β-Defensin 2 is a responsive biomarker of IL-17A–driven skin pathology in patients with psoriasis

Frank Kolbinger, Dr rer nat,[a]* Christian Loesche, MD,[a]* Marie-Anne Valentin, PhD,[a] Xiaoyu Jiang, PhD,[a]‡ Yi Cheng, PhD,[b] Philip Jarvis, MSc,[b] Thomas Peters, Dr rer nat,[a] Claudio Calonder, PhD,[a] Gerard Bruin, PhD,[a] Florine Polus, PhD,[a] Birgit Aigner, MD,[c] David M. Lee, MD, PhD,[a]§ Manfred Bodenlenz, PhD,[c] Frank Sinner, PhD,[c] Thomas Rudolf Pieber, MD, PhD,[c] and Dhavalkumar D. Patel, MD, PhD[a] *Basel, Switzerland, Cambridge, Mass, Shanghai, China, and Graz, Austria*

Background: IL-17A is a key driver of human autoimmune diseases, particularly psoriasis.
Objective: We sought to determine the role of IL-17A in psoriasis pathogenesis and to identify a robust and measurable biomarker of IL-17A–driven pathology.
Methods: We studied 8 healthy subjects and 8 patients with psoriasis before and after administration of secukinumab, a fully human anti–IL-17A mAb, and used a combination of classical techniques and a novel skin microperfusion assay to evaluate the expression of 170 proteins in blood, nonlesional skin, and lesional skin. For validation, we also tested stored sera from 601 patients with a variety of autoimmune diseases.
Results: IL-17A was specifically expressed in lesional compared with nonlesional psoriatic skin (9.8 vs 0.8 pg/mL, $P < .001$). Proteomic and gene transcription analyses revealed dysregulated antimicrobial peptides, proinflammatory cytokines, and neutrophil chemoattractants, levels of which returned to normal after treatment with secukinumab. β-Defensin 2 (BD-2) was identified as a biomarker of IL-17A–driven pathology by comparing protein expression in patients with psoriasis versus that in healthy subjects (5746 vs 82 pg/mL in serum, $P < .0001$; 2747 vs <218 pg/mL in dermis, $P < .001$), responsiveness to secukinumab therapy, and synergistic induction by IL-17A and TNF-α in epidermal keratinocytes. In a validation set of sera from 601 patients with autoimmune diseases thought to be IL-17A driven, we found that BD-2 levels are most highly increased in patients with psoriatic skin lesions, and in patients with psoriasis, BD-2 levels correlated well with IL-17A levels ($r = 0.70$, $n = 199$, $P < .001$) and Psoriasis Area and Severity Index scores ($r = 0.53$, $n = 281$, $P < .001$).
Conclusion: IL-17A is a primary driver of skin pathology in patients with psoriasis, and serum BD-2 is an easily measurable biomarker of IL-17A–driven skin pathology. (J Allergy Clin Immunol 2017;139:923-32.)

Key words: *IL-17, psoriasis, secukinumab, β-defensin 2, biomarker, dermal interstitial fluid, microperfusion, psoriatic arthritis, ankylosing spondylitis, rheumatoid arthritis, multiple sclerosis, autoimmunity*

T_H17 cells and the IL-17 family of cytokines are key drivers of autoimmune diseases, such as psoriasis, psoriatic arthritis (PsA), ankylosing spondylitis (AS), rheumatoid arthritis (RA), and multiple sclerosis (MS).[1] Psoriasis is the best studied of these diseases, with multiple interventions targeted at T_H17 cells and their cytokines showing efficacy in its treatment.

The IL-17 family of cytokines includes 6 members (IL-17A to IL-17F) that function as homodimers or heterodimers to signal through a family of cytokine receptors with multiple chains (IL-17RA to IL-17RE), with IL-17A and IL-17F signaling through a receptor complex composed of IL-17RA and IL-17RC.[2,3] Circulating levels of IL-17A protein have been shown to be higher in patients with psoriasis than in healthy control subjects and correlate with disease severity,[4] whereas mRNA expression of the *IL17A*, *IL17C*, and *IL17F* genes is higher in psoriatic lesional tissue than nonlesional tissue.[5-7] Indeed, therapies that target either IL-17A or IL-17RA are similarly highly effective in reducing disease in patients with psoriasis.[1,8-11] Although these numerous lines of evidence implicate an important role for IL-17A, a nonredundant role for IL-17F has not been precluded.

