Introduction

Atopic eczema (AE) or atopic dermatitis (AD) is a chronic relapsing, inflammatory skin disease, characterized by typical distributed eczematous skin lesions, dry skin, intense pruritus and a wide variety of pathophysiologic aspects. AD has a strong impact on the quality of life of patients and their families ***(Wüthrich et al., 2007)***.

AD becomes manifest within the first 5 years of life and is often associated with other atopic disorders like asthma, allergic rhinitis, conjunctivitis, and urticaria. Its prevalence has more than doubled in the last three decades and is most frequent in early infancy and childhood: the disease now affects 10-20% of children and 1-3% of adults in industrialized countries. ***(Buggiani et al., 2008)***.

Various immunologic parameters are altered in patients with AD (decreased number of circulating T cells; impaired lymphocyte response to mitogens; decreased cytotoxic potential of several cell types, such as monocytes and natural killer cells; decreased chemotactic responsiveness; peripheral blood eosinophilia), suggesting an immune dysregulation to be involved in the genesis of the disease. An abnormality of Th-2 cells results in an increased production of IL-4, IL-5, IL-6, IL-10, and IL-13, which leads to increased IgE and decreased IFN-γ production by circulating CD4+ and CD8+ cells. This imbalance of Th-2 cells occurs mainly in the acute process, with a switch toward Th-1 cells in the chronic stages of the disease ***(Buggiani et al., 2008)***.

A second mechanism involves environmental antigens (house dust mites, irritants, dietary allergens. Third mechanism involves a heritable defect in the skin barrier that could facilitate transepidermal penetration of allergens and promote secondary development of an allergic response ***(Buggiani et al., 2008)***.

A proliferation-inducing ligand (APRIL), also known as TALL-2 and TRDL-1, is a tumor necrosis factor (TNF) superfamily member (TNFSF13A) with close homology to B-cell-activating factor (BAFF), another TNF (TNFSF13B) also known as BLyS, TALL-1 and THANK. APRIL shares many functions in common with BAFF. BAFF and APRIL are produced by several cell types, including monocytes, macrophages, neutrophils, denderitic cells and T lymphocytes ***(Lopez-Fraga et al., 2001)***.

BAFF and APRIL share two TNF receptor superfamily (TNFRSF) members, transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI/ TNFRSF 13B) and B-cell maturation antigen (BCMA/ TNFRSF17) ***(Yu et al., 2000)***. BCMA play an important role for the survival of plasma cells (O'Connor et al., 2004). BAFF-specific receptor is BAFF receptor (BAFFR/BR3/TNFRSF13C) on T cells as well as B cells and APRIL-specific receptor is heparin sulphate proteoglycan (HSPG) on activated T cells, B cells and tumor cells ***(Sakurai et al., 2007)***.

BAFF and APRIL modulate the survival of developing B cell in the bone marrow and the expression of receptors for

BAFF and APRIL is developmentally regulated among B cells at various differentiation stages. Furthermore, both recombinant BAFF and APRIL markedly enhance the survival of precursor B cells whereas only BAFF can suppress apoptosis of immature B cells ***(Zhang et al., 2004)***.

APRIL costimulates B cells in vitro and in vivo (Yu et al., 2000). In addition, BAFF and APRIL also have an important role in T-cell activation and survival (Stein et al., 2002). BAFF and APRIL activate IgG, IgA and IgE isotype switching in B cells, independently of CD40–CD40L pathway ***(Litinskiy et al., 2002)***.

APRIL promotes the generation of rapidly dividing plasmablasts from activated human memory B cells by enhancing their survival in vitro, ***(Avery et al., 2003)***. It significantly enhances B-cell antigen presentation to T cells, an effect mediated via BCMA ***(Yang et al., 2005)***.

A recent study found that serum levels of APRIL, but not BAFF, were significantly elevated in patients with AD and strongly suggested that APRIL may have an important role in the pathogenesis of AD ***(Matsushita et al., 2007)***.

