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The Tryptophan Metabolism Enzyme, L-Kynureninase, is a Novel Inflammatory Factor in Psoriasis and other Inflammatory Diseases

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Abstract

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 via the enzyme indoleamine 2,3 dioxygenase (IDO) and subsequent production of kynurenine. However, in chronic inflammatory skin disease such as psoriasis and atopic dermatitis, another tryptophan metabolism enzyme downstream of IDO, L-kynureninase (KYNU), is heavily upregulated. The role of KYNU has not been explored in these skin diseases, or in general human immunology.

Objective—To explore the expression and potential immunological function of the tryptophan metabolism enzyme, L-kynureninase, in inflammatory skin disease and its potential contribution to general human immunology.

Methods—Psoriatic skin biopsies, as well as normal human skin, blood, and primary cells were used to investigate the immunological 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 upon successful treatment of psoriasis or atopic dermatitis. Tryptophan metabolites

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downstream of KYNU upregulate several cytokines, chemokines, and cell adhesions. By mining data on several human diseases, we found that in cancers, IDO is preferentially upregulated compared to KYNU, whereas in inflammatory diseases such as atopic dermatitis, KYNU is preferentially upregulated compared to IDO.

Conclusion—Our results suggest that tryptophan metabolism may 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 may qualitatively affect the immune response in that disease.

Keywords

Psoriasis vulgaris; tryptophan metabolism; L-kynureninase; inflammation

Introduction

Psoriasis vulgaris is a chronic inflammatory skin disease, which effects about 1-3% of the North American population (1, 2). This condition arises from interactions between hyper-proliferative keratinocytes (KCs) and infiltrating immune cells, specifically IL-17- and IFNγ-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, as skin disease provides non-invasive access to affected tissue, and the efficacy of immunomodulatory treatments can be easily monitored.

One gene found prominently upregulated in microarray studies of psoriasis is L-kynureninase (KYNU), an enzyme within the tryptophan metabolism pathway (5-8). KYNU is highly upregulated (over 20 fold) in lesional psoriatic skin compared to non-lesional skin. Additionally, KYNU is a gene within the “psoriasis classifier”, a set of genes that can correctly identify lesional versus non-lesional skin (6). The methylation pattern of KYNU is also altered in psoriasis patients, and this epigenetic modification predicts increased expression in psoriatic patients compared to normal individuals (9). KYNU has also been identified as a “molecular scar gene”, indicating that even after successful treatment of psoriasis, it remains slightly upregulated in non-lesional skin compared to normal skin (10). Moreover, KYNU was determined to be a gene synergistically enhanced by IL-17 and TNFα (11), and is considered one of the ‘Top-25’ psoriasis genes (12). However, the role of KYNU and tryptophan metabolism in 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 two, non-mutually exclusive mechanisms (13, 15). Firstly, IDO degrades tryptophan into kynurenine, thus depleting the microenvironment of

this amino acid and “starving” immune cells (15, 17). Secondly, the kynurenine produced by IDO is actively immune suppressive (15), through mechanisms such as inducing regulatory T-cells via kynurenine binding to the aryl-hydrocarbon receptor (AhR) (18, 19). This second mechanism of immune suppression by IDO may be the most potent, as 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 may be a mechanism for initiating “immune turn-off”, and indeed many proinflammatory signals, such as IFNγ, 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 two 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 points to a novel and actively inflammatory role of tryptophan metabolism in not only psoriasis, but in other human inflammatory diseases as well.

Methods

Skin and Blood Samples

Skin biopsies from normal volunteers and psoriasis patients, as well as blood samples from normal 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. De-identified surgical skin waste was utilized to obtain whole normal skin biopsies for culture. Normal blood samples were used to obtain PBMCs and T-cells. PBMCs were isolated using Ficoll-paque gradient centrifugation. T-cells were isolated from PBMCs using RosetteSep (Stem Cell Technologies), following manufactures instructions. Myeloid cell populations were FACS sorted from PBMCs using the antibody panel in Table S1.

Immunohistochemistry and Immunofluorescence

Immunohistochemistry and immunofluorescence were performed as previously described (24, 25). Antibodies are listed in Table S2. For immunohistochemistry and immunofluorescence experiments, n =3 or more per group; representative pictures are shown. For cell counts, images were quantified using ImageJ software. 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 RT-PCR was performed on samples as previously described (26). Primers were purchased from Applied Biosystems and are listed in Table S3. Gene expression was normalized to the house-keeping gene, human acidic ribosomal protein (*hARP*).

Cell Culture

Primary human keratinocytes (KC) and human dermal blood endothelial cells (EC) were cultured as previously described (25, 27). For cytokine experiments, KCs, ECs, and PBMCs were cultured in 12 well plates with 2mL of the appropriate cell-type medium. IL-17 (100 ng/ml), IFNγ (20 ng/mL), TNFα (20 ng/ml), IL-10 (40 ng/ml), and IL-13 (50 ng/ml) was added for 12 hours; cell lysate was collected and prepared for subsequent RNA isolation using Qiagen RNA isolation kits per manufacturer's instructions. For whole normal skin experiments, biopsies were cultured with 50ng/ml TNFα, 100ng/ml IFNγ, or both for 24 hours (RNA), or 3 days (IHC). For tryptophan metabolite experiments, KCs and ECs were cultured until 60–80% confluent in a 12 well plate with 2mL of appropriate media. T-cells were activated with anti-CD3/anti-CD28 beads (Dynabeads). Cells were then exposed to 50μM of tryptophan, kynurenine, 3-hydroxyanthranilic acid (3HAA), or quinolinic acid (QA) for three days (all from Sigma-Aldrich).

