Kynurenine Pathway in Respiratory Diseases

Guillaume Pamart, Philippe Gosset, Olivier Le Rouzic, Muriel Pichavant and Odile Poulain-Godefroy

Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 -CIIL - Center for Infection and Immunity of Lille, Lille, France.

International Journal of Tryptophan Research
Volume 17: 1–17

DOI: 10.1177/11786469241232871

ABSTRACT: The kynurenine pathway is the primary route for tryptophan catabolism and has received increasing attention as its association with inflammation and the immune system has become more apparent. This review provides a broad overview of the kynurenine pathway in respiratory diseases, from the initial observations to the characterization of the different cell types involved in the synthesis of kynurenine metabolites and the underlying immunoregulatory mechanisms. With a focus on respiratory infections, the various attempts to characterize the kynurenine/tryptophan (K/T) ratio as an inflammatory marker are reviewed. Its implication in chronic lung inflammation and its exacerbation by respiratory pathogens is also discussed. The emergence of preclinical interventional studies targeting the kynurenine pathway opens the way for the future development of new therapies.

KEYWORDS: Kynurenine, lung, immunity, COPD, asthma, respiratory infection, indoleamine 2 3-dioxygenase

RECEIVED: July 25, 2023. **ACCEPTED:** January 28, 2024.

TYPE: Club « Trypto'Fan » Amphi IBL- Institut Pasteur de Lille - Review

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Odile Poulain-Godefroy, Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017-CIIL-Center for Infection and Immunity of Lille, 1 Rue du Pr Calmette, BP245, Lille 59019, France. Email: odile.poulain@pasteur-lille.fr

Introduction

The kynurenine pathway (KP) is the major route of tryptophan catabolism in mammalian cells, and many of the intermediates and products of this pathway are involved in the modulation of the immune response. This pathway is activated by infectious agents and inflammatory mediators, which trigger the expression and activity of key enzymes and lead to the production of metabolites, collectively termed "kynurenines," which are bioactive and can induce contrasting immune responses in both protective and destructive ways.[1] Indoleamine 2,3-dioxygenase (IDO), the first enzyme of KP, converts tryptophan to kynurenine and has 2 isoforms: IDO1, which is widely expressed in both immune and non-immune cells, and IDO2, which has been described as restricted to liver, kidney and dendritic cells. Another enzyme expressed mainly in the liver, tryptophan dioxygenase (TDO2), is also capable of this conversion. Dioxygenase activity is estimated by the kynurenine/tryptophan (K/T) ratio, which is often a marker of inflammation.

Kynurenine is a substrate for 2 different pathways. From kynurenine, the kynurenine aminotransferase enzymes (KAT I, II, III, and IV) lead to the formation of kynurenic acid for the first branch of the kynurenine pathway. Alternatively, kynurenine can be converted by kynureninase (KYNU) to anthranilic acid or by kynurenine monooxygenase (KMO) to 3-hydroxykynurenine and then to 3-hydroxyanthranilic acid, quinolinic acid. Quinolinic acid is then finally catabolized to NAD+ by quinolinate phosphoribosyltransferase (QPRT). Kynurenic acid and quinolinic acid have been proposed to play opposite roles in the immune system, with kynurenic acid being primarily anti-inflammatory and quinolinic acid being pro-inflammatory (Figure 1).

Kynurenines are emerging as important mediators affecting cognition, pain, metabolic function, aging and immune homeostasis, and have been described as important mediators of inter-organ cross-talk. The imbalance of downstream metabolites has been suggested to lead to impairment in many physiological processes, particularly those related to inflammation.[2] Nevertheless, alteration of KP may induce both pathological and compensatory mechanisms, and caution must be exercised in interpreting these observations. Initially studied mainly in relation to brain and mental health,[3] KP is emerging as an important pathway in metabolic diseases such as obesity[4] or atherosclerosis[5] and mediates immune escape in cancer.[6] KP in the gut has mainly been studied as one component of the 3 major pathways of tryptophan metabolism found in the gastrointestinal tract, where direct tryptophan conversion is partly performed by intestinal microorganisms that make up the microbiota or by enterochromaffin cells that produce serotonin.[7]

Literature on KP alterations in lung disease has remained scarce over the last decade. However, there has been a growing interest in targeting this pathway to treat patients with lung cancer.[8] A breakthrough in the "lung/KP" literature was also made during the SARS-CoV2 pandemic with the demonstration of a significant increase in the serum kynurenine/tryptophan ratio in COVID-19 patients compared to controls, as a predictable consequence of the "cytokine storm" encountered in this pathology.[9] We deliberately excluded the review of studies related to lung cancer, which are underlined by specific carcinogenic mechanisms, and the majority of studies related to SARS-CoV2, which are extensively referenced elsewhere, except for some pertinent histological observations or to refer

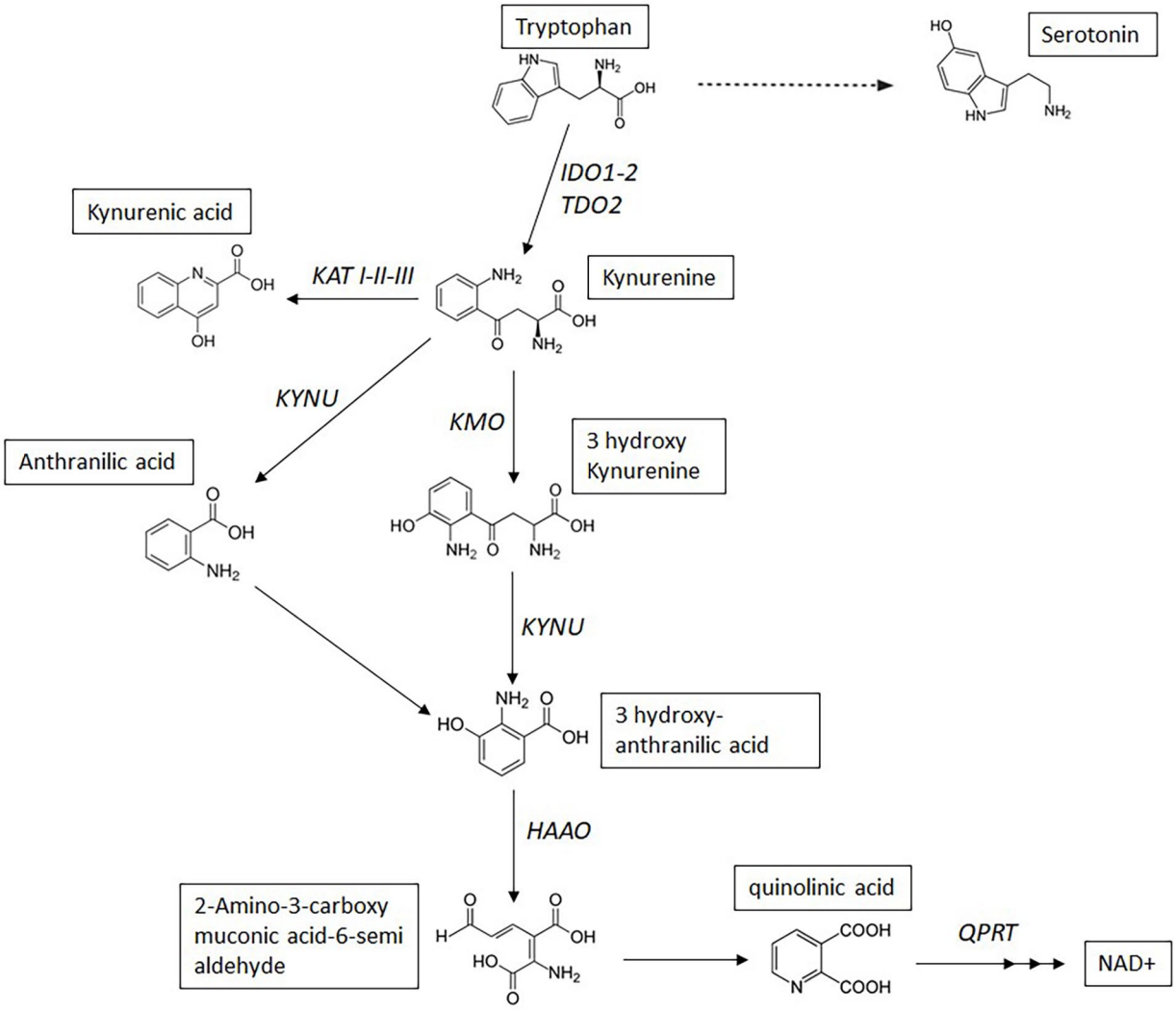

Figure 1. Schematic description of the kynurenine pathway.
HAAO, 3-hydroxyanthranilate 3,4-dioxygenase; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine amino transferase; KMO, kynurenine monooxygenase; KYNU, Kynureninase; QPRT, quinolinate phosphoribosyltransferase; TDO, tryptophan dioxygenase.

to recent reviews.[9] The induction of immunological tolerance by IDO1 has also made it an interesting molecule in organ transplantation studies: this topic, which also involves the lung, has been described elsewhere and will not be reviewed here.[10]

As inflammation is a major activator of KP, the aim of the current study is to review the data on KP in the respiratory system and its behavior in disease. This review focuses on lung diseases associated with exposure to environmental factors, namely asthma and chronic obstructive pulmonary disease (COPD), and infectious diseases.

