The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased T_H17 polarization

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Background: Atopic dermatitis (AD) shows very high prevalence in Asia, with a large unmet need for effective therapeutics. Direct comparisons between European American (EA) and Asian patients with AD are unavailable, but earlier blood studies detected increased IL-17$^+$–producing cell counts in Asian patients with AD.

Objective: We sought to characterize the Asian AD skin phenotype and compare it with the EA AD skin phenotype.

Methods: We performed genomic profiling (real-time PCR) and immunohistochemistry on lesional and nonlesional biopsy specimens from 52 patients with AD (25 EAs and 27 Asians), 10 patients with psoriasis (all EAs), and 27 healthy subjects (12 EAs and 15 Asians).

Results: Although disease severity/SCORAD scores were similar between the AD groups (58.0 vs 56.7, $P = .77$), greater acanthosis, higher Ki67 counts, and frequent parakeratosis were characteristics of lesional epidermis from Asian patients with AD ($P < .05$). Most (24/27) Asian patients had high IgE levels. A principal component analysis using real-time PCR data clustered the Asian AD phenotype between the EA AD and psoriasis phenotypes. T_H2 skewing characterized both Asian and EA patients with AD but not patients with psoriasis. Significantly higher T_H17 and T_H22 (*IL17A, IL19*, and *S100A12* in lesional and IL-22 in nonlesional skin; $P < .05$) and lower T_H1/interferon (*CXCL9, CXCL10, MX1*, and *IFNG* in nonlesional skin; $P < .05$) gene induction typified AD skin in Asian patients.

Conclusion: The Asian AD phenotype presents (even in the presence of increased IgE levels) a blended phenotype between that of EA patients with AD and those with psoriasis, including increased hyperplasia, parakeratosis, higher T_H17 activation, and a strong T_H2 component. The relative pathogenic contributions of the T_H17 and T_H2 axes in creating the Asian AD phenotype need to be tested in future clinical trials with appropriate targeted therapeutics. (J Allergy Clin Immunol 2015;136:1254-64.)

Key words: *Atopic dermatitis, T_H1, T_H2, T_H17, T_H22, acanthosis, parakeratosis, psoriasis, Asian, European American*

Atopic dermatitis (AD) is the most common inflammatory skin disease,[1] with a prevalence of 3% to 10%[2-5] and a large unmet need for effective therapeutics.[6] Increased IgE levels are found in approximately 80% ("extrinsic") and normal IgE levels ("intrinsic") are detected in 20% of patients with AD.[7] AD and psoriasis, another common skin disease, are recognized as polar inflammatory skin diseases.[8] With evolving molecular phenotyping, psoriasis is emerging as an IL-23/T_H17–skewed disease, whereas AD is considered a T_H2-centered disease, both sharing a common T_H22 component.[9-12] These distinctions are highly relevant to emerging therapies because psoriasis responds remarkably well to IL-23/IL-17 antagonists,[13-16] whereas AD responds to T_H2 antagonists.[17,18]

To date, the characterization of AD in different populations has made the assumption that disease mechanisms are similar across different populations. However, some findings potentially negate a unified pathogenic model. We have shown distinct differences in patients with intrinsic versus extrinsic AD, as judged by skin

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Abbreviations used
AD: Atopic dermatitis
DC: Dendritic cell
EA: European American
FCH: Fold change
FLG: Filaggrin
IHC: Immunohistochemistry
LC: Langerhans cell
PCA: Principal component analysis
RT-PCR: Real-time PCR
STAT1: Signal transducer and activator of transcription 1

profiling.[10] In patients with intrinsic AD, we detected significantly increased expression of *IL12B* (p40), *IL17A, IL22*, and respective keratinocyte-induced products (ie, S100As), whereas similar expression of T_H2-related products was observed in both patients with extrinsic and those with intrinsic AD[10]; indeed, these subtypes show similar responses to IL-4 receptor antagonism.[17,18]

Although a direct comparison is unavailable, a few findings suggest that Asian patients with AD might have different phenotypic characteristics. In Asia there is a much higher prevalence of AD in adults (7% to 10%),[19-21] and prominent T_H17 activation has been observed in blood and acute AD skin lesions.[22] In contrast, no increases were seen in IL-17 activation in European American (EA) patients with extrinsic AD.[10,11,23] Thus we sought to directly compare AD in EA and Asian (Japanese and Korean) AD populations, focusing mainly on extrinsic subsets and with a comparison with psoriasis vulgaris. Our report classifies the Asian AD phenotype as a mixed phenotype between the EA AD and psoriasis phenotypes, with highly atypical features for AD, including parakeratosis and a unique cytokine profile with coactivation of the T_H2 and T_H17 axes. The relative pathogenic contributions of the T_H2 versus T_H17 axes in Asian patients with AD will need to be established through clinical trials with pathway-selective antagonists.[17,18]

