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Diverse Regulation of Claudin-1 and Claudin-4 in Atopic Dermatitis

Robert Gruber,*† Christian Börnchen,‡ Katharina Rose,‡ Anne Daubmann,§ Thomas Volksdorf,‡ Ewa Wladykowski,‡ Sabine Vidal-y-Sy,‡ Eva M. Peters,¶‖ Mogbekeloluwa Danso,** Joke A. Bouwstra,** Hans C. Hennies,*†,†† Ingrid Moll,‡ Matthias Schmuth,* and Johanna M. Brandner‡

From the Department of Dermatology, Venerology, and Allergology, and the Division of Human Genetics,† Medical University of Innsbruck, Innsbruck, Austria; the Departments of Dermatology and Venerology‡ and Medical Biometry and Epidemiology,§ University Hospital Hamburg-Eppendorf, Hamburg, Germany; the Department of Psychosomatic Medicine,¶ Psychoneuroimmunology Laboratory, Justus-Liebig University, Giessen, Germany; the Center for Internal Medicine and Dermatology,‖ Charité-University Medicine, Berlin, Germany; the Department of Drug Delivery Technology,** Leiden Academic Center for Drug Research, Leiden University, Leiden, the Netherlands; and the Cologne Center for Genomics,†† University of Cologne, Cologne, Germany*

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Address correspondence to Johanna M. Brandner, Ph.D., Department of Dermatology and Venerology, University Hospital Hamburg-Eppendorf, Martinistrasse 52, 22303 Hamburg, Germany; or Robert Gruber, M.D., Department of Dermatology and Venerology and Division of Human Genetics, Medical University of Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria. E-mail: brandner@uke.de or r.gruber@i-med.ac.at.

Tight junctions are important for skin barrier function. The tight junction protein claudin 1 (Cldn-1) has been reported to be down-regulated in nonlesional skin of atopic dermatitis (AD) patients. In contrast, we did not observe a significant down-regulation of Cldn-1 in nonlesional skin of the AD cohort used in this study. However, for the first time, a significant down-regulation of Cldn-1 in the upper and lower epidermal layers of lesional skin was detected. In addition, there was a significant up-regulation of Cldn-4 in nonlesional, but not lesional, AD skin. For occludin, no significant alterations were observed. In an AD-like allergic dermatitis mouse model, Cldn-1 down-regulation in eczema was significantly influenced by dermal inflammation, and significantly correlated with hallmarks of eczema (ie, increased keratinocyte proliferation, altered keratinocyte differentiation, increased epidermal thickness, and impaired barrier function). In human epidermal equivalents, the addition of IL-4, IL-13, and IL-31 resulted in a down-regulation of Cldn-1, and *Cldn1* knockdown in keratinocytes resulted in abnormal differentiation. In summary, we provide the first evidence that Cldn-1 and Cldn-4 are differentially involved in AD pathogenesis. Our data suggest a role of Cldn-1 in AD eczema formation triggered by inflammation. *(Am J Pathol 2015, 185: 2777–2789; http://dx.doi.org/10.1016/j.ajpath.2015.06.021)*

Tight junctions (TJs) are cell-cell junctions, which are known from simple epithelia to be important for barrier function, as well as cell proliferation and differentiation.[1,2] Within the epidermis, TJs are located in the granular cell layers,[3,4] forming a barrier for molecular tracers in both mouse[5] and humans.[6] The TJ transmembrane protein claudin-1 (Cldn-1) is essential for murine skin barrier function. A knockout of Cldn-1 results in increased transepidermal water loss (TEWL), leading to death of mice within the first day of life.[5] This increase in TEWL is not because of decreased TJ water barrier function,[7] but because of increased stratum corneum water permeability.[8] In cultured human keratinocytes, Cldn-1 forms a permeability barrier for ions and (macro)-molecules sized between 332 and 40,000 Da, including the size of typical allergens.[7] The TJ transmembrane proteins Cldn-4 and occludin (Ocln) are involved in barrier formation for ions and molecules between 332 and 4000 Da in cultured keratinocytes.[7] Their role in skin barrier function remains unclear, but they are altered in the inflammatory skin disease psoriasis.[4,9,10]

Atopic dermatitis (AD) is a common, chronic, relapsing inflammatory skin disease, characterized by dry eczematous

