q21,[16] 1q21, 16q, 17q25, 20p[17] and 3p26.[18] An appealing aspect for the researchers involved in AD is the role of AD in the "allergic march": recently Burgess *et al*. posed the question: "Does eczema lead to asthma?".[19]

Interestingly, the phase Ia of the ISAAC study, showing the clear association among eczema, rhinitis and asthma, suggested the possible occurrence of the allergic march.[20] In a comprehensive review of AD, Wüthrich noted a reported high prevalence of AD (10% to 15%) that had been increasing in recent decades, a high persistence beyond puberty (40% to 60%), and a high rate of developing allergic rhinitis and/or asthma over time in children with AD (40% to 60%).[21]

In fact, AD precedes the development of asthma and allergic rhinitis, suggesting that AD is an "entry point" for subsequent allergic disease.[22] Important observations were provided by the German Multicenter Atopy Study (MAS), which recruited 1,314 newborns of whom 499 were at increased risk of atopic disease. In terms of early AD (onset before the age of 2 years) and subsequent asthma, only those children with early AD and concomitant early wheeze (in the first 3 years of life) were at increased risk of current wheeze or bronchial hyper-responsiveness (BHR) at the age of 7 years. In contrast, the majority of children with early AD and no early wheeze were not at increased risk of current wheeze or BHR at that age. Furthermore, in those children with concomitant AD and early wheeze, half had AD beginning before or with wheeze and half began wheezing before AD. The authors concluded that "rather than early AD being a risk factor for subsequent asthma in a progressive atopic march, it seems more likely that a certain phenotype exists as co-expression of asthma and AD characterized in early life by AD plus either wheezing or a specific pattern of atopy sensitization."[11]

Additionally, the data from the Melbourne Atopic Cohort Study (MACS) help understand the situation yet further: MACS recruited before birth 620 infants born between 1990 and 1994 in Melbourne, Australia, who had a family history of allergic disease. The authors found that infants who developed eczema within the first 6 months of life were at increased risk of new sensitization,[23] a finding later replicated within another Australian birth cohort.[24] Moreover, Lowe *et al*.[25] found that infants with atopic eczema (eczema plus sensitization) were at greater risk at ages 6 and 7 years of having childhood asthma (OR 3.52; 95% CI 1.88-6.59), allergic rhinitis (2.91; 1.48-5.71), and both childhood asthma and allergic rhinitis (6.30; 2.35-16.88) than infants with non-atopic eczema, even after excluding infants with early wheeze. The authors also found that the strongest association between eczema and asthma were for eczema that started in the first 6 months of life and was severe, particularly in boys. In short, these findings were consistent with the atopic march and at odds with the interpretation of Illi *et al*..[11] Perhaps, the lack of assessment of the temporal sequence of eczema, sensitization and wheeze, and the lack of stratification by sex in the German data led to the questionable conclusion that the atopic march was not a real phenomenon.

Another study analyzed the relationship between childhood eczema and asthma over time using data from the Tasmanian Longitudinal Health Study[26]: this was the first study to investigate the influence of eczema on the development of asthma from childhood to middle-age. Burgess *et al*. found that childhood eczema was significantly associated with new-onset asthma in three separate life stages: pre-adolescence (hazard ratio 1.70; 95% CI 1.05-2.75), adolescence (2.14; 1.33-3.46), and adult life (1.63; 1.28-2.09) as well as over the life span from the ages of 8 to 44 years (1.73; 1.42-2.12). These results provide strong evidence supporting the view that the atopic march continues well past childhood. Thus the weight of evidence now favors the atopic march as being a real

phenomenon, particularly in boys. Evidence for impaired skin-barrier function in eczema, together with recent developments in eczema genetics, strengthens the argument favoring a progressive atopic march.

AD causes physical and emotional distress for patients and their families. The impact is greater than with psoriasis and is equivalent to other serious medical conditions such as early onset of diabetes mellitus.[27] AD can have a profound impact on the quality of life of pediatric patients.[28-31] Children with AD are adversely affected in terms of disrupted sleep, school performance, social activities, and participation in sports[28,29] and may suffer embarrassment, peer rejection, teasing, and bullying.[29] Parents report loss of sleep, stress, and loss of leisure time[30]; families can become socially isolated by avoiding interactions with relatives and friends to minimize awkward situations,[31] and overprotection of a child with AD can provoke feelings of jealousy in spouses and siblings.[32] In AD patients evaluated by polysomnography, actigraphy, TNF, IL-6, and IL-10 a positive correlation between increase of AD severity and itching and increase of IL-6 and reduced quality of sleep was found.[33]

