Derma-Hc

Subjects: Others
Contributor: Woong Mo Yang

Atopic dermatitis (AD) is a chronic cutaneous disorder that is characterized by severe eczematous inflammation, swelling, and lichenification. Activation of T helper (Th)-22 cells by allergens leads to epidermal hyperplasia with hyperkeratosis at the chronic phase of AD. Derma-Hc is composed of five natural herbs with anti-AD effects, such as Astragalus membranaceus BUNGE, Schizonepeta tenuifolia Briq., Cryptotympana pustulata Fabr., Angelica sinensis Diels, Arctium lappa L. In this study, the ameliorative effect of Derma-Hc on cutaneous lichenification in 2,4-dinitrochlorobenzne (DNCB)-induced AD was investigated. The dorsal skin of mice was sensitized with DNCB to induce AD-like skin lesions. The dermatitis score and frequency of scratching were evaluated. Thickness of epidermis and dermis was measured by staining with H&E. In addition, infiltration of the mast cell was observed by staining with toluidine blue. Then, desmosomal cadherin, DSC1 was examined by immunofluorescence. Pathological mechanisms involved in lichenification were analyzed in AD-like skin lesions and TNF-α + IFN-γ-treated with human keratinocytes including keratinocyte differentiation genes and JAK1-STAT3 signaling pathway with IL-22 by RT-PCR and western blotting. Topical treatment of Derma-Hc improved AD-like symptoms such as dryness, edema and lichenefication and decreased the number of scratches. Histopathological analysis demonstrated that Derma-Hc significantly inhibited epidermal hyperplasia, hyperkeratosis, and mast cells infiltration.

Keywords: Derma-Hc ; atopic dermatitis ; lichenification ; dry skin ; keratinocyte

1. Introduction

Atopic dermatitis (AD) is multifactorial inflammatory skin disease characterized by pruritus, edema, eczema, xerosis cutis, and lichenification [1][2]. The cause of AD has not been fully understood, however, genetic factors, hygiene theory, allergen and abnormalities in the immune system are highly involved in pathogenesis of AD [3]. The incidence of AD has been increasing, affecting up to 20% of children and 10% of adult in industrialized countries [4]. The psychological and economic burdens lead to deterioration of the quality of AD patient's life [5]. Exacerbation of itching-scratching cycle result in lichenification [6]. Atopic skin becomes leathery, scaly, and thickened by continuously scratching or rubbing skin lesions due to chronic irritation [7]. Therefore, molecular mechanisms in lichenified skin lesions should be elucidated to manage chronic AD.

The pathomechanism of AD is featured by dysregulation of immune responses that are related to T-cell dominant inflammation and allergen hypersensitivity [8]. Especially, T cells, such as T helper (Th)1, Th2, Th17, and Th22 cells are critical mediators which initiate progression of AD in response to an allergen [9]. Upon sensitization to an allergen, IgE produced by B cell bound high affinity-IgE receptors and increased mast cell degranulation to release histamine and cytokines [10][11]. Th2/Th22 cell polarization produces cytokines, interleukin (IL)-4, IL-13, IL-31, and IL-22 in the acute phase of AD. On the other hand, Th1, Th17, and Th22 cells are activated, and associated cytokines were secreted in the chronic phase [12]. In addition, Th22 cell is highly contributed to chronic pruritus, dermatitis and skin barrier impairment by amplifying inflammation on keratinocytes [8]. IL-22 derived from Th22 cells specifically binds the heterodimeric receptor and activates janus kinase 1 (JAK1), signal transducer and activator of transcription 3 (STAT3) leading to the inhibition of terminal differentiation of keratinocytes and induces epidermal hyperplasia [13]. Stimulation of IL-22 in keratinocytes downregulates the expression of filaggrin (FLG), which further worsens activity between protease and protease inhibitor such as kallikrein-related peptidase 5 (KLK5), kallikrein-related peptidase 7 (KLK7) and serine protease inhibitor Kazal-type 5 (SPINK5) [14]. Then, KLK5 and KLK7 induced proteolysis of desmocollin 1 (DSC-1), one of corneodesmosonal components, which is involved in hyperkeratosis [15]. In this study, IL-22/Th22 cells-mediated epidermal thickening and the underlying mechanism as a major route of lichenification observed in chronic AD phase was investigated in vivo and in vitro models.

