Pathogenesis of Atopic Dermatitis (AD) and the role of allergic factors

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Abstract. – *Objective*. In this paper we will demonstrate that the exact pathogenesis of atopic dermatitis (AD) remains enigmatic, however the central defect is genetically determined, and the several dysfunctions we will highlight all point to a vicious cycle of allergen exposure, allergen-specific IgE production, and chronic Th2 cell stimulation. An important role is played by the late phase of IgE-mediated hypersensitivity, and evidence is accumulating that eosinophils actively participate in late phase-allergic reactions also in the skin.

Observations. AD is the first atopic disease to appear in the absolute sense: dendritic cells (DC) develop firstly in the skin and then in lung, in addition to homing receptors for T lymphocytes that are selective for skin localizations and not for lung. Among the DC, a primary role is reserved to Langerhans cells (LC) that express E-cadherin, a homophilic adhesion molecule that is prominently represented in epithelia. In addition keratinocytes and the interleukins (IL) they express are capable of activating a host of IgE-bearing cells.

Conclusion. Although much new information regarding the pathogenesis of AD has evolved over the past several years, the basic underlying etiology of this disorder remains elusive. Preventive measures are the only treatment for AD. We hope that the coming years will witness the development of new strategies for the treatment of AD, aimed at specific targets based on a thorough understanding of its pathogenesis.

Key Words:

Atopic dermatitis, Children, Skin immune system, T cells, IgE antibodies, Langerhans cells, Preventive measures.

Opening the Scenario: the Cells Orchestrating Cutaneous Inflammation

The principal cellular constituents of skin immune system (SIS)[1] are as follows: mast cells, T lymphocytes, LC expressing E-cadherin[2], keratinocytes, high endothelial venules (HEV), and several adhesion molecules[3]. Those cells together with eosinophils are strictly linked to organize the allergic sensitization. Contrary to general findings stressing that the normal skin contains [a] 8.000 mast cells/mm³, Irani et al.[4] have demonstrated that in AD they are [a] 20.000-40.000/mm³, and 94% are TC (tryptase and chymase). Mast cells participate in the IgE-mediated hypersensitivity reactions, and have been identified in the epidermis of AD patients[5]. Mast cells of the human skin, but not those of other tissues, have been found to be able to mount a secretory response (histamine and other mediators) to a host of non immunological stimuli, such as neuropeptides, including substance P (SP), vasoactive intestinal peptide (VIP), somatostatine, etc.[6], but not to eosinophil granule proteins, two of which, MBP (major basic protein) and EPO (eosinophil peroxidase), inhibit SP-induced histamine release from human skin mast cells[7]. Dermal mast cells contain and release IL, among which TNF-α (tumor necrosis factor-α)[8,9] which induces endothelial leukocyte adhesion molecule 1 (CD62E) due to cross-linking with the high-affinity receptor for IgE (FcϵRI)[9]. The direct activation of mast cells by IgE and the interactions also independent of CD40, and CD40L, which is expressed by both metachromatic cells, become apparent with the potential induction of IL$_4$, a clear explanation of the amplification and propagation of Th2-response[10]. This finding demonstrates that IgE-sensitization is a clear-cut reality.

Immunohistochemical staining of acute and chronic skin lesions in AD reveal a scarce number of infiltrating lymphocytes which consist predominantly of T cells (CD3, CD4,

CD45RO) and HLA-DR surface antigens with only occasional CD8+ lymphocytes[11]. It is tempting to speculate that the several immunologic and perhaps functional similarities between the thymic epithelium and the epidermis may explain the potential role of skin in the maturation of specific subpopulations of lymphocytes: even if the SIS lymphocytes express the two phenotypes, B and T, the great majority of cells locally present are T lymphocytes and perhaps no additional affection is associated with an exclusive infiltration of T cells[12]. In contrast, B cells are virtually absent[13]. In addition, there are recirculating memory cells (CD45RO+)[14], all with CLA (cutaneous lymphocyte-associated antigen), expressed by 45% of skin T cells[15]. The CD45 are activated, thus suggesting a previous contact with allergens, since virgin T cells localize poorly in skin[16]. Other studies have provided crucial data regarding vascular endothelial cells expressing high concentrations of skin-homing memory cells such as CD62E, CD54 (ICAM-1), and CD106 (VCAM-1)[16,17], with the HEV also expressing CD62E[18]. CD62E serves as a major skin-specific addressin in sites of chronic inflammation and interacts with CLA[18]. Additional counterreceptors on lymphocytes are α4fl1 = CD49d/CD29 for CD106 and CD11a = LFA-1 for CD54[16]. In the multistep paradigm[18], MCP-1 (monocyte chemotactic protein-1) is an additional important chemoattractant for T cells, and in this specific case is the receptor associated to Ga1 proteins, whereas CD49d/CD29 and CD11a/CD18 link respectively CD106 and CD54/102 (ICAM-2)[18]. Therefore adhesion molecules specific for the skin compartment bind preferentially T CLA+ (or MCP-1+) cells, thus favoring their accumulation in the skin chronic lesions, while the regional lymph nodes draining secondary lymphoid tissues link the cutaneous compartments with blood through afferent and efferent pathways[3,19]. In particular, TNF-α[18,19] has the capacity to act for selective subpopulations of circulating T cells, especially CD4+CD45RO+ expressing CLA or MCP-1 favoring T cells homing in skin, and their adhesion to endothelium[15]. The conclusion is that all these molecules favor CLA localization specifically in skin sites, while CLA negative is represented in lung tissues[20], therefore the lymphocyte T subsets are the inducers of atopic dysregulation firstly in

skin tissues, and secondly in lung, thus paralleling the LC absence in lung in the first moths of life[21].

Keratinocytes make up about 95% of the epidermal cells, and represent the main cutaneous cells releasing ILs. They may act as signal transducers, capable of converting exogenous stimuli, such as local injuries, mechanical irritations, UV radiations, into the production of epidermal proinflammatory ILs, integrins and chemotactic factors, as well as LCs, and as a consequence may initiate and exacerbate cutaneous inflammation[22,23]. The first cells to encounter the allergens, are now believed to play an active part in initiating and perhaps directing the mucosal immune response[22] by the release of various ILs[23]. Keratinocytes exert an active immunoregulatory role in concert with infiltrating peripheral blood mononuclear cells (PBMC); they aberrantly express class II HLA-DR antigens and CD54, correlated with lymphocyte infiltration, CD36 antigen[22], and cutaneous platelet-derived growth factor (PDGF)[24], in addition to releasing adhesion molecules. Skin keratinocytes do not normally express the HLA-DR antigens, but can be induced to do so in the presence of activated T lymphocytes in correspondence of primary sensitization. However, the lack of HLA-DR expression by keratinocytes may play a role in the induction of the T-cell stimulation leading to the differentiation of allergen-specific Th2 lymphocytes and the development of allergy[25]. Further studies will elucidate whether the Th2 differentiation precedes the lack of HLA-DR induction, or alternatively the lack of HLA-DR is the primary factor leading to Th2 activation[25]. On the contrary epidermal keratinocytes constitutively expressing CD80 can be transformed into efficient APC (antigen presenting cells), and play an important role in the resolution of DTH (delayed-type hypersensitivity) reactions in the skin based on their ability to induce T-helper cell clonal anergy[26]. Altered regulation of CD80 gene expression by epidermal cells may account for skin "hyperresponsiveness" encountered in chronic AD[26].

