Reduced Inflammatory Threshold Indicates Skin Barrier Defect in Transglutaminase 3 Knockout Mice

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Recently, a transglutaminase 3 knockout (TGM3/KO) mouse was generated that showed impaired hair development, but no gross defects in the epidermal barrier, although increased fragility of isolated corneocytes was demonstrated. Here we investigated the functionality of skin barrier *in vivo* by percutaneous sensitization to FITC in TGM3/KO ($n=64$) and C57BL/6 wild-type (WT) mice ($n=36$). Cutaneous inflammation was evaluated by mouse ear swelling test (MEST), histology, serum IgE levels, and by flow cytometry from draining lymph nodes. Inflammation-induced significant MEST difference ($P<0.0001$) was detected between KO and WT mice and was supported also by histopathology. A significant increase of CD4+ CD25+-activated T cells ($P<0.01$) and elevated serum IgE levels ($P<0.05$) in KO mice indicated more the development of FITC sensitization than an irritative reaction. *Propionibacter acnes*–induced intracutaneous inflammation showed no difference ($P=0.2254$) between the reactivity of WT and KO immune system. As *in vivo* tracer, FITC penetration from skin surface followed by two-photon microscopy demonstrated a more invasive percutaneous penetration in KO mice. The clinically uninvolved skin in TGM3/KO mice showed impaired barrier function and higher susceptibility to FITC sensitization indicating that TGM3 has a significant contribution to the functionally intact cutaneous barrier.

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INTRODUCTION

Our knowledge on epidermal differentiation and keratinization has significantly grown within the last decade. Recent studies provide a large amount of information on proteins and enzymes of the skin barrier; however, their significance in the clinical setting is only partially understood.

The epidermal barrier has a key role in skin homeostasis and mostly environmental antigens penetrating this barrier are able to induce reactions in the skin-associated adoptive and/or innate immune system and promote irritative or allergic contact dermatitis.

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Abbreviations: CH, contact hypersensitivity; DBP, dibutyl-phtalate; ET, ear thickness; KO, knockout; LN, lymph node; MEST, mouse ear swelling test; PBS, phosphate-buffered saline; SPINK5, serine protease inhibitor Kazal-type 5; TG, transglutaminase (protein); TGM, transglutaminase (gene); TGM3/KO, transglutaminase 3 knockout; WT, wild type

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Transglutaminases (TG) are a group of Ca 2+-dependent enzymes, which catalyze formation of covalent isopeptide bonds and produce stabilizing cross-links between protein networks. Some of these structures contribute to an effective barrier in the skin and mucosa. TGs were first described in 1959 (Clarke *et al.*, 1959), but the exact biological function of a TG enzyme, Factor XIIIa, in the process of blood coagulation was only discovered in 1968 (Pisano *et al.*, 1968). So far, eight TGs have been identified (Hitomi *et al.*, 2001; Griffin *et al.*, 2002; Eckert *et al.*, 2005). In the outer layers of the skin and hair follicle, mainly keratinocyte-type TG (TG1) and epidermal-type TG (TG3) are expressed. Defective cross-linking of the cell envelope because of mutations in the *TGM1* gene has been found in patients suffering from lammelar ichthyosis (Huber *et al.*, 1995; Russel et al., 1995). Accordingly, TGM1 knockout mice develop an erythrodermic skin with impaired barrier function (Matsuki *et al.*, 1998). Although no *in vivo* role for TG3 has been described, it is highly expressed in the late differentiating keratinocytes, corneocytes, and the hair follicles. TG3 is also a major antigen in dermatitis herpetiformis where TG3-IgA immune complexes are deposited in the papillary dermis (Sardy *et al.*, 2002).

Recently, a transglutaminase 3 knockout (TGM3/KO) mouse was generated that showed impaired hair development, but no gross defects in the epidermal barrier (John *et al.*, 2012). Here we further investigated in these mice the functionality of the skin barrier by a generally accepted and widely used Th2

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