Gene Omnibus Mining

Publically available gene array studies were mined for expression of *IDO1, TDO*, and *KYNU*. Studies were chosen which included comparison to normal or non-diseased tissue. The average fold change of *IDO1* and *TDO*, as well as the average fold change of *KYNU*, for a given disease compared to normal or non-diseased 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 S4.

Statistics

All data were analyzed using GraphPad Prism 5. qRT-PCR expression values were normalized to housekeeping gene hARP and log2-transformed for analysis. Measurements under the limit of detections were imputed as 20% of the minimum value observed for that gene. qRT-PCR expression data was modeled using a mixed effect model with Groups as a fixed effect and a random intercept for each pool/replication. This formulation intrinsically models the within patient correlation structure as in the case of a paired t-test. P values were adjusted across markers by the Benjamin-Hochberg approach. The Dunnet's p-value and the FDR are provided for experiment which include multiple comparisons (Figure 3, Figure 5, and Figure S6) are found in Table S5. The Spearman correlation test was used to determine significant correlations between *IDO* or *KYNU* and 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 standard error of the mean (SEM). P-values less than 0.05 were considered significant. *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

Results

The Tryptophan Metabolism Pathway is Upregulated in Psoriasis

KYNU is one of the psoriasis classifier genes, a “molecular scar” gene, highly upregulated in microarray studies, a ‘Top 25” psoriasis gene, synergistically enhanced by IL-17 and TNFα, and has a differential DNA methylation pattern in psoriatic patients (6). Interestingly, KYNU is the only gene that is found in all of these categories (Figure 1A), highlighting it as

an important gene to interrogate in psoriasis pathogenesis. Ingenuity Pathway Analysis (IPA) was used to explore upregulation of the tryptophan metabolism pathway in two meta-analysis psoriasis transcriptomes (6) (Figure 1B). Tryptophan metabolism was found to be significantly upregulated, despite the fact that only two enzymes in the pathway were upregulated, *IDO* and *KYNU*. *KYNU* is heavily upregulated, while *IDO* expression is only mildly increased (Figure S1).

IDO and KYNU Expressing Cells are found in Psoriasis Lesions

To confirm microarray data that *IDO* and *KYNU* are increased in psoriatic skin, quantitative real-time-PCR (qRT-PCR) analysis of RNA from normal, non-lesional (NL), and lesional (LS) biopsies was used to quantify mRNA expression of these enzymes (Figure 1C). Both *IDO* and *KYNU* were increased in LS skin compared to NL and normal skin.

Next, protein expression of IDO and KYNU was determined by immunohistochemistry (Figure 1D). 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. LS epidermis had significantly more area of IDO and KYNU positive staining compared to normal and NL epidermis, due to epidermal hyperproliferation (Figure 1E). Quantification of dermal cells showed the number of IDO^+ cells was similar between all groups. However, LS skin had a significantly higher numbers of $KYNU^+$ dermal cells compared to normal and NL skin (Figure 1F). This suggests that psoriasis LS skin is associated with increased numbers of $KYNU^+$, but not IDO^+ dermal cells.

Several Cell Types Express KYNU and IDO in Human Skin

To elucidate which dermal cells in human skin express KYNU and IDO, two-color immunofluorescence studies were conducted to query various cell populations. IDO expression in myeloid cells has been previously described (13) and therefore expression of these tryptophan metabolism enzymes within $CD11c^+$ dendritic cells (DC), BDCA-1^+ resident DCs, Langerhans cells, and $CD163^+$ macrophages was first evaluated. As expected, IDO was found to colocalize with some $CD11c^+$ cells (Figure 2A), however there were both IDO^+ and IDO^- DCs present in LS skin (see inset). Almost all $CD11c^+$ DCs in LS skin expressed KYNU. $CD163^+$ macrophages also heavily colocalized both IDO and KYNU in lesional skin (Figure 2B). Regarding specific DC subsets, BDCA-1^+ resident DCs were found to express both IDO and KYNU (Figure 2C), and Langerhan$^+$ cells predominantly IDO positive (Figure 2D). In contrast, $CD3^+$ T-cells and neutrophils (neutrophil elastase$^+$ cells) did not colocalize with KYNU or IDO (Figure 2E-F).

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

Cytokines in the Psoriatic Milieu Upregulate KYNU and IDO Expression

To determine how *KYNU* and *IDO* might be induced in psoriatic lesional skin, various cell types were exposed to an array of cytokine stimuli and expression of *KYNU* and *IDO* was assessed. Additionally, these experiments served as confirmation of the IDO and KYNU expression patterns in the various cell types seen by immunofluorescence (Figure 2). ECs, KCs, and peripheral blood mononuclear cells (PMBCs) were exposed to cytokines heavily present in the psoriatic microenvironment (IL-17, IFNγ, TNFα), the immunosuppressive cytokine IL-10, or the T_H2 associated cytokine IL-13 (Figure 3A-C).