Recent studies indicate a role for eosinophil disruption and degranulation in modulating tissue destruction[27]. CD40 ligand (CD154) is functionally expressed on human eosinophils[28], thus promoting the shifting of

B lymphocytes to the IgE phenotype. Eosinophils are not only active in mediating allergic inflammation, but intervene in cellular networks with APCs, mast cells, and T lymphocytes[29]. Several potent, toxic and cationic proteins, have been observed in the eosinophil granules, including MBP, eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), and EPO. It was found that these proteins are implicated in tissue damage associated with skin inflammation however their role in the pathophysiology of AD is still unclear[27,29].

It was shown that some of these proteins are elevated in the peripheral blood of patients with AD[27]. Usually, serum levels of MBP are elevated in patients affected by various disorders associated with eosinophilia and correlate significantly with the number of peripheral blood eosinophils[30]. ECP and MBP levels are low in serum and/or in the skin of healthy subjects, and high in those affected (MBP 454 ± 90 versus 687 ± 299 ng/ml)[31,32]. However, both increased levels of MBP in the peripheral blood and peripheral activation of eosinophils have been demonstrated. Although peripheral blood eosinophilia is a common feature of AD, accumulation of tissue eosinophils is not prominent[33]. Several studies have shown eosinophil disruption and loss of morphological identity in the skin of patients with AD[32-34]. More recently, it has been studied the eosinophil degranulation in human skin tissues. Immunofluorescent staining of the affected skin demonstrated extensive deposition of MBC in the absence of many tissue eosinophils suggesting that such cells degranulate in the skin. In more than half of AD specimens examined, Leiferman et al. also found MBP deposition in a granular pattern deeper in the dermis. Extracellular MBP deposition was much more diffuse in the involved areas than in the uninvolved areas of the skin[34]. Interestingly, extensive MBP deposition in the skin was demonstrated in 2 children who experienced eczematous lesions after DBPCFC (double-blind, placebo-controlled food challenge), thus indicating again the role of food allergy (FA) and eosinophils in AD[34].

EDN deposition was studied by Leiferman in patients with AD[27]. AD skin specimens showed extensive granular extracellular EDN deposition in the upper dermis, thus producing further evidence for EDN role in AD. Electron microscopy examination showed eosinophil degeneration and disruption with many free granules in the dermis, thus corroborating the evidence of eosinophil degranulation in AD[27]. In addition, this study seems to indicate that peripheral blood EDN may be a more sensitive marker of eosinophil degranulation than peripheral blood MBP[27].

According to other authors, elevated serum levels of ECP in children with AD were found[35]. Serum levels of ECP were 12.2 mg/l ± 9.6 in children with AD, and 6.6 mg/l ± 3.7 in normal children ($p < 0.001$). Despite the absence of correlation between ECP serum levels and total IgE as well as between the absolute number of peripheral blood eosinophils and ECP serum levels[35], it seems likely that elevated ECP serum concentrations in patients with AD may reflect the eosinophil activation in the skin. However it has been reported that *in vitro* ECP can induce an increased histamine release[36] and can suppress T-lymphocytes function via a non-toxic mechanism[37]. It is therefore tempting to speculate that eosinophil cationic proteins, in addition to noxious effects for the skin, may contribute to the profound immunologic abnormalities described in patients with AD. The detection of ECP levels raised in serum of AD patients and correlated with disease severity[38] represents only an indirect measure of the pathological process taking place in the skin. Therefore ECP measurement may represent a non invasive tool to assess the clinical activity of AD in relation to eosinophil involvement in AD[35,38].

LC and macrophages

LC = CD1a+ (3-4%), the epidermal contingent belonging to the family of potent accessory cells termed DC, act as APC, express HLA class II antigens, and CD1a and CD4 antigens[39,40]. The function of the cytoplasm granules of Birbeck is as yet poorly known[41]. LC located in the suprabasal layer of epidermidis express E-cadherin, the homophilic adhesion molecule that mediates their adhesion to keratinocytes *in vitro*[2]. LC express also CD11a, CD11b, CD36 and HLA-DR in chronic lesions, at variance with normal skin[42,43], and in addition CD54[44], CD80 and CD86[45]. A major breakthrough in our understanding of AD pathogenesis oc-

curred with the demonstration of membrane-bound IgE on epidermal LC[42,46]. Studies with CD1+ have demonstrated that LC in AD bind both FceRI and FceRII = CD23[46], in different proportions: 6,63 ± 1,92 versus 0,67 ± 1,12 cells, respectively[47], an upregulation of FceRI not present in allergic contact eczema[48]. However the functional role for CD23 should not be undervalued, since its expression is upregulated by IL_4[15]. In addition a facilitated antigen processing through CD23 has been found. Specific T-cell responses could be detected using 1000-fold lower levels of serum IgE complexed allergens in a CD23-dependent system[49]. Mudde et al.[50] have shown that IgE+ LC but not IgE- LC were capable of presenting Der p allergens to T cells, thus suggesting that cell-bound IgE on LC may facilitate binding of allergens to LCs prior to their processing and presentation. As a consequence, the expression of IgE-bearing LC in AD may have serious pathogenetic outcomes.

Macrophages infiltrating into the AD skin lesions have been shown to bear CD23 on their cell surfaces[51] expressed in response to IL_4[52] or GM-CSF[53], and are recruited by monocyte chemotactic protein-3 (MCP-3) and RANTES (regulated on activation normal T expressed and secreted) in the skin of human atopic subjects[54]. It has also been demonstrated that allergens activate IgE-bearing macrophages in an IgE-dependent manner, with formation of leukotrienes, PAF (platelet activating factor), IL_1, and TNF[55,56]. IgE-bearing macrophages may also been activated by autoantibodies to IgE, which can be present in patients with AD[57]. Taken together, these data suggest that although IgE-bearing LC and macrophages can be found in other inflammatory skin conditions, such as psoriasis, these skin diseases are not associated with the production of allergen-specific IgE[13,42].

LC as APC and T lymphocytes

LC are the most potent cells in the epidermis as regards the presentation of allergens. The presentation of inhalant allergens in patients with AD can be facilitated by the binding of allergen to LC-bound IgE. After contact with allergen, some LC become activated, exit the epidermis, thus the LC-bound allergen is transferred to the dermis, finally the LC migrate to T-cell-dependent regions of regional lymph nodes where they localize as mature LC[58-60]. LC process protein antigens, express high levels of HLA class I and II and present Der p allergen to naive T cells, inducing their activation[50,61], and to mast cells, stimulating the release of mediators. As to the pathogenesis of AD, the lymphocyte activation must be viewed as the most significant reaction-sequence[62]. Without doubt the allergen-specific T cells activated by allergen-IgE+ LC release IL_4 and IL_5, consequently B lymphocytes in the afferent lymph nodes synthesize IgE and the bone marrow forms eosinophils. Therefore, high serum IgE levels and peripheral blood eosinophilia are crucial phenomena in AD related to the stimulation of T cells by IgE-bearing LC after the binding of allergens[41]. Since no B cells are found in AD patients[42], the T lymphocyte activation must be translated systemically rather than locally, thus explaining the strongly elevated serum IgE levels and peripheral blood eosinophilia in such subjects[62]. The IgE thus formed will provide LC with cell-bound IgE: as a result LC will unremittingly stimulate allergen-specific Th2 lymphocytes rather than mast cells[41]. It is possible that the forces that drive differentiation into Th2 cells in AD patients are related to an abnormality in APCs[15], as recently confirmed by studies on TAP[63]. Th2 amplification favoring decreased IFN-γ and the resulting high IL_4 production in AD promote IgE synthesis[64].