Therefore, therapeutic management of AD has been practiced through targeting T cell pathways that block specific molecules involved in itching and inflammatory cascade [16][17]. Treatment with the corticosteroids and monoclonal antibodies classified into immunosuppressive drugs have been prescribed to reduce itching and skin inflammation for AD

patients [18][19]. However, long-term use of corticosteroids induces side effects including local skin atrophy, thinning skin, osteoporosis, and thrombosis [20]. Although calcineurin inhibitors, alternatives to corticosteroids, exert anti-inflammatory effects without local skin atrophy, the risk of malignancy, gingival hyperplasia and facial flushing is increased by prolonged usage [21]. Therefore, complementary therapies with natural herbs might be pivotal strategies for the treatment of AD.

Derma-Hc consists of five herbs including *Astragalus membranaceus* BUNGE (AM), *Schizonepeta tenuifolia* Briq. (ST), *Cryptotympana pustulata* Fabr. (CP), *Angelica sinensis* (Oliv.) Diels (AS), and *Arctium lappa* L. (AL), which have been traditionally used for treating skin disorders in Korea. In addition, the above five medicinal herbs have efficacy in maintaining coordination and balance by inhibiting blood heat and blood stasis in AD-like skin in terms of traditional Korean medicine [22]. Previous study has reported that Derma-H, mixture of AM and ST exerted anti-inflammatory and anti-pruritus effects by inhibiting the production of cytokines on AD skin [23]. In particular, AM reduced epidermal hyperplasia and hyperkeratosis in AD skin lesions [24]. ST topical treatment suppressed inflammatory factors in the serum of AD [25]. Furthermore, CP, AS, and AL were determined addition to Derma-H based on our research. CP has been known for treatment of allergic skin diseases according to analyzed prescription database [26]. In addition, AS improved skin inflammation by downregulating secretion of cytokines [27]. AL has anti-allergic activity on IgE-mediated hypersensitivity [28]. Therefore, Derma-Hc consisting of five medicinal ingredients might be expected to verify the phytotherapeutic potential of AD. We put emphasis on the IL-22-associated mechanism including characterized keratinocyte differentiation markers as well as AD-like phenotype.

2. Discussion

AD is the most common chronic inflammatory cutaneous disease related with skin hyper-reactivity to pathogenic triggers [29]. AD is caused by complex immunological pathways including skin barrier dysfunction, genetic susceptibility and dysregulation of the immune system [30]. Clinically, consequent symptoms such as pruritus, facial or extensor eruptions, relapsing dermatitis and lichenification were described as a diagnostic standard of AD by Hanifin and Rajika [31]. Alternative treatments from natural herbs have been suggested to prevent and improve AD in recent studies [32]. In our previous study, Derma-H as an effective topical ointment reduced pruritus and inflammation by inhibiting NGF-TrKA signal pathway in DNCB-induced AD skin lesions [23]. A polysaccharide from *Cryptotympana pustulata* of Derma-Hc has been used for retaining the moisture in skin [33]. In addition, another study demonstrated that *Angelica sinensis* has an effect on preventing abnormal epidermal proliferation [27]. In addition, *Arctium lappa* has been known for improving quality and texture of skin by circulating blood into the skin surface [34]. For that reason, Derma-Hc including Derma-H with three herbs was assumed to improve chronic AD symptoms such as hyperkeratosis and lichenification, resulting from severe itching.

The key signs of AD including erythema/hemorrhage, scarring/dryness, edema, and excoriation/erosion were evaluated by proportionally assigned dorsal skin to assess the accurate development of AD-like skin lesions [35]. The sum of individual scores was increased in the negative control group. The administration of Derma-Hc remarkably improved signs of AD by approximately half. These results demonstrated that Derma-Hc suppressed the development of AD by inhibiting the symptomatic intensity of AD. In addition, itching, a typical feature in AD, is an uncontrollable sensation which provokes scratching [36]. A constant ‘itch-scratch cycle’ causes more inflammation and rash, which further disrupts the cutaneous barrier [37]. In this study, topical treatment of Derma-Hc reduced scratching movement resulting in ameliorating pruritic symptoms.