A recent study prospectively investigated the relationship between infant eczema (within first 2 years of age), infant sleeping problems (within first 2 years of age), and the risk of mental health problems at 10 years of age. A population-based birth cohort was recruited in Germany and the subjects were followed until 10 years of age. Physician-diagnosed eczema, parent-reported sleeping problems, and known environmental risk factors for atopy were regularly assessed until 10 years of age. Mental health was measured using the Strenghts and Difficulties Questionnaire (parent version) at 10 years of age. In the fully adjusted model, children with infant eczema were at increased risk of hyperactivity/inattention at 10 years of age (odds ratio 1.78; 95% CI 1.02-3.09). Infant eczema with concurrent sleeping problems predicted emotional problems (OR 2.63; 95%CI 1.20-5.76) and conduct problem (OR 3.03; 95%CI 1.01-9.12) at 10 years of age. Infant eczema with concurrent sleeping problems appears to be a risk factor for the development of mental health problems.[34] Patients with chronic relapsing AD often suffer from stress-related exacerbations, exhaustion, depression, anxiety and helplessness. They may feel to be unable to influence the course of their disease, and often develop problems in social interaction. These case reports focus on the different possibilities in addition to classical dermatologic therapy-empathic psychosomatic-orientated conversations, strategies of behavioural medicine and the effect of long-term psychodynamic psychotherapy.[35]

PATHOPHYSIOLOGY OF AD

AD originates from a late, T-cell mediated reaction associated to the formation and production of memory T-cell of Th2 type, occurrence of homing receptor at skin level and CLA antigens.[36] Extrinsic or allergic AD shows high total serum IgE levels and the presence of specific IgE for environmental and food allergens, whereas intrinsic or non-allergic AD exhibits normal total IgE values and the absence of specific IgE. While extrinsic AD is the classical type with high prevalence, the incidence of intrinsic AD is approximately 20% with female predominance. The clinical features of intrinsic AD include relative late onset, milder severity, and Dennie-Morgan folds, but no ichthyosis vulgaris or palmar hyperlinearity. The skin barrier is perturbed in the extrinsic, but not intrinsic type. Filaggrin gene mutations are not a feature of intrinsic AD. The intrinsic type is immunologically characterized by the lower expression of interleukin (IL)-4, IL-5, and IL-13, and the higher expression of interferon-gamma. It is suggested that intrinsic AD patients are not sensitized to protein allergens, which induce Th2 responses, but with other antigens, and metals might be one of the candidates of such antigens.[37]

To understand the pathophysiology of AD requires careful consideration of the role of skin: an intact epidermal compartment is a prerequisite for the skin to function as a physical and chemical barrier.[38] The skin of atopic dermatitis patients is “dry” and “rough”, suggesting disruption of epidermal barrier function. Ichthyosis vulgaris is characterized by dry and rough skin with fish-like scales and has been known to be frequently accompanied by AD.[39]

The role of a genetically-impaired epidermal barrier as the primary cause of the rapid increase in prevalence of AD and respiratory atopy is important, based on available clinical and experimental data. The subsequently increased exposure to irritants and allergen post-natally in predisposed individuals would lead in a subset of these to a specific TH2 cell activation favouring the development of IgE responses to allergens. Other routes of sensitization are probably significant, but skin offers a good target to implement prevention strategies, so far completely ignored in the prophylactic recommendations given to high-risk families. Candidate genes for skin-barrier impairment are possibly those associated with ichthyosis vulgaris and X-linked hypohidrotic ectodermal dysplasia.[40] Also pivotal is the role of Langherans cells. Skin eruption in AD occurs mainly in the epidermis, whose barrier function and cytokine expression have been revealed to be abnormal. In fact, the epidermis contains Langerhans cells, a type of antigen-presenting cells, which could be considered the sentinel of the immune system. Some AD patients were found to have mutations or SNPs (single-nucleotide polymorphisms) in the filaggrin gene, which affect the epidermal barrier function. Proteinases in the epidermis are of importance in maintaining the epidermal barrier,