Aim of the Work

The present study aims to estimate serum APRIL levels in AD patients with various severity grades (mild AD, moderate AD and severe AD), before and after treatment compared to control subjects in an attempt to determine its involvement in pathogenesis of AD.

Atopic Dermatitis

Introduction:

Atopic dermatitis (AD) is a pruritic; inflammatory skin disease that typically occurs in early childhood. AD follows a chronic and relapsing course, and is considered part of an atopic state. Clinical manifestation of this atopic state includes asthma, allergic rhinitis, allergic conjunctivitis, and food allergies. Usually AD precede these allergic disorders ***(Goules et al., 2008)***. Two forms of AD have been delineated; extrinsic and intrinsic. Both forms are characterized by associated eosinophilia ***(Novak and Bieber, 2003)***. The extrinsic form involves 70-80% of the patients and is associated with IgE-mediated sensitization. Environmental allergens, aeroallergens and food may affect the disease and hereditary factors contribute less to the disease. In contrast, the intrinsic form involves only 20-30 % of patients and does not seem to be associated with IgE- mediated sensitization mechanisms. In this form of dermatitis hereditary and other, internal mechanisms are apparently involved ***(Goules et al., 2008)***.

Prevalence:

Epidemiological studies on representative population have demonstrated that the prevalence of AD has more than doubled in the last three decades and is most frequent in early infancy and childhood. The disease now affects 10-20% of children and 1-3% of adults in industrialized countries particularly in upper social classes and in urban regions ***(Buggiani et al., 2008)***.

The disease has a complex genetic background with many genes involved (multigenic). When both parents are affected by AD, up to 81% of their children manifest the disease. When one parent is affected, the prevalence drops to 56% ***(Van Leent and Bos, 2003)***.

Atopic dermatitis frequently starts in early infancy (so called early –onset atopic dermatitis). A total of 45% of all cases of atopic dermatitis begin within the first 6 months of life, 60% begin during the first year, and 85% begin before 5 years of age. More than 50% of children who are affected in the first 2 years of life do not have any sign of IgE sensitization but they become sensitized during the course of the disease ***(Von Mutius et al., 2004)***.

Up to 70% of children affected by AD have spontaneous remission before adolescence. The disease can also start in adults (so-called late onset atopic dermatitis), and in a substantial number of these patients there is no sign of IgE –mediated sensitization ***(Novak and Bieber, 2003)***.

It has been shown that both sexes are affected. In adults, female to male ratio was found to be 2:1 and in infants and children, males were found to be more affected in a ratio of 1.2:2 ***(Diepgen and Fartasch, 1992)***.

Histopathology:

The histopathologic features depend on the stage of the lesion. Early lesion demonstrates acanthosis, marked intracellular edema (spongiosis) of the epidermis with perivascular

infiltrates of activated T lymphocytes, monocytes macrophages, dendritic cells, and a few eosinophils in the dermis ***(Bieber, 2008)***.

In chronic skin lesions, hyperplasia of the epidermis occurs with prominent hyperkeratosis, parakeratosis and dermal fibrosis. Inflammatory infiltrate of the dermis in chronic lesion is mainly surrounding the vessels. There is an increased number of IgE-bearing Langerhans' (LCs) and inflammatory dendritic epidermal cells (IDEC) in the epidermis and macrophages dominate the dermal mononuclear cell infiltrate. Eosinophils also contribute to the inflammatory response, and T cells remain present in smaller numbers than seen in acute inflammation ***(Toda et al., 2003)***.

Clinically unaffected skin in AD is not normal. Unaffected AD skin contains a sparse perivascular T cell infiltrate not seen in normal healthy skin ***(Hamid et al., 1994)***.

Atopy as a systemic disease:

AD is often the initial cutaneous manifestation of an atopic systemic disorder with the subsequent development of asthma, food allergy and allergic rhinoconjunctivitis in the majority of patients with AD, the so-called "atopic march". The diseases are characterized by increased IgE serum levels and eosinophilia ***(Leung et al., 2006)***.