This review describes the first observations of KP activation in the lung. The different cell types that have been shown to express KP enzymes are then mentioned. We will then outline how kynurenines have been suggested to play an important role in homeostasis and defense mechanisms in the lung. Next, we will focus on several specific infectious or inflammatory lung pathologies. Finally, the effects of pharmacological modulation of KP in lung disease will be reviewed and the implications for potential therapeutics will be discussed.

Initial Observations of Kynurenine Pathway Activation in the Lung

The lungs are made up of airways and lung tissue, and provide gas exchange to take in oxygen and remove carbon dioxide from the body. The respiratory system is constantly exposed to environmental factors that can alter the lungs and their function. These factors include pollutants, pollen and pathogens, both viruses and bacteria, which can cause inflammation and infection.

In the 1980s, the presence of IDO in the mouse lung was demonstrated, and its expression is induced by interferon or lipopolysaccharide (LPS).[11-15]. IDO is the first KP enzyme to convert tryptophan into kynurenine. Its activity has often been assessed by the difference between the kynurenine content after 30 and 60 minutes of incubation.

In 1983, IDO expression was shown to be induced by LPS in murine alveolar interstitial cells.[16] Among mammalian species, LPS induces pulmonary IDO activity in rodents, with rabbits having the highest pulmonary IDO activity of all

species studied, 170 times that of rats or mice. This justifies the use of KP as an antioxidant scavenger in these species.[17] This species heterogeneity was further confirmed by analysis of IDO, KYNU and KMO activities in rat, rabbit, gerbil and mouse lungs, which showed that rabbit lung IDO activity was 146 to 516 times higher than that of the other species studied.[18]

In 1985, kynurenine was detected by immunolabelling in epithelial cells from the lungs of newborn hamsters.[19] Lung extracts from mice given intraperitoneal injections of interferon-gamma (IFNγ),[20] pokeweed mitogen[21] or LPS,[22] showed higher IDO activity than controls. In addition, infection with type-D retrovirus in macaques strongly increased IDO activity in the lungs.[23] Intraperitoneal injection of LPS (1 mg/kg) into gerbils was shown to increase lung IDO activity and quinolinate production 24 hours after injection. However, the same experiments in Sprague-Dawley rats showed no detectable change in pulmonary quinolinate production and only a modest increase in IDO activity.[24] IFNγ has been identified as the mediator responsible for IDO induction by LPS, poly(l-C), or pokeweed mitogen.[14] It has also been shown that glucocorticoids are effective in suppressing IFN-γ induced IDO in mouse lung slices.[25]

In human, IDO1 inducibility by IFNγ was validated in adenocarcinomic respiratory epithelial cells including A549 and Calu-3 cells[26,27] Interestingly, a comparative study of different cell lines from different origins showed that only macrophage-type cells (peripheral blood mononuclear cells; THP-1, U-937) and certain liver cells were able to synthesize quinolinic acid, whereas lung epithelial cells (MRC-9) or B lymphocytes were not.[28]

The biological activity of kynurenine and its metabolites is now well recognized in several pathologies and displays diverse biological functions throughout the body[29] This review focuses on the more recent studies of the respiratory tract.

Kynurenine Pathway Induction by Systemic Inflammation

Tryptophan metabolism has been shown to be increased in chronic inflammatory lung diseases such as interstitial lung disease, idiopathic pulmonary fibrosis or fibrosing alveolitis.[30] The interstitium is the network of tissue around the air sacs in your lungs where oxygen and other gases go in and out of your bloodstream. Interstitial lung diseases affect this tissue network. Idiopathic pulmonary fibrosis (IPF), or (formerly) fibrosing alveolitis, is a rare, progressive disease of the respiratory system characterized by the thickening and stiffening of the lung tissue associated with the formation of scar tissue. In these pathologies, tryptophan metabolism was assessed by measuring circulating levels of tryptophan and kynurenine in peripheral blood and by measuring IDO activity of bronchoalveolar cells. The ratio of serum tryptophan levels to serum kynurenine levels was significantly reduced in patients with idiopathic pulmonary fibrosis, fibrosing alveolitis associated with collagen vascular disease or sarcoidosis compared with the ratio in normal subjects.[30]

In the lungs of mice treated intraperitoneally with LPS, or dexamethasone, a potent, synthetic member of the glucocorticoid class, IDO1 and IDO2 expression was increased by LPS and decreased by dexamethasone. LPS had no effect on TDO2 expression but dexamethasone increased TDO2 expression.[31]

Piglets injected intravenously with complete Freund's adjuvant, which reproduced interstitial pneumonia lesions, or with LPS showed IDO activation in plasma and lung and had significantly lower plasma tryptophan concentrations than pair-fed healthy piglets.[32,33]

Pulmonary exposure to zinc oxide nanoparticles, which are widely used in everyday life such as in sunscreens and electronic nanodevices, caused acute lung inflammation. Oropharyngeal administration of zinc oxide nanoparticles was performed in mice. IDO1 mRNA levels were significantly increased in the lungs of exposed mice on day 2. IDO1 protein was detected by immunocytochemistry in the lungs and was increased in bronchial epithelial cells, bronchiolar epithelial cells, type II cells and immune cells of zinc oxide-treated mice.[34]

Exposure to silica, which is associated with inflammation and oxidative stress, has also been shown to induce activation of KP with an increase in serum kynurenine in silicosis patients compared to controls.[35,36]

Interestingly, acute psychological stress in mice increased IDO1 mRNA expression in the lungs and a transient depletion of plasma tryptophan was measured 6 hours after stress, while kynurenine levels increased. This was suggested to play a central role in the development of stress-induced immunosuppression.[37]

Therefore, the expression of KP is mainly induced by pro-inflammatory and psychological stress and has been described to be induced in different types of both structural and immune cells present in the lung.

Lung Cell Types Expressing Kynurenine Pathway Enzymes or Metabolites

Here are the studies that have specifically identified KP expression or activation in specific cell types.

Epithelial cells

Airway epithelial cells were the first cell type described to be implicated in KP, as kynurenine was detected by immunolabelling in epithelial cells from the lungs of neonate hamsters.[19]

In addition, IDO expression by various lung epithelial cell types has been documented in humans and various mouse models (Table 1). Most of the studies have involved expression induced by a pathogen, an external stress (zinc nanoparticles) or by IFNγ. However, constitutive expression of IDO1 has also been described by Western blot in human cells.[38]

Table 1. Expression of the kynurenine pathway in airway epithelial cells.

CELL TYPE	TECHNIQUE	PATHOGEN/ STIMULUS	MOLECULE	SPECIES	REFERENCES
Lung epithelial cells of newborns	Immunohistolabeling	None	Kynurenine	hamster	Keith and Brownfield[19]
Alveolar and bronchial epithelial cells	Immunohistolabeling	TLR9 ligands	IDO	Mouse (BALB/c)	Hayashi et al[41]
Airway epithelial cells	Immunohistolabeling	*M. tuberculosis*	IDO1	Mouse (C57BL/6J)	Desvignes and Ernst[42]
Bronchial epithelial cells, bronchiolar epithelial cells, type II cells	Immunohistolabeling	Zinc oxide nanoparticles	IDO1	Mouse (C57BL/6J)	Ho et al[34]
Bronchial and alveolar epithelial cells	Immunohistolabeling	Chlamydia	IDO1 &IDO2	Mouse (BALB/c)	Virok et al[39]
Airway epithelial cells	Immunohistolabeling	herpes virus	IDO1	Mouse (C57BL/6J)	Gurczynski et al[43]
Primary epithelial cells	Western blot	Aspergillus	IDO1	Mouse (C57BL/6J)	de Luca et al[44]
Normal human bronchial epithelial (NHBE)	Immunohistolabeling	Influenza A virus	IDO1	Human	Fox et al[45]
Immortalized mouse lung epithelial cells (MLE-15)	Immunohistolabeling	Influenza	IDO1	Mouse cell line	Fox et al[45]
Human primary bronchial epithelial cells	Western blot	IFN-γ	IDO1	Human	Zegarra-Moran et al[46]
Human bronchial epithelial cells	Immunohistolabeling & western blot	Aspergillus, poly (I:C)	IDO1	Human	Iannitti et al[47]
Human airway epithelial cells	Western blot	None, constitutive, decreased by LPS	IDO1	Human	Aldajani et al[38]
Type 1 and type 2 pneumocytes	Immunohistolabeling	SARS-CoV-2	IDO2	Human	Guo et al[40]
A549 and HBE4-E6/E7 cells	Enzymatic assay	IFN-γ	IDO	Human	Heseler et al[48]
BEAS-2B cells	Western blot	IFN-γ	IDO1	Human	Chacko et al[49]

When specified, the majority of studies described IDO1 induction in lung epithelial cells and 2 studies reported IDO2 induction in lung epithelial cells: in mice following Chlamydia infection[39] and in lung tissue from patients who died of SARS-CoV-2 infection.[40] Notably, in the Chlamydia study, immunohistochemistry revealed IDO1-2 positive bronchial epithelial cells even in uninfected non-infiltrated lung tissue.

Influenza infection increases IDO1 expression in immortalized mouse lung epithelial cells (MLE-15). This effect has also been described in normal human bronchial epithelial (NHBE) cells maintained at the air-liquid interface and secreting kynurenine in the basal medium. Interestingly, influenza infection of NHBE cells also induced a significant IFN-λ and IFN-α/β production- As recombinant IFN-λ was associated with an increase of kynurenine production at the basal side, this suggested that the kynurenine production was related to the influenza-induced IFN-λ production.[45]

Endothelial cells

IDO1 expression has been observed in normal human lung endothelial cells[50,51] but also during infection, hypertension or hypoxia (Table 2).