and their abnormalities have been reported in AD. Defects of various cytokines and chemokines produced by keratinocytes have also been reported. Thymic stromal lymphopoietin (TSLP) produced by keratinocytes has recently been a focus in AD. Adrenergic/cholinergic responses in the epidermis could also influence the pathogenesis of AD. Considering epidermal keratinocytes as a trigger of immune abnormalities, and not only as a peripheral effector, would be important to further disclose the pathogenesis of this enigmatic disorder.[41] This highlights how in clinical practice the role of skin is fundamental in understanding the atopy status: in fact, the first manifestations of atopy take place in the skin, with particular importance for the transepidermal water loss (TEWL) and the decrease of ceramides. Gupta *et al*. stated that "Skin barrier function as assessed by TEWL is intrinsically compromised in children with AD but not in children with other allergic conditions. The magnitude of skin barrier dysfunction correlates with AD disease severity".[42] Of interest, over the past few years, skin barrier dysfunction has emerged as a critical driving force in the development and progression of AD.[43] The barrier function depends upon the horny layers (*stratum corneum*), obtained by the differentiation of keratinocytes and composed by cross-linked proteins including filaggrin (FLG), which is produced by pro-filaggrins by the enzyme capsase-14.

FGL is a protein contained in the granular layer of the epidermidis that plays an important role in the aggregation of keratin filaments to form a rigid marginal band in *stratum corneum*, the indispensable stage in keratinocyte terminal differentiation.[44] Mutation in the filaggrin gene causes decreased barrier function of the horny layers of the epidermidis.[45] The expression level of filaggrin has been known to be decreased in atopic dermatitis patients at both the protein and mRNA levels.[46] To the integrity of skin barrier cooperate sphyngosines and ceramides, that are lipids scarcely present in the eczematous skin. Reduced levels of ceramides cause and increase TEWL, dry skin and fissuration with enhanced passage of antigens through the skin.[47] Identification of loss-of-function mutations in the gene encoding epidermal structural protein filaggrin as a major risk factor for AD has shed new light on disease mechanism in AD.[48] It has been widely replicated that FLG gene mutations are associated with more severe AD, early onset of the disease, enhanced systemic allergen sensitization, and an increased risk of asthma in patients with a previous history of eczema.[49,50] The critical link between abnormal skin barrier in FLG-deficient patients with AD and Th2 polarization might be explained in part by enhanced allergen penetration through the skin accompanied by increased production of TLSP. Insults from allergens or microbes can trigger keratinocytes to produce TLSP, an IL-7-like cytokine that is overexpressed in epidermidis of patients with AD, and signal immature myeloid dendritic cells to induce development of Th2 cells by inducing upregulation of OX40 ligand in the absence of IL-12 production.[51]

This interaction, in which dendritic cells seem to play a pivotal role,[52] leads to a cell response in the skin initially of T helper type 2 (Th2) but later of Th1, and a systemic Th2 response inducing the isotype switching to IgE synthesis and the involvement of eosinophils.[53,54]

When Th2 cells are activated, the production of a proinflammatory cytokines and chemokines pattern sustains the persistence of inflammation. Known AD-related cytokines are IL-5, IL-13 and tumor necrosis factor (TNF)-α, with emerging importance for IL-17, which seems to drive airway inflammation following cutaneous exposure to antigens,[55] and IL-31, which is expressed primarily in skin-homing Th2 cells.[56] Skin-homing is another crucial event in AD, mediated by the cutaneous lymphocyte-associated antigens (CLA) receptor, which characterizes T cell subpopulations with different roles in AD and asthma.

The epidermis is not only a physical barrier, but also functions as a chemical and immunological barrier, producing various cytokines and antimicrobial peptides. In AD patients, cathelicidine (LL37) and β-defensin (HBD-2), antimicrobial peptides, are decreased, which is considered to be the cause of frequent skin infections by *Staphylococcus* and *Streptococcus*.[57] The altered skin structure and a deficiency in antimicrobial peptides favour colonization, in particular yeast colonization (especially *Malassezia* spp.). Sensitization to the yeast occurs almost exclusively in AD patients. *S. aureus* enterotoxins with superantigenic activity stimulate activation of T cells and macrophages. So far, AD skin lesions are orchestrated by the local tissue expression of proinflammatory cytokines and chemokines with activation of T lymphocytes, dendritic cells, macrophages, keratinocytes, mast cells, and eosinophils which lead to the skin inflammatory responses.[58]