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

To determine if the psoriatic cytokine milieu directly results in upregulation of these tryptophan metabolism enzymes in skin resident cells, normal skin biopsies were cultured with IFNγ, TNFα, or the combination of IFNγ and TNFα (Figure 3D). IFNγ significantly upregulated *IDO* in normal skin biopsies, while TNFα only slightly, but still significantly, also upregulated *KYNU*. Interestingly, although the combination of IFNγ and TNFα significantly enhanced *IDO* upregulation even further in normal skin biopsies, *KYNU* was only modestly, but significantly upregulated, and expression of *IDO* was still over 100-fold greater than *KYNU*. Additionally, immunohistochemical staining of normal skin biopsies exposed to the cytokines did not reveal an increased number of IDO^+ or $KYNU^+$ cells (Figure S2). As shown in Figure 1, LS skin is characterized by an almost 10-fold greater expression of *KYNU* compared to *IDO*, as well as a significant increase in the number of $KYNU^+$ dermal cells. Therefore, exposure of normal skin to key psoriatic cytokines (i.e. IFNγ and TNFα) could not recapitulate the pattern of expression of tryptophan metabolism enzymes seen in LS skin.

Myeloid Cell Subsets Express the Highest Amount of KYNU

As we found that PBMCs and KCs constitutively express *KYNU*, the massively increased *KYNU* expression in psoriatic LS skin may be a result of infiltrating immune cells and/or KC hyper-proliferation. However, infiltrating immune cells are likely the predominant source of upregulated *KYNU* in psoriatic skin, as PBMCs expressed 100-fold more *KYNU* mRNA compared to ECs, KCs, and whole skin (Figure S3).

To further elucidate which populations in PBMCs were predominantly expressing these enzymes, RNA from FACS sorted monocyte, DC, and T-cell populations was assessed for expression of *IDO* and *KYNU* (Figure 3E-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 *KYNU* at steady state. Additionally, primary human dermal DCs from healthy skin, as well as monocyte-derived DCs and macrophages, also highly expressed *KYNU*. Therefore, it is likely that the $KYNU^+$ cells which infiltrate

psoriatic lesions are derived from circulating myeloid cells, and may differentiate into inflammatory DCs and macrophages, known to play a critical role in psoriasis pathogenesis (31, 32).

Tryptophan Metabolism is Reduced with Successful Response to Treatment in both Psoriasis and Atopic Dermatitis

The finding of increased tryptophan metabolism in 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 may have been thought to be beneficial rather than detrimental to psoriasis. However, we found a significant positive correlation between disease severity (PASI score) and expression of *KYNU* and *IDO*, with *KYNU* expression being more significantly associated with PASI than *IDO* (Figure 4A). Furthermore, expression of *KYNU* and *IDO* positively and significantly correlated with expression of many key inflammatory molecules in psoriasis pathogenesis, including IL17, IL22, IFNγ, CCL20, IL23p19, iNOS, MX1, IL12/23p40, K16, DEFB4, and *IL20* (Figure S4 and S5).

To determine if tryptophan metabolism was reduced upon successful treatment of psoriasis, the expression of *IDO* and *KYNU* mRNA was quantified in post-treatment biopsies of non-responding and responding patients to narrow-band ultra-violet B (NB-UVB) treatment (26) (Figure 4B). In patients not responding to NB-UVB treatment, expression of *KYNU* and *IDO* was not significantly decreased; however, responding patients had significantly reduced levels of these enzymes. *KYNU* expression was also significantly decreased in microarray analysis of patients treated for 12 weeks with biological therapies, including etanercept (anti-TNFα) (33) and usekizumab (anti-IL-12/23p40) (Figure 4C). These data raise the possibility that the tryptophan metabolism pathway may play an actively inflammatory rather than a regulatory role in psoriasis pathogenesis.

KYNU is downstream of IDO in the tryptophan metabolism pathway and degrades the IDO produced, immunosuppressive metabolite, kynurenine (34). Therefore, we hypothesized that the preferential increase of KYNU in psoriasis may play an inflammatory role by depleting kynurenine. Therefore, the *KYNU*:*IDO* ratio was determined in normal skin, NL, and LS skin (Figure 4D). Psoriatic lesional skin had a significantly higher *KYNU*:*IDO* ratio compared to normal skin. NL skin also had a significant increase in the *KYNU*:*IDO* ratio compared to normal skin, although the absolute amount of these enzymes in NL skin is lower than LS skin (Figure 1C). This suggests that tryptophan metabolism may be dysregulated even in histologically normal-looking skin of individuals with psoriasis.

Metabolites Downstream of KYNU Induce Inflammatory Gene Expression

Due to the fact that expression of the tryptophan metabolism enzymes *KYNU* and *IDO* are 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 3-hydroxyanthranilic acid (3HAA) (34), and it is possible that metabolites downstream of KYNU have direct inflammatory effects.

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

KCs cultured with 3HAA and QA had significantly enhanced expression of inflammatory genes, including *IL20* and *IL8* (Figure 5A and Figure S6A). Conversely, kynurenine decreased expression of *fillagrin*. Although not statistically significant, kynurenine also suppressed expression of *S100A12* and *IL8* compared to 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 (Figure 5B). Kynurenine upregulated *Foxp3*, as had been previously reported (18). Both 3HAA and QA significantly suppressed *IL4* expression (Figure 5B), and 3HAA preferentially promoted expression of the T_H1 master transcriptional regulator *Tbet*, compared to other T-cell polarization factors (Figure S6B). Regarding ECs, several tryptophan metabolites upregulated inflammatory genes, with 3HAA having the largest effect, including upregulation of *CCL2, CXCL1, CCL5, E-selectin, IL8, ICAM*, and *VCAM* (Figure 5C and Figure S6C).