METHODS

Skin samples

We included several cohorts of EA patients with AD (n = 25) and psoriasis (n = 10) previously published by our group,[24-26] as well as 27 East Asian patients with AD (12 Japanese and 15 Korean), which we refer to as the Asian AD patients throughout the article. Fifteen Asian (7 Japanese and 8 Korean) and 12 EA healthy control subjects were included for comparison. Japanese and Korean AD samples were obtained from patients with AD living in their respective countries. Lesional and nonlesional (>10 cm from active lesions) AD skin biopsy specimens were collected for these studies from areas with representative AD lesions (example shown in Fig E1 in this article's Online Repository at www.jacionline.org) under institutional review board–approved protocols, according to the Declaration of Helsinki principles. Nonlesional samples were unavailable from 7 Japanese patients with AD. Patients were allowed to use emollients only and avoided using systemic immunomodulators and phototherapy within the prior 4 weeks and topical steroids or calcineurin inhibitors within the prior 8 days. Patients were stratified into extrinsic (24 in Asian patients with AD and 14 in EA patients with AD) and intrinsic AD categories, with IgE levels of greater than 200 kU/L defining extrinsic AD and values of less than 200 kU/L defining intrinsic AD. No heterozygous filaggrin (FLG) mutations (R501X and 2282del4 allele) were found in any Korean patients with AD (all with extrinsic AD) and 6 EA patients with AD (1 with extrinsic and 5 with intrinsic AD, see Table E1 in this article's Online Repository at www.jacionline.org). The FLG mutation status was unavailable for the rest of the patients and healthy control subjects. Patients' characteristics are summarized in Table I, and the whole patient list is available in Table E1.

Quantitative real-time PCR

RNA was extracted for real-time PCR (RT-PCR) with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY). Reverse transcription to cDNA from RNA was carried out by using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, Calif). cDNA was amplified with TaqMan PreAmp Master Mix (Applied Biosystems), and the preamplified cDNA product was analyzed with TaqMan Gene Expression Master Mix. The primers used in this study are listed in Table E2 in this article's Online Repository at www.jacionline.org. Expression values were normalized to human acidic ribosomal protein *(hARP)*. Although data have been published on the EA AD cohort,[25] tissue samples from these patients were run together with all other samples for RT-PCR, eliminating a batch effect. RNA samples were unavailable from 1 EA patient and 2 Japanese patients with AD and 4 Korean, 3 Japanese, and 4 EA healthy subjects.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on frozen skin sections. The antibodies used in this study are listed in Table E3 in this article's Online Repository at www.jacionline.org. Epidermal thickness and positive cells per millimeter were quantified for IHC by using ImageJ V1.42 software (National Institutes of Health, Bethesda, Md). Skin tissue was unavailable for IHC from lesional samples of 2 Japanese patients with AD and samples of 2 Korean healthy subjects.

Statistical analysis

All analyses were carried out with the statistical language R (www.R-project.org).

The RT-PCR values normalized to human acidic ribosomal protein were transformed to the $\log_2$ scale. Both expression values (in $\log_2$) and cell counts were modeled by using a linear mixed-effects model with a fixed effect for tissue and ethnicity and patient as a random effect. Once the model was estimated, the comparisons of interests were tested by using contrast/*lsmeans*. Data are presented as (least squares) means and 95% CIs. Similar approaches were used in the subgroup analysis including only extrinsic patients.

For the RT-PCR data, principal component analysis (PCA) was carried out by using 25 variables and 129 lesional, nonlesional, and normal tissue samples. Because some of the values were missing, we used probabilistic PCA,[27] an iterative method that is tolerant to amounts of missing values between 10% to 15%, as in our case. Probabilistic PCA combines an expectation-maximization approach for PCA with a probabilistic model. The expectation-maximization approach is based on the assumption that the latent variables, as well as the noise, are normally distributed. We used the implementation available in the R package *pcaMethods*.[28,29]

Lesional versus nonlesional differences for all markers are represented by using heat maps. Unsupervised clustering of all lesional versus nonlesional $\log_2$ fold changes (FCHs) were carried out by using the Euclidean distance and average agglomeration criteria.

RESULTS

Patients' characteristics

This study included 27 Asian patients with AD (5 female and 22 male patients; age, 18-74 years [median, 27 years]) with SCORAD scores from 23 to 100 (mean, 58; SD, 20.2) and serum eosinophil counts of 2.3% to 33% (median, 8.2%); 25 EA patients with AD (9 female and 16 male patients; age, 23-73 years [median, 45 years]) with SCORAD scores from 33 to 77 (mean, 56.7; SD, 12.2) and serum eosinophil counts of 0.6% to 11.8% (median, 4.3%); 10 EA patients with psoriasis; and 15 Asian and 12 EA healthy control subjects. Among Asian patients