Abstract

Atopic dermatitis (AD) is a chronic inflammatory disease characterized by significant barrier disruption and intense pruritus. In recent years, there has been a growing number of targeted therapies in clinical development with a predominant focus on antagonizing Th2-mediated inflammation; however, these therapies are effective (IgA §1) in less than 50% of AD patients. We hypothesized that baseline expression of key inflammatory genes would identify potential subsets of AD patients for a more targeted therapeutic intervention with monoclonal antibody-based therapies. Epidermal skin samples were non-invasively collected from the lesional skin of 31 patients with moderate to severe AD using the ‘Smart Sticker’ adhesive skin collection kit. RNA was subsequently isolated and analyzed by RT-qPCR for pre-identified genes important to AD disease pathogenesis. IL-4Ra and IL-1Ra genes were expressed in 100% of AD patients. Similarily, CCL17/TARC, a biomarker of AD disease severity, was expressed in 96.8% (30/31) of AD patients. Interestingly, the Th2 cytokine IL-13 was expressed in 54.8% (17/31) while IL-31 was expressed in 29.0% (9/31). Additionally, the Th17 associated genes IL-22 and IL-23 were expressed in 51.6% (16/31) and 58.1% (18/31), respectively. Overall, this study demonstrates the potential utility of non-invasive skin sampling to stratify AD patients based on their dominant inflammatory signature and suggests the incorporation of this clinically valuable technique in the personalized treatment of AD patients with targeted therapies.

Methods

Subjects

Thirty-one subjects at least 18 years of age with clinically diagnosed moderate to severe atopic dermatitis and 20 subjects with healthy looking skin (normal skin) were enrolled in this study. Non-invasive skin samples were collected from all enrolled subjects using the DermTech adhesive skin collection kit (DermTech, Inc., La Jolla, CA) as described below. The study was reviewed and approved by Aspire IRB (San Diego, CA). All subjects provided written consent prior to enrollment.

Skin Sampling

The DermTech adhesive skin collection kit (DermTech, La Jolla, CA) was used to collect skin samples from lesional skin and nearby non-lesional skin. Prior to application of the Smart Sticker™ (Figure 1), the target skin was prepped with an alcohol pad to remove oils and then dry-wiped with a gauze pad to remove any remaining moisture. Each kit contains a total of 4 Smart Stickers™ for sample collection. Smart Stickers™ were applied individually to the designated area and 5 circular motions with the thumb were used to ensure adhesion to the targeted skin. Using a pen, small marks were made on the skin after application of the first tape strip to ensure consistent placement of each subsequent tape strip. Smart Stickers™ were removed slowly using standard precautions to prevent folding and eliminate potential sources of contamination. Once removed, the Smart Stickers™ were then placed onto the tri-fold sample collector. This process was repeated with the 2nd, 3rd, and 4th Smart Stickers™ on the same lesional/non-lesional skin. Once all 4 Smart Stickers™ were in the tri-fold collector, the collector was carefully folded and placed in a re-sealable bag for overnight shipment (the Smart Sticker™ and tri-fold collector are stable for 10 days at room temperature). Upon arrival at DermTech, samples were stored at -70°C or colder until RNA extraction.

RNA Extraction and Gene Expression Analysis

RNA was extracted from Smart Stickers™ using a closed-tube, bead-based method. Briefly, Smart Stickers™ were enzymatically digested to extract the genomic material from the adhesive and acidic acid isolated using magnetic beads on a kingfisher flex instrument (ThermoFisher Scientific). RNA was converted to cDNA for subsequent analysis of genes outlined below using an ABI7500. Cycle threshold (Ct) values of all qPCR reactions were measured and gene expression levels of each target gene were presented as ∆Ct (calculated as: ∆Ct = Ct (target)-Ct (ACTB, from the same sample)). Differences between normal skin and non-lesional AD skin (∆Ct) were calculated as: ∆Ct = Ct (lesional AD) - Ct (normal skin). Data is presented as normalized gene expression.

Conclusions

- Non-invasive skin sampling with the Smart Sticker™ is a viable method to assess inflammatory signatures in lesional AD skin
- Non-invasively assessed inflammatory gene expression has the potential to stratify AD subjects into different subtypes
- Additional studies are needed to evaluate the relationship between baseline inflammation and clinical response to targeted therapies

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