



ROLE OF TIGHT JUNCTIONS AND ITS PROTEIN EXPRESSION IN ATOPIC DERMATITIS: A REVIEW

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disease with xerosis, itchiness, as well as interconnection with immunoglobulin E (Ig E), mediated foods including airborne allergies. AD is not only related to the diminished stratum corneum barrier but also presents with an unusual expression of tight junctions (TJs) proteins. TJ barrier dysfunction leads to impairment in the stratum corneum (SC) barrier. The significant role of TJs in the epidermal barrier as indicated by Claudin-1 (Cldn-1) deficient mice that undergo high transepidermal water loss (TEWL) and skin dehydration. In atopic dermatitis, downregulation of Cldn-1 was observed due to inflammation. Still, a lack of distinct understanding exists in considering tight junction barrier impairment as a cause or outcome in atopic dermatitis. This review summarizes TJs main role in skin barrier function and TJ proteins (TJPs) expression observed in AD patients.

Keywords: Atopic Dermatitis, Tight Junctions, Claudin .

INTRODUCTION

AD or atopic eczema is a long-standing (chronic) inflammatory skin disease with dry skin, itchiness and interconnection to immunoglobulin E (Ig E) mediated foods as well as airborne allergies [1]. The possibility of developing asthma (the atopic march) or allergic rhinitis is found in more than 60% of children suffering from AD [2]. Its development might be associated with skin barrier defects, psychological and environmental factors because of genetic susceptibility associated with hypersensitivity and dysfunction of the immune system [3]. In atopic dermatitis, skin barrier defect has been considered as an important feature. Stratum corneum (SC) and TJs are essential for epidermal barrier function. In stratum granulosum, the intercellular spaces are blocked by TJs building closeness which represents fundamental potent along with physiological skin functioning. Significance of tight junctions in epidermal barrier function was indicated by Cldn-1 deficient mice that undergo high transepidermal water loss (TEWL) as well as skin dehydration [4]. Tight junction functions as 'gate' by controlling the movement of intercellular substances like cytokines, hormones, and electrolytes and contributes in particular permeability of epidermis [5].

TJ function is regulated by claudins which strengthen the barrier like Cldn-1 which contributes to epithelial barrier tightening [4] or like claudin-2 which impairs the epithelial barrier tightening by disturbing the barrier [6]. In AD, tight junction protein expression is downregulated [7]. The current data suggest the impairment of tight junction, contributing to immune dysregulation and barrier dysfunction observed in AD patients. This review summarizes the barrier function of TJs in AD along with the regulation of TJPs in AD individuals. It also briefly explains the components of innate immunity like Antimicrobial peptides (AMPs), Toll-like receptor 2 (TLR2) activation contributing to improving the barrier function of TJs in AD.

Structure of Tight Junctions:

Tight junctions are multiprotein junctional complex situated at the apical surface of epithelial cells, intervenes cell adhesion as well as firmly controls paracellular transport of particles, water including different atoms [8,9]. The barrier function of epithelium opposed to outside trespassers, for example, allergens, particles, and pathogens are structured by tight junction [10].

TJs are formed by three sorts of transmembrane proteins which include Claudin (Cldn), Occludin (Ocln) and junction adhesion molecules (JAMs) [8]. Moreover, structures of the tight junction are bolstered with the help of connector proteins, for example, zonula occludens (ZO) proteins "Figure 1:" [8,11].