Studies suggested an association of active AD, asthma and allergic rhinitis with increased level of high affinity IgE receptors (FcεRI) on IgE-expressing LCs relative to inactive

AD, asthma, and allergic rhinitis. This correlation implies a systemic regulation of active allergic disease, which is made worse by the local inflammatory response in AD ***(Semper et al., 2003)***.

Pathogenesis of atopic dermatitis:

The manifestations of AD result from a complex interaction between environmental factors, skin barrier dysfunction, susceptibility genes and immunological abnormalities ***(Boguniewicz, and Leung, 2006)***.

Two hypotheses concerning the mechanism of AD have been proposed, one holds that the primary defect resides in an immunologic disturbance that causes IgE-mediated sensitization with epithelial-barrier dysfunction regarded as a consequence of the local inflammation. The other proposed that an intrinsic defect in the epithelial cells leads to the barrier dysfunction and the immunologic aspects are considered an epiphenomenon ***(Bieber, 2008)***.

Genetics of atopic dematitis:

AD is a genetically complex disease that has a high familial occurrence. Twin studies of AD have shown concordance rate of 0.72-0.87 in monozygotic, and 0.21-0.23 in dizygotic twins, indicating that genetic factors play an important role in the development of this disease ***(Wuthrich et al., 1981)***.

Several candidate genes have been identified in AD, notably on chromosome 5q31-33. All of them encode cytokines

involved in the regulation of IgE synthesis: IL-4, IL-5, IL-12, IL-13, and granulocyte-macrophage colony stimulating factor (GM-CSF) ***(Morar et al., 2006)***.

Polymorphisms of gene encoding the IL-18 that contributes to shift of Th1 and Th2 cross-regulation toward Th1-mediated responses (so-called Th1 polarization) may contribute to the imbalance between Th1 and Th2 immune responses in atopic dermatitis ***(Lange et al., 2005)***.

It was recently demonstrated that filaggrin gene (FLG) on chromosome 1q21.3 which encodes a key protein in epidermal differentiation is a major player in AD, showing that a heritable epidermal barrier defect is responsible in many cases of AD. In addition, FLG mutations are major risk factors for eczema–associated asthma ***(Marenholz et al., 2006; Sandilands et al., 2007)***.

The regions on chromosomes 1q21, 3q21, 17q25and 20p linked to AD seem to overlap with psoriasis susceptibility loci. The co-localization of eczema and psoriasis genes support the concept that specific skin genes may influence the eczema phenotype via controlling epidermal function ***(Cookson, 2004)***.

Immunopathogenic mechanisms of AD:

The inflammatory infiltrate of AD consists of CD4 lymphocytes, macrophages, dendritic cells, eosinophils and mast cells. CD4 lymphocytes express the memory immunophenotype and are located in intraepidermal and subepidermal spaces. Two distinct subset of dendritic cells have been described in AD, the LCs and the IDEC, which are recruited to the site of inflammation ***(Wollenberg et al., 1996)***.

In patients with early onset AD, IgE mediated sensitization occurs several weeks or months after the lesions appear, suggesting that the skin is the site of sensitization ***(Illi et al., 2004)***. Molecules in pollens and some food allergens drive dendritic cells (DCs) to enhance Th2 polarization ***(Shreffler et al., 2006)***. Moreover, keratinocytes in AD produce high level of the thymic stromal lymphopoietin (TSLP) that signals dendritic cells to drive Th2 polarization ***(Soumelis et al., 2003)***.

Widespread skin inflammation can affect adaptive immunity, alter the phenotype of circulating monocytes, and increase the production of prostaglandin E2 in AD by inducing the production of large amount of cytokines such as GM-CSF or chemokines. All these factors provide signals required for driven Th2 polarization and for this reason, the skin acts as the point of entry in atopic sensitization ***(Bieber, 2008)***.

In non-IgE-mediated inflammation, epidermal-barrier dysfunction, mechanical irritative signals, or T-cell-mediated events that do not involve IgE lead to an initial inflammatory reaction accompanied by an alteration of the function of resident dendritic cells. These cells are also subjected to locally produced cytokine, TSLP and the pollen-derived mediators. As a result, the dendritic cells migrate to the regional lymph nodes and induce an allergen-specific Th2 polarization. The inflammatory reaction may also have a substantial systemic effect on the adaptive immune system, favoring the development of IgE-mediated sensitization ***(Bieber, 2008)***, Figure (1).