THE CAUSES OF AD

THE ROLE OF FOODS

AD is characterized by elevated levels of serum IgE and sensitization to environmental allergens including foods, indoor inhalants, and outdoor inhalants.[59] Epidemiological studies have shown a progression of atopic sensitization from food allergens to inhalant allergens. The German MAS (cited above), which prospectively followed 1314 infants >10 years, demonstrated that approximately 10% of infants were sensitized to food allergens at 1 year of age; however, allergic reactions to food were seen in 8% of children. By age 6, only 3% of children were sensitized to food allergens. Conversely, 1.5% of infants were sensitized to inhalant allergens at 1 year; at 5 years, 30% of children were sensitized to inhalant allergens.[60] This

study also demonstrated that the combination of AD, atopic family history, and food sensitivity is highly predictive of future respiratory allergy and asthma.[61] AD and food allergy (FA) frequently herald the allergic march. They commonly co-exist with severe and persistent AD, particularly in those with early onset.[62] FA is emerging as a major clinical and public health problem worldwide. It affects approximately 5-8% of children - with greatest prevalence in the first few years of life and gradual decrease during the first decade as tolerance develops - and 1-5% of adults. Such prevalence has risen substantially over the past decade, in parallel to the rise in prevalence previously seen for other atopic conditions.[63-66] Despite this, our current understanding on the etiology and biological mechanisms of FA is still incomplete. It is generally believed that FA, like the other allergic diseases such as asthma and AD, is determined by both environmental and genetic factors.[67-69]

Five foods - eggs, milk, soy, peanut, and wheat - are responsible for the highest percentage, 90%, of reactions in young children.[67] Obviously, the natural course of FA is different for each allergen. Allergies to peanuts, nuts, and seafood are more likely to persist, with a small fraction of patients developing tolerance, whereas allergies to milk, eggs, wheat, and soy generally disappear by late childhood.[68]

Foods as a trigger of AD have long been a subject of debate.[70-72] The first documented description of food allergy as a cause of AD dates back to 1915, when Scholoss reported an eczematous rash caused by foods which improved after their elimination from the diet.[73] Currently, foods are believed one of the most common cause of AD, but the association between foods and AD is stark. Though sensitization to foods frequently occurs in children and is more prevalent in children with AD, its causative role in the disease is often not as significant as often believed.[74] As a matter of fact, contrasting results in previous studies on the role of foods in pathogenesis of AD in children were reported. Some authors stated that foods often deteriorate skin lesions in children with AD,[75-77] but others report that foods are not usually involved in exacerbations of the disease in children with AD.[78,79] It is important to note that patients with AD often show unpredictable, irregular aggravation of skin lesions.[80] In the current practice, more than 90% of parents and more than 60% of physicians spot in food allergy the culprit of DA and this leads to an excess of allergy specialist consultation and allergy testing.[81] Indeed, the role of diet both as cause and management of AD is controversial, with pediatricians and allergists in favour and dermatologists contrary.[82] On one hand, it is clear that foods may directly provoke flares of AD in sensitized infants,[83] on the other hand, true food-induced AD is rare.[84] Such opposite points of view can cause confusion in parents and lead to elimination diets without appropriate guide by a nutritionist. Certainly, also for AD "it can be argued that the earlier interventions can be made, the greater the effect may be on the disease".[85] The relationship between food and AD is complex. A common misunderstanding is that food allergies have a significant impact on the course of AD, resulting in uncontrolled attempts at elimination diets and undertreatment of the skin itself.[86] Surely, to better understand the cutaneous reactions induced by FA it is important to recognize that just as AD can be associated with or without elevated immunoglobulin E (IgE) levels, FA can also be IgE or non-IgE mediated.[63] Indeed, the overestimation of the effect of FA on AD most probably stems from the observation of more common, immediate-type, IgE-mediated skin reactions, such as urticaria and erythema. These reactions are more visible and readily attributable to food exposure because of their rapid development, but they do not represent flares of AD. By contrast, eczematous flares that occur as delayed-type hypersensitivity reactions are generally non-IgE-mediated. These reactions may be overlooked because they develop 2 or more hours after food challenges, and this delay may render the correlation between the food exposure and reaction more difficult.[83]

It is of the utmost importance to avoid the many mistakes of the past, such as dietetic interventions during pregnancy,[87-89] to reach correct diagnosis. Moreover, restricted diets may even be potentially harmful for the fetus, with a significantly lower maternal weight gain during pregnancy and a non significant reduction in weight at birth and an increased risk of preterm birth.[90] Studies with follow-up beyond age 1 to 2 years have found a poor influence of prenatal diet on infants with AD, and the critical revision of elimination diets in the mother during pregnancy or lactation did not draw to any conclusions on their possible effect in preventing or improving AD or other atopic disorders.[90]