Excessive scratching and rubbing also induce epidermal hyperplasia in lichenified AD skin [38][39]. Epidermal hyperplasia, called acanthosis, is described as excessive proliferation of keratinocytes in the chronic phase of AD [40]. Hyperkeratosis associated with hyperplasia is dominant in the stratum corneum, which is the outermost layer of the epidermis [41]. Therefore, thickness of epidermis and dermis was measured to evaluate the effect of Derma-Hc on the extent of epidermal hyperplasia and hyperkeratosis by staining with H&E. Moreover, mast cells play an important role in the immediate hypersensitivity to allergic diseases [42]. It has been known that the activation of mast cells increased the number of mast cells [43]. We further performed toluidine blue staining to confirm the effect of Derma-Hc on the infiltration of mast cells. Our data provided that Derma-Hc has an inhibitory effect on the release of mast cells and epidermal and dermal thickening.

During the process of epidermal differentiation, expression of desmosomal cadherin, also called desmocollin, has an effect on the cutaneous morphological phenotypes [44]. One of the components of intracellular desmosome junctional protein, DSC1 is expressed between granular and spinous layers mediating the cohesion of HaCaT cells [45]. However, according to activation of protease such as KLK5 and KLK7, the junctional structures are degraded in cell-cell adhesion [15]. Consequently, loss of keratinocyte adhesion was correlated with hyperkeratosis in the uppermost corneocytes [46]. Therefore, we hypothesized that the expression of DSC1 affects AD-like skin lesions by activating KLK-associated

peptidases. In this study, the expression level of DSC1 is downregulated in the epidermis of DNCB-induced skin, whereas, distribution of DSC1 is increased by Derma-Hc treatment. This result confirmed that Derma-Hc restored the expression of DSC1 by inhibiting induction of weakened adhesion.

In regard to skin barrier function, the epidermal barrier is composed of highly flattened and differentiated keratinocytes, thereby protecting against pathogens and avoiding loss of moisture from skin [47]. *FLG* is a critical gene for the structure of the epidermis which is located in epidermal differentiation complex (EDC) [48]. FLG degraded by proteolysis maintains epidermal hydration, the normal pH gradient of epidermis and skin barrier function [49]. FLG deficiency or null mutation leads to epidermal dysfunction, resulting in vulnerability and sensitization to allergen and epidermal hyperplasia [30][50]. In this study, the mRNA level of FLG was decreased in DNCB treated with dorsal skin of mice and T+I-treated HaCaT cells. However, Derma-Hc recovered the expression of FLG both in vivo and in vitro level. These results suggested that Derma-Hc facilitated keratinocyte differentiation by restoring the EDC marker.

Recent studies have illustrated that FLG involved in proteases and protease inhibitor become dysregulated in keratinocytes of AD, altering the balance between them, inversely [51]. Hyperkeratosis in epidermis is thoroughly regulated by serine proteases such as KLK5 or KLK7 and serine protease inhibitors such as SPINK5 [14]. KLK-associated peptidases, also known as stratum corneum enzyme are activated at a slightly alkaline pH beyond the normal pH range of 4.5 to 5.5 [52]. KLK5 has a tryptic activity regulating the KLK cascade in the stratum corneum which induce increases of skin permeability and inflammation [53]. In addition, KLK7 is a key chymotryptic enzyme in AD-like lesion that destroys the epidermal barrier homeostasis [53]. Conversely, SPINK5, also known as lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI) is responsible for pH-dependent regulation in allergic manifestation [54]. Overall, the pH value of skin is increased by lowering the expression of FLG. Activation of KLK families is elevated, thereby SPINK5 is downregulated in response to pH dependency. Expressions of mRNA levels of serine protease enzymatic activities including KLK5 and KLK7 were increased, thereby the expression of SPINK5 was declined in DNCB-treated mice and human keratinocyte treated with TI, whereas Derma-Hc treatment down-regulated the gene expressions of KLK5 and KLK7. In addition, Derma-Hc restored SPINK5 level in dorsal skin and HaCaT cells. From these findings, Derma-Hc exhibited ameliorative effect against lichenification by modulating epidermal barrier homeostasis.

