A Multifaceted Role of Tryptophan Metabolism and Indoleamine 2,3-Dioxygenase Activity in *Aspergillus fumigatus*–Host Interactions

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Aspergillus fumigatus is the most prevalent filamentous fungal pathogen of humans, causing either severe allergic bronchopulmonary aspergillosis or often fatal invasive pulmonary aspergillosis (IPA) in individuals with hyper- or hypo-immune deficiencies, respectively. Disease is primarily initiated upon the inhalation of the ubiquitous airborne conidia—the initial inoculum produced by *A. fumigatus*—which are complete developmental units with an ability to exploit diverse environments, ranging from agricultural composts to animal lungs. Upon infection, conidia initially rely on their own metabolic processes for survival in the host's lungs, a nutritionally limiting environment. One such nutritional limitation is the availability of aromatic amino acids (AAAs) as animals lack the enzymes to synthesize tryptophan (Trp) and phenylalanine and only produce tyrosine from dietary phenylalanine. However, *A. fumigatus* produces all three AAAs through the shikimate–chorismate pathway, where they play a critical role in fungal growth and development and in yielding many downstream metabolites. The downstream metabolites of Trp in *A. fumigatus* include the immunomodulatory kynurenine derived from indoleamine 2,3-dioxygenase (IDO) and toxins such as fumiquinazolines, gliotoxin, and fumitremorgins. Host IDO activity and/or host/microbe-derived kynurenines are increasingly correlated with many *Aspergillus* diseases including IPA and infections of chronic granulomatous disease patients. In this review, we will describe the potential metabolic cross talk between the host and the pathogen, specifically focusing on Trp metabolism, the implications for therapeutics, and the recent studies on the coevolution of host and microbe IDO activation in regulating inflammation, while controlling infection.

Keywords: *Aspergillus fumigatus*, tryptophan metabolism, IDO, kynurenines, toxins, non-ribosomal peptides, peripheral tolerance, Th17 cells

INTRODUCTION

Aspergillus fumigatus is a saprophytic fungus that has a worldwide distribution. The asexual spores (called conidia) are ubiquitous and individuals inhale hundreds of spores daily. While most inhaled conidia are cleared by individuals with a healthy immune system, *A. fumigatus* can act as an opportunistic human pathogen in individuals with altered immune functions. Disease presentation can vary on the status of the host's immune system; *A. fumigatus* can cause allergic bronchopulmonary aspergillosis, a severe allergenic response, in the hyper-immune, or the fatal invasive growth

invasive pulmonary aspergillosis (IPA) in the hypo-immune, or in individuals with other susceptibilities such as patients unable to mount the necessary oxidative defenses such as in individuals with chronic granulomatous disease (CGD) (1).

The manifestation of disease is dependent not only on the host's immune status but also fungal factors including strain heterogeneity (2). *A. fumigatus* growth in the mammalian lung, following survival of resident pulmonary defenses, requires the fungus to adapt to a hypoxic and nutritionally scarce environment. *Aspergillus* mutants unable to synthesize primary metabolites necessary for growth are generally impaired in virulence. For example, deletion of *cpcA*, a transcription factor that globally modulates amino acid biosynthesis in the fungus led to a less virulent phenotype in a murine model of IPA (3). Additional studies have shown that mutants in sulfur utilization (4), uracil/uridine synthesis (5), zinc uptake, iron acquisition, and many more (6, 7) are also decreased in virulence.

To complicate disease progression further, there is an alarming rise in antifungal resistance strains of *A. fumigatus* (8, 9). Therefore, an understanding of *A. fumigatus* and host metabolic pathways is important in identifying nutrient limitations. One critical metabolic pathway is the biosynthesis of aromatic amino acids [AAAs, tryptophan (Trp), phenylalanine, and tyrosine], which are required not only for growth of *A. fumigatus* but are also precursors for several toxins (**Table 1**). The host relies on dietary sources for all AAAs while *A. fumigatus* synthesizes all

three. However, the host and *A. fumigatus* both possess AAA catabolic enzymes. In particular, one key enzyme important in immune homeostasis is indoleamine 2,3-dioxygenase (IDO), which converts Trp to kynurenine and related metabolites in both organisms. Historically, host IDOs activity has been described as an effective antimicrobial control for pathogens that are natural Trp auxotrophs such as *Staphylococcus aureus*, *Chlamydia* spp., and *Toxoplasma gondii*, presumably by Trp starvation (10). However, *A. fumigatus* can synthesize its own Trp and thus the Trp starvation may not be an effective pathogen control for those microbes able to synthesize their own Trp pools. Although, IDOs also play additional roles in host defenses through modifying kynurenine levels and subsequent cytokine responses as described below. In this review, we will summarize the recent studies describing the anabolic and the catabolic pathways of Trp metabolism, the implications for therapeutics, and the host–pathogen interaction.