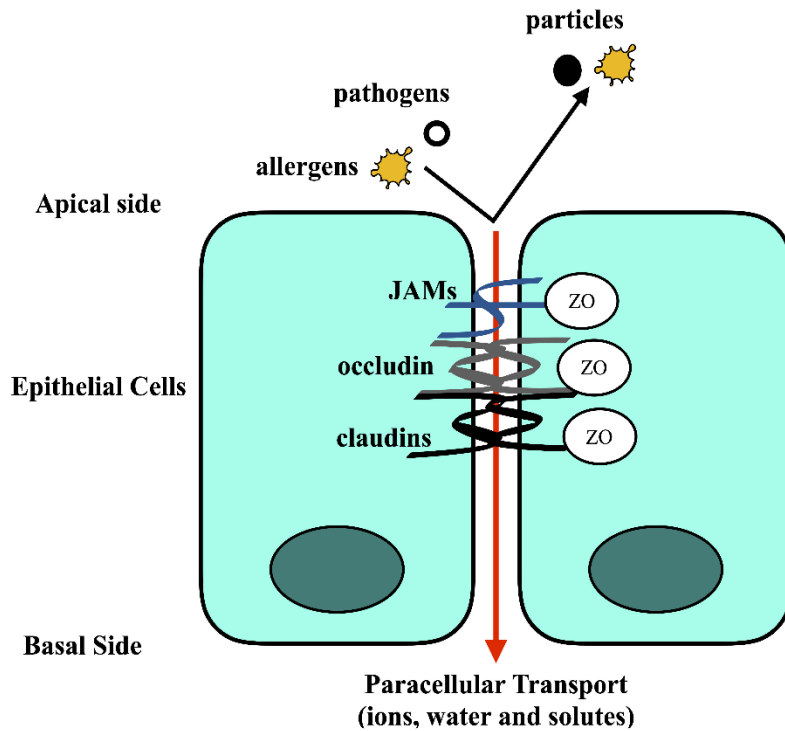


Figure 1: Three types of transmembrane proteins found in TJs: occludins, claudins, along with junction adhesion molecules (JAMs) including ZO-1 proteins.

Claudins being a family of proteins encodes four transmembrane domains consisting of a pair of very conservative extracellular loops. Barrier function is conducted by the extracellular loop 1 while extracellular loop 2 is responsible for narrowing the paracellular cleft [12].

Occludins are a group of proteins with five extracellular or intracellular domains and four transmembrane domains. The barrier function is conducted by C-terminus of this family and also conducts cell survival by interacting with other several proteins while N-terminus shows its correspondence to the tight junction barrier. The penetrability of the TJ barrier is regulated by extracellular loops [13].

ZO-proteins are also considered as a foundation for TJ because of its different unbreakable areas for TJ proteins and actin. It also links claudins or occludins to cytoskeletal actin [14].

Barrier Function of TJs in Skin:

TJ is found in simple and stratified mammalian epithelia which are made by intracellular and transmembrane proteins. They are present in stratum granulosum and after injury, its expression gets elevated. They are implicated in the keratinization of epidermal cells along with its differentiation [15].

The granular cell layer presents with the accumulation of distinctive tight junction structures accompanying with the barrier function of TJ. For a 557-Da tracer, barrier function could be specified and barrier function was shown after pre-digestion of skin with exfoliative toxin for 32-kDa tracer [4,16,17]. While

applying both of them from the dermis, they came to an end in the course of their journey from inside to outside. Similarly, both of them were terminated by stratum corneum and do not arrive at TJs when they were applied to the upper layer of the epidermis. Nevertheless, TJs are bidirectional [18], suggesting if TJs becomes leaky for a matter, the direction of permeation is dependent on the gradient of a molecule on each side. Hence, if TJs form a barrier, the barrier is created on each side. From outside to inside, if these molecules would arrive at TJ, which can occur once there is damage to the stratum corneum barrier, they likely get stopped. In the epidermis, TJs barrier function for alternative molecules, as well as water or alternative ions has not been signified. However, a barrier is formed by TJs to water and Ca^{2+} , Cl^- and Na^+ in cultured keratinocytes [19], showing a parallel part in the epidermis. Still, more research answering queries related to the barrier function of TJ/ permeability considering various solutes in the epidermis is surely required.

Furthermore, the significance of TJPs like Cldn-1, Cldn-4, ZO-1, Ocln for tight junction barrier to 4-kDa tracer along with the ions (Na^+ , Cl^- , Ca^{2+}) was indicated in the experiments with normal human epidermal keratinocytes (NHEKs) [19,20]. Additionally, ZO-1 and Cldn-1 are essential to maintain barrier integrity to larger molecules like (40 kDa) [19]. In 557-Da tracer, Cldn-1 knockout mice signified a barrier leakage which expressed claudin-1 significant role in the TJ barrier through Vivo demonstration [4,21]. The study performed via restored human skin explains the tight junctions gap by C-terminal Clostridium perfringens enterotoxin (cCPE) marking claudin-4 along with additional claudins reduced tight junction barrier to this tracer [22].

