

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 2015;■■■:■■■-■■■.)

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

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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 of KYNU is also altered in patients with psoriasis, and this epigenetic

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

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 suppressive¹⁵ 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 turnover,” 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 μ mol/L tryptophan, kynurenine, 3-hydroxyanthranilic acid (3HAA), or quinolinic acid (QA) for 3 days (all from Sigma-Aldrich, St Louis, Mo).

Gene Omnibus mining

Publicly 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

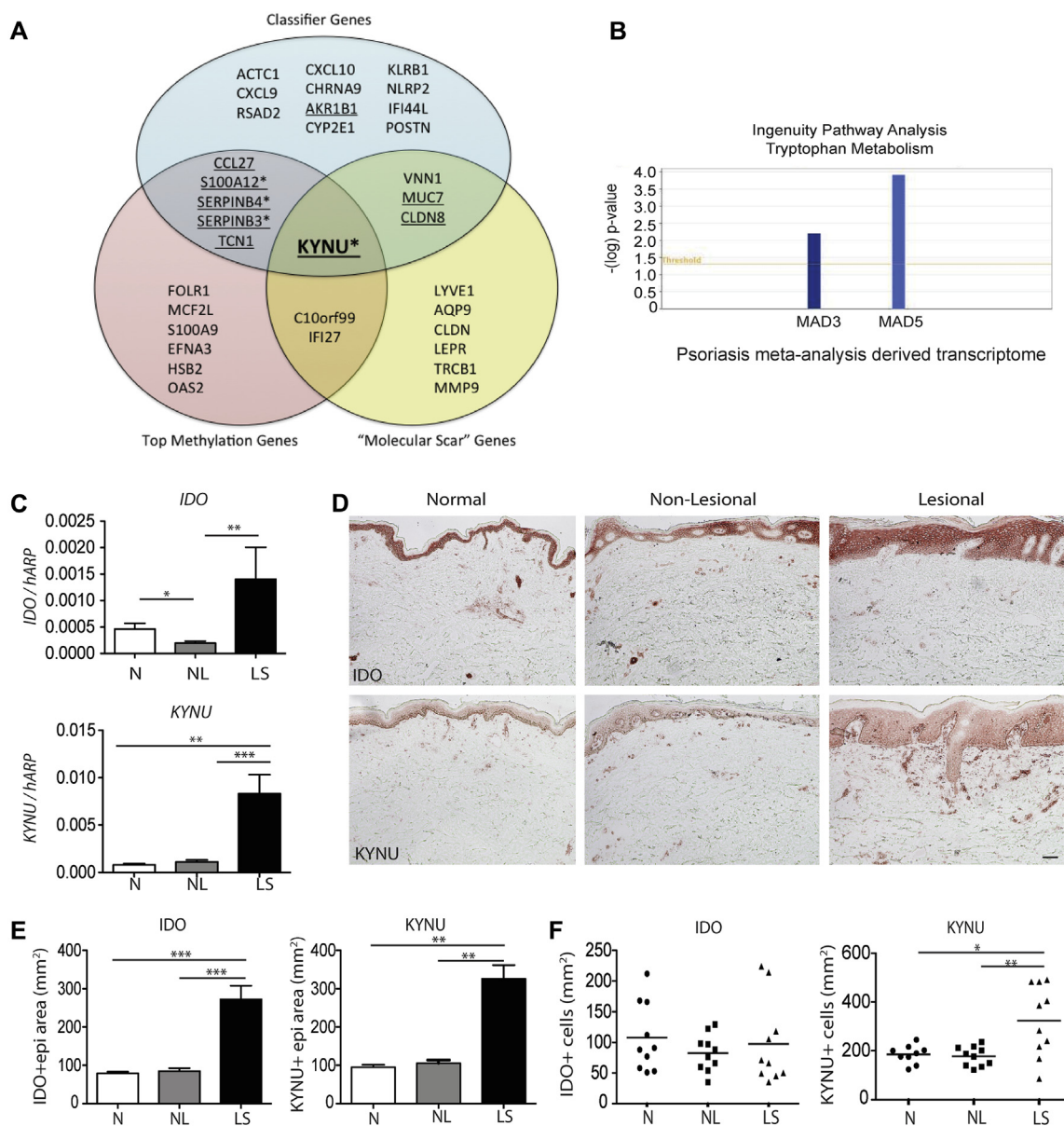


FIG 1. Expression of the tryptophan metabolism enzymes IDO and KYNU in psoriasis lesional skin. **A**, Venn diagram of psoriasis classifiers genes (blue circle), top psoriasis methylation genes (red circle), and "molecular scar" genes (after treatment with anti-TNF, yellow circle). Underlined genes are in the top 25 psoriasis genes; genes with an asterisk indicate synergistic expression with IL-17 and TNF. Adapted from Fig 5 from Tian et al.⁶ **B**, Significant upregulation of the tryptophan metabolism pathway in previously published meta-analysis derived (MAD) psoriasis transcriptomes,⁶ as determined by using Ingenuity Pathway Analysis. **C**, Expression of IDO and KYNU mRNA in skin biopsy specimens, as determined by using qRT-PCR. **D**, Immunohistochemistry of protein expression of IDO and KYNU in normal (N), nonlesional (NL), and lesional (LS) psoriatic skin. **E**, Quantification of IDO and KYNU epidermal expression. **F**, Quantification of dermal IDO⁺ and KYNU⁺ cells. Scale bar = 10 μ m. For qRT-PCR of IDO and KYNU, n = 5-13 samples per group. For immunohistochemistry of IDO and KYNU, n = 6-10 samples per group. * $P < .05$, ** $P < .01$, and *** $P < .001$.

to the housekeeping gene encoding human acidic ribosomal protein and log₂-transformed for analysis. Measurements of less than the limit of detection were imputed as 20% of the minimum value observed for that gene. qRT-PCR expression data were modeled by using a mixed-effect model, with group as a fixed effect and a random intercept for each pool/replication. This formulation intrinsically models the within-patient correlation structure, as with a paired *t* test. *P* values were adjusted across markers by using the Benjamin-Hochberg approach. The Dunnett *P* value and the false

discovery rate are provided for experiments that include multiple comparisons and are shown in Table E5 in this article's Online Repository at www.jacionline.org. The Spearman correlation test was used to determine significant correlations between IDO or KYNU expression and the Psoriasis Area and Severity Index (PASI) score or various inflammatory parameters, as well as the correlation between IDO-TDO and KYNU expression in various human diseases. All error bars represent the SEM. *P* values of less than .05 were considered significant.

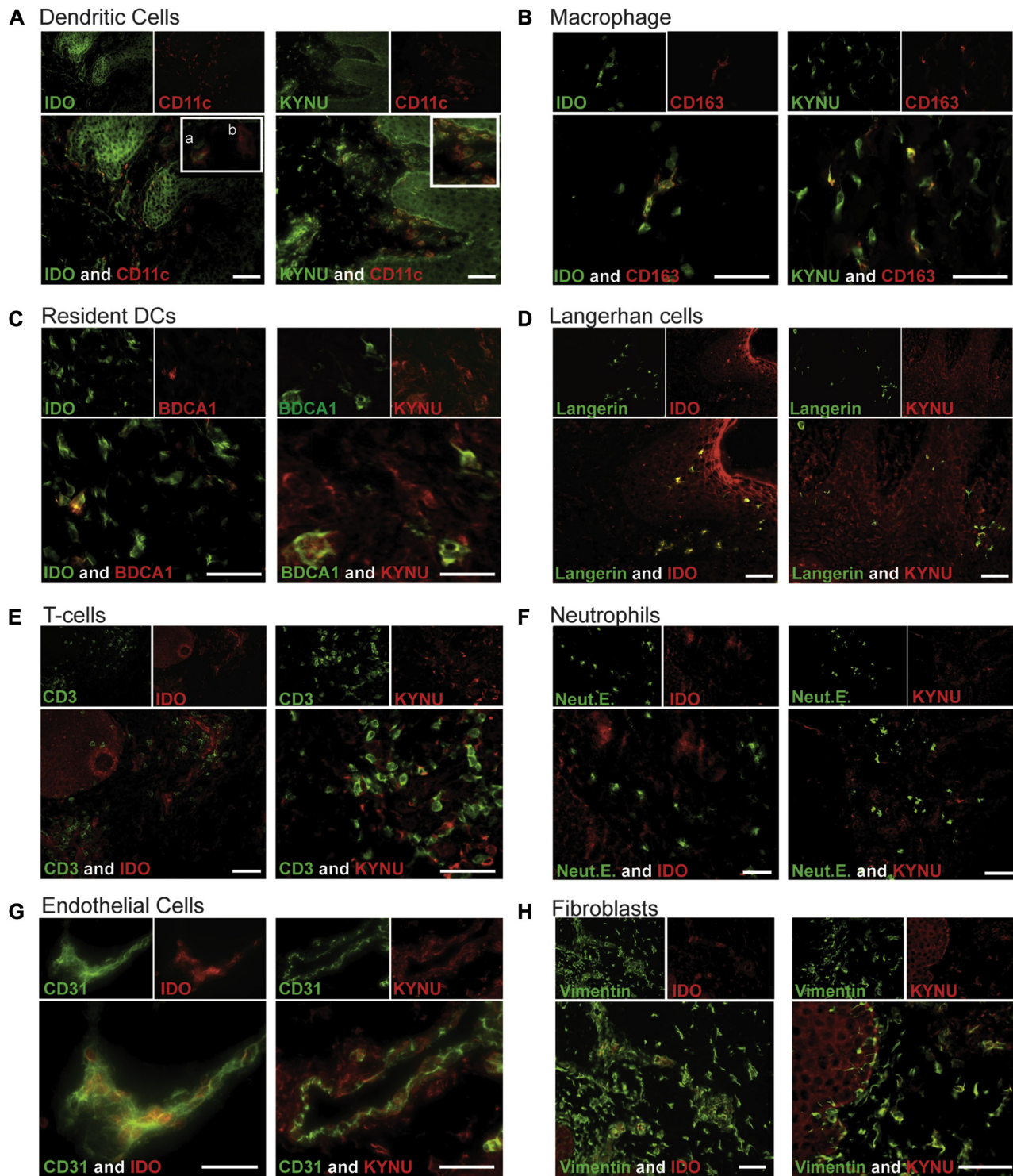


FIG 2. Identification of KYNU- and IDO-expressing cells in psoriatic skin. Double immunofluorescence staining of IDO and KYNU versus CD11c⁺ DCs (*white inset* highlights the presence of IDO⁺ [a] and IDO⁻ [b] CD11c⁺ DCs at *left* and KYNU⁺ CD11c⁺ DCs at *right*; **A**), CD163 macrophages (**B**), BDCA-1⁺ resident DCs (**C**), Langerin⁺ cells (**D**), CD3⁺ T cells (**E**), neutrophil elastase-positive cells (**F**), CD31⁺ ECs (**G**), and vimentin-positive cells (**H**). Scale bar = 5 μ m.