Trp SYNTHESIS AND POTENTIAL THERAPEUTIC TARGETING

Chorismic acid derived from the shikimic acid pathway is a key intermediate in producing Trp, phenylalanine (Phe), and tyrosine (Tyr) in microorganisms including *A. fumigatus* (**Figure 1**). Trp and Phe are classified as essential amino acids, whereas mammals acquire them from diet, whereas Tyr is synthesized *via* the hydroxylation of Phe (23, 24). The absence of the AAA biosynthetic enzymes and the low bioavailability of Trp in humans makes the Trp biosynthetic enzymes attractive targets for antifungals (25).

Fungal Trp Anabolic Pathway

Aromatic amino acid synthesis has been extensively studied in *Saccharomyces cerevisiae* and provides the basis for the functional characterization of orthologous enzymes in filamentous fungi (23, 24, 26–28). The shikimic acid pathway is a 7-enzymatic step reaction that initiates with two substrates, phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P), which are intermediates of glycolysis and pentose phosphate pathways, respectively (29) (**Figure 1**). The first step of the shikimic acid pathway is catalyzed by 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase to convert PEP and E4P to DAHP. In *S. cerevisiae* and *A. nidulans*, there are two DAHP synthases, Aro3 and Aro4, which are allosterically inhibited by phenylalanine and tyrosine, respectively (24). Steps 2–6 in filamentous fungi such as *A. nidulans* and *A. fumigatus* are completed by the pentafunctional enzyme AroM, or Aro1 in the model organism *S. cerevisiae* (30). The shikimate pathway culminates in the production of chorismic acid synthesized by the enzyme chorismate synthase (AroB) from 5-enolpyruvylshikimate-3-phosphate (EPSP) (31).

The synthesis of Trp from chorismate is initiated by an anthranilate synthase (AS), which converts chorismate to anthranilate, followed by three enzymatic steps as presented in **Figure 1** with the respective functions outlined in **Table 2**. AS(s) in *S. cerevisiae* have been characterized, and it consists of two subunits: anthranilate synthase subunit I (AAS-I), which binds chorismate and is subject to feedback inhibition by Trp and anthranilate synthase

TABLE 1 | *Aspergillus fumigatus* non-ribosomal peptides containing aromatic amino acids (AAAs) in their peptide structure.

Toxin	AAA[a]	Interaction with host[b–i]
Fumigaclavine C[b]	Tryptophan (Trp)	Downregulation of Th1 cytokines including TNF-α, IL-1β, and IL-17A. Induction of host cell apoptosis Decrease activation of caspase-1
Fumiquinazoline C[c]	Anthranilate Trp	Cytotoxic
Fumisoquin[d]	Tyrosine	Nothing reported
Fumitremorgin[e] Tryprostatin A[f] Verruculogen[g]	Trp	Neurotoxic and produces tremors in mice Tryprostatin causes the inhibition of microtubule assembly Verruculogen causes modification of the electrophysiological properties of HNEC
Gliotoxin[h]	Phenylalanine	Virulent in a steroid murine model of IPA Induction of host cell apoptosis and causes epithelial cell damage Inhibition of phagocytosis and oxidative bursts
Hexadehydroastechrome[i]	Trp	Overexpression resulted in a significantly higher virulence in a neutropenic murine model of IPA[b]

