An IL-17–dominant immune profile is shared across the major orphan forms of ichthyosis

Amy S. Paller, MD, MS,[a] Yael Renert-Yuval, MD,[b] Maria Suprun, MPH,[c] Hitokazu Esaki, MD,[b,d] Margeaux Oliva, BA,[b] Thy Nhat Huynh, MD,[a] Benjamin Ungar, BA,[d] Norma Kunjravia, MD,[d] Rivka Friedland, MD,[a] Xiangyu Peng, MSc,[b] Xiuzhong Zheng, MSc,[d] Yeriel D. Estrada, BSc,[b] James G. Krueger, MD, PhD,[c] Keith A. Choate,[e] Mayte Suárez-Fariñas, PhD,[b,c,d,f] and Emma Guttman-Yassky, MD, PhD[b,d] *Chicago, Ill, New York, NY, and New Haven, Conn*

Background: The ichthyoses are rare genetic disorders associated with generalized scaling, erythema, and epidermal barrier impairment. Pathogenesis-based therapy is largely lacking because the underlying molecular basis is poorly understood.
Objective: We sought to characterize molecularly cutaneous inflammation and its correlation with clinical and barrier characteristics.
Methods: We analyzed biopsy specimens from 21 genotyped patients with ichthyosis (congenital ichthyosiform erythroderma, n = 6; lamellar ichthyosis, n = 7; epidermolytic ichthyosis, n = 5; and Netherton syndrome, n = 3) using immunohistochemistry and RT-PCR and compared them with specimens from healthy control subjects, patients with atopic dermatitis (AD), and patients with psoriasis. Clinical measures included the Ichthyosis Area Severity Index (IASI), which integrates erythema (IASI-E) and scaling (IASI-S); transepidermal water loss; and pruritus.
Results: Ichthyosis samples showed increased epidermal hyperplasia (increased thickness and keratin 16 expression) and T-cell and dendritic cell infiltrates. Increases of general inflammatory (IL-2), innate (IL-1β), and some T_H1/interferon (IFN-γ) markers in patients with ichthyosis were comparable with those in patients with psoriasis or AD. TNF-α levels in patients with ichthyosis were increased only in those with Netherton syndrome but were much lower than in patients with psoriasis and those with AD. Expression of T_H2 cytokines (IL-13 and IL-31) was similar to that seen in control subjects. The striking induction of IL-17–related genes or markers synergistically induced by IL-17 and TNF-α (IL-17A/C, IL-19, CXCL1, PI3, CCL20, and IL36G; $P < .05$) in patients with ichthyosis was similar to that seen in patients with psoriasis. IASI and IASI-E scores strongly correlated with IL-17A ($r = 0.74$, $P < .001$) and IL-17/TNF–synergistic/additive gene expression. These markers also significantly correlated with transepidermal water loss, suggesting a link between the barrier defect and inflammation in patients with ichthyosis.
Conclusion: Our data associate a shared T_H17/IL-23 immune fingerprint with the major orphan forms of ichthyosis and raise the possibility of IL-17–targeting strategies. (J Allergy Clin Immunol 2017;139:152-65.)

Key words: *Epidermis, ichthyosis, inflammation, autosomal recessive congenital ichthyosis, congenital ichthyosiform erythroderma, lamellar ichthyosis, Netherton syndrome, epidermolytic ichthyosis, skin, IL-17, TNF-α*

Ichthyoses are genetically and clinically heterogeneous disorders with generalized skin scaling, thickening, and erythema. Other than ichthyosis vulgaris and recessive X-linked ichthyosis subtypes,[1-7] the ichthyoses each occur in less than 1:100,000 persons. Affected subjects have an extremely compromised quality of life because of disfigurement and the accompanying itching, pain, and functional limitation.[8,9] The epidermal barrier is abnormal, with defects in lipids and differentiation resulting in increased transepidermal water loss (TEWL).[10-12]

Treatment for ichthyosis is largely supportive and unsatisfactory. For more severely affected subjects, oral retinoids, vitamin A analogues, are often administered to improve the hyperkeratosis.[13-15] However, retinoids can worsen skin inflammation and pruritus and have deleterious effects (hypertriglyceridemia, teratogenicity, and hyperostosis),[16] limiting their use. Topical anti-inflammatory medications (ie, steroids and calcineurin inhibitors) are often ineffective and easily absorbed systemically, restricting chronic use.[17,18] Thus a huge unmet need exists for safe and more effective treatments that will ideally also target the erythema/inflammation.