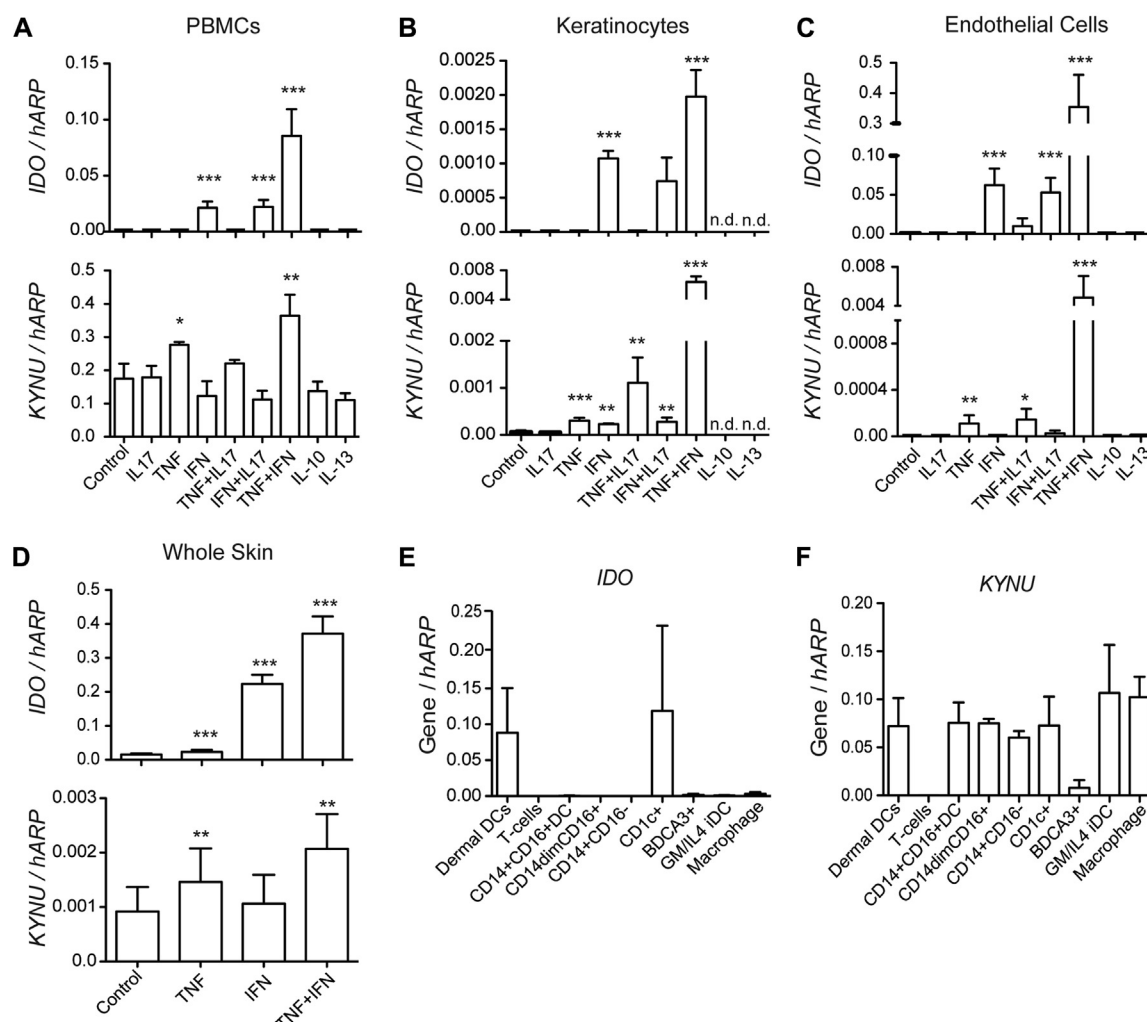


FIG 3. IFN- γ and TNF- α drive expression of *IDO* and *KYNU*, and myeloid cell subsets express the highest amount of *KYNU*. **A-C**, PBMCs (Fig 3, A), keratinocytes (KCs; Fig 3, B), and ECs (Fig 3, C) were cultured with or without various cytokines (IL-17, TNF- α , IFN- γ , TNF- α + IL-17, IFN- γ + IL-17, TNF- α + IFN- γ , IL-10, or IL-13) for 12 hours and assessed by using qRT-PCR for expression of *IDO* and *KYNU*. **D**, Whole normal skin biopsy specimens were cultured for 24 hours with TNF- α , IFN- γ , or both. For PBMC experiments, $n = 5$ samples per condition; for keratinocyte experiments, $n = 3$ samples per condition; for EC experiments, $n = 6-8$ samples per condition; for whole skin, $n = 3-5$ samples per condition. **E and F**, Expression of *IDO* (Fig 3, E) and *KYNU* (Fig 3, F) was determined by using qRT-PCR in dermal DC crawl-outs, as well as sorted primary peripheral blood cells, including CD3 $^{+}$ T cells, CD14 $^{+}$ CD16 $^{+}$ monocytes, CD14 ^{dim} CD16 $^{+}$ monocytes, CD14 $^{+}$ CD16 $^{-}$ DCs, CD1c $^{+}$ (BDCA-1 $^{+}$) DCs, and BDCA-3 $^{+}$ DCs. Additionally, expression of *IDO* and *KYNU* was determined in PBMC-derived DCs (GM-CSF and IL-4) and macrophages (colony-stimulating factor; $n = 3-4$ samples per group). * $P < .05$, ** $P < .01$, and *** $P < .001$.

RESULTS

Tryptophan metabolism pathway is upregulated in patients with psoriasis

KYNU is one of the psoriasis classifier genes, a “molecular scar” gene, highly upregulated in microarray studies, a top 25 psoriasis gene, and synergistically enhanced by IL-17 and TNF- α and has a differential DNA methylation pattern in patients with psoriasis.⁶ Interestingly, *KYNU* is the only gene found in all of these categories (Fig 1, A), highlighting it as an important gene to interrogate in psoriasis pathogenesis. Ingenuity Pathway Analysis was used to explore upregulation of the tryptophan metabolism pathway in 2 meta-analyses of psoriasis transcriptomes (Fig 1, B).⁶ Tryptophan metabolism was found to be

significantly upregulated, despite the fact that only 2 enzymes in the pathway were upregulated, *IDO* and *KYNU*. *KYNU* expression is heavily upregulated, whereas *IDO* expression is only mildly increased (see Fig E1 in this article’s Online Repository at www.jacionline.org).

IDO- and *KYNU*-expressing cells are found in psoriatic lesions

To confirm microarray data showing that *IDO* and *KYNU* expression is increased in psoriatic skin, quantitative real-time-PCR (qRT-PCR) analysis of RNA from normal, nonlesional, and lesional biopsy specimens was used to quantify mRNA expression of these

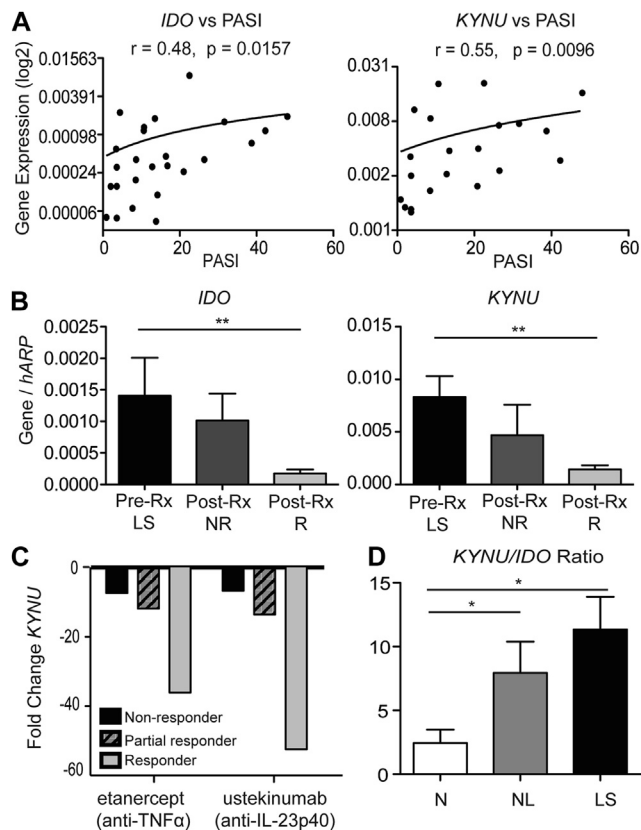


FIG 4. Reduction in tryptophan metabolism is associated with successful response to psoriasis treatment. **A**, Spearman correlation of *IDO* and *KYNU* expression with disease severity (PASI score). **B**, RNA from pretreatment and posttreatment lesional skin biopsy specimens of patients with psoriasis receiving narrow-band UVB therapy were assessed for expression of *IDO* and *KYNU* and stratified by response to treatment. **C**, Reduction in *KYNU* expression from microarray analysis of other psoriasis treatment regimens. **D**, Ratio of *KYNU/IDO* expression in normal, nonlesional, and lesional skin. For qRT-PCR results, $n = 3$ -12 samples per group. * $P < .05$ and ** $P < .01$.

enzymes (Fig 1, C). Expression of both *IDO* and *KYNU* was increased in lesional compared with nonlesional and normal skin.