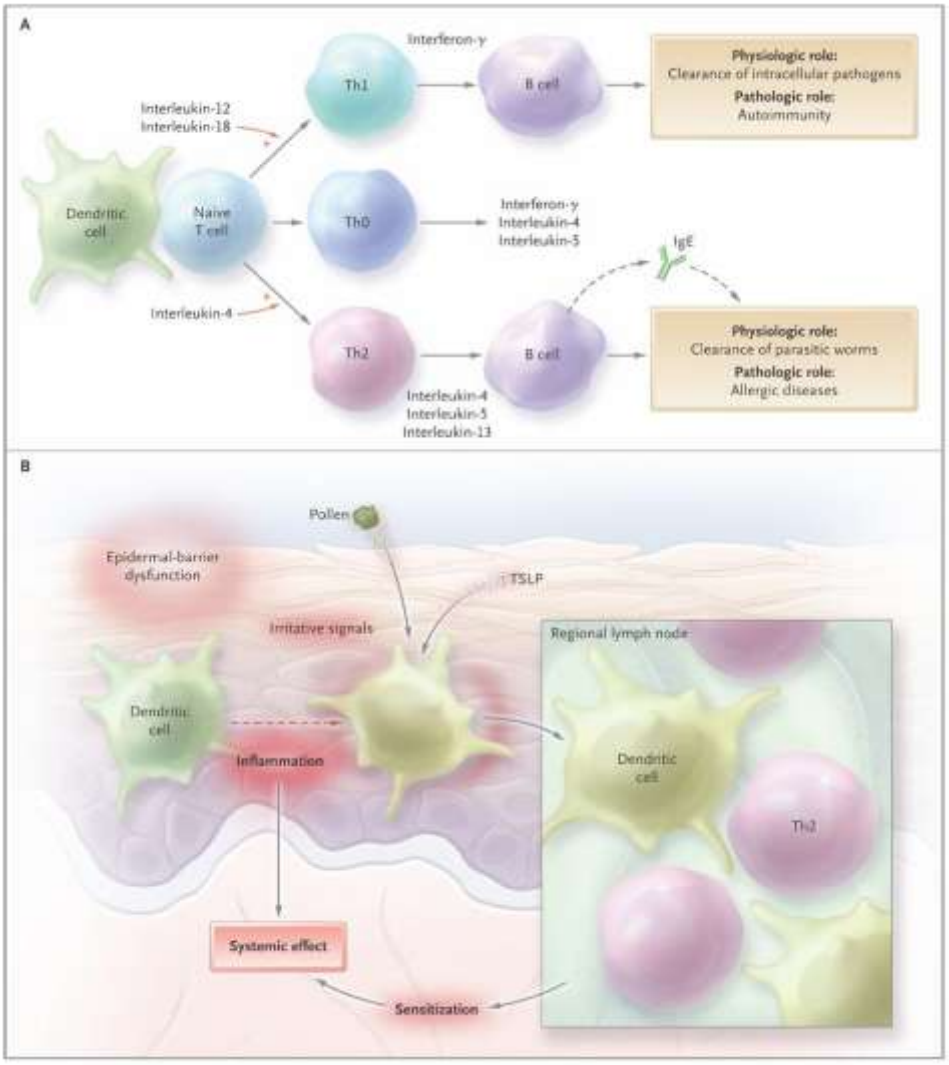

Figure (1): The Th1–Th2 Paradigm and Its Role in Allergy and the Skin as the Site of Initiation for Sensitization. Panel A shows that the outcome of helper T cell (Th) differentiation is dictated by the type of dendritic cell, the microenvironment, or both. On antigen presentation, naive T cells are subjected to either interleukin-12 and interleukin-18 or interleukin-4, which polarizes them to Th1 or Th2 helper cells, respectively. Th1 cells produce interferon-γ, whereas Th2 cells produce interleukin-4, interleukin-5, and interleukin-13. Th0 cells produce both Th1 and Th2 cytokines, probably in response to less stringent polarizing signals. Both helper T-cell types have distinct physiologic roles. However, a strong Th2 predominance leads to pathologic conditions such as overproduction of IgE and allergic diseases. Panel B shows non–IgE-mediated inflammation ***(Bieber, 2008).***