The Ratio of Tryptophan Metabolism Enzymes Corresponds to the Immunological Environment in Human Diseases

The observation that increased expression of KYNU compared to IDO promotes an inflammatory environment may not only apply to psoriasis, but may 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 (i.e. IDO, or the analogous enzyme, tryptophan dioxygenase [TDO]) compared to later enzymes (i.e. KYNU), resulting in accumulation of immunosuppressive metabolites, such as kynurenine. Conversely, inflammatory diseases may have higher expression of later tryptophan metabolism enzymes, resulting in a depletion of kynurenine and the production of putatively inflammatory metabolites, such as 3HAA or QA.

To assess this hypothesis, publically available gene array data from over 40 human diseases was 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* (left) versus *KYNU* (right) (Figure 6A). 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* (Spearman correlation $p<0.005$), a significant stratification between cancers versus proinflammatory diseases was found with regard to the relative upregulation of these enzymes (Figure 6B). 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 to cancers (Figure 6C), suggesting that although the tryptophan metabolism pathway may be upregulated in both groups of diseases, there is preferential enzyme expression, which may result in a specific metabolite milieu and lead to distinct immunological outcomes.

Altered Tryptophan Metabolism in Atopic Dermatitis and Reduction Upon Treatment

We further investigated the upregulation of these enzymes specifically in atopic dermatitis (AD), as a previous study demonstrated increased expression of tryptophan metabolism genes in AD as well as psoriasis (8). Gene array data from multiple groups all demonstrated that AD LS, although containing both increased *IDO-TDO* and *KYNU*, always expressed higher levels of *KYNU* compared *IDO-TDO* (Figure S7A). Furthermore, NL AD skin also had elevated *KYNU* compared to normal skin. This finding of aberrant tryptophan metabolism enzyme expression even in NL skin was akin to our psoriasis results (Figure 1 and Figure 4), demonstrating that even histologically normal-looking skin of either AD or psoriasis may have a skewed tryptophan metabolism profile and predispose these individuals to cutaneous inflammation.

The reduction of *KYNU* post treatment in AD was also explored. Many different treatments reduced expression of *KYNU* in AD LS (Figure S7B). Dupilumab demonstrated the most drastic reduction in *KYNU* expression, which may 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*. Results from this study demonstrated that pimecrolimus mainly improved LS 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 to keratinocytes, and likely plays a role directly in immune modulation rather than barrier function.

Discussion

Here we describe the expression and potential inflammatory function of the tryptophan metabolism enzyme, KYNU, 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 may dichotomously regulate immune responses is shown in Figure S8. Interestingly, dichotomous actions of tryptophan metabolism have been described in neurobiology, with early tryptophan metabolites (such as kynurenine) being considered neuroprotective and late tryptophan metabolites (such as QA) demonstrating neurotoxic activity (37-39). Therefore, the relationship between tryptophan metabolism and the immune system may be more complex than previously described, and analogous to the interaction of tryptophan metabolites with the nervous system.

In the late 1960's, clinical trials utilized 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 towards the production of late metabolites, may promote inflammation. We found that even NL skin of both atopic dermatitis and psoriasis contained an altered ratio of tryptophan metabolism enzyme expression (Figure 4D and Figure S7A). Therefore, NL skin in both of these diseases may have mild but biologically meaningful accumulation of inflammatory metabolites downstream of KYNU. This hypothesis is in accordance with previous work, which found that NL skin has a slightly elevated inflammatory profile compared to normal skin (42).

In this manuscript, only IDO and KYNU were assessed due to the fact these are the only two tryptophan metabolism enzymes significantly upregulated in psoriatic lesional skin. However, the tryptophan metabolism pathway is complex, and future studies which incorporate all of the enzymes within the pathway will provide a deeper understanding of how modulation of this pathway impacts inflammation. Additionally, there may be other metabolic reasons and biological consequences surrounding upregulated tryptophan metabolism in 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 psoriatic patients (44), and it is possible that dysregulated tryptophan metabolism could play a role in this susceptibility. Additionally, the hyper-proliferative state of KCs in psoriasis may 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 KCs contained the highest expression of *KYNU*, suggesting a likely immunomodulatory role for this enzyme.

As the dual nature of the tryptophan metabolism pathway has already been described in neurobiology, it is possible that dysregulation of this pathway in skin may 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 play 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 LS 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 atopic dermatitis.

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 the AhR, and activation of this receptor has been implicated in a variety of immunological studies (46-49). A recent publication demonstrated a critical role of the AhR in controlling skin inflammation (50). Specifically, they found that *AhR−/−* mice exhibited exacerbated cutaneous inflammation. In our study, we found that kynurenine reduced expression of some inflammatory genes in KCs and promoted *Foxp3* expression in T-cells (Figure 5). Therefore, loss of endogenous kynurenine-AhR signaling in psoriasis (via high upregulation of KYNU and degradation of kynurenine) may also lead to excessive inflammation.

In conclusion, we have provided the initial evidence that the tryptophan metabolism enzyme, L-kynureninase (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 may play a distinct role in both inflammatory diseases and cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

3HAA	3-hydroxyanthranilic acid
AD	atopic dermatitis
AhR	aryl hydrocarbon receptor
DC	dendritic cell
EC	endothelial cell
KC	keratinocyte
KYNU	L-kynureninase
LS	lesional
NL	non-lesional
PASI	psoriasis area and severity index
PBMC	peripheral blood mononuclear cell
QA	quinolinic acid
TDO	tryptophan dioxygenase

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Key Message

- The tryptophan metabolism enzyme, L-kynureninase (KYNU) is a highly upregulated but previously unexplored enzyme in chronic, inflammatory skin disease

- Tryptophan metabolites downstream of KYNU induce inflammatory gene expression

- Many human autoimmune and autoinflammatory diseases have elevated KYNU expression, suggesting this pathway may play a general role in inflammation

Capsule Summary

KYNU is highly expressed in inflammatory skin disease, however the role of this tryptophan enzyme been unexplored. Here we present evidence that KYNU promotes inflammation and may be a novel anti-inflammatory drug target.