JAK1-STAT3 signaling is greatly involved in terminal differentiation in keratinocytes [55]. JAK-STAT signal pathway has been known to induce epidermal hyperplasia and hyperkeratosis [56]. Hyperactivation of JAK1 is followed by increasing the expressions of KLK families [57]. In addition, STAT3 regulates genes associated with EDC markers including FLG [58]. For that reason, targeting JAK1-STAT3 signal pathway could interrupt the progression of AD. JAK1 was mainly bound on receptors of IL-22 to initiate phosphorylation of JAK1. Then, phosphorylated JAK1 phosphorylates STAT3 in sequence, thereby phosphorylated STAT3 translocate into the nucleus in keratinocyte [59]. Topical application of Derma-Hc downregulated expressions of phosphorylated JAK1 and STAT3 compared to the control group. In addition, Derma-Hc treatment dose-dependently decreased the p-form of JAK1 and STAT3 in HaCaT cells. These data suggested that Derma-Hc effectively prevents transducing signals as an inhibitor of JAK1 and STAT3 pathway.

Stimulation of the JAK1-STAT3 signal pathway upon IL-22 derived from the Th22 cell is predominantly activated in chronic AD skin [60]. Conversion of the acute phase to the chronic phase in AD is demonstrated by not only activation of Th1 and Th17 cell but also Th22 cell activity [61]. In particular, Chronic AD is characterized by recruitments of Th1, Th22, and Th17 subsets, which disrupt activation of keratinocyte differentiation markers [30]. The role for IL-22 in pathological AD promoted abnormal epidermal hyper-proliferation and impaired keratinocyte differentiation, resulting in epidermal hyperplasia and skin barrier dysfunction [58][62]. Thus, the molecular level of IL-22 was evaluated in dorsal skin tissues and human keratinocytes. Derma-Hc treatment significantly reduced the expression of IL-22 compared with DNCB-induced mice and T+I-treated HaCaT cells, respectively. Our study demonstrated that Derma-Hc inhibited the main driver of chronic AD as for IL-22.

References

1. Jeon, J.H.; Kwon, S.C.; Park, D.; Shin, S.; Jeong, J.H.; Park, S.Y.; Hwang, S.Y.; Kim, Y.B.; Joo, S.S. Anti-allergic effects of white rose petal extract and anti-atopic properties of its hexane fraction. Arch. Pharm. Res. 2009, 32, 823–830.
2. Nettis, E.; Ortoncelli, M.; Pellacani, G.; Foti, C.; Di Leo, E.; Patruno, C.; Rongioletti, F.; Argenziano, G.; Ferrucci, S.M.; Macchia, L.; et al. A Multicenter Study on the Prevalence of Clinical Patterns and Clinical Phenotypes in Adult Atopic D ermatitis. J. Investig. Allergol. Clin. Immunol. 2020, 30, 448–450.
3. Lee, J.H.; Ki, H.H.; Kim, D.K.; Lee, Y.M. Triticum aestivum sprout extract attenuates 2,4dinitrochlorobenzeneinduced at opic dermatitislike skin lesions in mice and the expression of chemokines in human keratinocytes. Mol. Med. Rep. 201

8, 18, 3461–3468.