Next, protein expression of *IDO* and *KYNU* was determined by means of immunohistochemistry (Fig 1, D). Prominent staining of epidermal keratinocytes, as well as staining of dermal cells, was noted for both enzymes. Immunohistochemistry allowed for the quantification of both epidermal protein expression area and dermal cell counts. Lesional epidermis had a significantly greater area of *IDO*⁺ and *KYNU*⁺ staining compared with normal and nonlesional epidermis because of epidermal hyperproliferation (Fig 1, E). Quantification of dermal cells showed *IDO*⁺ cell numbers were similar between all groups. However, lesional skin had significantly higher numbers of *KYNU*⁺ dermal cells compared with normal and nonlesional skin (Fig 1, F). This suggests that lesional psoriatic skin is associated with increased numbers of *KYNU*⁺, but not *IDO*⁺, dermal cells.

Several cell types express *KYNU* and *IDO* in human skin

Two-color immunofluorescence studies were conducted to query various cell populations and elucidate which dermal cells in human skin express *KYNU* and *IDO*. *IDO* expression in

myeloid cells has been previously described,¹³ and therefore expression of these tryptophan metabolism enzymes within *CD11c*⁺ DCs, blood dendritic cell antigen 1 (BDCA-1)⁺ resident DCs, Langerhans cells, and *CD163*⁺ macrophages was first evaluated. As expected, *IDO* was found to colocalize with some *CD11c*⁺ cells (Fig 2, A); however, there were both *IDO*⁺ and *IDO*[−] DCs present in lesional skin (see inset). Almost all *CD11c*⁺ DCs in lesional skin expressed *KYNU*. *CD163*⁺ macrophages also heavily colocalized both *IDO* and *KYNU* in lesional skin (Fig 2, B). Regarding specific DC subsets, BDCA-1⁺ resident DCs were found to express both *IDO* and *KYNU* (Fig 2, C), and Langerhan-positive cells were predominantly *IDO*⁺ (Fig 2, D). In contrast, *CD3*⁺ T cells and neutrophils (neutrophil elastase-positive cells) did not colocalize with *KYNU* or *IDO* (Fig 2, E and F).

Previous studies have also found expression of *IDO* in ECs.^{28,29} Similarly, we found that *CD31*⁺ ECs in lesional skin colocalized with both *IDO* and *KYNU* (Fig 2, G). Additionally, fibroblasts have been described to express these enzymes,³⁰ and colocalization of *KYNU* and *IDO* with some vimentin-positive cells was also found, but many vimentin-positive cells did not stain positive for these enzymes (Fig 2, H).

Cytokines in the psoriatic milieu upregulate *KYNU* and *IDO* expression

Various cell types were exposed to an array of cytokine stimuli and expression of *KYNU* and *IDO* was assessed to determine how *KYNU* and *IDO* can be induced in psoriatic lesional skin. Additionally, these experiments served as confirmation of the *IDO* and *KYNU* expression patterns in the various cell types seen by using immunofluorescence (Fig 2). ECs, keratinocytes, and PBMCs were exposed to cytokines heavily present in the psoriatic microenvironment (IL-17, IFN- γ , and TNF- α); the immunosuppressive cytokine IL-10; or the T_H2-associated cytokine IL-13 (Fig 3, A-C).

IFN- γ significantly induced expression of *IDO* in ECs, PBMCs, and keratinocytes. TNF- α alone moderately, but not significantly, upregulated *KYNU* expression in these cell types. However, the combination of IFN- γ and TNF- α significantly enhanced expression of both *IDO* and *KYNU* in all cell types. Although ECs only expressed *KYNU* on exposure to TNF- α or IFN- γ , both PBMCs and keratinocytes constitutively expressed *KYNU*. Exposure to IL-17, IL-10, and IL-13 did not significantly modulate *IDO* or *KYNU* expression in all cell types.

Normal skin biopsy specimens were cultured with IFN- γ , TNF- α , or the combination of IFN- γ and TNF- α to determine whether the psoriatic cytokine milieu results directly in upregulation of these tryptophan metabolism enzymes in skin-resident cells (Fig 3, D). IFN- γ significantly upregulated *IDO* expression in normal skin biopsy specimens, whereas TNF- α only slightly, but still significantly, also upregulated *KYNU* expression. Interestingly, although the combination of IFN- γ and TNF- α significantly enhanced *IDO* upregulation even further in normal skin biopsy specimens, *KYNU* expression was only modestly but significantly upregulated, and expression of *IDO* was still more than 100-fold greater than *KYNU*. Additionally, immunohistochemical staining of normal skin biopsy specimens exposed to the cytokines did not reveal increased numbers of *IDO*⁺ or *KYNU*⁺ cells (see Fig E2 in this article's Online Repository at www.jacionline.org). As shown in Fig 1, lesional skin is

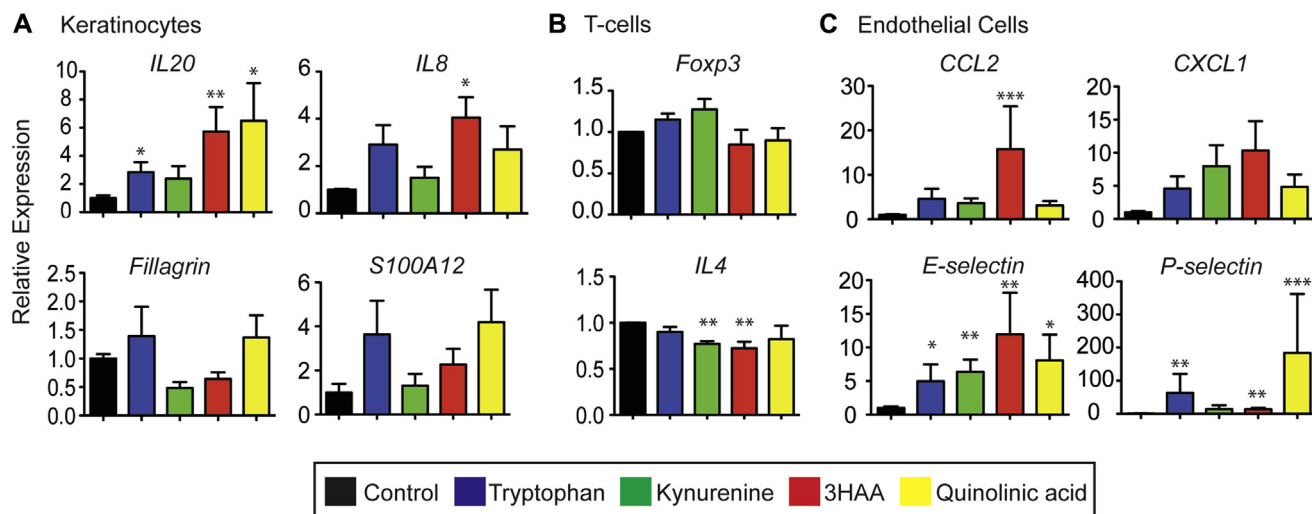


FIG 5. Tryptophan metabolites downstream of KYN upregulate inflammatory gene expression in several cell types important in psoriasis pathogenesis. Keratinocytes (KCs; **A**), activated T cells (**B**), and ECs (**C**) were cultured with 50 μ M/L tryptophan (TRYP, blue), kynurenine (KYN, green), 3HAA (red), and QA (yellow) or no metabolite (Control, black), as described in the experimental procedures. Expression of inflammatory genes normalized to control values is shown. For keratinocyte experiments, $n = 5-8$ samples per condition; for T-cell experiments, $n = 5-7$ samples per condition; for EC experiments, $n = 6-10$ samples per condition. * $P < .05$, ** $P < .01$, and *** $P < .001$.

characterized by an almost 10-fold greater expression of KYN compared with IDO, as well as a significant increase in the number of KYN⁺ dermal cells. Therefore exposure of normal skin to key psoriatic cytokines (ie, IFN- γ and TNF- α) could not recapitulate the pattern of expression of tryptophan metabolism enzymes seen in lesional skin.

Myeloid cell subsets express the highest amount of KYN

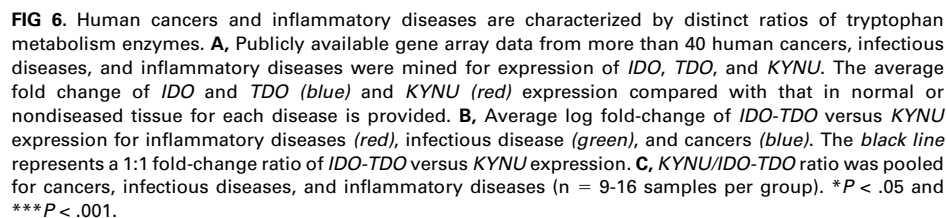
Because we found that PBMCs and keratinocytes constitutively express KYN, the massively increased KYN expression seen in psoriatic lesional skin might be a result of infiltrating immune cells, keratinocyte hyperproliferation, or both. However, infiltrating immune cells are likely the predominant source of upregulated KYN expression in psoriatic skin because PBMCs expressed 100-fold more KYN mRNA compared with ECs, keratinocytes, and whole skin (see Fig E3 in this article's Online Repository at www.jacionline.org).