It is apparent that the study of AD caused by true FA is confusing and controversial. It is often difficult to conclusively prove that specific foods induce dermatitis because clinical cases do not correlate well with skin prick test (SPT) and specific IgE measurement in serum.[74] Before one solicits tests to evaluate FA, the distinction between sensitization and allergy needs to be recognized. The presence of food-specific IgEs supports sensitization, but it does not necessarily translate to FA, which is a clinical response upon exposure to a specific food. Children who are sensitized may not necessarily develop a clinical reaction. For example, a child who has developed tolerance to cow's milk may have persistent milk-specific IgE but may be able to ingest cow's milk without any clinical symptoms.[86] The first suspicion for FA usually arises from patients. Unless major immediate anaphylactic reactions have occurred, however, history has proven to be an unreliable way to diagnose FA.[91] A number

of studies have reported poor correlation, with only 25% to 48% sensitivity and 72% to 97% specificity.[63,77,92-94] Many of the symptoms of FA are IgE-mediated, and therefore initial testing in an outpatient setting is determined on the basis of the presence of food-specific IgEs. The SPT has a high negative predictive value (>95%) and is most informative when it is negative. However the positive predictive value ranges between 30% and 50%.[93] Therefore, the SPT is useful for excluding immediate food hypersensitivity, but a positive result may only suggest such hypersensitivity.[63,91,95] Laboratory testing for food-specific IgE also has a high negative predictive value, estimated to be 75%, but the positive predictive value if often low, ranging from 20% to 60%.[83,94] Recently, diagnostic levels of food-specific IgEs have been determined, and specific IgEs above these levels may offer a positive predictive value of >95% for FA. These levels are available for certain foods, most reliably for hen's egg, cow's milk, fish, and peanut, and they are applicable to young children younger than 2 years of age.[95] However, the decision points are not reliable for wheat and soy.[95] Furthermore, the clinician must pay attention to the type of assay used because the actual diagnostic levels may differ as a result of technical discrepancies and differences in allergen sources among the different assays. Finally, these decision points are for immediate-type reactions and are not meant to predict risk for late eczematous reactions to foods.[96] Although SPT and serum IgE measurements can confirm sensitization, it can not prove clinical allergy to a specific food on its own with reliability. The diagnostic levels for specific IgE can help to avoid oral food challenges (OFCs), but the utility of this tests is limited for certain foods in young children, as long as the assay used is the same as that in published studies. The "gold standard" test to confirm or disprove food allergy is the oral food challenge (OFC), particularly in the form of double-blind, placebo-controlled OFCs.[63,97] OFCs are time-consuming and potentially able to elicit severe reactions, and they should be performed by experienced health-care professionals who have access to emergency equipment. Despite the mentioned caveats, OFCs are especially useful because observation after food exposure for 24 hours or more can allow both IgE and non-IgE-mediated processes to be assessed.[98] Eczematous allergic reactions to foods have similarities to allergic contact dermatitis in that both are T-lymphocyte mediated, with the former being associated with food-specific T-lymphocytes.[99] In addition, their clinical morphologies resemble each other. These shared features have led to the introduction of the atopy patch test (APT) as a way of investigating food-induced eczema. APT is carried out in a similar fashion to patch tests performed in dermatology clinics, by the application of small amounts of food allergens to a clear area on the patient's back. The application sites are checked for contact urticaria at 20 minutes and again at 48 and 72 hours.[100] There are reports supporting the use of APT in combination with IgE testing to increase the positive predictive value for diagnosing FA, thereby bypassing the need for OFCs.[74] However, the authors of other prospective studies have reported that APT offers only a small added benefit, if any, to standard SPT and serum IgE measurements.[101] Furthermore, the methodology appears to require more standardization and therefore in not yet generally recommended for routine diagnosis of food-induced AD.[102]