4. De Benedetto, A.; Agnihothri, R.; McGirt, L.Y.; Bankova, L.G.; Beck, L.A. Atopic dermatitis: A disease caused by innate i mmune defects? J. Investig. Dermatol. 2009, 129, 14–30.
5. Bylund, S.; von Kobyletzki, L.B.; Svalstedt, M.; Svensson, A. Prevalence and Incidence of Atopic Dermatitis: A Systema tic Review. Acta Derm. Venereol. 2020, 100, adv00160.
6. Yarbrough, K.B.; Neuhaus, K.J.; Simpson, E.L. The effects of treatment on itch in atopic dermatitis. Dermatol. Ther. 201 3, 26, 110–119.
7. Carter, K.F.; Dufour, L.T.; Ballard, C.N. Identifying secondary skin lesions. Nursing 2004, 34, 68.
8. Lou, H.; Lu, J.; Choi, E.B.; Oh, M.H.; Jeong, M.; Barmettler, S.; Zhu, Z.; Zheng, T. Expression of IL-22 in the Skin Caus es Th2-Biased Immunity, Epidermal Barrier Dysfunction, and Pruritus via Stimulating Epithelial Th2 Cytokines and the GRP Pathway. J. Immunol. 2017, 198, 2543–2555.
9. Eyerich, K.; Eyerich, S. Th22 cells in allergic disease. Allergo J. Int. 2015, 24, 1–7.
10. Kawakami, T.; Ando, T.; Kimura, M.; Wilson, B.S.; Kawakami, Y. Mast cells in atopic dermatitis. Curr. Opin. Immunol. 20 09, 21, 666–678.
11. Amin, K. The role of mast cells in allergic inflammation. Respir. Med. 2012, 106, 9–14.
12. Wang, A.X.; Xu Landen, N. New insights into T cells and their signature cytokines in atopic dermatitis. IUBMB Life 201 5, 67, 601–610.
13. Fujita, H. The role of IL-22 and Th22 cells in human skin diseases. J. Dermatol. Sci. 2013, 72, 3–8.
14. Rerknimitr, P.; Otsuka, A.; Nakashima, C.; Kabashima, K. The etiopathogenesis of atopic dermatitis: Barrier disruption, i mmunological derangement, and pruritus. Inflamm. Regen. 2017, 37, 14.
15. Caubet, C.; Jonca, N.; Brattsand, M.; Guerrin, M.; Bernard, D.; Schmidt, R.; Egelrud, T.; Simon, M.; Serre, G. Degradati on of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK 7. J. Invest. Dermatol. 2004, 122, 1235–1244.
16. Simpson, E.L. Atopic dermatitis: A review of topical treatment options. Curr. Med. Res. Opin. 2010, 26, 633–640.
17. Tidwell, W.J.; Fowler, J.F., Jr. T-cell inhibitors for atopic dermatitis. J. Am. Acad. Dermatol. 2018, 78, S67–S70.
18. Thomsen, S.F. Atopic dermatitis: Natural history, diagnosis, and treatment. ISRN Allergy 2014, 2014, 354250.
19. Dattola, A.; Bennardo, L.; Silvestri, M.; Nistico, S.P. What’s new in the treatment of atopic dermatitis? Dermatol. Ther. 2 019, 32, e12787.
20. Mostow, E. A systematic review of the safety and efficacy of systemic corticosteroids in atopic dermatitis. J. Am. Acad. Dermatol. 2019.
21. Thaci, D.; Salgo, R. Malignancy concerns of topical calcineurin inhibitors for atopic dermatitis: Facts and controversies. Clin. Dermatol. 2010, 28, 52–56.
22. Jeon, Y.C. Treatment for an Adult Patient with Psoriasis with Traditional Korean Medicine, Especially Sa-Am Acupunctu re and Herbal Medicine. J. Acupunct. Meridian Stud. 2016, 9, 88–92.
23. Jo, S.Y.; Kim, M.H.; Lee, H.; Lee, S.H.; Yang, W.M. Ameliorative and Synergic Effects of Derma-H, a New Herbal Form ula, on Allergic Contact Dermatitis. Front. Pharmacol. 2020, 11, 1019.
24. Kim, J.H.; Kim, M.H.; Yang, G.; Huh, Y.; Kim, S.H.; Yang, W.M. Effects of topical application of Astragalus membranace us on allergic dermatitis. Immunopharmacol. Immunotoxicol. 2013, 35, 151–156.
25. Choi, Y.Y.; Kim, M.H.; Kim, J.H.; Jung, H.S.; Sohn, Y.; Choi, Y.J.; Hwang, M.K.; Kim, S.H.; Kim, J.; Yang, W.M. Schizone peta tenuifolia inhibits the development of atopic dermatitis in mice. Phytother. Res. 2013, 27, 1131–1135.
26. Chen, H.Y.; Lin, Y.H.; Huang, J.W.; Chen, Y.C. Chinese herbal medicine network and core treatments for allergic skin di seases: Implications from a nationwide database. J. Ethnopharmacol. 2015, 168, 260–267.
27. Lee, J.; Choi, Y.Y.; Kim, M.H.; Han, J.M.; Lee, J.E.; Kim, E.H.; Hong, J.; Kim, J.; Yang, W.M. Topical Application of Angel ica sinensis Improves Pruritus and Skin Inflammation in Mice with Atopic Dermatitis-Like Symptoms. J. Med. Food 201 6, 19, 98–105.
28. Sohn, E.H.; Jang, S.A.; Joo, H.; Park, S.; Kang, S.C.; Lee, C.H.; Kim, S.Y. Anti-allergic and anti-inflammatory effects of butanol extract from Arctium lappa L. Clin. Mol. Allergy 2011, 9, 4.
29. Petersen, T.K. In vivo pharmacological disease models for psoriasis and atopic dermatitis in drug discovery. Basic Clin. Pharmacol. Toxicol. 2006, 99, 104–115.

