Comparative proteomic profiling of patients with atopic dermatitis based on history of eczema herpeticum infection and *Staphylococcus aureus* colonization

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Background: Atopic dermatitis (AD) is the most common inflammatory skin disorder in the general population worldwide, and the majority of patients are colonized with *Staphylococcus aureus*. Eczema herpeticum is a disseminated herpes simplex virus infection that occurs in a small subset of patients.

Objectives: The goal was to conduct proteomic profiling of patients with AD based on *S aureus* colonization status and history of eczema herpeticum. We hoped to identify new biomarkers for improved diagnosis and prediction of eczema herpeticum and *S aureus* susceptibility and to generate new hypotheses regarding disease pathogenesis.

Methods: Skin taping was performed on nonlesional skin of nonatopic control subjects and on lesional and nonlesional skin of patients with AD. Subjects were classified according to the history of eczema herpeticum and *S aureus* colonization. Proteins were analyzed by using mass spectrometry; diagnostic groups were compared for statistically significant differences in protein expression.

Results: Proteins related to the skin barrier (filaggrin-2, corneodesmosin, desmoglein-1, desmocollin-1, and transglutaminase-3) and generation of natural moisturizing factor (arginase-1, caspase-14, and gamma-glutamyl cyclotransferase) were expressed at significantly lower levels in lesional versus nonlesional sites of patients with AD with and without history of eczema herpeticum; epidermal fatty acid–binding protein was expressed at significantly higher levels in patients with methicillin-resistant *S aureus*.

Conclusion: This noninvasive, semiquantitative profiling method has revealed novel proteins likely involved in the pathogenesis of AD. The lower expression of skin barrier proteins and enzymes involved in the generation of the natural moisturizing factor could further exacerbate barrier defects and perpetuate water loss from the skin. The greater expression of epidermal fatty acid–binding protein, especially in patients colonized with methicillin-resistant *S aureus*, might perpetuate the inflammatory response through eicosanoid signaling.

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Key words: *Atopic dermatitis, mass spectrometry, proteomics, natural moisturizing factor, eczema herpeticum, tape stripping, skin barrier, filaggrin-2, epidermal fatty acid binding protein, methicillin-resistant* Staphylococcus aureus

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that affects nearly 17% of children and can persist into adulthood,[1] significantly compromising quality of life.[2] AD is a multifactorial skin disease characterized by defects in the skin barrier and immune system.[3] Numerous factors modulate disease severity on an individual basis, including genetic susceptibility,[4] immune response,[5] and diverse environmental factors.[3] Patients with AD are prone to skin infections, including eczema herpeticum (EH), a disseminated herpes simplex virus 1 or 2 infection that occurs in a subset of patients with AD.[6] EH can be complicated by keratoconjunctivitis, viremia, meningitis, and encephalitis.[7] Patients with EH tend to have early-onset AD, more severe disease, increased risk of asthma, increased allergen sensitization, increased T_H2 polarity, and more frequent skin infections.[8] Additionally, it has been shown that up to 90% of patients with AD are colonized with *S aureus*[9] and 16% are colonized with methicillin-resistant *Staphylococcus aureus* (MRSA).[10] Patients with a history of EH have a higher risk of MRSA colonization.[8]

In addition to an increased susceptibility to skin infection, patients with AD have numerous abnormalities in their epidermis, which acts as a critical mechanical barrier against microbes and serves to maintain proper skin hydration.[11] The epidermis is comprised of 4 distinct layers: basal (the deepest layer), spinous, granular, and cornified (the uppermost layer). Epidermal differentiation begins with the migration of proliferating keratinocytes from the basal layer and ends with their terminal differentiation into corneocytes (dead keratinocytes). The stratum corneum, or cornified layer, is a flattened sheet of corneocytes tightly connected by corneodesmosomes and embedded in an intercellular matrix of nonpolar lipids.[12] This layer of dead cells is the key physical and permeability barrier against the environment and is continuously shed and renewed by differentiating keratinocytes. Recent work suggests that abnormal epidermal differentiation, including defective corneocyte compaction, cornification, and lipid release, play a key role in the pathogenesis of AD.[13]

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Abbreviations used
AD: Atopic dermatitis
EASI: Eczema Area and Severity Index
e-fabp: Epidermal fatty acid binding protein
EH: Eczema herpeticum
EH−: AD without a history of eczema herpeticum
EH+: AD with a history of eczema herpeticum
flg-2: Filaggrin-2
GGCT: Gamma-glutamyl cyclotransferase
MRSA: Methicillin-resistant *Staphylococcus aureus*
MSSA: Methicillin-sensitive *Staphylococcus aureus*
NMF: Natural moisturizing factor
PCA: 2-Pyrrolidone-5-carboxylic acid
TG3: Transglutaminase-3 (protein-glutamine gamma-glutamyltransferase E)

The goal of this exploratory, hypothesis-generating proteomics study funded by the National Institutes of Health/National Institute of Allergy and Infectious Diseases' Atopic Dermatitis Vaccinia Network was to identify unique patterns of biomarkers associated with AD pathogenesis and EH/*S aureus* susceptibility. Samples were collected from nonatopic subjects and patients with AD by means of tape stripping, and proteomic profiling was performed. Samples were analyzed in triplicate by means of mass spectrometry, and a custom-designed, in-house Java Application was developed to process the data.[14] Differences in protein expression between diagnostic groups were estimated, and statistical significance was evaluated based on a linear mixed model.