Inflammation in AD is biphasic: the initial phase (acute) allergens penetrate the damaged skin and are captured by FcεR1 bound IgE molecules on dendritic cells, which are activated by the FcεR1 receptors, and exhibit enhanced antigenic presentation capacity. Subsequently, dendritic cells display these specific antigens (allergens) to memory Tcells that are also recruited to the inflamed atopic dermatitis ***(Akdis et al., 2002; Akdis et al., 2003)***.

This initial phase is characterized by the polarization of naïve Tcells (Th0) into Th2 cells and the production of associated cytokines such as IL-4, IL-13, and IL-5. In addition, antigen-specific IgE complexes activate mast cells, inducing the inflammatory response ***(Goules et al., 2008)***. IL-4 and IL-13 cytokines induce the isotype switching IgE synthesis, whereas IL-5 plays an important role in eosinophil expansion and survival ***(Leung et al., 2004)***.

The early events of AD are characterized by marked upregulation of several proinflammatory cytokines, such as IL1, tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-18. This over production attributes to pruritus and mechanical trauma as will as colonization of atopic skin by microbes (Goules et al., 2008). IL-31 is mainly produced by Th2 cells and has been associated with pruritus ***(Dillon et al., 2004)***.

These proinflammatory cytokines together with chemokines direct the recruitment of different subsets of leukocytes including memory T cells expressing the homing cutaneous lymphocyte-

associated antigen (CLA). CLA defines a subset of circulating memory T cells and interacts with E-selectin on inflamed postcapillary dermal venules, mediating the rolling of leukocytes along the endothelium ***(Campbell and Butcher, 2000)***.

Cytokines as IL-1 and TNF-α from resident cells (keratinocytes, mast cells, and DCs) bind to receptors on vascular endothelium, activating cellular signaling including the NF-κB pathway and inducing expression of vascular endothelial cell adhesion molecules. These events initiate the process of activation and adhesion to the endothelium followed by extravasation of inflammatory cells. Once the inflammatory cells have infiltrated into the tissues, they respond to chemotactic gradients established by chemoattractant cytokines and chemokines, which emanate from the site of injury or infection ***(Ono, 2003)***, Figure (2).

The late phase (chronic): is characterized by Th1 switch, which accounts for the chronicity of AD, and increased production of its related cytokines such as IFN-γ and IL-12 ***(Grewe et al., 1995)***. IFN- γ is the predominant cytokine that may induce apoptosis in keratinocytes, which may be a key event in the eczematous process in AD. Importantly, Th1and Th2 phases are not mutually exclusive, as acute exacerbation may occur in chronic phase of AD ***(Wuthrich et al., 2007)***.

The switch from Th2 to Th1 is due to production of IL-12 and IL-18 from IDEC, which leads to the chronic phase of the disease ***(Bieber, 2008)***. Persistent skin inflammation in

chronic lesions may be also due to elevated IL -5 and GM-CSF expression in the skin leading to enhanced survival of eosinophils and monocytes-macrophages as well as LCs. In addition, extracellular matrix molecules deposited into chronic skin lesions have been found to enhance the survival of memory T cells ***(Akdis et al., 2003)***, Figure (2).