Much of our current understanding of psoriasis pathophysiology and the effects of IL-17A blockade has largely been based on studies evaluating gene expression from skin biopsy specimens.[6,12-14] Although these studies have provided significant insights into the pathways that might be involved in IL-17A–mediated pathogenesis, they are limited by the frequent incongruence between changes in mRNA and protein expression. Furthermore, they do not quantify the early microanatomically specific changes in protein expression, in particular large proteins,

From [a]Novartis Institutes for BioMedical Research and [b]Novartis Pharma, Basel, Switzerland, and Shanghai, China; and [c]HEALTH–Institute for Biomedicine and Health Sciences, Joanneum Research Forschungsgesellschaft m.b.H., and the Division of Endocrinology and Metabolism, Department of Internal Medicine, Medical University Graz.
*These authors contributed equally to this work.
‡Dr Jiang is currently affiliated with Merrimack Pharmaceuticals, Cambridge, Mass.
§Dr Lee is currently affiliated with Roche, Basel, Switzerland.
This clinical trial was sponsored by Novartis Pharma AG.
Disclosure of potential conflict of interest: F. Kolbinger, C. Loesche, M.-A. Valentin, Y. Cheng, P. Jarvis, T. Peters, C. Calonder, G. Bruin, and F. Polus are employees of Novartis. X. Jiang is an employee of Merrimack Pharmaceuticals. D. M. Lee is an employee of Roche. M. Bodenlenz, F. Sinner, and T. R. Pieber receive personal fees from Joanneum Research. D. D. Patel is an employee of Novartis and receives royalties from Duke University. B. Aigner declares no relevant conflicts of interest.
Received for publication January 11, 2016; revised May 18, 2016; accepted for publication June 16, 2016.
Available online August 5, 2016.
Corresponding author: Dhavalkumar D. Patel, MD, PhD, Forum 1, Basel CH-4002, Switzerland. E-mail: dhavalkumar.patel@novartis.com.
The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections
0091-6749

http://dx.doi.org/10.1016/j.jaci.2016.06.038

Abbreviations used
AS: Ankylosing spondylitis
BD-2: β-Defensin 2
CD: Crohn disease
dISF: Dermal interstitial fluid
GM: Geometric mean
IHC: Immunohistochemistry
LLOQ: Lower limit of quantification
MMP: Matrix metalloproteinase
MS: Multiple sclerosis
qRT-PCR: Quantitative RT-PCR
PASI: Psoriasis Area and Severity Index
PsA: Psoriatic arthritis
RA: Rheumatoid arthritis
RQ: Relative quantity

such as cytokines and chemokines. To date, protein expression response to therapy in psoriatic skin has been mostly demonstrated on a qualitative rather than quantitative level through the use of histology and immunohistochemistry (IHC).[12,14-16]

To increase our understanding of the inflammatory pathophysiology in psoriatic skin at a protein expression level and to identify a protein biomarker of IL-17A–mediated pathology, we conducted an exploratory study in 8 healthy subjects and 8 patients with psoriasis to quantify and compare the soluble proteomic profiles of lesional versus nonlesional skin before and after single-dose systemic treatment (300 mg administered subcutaneously) with secukinumab (a fully human anti–IL-17A mAb with a half-life of approximately 27 days) and/or healthy skin. Dermal open flow microperfusion, a novel technique that provides minimally invasive access to dermal interstitial fluid (dISF), was used to sample the dermis.[17-19] Dermal (dISF) samples were then analyzed for 170 proteins encompassing cytokines, chemokines, growth factors, cell adhesion molecules, and soluble receptors. Early proteomic changes that occur within the dermis after secukinumab treatment were complemented with gene expression changes extracted from full skin biopsy specimens. Our aim was to identify which pathways might be central to psoriasis pathology in skin and whether the antimicrobial peptide β-defensin 2 (BD-2) might be a protein biomarker of IL-17A–mediated pathology.

METHODS

Study design

This single-center clinical study was conducted according to ethical principles and good clinical practice at the Medizinische Universität in Graz, Austria, after being approved by the ethics committee and Austrian health authority. Enrolled patients needed to sign informed consent forms and comply with the protocol requirements (ClinicalTrials.gov Identifier NCT01539213).