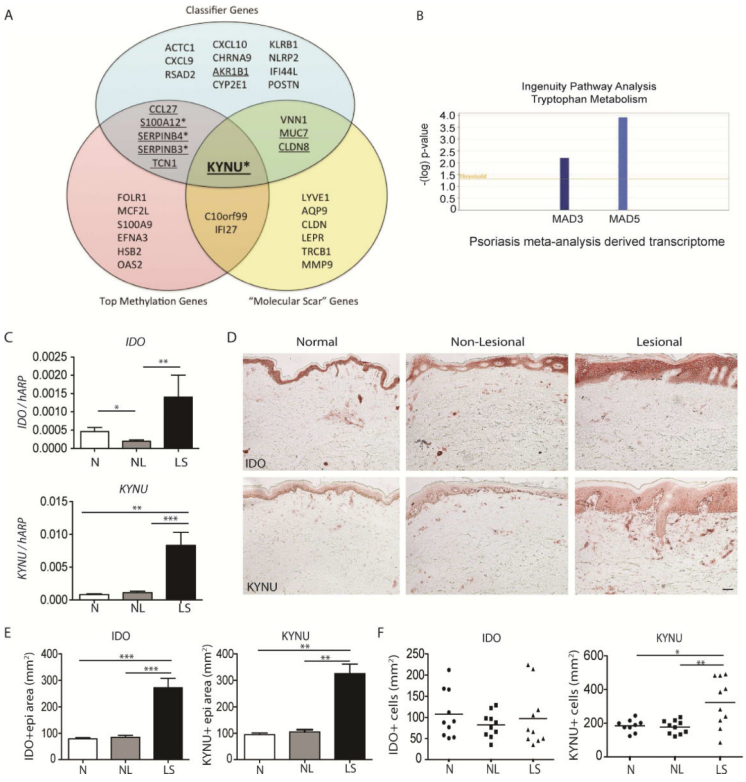

Figure 1. Expression of the tryptophan metabolism enzymes IDO and KYNU in psoriasis lesional skin

(A) Venn Diagram of psoriasis classifiers genes (blue circle), top psoriasis methylation genes (red circle), and "molecular scar" genes (post-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 Figure 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 Ingenuity Pathway Analysis. (C) Expression of *IDO* and *KYNU* mRNA in skin biopsies as determined by qRT-PCR. (D) Immunohistochemistry of protein expression of IDO and KYNU in normal (N), non-lesional (NL), and lesional (LS) psoriatic skin. (E) Quantification of IDO and KYNU epidermal expression. (F) Quantification of dermal IDO^+ and $KYNU^+$ cells. Scale bar = 10μm. For qRT-PCR of *IDO* and *KYNU*, n = 5–13 per group. For immunohistochemistry of IDO and KYNU, n = 6–10 per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