In the stratum granulosum, TJPs colocalization takes place at TJ barrier-forming structures, but strangely, all of the TJPs are not limited to these structures of barrier formation including stratum granulosum. Ocln is found to be present in different structures in stratum granulosum [23]. Studies show that in every viable cell layers like (claudin-1, claudin-7, junctional adhesion molecule-A (JAM-A) or in the stratum spinosum like (claudin-4 or zonula occludens-1 (ZO-1) and ZO-2) further TJ and TJ associated proteins were also found [24].

In <5kDa tracer, the experiment performed in chronic AD showed decreased barrier function which was not similar for >30kDa tracers [25]. Research shows that the knockdown of occludin can decrease sensitivity to initiation of cell death and cell adherence [17]. Hence TJ protein is involved in various functions along with the barrier function.

Atopic Dermatitis:

Atopic dermatitis (AD) is a chronic, inflammatory skin disease. Its growing incidence rate ranges from 3% in adults to 10% - 20% in children [26]. AD presents with xerosis and scaling skin (non-lesional) frequently indicating severity with itchy eczema (lesional) [27]. AD is not only limited as an inflammation to the skin but also considered mostly as a Th2-driven systemic disease [28]. Barrier defects are considered as the earliest steps for the progression of AD [29]. AD inflammation represents elevated levels of serum IgE and inflammatory infiltrates (macrophages, lymphocytes along dendritic cells) giving rise to (pro-)inflammatory cytokines (Interleukin-4, Interleukin-13, Interleukin-31). Acute AD lesions show a T-helper type 2 (Th2) cytokine and IL-4. In chronic AD lesions Interleukin-17(IL-17), Interleukin-22 (IL-22) as well as Interferon ($\text{IFN-}\gamma$) dominates. [30].

AD pathogenesis involves altered innate plus adaptive immune responses along with barrier dysfunction in the skin. Bacterial superinfection is correlated with an elevation of total IgE levels in AD [31]. Non-lesional AD presents with high transepidermal water loss (TEWL) disturbing barrier function (inside-outside) whereas, in lesional AD, it is more pronounced [32].

AD lesions are more prone to get infected with *Staphylococcus aureus* [33]. *S.aureus* is found in 30-100% of non-lesional AD patients as compared to lesional AD patients where *S.aureus* is found in 75-100% [34]. Among various *S.aureus* strains, the extracellular serine protease A (SspA) or V8 protease most commonly gets expressed [35]. In vivo, V8 protease is seen affecting stratum corneum along with damaging murine skin integrity. The previous study explains that extracellular serine protease splits TJPs such as claudin, Ocln, ZO-1 and cadherins [36] ultimately leading to skin barrier dysfunction. Increasing evidence suggests a vital role of tight junction in AD pathogenesis.

Expression of TJPs in AD:

In the skin lesion of AD individuals, Claudin-1 protein expression is down-regulated due to inflammation [37]. The amount of Cldn-1 downregulation corresponds markedly with the substance of dermal infiltrate following the upper and lower layer of the epidermis. The study in mice showed the deficiency of claudin 1 leading to high transepidermal water loss TEWL, liver abnormalities and ultimately death [5]. Decrease mRNA of claudin-1 by AD-like inflammation is found by the study done in FLG knockout mice and AD-like dermatitis NC/Nga mouse model [37]. Tokumasu et.al study on mouse models showed the reduced claudin-1 expression with AD characteristics such as elevation of Interleukin-10 and Interferons, increased epidermal thickness, dry skin, and infiltration of macrophages [38]. Similarly, Cldn-1 was downregulated in skin diseases like psoriasis and squamous cell carcinoma. Skin lesions in psoriasis show claudin-1 downregulation in the upper and bottom surface whereas it is completely lost in other areas [39].