RNA from monocytes, DCs, and T-cell populations undergoing fluorescence-activated cell sorting was assessed for IDO and KYN expression to further elucidate which populations in PBMCs were predominantly expressing these enzymes (Fig 3, E and F). Many myeloid cell subsets, including CD14⁺CD16⁺ monocytes, CD14^{dim}CD16⁺ monocytes, CD14⁺CD16⁺ DCs, and CD1c⁺ (BDCA1⁺) DCs, all expressed high levels of KYN at steady state. Additionally, primary human dermal DCs from healthy skin (dermal DC crawl-outs), as well as monocyte-derived DCs and macrophages, also expressed high levels of KYN. Therefore it is likely that the KYN⁺ cells that infiltrate psoriatic lesions are derived from circulating myeloid cells and might differentiate into inflammatory DCs and macrophages, which are known to play a critical role in psoriasis pathogenesis.^{31,32}

Tryptophan metabolism is reduced with successful response to treatment in both patients with psoriasis and those with AD

The finding of increased tryptophan metabolism in patients with psoriasis is intriguing because tryptophan metabolism is associated with immune suppression, such as during fetal-maternal tolerance and in tumor microenvironments.^{13,14} Therefore upregulation of this pathway might have been thought to be beneficial rather than detrimental to patients with psoriasis. However, we found a significant positive correlation between disease severity (PASI score) and expression of KYN and IDO, with KYN expression being more significantly associated with PASI scores than IDO expression (Fig 4, A). Furthermore, KYN and IDO expression positively and significantly correlated with expression of many key inflammatory molecules in psoriasis pathogenesis, including *IL17*, *IL22*, *IFNG*, *CCL20*, *IL23p19*, inducible nitric oxide synthase, *MX1*, *IL12/23p40*, *K16*, *DEFB4*, and *IL20* (see Figs E4 and E5 in this article's Online Repository at www.jacionline.org).

To determine whether tryptophan metabolism was reduced on successful treatment of psoriasis, expression of IDO and KYN mRNA was quantified in posttreatment biopsy specimens of patients not responding and patients responding to narrow-band UVB treatment (Fig 4, B).²⁶ In patients not responding to narrow-band UVB treatment, expression of KYN and IDO was not significantly decreased; however, responding patients had significantly reduced levels of these enzymes. KYN expression was also significantly decreased in microarray analysis of patients treated for 12 weeks with biological therapies, including etanercept (anti-TNF- α)³³ and ustekinumab (anti-IL-12/23p40; Fig 4, C). These data raise the possibility that the tryptophan metabolism pathway might play an actively inflammatory rather than a regulatory role in psoriasis pathogenesis.



Because expression of the tryptophan metabolism enzymes *KYNU* and *IDO* is associated with psoriasis, particularly with the ratio of these enzymes being in favor of *KYNU*, we hypothesized that this might result in accumulation of specific tryptophan metabolites. *KYNU* degrades kynurenine to produce 3HAA,³⁴ and it is possible that metabolites downstream of *KYNU* have direct inflammatory effects.

Various human cell types, including keratinocytes, T cells, and ECs, were exposed to metabolites upstream and downstream of KYNU and changes in inflammatory gene expression were quantified to determine the capacity of different tryptophan metabolites to induce inflammation. In general, metabolites downstream of KYNU (3HAA and QA) induced inflammatory gene expression, whereas kynurenine had minimal inflammatory capacity (Fig 5).

KCs cultured with 3HAA and QA had significantly enhanced expression of inflammatory genes, including *IL20* and *IL8* (Fig 5, A, and see Fig E6, A, in this article's Online Repository at www.jacionline.org). Conversely, kynurenine decreased expression of filaggrin. Although not statistically significant, kynurenine also suppressed expression of *S100A12* and *IL8* compared with tryptophan, and this suppression was lost with 3HAA (for *IL8*) and QA (for *S100A12*). Tryptophan metabolites had mild but noticeable effects on activated T cells (Fig 5, B). Kynurenine upregulated forkhead box protein 3, as has been previously reported.¹⁸ Both 3HAA and QA significantly suppressed *IL4* expression (Fig 5, B), and 3HAA preferentially promoted expression of the T_H1 master transcriptional regulator T-bet compared with other T-cell polarization factors (see Fig E6, B). Regarding ECs, several tryptophan metabolites upregulated inflammatory genes, with 3HAA having the largest effect, including upregulation of *CCL2*, *CXCL1*, *CCL5*, E-selectin, *IL8*, intercellular adhesion molecule (*ICAM*), and vascular cell adhesion molecule (*VCAM*; Fig 5, C, and see Fig E6, C).

Ratio of tryptophan metabolism enzymes corresponds to the immunologic environment in human diseases

The observation that increased expression of KYNU compared with IDO promotes an inflammatory environment might not only apply to psoriasis but might also be applicable to other inflammatory diseases. If this is correct, immunosuppressive microenvironments, such as cancer, should have higher expression of early tryptophan metabolism enzymes (ie, IDO or the analogous enzyme TDO) compared with later enzymes (ie, KYNU), resulting in accumulation of immunosuppressive metabolites, such as kynurenine. Conversely, inflammatory diseases might have higher expression of later tryptophan metabolism enzymes, resulting in depletion of kynurenine and production of putatively inflammatory metabolites, such as 3HAA or QA.

To assess this hypothesis, publically available gene array data from more than 40 human diseases were mined for expression of IDO, TDO, and KYNU. The average fold change of IDO and TDO versus KYNU for each disease is shown, with diseases organized by preferential upregulation of IDO-TDO (Fig 6, A, left) versus KYNU (Fig 6, A, right). In almost all cases, cancers were located to the left of the graph, indicating preferential upregulation of IDO-TDO, whereas the opposite was true for proinflammatory situations, which mainly clustered on the right side of the graph and contained higher upregulation of KYNU. Although most diseases upregulated both IDO-TDO and KYNU ($P < .005$, Spearman correlation), a significant stratification between cancers versus proinflammatory diseases was found with regard to the relative upregulation of these enzymes (Fig 6, B). Most infectious diseases had more modest upregulation and are located more centrally on the chart. From these data,

the KYNU/IDO-TDO ratio was obtained for each disease, and diseases were grouped according to cancer, infectious disease, and inflammatory disease. Inflammatory diseases displayed a significantly higher KYNU/IDO-TDO ratio compared with cancers (Fig 6, C), suggesting that although the tryptophan metabolism pathway might be upregulated in both groups of diseases, there is preferential enzyme expression that could result in a specific metabolite milieu and lead to distinct immunologic outcomes.

Altered tryptophan metabolism in patients with AD and reduction on treatment

We further investigated the upregulation of these enzymes specifically in patients with atopic dermatitis (AD) because a previous study demonstrated increased expression of tryptophan metabolism genes in both patients with AD and those with psoriasis.⁸ Gene array data from multiple groups demonstrated that lesional AD skin, although containing both increased IDO-TDO and KYNU expression, always expressed higher levels of KYNU compared with IDO-TDO (see Fig E7, A, in this article's Online Repository at www.jacionline.org). Furthermore, nonlesional AD skin also had increased KYNU expression compared with normal skin. This finding of aberrant tryptophan metabolism enzyme expression, even in nonlesional skin, was akin to our results in patients with psoriasis (Figs 1 and 4), demonstrating that even histologically normal-looking skin of either patients with AD or those with psoriasis can have a skewed tryptophan metabolism profile and predispose these subjects to cutaneous inflammation.

The reduction of KYNU expression after treatment in patients with AD was also explored. Many different treatments reduced KYNU expression in lesional AD skin (see Fig E7, B). Dupilumab demonstrated the most drastic reduction in KYNU expression, which might correlate to the highly successful nature of this therapeutic.³⁵ Interestingly, in contrast to all other treatments analyzed, pimecrolimus did not result in reduction of KYNU expression. Results from this study demonstrated that pimecrolimus mainly improved lesional skin through restoration of barrier function, whereas inflammatory components were minimally altered.³⁶ This agrees with our finding that KYNU is much more highly expressed in immune cells compared with keratinocytes and likely plays a direct role in immune modulation rather than barrier function.

DISCUSSION

Here we describe the expression and potential inflammatory function of the tryptophan metabolism enzyme KYNU, which is encoded by a highly upregulated gene in chronic inflammatory skin. To our knowledge, this is the first exploration of KYNU and a role for this enzyme in the pathogenesis of inflammatory skin disease. Our model for how tryptophan metabolism can dichotomously regulate immune responses is shown in Fig E8 in this article's Online Repository at www.jacionline.org. Interestingly, dichotomous actions of tryptophan metabolism have been described in neurobiology, with early tryptophan metabolites (eg, kynurenine) being considered neuroprotective and late tryptophan metabolites (eg, QA) demonstrating neurotoxic activity.³⁷⁻³⁹ Therefore the relationship between tryptophan metabolism and the immune system might be more complex

than previously described and analogous to the interaction of tryptophan metabolites with the nervous system.