In early phase SARS-CoV2 pneumonia, diffuse IDO expression was documented in endothelial cells in both interstitial capillaries and post-capillary venules.[54,55] In an autopsy cohort of SARS-CoV2 infected patients, an extensive accumulation of the tryptophan degradation products 3-hydroxyanthranilic acid and quinolinic acid was found in the lungs. Immunohistology showed that IDO1 expression was sparse. However, IDO2 expression was abundant in the cytoplasm of

Table 2. Expression of the kynurenine pathway in respiratory endothelial cells.

CELL TYPE	TECHNIQUE	PATHOGEN/STIMULUS	MOLECULE	SPECIES	REFERENCES
Vascular endothelium of lung	Immunohistolabeling	Plasmodium	IDO	Mouse (CBA/T6)	Hansen et al[52]
Endothelial cells	Immunohistolabeling	*M. tuberculosis*	IDO1	Mouse (C57BL/6J)	Desvignes and Ernst[42]
Lung endothelial cells	Western blot	Constitutive and induced by hypoxia	IDO	Mouse (C57BL/6J)	Xiao et al[53]
Lung endothelial cells	Immunohistolabeling	Normal human lung	IDO1	Human	Théate et al[51]
Pulmonary artery	qPCR, immunohistolabeling	Control & pulmonary arterial hypertension	IDO1	Human	Nagy et al[50]
Endothelium	Immunohistolabeling	SARS-CoV-2	IDO	Human	Chilosi et al[54], Doglioni et al[55]
Endothelial cells	Immunohistolabeling	SARS-CoV-2	IDO1 & IDO2	Human	Guo et al[40]
Endothelial cells	Immunohistolabeling	SARS-CoV-2 early/ mild pneumonia	IDO1	Human	Chilosi et al[56]
Endothelial cells	Immunohistolabeling	SARS-CoV-2 severe/ fatal cases	IDO2	Human	Chilosi et al[56]

endothelial cells, and type 1 and type 2 pneumocytes. Both 3-hydroxy-anthranilic acid and quinolinic acid co-stained with IDO2.[40] The authors suggested a role for these metabolites in apoptosis; indeed 3-hydroxyanthranilic and quinolinic acids have been shown to induce the selective apoptosis of murine Th1 thymocytes in vitro. This apoptosis; did not require Fas/Fas ligand interactions, and was associated with the activation of caspase-8 and the release of cytochrome c from mitochondria.[57] Therefore, the production of these metabolites in the lungs may influence the SARS-Cov2 pneumonia. In addition, 3-hydroxyanthranilic acid and quinolinic acid have respectively antioxidant and pro-oxidant properties that may impair redox homeostasis and immune response.[58]

In pulmonary blood vessels, IDO1 is predominant in early/mild pneumonia and in lung tissue from patients with long COVID-19, whereas IDO2 is predominant in severe/fatal cases. It has been suggested that IDO1 is necessary for the proper control of pulmonary vascular tone and its deficiency in SARS-CoV2 infection may be related to the progression of the syndrome toward vascular dysfunction.[56]

Immune cells

Antigen presenting cells. Upon stimulation with inflammatory or infectious signals, IDO was shown to be increased in several immune cell types of myeloid lineage: macrophages and dendritic cells (Table 3). IDO was induced in F4/80-positive cells (monocytes/macrophages) in the lungs of Balb/c mice injected with immunostimulatory sequence oligodeoxynucleotides (ISS-ODN: a TLR9 ligand).[41] IDO was also detected in macrophages and dendritic cells after infection with *Mycobacterium tuberculosis*.[42] or exposure to zinc nanoparticules.[34] In horses, in vitro stimulation of macrophages with LPS has shown that alveolar but not peritoneal macrophages can express IDO.[59] These different observations suggest that IDO expression is differentially regulated in different animal models and cell types.

Other kynurenine pathway enzymes were also detected in lung macrophages. IDO2 protein (and IDO1) was detected by immunohistolabeling 7 days after infection with *Chlamydia muridarum* and *Chlamydia pneumoniae* in mice, with moderate/strong positivity often detected in lung macrophages.[39] Notably, KMO mRNA expression was detected in lung BAL cells from influenza-infected mice.[62]

Other immune cells. In addition, myeloid-derived suppressor cells (MDSC) were able to express IDO in several contexts. In murine paracoccidioidomycosis, MDSC expressing IDO1 infiltrated the lungs, an effect associated with more severe disease and impaired Th1 and Th17 protective responses. It has also been shown that blood eosinophils from atopic patients constitutively express IDO1, as do eosinophils infiltrating the airway mucosa of asthmatic patients.[66]

Fibroblasts and muscle cells

Isolated lung fibroblasts infected with gamma herpes virus expressed the enzyme tryptophan dioxygenase (TDO2). Notably, IDO1 was also expressed in vascular smooth muscle cells in control lungs.[43]

Table 3. Immune cells, fibroblasts and muscle cells expressing the kynurenine pathway in the airways.

CELL TYPE	TECHNIQUE	PATHOGEN/STIMULUS	MOLECULE	SPECIES	REFERENCES
Macrophages	Immunohistolabeling	TLR9 ligands	IDO	Mouse (BALB/c)	Hayashi et al[41]
Macrophages and dendritic cells	Immunohistolabeling	*Mycobacterium tuberculosis*	IDO1	Mouse (C57BL/6J)	Desvignes and Ernst[42]
Macrophages	Immunohistolabeling	Zinc oxide nanoparticles	IDO1	Mouse (C57BL/6J)	Ho et al[34]
Alveolar macrophages	qPCR	IFN-γ, LPS	IDO1 not inducible	Mouse (C57BL/6)	Swanson et al[60]
$CD68^+$ cells	Immunohistolabeling	Naive	IDO1	Mouse (C57BL/6J)	Gurczynski et al[43]
$CD45^+$ cells	Immunohistolabeling	Gamma-herpes virus	IDO1	Mouse (C57BL/6J)	Gurczynski et al[43]
Alveolar macrophages	Immunohistolabeling, flow cytometry	hematopoietic stem cell transplantation	IDO	Mouse (C57BL/6)	Lee et al[61]
Lung macrophages	Immunohistolabeling	Chlamydia	IDO1 &IDO2	Mouse (BALB/c)	Virok et al[39]
BAL cells	qPCR	Influenza A virus	IDO1 & KMO	Mouse (BALB/c)	Cho et al[62]
Lung $CD11c^+$ dendritic cells	qPCR, Immunohistolabeling	Constitutive and inducible by IFNγ	IDO1	Mouse (C57BL/6)	Swanson et al[60]
Lung dendritic cells	qPCR	Aspergillus	IDO1	Mouse (C57BL/6)	Iannitti et al[47]
Dendritic cells	Flow cytometry	Paracoccidioidomycosis	IDO1	Mouse (C57Bl/6)	de Araújo et al[63]
Myeloid-derived suppressor cells	Flow cytometry	Paracoccidioidomycosis	IDO1	Mouse (C57BL6/J)	Preite et al[64]
Alveolar macrophages	qPCR	LPS	IDO	Horse	Karagianni et al[59]
Monocyte-derived dendritic cells	Flow cytometry	Respiratory syncytial virus	IDO1	Human	Ajamian et al[65]
Eosinophils	Immunohistolabeling	Constitutive in atopic patients	IDO1	Human	Odemuyiwa et al[66]
Eosinophils	Immunohistolabeling	Allergic patients	IDO1	Human	Odemuyiwa et al[66]
Fibroblasts	Immunohistolabeling	Gamma-herpes virus	TDO2	Mouse (C57BL6/J)	Gurczynski et al[43]
Vascular smooth muscle cells	Immunohistolabeling	Gamma-herpes virus	IDO1	Mouse (C57BL6/J)	Gurczynski et al[43]
Alveolar interstitial cells	Immunohistolabeling	LPS	IDO	Mouse (S1c:ICR)	Urade et al[16]

In summary, these data demonstrate the broad spectrum of KP component expression in different lung cell types. However, in the airways, most studies focus on the first enzymatic reaction of the kynurenine pathway (IDO1, IDO2, and TDO2) and do not address downstream reactions, despite the potential metabolic activity of downstream metabolites described in other diseases.[1]

Mechanisms of Action of Kynurenine Metabolites

Several mechanisms of action have been proposed to explain the role of KP activation in the maintenance of pulmonary homeostasis and immune defense. Historically hypothesized to act through tryptophan depletion and antioxidant control, KP has been defined as an important player implicated in modulation of the T-cell response and also in endothelial control of vascular tone.