Cytokines and AD

Summing-up the data as yet reviewed on the role of ILs released by epidermal cells in the pathogenesis of AD, we show the origins of skin ILs in Table I[23,65]. TGF-β and IL_{10} have been identified as inhibitors of several ILs[66]. In addition TNF-α expressed by mast cells up-regulates CD62E on keratinocytes,

Table I. Cells producing skin cytokines.

- LC: IL_1, IL_6, IL_8, IL_{10}, G-CSF, GM-CSF, M-CSF, TNF-α;
- Keratinocytes: IL_1, IL_3, IL_6-IL_8, IL_{10}, G-CSF, GM-CSF, M-CSF, PDGF, TGF-α and -β, TNF-α;
- Melanocytes: IL_1, IL_6, IL_8, G-CSF, GM-CSF, M-CSF, PDGF, TGF-α and -β, TNF-α

Data from 23, 65.

facilitating their interactions with CD11a/CD18-T cells[67]. The IL production by T-lymphocyte clones in AD is summarized in Table II[68,69].

The Background: Genetic Factors

The genetic factors form the background of the *atopic diathesis of AD* which is known since 1916[70]. Positive associations with the HLA system have been thoroughly examined but not fully established[71]. To date no definite consistent association has been found with HLA-A, -B, -C and -DR antigens[72], neither with a gene on 11q13[73], nor in children with cow milk (CM) allergy (CMA) and in their parents with alleles HLA-A, HLA-B and HLA-DR, or with alleles of HLA-D domain, such as DQ and DR52/53[74]. A recent study in children with AD and CMA found a positive association with HLA-DQ, and that DQ+ children had a prevalence of humoral rather than cellular responses[75]. Additional positive associations have been established with DPB, DRB[76], HLA-A1 and -B5 in patients with DA, but complicated by asthma and/or allergic rhinitis (AR)[77]. Kuwata et al.[63] studied the transporter (TAP genes) polymorphism associated with antigen-processing genes in AD and reported a tendency toward an increased frequency of TAP-1 637Asp in subjects with AD. This, they concluded, may contribute in combination with DRB1*1302/DQB1*0604 to the pathogenesis of AD[63]. An association between AD and genetic variants of mast-cell chymase (MCC) located on chromosome 14q11.2 has been found[78]. Since there is no association of MCC with atopic asthma, AR, or non-atopic asthma, these findings suggest that the genetic basis of AD may involve the interaction of variants of MCC (promoting skin inflammation)[78] and FceRIβ on chromosome 11q (promoting enhanced IgE responses and atopy)[79]. In severe AD patients was found an allele for the a subunit of the IL_4R that segregates with atopy, thus predisposing persons to allergic diseases[80]. Further, AD patients produce IgE antibodies against environmental allergens regulated by CD4+ HLA-class II restricted, although cytotoxic CD8+ HLA-class I restricted subpopulations may predominate in the infiltrates in AD lesions[81].

As regards the genetic inheritance[82], the available evidence suggests that AD is inherited with an autosomal dominant or recessive pattern, always associated with abnormal IgE production, however the etiology of this disorder remains enigmatic[83]. The mode of inheritance is not consistent and may display autosomal dominant and autosomal recessive patterns, although the autosomal dominant trait has been reported more frequently, in association with a maternal pattern of transmission[73,84]. Accordingly, AD is less likely a monogenic disorder with essentially weak penetrance, but the interaction of multiple genes and multiple exogenous or environmental factors outline a multifactorial inheritance pattern with a polygenic component, which better encompasses the variety of biochemical and immune abnormalities involved in the phenotypic expression of disease. Nonetheless, bone marrow transplantation experience has demonstrated clearing of AD with normalization of IgE serum levels in patients with Wiskott-Aldrich syndrome (WAS) following

Table II. Cytokine production by T-lymphocyte clones in AD.

Cytokines	Dpt-specific CD4+ Atopic donors	T-lymphocyte clones non-atopic donors
IL_2	+	+++
IL_4	+++	–
IL_5	+++	+
IL_6	+++	++
GM-CSF	+++	+++
IFN-γ	+	+++
TNF-α	+++	+++

Modified by 68, 69.

successful engraftment[85]. Conversely, transfer of AD, and latent atopy, and antigen-specific IgE has also been observed after successful reconstitution with atopic donor bone marrow[86]. As Sampson points out[13], taken together these reports suggest that a genetically inherited bone marrow-derived cell is central to the immunopathogenesis of AD.

The role of atopic heredity in the development of AD is well established, and is a significant risk factor (OR > 1)[82]. In monozygotic (MZ) twins the pairwise concordance rate is 0.72 versus 0.23 in dizygotic (DZ) pairs[72], and in MZ boys is 0,54 versus 0.35 of DZ boys while for girls is 0.73 (MZ) versus 0,40 (DZ)[87]. Twin studies also demonstrate that offsprings of parents who had AD are at higher risk for AD than even those with parents having other atopic disease[72]. Genetic effects may account for 33-76% of the variation in liability to atopic diseases, however twin girls have a higher risk of being diagnosed with AD than boys[87]. Regarding the risk factors in the progeny, parental AD significantly increases the risk of early development of AD (OR 2,5-3,4), compared with children with parental asthma or AR (OR 1,4-1,5)[82]. In addition, when both parents have atopic disease of the same sort, the risk of atopic disease in their child is 80%; if parents have different atopic disease the child has a risk of 61% of developing a similar phenotype, and when only one parent is atopic the risk at 2 years is 38%[88,89]. Available evidences call attention to a possible parallel between AD and T-cell PID (primary immune deficiencies) characterized by high IgE levels, thus leading to the proposal that AD might fit a pattern of PID[85,90]. However such an assumption can be rejected since clinically in patients with AD no firm evidence for systemic immunosuppression has been consistently put forward[15], in addition the analysis reveals that AD itself is not a manifestation of decreased CMI (cell mediated immunity), neither it is precipitated by an increased susceptibility to generalized infections[91]. However, the prevalence of severe skin infections, bacterial, viral and possibly by fungi and yeasts[15], as well as the decreased responsiveness to contact allergens[92], the cutaneous anergy to skin prick tests (SPT) with several antigens, and a reduced dinitrochlorobenzene sensitization[42], point to an abnormal regulation of SIS[90].

In AD children *several immunologic abnormalities* (Table III)[15,42] have been found which can be regarded as the result of decreased activity of cyclic nucleotides, such as reduced T-suppressor activity, exaggerated IgE concentrations, increase of cyclic-AMP PDE (phosphodiesterase) activity, abnormal cutaneous permeability barrier, where abnormalities of EFA (essential fatty acids) metabolism may explain the dry skin and the increase in trans-epidermal water loss characteristic of AD[93] (*biochemical abnormalities*).

Implications for the Pathogenesis of AD

Immune abnormalities

Several lines of evidence suggest that a variety of qualitative and/or quantitative im-

Table III. Genetic-immunologic and clinical features of AD.