IPA, invasive pulmonary aspergillosis; HNEC, human nasal epithelial cells.
[a]Only the AAA is designated. Other amino acids are also in the structure of these metabolites.
[b–i]Biosynthesis and host interactions based from the following sources: [b](11, 12); [c](13); [d](14); [e](15, 16); [f](17); [g](18, 19); [h](7, 20); [i](21, 22).
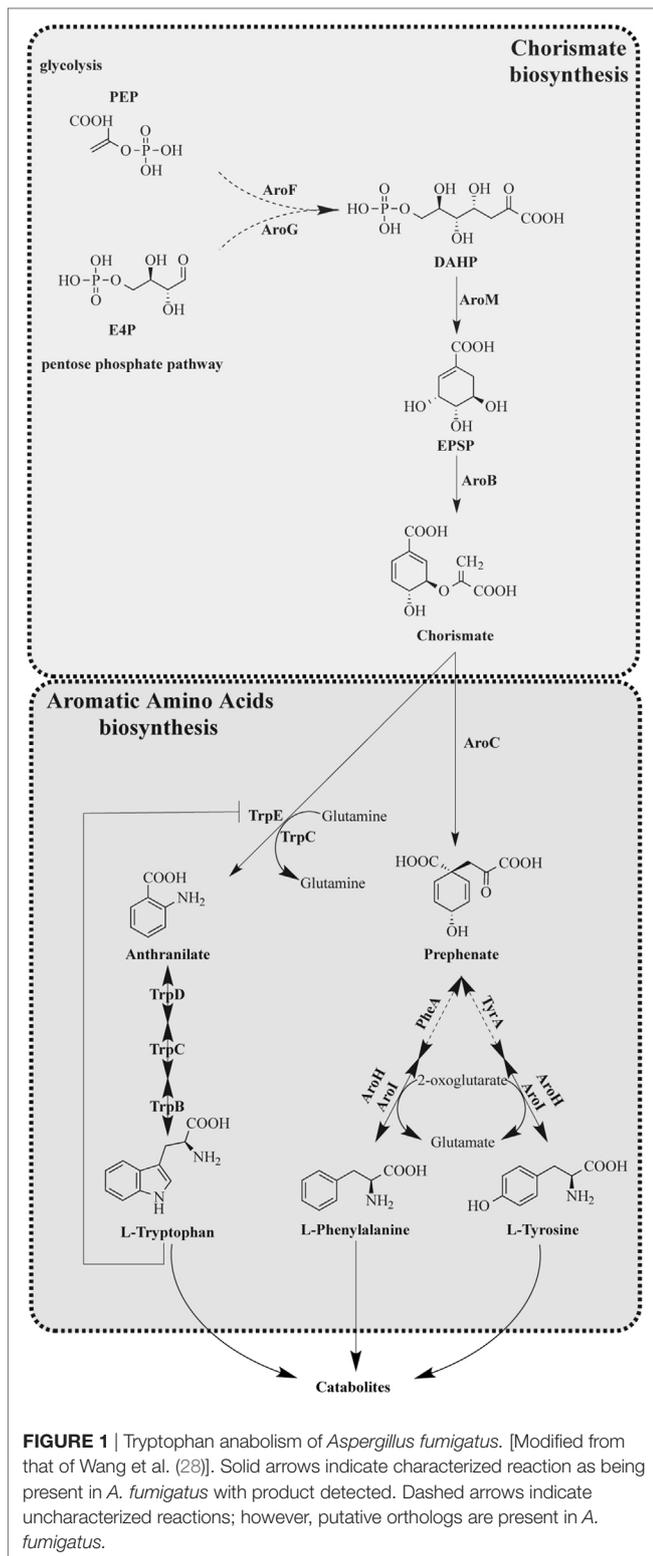
FIGURE 1 | Tryptophan anabolism of *Aspergillus fumigatus*. [Modified from that of Wang et al. (28)]. Solid arrows indicate characterized reaction as being present in *A. fumigatus* with product detected. Dashed arrows indicate uncharacterized reactions; however, putative orthologs are present in *A. fumigatus*.

subunit II (AAS-II) which is a glutamine amidotransferase (32). The *A. nidulans trpC*, an AAS-II encoding gene, was characterized in 1977 (33) and found exchangeable with an *A. fumigatus trpC* in 1994 (34). Wang et al. (28) recently characterized *trpE*, the AAS-I

encoding gene in *A. fumigatus*. Wang et al. (28) explored the functions of two putative AAS-Is termed *trpE* and *icsA* by creating null mutants. The deletion of *trpE* led to a Trp auxotrophic strain, whereas the deletion of *icsA* did not. To ensure that *icsA* did not serve a redundant role for Trp synthesis, the group overexpressed *icsA* in a *trpE* deletion and showed that the overexpression of *icsA* does not reverse the Trp auxotrophy concluding that TrpE is the only AS in *A. fumigatus*. Interestingly, the group showed that IcsA is an active enzyme in *A. fumigatus* as the precursor-chorismate pool is altered in the absence or overproduction of IcsA; however, the product is not known (28). Sasse et al. also confirmed that deletion of *trpE* (they termed *trpA*) results in an *A. fumigatus* Trp auxotrophy (31).

The Shikimate Pathway As Potential Antifungal Targets

Currently, there are four major classes of antifungals: azoles and amphotericin B targeting ergosterol, 5-fluorocytosine targeting DNA synthesis, and echinocandins targeting cell wall synthesis. These antifungals either exhibit high toxicity to the mammalian cell (particularly amphotericin B and 5-fluorocytosine) or lose efficacy due to the emergence of drug resistant strains (azoles and echinocandins) (9). With *A. fumigatus* being a eukaryotic pathogen and sharing many proteins with mammalian hosts, there are limitations to developing effective and safe antifungals and therefore a great need for treatments that are fungal specific. Since Trp is a human-essential amino acid and the enzymes in the biosynthesis are fungal specific, several studies have suggested utilizing and finding drugs to target the enzymes of this pathway (35–38).

Targeting essential amino acid pathways have already shown potential for new classes of antifungals. Several groups have explored inhibitors of genes or enzymes involved in methionine biosynthesis. Azoxybacillin, a compound isolated from *B. cereus* targets methionine biosynthesis by interfering with expression of homoserine transacetylase and sulfite reductase encoding genes (39–41). Whereas azoxybacillin displayed a broad spectrum antifungal activity *in vitro*, *in vivo* activity was low possibly due to bioavailability in the host (41). R1-331, a natural product from *Streptomyces akiyoshiensis*, is an effective inhibitor of homoserine dehydrogenase involved in both methionine and threonine biosynthesis (42, 43). Yamaguchi et al. show that R1-331 was active against medically important fungi such as *Candida albicans* and *Cryptoccocus neoformans* and proved to be effective in the treatment of systemic murine candidiasis (42, 44).