Tryptophan depletion

Chlamydiae are obligate intracellular bacteria that proliferate in the epithelial cells of the respiratory and urogenital tracts. Tryptophan depletion via induction of IDO has been shown to be an important defense mechanism against human strains, which are more sensitive to tryptophan limitation than animal strains.[49] In addition, IDO1 mRNA was expressed at very low levels in resting human primary bronchial epithelial cells, and its expression was strongly up-regulated by treatment with IFN-γ. When grown as a polarized epithelium on permeable supports, transepithelial transport of tryptophan and kynurenine from the apical to the basolateral media was demonstrated. This was proposed as a mechanism to maintain low apical tryptophan concentrations and limit the growth of tryptophan-dependent pathogens by starving them of an amino acid.[46]

In another study, IFN-γ-stimulated lung cells were shown to be able to inhibit T-cell proliferation and restrict the replication of microorganisms such as *Toxoplasma gondii, Staphylococcus aureus* and Herpes Simplex Virus. This IFN-γ-dependent antimicrobial effect of HBE4-E6/E7 (human pulmonary bronchial epithelial cells) and A549 (human alveolar type II cells) cells was correlated with IDO1 activation and was inhibited by 1-methyltryptophan. Possible mechanisms involving IDO1 include the restriction of microbial growth by tryptophan depletion and the activation of regulatory T cells.[48]

Francisella tularensis is an intracellular gram-negative bacterium associated with fatal lung infections. IDO1 gene expression was highly induced in the lungs of mice infected with these bacteria, but not with heat-killed *Francisella.* In contrast, IDO1 gene expression remained at basal levels in infected livers and spleens. IDO2 and TDO2 were not induced in any organ. IDO1 has been proposed to exert an antimicrobial effect against bacteria by reducing intracellular levels of tryptophan.[67]

Anti-oxidant defenses

Induction of IDO in human airway epithelial cells was early described as a protective mechanism against oxidative stress induced during influenza infection.[68] Influenza infection was associated with oxidative stress and concomitant induction of lung IDO1 and production of kynurenine and 3-hydroxykynurenine. IDO1 required superoxide anion for its catalytic activity, suggesting that IDO activity may act as a local antioxidant defense.[69] KP metabolites are involved in redox reactions and their effect on cellular redox homeostasis is well documented.[70]

Modulation of the T cell response

The role of KP in modulating the T-cell response appears to be important. T cells belong to different subsets. The main function of T helper 1 (Th1) cells is to activate the cellular immune response, whereas the main function of T helper 2 (Th2) cells is to activate the antibody-mediated immune response. Among T helper cells, Th17 cells, which produce pro-inflammatory cytokines such as IL-17A, IL-17F, and IL-22, are involved in defense against extracellular bacteria and fungi. Regulatory T cells (Treg), including natural and inducible Treg, have been implicated in the prevention and control of autoimmune diseases by maintaining self-tolerance, suppressing allergy, asthma and pathogen-induced immunopathology. Among T cell populations, Treg and pro-inflammatory Th17 cells have provided a better understanding of the pathophysiology of several diseases through their role in the physiological balance between inflammatory and immunosuppressive immune responses.[71] Acting through the aryl hydrocarbon receptor, kynurenine metabolites cause T-cell anergy and apoptosis, proliferation of both Treg and Th17 cells, and reorientation of the Th1/Th2 response.[72] In the lung, the effects of kynurenines on T cells have been described in many publications and are listed in Table 4.

Reduction of T cell proliferation. Expression of IDO by epithelial cells was able to reduce T-cell proliferation in several models. When co-cultured with peripheral blood mononuclear cells (without cell-cell contact), human epithelial cells constitutively express IDO1 under resting conditions. After stimulation with tuberculin, T cells proliferate and produce IFN-γ, but the presence of epithelial cells inhibits this proliferation. When stimulated with different TLR ligands, the ability of epithelial cells to suppress T-cell proliferation was reduced. This was associated with a reduction in IDO1 induction. Using gene silencing, it was further shown that IDO1 plays a key role in epithelial cell-mediated suppression of T cell proliferation. Thus, epithelial cells can lose their inhibitory effect on T cell activation in response to various TLR agonists that mimic bacterial or viral infections.[38]

Another study used a mouse transformed airway epithelial cell line overexpressing IDO1. In vitro, IDO1 epithelial expression was able to reduce the proliferation of CD4+ T cells. A triple transgenic mouse was therefore created in which IDO was overexpressed only in the non-ciliated airway epithelial cells of the lung (CC10-IDO). When these mice were sensitized and challenged with *Aspergillus fumigatus* hyphal antigens to induce a hypersensitivity reaction, a significant induction of IDO1 was observed in their lungs, but they showed no alterations in pulmonary methacholine hyperresponsiveness. However, the number of CD4+ T cells in the inflamed lung was reduced and the ability of antigen-specific splenic CD4+ effector T cells to secrete IL-4, IL-5, IL-13 and IFN-γ was impaired.[75]

In addition, systemic blockade of IDO1 resulted in spontaneous lung T-cell proliferation and lung inflammation[60] confirming the key role of IDO1 in controlling T-cell proliferation in the lung.

Modulation of the Th1 and Th17 responses. Interferon gamma (IFN-γ) is essential for limiting progressive, lethal infection with Mycobacterium tuberculosis. Tuberculosis is a pulmonary pathology in which inhibition of the Th17 response by kynurenine metabolites has been documented.[42] It has been shown that IFN-γ-responsive lung epithelial and endothelial cells are also required for protective immunity against tuberculosis. Using chimeric mice reconstituted with bone marrow cells, it has been shown that when hematopoietic cells, including macrophages, are unable to respond to IFN-γ, the response of non-hematopoietic cells (epithelial and endothelial cells) to IFN-γ is detrimental. In contrast, bone marrow chimeric mice with IFN–γ-unresponsive lung epithelial and endothelial cells are able to survive the acute phase of infection. Non-hematopoietic cells contribute to the IFN-γ-dependent recruitment of myeloid cells to the lung during

Table 4. Mechanisms of action of kynurenine metabolites.

	MECHANISM	PATHOLOGY/STIMULUS	REFERENCES
Tryptophan depletion			
Induction of IDO	Tryptophan depletion	Chlamydiosis, Tularemia	Chacko et al[49], Peng and Monack[67]
IDO1 in human primary bronchial epithelial cells	Depletion of apical tryptophan (antimicrobial mechanism)	None	Zegarra-Moran et al[46]
IDO1 induction in airway epithelial cells	Antimicrobial effect against *T. gondii, S. aureus* and HSV	Stimulation with IFN-γ	Heseler et al[48]
Anti-oxidant defenses			
5-hydroxytryptophan, 3hydroxykynurenine, xanthurenic acid, or 3-hydroxyanthranilic acid, IDO	Anti-oxidant	Viral pneumonia, influenza A virus	Christen et al[69], Jacoby and Choi[68]
Modulation of T cell response			
IDO activation by TLR9 ligands	Th2 cell death	Experimental asthma	Hayashi et al[41]
IDO1 in eosinophils	sustain Th2 proliferation, inhibit Th1 proliferation	Asthma, allergy	Odemuyiwa et al[66]
Prevention of sensitization to allergen	kynurenine and 3-HAA inhibit T cell proliferation	Experimental sensitization	Odemuyiwa et al[73]
IDO1 in dendritic cells	RSV-induced IDO activity via Rig-I, suppression of Th1 but not of Th2 related factors	Respiratory syncytial virus	Ajamian et al[65]
IDO1 expression in leukocytes	Regulation of Th17- and Th1-type neutrophilic inflammation	Rhinovirus-induced asthma model	Hossain et al[74]
IDO1 in airway epithelial cells	Suppression of T cell proliferation	TLR ligands	Aldajani et al[38]
IDO1 in airway epithelial cells	Reduction of the proliferation of $CD4^+$ T Cells	Aspergillosis	Paveglio et al[75]
IDO expression in plasmacytoid dendritic cells	Stimulation of Treg proliferation	Paracoccidioidomycosis	Araújo et al[76]
IDO1–/– mice	Reduction in pulmonary Treg	paracoccidioidomycosis	de Araújo et al[63]
Lung epithelial and endothelial cells production of tryptophan catabolites	Inhibition of Th17 cell differentiation	*M. tuberculosis*	Desvignes and Ernst[42]
IDO1 inhibition	Modulation of memory T cell response	influenza A virus	Sage et al[77]
Modulation of vascular tone			
IDO activation in endothelial cells	Protective mechanism against pulmonary hypertension	hypertension	Xiao et al[53]
Kynurenine production by pulmonary artery	Increase in acute pulmonary vasodilation (feedback loop)	hypertension	Nagy et al[50]

M. tuberculosis infection. Gene expression profiling shows that IDO1 is underexpressed in airway epithelial and vascular endothelial cells in the absence of IFN-γ. Mice with IFN-γ unresponsive non-hematopoietic cells developed an excessive IL-17 response. Kynurenine metabolites were able to inhibit Th17 cell differentiation, suggesting that their absence triggers excessive IL-17 production[42];

In a model of rhinovirus (RV)-induced neutrophilic airway inflammation, IDO ablation enhanced allergen-specific Th17- and Th1-biased CD4+ T cell responses following human RV infection. IDO activity in hematopoietic stem cell-derived leukocytes was required to regulate Th17- and Th1-type neutrophilic airway inflammation during asthma exacerbations in this mode.[74]

Promotion of the Th2 response. Induction of pulmonary IDO activity by TLR9 ligands inhibited experimental asthma in mice via a Th2 cell death mechanism.[41] Eosinophils are consistently increased in number in allergic asthma. Blood eosinophils from atopic patients have been shown to constitutively

express IDO1, in contrast to monocytes and macrophages, which do not express IDO1 mRNA transcripts until activated with IFN-γ. In addition, eosinophils infiltrating the airways of asthmatic patients express IDO1. Coculture of eosinophils with a Th1 cell line activated with anti-CD3 antibodies resulted in inhibition of their proliferation, whereas this was not the case for Th2 cells. It has therefore been suggested that IDO1 expression in eosinophils maintains the Th2 polarization seen in diseases characterized by eosinophilic inflammation, including asthma.[66]

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract (LRT) disease in infancy and early childhood. Severe RSV bronchiolitis in infancy is considered a risk factor for the development of allergic asthma and early-onset COPD. RSV infection of human mesenchymal stem cells upregulates IFN-β and IDO and affects immune cell proliferation, which may explain the lack of protective RSV immunity and the chronicity of RSV-associated lung diseases such as asthma and COPD.[78] By triggering Th2-biased immune responses, RSV activation of IDO has been suggested as a potential mechanism for the development of allergic disease.[65] Infants who develop severe bronchiolitis due to RSV have an increased risk of developing asthma later in life. RSV is able to infect human dendritic cells derived from circulating monocytes. Supernatants from these infected cells produced higher levels of kynurenine compared to uninfected cells or cells infected with inactivated virus. Treatment with IDO inhibitors reduced the activity of the enzyme IDO1 and the release of kynurenine in response to RSV. Induction of IDO1 required active virus replication and was blocked by ribavirin, an inhibitor of virus replication. Coculture of RSV-infected monocyte-derived dendritic cells with activated T cells suppressed expression of T-bet (a Th1-associated factor) but not GATA3 (a Th2 regulator) through activation of the retinoic acid-inducible gene-I (RIG-I)-related pathway via NF-κB and p38 MAPK.[65] Overall, IDO activation will promote the Th2 response and the development of an allergic lung reaction.

