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Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6

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Abstract Atopic dermatitis (AD) is characterized by a defective skin barrier which allows increased allergen and pathogen penetration. Loricrin (LOR) and involucrin (IVL) are proteins important for skin barrier formation and integrity. In this study, we demonstrate that the gene and protein expression of LOR and IVL is significantly decreased in involved (LOR: $p<0.001$; IVL: $p<0.001$) and uninvolved (LOR: $p<0.001$; IVL: $p<0.001$) skin of AD subjects, as compared to skin from healthy subjects. Using primary keratinocytes, we further demonstrate the down-regulatory effect of IL-4 and IL-13 – which are over-expressed in the skin of AD patients – on LOR and IVL expression in keratinocytes. Additionally, skin biopsies from signal transducer and activator of transcription (STAT)-6 transgenic mice were deficient in the expression and production of LOR and IVL. This study suggests that Th2 cytokines inhibit expression of LOR and IVL through a STAT-6 dependent mechanism.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects up to 20% of children and significantly disrupts the quality of life for each individual affected by the disease [1]. In recent years, it has been suggested that the epidermal skin barrier plays a significant role in AD disease susceptibility and severity [2–5]. Using a murine model of AD, it has been shown that skin barrier dysfunction enhances allergen sensitization leading to increased IgE levels and airway hyper-reactivity [6]. This supports the notion that absorption of allergens through the skin may be the first step of the atopic march.

Loricrin (LOR) and involucrin (IVL) are important proteins that facilitate terminal differentiation of the epidermis and formation of the skin barrier [7–12]. Human LOR is an insoluble protein initially expressed in the granular layer of the epidermis during cornification and comprises 80% of the total protein mass of the cornified envelope (CE) [7,13–16]. Additionally, LOR functions as a main reinforcement protein for the CE and is deposited onto a scaffold of IVL and other

Abbreviations: AD, atopic dermatitis; CE, cornified envelope; EDC, epidermal differentiation complex; IL, interleukin; IVL, involucrin; LOR, loricrin; STAT, signal transducer and activator of transcription.

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calcium-binding proteins [17]. IVL is an also common component of the CE and provides a scaffold to which other proteins subsequently become cross-linked [8]. In the CE structure, IVL is adjacent to the cell membrane and forms the exterior surface of the CE [18].

The current studies were therefore conducted to investigate the expression of LOR and IVL in eczematous skin lesions and non-lesional skin from AD subjects and investigate the role of IL-4 and IL-13, cytokines over-expressed in AD skin [19, 20], on the expression of LOR and IVL.

Materials and methods

Patients

Subjects included 13 healthy persons with no history of skin disease (mean age: 34.3 years) and 14 patients with moderate-to-severe atopic dermatitis (mean age: 33.6 years; 20–60% skin involvement). None of the patients had previously received systemic corticosteroids or cyclosporine, and none had received topical corticosteroid or calcineurin inhibitors for at least 1 week before enrollment. This study was approved by the Institutional Review Board at National Jewish Medical and Research Center in Denver and conducted according to the Declaration of the Helsinki Guidelines. All subjects gave written informed consent prior to participation in this study.

Two-millimeter punch biopsies were collected from erythematous lesions that were less than 3 days old (involved atopic dermatitis) and uninvolved skin from the same AD patients, and normal healthy skin. The skin biopsies were immediately submerged in 1 ml Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH) and frozen at −80 °C for future RNA isolation or immediately in 1 ml 10% buffered formalin for immunohistochemistry.

Mice

Heterozygous STAT-6 transgenic mice were obtained from Mark Kaplan (Indiana University, Indianapolis, IN). These mice constitutively express STAT-6. Upon arrival, heterozygous mice were bred with C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) to generate additional transgenic mice and wild-type controls. All animal protocols were approved by the Institutional Animal Care and Use Committee at the National Jewish Medical and Research Center. This institution has an animal welfare assurance number (A3026-1) on file with the Office of Protection and Research Risks.