In the late 1960s, clinical trials used a low-tryptophan diet as a novel treatment for psoriasis.^{40,41} Although some of the trials demonstrated efficacy, these studies were not continued, and the mechanism was not explored. It is interesting that almost half a century later, with the explosion of transcriptomics, epigenomics, and bioinformatics tools, one of the genes most heavily associated with psoriasis is in the tryptophan metabolism pathway. Our findings suggest that feeding the tryptophan metabolism pathway, particularly when it is skewed toward production of late metabolites, might promote inflammation. We found that even nonlesional skin of both patients with AD and those with psoriasis contained an altered ratio of tryptophan metabolism enzyme expression (Fig 4, D, and see Fig E7, A). Therefore nonlesional skin in patients with either of these diseases can have mild but biologically meaningful accumulation of inflammatory metabolites downstream of KYNU. This hypothesis is in accordance with previous work, which found that nonlesional skin has a slightly increased inflammatory profile compared with normal skin.⁴²

In this article only IDO and KYNU were assessed because these are the only 2 tryptophan metabolism enzymes significantly upregulated in psoriatic lesional skin. However, the tryptophan metabolism pathway is complex, and future studies that incorporate all of the enzymes within the pathway will provide a deeper understanding of how modulation of this pathway affects inflammation. Additionally, there might be other metabolic reasons and biological consequences surrounding upregulated tryptophan metabolism in patients with psoriasis. The catabolism of tryptophan also feeds into the serotonin and melatonin pathways.^{39,43} There is an increase in the incidence of depression in patients with psoriasis,⁴⁴ and it is possible that dysregulated tryptophan metabolism could play a role in this susceptibility. Additionally, the hyperproliferative state of keratinocytes in patients with psoriasis can result in altered metabolic activities to meet the energy demands of these cells. However, in this study it was found that myeloid cells and not keratinocytes contained the highest expression of KYNU, suggesting a likely immunomodulatory role for this enzyme.

Because the dual nature of the tryptophan metabolism pathway has already been described in neurobiology, it is possible that dysregulation of this pathway in skin might also affect cutaneous nerves, such as nociceptors. A recent publication demonstrated a critical role for nociceptors in imiquimod-induced skin inflammation.⁴⁵ Nociceptors were in close contact with dermal DCs, and the authors suggest that interplay between DCs and nerves plays a crucial early role in skin inflammation, although the mechanism was not explored.⁴⁵ From our studies, we found that myeloid cells, including dermal DCs, were the predominant cell type expressing KYNU in lesional skin. Microglia, the predominant myeloid cell type in the brain, can produce significant amounts of late tryptophan metabolites, such as QA, and result in neurotoxicity.³⁹ Therefore it is possible that late tryptophan metabolites produced by dermal myeloid cells could exert effects on cutaneous nerves, resulting in the itch associated with psoriasis and AD.

Although we found that tryptophan metabolites can differentially modulate inflammatory gene expression, the molecular underpinnings of how these metabolites induce changes in gene expression are not well understood. Kynurenine is a known ligand

for AhR, and activation of this receptor has been implicated in a variety of immunologic studies.⁴⁶⁻⁴⁹ A recent publication demonstrated a critical role of AhR in controlling skin inflammation.⁵⁰ Specifically, these researchers found that AhR^{-/-} mice exhibited exacerbated cutaneous inflammation. In our study we found that kynurenine reduced expression of some inflammatory genes in keratinocytes and promoted forkhead box protein 3 expression in T cells (Fig 5). Therefore loss of endogenous kynurenine-AhR signaling in patients with psoriasis (through high upregulation of KYNU and degradation of kynurenine) can also lead to excessive inflammation.

In conclusion, we have provided initial evidence that the tryptophan metabolism enzyme KYNU plays a proinflammatory role in human disease. This study lays the foundation for an exciting new connection between tryptophan metabolism and immune regulation, indicating that this pathway is more complicated than previously appreciated and might play a distinct role in both patients with inflammatory diseases and those with cancers.

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Key messages

- The tryptophan metabolism enzyme KYNU is a highly upregulated but previously unexplored enzyme in patients with chronic inflammatory skin disease.
- Tryptophan metabolites downstream of KYNU induce inflammatory gene expression.
- Many human autoimmune and autoinflammatory diseases have increased KYNU expression, suggesting this pathway might play a general role in inflammation.

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Supplementary Table 1 - FACS Sorting Antibody Panel.

Color	Antigen	Manufacturer	Clone	Isotype	Dilution
FITC	CD3	BD Pharmigen	SK7	IgG1	1:20
FITC	CD20	BD Pharmigen	L27	IgG1	1:20
FITC	CD56	BD Pharmigen	NCAM16.2	IgG2b	1:20
PE	CD1c	Miltenyi	AD5-8E7	IgG2a	1:50
PerCP-Cy5.5	CD11c	Biolegend	Bu15	IgG1	1:50
PE-Cy7	CD16	BD Pharmigen	B73.1	IgG1	1:20
APC	BDCA3	Miltenyi	AD5-14H12	IgG1	1:20
AF-700	HLA-DR	eBioscience	L243	IgG2a	1:1000
Pacific Blue	CD14	BD Pharmigen	M5E2	IgG2a	1:100

Supplementary Table 1 - FACS Sorting Antibody Panel - T-cells, B-cells, and NK cells were stained with FITC-conjugated CD3, CD20, and CD56, respectively, and depleted. Monocytes were selected based upon CD14 and CD16 expression, while DCs were selected based on positive expression of CD11c and HLA-DR, and further sorted upon CD1c and BDCA3 positivity.

Supplementary Table 2 - Antibodies used for immunohistochemistry/immunofluorescence

Antigen	Manufacturer	Clone	Dilution	Amplification/detection (2 ^o antibodies)
KYNU	Sigma-Aldrich	1G2	1:50	Goat anti-mouse IgG2a A-488 or A-586
IDO	AbD Serotec	4D2	1:100	Goat anti-mouse IgG2b A-488 or A-586
CD11c	BD	B-ly6	1:100	Goat anti-mouse IgG1 A-488
BDCA-1	Miltenyi Biotec	AD5-8E7	1:100	Goat anti-mouse IgG2a A-488
CD163-FITC	Acris	5C6-FAT	1:100	Goat anti-mouse IgG - FITC
CD3	BD	SK7	1:100	Goat anti-mouse IgG1 A-488
CD31	BD	WM59	1:100	Goat anti-mouse IgG1 A-488 or A-647
Vimentin	Thermo Scientific	Ab2	1:100	Goat anti-mouse IgG1 A-488
Neutrophil Elastase	Dako	NP57	1:100	Goat anti-mouse IgG1 A-488
Langerin	Immunotech	DCGM4	1:100	Goat anti-mouse IgG1 A-488

Supplementary Table 3 - Quantitative Real-Time PCR Primer Probes

Gene	Assay ID
<i>KYNU</i>	Hs01114099_m1
<i>IDO1</i>	Hs00984148_m1
<i>Foxp3</i>	Hs01085834_m1
<i>Tbet</i>	Hs00203436_m1
<i>GATA3</i>	Hs01076122_m1
<i>IFN-gamma</i>	Hs00989291_m1
<i>S100A7</i>	Hs00161488_m1
<i>S100A12</i>	Hs00194525_m1
<i>K16</i>	Hs00955088_g1
<i>IL-8</i>	Hs99999034_m1
<i>Fillagrin</i>	Hs00856927_g1
<i>IL1a</i>	Hs00174092_m1
<i>IL1b</i>	Hs00174097_m1
<i>CXCL1</i>	Hs00236937_m1
<i>IL20</i>	Hs00218888_m1
<i>IL6</i>	Hs00985639_m1
<i>Eselectin</i>	Hs00950401_m1
<i>Pselectin</i>	Hs008927900_m1
<i>ICAM</i>	Hs00164932_m1
<i>VCAM</i>	Hs01003372_m1
<i>CXCL10</i>	Hs99999049_m1
<i>CCL2</i>	Hs00234140_m1
<i>CCL5</i>	Hs00174575_m1
<i>PAI1 (SERPIN)</i>	Hs01126604_m1
<i>DEFB4</i>	Hs00175474_m1