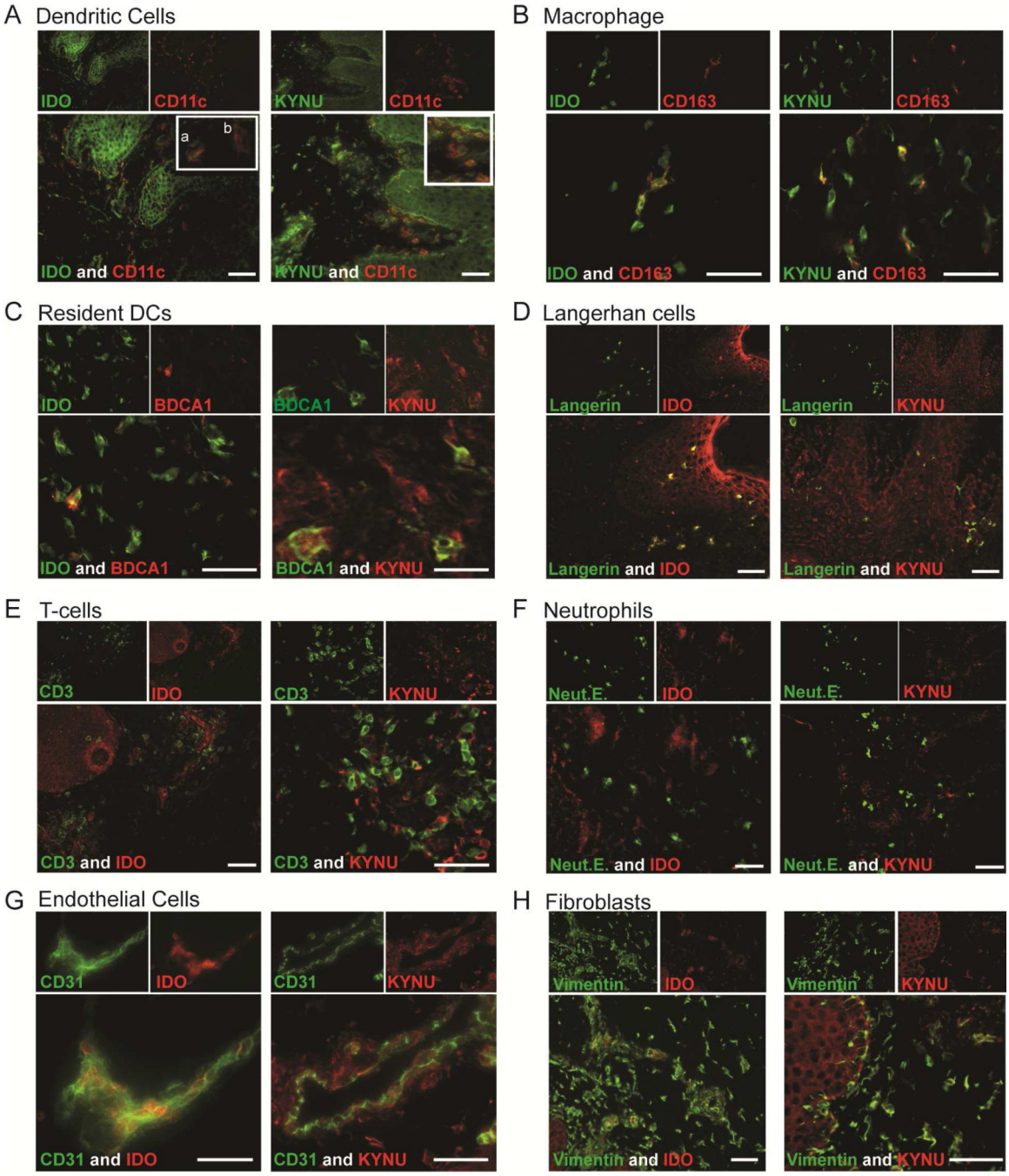

Figure 2. Identification of KYNU and IDO expressing cells in psoriatic skin

Double immunofluorescence staining of IDO and KYNU versus (A) $CD11c^+$ dendritic cells (DCs) (white inset highlights the presence of IDO^+ 'a' and IDO^- 'b' $CD11c^+$ DCs at left, and $KYNU^+$ $CD11c^+$ DCs at right), (B) CD163 macrophages, (C) $BDCA1^+$ resident DCs, (D) $Langerin^+$ cells, (E) $CD3^+$ T-cells, (F) neutrophil $elastase^+$ cells, (G) $CD31^+$ endothelial cells, and (H) $vimentin^+$ cells. Scale bar = 5μm.