Genetic-immunologic
Genetic background of atopic syndrome
Increased levels of allergen-specific IgE in serum and skin
Normal serum IgA, IgG and IgM concentrations
Preferential expression of allergen-specific Th2 lymphocytes
Decreased CD8 suppressor/cytotoxic number and function
Increased expansion on CD23 on B and mononuclear cells (PBMC)
Increased production of IL_4/IL_5/IL_{10} and other Th2-like IL
Decreased levels of IFN-γ/IL_{12}/IL_{18} and other Th1-like IL
Deficit of PBMC not secreting IFN-γ/IL_{12}
Increased basophil releasability
Persistent macrophages activation with hypersecretion of GM-CSF, PGE_2, IL_{10}
Autonomic nervous system dysregulation
Disturbed essential fatty acid metabolism
Role of foods and food additives
Role of aeroallergens
Role of infections
Clinical
Typical flexural localization of skin lesions
Clinical course irregular and unpredictable
Severity of pruritus and inflammation
Skin hyperirritability
Exacerbations by stress and irritant factors

Adapted from 15, 42.

mune abnormalities demonstrated *in vitro* and *in vivo* (Table III) are correlated with mostly decreased proliferative responses of PBMCs to mitogens and antigens[94]. As a consequence, T cells express in AD patients low levels of ILs[95] and less IL_1 than healthy subjects[96], whereas high concentrations of IL2R are frequently observed when the eczema is most florid, and are correlated with the extension and the severity of the clinical pattern[97].

Perhaps more importantly the absolute number of T cells appears to be within the normal range but it is decreased that of T-cell subsets, especially the Th1-subpopulation: 52% of T cells were Th2, 44% Th0, and only 4% Th1[98]. Studies on skin-derived Der p-specific T cells in AD subjects reveal that 42,2% of T clones express the Th2 phenotype and only 11,5% that of Th1[99], or 70% the Th2 and 15% the Th0 subpopulations[100]. The CD4/CD8 ratio in T-cell clones may be increased till to 200% compared to controls[101], however other workers were unable to reproduce these findings[91,102]. The Th1 dysregulation appears to reside in the CD29+ memory subset, in this case not correlated to the severity, or the extension of eczema, but principally to the decreased CD8 subpopulation, which in the skin infiltrate is definitely less than that of CD4 cells[94]. Studies with AMLR (autologous mixed lymphocyte reaction), which is thought to represent an inducer circuit for the activation of CD8+ effector cells[83] have found a marked deficiency or decreased responsiveness in all studied patients with AD, but also in individuals with no known evidence of skin disease[103]. Since the defect was associated with a reduced number of circulating T cell bearing the surface marker CD29, the impaired generation of CD8+ is considered secondary to this defect. The quantitative deficit of CD8 has been also found in T allergen-specific clones of atopic donors: 92% were CD4 and only 8% CD8 (11,5:1). In a subsequent study employing flow cytometry and hemocytometry, the ratio was 4:1, with the percentages of CD4 cells significantly higher in chamber fluids than in peripheral blood, while the reverse was true for the CD8 lymphocytes[104].

From a *functional* point of view, the regulation of *in vitro* IgE synthesis by CD8 cells, and the generation of Con-A-activated T lymphocytes in atopic subjects appears to be abnormal[91]. However the significantly reduced proliferation index in PBMC from AD patients compared with normal PBMC may be consistent with previous studies[105]. Such a deficit could occur in atopic individuals because of the altered PGE_2 regulation of CD8 subsets[105], even if the CD8 impairment might also be due to LTB4[106]. The flow cytometry shows a decrease in cytotoxic CD8 T cells expressing S6F1bright[107], a characteristic that, along with the similar impairment of NK-cell activity, and according to the AD active and quiescent clinical stages as seen from Table IV[108], may partially account for the increased susceptibility to viral infections, particularly

Table IV. Cytokine production by T-lymphocyte clones in AD.

	Active phase	Quiescent phase	Normals
	Mean ± SD	Mean ± SD	Mean ± SD
Chemotaxis	58,18 ± 18,75	57,13 ± 14,93	89,03 ± 5,19
Cytotoxic activity	20,42 ± 4,41	26,61 ± 4,71	44,74 ± 5,18
Severity of AD (mean ± SD)			
	Mild	Moderate	Severe	Normal
Cytotoxic activity	25,04 ± 1,87	20,39 ± 3,29	16,24 ± 2,77	44,74 ± 5,18

p (patients versus controls) < 0.0001; SD = standard deviation.
Adapted from 108.

with *Herpes simplex* (HSV)[109]. The selective alterations of NK-cell activity may be the result of the severity of AD more than the dysregulation of IgE antibodies, or of T-cell subpopulations[94]. In this regard, NK-cell activity can be reduced *in vitro* by adding PGE_2 and restored by IFN-β and -γ addition[110].

Suggestive findings support the concept of a *hereditary nature* of the abnormalities of cell-mediated immunity (CMI), since in neonates with a family history of atopy have been observed lower numbers of T cells than in babies with no genetic predisposition for atopy[111], as well as lower CD8 counts in 1-2-month-old infants who developed AD than babies who did not[112]. No univocal results have confirmed that the T-cell imbalance in AD is of primary nature[94]. Recent data suggest that there are no definite data corroborating such a defect: it is true that in healthy infants with AD aged 0-1 years the mean level of CD8 cells was 5,5%, but this concentration subsequently increased up to 7,5%[113], in older children aged 0-6 years there were no quantitative deficits of CD3, CD4, CD8 and CD19[114]. However CD8 levels are 21%, less than a half of CD4 levels (41%), ratio = 1,9, and B cells only 22,5%, evident especially in the first year of life in healthy infants[115]. Consequently the Th2 lymphocytes, facing the CD8 deficiency, trigger a vigorous proliferative response, leading to hyper IgE and the skin lesions. On the other side, from a general point of view it is difficult to demonstrate that AD develops earlier when at birth or during the first months of life there is a CD8 T cell number and function deficiency with parallel increase of the CD4/CD8 ratio. Based on this background, much remains to be clarified in relation to the development of AD.

More crucial are the *qualitative deficits*; several data suggest an immune imbalance in lymphocyte activation, such as the decreased function of cytotoxic T subsets (which again may account for the increased frequency of viral infections), in addition to the increased numbers of non T non B cells and the restoration *in vitro* of both the number and function of lymphocytes[116]. However, AD cannot be simply defined as a clinical manifestation of decreased CMI. More importantly, immunohistologic studies of the cellular infiltrate and studies directed at circulating parameters of CMI indicate a vigorous T-cell activity in AD lesional skin[83]. The increased T-cell reactivity is correlated to the predominant Th2 phenotype, which *downregulates the function and activation of the Th1 cells:* Th2 lymphocytes secrete a wide spectrum of ILs, especially skin-derived IL_4 that affects the switch of immature B lymphocytes to antibody-secreting cells, and activates IgE receptors on LC and monocytes infiltrating lesional skin, automatically antagonizing the expression of Th1 and IL_2R. It is of note that a reduced production of IFN-γ (on chromosome 12q22-24) has been found in cord blood mononuclear cells cultures of neonates with positive family history, therefore at high risk of developing atopic disease compared with neonates without family history of atopy[117-121].

It is not known as yet exactly how the different types of T cell contribute to AD pathogenesis[95], nor which are their relationships with IgE antibodies. However both Th2 and IgE predominance are coordinated. IgE synthesis is promoted by IL_4 and inhibited by IFN-γ[68,122]. In addition, there is *a significant relationship between the increase of IgE and IL_4 levels*: consequently several factors are likely to play a role:

- increase of Th2 subsets secreting IL_4 and IL_{10} induced by IL_1;
- establishment of a Th2-like pattern of IL production, due to specific clones of allergen-specific CD4+ T lymphocytes with an excess of Th2[123], which drive an uninterrupted production of both IgE and ILs[124];
- increased synthesis of IL_4 and IL_{10} and the ensuing inhibition of IL_{12}, are the critical factors underlying the aberrant IL production profile that promotes the suppression of IFN-γ production[125,126];
- skin-infiltrating Th2 cells after allergen exposure secrete IL_3-IL_6 and GM-CSF which favor the migration, differentiation, and survival of IgE and eosinophils;
- over-expression of IL_4 by allergen-stimulated mast cells, and of IL_4 and PGE_2 by monocyte-macrophages infiltrating the chronic AD lesion, either of which inhibits IFN-γ, thus further amplifying the Th2 response[122,127,128];
- increase of PDE activity by stimulated T cells, which has been suggested to direct differentiation into Th2 type cells, with a high IL_4 production[90].