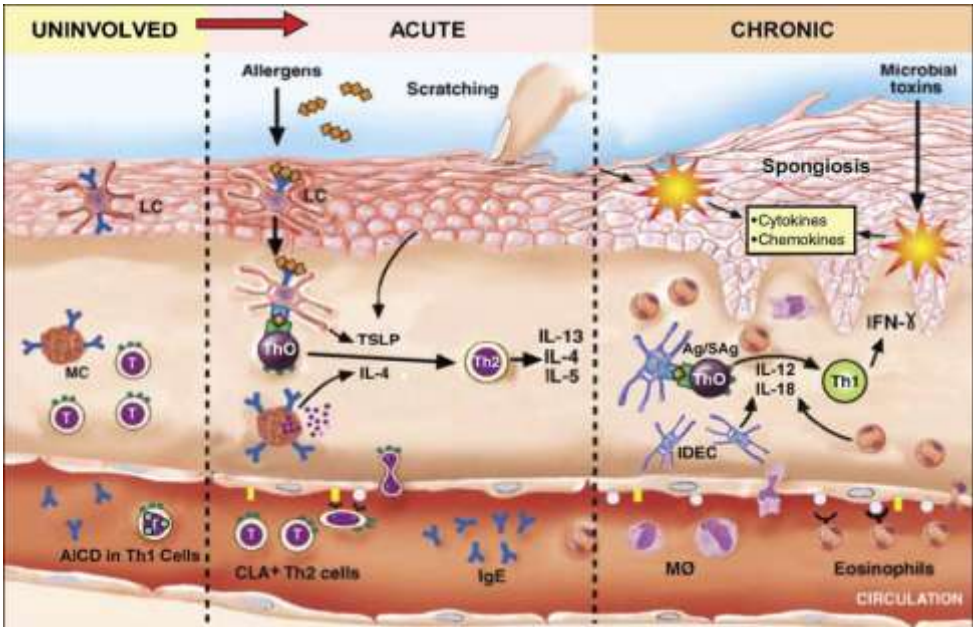

Figure (2): Immunologic pathways in AD. Th2 cells circulating in the peripheral blood of AD patients result in elevated serum IgE and eosinophils. These T cells express the skin homing receptor, CLA, and recirculate through unaffected AD skin where they can engage allergen-triggered IgE+ LCs and mast cells (MCs) that contribute to Th2 cell development. Skin injury by environmental allergens, scratching, or microbial toxins activates keratinocytes to release proinflammatory cytokines and chemokines that induce the expression of adhesion molecules on vascular endothelium and facilitate the extravasation of inflammatory cells into the skin. Keratinocyte-derived thymic stromal lymphopoietin (TSLP) and DC-derived IL-10 also enhance Th2 cell differentiation. AD inflammation is associated with increased Th2 cells in acute skin lesions, but chronic AD results in the infiltration of inflammatory IDECs, macrophages (Mφ), and eosinophils. IL-12 production by these various cell types results in the switch to a Th1-type cytokine milieu associated with increased IFN-γ expression ***(Leung, 2000)***.

Chemokines in AD:

Several chemokines have been found to participate in leukocyte recruitment to the site of inflammation in AD.

- CCL27 is produced by keratinocytes and is considered a skin-specific chemokine. Its receptor, CCR10 is expressed on the surface of memory T cells and seems to regulate their recruitment to the skin ***(Homey et al., 2002)***. Memory T cells also express the receptor CCR4, which can interact with both CCR10 ligand and CCL17, a chemokine produced by vascular endothelium. CCL17 and CCL27 may act synergistically to enhance memory T-cell recruitment to the skin ***(Goules et al., 2008)***.
- CCL18 has been found to be highly expressed by LCs and dendritic epidermal cells in the epidermis of patients with AD, but not in other inflammatory skin or autoimmune diseases, such as psoriasis and cutaneous lupus erythematous, or in normal skin ***(Gunther et al., 2005)***. CCL18 can interact with CLA-bearing T cells in the peripheral blood, contributing to the recruitment of T cells to the site of atopic skin inflammation. In addition, exposure to S.aureus enterotoxin B and allergens seems to upregulate the expression of CCL18 ***(Pivarcsi et al., 2004)***.
- CCL1 is also significantly upregulated in patients with AD, in comparison with the levels seen in patients with psoriasis and cutaneous lupus erythematosus and healthy skin. CCL1 can interact with the receptor CCR8 on a subset of T cells and dendritic cells, suggesting a role in cutaneous inflammatory response in AD ***(Gomber et al., 2005)***.