30. Kim, J.; Kim, B.E.; Leung, D.Y.M. Pathophysiology of atopic dermatitis: Clinical implications. Allergy Asthma Proc. 2019, 40, 84–92.

31. Sharma, L. Diagnostic clinical features of atopic dermatitis. Indian J. Dermatol. Venereol. Leprol. 2001, 67, 25–27.

32. Vender, R.B. Alternative treatments for atopic dermatitis: A selected review. Skin Ther. Lett. 2002, 7, 1–5.

33. Zhu, Y.; Yu, X.; Ge, Q.; Li, J.; Wang, D.; Wei, Y.; Ouyang, Z. Antioxidant and anti-aging activities of polysaccharides fro m Cordyceps cicadae. Int. J. Biol. Macromol. 2020, 157, 394–400.

34. Chan, Y.S.; Cheng, L.N.; Wu, J.H.; Chan, E.; Kwan, Y.W.; Lee, S.M.; Leung, G.P.; Yu, P.H.; Chan, S.W. A review of the pharmacological effects of Arctium lappa (burdock). Inflammopharmacology 2011, 19, 245–254.

35. Hanifin, J.M.; Thurston, M.; Omoto, M.; Cherill, R.; Tofte, S.J.; Graeber, M. The eczema area and severity index (EASI): Assessment of reliability in atopic dermatitis. EASI Evaluator Group. Exp. Dermatol. 2001, 10, 11–18.

36. Ikoma, A.; Steinhoff, M.; Stander, S.; Yosipovitch, G.; Schmelz, M. The neurobiology of itch. Nat. Rev. Neurosci. 2006, 7, 535–547.

37. Rinaldi, G. The Itch-Scratch Cycle: A Review of the Mechanisms. Dermatol. Pract. Concept. 2019, 9, 90–97.

38. Arima, K.; Ohta, S.; Takagi, A.; Shiraishi, H.; Masuoka, M.; Ontsuka, K.; Suto, H.; Suzuki, S.; Yamamoto, K.; Ogawa, M.; et al. Periostin contributes to epidermal hyperplasia in psoriasis common to atopic dermatitis. Allergol. Int. 2015, 64, 41–48.

39. Matsunaga, Y.; Ogura, Y.; Ehama, R.; Amano, S.; Nishiyama, T.; Tagami, H. Establishment of a mouse skin model of th e lichenification in human chronic eczematous dermatitis. Br. J. Dermatol. 2007, 156, 884–891.

40. Nakahigashi, K.; Kabashima, K.; Ikoma, A.; Verkman, A.S.; Miyachi, Y.; Hara-Chikuma, M. Upregulation of aquaporin-3 is involved in keratinocyte proliferation and epidermal hyperplasia. J. Investig. Dermatol. 2011, 131, 865–873.

41. Gu, H.; Kim, W.H.; An, H.J.; Kim, J.Y.; Gwon, M.G.; Han, S.M.; Leem, J.; Park, K.K. Therapeutic effects of bee venom o n experimental atopic dermatitis. Mol. Med. Rep. 2018, 18, 3711–3718.

42. Irani, A.M.; Sampson, H.A.; Schwartz, L.B. Mast cells in atopic dermatitis. Allergy 1989, 44 (Suppl. 9), 31–34.

43. Sehra, S.; Serezani, A.P.M.; Ocana, J.A.; Travers, J.B.; Kaplan, M.H. Mast Cells Regulate Epidermal Barrier Function a nd the Development of Allergic Skin Inflammation. J. Investig. Dermatol. 2016, 136, 1429–1437.

44. Donetti, E.; Bedoni, M.; Boschini, E.; Dellavia, C.; Barajon, I.; Gagliano, N. Desmocollin 1 and desmoglein 1 expression in human epidermis and keratinizing oral mucosa: A comparative immunohistochemical and molecular study. Arch. Der matol. Res. 2005, 297, 31–38.

45. Totsuka, A.; Omori-Miyake, M.; Kawashima, M.; Yagi, J.; Tsunemi, Y. Expression of keratin 1, keratin 10, desmoglein 1 and desmocollin 1 in the epidermis: Possible downregulation by interleukin-4 and interleukin-13 in atopic dermatitis. Eu r. J. Dermatol. 2017, 27, 247–253.