Compounds targeting AAA pathways are limited with the most famous being the herbicide Roundup, where the active ingredient glyphosate inhibits EPSP synthase, one of the first enzymes initiating the shikimate pathway (45) (**Figure 1**). Glyphosate has shown to inhibit growth of several fungi including *Candida maltose* (46), *Pneumocystis* (47), and *Cryptococcus neoformans* where glyphosate delayed fungal melanization *in vitro* and *in vivo* and prolonged mice survival during infection (48). Another inhibitor of AAA pathway is a fluorinated anthranilate moiety, 6-FABA, which targets the TrpE enzyme and showed bactericidal activity when used on *Mycobacterium tuberculosis* (49). The studies of

TABLE 2 | *Aspergillus fumigatus* tryptophan (Trp) metabolism genes and putative protein function.

Protein	Ortholog in *Saccharomyces cerevisiae*	Gene name in *A. fumigatus*	Protein function[a]	Ortholog in mammals
Chorismate biosynthesis				
AroF	Aro3	Afu1g02110	DAHP synthase	–
AroG	Aro4	Afu7g04070		
AroM	Aro1	Afu1g13740	EPSP synthase	–
AroB	Aro2	Afu1g06940	Chorismate synthase	–
Aromatic amino acid (AAA) biosynthesis				
TrpE	Trp2	Afu6g12580	Anthranilate synthase	–
TrpC	Trp3	Afu1g13090		
TrpD	Trp4	Afu4g11980	Anthranilate phosphoribosyltransferase	–
TrpC	Trp1	Afu1g13090	Phosphoribosylanthranilate isomerase	–
TrpB	Trp5	Afu2g13250	Trp synthase	–
IcsA	–	Afu6g12110	Isochorismate synthase	–
AroC	Aro7	Afu5g13130	Chorismate mutase	–
PheA	Pha2	Afu5g05690	Prephenate dehydratase	–
TyrA	Tyr1	Afu2g10450	Prephenate dehydrogenase	–
AroH	Aro8	Afu2g13630	AAA transaminase	–
AroI	Aro9	Afu5g02290		
Trp degradation				
IdoA	Bna2	Afu3g14250	Indoleamine 2,3-dioxygenases	IDO1
IdoB	Bna2	Afu4g09830		IDO2
IdoC	Bna2	Afu7g02010		TDO
FmdS	Bna7	Afu1g09960	Kynurenine formamidase	AFMID
Bna4	Bna4	Afu6g07340	Kynureninase	
Bna5	Bna5	Afu4g09840		KYNU
AroH	Aro8	Afu2g13630	AAA transaminase	LAAO
AroI	Aro9	Afu5g02290		
AadA	–	Afu3g02240	Trp carboxylase	AADC
MaoN	–	Afu3g00100	Monoamine oxidase	MAOA
AldA	Ald4	Afu2g00720	Aldehyde dehydrogenase	ALDH
	Ald5	Afu7g01000		
		Afu6g11430		