From [a]the Departments of Dermatology and Pediatrics, Northwestern University Feinberg School of Medicine, Chicago; the Departments of [b]Dermatology and [c]Population Health Science and Policy and [f]the Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York; [d]the Laboratory for Investigative Dermatology, Rockefeller University, New York; and [e]the Department of Dermatology, Yale University School of Medicine, New Haven.

Supported by the Foglia Family Foundation Endowment and the National Psoriasis Foundation (RF fellowship).

This research was supported by the Foglia Family Foundation Endowment and the National Psoriasis Foundation (RF fellowship). We acknowledge Core resources provided by the Northwestern University Skin Disease Research Center (NIAMS P30AR057216).

Disclosure of potential conflict of interest: A. S. Paller has received consultancy fees from Anacor, Galderma, Stiefel/GlaxoSmithKline, Novartis, Regeneron, and Vitae Pharmaceuticals and has received grants from Anacor, Astellas, and LEO Pharma. J. G. Krueger has received personal fees and/or fees to his institution from Novartis, Pfizer, Amgen, Lilly, Merck, Kadmon, Dermira, Boehringer, Innovaderm, Kyowa, BMS, Janssen, Serono, Biogen Idec, Delenex, AbbVie, Sanofi, Baxter, Paraxel, Xenoport, and Kineta. K. A. Choate has received consultancy fees from Alderya Therapeutics and payment for lectures from Abbvie and Janssen. M. Suárez-Fariñas has received grants from Pfizer, Quorum Consulting, and Genisphere. E. Guttman-Yassky has received board memberships from Sanofi Aventis, Regeneron, Stiefel/GlaxoSmithKline, MedImmune, Celgene, Anacor, Leo Pharma, AnaptysBio, Celsus, Dermira, Galderma, Novartis, Pfizer, and Vitae; consultancy fees from Regeneron, Sanofi Aventis, Medimmune, Celgene, Stiefel/GlaxoSmithKline, Celsus, BMS, Amgen, Drais, AbbVie, Anacor, AnaptysBio, Dermira, Galderma, Leo Pharma, Novartis, Pfizer, Vitae, Mitsubishi Tanabe, and Eli Lilly; and grants/grants pending from Regeneron, Celgene, BMS, Janssen, Dermira, Leo Pharma, Merck, and Novartis. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication May 2, 2016; revised June 18, 2016; accepted for publication July 19, 2016.

Available online August 20, 2016.

Corresponding author: Amy S. Paller, MD, MS, Departments of Dermatology and Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL 60611. E-mail: apaller@northwestern.edu.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.07.019

Abbreviations used
AD: Atopic dermatitis
AMP: Antimicrobial peptide
ARCI: Autosomal recessive congenital ichthyosis
CIE: Congenital ichthyosiform erythroderma
CISI: Congenital Ichthyoses Severity Index
DC: Dendritic cell
DC-LAMP: Dendritic cell lysosomal-associated membrane protein
DEFB4: β-Defensin-B4
EI: Epidermolytic ichthyosis
FLG: Filaggrin
hARP: Human acidic ribosomal protein
IASI: Ichthyosis Area Severity Index
IASI-E: Ichthyosis Area Severity Index–Erythema
IASI-S: Ichthyosis Area Severity Index–Scaling
IHC: Immunohistochemistry
K16: Keratin 16
LCN2: Lipocalin 2
LI: Lamellar ichthyosis
LOR: Loricrin
NS: Netherton syndrome
PAR2: Protease-activated receptor 2
PPL: Periplakin
TEWL: Transepidermal water loss
TSLP: Thymic stromal lymphopoietin

Despite elucidation of the genetic basis for the various forms of ichthyosis, their underlying molecular mechanisms are poorly understood, with our knowledge predominantly based on culture and animal models.[19-29] These model systems chiefly focus on abnormal barrier function and lipid homeostasis, with little attention paid to immune disturbances.[6,30,31] Human studies, largely limited to Netherton syndrome (NS) and the lamellar ichthyosis (LI) phenotype of autosomal recessive congenital ichthyosis (ARCI), have examined just a few cytokines.[32-38] Blood analyses found inconsistent T_H2 skewing[39] and increases in levels of proinflammatory cytokines (TNF-α, IL-1β, IL-2, and IL-18).[40-42] Skin studies showed increased expression of TNF-α and IL-1β in patients with ARCI-LI[35] and of protease-activated receptor 2 (PAR2),[32] thymic stromal lymphopoietin (TSLP), TNF-α, IL-8,[43] and the T_H2 cytokine IL-33 in patients with NS,[38] which are often coupled with increased expression of terminal differentiation products (ie, filaggrin [FLG], loricrin [LOR], and involucrin), and lipid impairement.[32,35,37,38] Studies of response to systemic treatments, including retinoids (n = 11), anti-TNF (n = 1), and oral corticosteroids combined with omalizumab (n = 1), in patients with ARCI-LI and those with NS, respectively,[33-35] have only assessed a few cytokines. Therapy-induced decreases in IL-1β, IL-8, TSLP, IL-5, and IL-17A levels were found in patients with NS, whereas IL-1α and TNF-α levels were decreased (nonsignificantly) in patients with ARCI-LI.