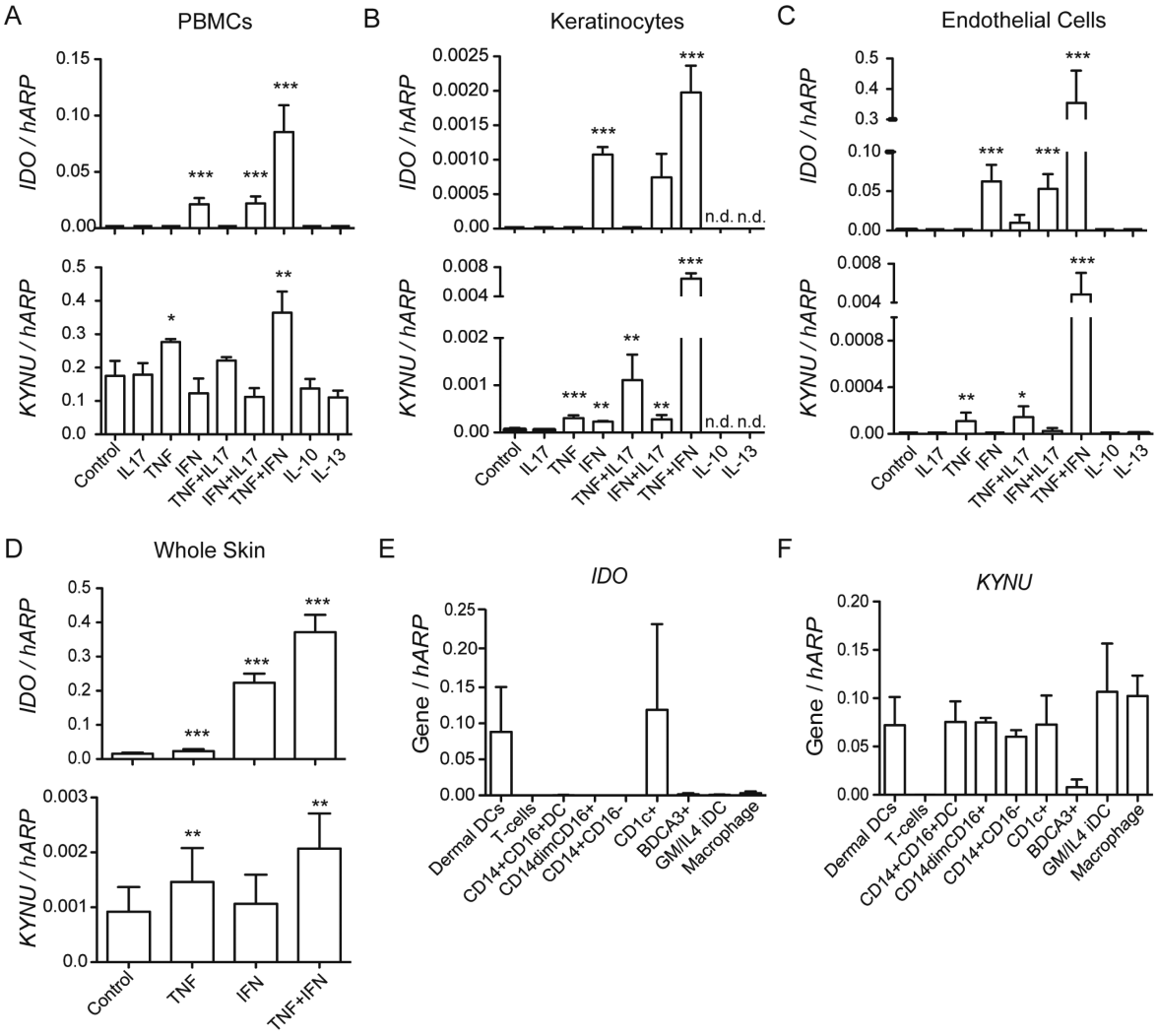

Figure 3. IFNγ and TNFα drive expression of *IDO* and *KYNU*, and myeloid cell subsets express the highest amount of *KYNU*

(A) Peripheral blood mononuclear cells (PBMCs), (B) keratinocytes (KCs), and (C) endothelial cells (ECs) were cultured with or without various cytokines: IL-17, TNFα, IFNγ, TNFα + IL-17, IFNγ + IL-17, TNFα + IFNγ, IL-10, or IL-13, for 12 hours and assessed by qRT-PCR for expression of *IDO* and *KYNU*. (D) Whole normal skin biopsies were cultured for 24 hours with TNFα, IFNγ, or both. For PBMC experiments, n = 5 per condition; for KC experiments, n = 3 per condition; for EC experiments, n = 6–8 per condition; for whole skin, n = 3–5 per condition. Expression of (E) *IDO* and (F) *KYNU* was determined by 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, $CD1c^+$ ($BDCA1^+$) DCs, and $BDCA3^+$ DCs. Additionally, expression of *IDO* and *KYNU* was determined in PBMC-derived DCs (GM-CSF and IL-4) and macrophages (CSF); n=3-4 per group. * $p < 0.05$.

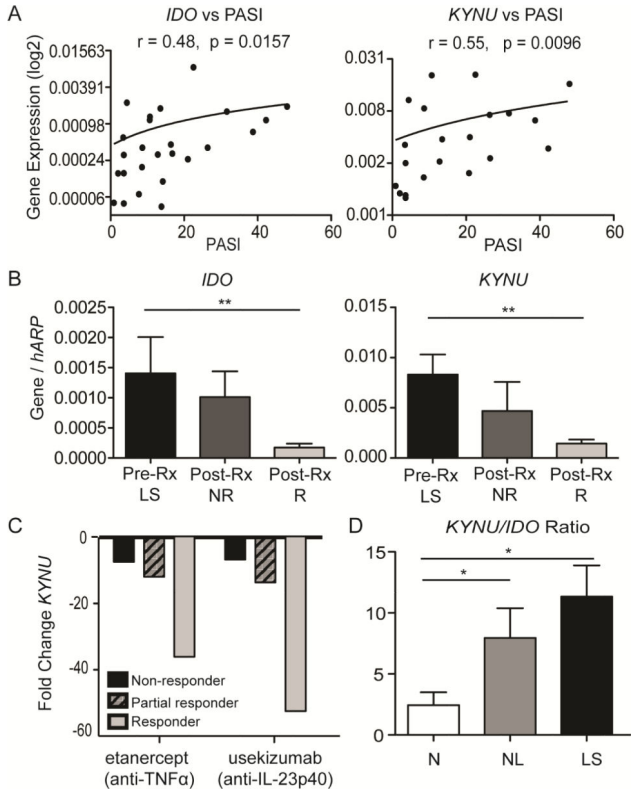

Figure 4. Reduction in tryptophan metabolism is associated with successful response to psoriasis treatment

(A) Spearman correlation of *IDO* and *KYNU* expression with disease severity (PASI score). (B) RNA from pre- and post-treatment lesional skin biopsies of psoriasis patients receiving narrow-band UVB therapy were assessed for expression of *IDO* and *KYNU*, stratified by response to treatment. (C) Reduction in *KYNU* from microarray analysis of other psoriasis treatment regimens. (D) The ratio of *KYNU:IDO* expression in normal, NL, and LS skin. For qRT-PCR results, n = 3–12 per group. * $p < 0.05$, ** $p < 0.01$.