We enrolled 8 healthy subjects (mean age, 26.1 years) and 8 patients with psoriasis (mean age, 38.8 years). All 16 subjects were white, and all patients with psoriasis were male, as were 6 healthy subjects. All subjects received a single 300-mg subcutaneous injection of secukinumab on study day 1. Skin biopsy specimens (4-mm skin punch), serum samples, and dermal samples were obtained on study days 1 (before secukinumab treatment), 8 and 15 (7 and 14 days after secukinumab treatment). Details of the dermal sampling methodology were described previously.[19]

In addition, baseline serum from patients with various inflammatory diseases sampled in multicenter trials conducted after ethics committee and health authority approval have been analyzed. Sera analyzed were from 289 patients with psoriasis (NCT01539213 and NCT00941031), 37 patients with PsA (NCT00809614), 56 patients with AS (NCT00809159), 68 patients with MS (NCT01051817), 94 patients with RA (NCT01426789), and 57 patients with Crohn disease (CD; NCT00584740).

Protein and RNA measurements

Free IL-17A and IL-17F levels in serum and dISF were quantified by using microparticle-based fluorescent sandwich immunoassays based on Erenna technology validated in human serum (Singulex IL-17A Human Immunoassay Kit, catalog no. 03-0017-05; Singulex IL-17F Human Immunoassay Kit, catalog no. 03-0018-03; Singulex, Alameda, Calif). The lower limit of quantification (LLOQ) for IL-17A was 0.64 pg/mL in dISF and 0.096 pg/mL in serum, and that for IL-17F was 96.6 pg/mL in dISF and 14.4 pg/mL in serum. BD-2 was quantified by using ELISA (Alpha Diagnostic, catalog no. 100-250-BD-2). The LLOQ for BD-2 was 218 pg/mL in dISF and 32.5 pg/mL in serum. Sinistrin served as a reference substance and was used to estimate the absolute concentrations of biomarkers in dISF.[19]

The chemiluminescent multiplex enzyme immunoassay platform from Aushon BioSystems (Billerica, Mass) was used to profile and quantify the levels of 170 proteins distributed over 43 panels. The panel of 170 proteins encompasses cytokines, cytokines receptor, chemokines, cell adhesion molecules, angiogenesis factors, matrix metalloproteinases (MMPs), growth factors, and neurotrophic factors (see Table E1 in this article's Online Repository at www.jacionline.org).

Total RNA was isolated from skin biopsy specimens by using the Qiagen RNeasy Micro Kit (Qiagen, Hilden, Germany). RNA isolated from commercial skin biopsy specimens (Asterand UK, Royston, United Kingdom) from healthy subjects (n = 10) served as controls for gene expression data. Gene expression analysis was done by using quantitative RT-PCR (qRT-PCR) or NanoString technology (NanoString Technologies, Seattle, Wash). See the Methods section and Table E2 in this article's Online Repository at www.jacionline.org for further details.

Cell culture

Human primary skin cells were obtained from PromoCell (Heidelberg, Germany). Epidermal keratinocytes (adult, pooled donors, passage 5, 30,300 cells/cm^2), dermal fibroblasts (adult, single donor, passage 6, 30,300 cells/cm^2), and dermal microvascular endothelial cells (juvenile, pooled donors, passage 5, 22,700 cells/cm^2) were seeded onto 96-well plates and incubated overnight. Thereafter, cells were incubated for another 20 hours in medium alone or 0.03 to 960 ng/mL IL-17A in the absence or presence of 1 ng/mL TNF-α, 0.03 to 960 ng/mL IL-17F in the absence or presence of 1 ng/mL TNF-α, or 0.03 to 960 ng/mL TNF-α. Supernatants were examined for the release of BD-2.

Statistical analysis

For the skin microperfusion study, log-transformed baseline (day 1) IL-17A and IL-17F protein levels were analyzed by using a mixed effects model with site (healthy volunteer or patient for serum levels and lesional or nonlesional psoriatic skin for skin levels) as a fixed effect and subject as a random effect, respectively. The adjusted geometric means (GMs) at each site were provided on the original scale together with the *P* values for comparisons between sites. Pearson correlation coefficients (denoted by *r*) between baseline protein levels (IL-17A and IL-17F) and efficacy scores (Psoriasis Area and Severity Index [PASI] score) were calculated together with the corresponding *P* values by site. Log-transformed baseline BD-2 levels in serum and skin were also analyzed by using a mixed effects model with site (healthy volunteer or patient for serum levels and healthy and lesional or nonlesional psoriatic skin for skin levels) and subject as a random effect, respectively. The adjusted GMs at each site were provided on the original scale together with the *P* values for comparisons between sites. IL-17F levels in serum from patients with psoriasis and BD-2 levels over time in serum and skin were log-transformed and analyzed by site by using a mixed effects model with visit day (baseline, day 8, and day 15) as a fixed effect and subject as a random effect, respectively. The adjusted GMs and 95% CIs at each visit