Interestingly, the demonstration at the skin level of the concomitant *release by PBMC of atopic donors of reduced quantities of IFN-γ and of substantial amounts of IL₄*[129], was detected only 2 h after the antigenic challenge in mite allergen-induced dermatitis in atopic subjects, even before the gene expression[130], and in highly atopic children aged 5.4 years (mean)[131]. Thus in all likelihood *the relevant expansion of allergen-specific Th2 cells, through the upregulation of IL₄ levels, have an ongoing impact on the chronic stimulation of IgE synthesis, on the induction of Fcε receptors on B cells and on molecules crucial for antigen presentation, such as monocytes and LC, as well as on the proliferation of mast cells and perhaps also of eosinophils through IL₅*. The dominant role of T cells has been confirmed *in vivo* in children with AD by the spontaneous expression of IL₄ mRNA as compared to controls, although in neonates and children under 10 years of age IL₄ secretion was found to be significantly reduced[132]. There is evidence of high frequency of IL₄-producing CD4+ allergen-specific T lymphocytes in AD lesional skin and not at all IFN-γ, contrary to the findings in healthy individuals[100]. Several workers have found in AD significantly lower IFN-γ concentrations[133-136], and much higher of IL₄ compared to controls ($p < 0.0001$)[136]. It has been subsequently shown that despite the reduced secretion of IFN-γ, atopic children have an increased percentage of IFN-γ-producing cells in unstimulated PBMC cultures compared with controls, thus evidencing a post-transcriptional defect[137]. Consequently, *the inability to secrete IFN-γ* due to a possible impaired IL₁₂ or IL₁₈ production[138] *together with the abnormal T lymphocyte function may contribute not only to the enhanced synthesis of IgE, but also to the high IgE levels in 80% of the children.* The significant abnormalities which sanction the exit of Th1 lymphocytes and Th1-like ILs may be of paramount importance to the pathogenesis either of atopy, or AD. A similar immune dysfunction is directly involved in children with WAS or AD[85].

The pathogenetic role of IgE antibodies (atopic status)

We will now consider the influence of total and specific IgE levels on the clinical manifestations of AD. There are no doubts that the major immunologic contribution to pathogenesis is the IgE-mediated sensitization. Table V[11,82,84-85,139-146] summarizes some important features which speak in favor of the *role of IgE antibodies*, which recently has been further strengthened in view of the following points:

• precisation of the function of CD154 which is known to play an important role in the induction of *the synthesis of IgE also in peripheral tissues, such as the skin*[10,28];

Table V. Evidences in favor of the role of IgE in AD.

1) Family history positive for AD in 42-90% of children
2) Statistically significant high IgE levels in about 80% of children
3) About 85% of patients have positive immediate SPT and/or IgEs to a variety of food and inhalant allergens
4) Serum IgE levels are highest in children with coexisting respiratory allergy
5) Substantial evidence supports the notion that IgE levels are low during remissions, and higher during flaring of AD
6) 50-80% of children has coexisting allergic IgE-mediated manifestations such as AR and/or asthma, and food allergy (FA)
7) Positive/negative effects of bone marrow transplantation
8) Several studies correlate eczematous flares with immediate and/or late-phase skin reactions after SPT and/or patch tests with inhalant and allergens, for example after application of mite extract to the abraded skin of subjects with PTC positive for Der p 1
9) Association between clinical manifestations and food allergens in 30-50% of children; in 33% of subjects undergoing DBPCFC
10) Tthe removal from potential allergens in infants' home environment (sea resorts) leads to clearing of eczematous skin lesions
11) Flaring of skin lesions after exposure to environmental allergens and resolution after allergen elimination
12) Reduced prevalence of AD in at risk babies if food allergens are eliminated during the first year of life

Adapted from 11, 82, 84, 85, 139-145.

- identification of LC *in vivo*[47];
- presence of IgE anti-*Staphylococcus aureus*[147,148] and anti-Candida[149] in patients with skin colonized by such agents.

In AD there are no definite data indicating a preferential expansion of IgE-mediated mechanisms, because the routine histological appearance of eczematous lesions rather shows a close resemblance to a classical type IV cell-mediated hypersensitivity reaction, certainly not dominated by IgE[15], although a not conventional one[35]. However, additional evidence of the role of IgE-mediated mechanisms is indirectly confirmed by the recent demonstration that:

- mechanisms involving IgE molecules in IgE-mediated skin reactions may also follow in the LPR (late-phase reaction), still involving allergen activation of cutaneous mast cells bearing FceRI on their surfaces[150],
- the release of IL_1 at sites of human cutaneous allergic reaction during the DTH also may be involved in the IgE-dependent inflammation of eczematous skin since it is associated with the activation of LC[96],
- children with AD developing reactions to a positive DBPCFC were found to have a rise in plasma histamine levels but not after placebo challenge[151] in addition to skin reactions[145],
- skin biopsy specimens of uninvolved sites obtained before DBPCFC have not revealed the eosinophils which instead were found to infiltrate the skin lesions 4 and 14 hr later[151],
- food allergen-induced mast cell activation has been shown to trigger either an immediate reaction or a LPR in the skin[151],
- higher rates of spontaneous histamine release from basophils in patients with AD and food hypersensitivity compared with control subjects[152],
- eosinophils link IgE molecules in skin lesion through both FcεRI and CD23[153],
- studies till yet referred to have evidenced the presence also of FcεRII on the surface of several cells, such as eosinophils, macrophages and platelets[154], thus ready to respond to IgE, and very plausibly involved in IgE-mediated skin reactions[150].

These, and other cells, as well IgE molecules constitute the inflammatory infiltrate. During the early phase of mast cell activation, patients developing a pruritic, erythematous, macular or morbilliform rash following relevant allergen exposure to a DBPCFC were found to have an increase in plasma histamine[139]. Four to eight hrs following initial mast cell activation, onset of an IgE-dependent LPR occurs: biopsies obtained at the 8-hr limit reveal 48% lymphocytes, 27% eosinophils, 9% neutrophils, and 5% monocytes[13]. These two phases are thus characterized by IgE-mediated hypersensitivity reactions. However the IgE system is not the only immune pathogenic mechanism in AD, even considering its place in LPR and FA: there are several pieces of observation against the role of IgE antibodies in the pathogenesis of AD. The role of anti-IgE is further enlightened by the points summarized in Table VI, often complementary to those analyzed in Table V[155,156]:

The role of Th2 cells

There are several mechanisms regulating selective migration of Th2-like lymphocytes into lesional AD skin. T cells (4×10^{12} in the skin of a normal adult), typical components of SIS[1], are the only cells within the involved skin which proliferate on recognition of pro-

Table VI. Evidences against a role of IgE in AD.

