Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin

Laurence Pellerin, PhD,[a,b,c] Julie Henry, PhD,[a,b,c] Chiung-Yueh Hsu, PhD,[a,b,c] Stéfana Balica, MD,[d] Catherine Jean-Decoster, PhD,[e] Marie-Claire Méchin, PhD,[a,b,c] Britta Hansmann, PhD,[f] Elke Rodriguez, PhD,[f] Stefan Weindinger, MD, PhD,[f] Anne-Marie Schmitt, MD, PhD,[e] Guy Serre, MD, PhD,[a,b,c] Carle Paul, MD, PhD,[a,b,c,d]* and Michel Simon, PhD[a,b,c]* *Toulouse, France, and Kiel, Germany*

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by a disturbed epidermal barrier. In a subset of patients, this is explained by nonsense mutations in the gene encoding filaggrin (FLG).

Objectives: We sought to evaluate the respective role of *FLG* mutations and proinflammatory cytokines and to assess the expression of FLG, hornerin (HRNR), and FLG2, 2 FLG-like proteins, which are involved in epidermal barrier functions, in normal skin and both lesional and nonlesional skin of patients with AD.

Methods: An *FLG*-genotyped cohort of 73 adults with AD and 73 aged-matched control subjects was analyzed by using immunohistochemistry and immunoblotting. Normal primary human keratinocytes were differentiated in either the absence or presence of IL-4, IL-13, and IL-25.

Results: Compared with control subjects, FLG, HRNR, and FLG2 were detected at significantly lower levels in the skin of patients with AD, irrespective of their *FLG* genotype. The reduction was greater in lesional compared with nonlesional skin. In addition, the proFLG/FLG ratio was found to be higher in the skin of wild-type patients than in control subjects. Cytokine treatment of keratinocytes induced a dramatic reduction in FLG, FLG2, and HRNR expression both at the mRNA and protein levels.

Conclusion: The stratum corneum of lesional but also clinically unaffected skin of adults with AD is abnormal, with reduced expression of FLG and FLG-like proteins. In addition to nonsense mutations, proinflammatory cytokines and some defects in the proFLG processing can contribute to the FLG downregulation. Our study suggests that skin inflammation reduces the expression of FLG-like proteins, contributing to the AD-related epidermal barrier dysfunction. (J Allergy Clin Immunol 2013;131:1094-102.)

Key words: *Atopic dermatitis, skin, keratinocytes, filaggrin, hornerin, stratum corneum, skin barrier, cytokine*

Atopic dermatitis (AD; OMIM #603165) is one of the most common chronic inflammatory skin diseases. It usually begins in early childhood and affects up to 20% of children and 3% of adults in industrialized countries. AD is characterized by erythematous skin lesions, pruritis, altered epidermal barrier, and marked mononuclear cell infiltrate in the dermis.[1] AD results from complex interactions between genetic and environmental factors. Two nonexclusive pathophysiologic models have been proposed and remain debated. Historically, it was thought that the primary defect resides in the immune system, leading to excessive inflammation and a secondary local epidermal barrier disruption (the inside-outside theory).[2] Loss-of-function mutations in the gene encoding filaggrin (FLG) are the strongest and most widely replicated risk factor for the disease (see Palmer et al,[3] Brown and McLean,[4] and the references cited therein), suggesting an alternative view of AD pathophysiology because FLG is an essential component of the stratum corneum.[5] A primary intrinsic alteration of the upper epidermis allows the entrance of pathogens and allergens and induces a subsequent immune response (the outside-inside theory).[1,6-9]

FLG is synthesized by granular keratinocytes as a large precursor called proFLG. ProFLG consists of a large repetitive central domain flanked by 2 unique N- and C-terminal domains. During the late steps of terminal differentiation, proFLG is cleaved. The generated basic FLG monomers aggregate the keratin cytoskeleton to form the corneocyte fibrous matrix. In the upper stratum corneum, FLG is completely proteolyzed into free amino acids that are essential for skin photoprotection and for acidification and hydration of the stratum corneum.[10-12] In turn, FLG deficiency has

From [a]UMR5165 CNRS, [b]U1056 INSERM, [c]the University of Toulouse, [d]the Department of Dermatology, University Hospital of Toulouse, and [e]Centre Européen de Recherche sur la Peau et les Epithéliums de Revêtement (CERPER), Pierre Fabre Dermo-Cosmétique, Toulouse, and [f]the Department of Dermatology, University Hospital Schleswig-Holstein, Kiel.

*These authors contributed equally to this work.

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Corresponding author: Michel Simon, PhD, CNRS-UPS UMR5165, CHU Purpan, Place du Dr Baylac TSA40031, 31059 Toulouse cedex 9, France. E-mail: michel.simon@udear.cnrs.fr.

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