Relationship between the beginning of atopic dermatitis with children not completing 5years of age and Cldn-1 single nucleotide polymorphism (SNP) was found in an Ethiopian cohort [40]. A hospital-based Korean study has shown a significant correlation between AD and CLDN-1 SNPs. But the study was only based in the hospital group not in the Korean population. Similarly, the study done in a Danish-based population group showed a link of single nucleotide polymorphism [41]. Immuno-intensity investigation performed in the non-lesional skin of the Austrian cohort showed no further differences in the expression of claudin-1. Hence, it also proves that Cldn-1 expression is based on genetic variations among different populations. Increased expression of claudin-4 was shown by two individual studies performed in the non-lesional skin of the European population [37]. As comparing with lesional to non-lesional skin, alteration of Claudin-8 was found with RNA seq [42]. In non-lesional skin, Western blot analyses were performed in three Japanese patients which shows decreased expression of claudin-4. Furthermore, in non-lesional skin downregulation of ZO-1 was observed with no changes of Cldn-1 [43].

Claudin-1 and claudin-23 were downregulated for non-lesional AD patients of North America contributing to impair the barrier function. The report also includes relation connecting the haplotype-tagging

SNPs in the area of Claudin 1 and AD. Studies have shown the association of Claudin-1 with atopic dermatitis. The risk of developing AD and disease severity in an African-American cohort is associated with the gene variants of Claudin 1(3q28) [44]. The role of CLDN1 variant rs893051 in the intronic region of CLDN1 has been established in the African-American population [39] being associated with the severe disease among AD patients in the African-American population [45].

Mutation of genes like filaggrin along with SPINK5 (serine protease inhibitor kazal- type 5) is connected with barrier formation of skin plus defective differentiation of epidermis [46]. In 2006, the study described the genetic predisposition showing inactive mutation of the filaggrin gene. A homo-or heterozygote filaggrin mutation was seen regarding one-third of European individuals with atopic dermatitis [47]. The loss of function mutations of filaggrin is found to be rare in African patients suffering from AD [48]. An increase in surface pH was observed in filaggrin-deficient patients. The key role of filaggrin protein is to form a skin barrier and support the terminal differentiation [49]. Increased antigen penetration was also seen in filaggrin knockout mice [50]. During keratinocyte differentiation, Interleukin-13 (IL-13) along with Interleukin-4 (IL-4) inhibits the filaggrin production in vitro, showing that Th2 inflammation worsens the skin barrier dysfunction along with acquired filaggrin deficiency [51].

The result of immunostaining the skin lesion of dogs with AD presented with disorganized staining of CLDN1 when treated with house dust mites [52]. A recent study by Kim and his team investigated the alteration of tight junction in the epidermis by immunohistochemistry in Canine models of AD compared with normal dogs. The study also revealed that the expression of TJ for ZO-1 plus Ocln is decreased in dogs with AD. The study observed so far does not describe the association of occludin and ZO-1 in AD progression in human being but are essential in the dog model of atopic dermatitis [53]. The previous study suggested that in AD individual's alteration of TJPs contributes to its progression and development [54].

Still, there is no distinct agreement in considering diminished barrier function of the tight junction as cause or outcome in atopic dermatitis. Skin inflammation and damaged physical skin barrier form a vicious cycle in the pathogenesis of AD. Immediately after dermatitis, skin inflammation leads to the leakage of TJ. Studies show that TJ barrier dysfunction leads to impairment in the SC barrier [21]. It is hypothesized that the SC (Stratum Corneum) barrier is impaired by dermatitis through the leakage of TJ barrier and disrupted keratinocytes differentiation making it easier for percutaneous sensitization initiating a vicious cycle between barrier deficiencies along with skin inflammation“Figure 2:” [25,55]. This process may give rise to chronic inflammation of atopic dermatitis.