Increasing Treg response. As a counterpart to Th17 inhibition, Treg induction by kynurenine metabolites has been demonstrated in several respiratory diseases. Plasma kynurenine concentrations and the kynurenine/tryptophan ratio correlated positively with inducible Treg/Th17 and inversely with Th17 subsets in blood samples from patients with pulmonary arterial hypertension.[79]

Paracoccidioidomycosis, a primary fungal infection, is acquired by inhalation of fungal particles of *Paracoccidioides brasiliensis.* Mice infected with *P. brasiliensis* have a high expression of IDO mRNA in their lungs.[80] In susceptible mice, IDO inhibition can lead to uncontrolled tissue pathology and mortality, demonstrating that the KP pathway may be involved in host defense.[76] IDO1 activation was shown to control disease severity in a mouse model of pulmonary paracoccidioidomycosis caused by the dimorphic fungus *P. brasiliensis*, whereas IDO1-deficient (IDO1-/-) C57BL/6 mice showed increased mortality rates associated with increased fungal burden and tissue damage. *P. brasiliensis* infection was also shown to induce an increased frequency and number of IDO-expressing plasmacytoid dendritic cells compared to uninfected mice. It has been suggested that these cells have a tolerogenic function that increases Treg activity in this infection.[81] An increased number of type 3 innate lymphoid cells (ILC3) was reported in the lungs of infected IDO1-/- mice, while a decreased expansion of ILC1 and NK cells was observed. A concomitant decrease in mRNA for the Foxp3 transcription factor was observed in IDO1-deficient mice. In contrast, RORγC and GATA3 mRNAs were significantly upregulated in IDO1-/- mice. Taken together, this led to a marked reduction in pulmonary Treg (CD4+ CD25+ Foxp3+) cells and an expansion of Th17 cells in IDO1-/- mice at 2 and 10 weeks of infection.[63]

The involvement of IDO activity in the control of T cell responses and lung physiopathology has also been reported during respiratory infection with influenza virus. Yoshida et al demonstrated a high induction of IDO in the trachea and lungs of mice exposed to influenza virus.[82] IDO mRNA increased in the lungs of influenza-infected mice.[83] The effect of IDO on the primary immune response to influenza virus infection was determined using the IDO inhibitor 1-methyl-D,L-tryptophan (1 MT). IDO inhibition had no effect on leukocyte infiltration or viral replication. Inhibition of IDO activity enhanced the Th1 cytokine response, enhanced the Th17 response and increased the number of influenza-specific CD8+ T cells.[84] Drug analog inhibition of IDO activity increased pro-inflammatory cytokine gene and protein expression at 24 and 48 hours post-influenza infection compared to control treated mice.[85] This inhibition reduced tissue damage associated with a lethal influenza challenge.[77] Surprisingly, the opposite effect was observed in another study using IDO KO mice. IDO1 KO mice showed significantly lower morbidity after influenza infection.[86] These seemingly contradictory observations suggest that in addition to its role during acute infection, IDO1 may also play a role in the development and shaping of the immune system.

Taken together, these data show that IDO activity can effectively control lung lesions during respiratory infections, but may also be involved in immune cell development.

Vascular tone control

Pulmonary arterial hypertension (PAH) is a form of hypertension of the pulmonary arteries in which the chronic inflammatory process is widely recognized as a prominent pathogenic component. In a mouse model of experimental PAH, overexpression of IDO in the pulmonary endothelium was shown to halt and attenuate vascular structural remodeling.[53] It has also

been reported that PAH is characterized by upregulated tryptophan metabolism and increased kynurenine biosynthesis.[79]

In another study using human lung explants from control and PAH patients, IDO localization by immunofluorescence showed the presence of IDO in laser-captured microdissected pulmonary arteries and in lung homogenates from both groups. However, tryptophan levels were significantly reduced in the lungs of PAH patients.[50] Serum kynurenine levels correlate strongly with mean pulmonary arterial pressure in PAH. Kynurenine has been shown to increase cAMP and cGMP in pulmonary arterial smooth muscle cells and to cause acute pulmonary vasodilatation in intact pulmonary arteries, isolated perfused lungs and animal models of PAH. Increased pulmonary production of kynurenines may therefore serve as a negative feedback mechanism in response to PAH.[50]

A recent study confirms these observations by reporting an activation of KP with lower levels of tryptophan and higher concentrations of kynurenine, 3-hydroxykynurenine, quinolinic acid, kynurenic acid and anthranilic acid in treatment-naïve PAH patients compared to controls. PAH therapy partially normalized this profile in survivors after 1 year. KP metabolites correlated with PAH severity at baseline and predicted mortality in PAH patients.[87]

Direct modulation of the kynurenine pathway by endogenous pathogen molecules and the role of host and pathogen polymorphisms

Interestingly, some pathogens are able to directly interfere with host KP regulation. The influenza protein NS1 has been shown to directly attenuate IDO1 and kynurenine production.[88] *Pseudomonas aeruginosa*, a Gram-negative bacterium, infects the lungs and is involved in healthcare-associated pneumonia with poor clinical outcome. *P. aeruginosa* carries the genes for the KP and therefore synthesizes kynurenine metabolites responsible for immunomodulatory crosstalk with the host.[89]

In aspergillosis, both host and pathogen genetic variations have been implicated in the severity of disease. Aspergillosis is an infection caused by inhalation of Aspergillus spores, a common indoor and outdoor mold. IDO expression is induced in epithelial cells from mice infected with *Aspergillus fumigatus*, while exogenous supply of kynurenines inhibits fungal growth and improves host defense.[44] In humans, genetic polymorphisms affecting IDO1 were associated with the risk of *Aspergillus* infection and appeared to down-modulate IDO1 expression. In contrast, IDO2 polymorphisms were differentially associated with the risk of aspergillosis in 2 cohorts of patients, as no association was found in cystic fibrosis patients compared to hematopoietic stem cell transplant recipients.[90] In aspergillosis, tryptophan catabolism from the host or from the microbe has co-evolved and cross-talks contribute to the regulation of inflammation and control of infection.[91] *A. fumigatus* possesses 3 ido genes that are expressed under hypoxic or tryptophan-rich conditions. Loss of these genes results in increased fungal pathogenicity and inflammation in a mouse model of aspergillosis. These results suggest that fungal IDOs enable the fungus to establish a close functional metabolic relationship with its mammalian host.[92]

In another study, single nucleotide polymorphisms (SNPs) in the promoters or coding regions of IDO2 were shown to affect its enzyme activities and to affect the K/T ratio in tuberculosis patients.[93]

Asthma and COPD: Defective Activation of the Kynurenine Pathway?

As reported above, pro-inflammatory signals are important inducers of IDO activation, which is involved in the resolution of inflammation. However, in chronic inflammatory lung diseases such as asthma and COPD, subjects have an exaggerated inflammatory response that is responsible for altering their lung function. This group of diseases causes airflow obstruction and progressive breathlessness associated with chronic bronchitis and emphysema, respectively. Our hypothesis is that these patients are in a state where the KP response is insufficient to ensure a return to homeostasis.

Asthma is an inflammatory disease of the airways leading to bronchial hyperreactivity and airway obstruction. First observations in 1970 described higher serum kynurenine in asthma and chronic bronchitis than in normal subjects.[94] However, in a rat model of asthma, kynurenine levels remain unchanged in the plasma, lungs or liver of asthmatic animals compared with controls.[95]

Tryptophan catabolism differs between healthy subjects and patients with allergic asthma. Pulmonary IDO activity, as assessed by tryptophan and kynurenine concentrations in exhaled breath condensate, was lower in patients with allergic asthma than in healthy subjects and was not increased by experimental rhinovirus infection. In contrast, these experimental rhinovirus infections significantly increased blood concentrations of tryptophan and its catabolites in patients with allergic asthma and were associated with eosinophilic inflammation and asthma symptom scores. The reduced pulmonary IDO activity in patients with allergic asthma at baseline may underlie the reduced control of viral infections.[96]

This high serum tryptophan concentration has been confirmed in other cohorts of patients with pollinosis, allergic rhinitis or asthma[97-99]. Booster immunotherapy in hay fever patients induced a decrease in serum tryptophan and kynurenine concentrations, demonstrating the involvement of tryptophan metabolism in the course of allergic responses.[100]

The effect of background treatment of chronic inflammatory respiratory diseases on KP has been little studied. However, corticosteroid treatment in asthmatic patients may also affect IDO activity. IDO activity was significantly increased by corticosteroid treatment in patients with mild intermittent and mild-to-moderate persistent asthma, and this increase was negatively correlated with sputum eosinophils.[101] At baseline, all patients had low IDO activity in induced sputum compared

to age-matched non-asthmatic subjects. Statin use enhanced the anti-inflammatory effects of inhaled corticosteroids in asthmatics by increasing IDO induction.[102]

COPD is a common respiratory disease characterized by progressive and incompletely reversible airflow obstruction. IDO activity and expression was reduced in the sputum of patients with COPD and inversely correlated with clinical severity. This suggests that inflammation in patients with COPD may be enhanced by the lack of IDO induction and that this may be a factor in determining disease severity and long-term disease progression.[103] IDO mRNA expression (fold change) and IDO activity (K/T ratio) were significantly increased during treatment with simvastatin, a compound with anti-inflammatory properties, compared with placebo in COPD patients.[104]

Taken together, these data show that these lung inflammatory diseases are associated with a defect in KP in relation to the level of local inflammatory response. This suggests that an alteration in this resolution mechanism may be involved in the development and progression of the disease, a process that is counteracted by anti-inflammatory treatments.