THE ROLE OF HOUSE DUST MITES

Recent research highlighted the important role played by house dust mites (HDM) as a cause of AD. Since 1967, HDM have been shown to be the main allergens blamed for household dust allergy[103] and are currently recognized worldwide as a major cause of allergic diseases.[104] The most frequently responsible mites are *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (belonging to the family *Pyroglyphidae*) which produce a wide array of allergens occurring in mite bodies and faeces. Currently, there are 22 defined mite allergens, some of them acting as major allergens based on the recognition by IgE from more than 50% of mite allergic patients.[105] Most of these allergens are proteolytic enzymes. It is apparent that the allergens most clinically important are Der p 1 and Der p 2 from *D. Pteronyssinus* and Der f 1 and Der f 2 from *D. farinae*. The group 1 allergens are cysteine protease and the group 2 allergens show structural homology with MD-2, a co-receptor of the Toll-like receptor whose ligand are lipopolysaccharides (LPS).[105]

The importance of dust mites in AD is suggested by their detection on the skin, clothes, and bedding of patients with AD[106,107] but especially by the increasing knowledge being compiled regarding the ability of airborne allergens to go through the skin.

As reported above, patients with AD have a baseline-impaired barrier function that allows proteins to enter into the viable epidermis.[42,43] In AD, airborne proteins have the ability to penetrate into the epidermis and worsen AD severity through 3 mechanisms: inherent proteolytic enzyme activity, activation of proteinase-activated receptors-2 (PAR-2), and immunoglobulin E (IgE) binding, that leads to inflammation.

Concerning the first mechanism, airborne proteins produced by HDM, but also by cockroaches, have direct proteolytic activity on the skin that contribute to barrier impairment and delayed barrier recovery in patients with AD.[108,109] Such proteins have been extensively investigated and consist primarily of cysteine and serine proteases,[108-110] which may directly disrupt epithelial tight junctions, induce degranulation of eosinophils, and activate keratinocytes, caus-

ing increased production of interleukin (IL)-6, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF).[110-112] These effects contribute to barrier impairment and induce local inflammation. In addition, the exogenous proteases alter the skin's natural equilibrium between endogenous proteases and endogenous protease inhibitors causing a delayed barrier recovery in the *stratum corneum*.[113,114] The altered barrier function allows not only easier access into the epidermis for airborne proteins, but also for microbes and irritants. When they have breached this barrier they can easily interact with local immune cells to initiate the Type-I-immediate and Type-IV-delayed hypersensitivity reactions common among patients with AD.[108,115] It was also shown that applying certain weed pollens, animal dander, and moulds onto asymptomatic skin caused an eczematous reaction in a subgroup of patients with AD.[112] However, the specific proteases associated with weed pollens, animal dander, and moulds are not as well described as those from dust mites.

The second mechanism through which airborne proteins may exacerbate AD is the direct activation of the PAR-2 receptor on epidermal keratinocytes and dermal unmyelinated nerve fibres. The PAR-2 receptor is crucial to neural transmission of the itch sensation, maintenance of the epidermal calcium ion gradient, and barrier recovery, although the exact mechanisms of action are not completely clear.[108,116] Biopsies from skin of AD patients characteristically show an increased density of PAR-2 receptors.[116] Proteins of HDM, as well as of cockroach, have been shown to activate PAR-2 receptors.[110] The PAR-2 receptor is also an increased nuclear factor kappa-light-chain enhancer of activated B cells (NF-kB) and increased production of leukotriene B4 and prostaglandin E2.[108,117] Therefore, chronic activation of PAR-2 receptors induces epidermal barrier dysfunction, chronic itch, and delayed skin barrier recovery.

The third mechanism is the classic IgE-mediated allergy. Airborne proteins bind to specific IgE antibodies and elicit the release of histamine and other inflammatory mediators from mast cells and basophils, which result in tissue damage.[118,119] In addition, IgE-mediated histamine release from mast cells exacerbates the itch-scratch cycle, which can further aggravate AD.[112] The skin of AD patients shows increased numbers of Langerhans cells expressing the high-affinity IgE receptor FcεRI compared with the skin of patients without AD.[118] Moreover, it was reported that skin biopsies taken from patients with AD while APT was being performed, have increased chemotactic signals and invasion of dendritic epidermal cells within 24 to 48 hours after protein application.[120] Nonetheless, the incomplete effectiveness of antihistamines in relieving AD-related itching suggests that other mechanisms at the same time at work, such as the proteolytic effects and the PAR-2 binding cited above.

