The tryptophan metabolism enzyme L-kynureninase is a novel inflammatory factor in psoriasis and other inflammatory diseases

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Background: Many human diseases arise from or have pathogenic contributions from a dysregulated immune response. One pathway with immunomodulatory ability is the tryptophan metabolism pathway, which promotes immune suppression through the enzyme indoleamine 2,3-dioxygenase (IDO) and subsequent production of kynurenine. However, in patients with chronic inflammatory skin disease, such as psoriasis and atopic dermatitis (AD), another tryptophan metabolism enzyme downstream of IDO, L-kynureninase (KYNU), is heavily upregulated. The role of KYNU has not been explored in patients with these skin diseases or in general human immunology.

Objective: We sought to explore the expression and potential immunologic function of the tryptophan metabolism enzyme KYNU in inflammatory skin disease and its potential contribution to general human immunology.

Methods: Psoriatic skin biopsy specimens, as well as normal human skin, blood, and primary cells, were used to investigate the immunologic role of KYNU and tryptophan metabolites.

Results: Here we show that $KYNU^+$ cells, predominantly of myeloid origin, infiltrate psoriatic lesional skin. KYNU expression positively correlates with disease severity and inflammation and is reduced on successful treatment of psoriasis or AD. Tryptophan metabolites downstream of KYNU upregulate several cytokines, chemokines, and cell adhesions. By mining data on several human diseases, we found that in patients with cancer, IDO is preferentially upregulated compared with KYNU, whereas in patients with inflammatory diseases, such as AD, KYNU is preferentially upregulated compared with IDO.

Conclusion: Our results suggest that tryptophan metabolism might dichotomously modulate immune responses, with KYNU as a switch between immunosuppressive versus inflammatory outcomes. Although tryptophan metabolism is increased in many human diseases, how tryptophan metabolism is proceeding might qualitatively affect the immune response in patients with that disease. (J Allergy Clin Immunol 2016;137:1830-40.)

Key words: *Psoriasis vulgaris, tryptophan metabolism, L-kynureninase, inflammation*

Psoriasis vulgaris is a chronic inflammatory skin disease that affects about 1% to 3% of the North American population.[1,2] This condition arises from interactions between hyperproliferative keratinocytes and infiltrating immune cells, specifically IL-17– and IFN-γ–producing T cells and inflammatory dendritic cells (DCs).[1-3] Additionally, several comorbidities are associated with psoriasis, such as cardiovascular disease, indicating the underlying pathogenesis of psoriasis is more than "skin deep."[4] In recent years microarray and genomic studies have elucidated several key genes associated with this disease.[1] This information has not only enriched the understanding of psoriasis pathogenesis but also general human immunology because skin disease provides noninvasive access to affected tissue and the efficacy of immunomodulatory treatments can be easily monitored.

One gene found to be prominently upregulated in microarray studies of psoriasis is L-kynureninase (KYNU), an enzyme within the tryptophan metabolism pathway.[5-8] KYNU is highly upregulated (>20-fold) in lesional psoriatic skin compared with nonlesional skin. Additionally, *KYNU* is a gene within the "psoriasis classifier," a set of genes that can correctly identify lesional versus nonlesional skin.[6] The methylation pattern

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Abbreviations used
AD: Atopic dermatitis
AhR: Aryl hydrocarbon receptor
BDCA-1: Blood dendritic cell antigen 1
DC: Dendritic cell
EC: Endothelial cell
3HAA: 3-Hydroxyanthranilic acid
IDO: Indoleamine 2,3-dioxygenase
KYNU: L-kynureninase
qRT-PCR: Quantitative real-time PCR
PASI: Psoriasis Area and Severity Index
QA: Quinolinic acid
TDO: Tryptophan dioxygenase

of *KYNU* is also altered in patients with psoriasis, and this epigenetic modification predicts increased expression in patients with psoriasis compared with healthy subjects.[9] *KYNU* has also been identified as a "molecular scar gene," indicating that even after successful treatment of psoriasis, it remains slightly upregulated in nonlesional compared with normal skin.[10] Moreover, *KYNU* was determined to be a gene synergistically enhanced by IL-17 and TNF-α[11] and is considered one of the "top 25" psoriasis genes.[12] However, the role of KYNU and tryptophan metabolism in patients with psoriasis has not been explored.