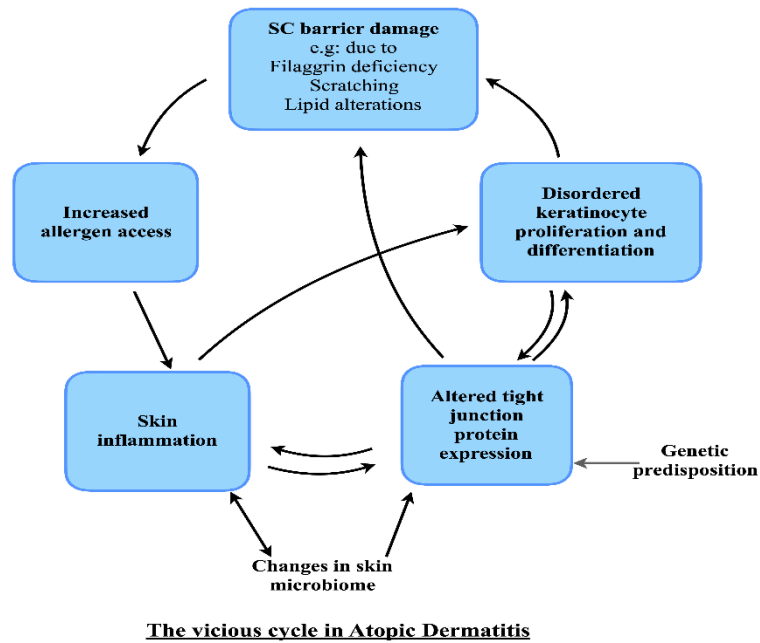


Figure 2: The vicious cycle in atopic dermatitis. Stratum corneum (SC) barrier impairment because of filaggrin deficit, laceration, lipid changes cause the greater approach of allergens. The outcome is the inflammation of the skin. It is further induced by skin microbiome changes giving rise to alteration of TJPs expression. Disturbed keratinocyte growth, induced due to inflammatory skin along with the alteration of TJPs expression, once more time results in impairment of SC barrier. That too could be straightly provoked by TJPs alteration.

TJ's BARRIER REPAIR IN AD:

Toll-like Receptor 2 Activation:

Toll-like receptor (TLR) 2 decreased expression on their macrophages including monocytes are seen in patients suffering from AD [56,57]. As compared to non- atopic groups, AD patients are frequently infected and colonized by *S. aureus* indicating that alteration of TLR2 promotes such susceptibility. The study performed in the human wound model along with TLR2 deficient mouse suggested TLR2 role for maintaining tight junction integrity responding to barrier insults, also in AD patients the barrier repair mechanism is possibly deficient [58].

Role of p63:

p63 suggests its vital part in barrier formation as well as keratinocyte adherence. p63 direct target is non-other than claudin-1 [59]. Experimental Δ Np63 knockout mice study has shown wound healing and reduced keratinocyte differentiation which demonstrates the basic role of Δ Np63 in the integrity of epidermis [60]. Increased claudin-4 plays an important role in an undefined restoration of impaired keratinocytes and

also provides supportive responses in the diminished barrier in AD [61]. In the epidermis of AD, higher claudin-4 expression with reduced Δ Np63 expression was observed at the cell membranes of keratinocytes when treated with Toll-like receptor 3 ligand [62].

Antimicrobial Peptides (AMPs):

In the skin innate immunity, AMPs have a significant role and it reacts as endogenous antibiotics. Among the various AMPs, β -defensin and Cathelicidin (LL37) are considered to be important [63]. In AD individuals, its release with decreased expression might cause greater vulnerability to skin infections caused due to bacteria, fungi or viruses [54]. Studies show that lesional skin of AD exhibits diminished induction of Host defense peptides (HDPs) or AMPs like LL-37 and human β -defensins (hBDs) in contrast with the lesional skin of psoriasis [64] describing the recurrent bacterial infections in AD individuals. Previous studies performing Western blot analysis explain the role of LL-37 which noticeably increases the TJ proteins expression like Cldn-1,4,3,9,7,14, Ocln and hBD-3 upgrades the regulation of claudin-1,3,4,14 and 23 including its membrane distribution. Furthermore, it promotes the TJ barrier function by decreasing paracellular flux in the layers of keratinocytes and elevating the transepithelial electrical resistance (TER) [65,66].

The recent study by Wang and his team established the role of IL-1 β by inducing keratinocyte protection against Staphylococcus aureus protease SspA/V8 indicated the role of IL-1 β in the upregulation of Cldn-1 expression in HaCat cells. In primary cells, the degradation of Cldn-1 is not prohibited along with safety independent of the Cldn-1 level. This process of protection against V8 protease was induced by human β -defensin 2 (hBD2) [67].