- A marked elevation of serum IgE is found in several diseases, not neces-sarily atopic
- About 15-20% of normal children have high concentrations of serum IgE without any clinical manifestation of atopy
- About 15-20% of children have AD without apparent signs of hyper IgE production
- The synthesis of IgE could be secondary to a functional imbalance of the immune mechanisms controlling it
- IgE antibodies are not a prerequisite for the development of skin lesions, since AD can manifest itself even in not atopic children
- The typical skin lesion, the pomphus, is not a specific marker of IgE-mediated allergic reaction of AD
- AD is present in patients with agammaglobulinemia, normal IgE levels, and PTC negative for allergens, as well as in WAS

Adapted from 155, 156.

cessed allergenic peptides presented by the APC, become activated upon presentation of allergens, consist predominantly of cells with immune memory, are enabled to proliferate and recirculate, to be rapidly recruited to migrate to the target tissue, the skin, during human cutaneous allergic LPR[1,116,157]. This tissue-selective homing is regulated in large part at the level of skin disease-related T cells by the significant expression of CLA, that we know as a skin homing receptor for T cells[10]. Influx of T lymphocytes into cutaneous sites of inflammation may be stimulated by increased expression of adhesion molecules on vascular endothelial cells, such as CD62E induced by IL_1 and TNF-α[10]. The importance of the release of these IL in the local accumulation of inflammatory cells is highlighted by the inhibition by neutralizing antisera to both IL_1 and TNF-α[158]. The ensuing transmigration of memory CLA-T cells is possibly modulated by interactions among CD11a/CD54, and CD49d/CD29/CD106[159]. The activation is substantiated by high levels of both CD62E and CD49d/CD29 expressed by > 90% of skin T lymphocytes[104]. This is, through the release of several IL, the *connecting point between the synthesis of IgE, eosinophils, mast cells, monocytes and basophils*[158]. Th2 cells, in addition to controlling the synthesis of IgE and the infiltration of the eosinophils, may play therefore a direct role in the inflammation, and on the production in the bone marrow and maturation of mast cells and particularly of eosinophils, also playing a prominent role in prolonging their local survival through IL_{3-5} and GM-CSF[66]. Th2 lymphocytes interacting with eosinophils are known to have upregulating effects on their growth, mobility, tissue localization and activation[160] because of their high eosinophilotropic IL_5 secretion[161]. The point reference for skin T cells is IL_4, their growth factor, the cellular infiltrate in allergen-induced skin LPR expresses increased mRNA for IL_3, IL_4, IL_5 and GM-CSF, yet no mRNA for IFN-γ[162]. However the secretion of IL significantly increases by adding IL_2 to the culture medium, demonstrating that T cells infiltrate skin sites after 12 hr[40] or after 12-24 hr[162]. Clearly IL_2 and IFN-γ are active in healthy individuals, but in patients with AD the levels are not significant[163]. The levels of IL_2 are reduced also due the poor production from PBMC[164], unlike

IL_4 and IL_5[165]. Immunohistochemical analysis of the inflammatory infiltrate in skin lesions revealed that the PBMC infiltrate in the epidermis consisted predominantly of T cells, with subsequent invasion of dermis following RANTES stimulation[54]; however 5 hr after antigen challenge also the levels of chemokines IL_8 and MCP-1 are significantly higher than the controls[166]. T cells bear the CD3+ /CD4+45RO+ surface antigens with class II HLA markers[11]. In a pediatric cohort the CD45RO were 87,9 ± 7,6% of CD4 versus 6 ± 3,7 of CD45RA[14], employing more sophisticated analysis the ratio was 9:1 and on peripheral blood about 2:1[140], with an inverse ratio in young infants[167], at the age of onset of AD[168]. The activated lymphocytes express IL_2R[169], an observation confirmed by the substantial expression of HLA-DR+ from endothelial cells and the CD4 antigen of LC[164]. In the absence of a definite association between the extension of clinical lesions and the number of cutaneous T cells, this is significant between the number of CD4+ and that of eosinophils after 24 hr, still noticeable 48 hr after onset of the reaction[169]. During the accumulation of T lymphocytes at the site of allergen-induced LPR *resident cells in the skin, such as keratinocytes,* rather than infiltrating leukocytes *appear to be the source of Th2-like IL*: IL_{3-5}, IL_1, and IL_8 chemotactic for lymphocytes, GM-CSF and IL_7 as growth factors for some IL[23].

It has become increasingly appreciated that additional cutaneous events are involved in the activation of T cells, such as IL_6 elicited during the allergen-induced immediate reaction and the LPR, closely correlated with the infiltration of eosinophils. Since the induction of IL_6 does not result in the appreciable migration of these inflammatory cells in injured skin tissue, this may be mediated by the predominant expression of another chemotactic factor, for example PAF[13]. Studies *in situ* of skin biopsies, in allergen-induced cutaneous LPRs in atopic subjects, have revealed the expression only of Th2-like IL[162], a not surprising occurrence, being the allergen-specific CD4 in lesional atopic skin by a large majority Th2[64,98,100,135]. Indeed PBMC of AD patients have low levels of IFN-γ and high of IL_4[64], while adding to the sopranatants of patients with AD and controls an anti-IL_4, the concentrations of IFN-γ increase significantly

only in the controls, whereas the concentrations of IgE in the patients are significantly higher than the levels of controls[64]. The inability to produce IFN-γ may further confirm the raised IgE synthesis and sustained T-cell activation observed in AD patients.

Surprisingly, the contemporary demonstration of *in situ* expression of Th1-like (IFN-γ and IL$_2$) and Th2-like (IL$_4$) in skin biopsy samples shows that skin lesions are not caused by the exclusive expression of either Th1- or Th2-like ILs[170]. Recent studies suggest a two-phase model for the pathogenesis of AD, with a switch from an initial Th2 to a Th1 response *in situ*, that is a cutaneous LPR mediated by memory Th2-like cells secreting IL$_4$ and IL$_5$, histologically indistinguishable from the Th1- and IFN-γ-modulated LPR[171]. How can we differentiate the two phases? These apparently contrasting findings may indicate the different kinetics of IL$_4$ and IFN-γ expression by T cells after allergen-specific stimulation[172]. Recent paradigms of the etiology of AD have suggested that in an early phase Th2-like ILs play a crucial role for initiating eczematous lesions[170], while with the subsequent influx of Th1 cells, IFN-γ may be critical for the induction of keratinocyte CD54 expression[170], also showing detrimental effects with the subsequent accumulation of inflammatory cells in injured skin[171]. The apparent contradiction of LPR mediated by both the Th1 and Th2 cells can be explained by a study showing that also Th2 lymphocytes may be crucial for the induction of atopic eczema[173], by attracting inflammatory cells and inducing IgE production[171].