METHODS

Study population and design

Participants with AD and nonatopic healthy control subjects aged 1 to 80 years were enrolled at National Jewish Health. AD was diagnosed according to standardized criteria developed by the National Institutes of Health/National Institute of Allergy and Infectious Diseases' Atopic Dermatitis and Vaccinia Network.[8] A total of 65 participants were enrolled: 29 patients with AD without a history of eczema herpeticum (EH− patients), 21 patients with AD with a history of eczema herpeticum (EH+ patients), and 15 nonatopic control subjects. Swabs were collected from nonlesional skin of all participants and lesional skin of participants with AD to determine *S aureus* colonization status (methicillin-sensitive *Staphylococcus aureus* [MSSA]/MRSA/no *S aureus* colonization). Skin tapings were collected from nonlesional and lesional skin (if applicable). Participants were required to discontinue the use of topical medications for 7 days and oral antibiotics for 10 days before sample collection.

Proteomic analysis was conducted on skin tapings from a subset of participants who were sex and age matched (±10 years and age = 21-year-old cutoff) across groups based on the Spectra MRSA screening assay result (Remel, Thermo Fisher Scientific, Lenexa, Kan): 6 EH+ patients colonized with MSSA, 5 EH+ patients colonized with MRSA, 6 EH+ patients with no *S aureus* colonization, 6 EH– patients colonized with MSSA, 6 EH− patients colonized with MRSA, 6 EH− patients with no *S aureus* colonization, and 5 nonatopic subjects with no *S aureus* colonization and 1 nonatopic subject colonized with MSSA. The more accurate Kirby-Bauer assay was then performed, and the results of this assay were used to assign *S aureus* colonization status for analysis (Table I).

The National Jewish Health Institutional Review Board approved this study. Written informed consent was obtained from each participant or from the parent or legal guardian in the case of minors. Participants aged 7 to 17 years provided assent.

Skin taping and storage

Skin tapings were collected from lesional (mostly chronic/>3 days old) and nonlesional sites of patients with AD and from nonlesional skin of nonatopic subjects, as described previously.[14] Samples were "heat killed" in a water bath at 70°C for 30 minutes to eliminate the risk of infectivity and then frozen in a −80°C freezer. Lack of colony growth on blood agar plates was confirmed in preliminary test samples subjected to 70°C for 30 minutes.

Protein extraction

Proteins were removed from the tape discs with an extraction buffer containing 0.01% 3-(3-[1,1-bisalkyloxyethyl]pyridin-1-yl)propane-1-sulfonate.[14] Extracts from tape discs corresponding to layers 1 to 5, 6 to 10, 11 to 15, and 16 to 20 were combined and processed as previously outlined.[14]

Protein digestion

Proteins were digested as previously described[14] and then purified with Oasis HLB μElution Plate (30 μm) and equipped with a vacuum manifold, according to the manufacturer's directions (Waters, Milford, Mass).

Mass spectrometry

Liquid chromatography and mass spectrometry were carried out as previously described.[14] Samples were run in triplicate on an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, Calif) and Agilent ETD ion trap (model 6340) mass spectrometer with an HPLC chip.

Database searching

Raw data were extracted and analyzed by using the Spectrum Mill database searching program (Rev A.03.03.080 SR1, Agilent Technologies), as previously described.[14] Data were searched against the SwissProt *Homo sapiens* database (UniProt Release 14).[14] Data were validated, and protein identifications were considered significant if the following confidence thresholds were met: minimum of 2 peptides per protein, protein score greater than 11, individual peptide scores of at least 7, and scored percentage intensity of at least 70%.

Protein selection

Protein database search results were compiled for the triplicate MS runs, pooled layers, subjects, and lesional/nonlesional sites through spectral counting by using an in-house developed Java application (Sun Microsystems, Santa Clara, Calif). Spectral counts were calculated as the sum of the spectra matched to peptides corresponding to a protein in the database to quantify relative protein amounts. Spectral counts were then normalized to the total number of spectra per MS run. Proteins were considered for statistical analysis only if they were present in 2 of 3 technical replicates and if there were at least 6 non-0 values across treatment groups. Spectral counts for each selected protein were averaged across technical replicates and across pooled layers yielding 1 mean spectral count per tape-stripping site (lesional/nonlesional sites for patients with AD and nonlesional sites for nonatopic subjects). All keratins were excluded from statistical analysis because of high homology, which rendered it impossible to distinguish isoforms with confidence.

Statistical methods

Descriptive statistics are presented to characterize all subjects included in the analysis. Categorical data are presented as enumerations and percentages. Continuous data are presented as arithmetic means ± SDs or as medians (25th-75th percentiles) if the distribution of the data is skewed.

Normalized mean spectral counts were modeled by using a linear mixed model with random intercepts to account for the correlation of multiple samples (lesional/nonlesional) for a single subject to compare protein levels between diagnostic groups. The predictors of interest were diagnostic group, *S aureus* colonization status (as measured by using the Kirby-Bauer assay), and sample type, but age and sex were included in the model to account for

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