Supplementary Table 4 - Gene Array Data Analyzed

GSE #	Study	IDO/TDO (avg. logFC)	KYNU (avg. logFC)
GSE32407	IFNg Injected Normal Skin	5.19	0.575
GSE12470	Ovarian Cancer	3.2032002	0.6659811
GSE42677	Squamous Cell Carcinoma	2.4352376	2.314963975
GSE40611	Sjogren's disease	1.81141281	0.744648326
GSE33113	Colon Cancer	1.7053416	1.114699033
GSE7670	Lung adenocarcinoma	1.183856285	0.173223368
GSE9750	Cervical Cancer	1.0507266	-0.0944289
GSE3325	Prostate Cancer	0.769052733	-0.2119177
GSE46517	Melanoma	0.7934783	0.626938325
GSE25534	Tuberculosis	0.63134675	0.583784673
GSE6344	Renal cancer	0.569011267	-0.8058893
GSE45918	Epstein Barr Virus Infection	0.4006373	-0.606530035
GSE42568	Breast Cancer	0.372986667	0.13658
GSE27864	NOMID	0.3885255	0.270234
GSE19750	Adrenocortical Carcinoma	0.153	0.063778
GSE58208	Hepatitis B	0.096875833	-0.35776075
GSE7586	Malaria	9.62E-02	7.95E-02
GSE3689	Endometrial Cancer	0.0313377	0.007632275
GSE29721	Liver Cancer	-0.4548912	-0.6093201
GSE29226	Type 2 diabetes	-0.37179097	-0.51741283
GSE42826	Pneumonia	-0.3045516	-0.7081979
GSE27448	Bladder Cancer	-1.65E-01	-1.57E+00
GSE38941	Acute Liver Failure	-1.201	-0.22452
GSE20129	Atherosclerosis	0.005314875	0.023757515
GSE20931	Obesity assoc. colon inflam.	-0.00338	0.03995
GSE54514	Sepsis-Survivor	-0.01077062	0.06443392
GSE36474	Multiple myeloma	-2.17E-01	1.53E-01
GSE34205	RSV Infection	-0.556456323	0.104295152
GSE40586	Bacterial meningitis	-4.31E-01	1.16E-01
GSE43190	West Nile Virus Infection	0.232412615	0.2463719
GSE7368	Asthma (steroid sens.vs resist.)	0.103449567	0.4294671
GSE54514	Sepsis - Lethal	0.0864	0.499
GSE38010	Multiple Sclerosis	-4.6	0.514
GSE6281	Nickel Allergy (96 hrs vs cont)	9.10E-02	0.5405
GSE37157	Pollen Allergy	1.47E-01	0.564825
GSE57376	Psoriatic Arthritis	0.273281667	0.567038
GSE40812	HCV Infection	-0.136839308	0.62509742
GSE57376	Rheumatoid Arthritis	0.21225	0.699280002
GSE42826	Sarcoidosis	7.94E-01	8.87E-01
GSE6475	Acne	0.9255	1.167875
GSE16161	Atopic Dermatitis	0.5277	1.397
GSE41078	Acute UV on Normal Skin	0.9895	1.578825
GSE52428	Influenza Infection	0.0241836	1.537666667
GSE52360	DPCP-injected skin	1.946719667	2.44975
GSE11903	Psoriasis	0.105669725	4.41290535
GSE52471	Lupus	3.332306	4.861295167

Supplementary Table 5 – Additional statistical analysis with adjustments for multiple groups

			p-value (versus control)							
Figure	Cell Type	Gene	IL17	TNF	IFN	TNF+IL17	IFN+IL17	TNF+IFN	IL10	IL13
3A	PBMC	IDO	1	1	3.59E-12	1	3.28E-12	1.45E-16	1	1
		KYNU	0.894	0.030	0.089	0.177	0.106	0.004	0.351	0.120
3B	KC	IDO	1	1.22E-07	6.07E-07	6.07E-07	1	1.22E-07	n.d.	n.d.
		KYNU	7.80E-05	5.75E-07	0.004	0.004	0.001	5.75E-07	n.d.	n.d.
3C	HDBEC	IDO	0.234	0.368	9.60E-11	0.615	7.99E-11	1.79E-14	0.195	0.195
		KYNU	1	0.009	1	0.030	0.373	5.86E-12	0.954	0.477
3D	Whole Skin	IDO	n.d.	2.95E-08	1.91E-07	n.d.	n.d.	2.95E-08	n.d.	n.d.
		KYNU	n.d.	0.004	0.4108	n.d.	n.d.	0.004	n.d.	n.d.

			Dunnet's p-value (versus control)							
Figure	Cell Type	Gene	IL17	TNF	IFN	TNF+IL17	IFN+IL17	TNF+IFN	IL10	IL13
3A	PBMC	IDO	1	1	2.85E-11	1	2.60E-11	8.93E-14	1	1
		KYNU	0.999	0.162	0.391	0.616	0.442	0.030	0.854	0.481
3B	KC	IDO	1	6.58E-07	3.25E-06	3.25E-06	1	6.58E-07	n.d.	n.d.
		KYNU	0.0004	3.09E-06	0.0218	0.0218	0.009	3.09E-06	n.d.	n.d.
3C	HDBEC	IDO	0.719	0.870	7.63E-10	0.979	6.35E-10	8.83E-13	0.654	0.654
		KYNU	1	0.055	1	0.167	0.874	4.74E-11	0.999	0.937
3D	Whole Skin	IDO	n.d.	8.45E-08	5.48E-07	n.d.	n.d.	8.45E-08	n.d.	n.d.
		KYNU	n.d.	0.011	0.716	n.d.	n.d.	0.011	n.d.	n.d.

			FDR (versus control)							
Figure	Cell Type	Gene	IL17	TNF	IFN	TNF+IL17	IFN+IL17	TNF+IFN	IL10	IL13
3A	PBMC	IDO	1	1	2.29E-11	1	2.29E-11	1.16E-15	1	1
		KYNU	0.894	0.210	0.538	0.5383	0.538	0.037	0.703	0.538
3B	KC	IDO	1	7.32E-07	3.03E-06	3.03E-06	1	7.32E-07	n.d.	n.d.
		KYNU	0.0003	3.45E-06	0.009	0.009	0.007	3.45E-06	n.d.	n.d.
3C	HDBEC	IDO	0.979	0.979	5.76E-10	0.979	5.59E-10	1.44E-13	0.979	0.979
		KYNU	1	0.061	1	0.183	1	4.69E-11	1	1
3D	Whole Skin	IDO	n.d.	8.85E-08	3.82E-07	n.d.	n.d.	8.85E-08	n.d.	n.d.
		KYNU	n.d.	0.0130	0.410	n.d.	n.d.	0.013	n.d.	n.d.

Figure	Cell Type	Gene	p-value (versus control)				Dunnet's p-value (versus control)				FDR (versus control)			
			TRYP	KYN	3HAA	QA	TRYP	KYN	3HAA	QA	TRYP	KYN	3HAA	QA
5A	KC	IL.20	0.040	0.316	0.005	0.012	0.127	0.671	0.020	0.041	0.081	0.316	0.023	
		IL.8	0.084	0.894	0.026	0.432	0.244	0.996	0.083	0.800	0.254	0.894	0.104	
		Fillagrain	0.246	0.310	0.347	0.663	0.569	0.663	0.710	0.947	0.985	0.985	0.985	
		S100A12	0.342	0.924	0.292	0.113	0.704	0.998	0.639	0.314	0.877	0.924	0.877	
5B	T-cell	FoxP3	0.468	0.221	0.123	0.281	0.834	0.532	0.340	0.627	0.664	0.664	0.494	
		IL4	0.427	0.070	0.022	0.058	0.799	0.211	0.074	0.177	0.427	0.174	0.090	
5C	EC	CCL2	0.120	0.304	0.0004	0.141	0.335	0.662	0.001	0.382	0.360	0.360	0.001	
		CXCL1	0.565	0.050	0.053	0.166	0.903	0.159	0.167	0.434	0.565	0.203	0.203	
		E.selectin	0.020	0.008	0.008	0.010	0.068	0.028	0.031	0.037	0.032	0.032	0.032	
		P.selectin	0.001	0.055	0.001	0.0006	0.004	0.173	0.006	0.002	0.003	0.055	0.003	

Figure	Cell Type	Gene	p-value (versus control)				Dunnet's p-value (versus control)				FDR (versus control)			
			TRYP	KYN	3HAA	QA	TRYP	KYN	3HAA	QA	TRYP	KYN	3HAA	QA
S6	KC	IL.1a	0.653	0.235	0.023	0.418	0.943	0.552	0.074	0.836	0.836	0.706	0.092	0.836
		K16	0.357	0.999	0.135	0.790	0.723	0.999	0.362	1	1	1	0.540	1
	T-cell	T.bet	0.391	0.183	0.966	0.497	0.764	0.464	0.999	1	1	0.732	1	1
		GATA3	0.601	0.460	0.086	0.602	0.921	0.828	0.252	1	1	1	0.346	1
		IFN γ	0.139	0.269	0.749	0.493	0.375	0.610	0.975	0.986	0.557	0.807	0.986	0.986
		IL22	0.142	0.567	0.612	0.545	0.381	0.903	0.926	1	0.571	1	1	1
		Tbet.GATA3	0.366	0.107	0.115	0.103	0.736	0.301	0.321	0.414	0.414	0.414	0.414	0.414
		Tbe.FOXP3	0.130	0.101	0.246	0.280	0.353	0.288	0.573	0.493	0.406	0.406	0.493	0.493
	EC	IL8	0.264	0.176	0.006	0.190	0.658	0.506	0.028	0.706	0.706	0.706	0.032	0.706
		CCL5	0.468	0.860	0.004	0.545	0.873	0.996	0.019	1	1	1	0.022	1
		ICAM	0.300	0.444	0.614	0.144	0.708	0.855	0.949	0.577	0.901	0.901	0.901	0.577
		VCAM	0.585	0.900	0.403	0.837	0.938	0.998	0.821	1	1	1	1	1
		PAI.I	0.617	0.727	0.511	0.174	0.950	0.980	0.900	0.873	1	1	1	0.873