The [Kynurenine]/[Tryptophan] Ratio in Respiratory Diseases

In this section we will focus on the potential use of monitoring the ratio of kynurenine to tryptophan concentrations (K/T ratio) as a valuable marker in respiratory disease. In in vitro studies, the activation of IDO was first evaluated by monitoring this ratio. The use of serum or plasma K/T ratio evaluation has been further extended in vivo to determine IDO activation, allowing this ratio to be defined as a marker of inflammation. However, determinants other than IDO may be involved in K/T variations in vivo.[105] Table 5 gives an overview of the different studies that have analyzed the K/T ratio in relation to lung diseases.

Infectious diseases

The most impressive increase in the K/T ratio was recently described in patients infected with SARS-CoV-2, which can cause coronavirus induced disease 2019 (COVID-19), a disease characterized by activated immuno-inflammatory pathways and, in the most severe cases, a cytokine storm. During infection, increased levels of IFN-γ, IL-1β, IL-6, and ROS can induce IDO, which activates tryptophan catabolism. A meta-analysis was performed including 14 articles comparing tryptophan and its catabolites in COVID-19 patients versus non-COVID-19 controls, as well as severe/critical versus mild/moderate COVID-19. Increased IDO enzyme activity was demonstrated in COVID-19 and severe/critical patients, and the involvement of tryptophan catabolites in the pathophysiology and progression of COVID-19 was suggested.[9]

Pulmonary tuberculosis (TB) is a serious infection caused by Mycobacterium tuberculosis that affects the lungs but can spread to other organs. In an attempt to find new biomarkers, tryptophan and kynurenine were analyzed in the sera of patients with pulmonary tuberculosis. The data showed a significant increase in kynurenine concentrations and a significant decrease in tryptophan concentrations compared to controls. Among TB patients, non-survivors had significantly higher kynurenine concentrations and significantly lower tryptophan concentrations, resulting from a significant increase in IDO activity compared to survivors.[125] Since this study, several other groups have confirmed the link between IDO activity and tuberculosis diagnosis or severity.[106,107,109,110,126] In addition, IDO1 may have diagnostic value in the early identification of multidrug-resistant (MDR) TB patients, as IDO1 has been positively correlated with cavitary lung lesions in TB patients, potentially indicating a more severe infectious state and a higher risk of developing MDR-TB.[111] These studies were also validated by metabolomics analysis, which showed that increased IDO activity helps to distinguish TB patients from healthy individuals.[108]

Seasonal influenza is an acute respiratory infection caused by influenza viruses circulating in all parts of the world. Influenza infection increases IDO activity (K/T ratio) in the lungs and sera of influenza-infected mice.[84] Increased IDO activity Is associated with poor clinical outcome in adults hospitalized with influenza.[112]

Chronic inflammatory pulmonary diseases and exacerbations

Among cancer-free adults, current smokers had lower levels of almost all KP components compared with never-smokers, but a similar K/T ratio. Conversely, former smokers showed a small increase in the K/T ratio compared with never smokers.[114] Similar results have been observed in another cohort.[115] This may indicate that in former smokers, smoking cessation revealed an acquired imbalance. Compared with controls, COPD patients who were predominantly active or former smokers had significantly higher plasma Kyn concentrations and K/T ratios. The K/T ratio was independently associated with COPD severity after adjustment for several clinical and demographic confounders.[116,127] These observations need to be reproduced, as another study showed that the K/T ratio was higher in non-smokers than in smokers, but similar to controls in COPD patients and higher in COPD patients who were ex-smokers.[117]

Discrepancies between these results may be explained by the small effect of COPD on the K/T ratio and its dependence on the small number of samples in some studies and the lack of adjustment for sex or age and/or the presence of confounders such as an additional infection. It should be noted that in another cohort, analysis of the serum of patients with smoking-associated COPD showed that the K/T ratio was not significantly different from that of the control group, whereas patients with tuberculosis-associated COPD had an

Table 5. K/T ratio in lung disease.

	OBSERVED ASSOCIATION	REFERENCES
Infectious diseases		
COVID-19		
Meta-analysis	K/T elevation signals a worsening outcome	Almulla et al[9]
Tuberculosis		
Pediatric tuberculosis	Diagnostic	Tornheim et al[106]
Progression from latent to active TB disease	Diagnostic	Adu-Gyamfi et al[107], Weiner et al[108], Collins et al[109]
Progression from latent to active TB disease	Macaque model	Gough et al[110]
Multi-drug resistant (MDR) tuberculosis	Discriminating MDR-TB patients from TB patients cutoff = 46.58 μM/mM	Shi et al[111]
Influenza		
Adults hospitalized with influenza A (H1N1)	Poor clinical outcome	Pett et al[112]
Pneumoniae		
Community acquired-pneumoniae	Increased K/T ratio and decreased with resolution	Arshad et al[113]
Chronic inflammatory lung disease and exacerbations		
Smoking and COPD		
Smokers versus never smokers	Similar K/T ratio	Zahed et al[114], Theofylaktopoulou et al[115]
Former smokers versus never smokers	K/T enhanced in former smoker	Zahed et al[114], Theofylaktopoulou et al[115]
COPD	K/T higher in COPD and associated with COPD severity	Zinellu et al[116], Arshad et al[113]
Non-smokers versus smokers	Higher K/T in smokers	Naz et al[117]
COPD versus control	Similar K/T -Higher K/T in former smokers	Naz et al[117]
Tuberculosis or HIV-associated COPD	Higher K/T in infected patients	Kim et al[118], Hodgson et al[119]
COPD exacerbation	Increased K/T ratio during exacerbations	Gulcev et al[120]
COPD: sputum analysis	Decreased K/T ratio in sputum	Maneechotesuwan et al[103]
Asthma and atopy		
Asthma and atopy	Decreased K/T in atopy, similar K/T in asthma	Luukkainen et al[121]
Childhood allergic asthma	Decreased K/T ratio	Hu et al[122]
Children asthma and allergic rhinitis	Decreased K/T ratio	Ünüvar et al[123]
Cystic fibrosis		
Exacerbation in CF	Increased K/T ratio and decrease with resolution	Muhlebach et al[124]

increase in the serum K/T ratio.[118] Similarly, K/T ratio was significantly higher in COPD patients with HIV, but this difference was not due to COPD status but rather to viral infection.[119] Exacerbations are a major cause of morbidity in COPD, mostly due to respiratory infections. Patients with acute exacerbations of COPD have a unique metabolomic signature that includes a decrease in plasma tryptophan levels, consistent with an increased K/T ratio.[120] In another study, an increased K/T ratio was observed in patients with COPD or community-acquired pneumonia on admission to hospital. The level of induction is higher in patients with pneumonia, but rapidly normalizes with clinical recovery and resolution of

inflammation, confirming that the K/T increase is influenced by pre-existing pulmonary infection.[113]

A unique study examined the K/T ratio in sputum from COPD patients and showed that the ratio was lower in these patients. Interestingly, this ratio was associated with clinical severity. Inflammation is enhanced in COPD patients and is a factor in determining disease severity and long-term disease progression. This study suggests that the lack of IDO activation in the lungs of COPD patients leads to a defect in the resolution of inflammation.[103] It also highlights that the local inflammatory response associated with COPD does not affect systemic parameters, at least as far as KP is concerned.

As in COPD, acute exacerbations in cystic fibrosis (CF) patients, mostly due to respiratory infections caused by Pseudomonas aeruginosa, are major life events associated with decline in lung function. Reduced tryptophan/kynurenine metabolism as a result of IDO enzyme deficiency has been demonstrated in murine CF and in human bronchial epithelial cells from CF patients.[47] The serum profile of metabolites in patients with cystic fibrosis was evaluated to differentiate between pre- and post-exacerbation states and to determine which pathways are affected during the process of recovery. In this study, the K/T ratio decreased after therapy, consistent with resolution of inflammation.[124]

Cohorts of asthmatic patients have shown a trend toward reduced IDO activation and K/T ratio in this disease. The first description of higher serum kynurenine levels in asthma and chronic bronchitis than in normal subjects was published in 1970.[94] However, tryptophan levels were not assessed in this study. In another cohort, low IDO activity, as assessed by serum K/T ratio, was associated with atopy but not with asthma.[121] In a cohort of children, peripheral blood IDO activity, as assessed by K/T ratio and induced sputum, was significantly lower in patients with childhood allergic asthma than in children in the control group.[122] Another cohort of children supported these observations with higher serum tryptophan and kynurenine levels and lower IDO1 enzyme activity in patients with asthma and allergic rhinitis compared to controls.[123]

Overall, these studies suggest that pulmonary infection increases the serum K/T ratio, whereas asthma tends to decrease it. The picture is less clear for COPD, as smoking or smoking history affects the K/T ratio. Nevertheless, serum K/T ratio is correlated with COPD severity and is also increased by exacerbations induced by respiratory pathogens.