[a]*Prediction of protein function based on AspGD (http://www.aspgd.org/) and KEGG (http://www.genome.jp/kegg/kegg2.html).*
DAHP synthase, 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase; EPSP synthase, enolpyruvylshikimate-3-phosphate synthase; AFMID, arylformamidase; KYNU,
kynureninase; LAAO, L-amino-acid oxidase; AADC, aromatic-L-amino-acid decarboxylase, MAOA, monoamine oxidase; ALDH2, aldehyde dehydrogenase family.
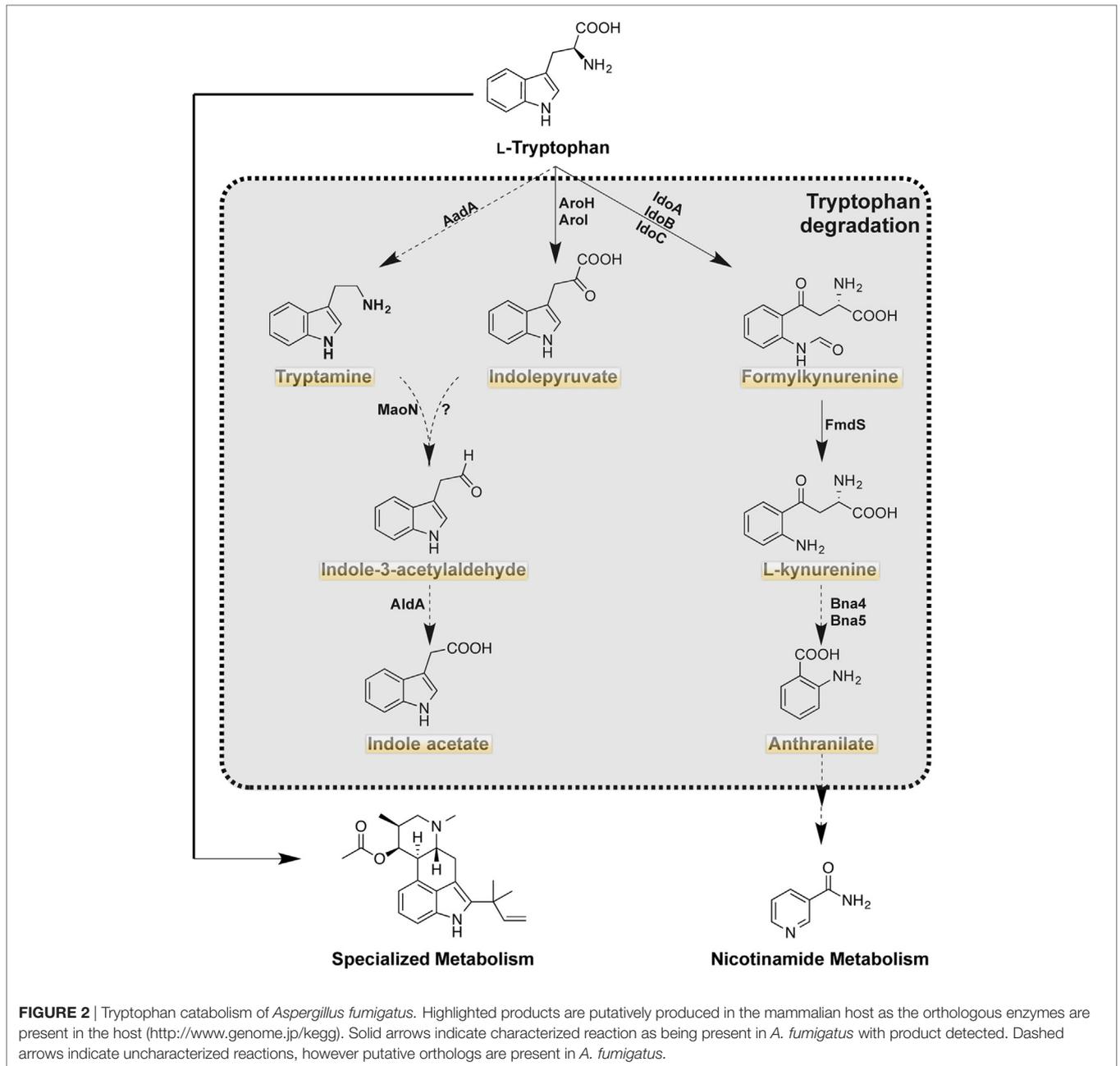
these inhibitors suggest that the Trp biosynthetic pathway could be fruitful in future antifungal drug design.

The value of AAA pathways as drug targets is supported by the findings that AAA auxotrophic mutants are less virulent in animal infection models. Sasse et al. explored the possibility of these pathways as potential drug target by testing the virulence of several AAA auxotrophic mutants in a murine IPA model (31). This study demonstrated that AroM (**Figure 1**) was required for *A. fumigatus* viability. The group also constructed a conditional AroB repression strain that was attenuated virulence. Both a Trp auxotroph (TrpE mutant) and Tyr/Phe auxotroph (AroC mutant) were severely attenuated in virulence for pulmonary infection. Interestingly, the group also unveiled a putative difference in AAA distribution within the host by conducting a systemic infection showing that in a bloodstream infection the TrpE and the AroC mutants although less virulent, can establish some infection (31). Taken together, these results suggest that inhibitors of AAA biosynthetic pathways can potentially be used against *A. fumigatus* as a standalone treatment in a localized pulmonary infection or as an additive treatment in a systemic infection. The result of the bloodstream infection observed by Sasse et al. also suggests that there are mechanisms for the fungus to sense Trp

in its environment and utilize it. In *S. cerevisiae*, the Trp specific permease, Tat2, is required for Trp uptake in yeast (50) and its closest homolog in *A. fumigatus* (Afu7g04290) is upregulated during fungal encounters with neutrophils (51) and dendritic cells (52). In *A. nidulans*, the G-protein coupled receptor (Gpr) H may be responsible for sensing Trp and glucose and GprH is conserved in *A. fumigatus* (53, 54). Perhaps, for a systemic infection, inhibition of specific permeases or development of a GprH antagonist would be useful in reducing infections by *Aspergillus*.

CATABOLIC Trp METABOLITES IN FUNGI AND HOST

Although the anabolic Trp pathway is absent in mammals, the common catabolic pathways exist in the mammalian host with possession of the same enzymes as *A. fumigatus* (**Figure 2**). Through the expression of Trp degradation enzymes, immune cells are both controlling inflammation and combating microbial infection. In addition to Trp degradation pathways conserved with animals, *A. fumigatus* can also direct Trp pools to secondary metabolites that may impact host health and response (**Table 1**).

FIGURE 2 | Tryptophan catabolism of *Aspergillus fumigatus*. Highlighted products are putatively produced in the mammalian host as the orthologous enzymes are present in the host (http://www.genome.jp/kegg). Solid arrows indicate characterized reaction as being present in *A. fumigatus* with product detected. Dashed arrows indicate uncharacterized reactions, however putative orthologs are present in *A. fumigatus*.