The role played by the cytokine TNF-α is clearly apparent through its capacity to regulate the expression of TSLP in keratinocytes.[121,122] Of interest, the overexpression of TSLP seems sufficient to drive the progression from AD to asthma, that is, the atopic march.[123] At the same time the role of Toll-like receptors (TLRs) is currently under investigation. As reported above, the co-receptor MD-2 of TLR has as ligand the LPS. Recent studies have shown that keratinocytes express TLRs 1-6 and 9, and that they respond to LPS through the production of a number of cytokines and chemokines.[124-126]

Other mechanisms possibly involved in the triggering of AD by dust mites are the ability to activate human dermal endothelial to express adhesion molecules and to secrete particular cytokines, and the activation of inflammasome. As far as the first aspect is concerned, mite extracts stimulated endothelial cells to express intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin and to secrete interleukin (IL)-6, IL-8, monocyte chemoattractant protein (MCP-1), and granulocyte/macrophage colony stimulating factor (GM-CSF). By contrast, *D. farinae* or *D. pteronyssinus* extracts depleted of endotoxin activity expressed only constitutive levels of ICAM-1, VCAM-1, and E-selectin.[127] Taking into account the inflammasome activation, *D pteronyssinus* activated caspase-1 and induced caspase-1-dependent release of IL-1β and IL-18 from keratinocytes, and stimulated assembly of the inflammasome by recruiting apoptosis-associated specklike protein containing a caspase-recruitment domain (ASC), caspase-1, and nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin-domain containing 3 (NLRP3) to the perinuclear region. Activation of the NLRP3 inflammasome by *D. pteronyssinus* was dependent on cysteine protease activity, leading the authors to suggest that dust mite allergens are danger signals for the skin and may play a pivotal role in the pathogenesis of AD.[128]

The knowledge of the mechanisms underlying the kind of sensitization and the type of clinical expression is far from exhaustive. It was long believed that the load of mite allergens which a subject is exposed to could be an essential factor in determining simple sensitization or clinical allergy. However, recent data have indicated that this relationship is not linear, as the highest exposure to dust mites was not directly associated with the risk of allergic outcome.[129] Instead, the mite species seem to have an influence on the type of allergy which subjects develop. In a large group of around 1,700 children, respiratory allergy was associated with both *D. pteronyssinus* and *D. farinae* exposure, while only the latter was associated with AD.[130] In a recent study, children with asthma and AD showed different sensitization profiles to HDM, with a sensitization pattern more extensive

and significantly higher IgE levels for Der p 1 and Der p 5 in the AD group.[131]

As far as allergy testing is concerned, the SPT or the measurement of specific IgE antibodies to HDM in serum is used to indicate sensitization, but they must be combined with the subject's case history to diagnose clinical allergy. The SPT and specific IgE strictly assess type-I IgE mediated allergic responses to a protein, without assessing the ability of the protein to induce inflammation. Patients with AD commonly show sensitization to a number of allergens, which increases with the severity of the disease.[132] In a cohort of 94 children with AD followed up from infancy to 7 years of age, 80% of the children became sensitized to aeroallergens and 75% of them noticed symptoms when exposed.[133] It seems conceivable that this phenomenon may be related to the mechanisms of skin barrier disruption, mucosal absorption of allergens, and consequent local inflammation described above. Among aeroallergens, HDM seems to play a major role.[134-136] However, the SPT has high sensitivity because of the high prevalence of sensitizations but it is nonspecific in distinguishing aeroallergen driven AD when compared to patient history. In fact, sensitivity for aeroallergens is comprised between 68% and 100% while specificity ranges between 33% and 71%. Thus, SPT is likely to detect a secondary phenomenon to the physiology of AD and is not a reliable marker to identify patients with aeroallergen-driven AD.[137]

The measurement of specific IgE level in serum has similar characteristics, regarding the linear association between increasing severity of AD and increasing levels of specific IgE to HDM and other aeroallergens,[138] the fact that patients with AD show higher HDM-specific IgE levels than patients with asthma,[139] and the diagnostic performance showing high sensitivity (65% to 92%) but low specificity (33% to 69%).[137] Instead, the APT assesses type-IV delayed hypersensitivity responses to a protein and was introduced in 1989 by Ring *et al.* as a tool to investigate the role of aeroallergens in atopic dermatitis.[140] Differently from SPT, the APT essentially assesses the patient's inflammatory response to a given protein. Such inflammation is thought to be primarily due to a delayed hypersensitivity reaction, but there are data suggesting that the direct proteolytic activity of the protein or binding of PAR-2 receptor may be relevant as well.[141]