Children with food-induced AD provide an unique opportunity for performing studies on the allergen-induced lymphocyte proliferation which have demonstrated that in addition to IgE-mediated mechanisms Th1 cells can release levels of IL$_2$ and IFN-γ higher than the controls[174]. Further evidence is shown in children with CM-induced AD, whose PBMC displayed significantly higher levels of CLA than reactive T cells from nonatopic control subjects or atopic children without AD[175]. Since the control subjects failed to express CLA one can postulate that the high CLA expression of casein-reactive cells may facilitate the localization in skin of such T cells, thus playing a key role in determining cutaneous lesions[175]. In addition fresh-

ly isolated circulating CLA+ T cells in patients with AD, but not in normal control subjects, selectively highlighted both evidence of activation (HLA-DR expression), and spontaneous production of IL$_4$ but not IFN-γ[176], thereby showing to be Th2 cells. Accordingly we can outline a second phase of inflammation when Th1 cells predominate in skin lesions[171], along with Th1-like ILs such as IL$_2$ (and sIL$_2$R) and IFN-γ but also IL$_4$[162,170,177]. Taken together, these observations strengthen the two-phase model of LPR, with an early phase dominated by Th2 cells followed by a switch in Th1 cells. This switch of profile is probably imposed by the gradual production of local IL$_{12}$ that selectively promotes the production of IFN-γ by T cells[178]. Thus far it is unclear which Th-like IL(s) contribute to perpetuate the local inflammation, otherwise the switch represents *an attempt by the body to restore homoeostasis in the skin, which however is unsuccessful in subjects with AD*[171]. The existence of two T phenotypes may be explained considering the Th2 effector of the missed attempt of inhibiting Th1, to limit the tissue damage following the activation of these T lymphocytes. We have till yet stressed the severe deficiency of IFN-γ in at risk neonates and infants: paradoxical results show on the one hand down-regulating effects on the IFN-γ response caused by the inhibition of IL$_{12}$ due to excess production of IL$_4$ and IL$_{10}$[179], on the other substantial amounts of cells producing IFN-γ in children with severe AD compared with normal controls[131]. Finally the data regarding T cells are not convincing, being in the majority of cases the Th2 in the first place, implicated in the atopic responses, while T-cell clones specific for *Candida albicans* and tetanus toxoid of atopic donors mainly produce Th1-like ILs[83], confirming that the differentiation of Th0 into Th1 or Th2 along with the distorted IL profile is determined by the characteristics of the allergens in addition to genetic factors[66]. In this regard, recent studies support the likelihood that a genetically determined aberrant expression of type-2 ILs may play a role in certain patients. Indeed the studies indicate an association between high levels of total IgE and increased expression of genes located in the region of chromosome 5q in position 31-33[180-182]. It is not without significance that the clus-

ter of genes of type-2 ILs IL_3, IL_4, IL_5 IL_9, IL_{13} is located in the same *locus*, along with GM-CSF, IL_6, CSF and CD14[183].

The role of inhalant allergens

Since 1918[184] there is mounting evidence that percutaneous absorption of allergens can contribute to AD pathogenesis. Studies have demonstrated that contact with or inhalation of aeroallergens can trigger the typical skin lesion of AD in some patients, who also experience eczematous flaring following exposure to ragweed pollen[185]. In addition, Cohen[186] did a very interesting observation, which definitively showed that pollens can reach cutaneous mast cells. His study clearly documented the rapid absorption of pollen through the respiratory mucosa and transported to distal skin mast cells[186]. Ragweed pollen was blown into the nostrils of 50 normal control subjects passively sensitized intracutaneously with serum from a ragweed allergic patient and serum from non-atopic controls. Within a mean of 20 minutes all test subjects developed a wheal-and-flare response at the sensitized site but not at the control site. Hopkins et al[187] were able to induce in a patient asthma and flares of AD following inhalation of an Alternaria spray. Tuft et al attempted to establish the pathogenic role of inhaled pollens in AD. They hypothesized that inhalation of pollen led to sweating, which was linked to the development of pruritus and subsequent AD lesions. Eczematoid changes developed which persisted for several days[188,189]. Tuft et al performed inhalation studies also with Alternaria: sweating and pruritus developed within minutes, and changes over 12-24 h developed in skin sites, which lasted for 4-5 days[190]. Rajka confirmed these data in 2/5 patients with "pure" AD, who developed skin lesions following the inhalation of a mould extract[191]. The inhalation studies of ragweed and Alternaria provided the first evidence for a pathogenic role of aeroallergens in patients with AD.

Several studies of the early '50 have also shown that house dust exposure may influence the clinical outcome of AD. However, it is of note that more than 50 years ago, Rost demonstrated that the skin lesions remarkably improved when patients with AD were kept in a dust-free environment[192]. Therefore inhalation of Der p allergen, as ragweed

could play a role in the pathogenesis of AD[185]. Mitchell et al first suggested that the skin lesions of AD could be provoked even by contact with Der p[193]. Repeated patch tests (PT) with aqueous Der p 1 extract on lightly abraded skin were performed in 10 adult patients with AD. Only patients with positive SPT to Der p had positive PT. PT was also positive in 4/6 atopic adults not suffering from AD. At 72 hrs epidermal changes, including focal spongiosis and microvesiculation were evident along with a significant increase in the number of basophils and eosinophils. Biopsy specimen of the positive lesions also showed mononuclear cell and neutrophil infiltration[193]. Eczematous lesions on not manipulated skin appeared in 3/17 patients with AD following PT employing Der p lyophilized commercial preparation. Biopsies of the positive test sites revealed an eczematous reaction with epidermal spongiosis and microvesiculation. Immunostaining of cryostat sections showed dermal cell infiltrates consisting of mainly T lymphocytes. There was a smaller proportion (0-30%) of LC cells and the number of mast cells and basophils was usually 5-10%[194]. Typical AD lesion occurred on non manipulated skin in 4/13 adults with AD by applying twice a day for 2-5 days an ointment containing Der f[195]. Gondo et al also demonstrated the penetration of Der f (which was linked with ferritin) into the stratum corneum, the epidermis and the dermis. However, the lesions were present only in typical areas and only with previous skin scratch. The authors hypothesized that AD, rather than being primary eruption, is likely to be the result of various repeated stimuli, combining reactions including type I and type IV immunoreaction with a primary irritant response to a combination of physical, chemical, and mechanical factors, including scratching due to persistent itching. In fact following the percutaneous challenge with mite allergen, a type I reaction occurred in the patients. A delayed type IV reaction occurred on repeated challenge[195].

Adinoff et al and Clark et al[196-198] elicited delayed cutaneous response in 18 patients with AD applying various aeroallergen extracts (20 w/v in 50% glycerin) on clinically uninvolved and not manipulated skin. Only patients with positive SPT response to Der p had positive PT response to Der p. Atopic

patients not suffering from AD failed to show positive PT responses. Norris et al[199] applied for 5 days 1 ml of a PT solution containing Der p on the unmanipulated antecubital or popliteal skin of atopic adults with or without AD. Worsening of the skin lesions occurred in 1/3 patients with AD and positive PT response to Der p. All patients with AD and negative SPTs to Der p had negative PT response. Bruijnzeel-Koomen et al[40] showed 70% positive PT response, applying house dust mite (and pollen allergens) on the back of AD adult patients, previously removing the superficial stratum corneum by 15 consecutive applications of adhesive tape. No positive responses were found in atopic patients without AD or in controls. Positive PT reactions were not found in normal controls or atopic patients without AD. These PTs caused eczematous lesions. Analysis of the cellular infiltrate demonstrated an influx of eosinophils into the dermis, starting from 2-6 hours after patch-testing. Immunostaining with antibodies against granular constituents of the eosinophils revealed that infiltrating eosinophils were in an activated state and had lost part of their granular contents. At 24 hours eosinophils also appeared in the epidermis. Histologically, a predominance of T cells of the helper/inducer phenotype have been observed. Recent data have confirmed the prevailing role of Der p in PT lesions, and the close association of the allergens with Th2 lymphocytes[100]. It has been speculated that immediately after PT-testing some allergens penetrate the epidermis, bind the IgE molecules on mast cells in the dermis and induce an immediate type reaction. Mast cells release eosinophil chemotactic factors and some of the infiltrating eosinophils become activated[50]. Activated eosinophils which have lost their granular contents are seen in PT lesions: electron microscopy showed that some epidermal eosinophils were in close contact with LCs, thus suggesting a cell-cell interaction[40].