Fungal Trp Catabolism

There are three putative pathways (**Figure 2**; **Table 2**) for the degradation of Trp in *A. fumigatus*. The kynurenine branch is catalyzed by IDOs that convert Trp into formylkynurenine. In *A. fumigatus*, there are 3 putative *ido* genes: *idoA*, *idoB*, and *idoC*, the orthologs of *A. oryzae idoα*, *idoβ*, and *idoγ*, respectively (55). Enzymatic studies of *Aspergillus oryzae* IDO enzymes suggest two of the three enzymes, IDOα, and IDOβ, may participate in Trp degradation as they have a higher affinity of its substrate. However the recent study by Wang et al., suggested IDOb might be the more dominant enzyme than IDOa as determined by gene expression of *A. fumigatus* grown on Trp amended media (28). Additionally,

idoC gene expression was slightly induced by the addition of Trp, but the authors note that IDOc had a closer relationship to bacterial IDOs than to fungal IDOs (28, 55). In *S. cerevisiae*, formylkynurenine is further oxidized to the immunomodulatory product kynurenine by a kynurenine formamidase denoted as Bna7, which has been described in *A. nidulans* and is predicted to be involved in NAD (+), biosynthesis (56). The kynurenine branch in fungi is involved in the *de novo* biosynthesis of NAD (+), a coenzyme that is required for oxidation-reduction reactions (57).

Tryptophan can also be metabolized via the indole pyruvate pathway, initiated through the transamination of Trp by aromatic

aminotransferases (termed Aro8 and Aro9 in *S. cerevisiae*). These aromatic aminotransferases are also involved in the synthesis of Phe and Tyr in *S. cerevisiae*, and their orthologs are present in *A. fumigatus* (28). In *S. cerevisiae*, the deletion of both Aro8 and Aro9 results in Phe and Tyr auxotrophies (24, 58, 59). In *Candida* spp., where filamentation and pigment production play a role in virulence, the products of these enzymes have been described to influence both phenotypes. The deletion of *aro8* in *C. glabrata* results in a reduced pigment production and leads to an increased sensitivity to hydrogen peroxide (27). The *aro8* and *aro9* mutants of *C. albicans* results in a decreased conversion of Trp to indole acetaldehyde, which is formed via decarboxylation of indole pyruvate. Filamentation of *C. albicans* increased with the exposure to indole acetaldehyde (60).

Indole acetaldehyde can also be produced via the third putative product of Trp degradation: tryptamine. Tryptamine is most famously known as the active compound in psilocybin and for its similarity to serotonins (61). Although the production of tryptamine has yet to be described in *A. fumigatus*, the downstream product of the tryptamine and indole pyruvate pathway, indole acetic acid has been described in several *Aspergillus* spp. including *A. fumigatus* (62, 63). Downstream metabolism of kynurenine, indole pyruvate, and tryptamine has not been explored further, but *A. fumigatus* does possess putative enzymes for the re-synthesis of anthranilate, the precursor to Trp (as denoted in **Figures 1** and **2**).

AAA Incorporation into *Aspergillus* Toxins

Many filamentous fungi, including *A. fumigatus*, produce bioactive small molecules that can have detrimental impacts on human health. Subsets of these toxins are small peptides synthesized by non-ribosomal peptide synthetases (NRPS). Several pathogenic *Aspergillus* species synthesize AAA derived peptides including gliotoxin (*Phe* and serine) (64), fumiquinazoline (*Trp*, *Anthranilate*, and Alanine) (13), fumigaclavine (*Trp*) (65), fumitremorgin (*Trp* and Proline) (66), hexadehydroastechrome (*Trp* and Alanine) (21), fumisoquin (*Tyr*, Serine, and Methionine) (14), DPP-IV inhibitor WYK-1 (*Trp*, *Tyr*, and Leucine) (67), cyclopiazonic acid (*Trp*) (68) and benzomalvin (*Phe* and *Anthranilate*) (69). **Table 1**

summarizes the known AAA derived secondary metabolites of *A. fumigatus* and their effect on the host.

Although, fumiquinazolines have yet to be assessed for virulence in an animal model, they are known to have cytotoxic properties (13). Fumigaclavines have been described to have immunosuppressive properties in several studies including the suppression of antifungal cytokines such as TNFα, IL-17, and IFN-γ (12). Fumitremorgin, verruculogen, and tryprostatin—all related products of the fumitremorgin pathway—induce tremorgenic activity in mice and act on the central nervous system (15, 66, 70, 71). Mutants in the hexadehydroastechrome pathway (21) and gliotoxin (20, 21) pathways have altered virulence in murine IPA models. Decreased virulence of the *gliP* mutant (GliP is the NRPS required for gliotoxin synthesis) is dependent on host immune status [reviewed in Ref. (7)]. Overexpression of *hasA* encoding the hexadehydroastechrome transcription factor and thus leading to increased hexadehydroastechrome production was more virulent than wild type *A. fumigatus* in a neutropenic model of IPA (21). Although the exact mechanism underlying the increased virulence of the OE::*hasA* strain is unknown, iron homeostasis and cross talk between metabolic pathways may contribute to the increased virulence of OE::*hasA* (22). These studies highlight the potential contribution of AAA derived toxins in virulence of *A. fumigatus*.