It has been reported that patients with an air-exposed pattern of AD have significantly higher rates of positive APT results when compared to patients with non air-exposed skin areas.[142] The APT shows a clear advantage over the SPT in terms of specificity, which ranges between 65% and 95%.[137,143] Factors influencing the results include patient selection by AD severity and distribution, use of skin abrasion in testing methodology, type of protein, and concentration of protein.[137]

Indeed, there is notable evidence supporting the capacity of the APT to reproduce the pathophysiologic events of AD. In biopsy-based studies, a Th2 cytokine pattern was found 24 hours after APT, but a shift to a Th1 pattern, as occurs in chronic AD skin lesions, was noted after 48 hours.[144,145] A more frequent positivity to APT was reported in patients with allergen-specific lymphocyte proliferation and expression of activation markers on peripheral blood T-cells following *in vitro* stimulation with house dust mite, cat or grass pollen allergens, than in patients without lymphocyte proliferation.[146] Application of the APT to skin of subjects with AD was followed by an influx of inflammatory dendritic epidermal cells.[147] A significant increase of TEWL was reported in the site of APT application, both after 48 and 72 hours, compared with the control skin site.[148] By immunohistochemical analysis, the presence of IgE on Langerhans cells was demonstrated in positive APT reactions to *Dermatophagoides* in patients with mite-associated AD.[149] Clinically, patients with a diagnosis of intrinsic AD because of negative IgE tests actually had a positive APT for dust mites.[150] This aspect is of particular interest, because AD patients with negative SPT and IgE measurement in serum should be defined as nonatopic unless APT is performed. A number of studies evaluated how common such patients are, with different observations. In one study the rate of positive APT in nonatopic patients was 23%,[151] while in another study comparing AD patients with extrinsic and intrinsic forms, the rate of positive APT was 47.4% and 66.6%, respectively.[152] In a European multicenter study, which included 314 patients with AD, the frequency of clear-cut positive APT reactions ranged from 39% with dust mites to 9% with celery. A remarkable observation from the study was that positive APT in face of all SPT and sIgE testing negative was found in 7% of the patients, whereas a positive APT without SPT or sIgE for the respective allergen was seen in 17% of the patients.[153] This lead the authors to conclude that, as no gold standard for aeroallergen provocation in AD is available, the relevance of aeroallergens for AD might be evaluated by APT in addition to SPT and specific IgE measurement. New observations increased the value of the APT. In children with respiratory symptoms an exclusive positivity to APT with dust mites was observed[154] and another study reported that in 63 children with mite-induced asthma and rhinitis, all with positive SPT and specific IgE in serum, 16 (25%) were positive to mite APT too, indicating that delayed hypersensitivity reactions were involved.[155]

These observations lead us to investigate the possible factors underlying the positive result of APT in subjects with respiratory symptoms. In our first study, conducted on 297 children, we could demonstrate that in subjects with asthma or rhinitis a posi-

tive APT to dust mite was strongly associated with the presence of current or past AD.[156] Most subjects with respiratory disease but a negative history for AEDS had a positive SPT. Multivariate analysis showed that there was an increased likelihood of a positive APT result in patients with AD (odds ratio 17.4), in patients with AD and respiratory disease (odds ratio 21.9), and in patients with past AD and respiratory disease (odds ratio 22.8). These findings were confirmed in a study on a large population of 465 children aged 0.4 to 17.6 years. They were divided into four groups: group A, current AD (40 patients); group B, current AD with respiratory symptoms (156 patients); group C, past AD with respiratory symptoms (203 patients); and the control group, respiratory symptoms with no history of AD (66 patients). The APT was significantly more frequently positive in groups with current AD (groups A and B) or past AD (group C) than in the control group, while SPT and specific IgE in serum were significantly more frequently positive in the control group.[157] Such significant differences in response to APT in patients with diverse clinical expressions suggest that distinctive immunologic mechanisms underlie the different manifestations of hypersensitivity to dust mites. It seems conceivable that in subjects with a negative history for AD sensitization occurs by respiratory route and leads to the development of a Th2 pattern of response with ongoing production of specific IgE and consequent positive SPT and in vitro IgE tests. By contrast, in cases where the mite allergens enter through the skin, as can occur during exposure to common indoor concentrations of the major allergen Der p 1,[158] such entering being facilitated by its proteolytic activity and in the presence of a filaggrin-dependent skin barrier dysfunction, a different chain of events is likely to take place. This is ultimately revealed by positive APT and negative SPT and *in vitro* IgE tests.

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