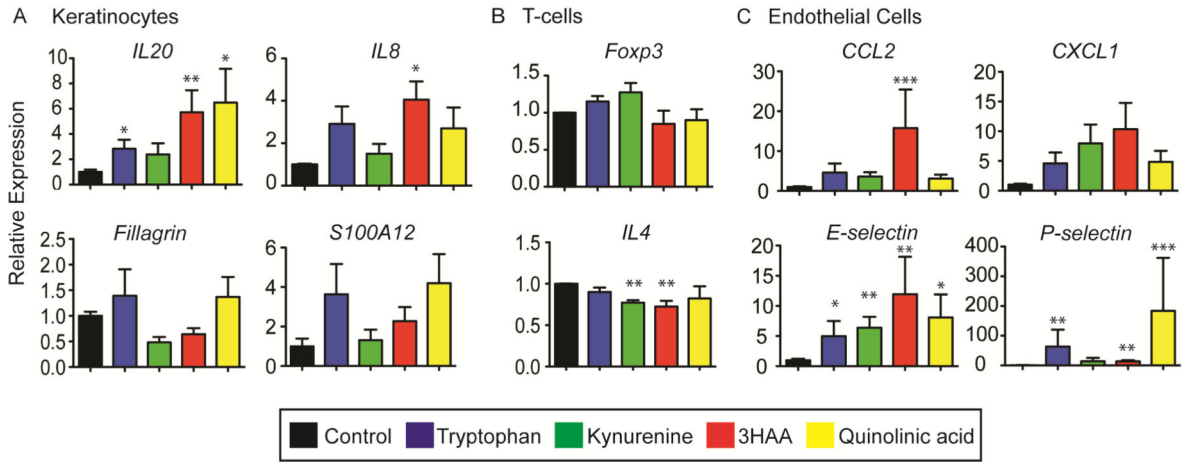

Figure 5. Tryptophan metabolites downstream of KYNU upregulatate inflammatory gene expression in several cell types important in psoriasis pathogenesis

(A) KCs, (B) activated T-cells, and (C) ECs were cultured with 50μm of tryptophan (TRYP, blue), kynurenine (KYN, green), 3-hydroxyanthranic acid (3HAA, red), and quinolinic acid (QA, yellow), or no metabolite (Control, black) as described in the Experimental Procedures. Expression of inflammatory genes, normalized to control, is shown. For KC experiments, n = 5–8 per condition; for T-cell experiments n = 5–7 per condition; for EC experiments, n= 6–10 per condition. * $p < 0.05$, ** $p < 0.01$, ***$p< 0.001$.

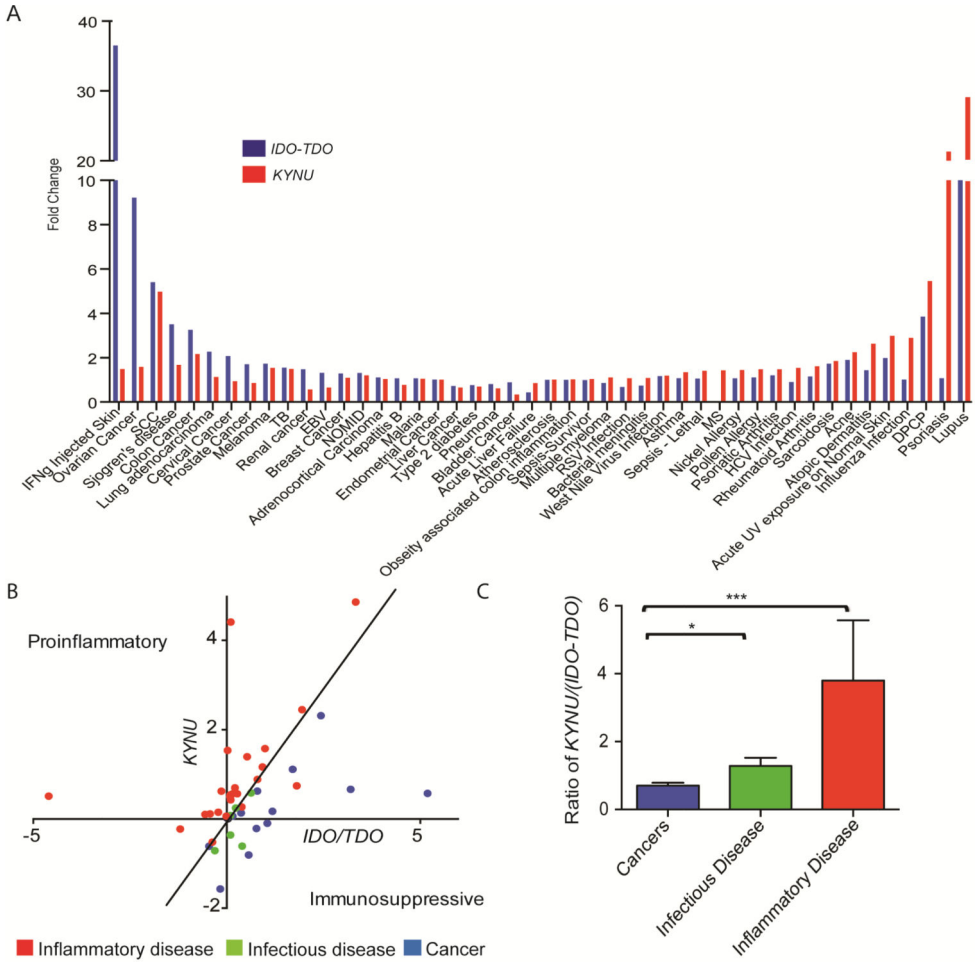

Figure 6. Human cancers and inflammatory diseases are characterized by distinct ratios of tryptophan metabolism enzymes

(A) Publicly available gene array data from over 40 human cancers, infectious diseases, and inflammatory diseases were mined for expression of *IDO, TDO*, and *KYNU*. The average fold change of *IDO* and *TDO* (blue) and *KYNU* (red), compared to normal or non-diseased tissue, for each disease is provided. (B) The average log fold-change of *IDO-TDO* versus *KYNU* for inflammatory diseases (red), infectious disease (green), and cancers (blue). Black line represents a 1:1 fold change ratio of *IDO-TDO* versus KYNU. (C) The ratio of *KYNU:IDO-TDO* was pooled for cancers, infectious diseases, and inflammatory diseases. n=9–16 per group. * $p < 0.05$, ** $p < 0.01$, ***$p< 0.001$.