The tryptophan metabolism pathway is typically associated with immune suppression.[13-15] The initial rate-limiting enzyme in the tryptophan metabolism pathway, indoleamine 2,3-dioxygenase (IDO), has been thoroughly studied and linked to cancer progression, generation of regulatory T cells, and immune tolerance.[13,14,16] IDO induces immune suppression through 2 non–mutually exclusive mechanisms.[13,15] First, IDO degrades tryptophan into kynurenine, thus depleting the microenvironment of this amino acid and "starving" immune cells.[15,17] Second, the kynurenine produced by IDO is actively immune suppressive[15] through such mechanisms as inducing regulatory T cells through kynurenine binding to the aryl hydrocarbon receptor (AhR).[18,19] This second mechanism of immune suppression by IDO might be the most potent because exogenous administration of kynurenine can directly induce tolerance independently of tryptophan depletion.[18] IDO is also upregulated in several inflammatory settings, ranging from delayed-type hypersensitivity reactions to sepsis.[20,21] In these situations upregulation of IDO might be a mechanism for initiating "immune turnoff," and indeed, many proinflammatory signals, such as IFN-γ, induce IDO expression.[13,22,23] However, it is not clear why IDO and tryptophan metabolism are upregulated in both immune-tolerant and proinflammatory environments.

In this study we investigated the 2 tryptophan metabolism enzymes (IDO and KYNU) found to be significantly upregulated in the chronic inflammatory skin disease psoriasis. We find that tryptophan metabolism, specifically when enriched for KYNU, promotes inflammation. Our data point to a novel and actively inflammatory role of tryptophan metabolism in not only patients with psoriasis but also those with other human inflammatory diseases.

METHODS

Skin and blood samples

Skin biopsy specimens from healthy volunteers and patients with psoriasis, as well as blood samples from healthy volunteers, were obtained under a Rockefeller University Institutional Review Board–approved protocol. Written informed consent was obtained, and the study was performed in adherence with the Declaration of Helsinki. Deidentified surgical skin waste was used to obtain whole normal skin biopsy specimens for culture. Normal blood samples were used to obtain PBMCs and T cells. PBMCs were isolated by using Ficoll-Paque gradient centrifugation. T cells were isolated from PBMCs by using the RosetteSep system (STEMCELL Technologies, Vancouver, British Columbia, Canada), according to the manufacturer's instructions. Myeloid cell populations underwent fluorescence-activated cell sorting from PBMCs by using the antibody panel in Table E1 in this article's Online Repository at www.jacionline.org.

Immunohistochemistry and immunofluorescence

Immunohistochemistry and immunofluorescence were performed, as previously described.[24,25] Antibodies are listed in Table E2 in this article's Online Repository at www.jacionline.org. For immunohistochemistry and immunofluorescence experiments, there were 3 samples per group; representative pictures are shown. For cell counts, images were quantified with ImageJ software (National Institutes of Health, Bethesda, Md). For epidermal quantification, an automated epidermal area quantification algorithm was created to load the .tiff images, find the epidermis, and output the mean epidermal thickness. The algorithm, written in the Matlab computing environment, can be found at http://dangareau.net/find-epi.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed on samples, as previously described.[26] Primers were purchased from Applied Biosystems (Foster City, Calif) and are listed in Table E3 in this article's Online Repository at www.jacionline.org. Gene expression was normalized to the housekeeping gene encoding human acidic ribosomal protein.

Cell culture

Primary human keratinocytes and human dermal blood endothelial cells (ECs) were cultured, as previously described.[25,27] For cytokine experiments, keratinocytes, ECs, and PBMCs were cultured in 12-well plates with 2 mL of the appropriate cell-type medium. IL-17 (100 ng/mL), IFN-γ (20 ng/mL), TNF-α (20 ng/mL), IL-10 (40 ng/mL), and IL-13 (50 ng/mL) were added for 12 hours, and cell lysates were collected and prepared for subsequent RNA isolation by using Qiagen RNA isolation kits (Qiagen, Hilden, Germany), according to the manufacturer's instructions. For whole normal skin experiments, biopsy specimens were cultured with 50 ng/mL TNF-α, 100 ng/mL IFN-γ, or both for 24 hours (RNA) or 3 days (immunohistochemistry). For tryptophan metabolite experiments, keratinocytes and ECs were cultured until 60% to 80% confluence in a 12-well plate with 2 mL of appropriate media. T cells were activated with anti-CD3/anti-CD28 beads (Dynabeads). Cells were then exposed to 50 μmol/L tryptophan, kynurenine, 3-hydroxyanthranilic acid (3HAA), or quinolinic acid (QA) for 3 days (all from Sigma-Aldrich, St Louis, Mo).

Gene Omnibus mining

Publically available gene array studies were mined for expression of *IDO1*, tryptophan dioxygenase *(TDO)*, and *KYNU*. Studies were chosen that included comparison with normal or nondiseased tissue. The average fold change of *IDO1* and *TDO* expression, as well as the average fold change of *KYNU* expression, for a given disease compared with normal or nondiseased tissue was obtained. *IDO* and *TDO* fold changes were averaged, and *KYNU* fold changes were averaged; GSE numbers for all studies used are provided in Table E4 in this article's Online Repository at www.jacionline.org.

Statistics

All data were analyzed with GraphPad Prism 5 software (GraphPad Software, La Jolla, Calif). qRT-PCR expression values were normalized