Recent studies show that in the skin lesions the predominant atopic patients' Der p-specific T-cell clones are Th2 cells expressing IL_4 and IL_5 and not IFN-γ. On the contrary non atopic Der p-specific T-cell clones all produced IFN-γ and only in some cases a minimal amount of IL_4[123,161]. Thus the dysregulation of IgE synthesis in AD reaches its apex,

in the absence of IFN-γ. IFN-γ is absent in the the cord blood of atopic neonates, however it is speculated that even the poor production of IFN-γ is predictive of AD development[200].

The role of food allergens

It has been suggested that chronic ingestion of the offending food(s) leads to cutaneous irritability, basophil releasability and activation[201]. Release of mediators as a consequence of an IgE reaction, and high levels of serum histamine have been shown in children with AD after a positive DBPCFC response[151]; but histamine release only (at the gastrointestinal and/or skin level) cannot completely explain the histology of the skin lesions, which is more indicative of a type IV cell-mediated response[151]. An important role is played by the late phase of IgE-mediated hypersensitivity, and evidence is accumulating that eosinophils actively participate in allergic LPR in different tissues, including the skin[27]. When the ingested food antigens come into contact with the skin mast cells, histamine and other chemoattractants are released into skin tissue. It is striking the negative effect on basophils and SBHR levels set forth by food allergen identification and elimination. The repeated ingestion of food allergens was discovered to be associated with a spontaneous production of histamine-releasing-factor (HRF) produced by PBMC *in vitro* and *in vivo*. By binding to surface-bound IgE molecules and basophils, HRF(s) may perpetuate histamine release and induce allergic reactions which are too delayed or too prolonged to be considered classic IgE-mediated reactions. The finding that IgE molecules from atopic patients bind HRF but that IgE antibodies from non-atopic subjects do not suggests that these molecules have a great deal of clinical significance. These HRF are able to promote a continuous histamine release from mast cells and basophils, and increased skin hyperirritability due to a variety of minor nonspecific stimuli including additives, detergents, heat, and cold[152]. It has been shown that spontaneous basophil histamine release (SBHR) is high in children with food induced AD while they are not on an restricted diet but it is close to normal when these children are on an elimination diet[152]. SBHR correlates to the HRF production from

PBMC and HRF may activate or decrease the activation threshold of both basophils and mast cells and could explain the high SBHR described in patients with AD[152].

The contributing role of food allergens in the pathogenesis of AD has been established by DBPCFCs[145,201-203] and confirmed by significant improvement after appropriate elimination diet[204-207]. We have evaluated the efficacy of a CM- and/or egg-free diet in 59 children aged 2-14 yr suffering from severe AD[206]. The elimination of CM and/or egg for 4 weeks resulted in the healing, or marked improvement of skin lesions in 96% of children[206]. Sampson who has devoted most of his work to original studies in the field of AD and FA has evaluated children aged 3 mo-25 yr: in the patients studied, 412 DBPCFC (64%) were negative and 235 (36%) were positive, and 130 (55%) reacted to at least one food. Overall, 174/235 reactions (75%) involved the skin[201]. In addition, it was shown that eggs, peanuts, CM, wheat, fish, and soybeans accounted for nearly 90% of the positive reactions in AD patients. Most of these children (78%) reacted to one or two of foods, 15% to three foods and only 3 children to four or more different food[145]. Thus food-induced AD appears to be rather specific in terms of the number and identity of the implicated foods.

In a subsequent study of ours[207], 146 children with AD aged 6 mo-10 yr underwent 154 challenge tests, 61 of which (42%) were positive. In detail 42/101 challenges with CM (42%) and 19/45 with egg (42%) were positive. The symptoms elicited were either immediate or delayed (Table VII)[207]. Employing two foods (CM and egg) for challenges, we have obtained positive reactions in 75% of children[207], a figure very similar to that of Sampson[145]. Foods frequently reported to induce hypersensitivity such as citrus fruit, chocolate, strawberries[145], did not elicit positive responses in our patients[207]. The high number of positive SPT and RAST to foods found in children with AD supports the frequent observation that children with AD are often allergic to a large variety of foods. Even if children consume a wide variety of different foods, the most common foods of the Italian diet such as CM, egg and wheat accounted for more than 93% of the positive responses. This data should be evaluated in order to eliminate the nutritional problems of too restrictive diets. As regards the allergenicity of soy, we have reviewed 6 studies employing challenge tests to soy, which were positive in 4,2% of 2496 children aged 0.4-18 years. In addition in SPT-RAST-oral food challenge/DBPCFC-based 17 epidemiological studies soy incidence attains 3%[208]. On the contrary, CM is so a potent allergen that a drop is enough to provoke anaphylaxis in allergic infants[209]. However, children developing tolerance can ingest normal quantities of foods without clinical manifestations, despite the fact that SPT and RAST usually remain positive[201].

The role of skin infections and bacterial superantigens

Microbial pathogens initiate disease through a number of pathways. Certain bacterial components may be directly responsible for disease, since they can stimulate large numbers of lymphocytes. The recently described family of microbial superantigens have the ability to function as potent immunoregulatory compounds. On the other side patients with AD have an increased susceptibility to a variety of microbial agents. Recently a greatest focus has been concentrated on the significance of Staphylococcus aureus colonization and infection to the severity of AD skin lesions[210]. More than 50% of the AD patients secreted staphylococcal enterotoxins and others such as[11]: SEA = staphylococcal enterotoxin A, SEB = staphylococcal enterotoxin B, SEC = staphylococcal enterotoxin C, SED = staphylococcal enterotoxin D, SEE = staphylococcal enterotoxin E and TSST-1 = toxic shock syndrome toxin-1.

Table VII. Clinical reactions elicited in 61 children positive to challenge tests.

Symptoms	N° of children (%)
AD worsening	30 (49%)
Pruritus	28 (46%)
Rash/erythema	26 (43%)
Urticaria	14 (23%)
Asthma	9 (15%)
Lip edema	5 (8%)
Diarrhea	4 (6%)
Vomiting	2 (3%)

Adapted from 207.

The capacity of bacterial toxins to bind MHC class II molecules or stimulate T cells bearing TcR Vß's suggest several mechanisms by which SEs could exacerbate AD.

1) SEs secreted at the skin surface could penetrate inflamed skin and engage HLA-DR on macrophages or LC to stimulate the production of IL_1 and TNF, IL with potent proinflammatory properties.
2) SEs could stimulate T cells via their TcR Vβ to divide and release ILs which modulate tissue inflammation.
3) Nearly 50% of AD patients have circulating IgE antibodies directed to SE, such as TSST-1 or SEB. These toxins have been identified on their skin[11].

Eight patients survivors of toxic shock syndrome had TSST-1 producing staphylococci and dermatitis, and four of them had increased IgE levels (median 290 kIU/l) suggesting AD. AD might therefore be a consequence of immune activation during exposure to SEs, in addition TSST-1 bound to keratinocytes may disturb the IL repertoire, thus enhancing IL_4 and IgE antibodies[211].

Concluding Remarks

AD is a disease of offsprings of atopic parents, infants and little children, who can be easily identified and subjected to preventative measures, the first treatment for AD[212,213]. While FA appears to be transitory, the frequency of positive SPT between 1 and 7 years of age increased for mite 14,3 and for pollens 31,5 times[214], probably for insufficient prevention[212]. It is conceivable that the coming years will witness the development of new strategies for the prevention and treatment of AD, aimed at specific targets based on a thorough understanding of its pathogenesis.

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