<http://dx.doi.org/10.1016/j.jaci.2015.09.055>

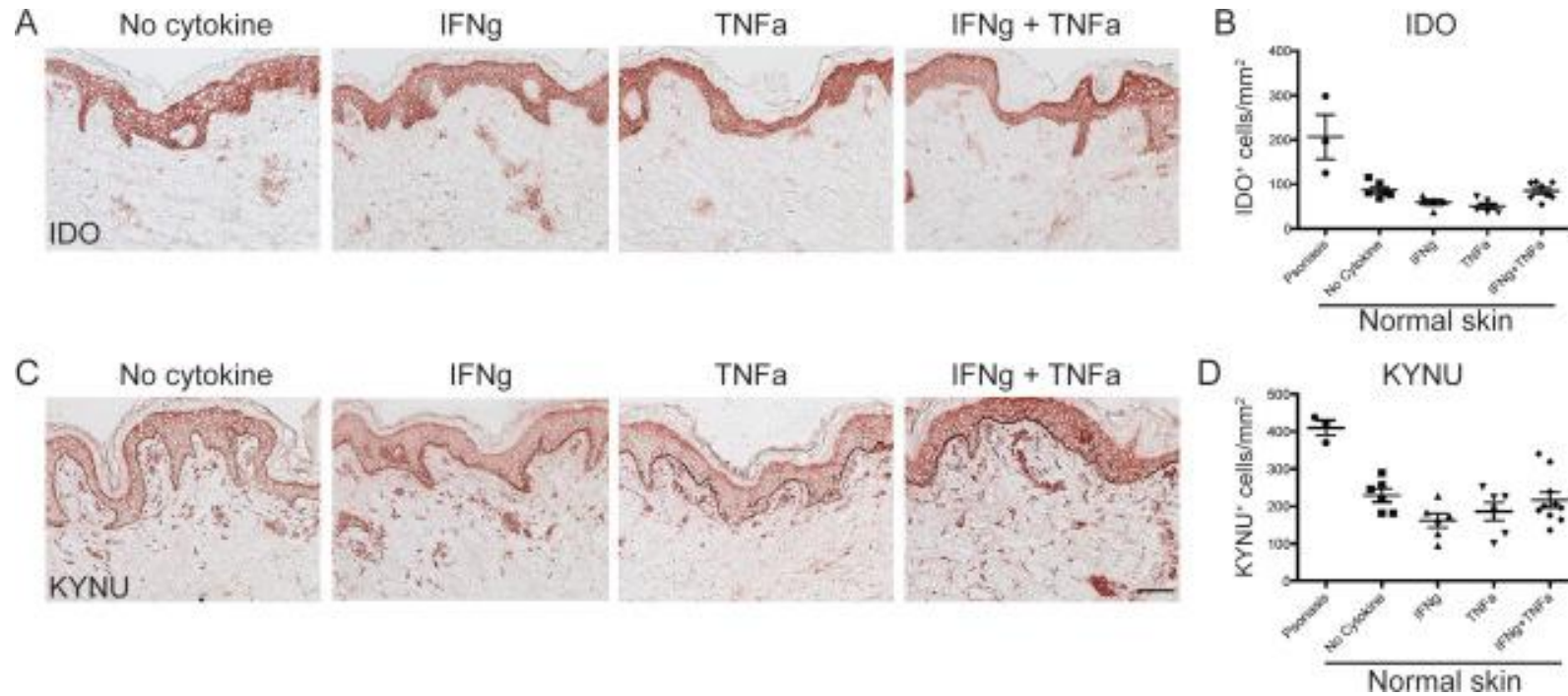


Fig E2 (wo legend).

Jamie L. Harden, Steven M. Lewis, Samantha R. Lish, Mayte Suárez-Fariñas, Daniel Gareau, Tim Lentini, Leanne M. Johnson-Huang, James G. Krueger, Michelle A. Lowes

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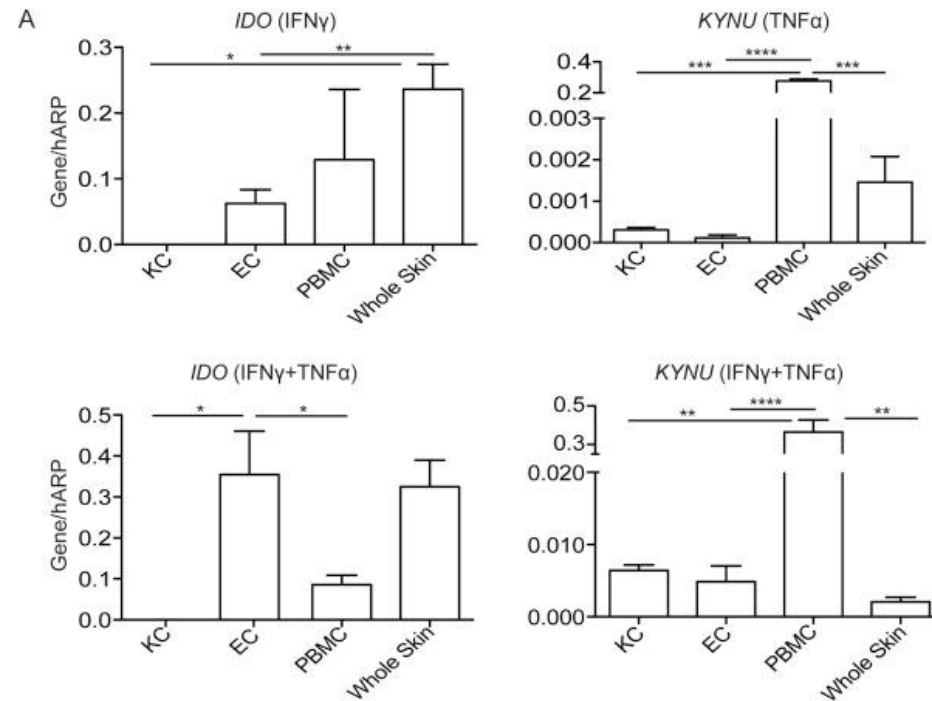


Fig E3 (wo legend).

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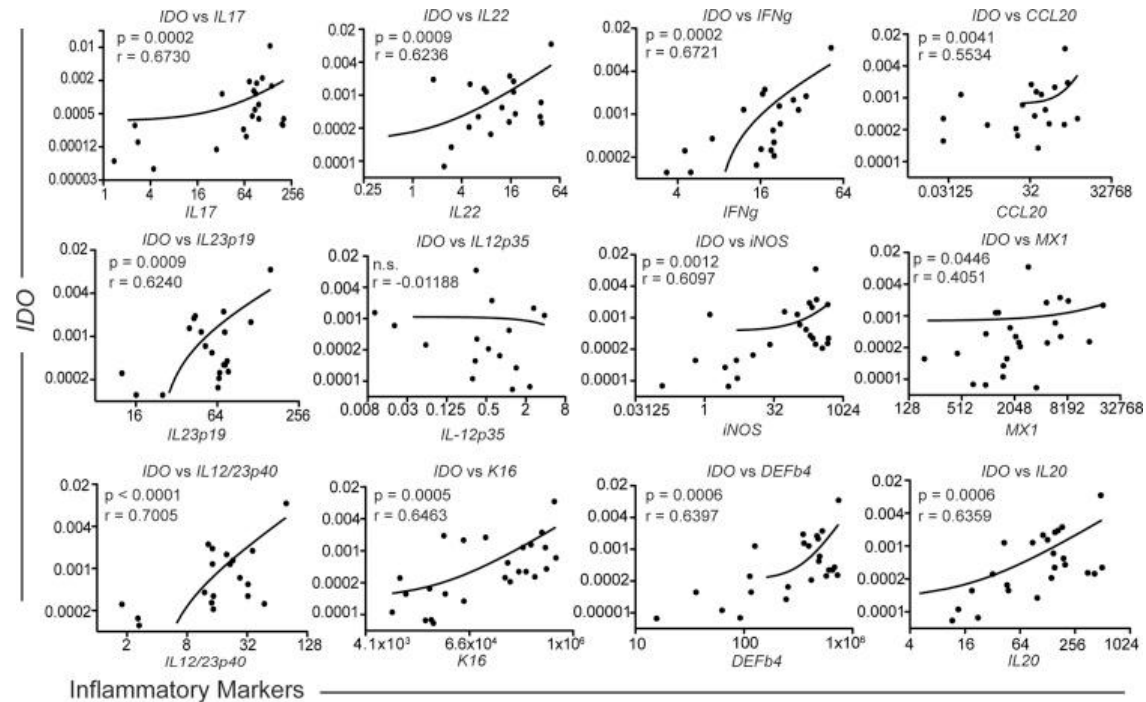


Fig E4 (wo legend).

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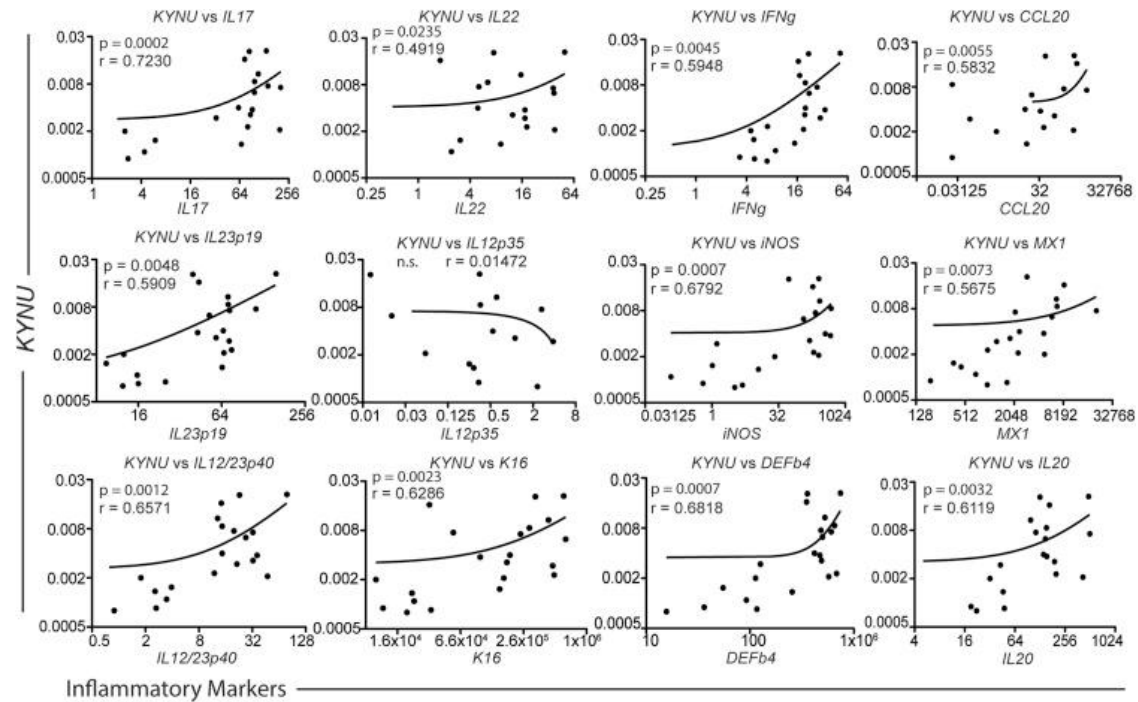


Fig E5 (wo legend).