The dorsal thoracic and lumbar regions of mice were clipped and treated with the depilatory agent Nair to remove hair. Seventy-two hours following hair removal, mice were euthanized via carbon dioxide asphyxiation. Four-millimeter biopsies were collected and immediately submerged in 1 ml Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH) for RNA isolation.

RNA preparation and real-time RT-PCR

Total RNA was isolated from skin biopsies by chloroform: phenol extraction and isopropanol precipitation according to manufacturer's guidelines (Molecular Research Center, Inc., Cincinnati, OH). RNeasy Mini Kits (Qiagen, Valencia, CA) were used according to the manufacturer's protocol to further purify RNA from skin biopsies and to isolate RNA from cell cultures. One microgram of RNA was reverse-transcribed in a 20-μl reaction containing Random Primers (Invitrogen, Carlsbad, CA), dNTP Mix (Invitrogen), 5×First Strand Buffer (Invitrogen), RNase Inhibitor (Invitrogen) and Superscript III enzyme (Invitrogen) for 60 min at 42 °C and then 70 °C for 15 min. Real-time RT-PCR was performed and analyzed by the dual-labeled fluorogenic probe method using an ABI Prism 7300 sequence detector (Applied Biosystems, Foster City, CA) as previously described [20]. Primers and probes for human GAPDH, LOR and IVL were purchased from Applied Biosystems. Relative expression levels were calculated by the relative standard curve method as outlined in the manufacturer's technical bulletin (Applied Biosystems). A standard curve was generated using the fluorescent data from 10-fold serial dilutions of total RNA of the highest expression sample. To allow for comparisons between samples and group, quantities of all targets in test samples were normalized to the corresponding GAPDH levels in the skin biopsies and cultured keratinocytes.

Immunohistochemistry

Paraffin-embedded tissues were cut at 5 μm and placed on frosted microscope slides. Using xylene and a series of ethanol

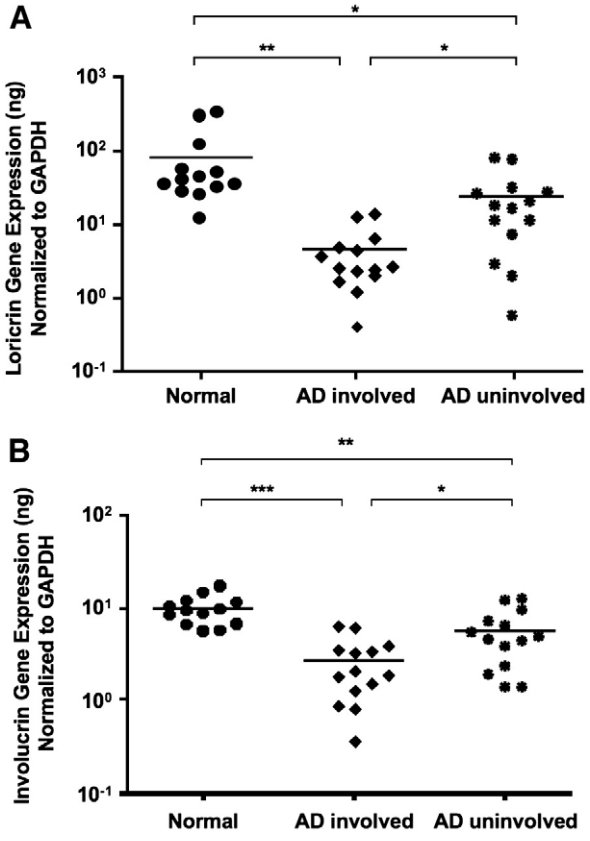

Figure 1 Gene expression of LOR and IVL in skin from normal subjects and AD patients. RNA was isolated from the skin of 13 normal subjects and 14 patients with AD, and the gene levels of LOR (A) and IVL (B) were evaluated by real-time RT-PCR. *$p<0.05$; **$p<0.01$; ***$p<0.001$.