Interventional Approaches Targeting the Kynurenine Pathway in Respiratory Disease?

Targeting KP in respiratory diseases has not yet been described in clinical trials. However, several reports based on preclinical models suggest that modulation of this pathway may be useful in several pathologies.

The role of IDO in the development of allergic sensitization leading to allergic inflammation and airway hyperresponsiveness was investigated in a mouse model of induction of mucosal tolerance to lipopolysaccharide-free ovalbumin. Slow-release pellets containing 10 mg of 1-methyl-dl-tryptophan (1-MT, an IDO inhibitor) were implanted subcutaneously and were able to inhibit tolerance induction. In addition, tolerance was reconstituted in mice receiving 1-MT after intraperitoneal injection of a mixture of kynurenine and hydroxyanthranilic acid, 2 downstream metabolites. Products of tryptophan catabolism therefore play an important role in the prevention of allergic diseases.[73] Similarly, allergen immunotherapy by subcutaneous administration of allergen extract is used to treat allergic diseases. In a mouse model of ovalbumin sensitization, inhibition of IDO by 1-methyl-DL-tryptophan during immunotherapy, but not during inhalation challenge, partially reversed the suppressive effects of immunotherapy on airway eosinophilia and Th2 cytokine levels. Mice were then treated with suboptimal immunotherapy and tryptophan or one of its metabolites or saline. Interestingly, administration of tryptophan or its metabolites, kynurenine, 3-hydroxykynurenine, and xanthurenic acid, but not 3-hydroxyanthranilinic acid, quinolinic acid, and kynurenic acid, during suboptimal immunotherapy potentiated the reduction of eosinophilia in this model. Therefore IDO activity as well as some tryptophan metabolites contributes to tolerance induction during allergen immunotherapy.[128]

However, the role of KP in infectious diseases appears to be more complex, involving different mechanisms depending on the type of pathogen, bacteria, viruses or fungi.

In some models, IDO1 expression had no effect on the outcome of infection. Although *M. tuberculosis* induces robust expression of IDO1 and activation of tryptophan metabolism, IDO1 deficiency does not affect immune control and the outcome of infection in the mouse model of tuberculosis.[129] Interestingly, IDO expression is highly induced in tuberculosis lesions in non-vaccinated macaques. IDO expression was largely found in the ring-wall structure of tuberculosis granulomas, within the macrophage-rich region.[130] In this model, IDO inhibition was shown to reduce the bacterial burden, pathology and clinical signs of TB disease, leading to increased host survival[131] and improved treatment of tuberculosis with chemotherapy by enhancing proliferative T-cell responses and recruitment of effector T-cells to the lung.[132] These data highlight discrepancies between the efficacy of mouse and macaque KP in controlling TB, where IDO expression appears to be deleterious in macaques and non-discriminatory in mice.

In another model, IDO1 expression was also detrimental to infection defense. Histoplasmosis is an infection caused by the fungus Histoplasma after inhalation of the microscopic fungal spores. In a mouse model of intranasal histoplasma infection, functional IDO was induced in the lungs of the mice. Mice treated with an IDO inhibitor had a lower fungal burden in the lungs and less IL-6 and IL-17 expression.[133]

However, IDO1 expression appears to be effective against influenza virus infection. Increased lung inflammation and cellular infiltration was observed in aged mice during influenza

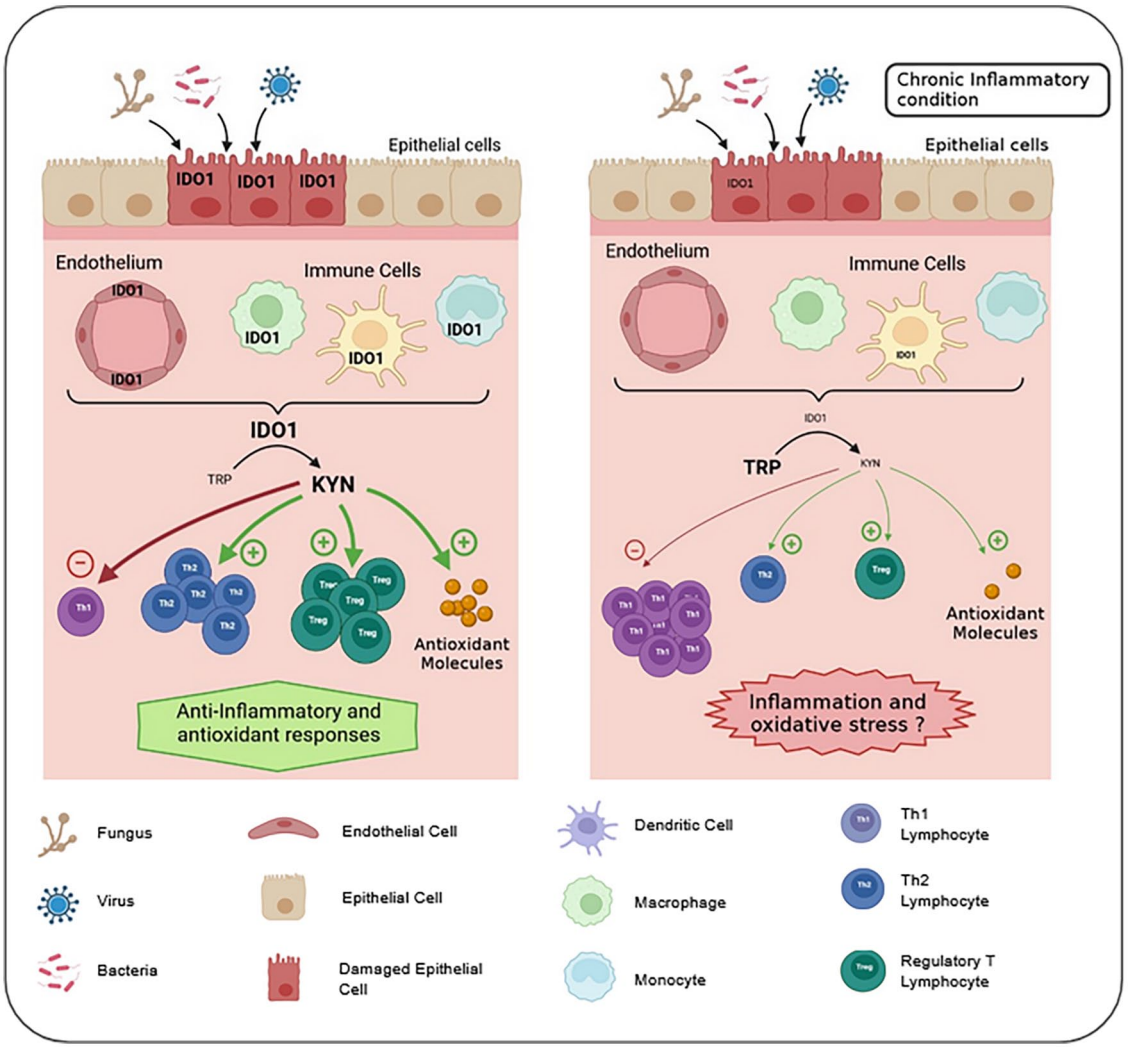

Figure 2. Activation of the kynurenine pathway in the airways.
During respiratory infection, fungi, bacteria or viruses induce IDO1 expression and enzymatic activity in epithelial, immune or even endothelial cells. Tryptophan is degraded to kynurenine metabolites, which modulate T-cell responses by downregulating Th1 and upregulating Th2 and Treg responses, and have antioxidant properties. Our hypothesis is that in chronic inflammatory conditions the IDO1 response is reduced and therefore the development of inflammation and oxidative stress is less controlled and may lead to exacerbations.

infection and was associated with reduced IDO activation. Daily in vivo treatment of young adult mice with an IDO1 inhibitor resulted in increased cellular infiltration, significantly greater weight loss and increased viral titers, as well as increased production of pro-inflammatory cytokines such as IL-6 and decreased production of anti-inflammatory cytokines such as IL-10. Compared with young lungs, aged BAL cells also showed reduced Ido1 and Kmo mRNA expression during influenza infection.[62] Inhibition of IDO resulted in a worsening of the pathology. Aged bone marrow-derived macrophages treated with mitoquinol, a mitochondrial-targeted antioxidant, showed a significant increase in IDO1 expression, an increased kynurenine-to-tryptophan ratio after stimulation with LPS and poly I:C, and a corresponding decrease in IL-6 production. Treatment of aged mice with mitoquinol after influenza infection reduced weight loss and viral titers, improved mitochondrial function and restored tryptophan metabolism.[62]

These studies demonstrated the need for a very precise knowledge of KP and the imbalance of kynurenine metabolites that can be either beneficial, harmful or neutral depending on the pathogen, the host or the time course of infection. However, we have noticed that other kynurenine pathway enzymes, apart from IDO1, are rarely studied in the respiratory tract and the possible activity of downstream metabolites is not even addressed. Further studies are essential to design a more appropriate pharmacological target. Future work is needed to determine if we can extend these observations to other bacterial and viral pathogens and to translate these data to the context of acute exacerbations associated with lung inflammatory diseases.