Host Trp Catabolism *via* IDO

The function of host IDO during mammalian infection was originally thought to center on the anti-proliferative effects of pathogenic microorganism via deprivation of Trp exerted by the host. IDO is up-regulated by interferon gamma (IFNγ) and depletes Trp (the least abundant essential amino acid) to inhibit pathogen expansion (72, 73), as demonstrated in the constraint of chlamydial growth (74). Numerous studies have since implicated IDO activity as important in fungal infections and have reported the relative outcomes of IDO expression on disease progression (**Table 3**). Accumulating data continues to support that IDO participates in the host–pathogen interaction in human epithelial cells; therefore, the co-evolution of host and microbe Trp metabolism has been investigated (75). The current consensus is that IDO activation is pivotal in regulating inflammatory processes directly

TABLE 3 | Summary of studies where IDO enzyme activity was found to be implicated in fungal infections.

Fungus	Mouse model	IDO	Outcome	Reference
Candida albicans	Gastrointestinal inf.	Upregulation	Protection of the host against fungus	(76)
C. albicans	Gastrointestinal inf.	Upregulation	Protection of the host against fungus	(77)
C. albicans	Gastrointestinal inf.	Upregulation	Protection of the host against fungus	(78)
C. albicans	*In vitro*	Downregulation	Not done	(79)
Aspergillus fumigatus	Keratitis	Upregulation	Protection of the host against fungus	(80)
A. fumigatus	Allergy	Overexpression	Protection of the host against fungus	(81)
A. fumigatus	IPA	Upregulation	Protection of the host against fungus	(82)
A. fumigatus	IPA in chronic granulomatous disease mice	Upregulation	Protection of the host against fungus	(83)
A. fumigatus	IPA in CF mice	Upregulation	Protection of the host against fungus	(84)
Paracoccidioides brasiliensis	Pulmonary infection	Upregulation	Protection of the host against fungus	(85)
P. brasiliensis	Pulmonary infection	Upregulation	Protection of the host against fungus	(86)
P. brasiliensis	Pulmonary infection	Upregulation	Protection of the host against fungus	(87)
Histoplasma capsulatum	Pulmonary infection	Downregulation	Protection of the host against fungus	(88)
H. capsulatum	Pulmonary infection	Upregulation	Protection of the host against fungus	(89)

via Trp depletion and indirectly via the IDO-mediated release of Trp catabolic secondary metabolites (namely, kynurenines).

Dietary Trp is catabolized by two different IDO protein isoforms, IDO1 and IDO2 that are expressed by immune cells, and TDO (Trp 2,3-dioxygenase) that is mainly expressed in the liver. Cells involved in the innate processes of the anti-microbial defense, such as dendritic cells (DCs), neutrophils, and macrophages express IDO1 upon microbial encounter mainly *via* toll-like receptor stimulation. How fungi specifically induce IDO expression is not known; however, induction by other pathogens is associated with pathogen associated molecular patterns, including lipopolysaccharides and CpG oligodeoxynucleotides (78, 90–92), underlining a role for kynurenine metabolism in microbial-induced inflammatory processes.

IDO-Mediated Tolerance: Impacts on Antimicrobial Responses

Evolutionary studies have shown that the host immune defense against microbes is characterized by three different mechanisms: avoidance, resistance, and tolerance (93). Modules of immunity provide resistance to limit pathogen burden and tolerance and host damage caused by the immune reaction *per se*. However, the inflammatory reaction, although largely considered beneficial for its antimicrobial functions, may also contribute to pathogenicity. Thus, rescue from infection pathology may not only depend on microbial colonization (and inactivation of resistance mechanisms) but also on the resolution of tissue inflammatory pathology through tolerogenic responses to pathogens (94).

Studies using a mouse model of mucosal or invasive *C. albicans* infection found that systemic inhibition of IDO *in vivo* reduced gastrointestinal inflammation and unexpectedly, elevated the levels of fungal colonization compared to control mice (77). Notably, tolerogenic responses toward *C. albicans* were abrogated when IDO was antagonized *in vivo*, as shown in various models of inflammatory disorders (95, 96). As with *C. albicans*, IDO and kynurenine production during *A. fumigatus* infection contributes to fungal pathogen eradication and the regulation of an unacceptable level of tissue damage (97). Indeed, IDO can increase kynurenine host levels to induce adaptive Treg expansion while limiting Th17 polarization (83, 96). In this context, the Th17 pathway, which downregulates Trp catabolism, may instead favor pathology and better explain the paradoxical correlation between fungal infection and chronic inflammation (98).