To elucidate the basis for the cutaneous inflammation seen in patients with ichthyosis and its correlation with clinical characteristics, we analyzed skin from 21 patients with the most prevalent orphan forms of severe ichthyosis: ARCI-LI, ARCI–congenital ichthyosiform erythroderma (CIE), epidermolytic ichthyosis (EI), and NS. All subtypes showed cutaneous skewing of T_H17 expression, which correlated with disease severity. This T_H17 profile most closely resembled that of psoriasis, in which IL-17 antagonism is highly effective in reversing the inflammation and epidermal pathology.[44-47] These data can lead to a new treatment paradigm targeting the T_H17/IL-23 pathway in patients with ichthyosis.

METHODS

Patients' characteristics

Twenty-one patients (aged 10-57 years) with ichthyosis and known mutations were enrolled (Tables I and II and see Table E1 and the Methods section in this article's Online Repository at www.jacionline.org). Written institutional review board–approved consent was provided by subjects (≥12 years) and parents (<18 years). Demographic information, medical history, physical examination, clinical severity scores, pruritus (5-D itch scale and Itch Numeric Rating Scale), photography, and TEWL measurement were captured. Few scoring instruments have been used for ichthyosis severity, and the only one tested for reliability is the Congenital Ichthyoses Severity Index (CISI). In addition to scoring erythema/redness and hyperkeratosis/scaling, CISI measures alopecia (not a feature in most patients) and does not score potential differences in body regions.[48] Given its limitations, we modified the CISI scale, eliminating alopecia and prorating intensity based on body region and extent to create a composite score similar to the Psoriasis Area and Severity Index.[49] This Ichthyosis Area and Severity Index (IASI) measures the severity of the erythema (Ichthyosis Area Severity Index–Erythema [IASI-E]) and scaling (Ichthyosis Area Severity Index–Scaling [IASI-S]), adding them together to a total IASI score (Tables I and II and see Table E2 and the Methods section in this article's Online Repository at www.jacionline.org).

Four-millimeter biopsy specimens were collected and assessed in parallel with tissue from healthy subjects, patients with atopic dermatitis (AD), and patients with psoriasis previously published by our group.[50-54] Genotyping for *FLG* mutations in the AD cohort was previously performed on 4 patients, and results were negative.[51] Four samples of healthy adolescents were included (see Table E3 in this article's Online Repository at www.jacionline.org) for comparison with the younger ichthyosis cohort. Patients' characteristics are presented in Tables I and II and Tables E1 and E3.

Quantitative RT-PCR

RT-PCR was performed, as previously described.[55,56] Expression values were normalized to human acidic ribosomal protein (hARP).

Immunohistochemistry

Immunohistochemistry (IHC) was performed on frozen sections, as previously described.[57] Antibodies are shown in Table E4 and cell counts are shown in Table E5 in this article's Online Repository at www.jacionline.org.

Statistical analyses

Except for RT-PCR expression values, no other missing value imputations were performed. All available observations were included in analyses, which were performed by using the statistical language R (www.R-project.org). Differences in expression values (in $\log_2$ scale), cell counts, and clinical variables were assessed by using linear models, which were age adjusted to account for significant differences in age distributions.

Unsupervised hierarchical clustering of variables or samples/patients was performed by using the Pearson correlation coefficient as a distance metric with the McQuitty agglomeration algorithm. The results are represented as a heat map with a dendrogram and a tree or phylogram (using R package *ape*). The uncertainty of the hierarchical clustering analysis was assessed by using multiscale bootstrap resampling (extended statistics are shown in the Methods section in this article's Online Repository).

RESULTS

Demographics and clinical characteristics of patients with ichthyosis

Twenty-one patients aged 10 years or greater with ARCI-CIE (n = 6), ARCI-LI (n = 7), EI (n = 5), or NS (n = 3) and with