46. Matsui, T.; Amagai, M. Dissecting the formation, structure and barrier function of the stratum corneum. Int. Immunol. 20 15, 27, 269–280.

47. Sandilands, A.; Sutherland, C.; Irvine, A.D.; McLean, W.H. Filaggrin in the frontline: Role in skin barrier function and dis ease. J. Cell Sci. 2009, 122, 1285–1294.

48. O'Regan, G.M.; Sandilands, A.; McLean, W.H.; Irvine, A.D. Filaggrin in atopic dermatitis. J. Allergy Clin. Immunol. 2009, 124, R2–R6.

49. Brown, S.J.; McLean, W.H. One remarkable molecule: Filaggrin. J. Investig. Dermatol. 2012, 132, 751–762.

50. Scharschmidt, T.C.; Man, M.Q.; Hatano, Y.; Crumrine, D.; Gunathilake, R.; Sundberg, J.P.; Silva, K.A.; Mauro, T.M.; Hu pe, M.; Cho, S.; et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory threshol ds to irritants and haptens. J. Allergy Clin. Immunol. 2009, 124, 496–506.e6.

51. Lee, H.J.; Lee, S.H. Epidermal permeability barrier defects and barrier repair therapy in atopic dermatitis. Allergy Asthm a Immunol. Res. 2014, 6, 276–287.

52. Ekholm, I.E.; Brattsand, M.; Egelrud, T. Stratum corneum tryptic enzyme in normal epidermis: A missing link in the desq uamation process? J. Investig. Dermatol. 2000, 114, 56–63.

53. Morizane, S. The Role of Kallikrein-Related Peptidases in Atopic Dermatitis. Acta Med. Okayama 2019, 73, 1–6.

54. Egawa, G.; Kabashima, K. Multifactorial skin barrier deficiency and atopic dermatitis: Essential topics to prevent the ato pic march. J. Allergy Clin. Immunol. 2016, 138, 350–358.e1.

55. Fridman, J.S.; Scherle, P.A.; Collins, R.; Burn, T.; Neilan, C.L.; Hertel, D.; Contel, N.; Haley, P.; Thomas, B.; Shi, J.; et a l. Preclinical evaluation of local JAK1 and JAK2 inhibition in cutaneous inflammation. J. Investig. Dermatol. 2011, 131,

1838–1844.

56. He, H.; Guttman-Yassky, E. JAK Inhibitors for Atopic Dermatitis: An Update. Am. J. Clin. Dermatol. 2019, 20, 181–192.
57. Yasuda, T.; Fukada, T.; Nishida, K.; Nakayama, M.; Matsuda, M.; Miura, I.; Dainichi, T.; Fukuda, S.; Kabashima, K.; Nakaoka, S.; et al. Hyperactivation of JAK1 tyrosine kinase induces stepwise, progressive pruritic dermatitis. J. Clin. Investig. 2016, 126, 2064–2076.
58. Brunner, P.M.; Pavel, A.B.; Khattri, S.; Leonard, A.; Malik, K.; Rose, S.; Jim On, S.; Vekaria, A.S.; Traidl-Hoffmann, C.; Singer, G.K.; et al. Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab. J. Allergy Clin. Immunol. 2019, 143, 142–154.
59. Welsch, K.; Holstein, J.; Laurence, A.; Ghoreschi, K. Targeting JAK/STAT signalling in inflammatory skin diseases with small molecule inhibitors. Eur. J. Immunol. 2017, 47, 1096–1107.
60. Lejeune, D.; Dumoutier, L.; Constantinescu, S.; Kruijer, W.; Schuringa, J.J.; Renauld, J.C. Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line. Pathways that are shared with and distinct from IL-10. J. Biol. Chem. 2002, 277, 33676–33682.
61. Bernard, F.X.; Morel, F.; Camus, M.; Pedretti, N.; Barrault, C.; Garnier, J.; Lecron, J.C. Keratinocytes under Fire of Proinflammatory Cytokines: Bona Fide Innate Immune Cells Involved in the Physiopathology of Chronic Atopic Dermatitis and Psoriasis. J. Allergy 2012, 2012, 718725.
62. Hanel, K.H.; Cornelissen, C.; Luscher, B.; Baron, J.M. Cytokines and the skin barrier. Int. J. Mol. Sci. 2013, 14, 6720–6745.