Another example of this paradox was demonstrated in the context of CGD, in which an NADPH oxidase defect results in reduced host production of antimicrobial ROS and extreme susceptibility to *Aspergillus* infections (1, 83). Although human studies have excluded a role for IDO in CGD (99), further investigations into the IDO pathway are warranted as such studies have failed to demonstrate functional IDO activity at sites of chronic inflammation. Measures of IDO functional activity during IPA have, however, been made in mouse models, and implicate defective IDO activity as a key mediator of chronic inflammation in CGD (83). An exaggerated Th17 pulmonary response was associated with reduced fungal clearance in mouse models of CGD that develop IPA. Here, reduced IDO function was directly related to NADPH/ROS deficiency, as ROS is essential for IDO catalytic activity in mammals (100). ROS deficiency as a result of reduced NADPH function, significantly enhanced IL-17 inflammation and fungal germination in the lung, thus further reducing neutrophil-mediated antimicrobial activities (83).

Since regulation of homeostasis and peripheral tolerance are extremely important in prevention of invasive Aspergillosis or allergy to *Aspergillus* antigens (97, 101), the role of IDO has been extensively studied in this model of fungal infection (81, 82, 102). These studies highlight the induction of the IDO metabolic pathway at different site of fungal colonization as keratinocytes or lung as well as the important anti-inflammatory activity of IDO in the tissue microenvironment (80–82, 84).

Aryl Hydrocarbon Receptor (AhR) Activation by IDO Metabolites in Mammals: Biological Consequences

The AhR is a ligand-activated transcription factor first identified for its role during embryonic development and induction of xenobiotic metabolizing enzymes as a response to environmental toxins, such as dioxin (103). More recently, AhR has been shown to play a critical role in immunity by acting as an immune modulator during fungal infection (85). The connection between the AhR and the immune response lies in part in the endogenous Ahr ligands, which comprise many Trp metabolites, including kynurenine (104). Microbial Trp-derived metabolites can activate the AhR, leading to adjustments in the immune response that may hinder disease development (105). The AhR–IDO axis has been recently demonstrated in fungal infection, highlighting a role for IDO-derived metabolites to trigger AhR target genes (85, 106). For example, one AhR target gene, *Il22*, has been widely studied in the context of fungal/microbial infections (105, 107–109). AhR activation by IDO metabolites can also mediate the expansion of peripheral Treg with anti-inflammatory properties. Using IDO-deficient mice, increased pulmonary disease caused by *Paracoccidioides brasiliensis* was associated with decreased Treg expansion and reduced AhR protein expression (85). In murine models of IPA, distinct Treg populations capable of mediating anti-inflammatory effects expand following exposure to *Aspergillus* conidia (97). Late in infection, tolerogenic adaptive Treg (with shared phenotypic identity with the Treg controlling autoimmune diseases or diabetes) produce IL-10 and TGFβ, inhibit Th2 cells, and prevent an allergic reaction to *Aspergillus* (97).

CONCLUSION

The interplay of Trp metabolic pathways and fungal/host interactions is intriguing with many unanswered questions of the exact nature of crosstalk of shared metabolites and consequences of activation of Trp degradative pathways. In *Aspergillus* infections in particular, not only does the pathogen synthesize and degrade Trp but it also can utilize this amino acid (and its precursor anthranilate or the other two AAAs Tyr and Phe) to yield several potentially damaging toxins (**Table 1**). Also, as both host and *Aspergillus* share catabolic IDO pathways, it is unclear which

organism may generate immunomodulatory Trp degradation products and if they respond to each other's products (e.g., kynurenine). Development of *A. fumigatus* IDO mutants for investigation of disease development could yield valuable information on this front. The research on the expression host IDOs exhibit the importance of an extremely coordinated immune response to mount the right inflammatory response for clearance of spores. However, while an increased IDO expression in the host can control inflammation, the suppression of the IDO-regulated antifungal Th17 responses can favor fungal growth. In this context, it will be critical to explore the entire IDO-mediated innate response, including the specific T cell regulatory subsets affected by IDO activity.

Although the Trp catabolic pathway is shared between host and pathogen, the anabolic pathway is unique to *A. fumigatus*. The antifungals currently used in treatment are becoming increasingly ineffective with emerging drug resistant strains; therefore, drugs targeting essential fungal specific pathways are needed. A proposal for fungal treatment has been highlighted through the studies of essential amino acids. As AAA mutants are auxotrophs and decreased in virulence (28, 31), investigations of drugs targeting these pathway enzymes could lead to novel antifungal compounds. Indeed, a few compounds have exhibited some efficacy in targeting Trp metabolic pathways in *M. tuberculosis* and several fungi and efforts to identify additional inhibitors are warranted.

AUTHOR CONTRIBUTIONS

TC, TZ, and NK have made a substantial, direct, and intellectual contribution to the work and LR provided intellectual insights to the revision. All authors have approved it for publication.

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