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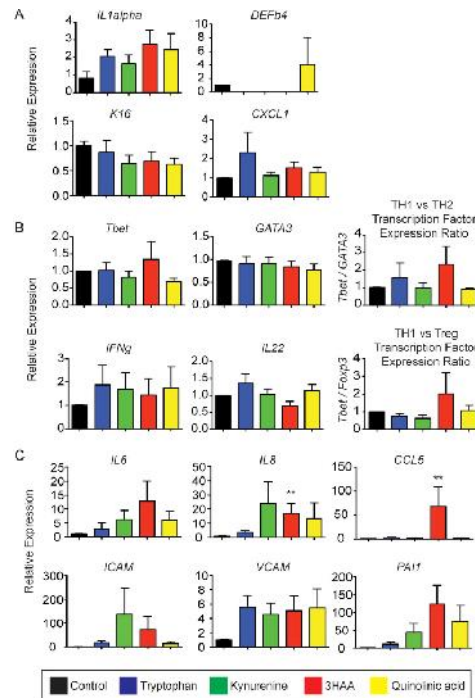


Fig E6 (wo legend).

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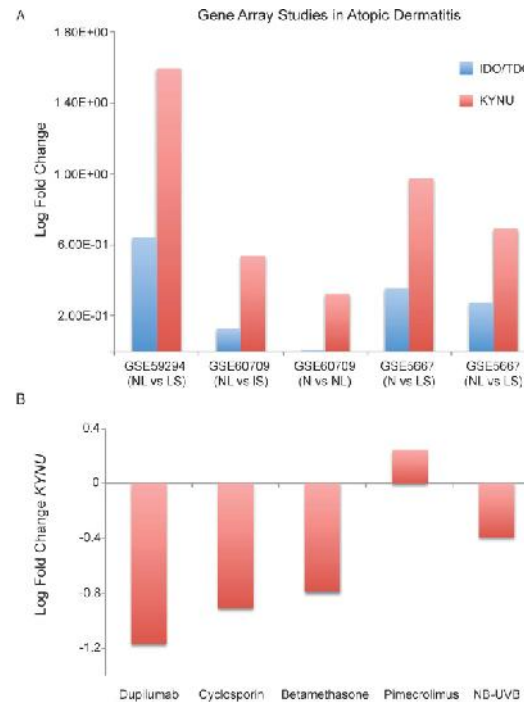


Fig E7 (wo legend).

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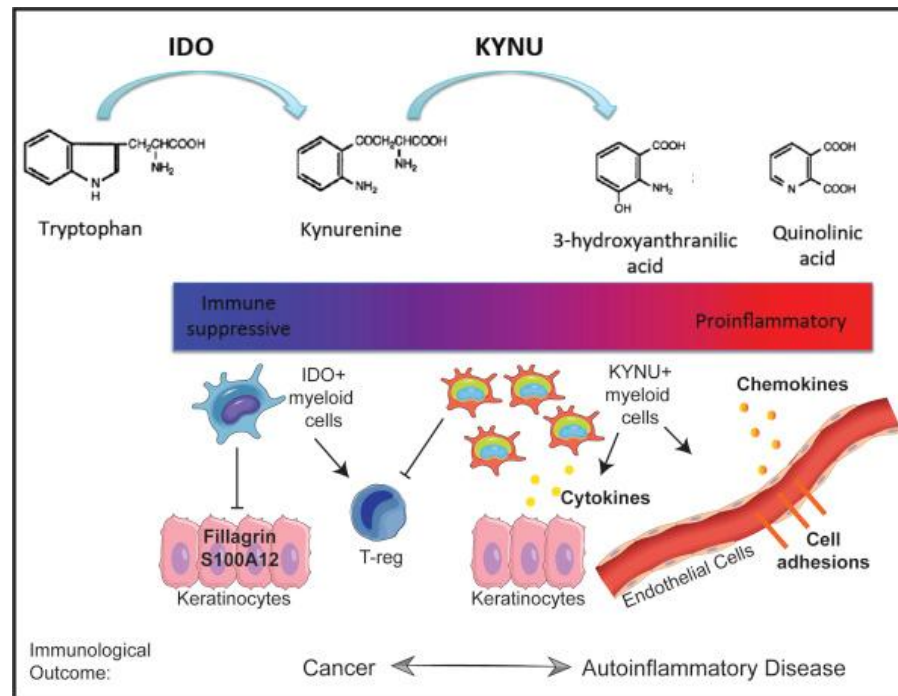


Fig E8 (wo legend).

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Figure S1. Ingenuity Pathway Analysis of the Tryptophan Metabolism Pathway in Psoriasis. The tryptophan metabolism pathway as curated in IPA. Two enzymes are significantly increased in the tryptophan metabolism pathway in psoriasis; IDO and KYNU. The darkness of the red diamond indicates the degree of upregulation of that gene. The faint red diamonds indicate IDO (mildly upregulated) and bright red diamonds indicate KYNU (heavily upregulated). Open symbols indicate genes with unchanged expression in psoriatic skin; green diamonds indicate genes with downregulated expression in psoriatic skin.

Figure S2. Cytokines alone can not increase the number of IDO+ or KYNU+ cells in normal human skin. Normal human skin (abdominoplasty surgical waste skin) was cultured with either no cytokines, IFN γ , TNF α , or the combination of IFN γ and TNF α , for 3 days. Representative images of immunohistochemical staining of (A) IDO and (C) KYNU in frozen skin sections and quantification of (B) IDO+ and (D) KYNU+ cells in these sections are shown. (A and C) Scale bar = 10 μ m. (B and D) Error bar represents the standard error of the mean. There was no statistically significant difference in the number of IDO+ or KYNU+ cells in normal skin cultured with cytokines.

Figure S3. Comparison of IDO and KYNU mRNA expression. (A) IDO and KYNU induction after cytokine exposure in keratinocytes (KC), dermal endothelial cells (EC), peripheral blood mononuclear cells (PBMC), and whole skin. n = 3-8 per group.

Figure S4. Expression of IDO positively correlates with expression of several key inflammatory molecules in psoriatic skin. Log2 expression of IDO and inflammatory gene expression in skin biopsies from both non-lesional and lesional psoriatic skin was assessed by qRT-PCR. The Spearman rank correlation test was used to determine a significant association between IDO expression and expression of various inflammatory genes. The p-value and the Spearman 'r' for each association is provided.

Figure S5. Expression of KYNU positively correlates with expression of several key inflammatory molecules in psoriatic skin. Log2 expression of KYNU and inflammatory gene expression in skin biopsies from both non-lesional and lesional psoriatic skin was assessed by qRT-PCR. The Spearman rank correlation test was used to determine a significant association between KYNU expression and expression of various inflammatory genes. The p-value and the Spearman 'r' for each association is provided.

Figure S6. Modulation of Immunologically Relevant Genes in KCs, T-cells, and HDBECs upon exposure to different tryptophan metabolites. (A) Keratinocytes, (B) activated T-cells, and (C) human dermal blood endothelial cells were cultured with 50 μ m of tryptophan (TRYP, blue) or various tryptophan metabolites: kynurenine (KYN, green), 3-hydroxyanthranic acid (3HAA, red), and quinolinic acid

(QA, yellow), or no metabolite (Control, black) as described in the materials and methods. Expression of various inflammatory genes, normalized to control, is shown. Data was analyzed using the Mann Whitney U (non-parametric) t-test. Error bars represent standard error of the mean. For keratinocyte experiments, n = 5-8 per metabolite condition; for T-cell experiments n = 5-7 per metabolite condition; for HDBECs, n= 6-10 per metabolite condition. * p < 0.05, ** p < 0.01, ***p< 0.001.

Figure S7. Atopic dermatitis presents with increased KYNU compared to IDO-TDO, and successful treatment modalities which modulate immune response result in decreased KYNU. (A) Gene array data from studies on atopic dermatitis was mined for expression of IDO, TDO, and KYNU as described in the Materials and Methods and in Figure 6. (B) The log fold reduction of KYNU in lesional skin post-treatment was determined from several gene array studies: Dupilumab (GSE59294), Cyclosporin (GSE58558), Betamethasone and Pimecrolimus (GSE32473), and NB-UVB (GSE27887).

Figure S8. Hypothesized mechanism for how altered tryptophan metabolism may dichotomously regulate immune responses. A simplified schematic of tryptophan metabolism at top, with the enzymes indoleamine 2,3-dioxygenase (IDO) and L-kynureninase (KYNU). Myeloid cells are a major contributor to tryptophan metabolism in human skin. IDO+ myeloid cells produce kynurenine, which promotes polarization of T-regs and reduce expression of inflammatory molecules. KYNU+ myeloid cells, which are significantly increased in psoriasis, may result in production of downstream metabolites such as 3HAA and QA. These later metabolites reduce T-reg polarization and induce expression of chemokines, cytokines, and adhesion molecules. Although tryptophan metabolism is increased in both cancer and inflammatory disease, the balance of tryptophan metabolism may dictate unique outcomes, depending on relative enzyme expression and accumulation of specific metabolites.