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skin with intense pruritus, as well as increased levels of allergen-specific IgE serum antibodies, and inflammatory infiltrates producing proallergic cytokines (IL-4, IL-13, and IL-31).[11,12] Both gene-gene and gene-environment interactions play a role in AD pathogenesis, and an impaired epidermal barrier is increasingly accepted as a key step in disease development. Although null mutations in the filaggrin (*FLG*) gene are strongly associated with AD in approximately one third of European AD patients,[13] two thirds of AD patients lack a *FLG* mutation. Recently, further susceptibility loci for AD have been identified, and the search for new molecules modulating the skin barrier is ongoing.[14–16]

Recently, decreased Cldn-1 expression was reported to contribute to impaired skin barrier function in nonlesional skin from Northern American AD patients.[17] An association between AD and haplotype-tagging single-nucleotide polymorphisms in the region of *CLDN1* was reported too.[17] We herein investigated the role of Cldn-1 and other TJ proteins in a central European AD cohort. First, we did not find a consistent down-regulation of Cldn-1 in nonlesional skin in our AD cohort. Instead, we found an up-regulation of Cldn-4. Second, we observed a significant down-regulation of Cldn-1 in lesional AD skin, which prompted us to perform further investigations in an AD-like allergic dermatitis (AID) mouse model (characterized by increased levels of IgE, IL-4, and IL-5 levels, increased numbers of eosinophils and CD4 T cells, and an increased type 2 helper T-cell/type 1 helper T-cell ratio),[18,19] and in reconstructed human epidermis (RHE).

Materials and Methods

Human Subjects

The study was approved by the Institutional Review Boards of the Innsbruck Medical University (Innsbruck, Austria) and the University Hospital Hamburg-Eppendorf (Hamburg, Germany), and complied with the Declaration of Helsinki principles. Twenty-five well-characterized adult whites with extrinsic AD and 12 age-, sex-, race-, and site-matched controls without carrying a *FLG* mutation were included between January 2012 and March 2013 (Table 1). All patients and 10 controls consented to a skin biopsy obtained from untanned lower back skin for immunohistochemistry. In eight patients, the biopsy taken in this location was from lesional skin; and in 17 patients, from nonlesional skin. Lower back skin was chosen because influences by sunlight are unlikely on this anatomical site. Lesional AD was defined as inflamed erythematous itching skin, but without signs of superinfection. None of the subjects used emollients or any other topical formulations on their backs for at least 5 days before a skin biopsy was performed. The clinical diagnosis of AD was made by two experienced dermatologists, in accordance with the common diagnostic criteria (R.G. and M.S.).[20] Patients with AD and concomitant endocrinologic disorders, cancer, and/or treatment with phototherapy, systemic immunosuppressives, retinoids, or chemotherapy were excluded. Blood samples for total serum IgE levels and genotyping were taken. All subjects provided written informed consent.

Functional Measurements

Functional measurements were performed on lesional and nonlesional skin of AD patients and of controls on both the lower arms and lower legs. Subjects were not allowed to use topical formulations on the test sites for at least 5 days before measurements. TEWL was measured with an evaporimeter (Servomed, Stockholm, Sweden), and skin surface pH was measured with a PH900 meter (Courage&Khazaka, Cologne, Germany). Study subjects underwent at least a 20-minute preassessment rest period. Measurements were taken in accordance with published guidelines.[21–23] Environment-related variables were as follows: ambient air temperature, 20°C to 24°C; skin

Table 1 Patient Characteristics

Characteristics	Atopic dermatitis patients	Controls
Sample size	25	12
Male subjects, No. (%)	9 (36)	6 (50)
Age, median, years	29	32.5
EASI, mean (95% CI)	11.76 (8.19–15.3)	NA
Total IgE, mean (95% CI)	1792 (1003–2581)	16.21 (5.9–26.52)
Filaggrin-null alleles, No. (%)	9 (36)	0 (0)
No. of specific filaggrin mutations	5× c.2282del4/wt; 4× p.R501X/wt	
Frequency of allergies		No allergies
Type I (%)	Pollen (72) House dust mite (44) Cat (32) Horse (24) Dog (20) Wheat (8) Nuts (16)	
Type IV (%)	Nickel (20)	

EASI, eczema area and severity index; NA, not applicable.