Interleukin-17A (IL-17A) Improves TJs Function:

The recent study performed with the human skin model as well as with primary human keratinocytes, IL-17A increased the progression of TJs barrier function of the epidermis by elevating the transepithelial electric resistance (TEER) and reducing paracellular flux "Figure 3:". This protective effect of IL-17A was inhibited when again treated with IL-4. This further adds the role of IL-17A which improves TJs barrier function however it gets offended by interleukin-4. Repairing the proportion of IL-17/IL-4 improves barrier function in the skin of AD individuals [68].

Clinical Indication in AD Treatment

AD treatment includes moisturizers like lipid mixtures, petrolatum, ceramide-controlling triple-physiologic lipid (ceramide: cholesterol: free fatty acids with a molar ratio of 3:1:1) [69,70,71]. Petrolatum enhances the barrier functions of skin by upregulating AMPs like HBD-2, LL-37, S100 proteins and elafin [70]. The use of coagulase-negative Staphylococcus strains reduces colonization by Staphylococcus aureus in AD individual's skin [72].

An experiment performed with AD induced mouse skin shows decreased eosinophils, mast cell infiltration and Th2 cytokines production by Lactobacillus strain, CJLP55, isolated from kimchi [73]. A recent study demonstrates the role of prebiotics and probiotics which enhances the symptoms in AD patients as well

as their disease severity and quality of life [74]. Genes that were involved in the barrier function of skin were upregulated by Dupilumab, anti-IL-4 R α monoclonal antibody [75].

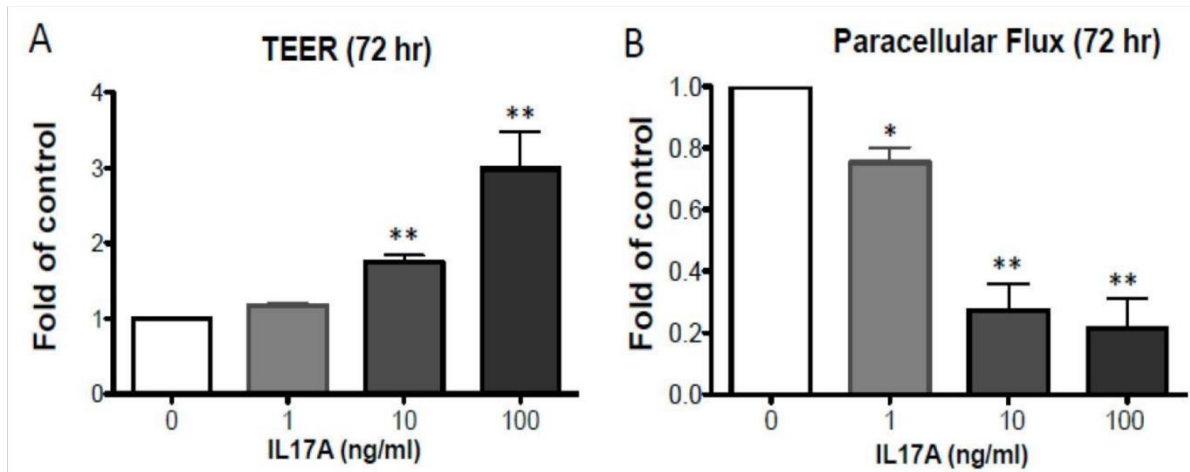


Figure 3: IL-17A improves epidermal TJ barrier function. In primary human keratinocytes, IL-17A dose-dependently (A) increased TEER (n = 3–6) and (B) decreased the paracellular flux of fluorescein (n = 3) 72 h after cytokine treatment. Data represent \pm SEM fold of control. Significance was calculated compared to untreated controls. * p \leq 0.05, ** p \leq 0.05.

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CONCLUSION

In summary, among the several skin barriers, the TJ barrier is essential for the barrier system as well as important for skin barrier integrity in skin inflammatory disease like AD. Alterations of TJPs can lead to the imbalance of the barrier system in the skin. Inflammation in AD leads to epidermal barrier leakage of tight junction and disturbing barrier formation of stratum corneum. Upregulation of TJPs enhances epidermal TJ barrier integrity and improves the TJ barrier function in AD, thus contributing to developing new strategies to better control the various inflammatory skin diseases like AD. In the future, the TJ barrier function and their clinical relevance need further investigation in most of the inflammatory skin diseases in more detail.

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