Conclusion

Kynurenines are produced in the airways following exposure to pathogens or inflammatory stimuli. In response to inflammation, epithelial, endothelial and myeloid immune cells are capable of expressing KP enzymes and producing bioactive kynurenine metabolites (Figure 2). The mechanisms involved range from tryptophan depletion and antioxidant defense to modulation of the T-cell response, with increased levels of Treg

cells and inhibition of Th17 differentiation. Numerous epidemiological studies have now demonstrated the validity of using the K/T ratio as a systemic marker of active infection, especially in tuberculosis. However, in the field of chronic inflammatory diseases such as asthma or COPD, the role of kynurenine enzymes and metabolites is less clear, with potentially opposing functions during the time course of pathology, ranging from the response to environmental factors, to the non-resolution of chronic inflammation, to the ability to control an exacerbation episode following infection. To date, and to the best of our knowledge, despite promising results in several preclinical models, no clinical pharmaceutical intervention has been carried out. All these data clearly show that more research is needed to fully understand the multiple biological functions of KP metabolites and their potential therapeutic applications.

REFERENCES

1. Badawy AA. Kynurenine pathway and human systems. *Exp Gerontol*. 2020; 129:110770.
2. Joisten N, Ruas JL, Braidy N, Guillemin GJ, Zimmer P. The kynurenine pathway in chronic diseases: a compensatory mechanism or a driving force? *Trends Mol Med*. 2021;27:946-954.
3. Savitz J. The kynurenine pathway: a finger in every pie. *Mol Psychiatry*. 2019; 25:131-147.
4. Favennec M, Hennart B, Caiazzo R, et al. The kynurenine pathway is activated in human obesity and shifted toward kynurenine monooxygenase activation. *Obesity*. 2015;23:2066-2074.
5. Metghalchi S, Ponnuswamy P, Simon T, et al. Indoleamine 2,3-dioxygenase fine-tunes immune homeostasis in atherosclerosis and colitis through repression of interleukin-10 production. *Cell Metab*. 2015;22:460-471.
6. Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. *Nat Rev Drug Discov*. 2019;18:379-401.
7. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe*. 2018;23:716-724.
8. Abd El-Fattah EE. IDO/kynurenine pathway in cancer: possible therapeutic approaches. *J Transl Med*. 2022;20:347.
9. Almulla AF, Supasitthumrong T, Tunvirachaisakul C, et al. The tryptophan catabolite or kynurenine pathway in COVID-19 and critical COVID-19: a systematic review and meta-analysis. *BMC Infect Dis*. 2022;22:615.
10. Yeung AW, Terentis AC, King NJ, Thomas SR. Role of indoleamine 2,3-dioxygenase in health and disease. *Clin Sci*. 2015;129:601-672.
11. Yoshida R, Hayaishi O. Induction of pulmonary indoleamine 2,3-dioxygenase by intraperitoneal injection of bacterial lipopolysaccharide. *Proc Natl Acad Sci*. 1978;75:3998-4000.
12. Hayaishi O. Properties and function of indoleamine 2, 3-dioxygenase1. *J Biochem*. 1976;79:13-21.
13. Yoshida R, Imanishi J, Oku T, Kishida T, Hayaishi O. Induction of pulmonary indoleamine 2,3-dioxygenase by interferon. *Proc Natl Acad Sci*. 1981;78:129-132.
14. Yoshida R, Oku T, Imanishi J, Kishida T, Hayaisi O. Interferon: A mediator of indoleamine 2,3-dioxygenase induction by lipopolysaccharide, poly(I) · poly(C), and pokeweed mitogen in mouse lung. *Arch Biochem Biophys*. 1986;249:596-604.
15. Bianchi M, Bertini R, Ghezzi P. Induction of indoleamine dioxygenase by interferon in mice: A study with different recombinant interferons and various cytokines. *Biochem Biophys Res Commun*. 1988;152:237-242.
16. Urade Y, Yoshida R, Kitamura H, Hayaishi O. Induction of indoleamine 2,3-dioxygenase in alveolar interstitial cells of mouse lung by bacterial lipopolysaccharide. *J Biol Chem*. 1983;258:6621-6627.
17. Daley-Yates PT, Powell AP, Smith LL. Pulmonary indoleamine 2,3-dioxygenase activity and its significance in the response of rats, mice, and rabbits to oxidative stress. *Toxicol Appl Pharmacol*. 1988;96:222-232.
18. Fujigaki S, Saito K, Takemura M, et al. Species differences in L-tryptophan-kynurenine pathway metabolism: quantification of anthranilic acid and its related enzymes. *Arch Biochem Biophys*. 1998;358:329-335.
19. Keith IM, Brownfield MS. Evidence for presence of kynurenine in lung and brain of neonate hamsters. *Histochemistry*. 1985;83:465-471.
20. Saito K, Markey SP, Heyes MP. Effects of immune activation on quinolinic acid and neuroactive kynurenines in the mouse. *Neuroscience*. 1992;51:25-39.
21. Saito K, Crowley JS, Markey SP, Heyes MP. A mechanism for increased quinolinic acid formation following acute systemic immune stimulation. *J Biol Chem*. 1993;268:15496-15503.
22. Saito K, Markey SP, Heyes MP. Chronic effects of gamma-interferon on quinolinic acid and indoleamine-2,3-dioxygenase in brain of C57BL6 mice. *Brain Res*. 1991;546:151-154.
23. Saito K, Lackner A, Markey SP, Heyes MP. Cerebral cortex and lung indoleamine-2,3-dioxygenase activity is increased in type-D retrovirus infected macaques. *Brain Res*. 1991;540:353-356.
24. Heyes MP, Saito K, Chen CY, et al. Species heterogeneity between gerbils and rats: quinolinate production by microglia and astrocytes and accumulations in response to ischemic brain injury and systemic immune activation. *J Neurochem*. 1997;69:1519-1529.
25. Sayama S, Yoshida R, Oku T, et al. Inhibition of interferon-mediated induction of indoleamine 2,3-dioxygenase in mouse lung by inhibitors of prostaglandin biosynthesis. *Proc Natl Acad Sci*. 1981;78:7327-7330.
26. van Wissen M, Snoek M, Smids B, Jansen HM, Lutter R. IFN-gamma amplifies IL-6 and IL-8 responses by airway epithelial-like cells via indoleamine 2,3-dioxygenase. *J Immunol*. 2002;169:7039-7044.
27. Werner-Felmayer G, Werner ER, Fuchs D, et al. Characteristics of interferon induced tryptophan metabolism in human cells in vitro. *Biochim Biophys Acta Mol Cell Res*. 1989;1012:140-147.
28. Saito K, Chen CY, Masana M, et al. 4-chloro-3-hydroxyanthranilate, 6-chlorotryptophan and norharmane attenuate quinolinic acid formation by interferon-gamma-stimulated monocytes (THP-1 cells). *Biochem J*. 1993;291:11-14.
29. Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: tryptophan's metabolites in exercise, inflammation, and mental health. *Science*. 2017;357: eaaf9794.
30. Meyer KC, Arend RA, Kalayoglu MV, et al. Tryptophan metabolism in chronic inflammatory lung disease. *J Lab Clin Med*. 1995;126:530-540.
31. Dostal CR, Gamsby NS, Lawson MA, McCusker RH. Glia- and tissue-specific changes in the kynurenine pathway after treatment of mice with lipopolysaccharide and dexamethasone. *Brain Behav Immun*. 2018;69:321-335.
32. Melchior D, Mézière N, Sève B, Le Floc'h N. Is tryptophan catabolism increased under indoleamine 2,3 dioxygenase activity during chronic lung inflammation in pigs? *Reprod Nutr Dev*. 2005;45:175-183.
33. Wirthgen E, Tuchscherer M, Otten W, et al. Activation of indoleamine 2,3-dioxygenase by LPS in a porcine model. *Innate Immun*. 2014;20:30-39.
34. Ho CC, Lee HL, Chen CY, et al. Involvement of the cytokine-IDO1-AhR loop in zinc oxide nanoparticle-induced acute pulmonary inflammation. *Nanotoxicology*. 2017;11:360-370.
35. Palabiyik SS, Girgin G, Tutkun E, Yilmaz ÖH, Baydar T. Immunomodulation and oxidative stress in denim sandblasting workers: changes caused by silica exposure. *Arch Ind Hyg Toxicol*. 2013;64:431-437.
36. Xue C, Wu N, Fan Y, Ma J, Ye Q. Distinct metabolic features in the plasma of patients with silicosis and dust-exposed workers in China: a case–control study. *BMC Pulm Med*. 2021;21:1-10.
37. Kiank C, Zeden JP, Drude S, et al. Psychological stress-induced, IDO1-dependent tryptophan catabolism: implications on immunosuppression in mice and humans. *PLoS One*. 2010;5:e11825.
38. Aldajani WA, Salazar F, Sewell HF, Knox A, Ghaemmaghami AM. Expression and regulation of immune-modulatory enzyme indoleamine 2,3-dioxygenase (IDO) by human airway epithelial cells and its effect on T cell activation. *Oncotarget*. 2016;7:57606-57617.
39. Virok DP, Raffai T, Kókai D, et al. Indoleamine 2,3-dioxygenase activity in Chlamydia muridarum and chlamydia pneumoniae infected mouse lung tissues. *Front Cell Infect Microbiol*. 2019;9:192.
40. Guo L, Schurink B, Roos E, et al. Indoleamine 2,3-dioxygenase (IDO)-1 and IDO-2 activity and severe course of COVID-19. *J Pathol*. 2022;256:256-261.
41. Hayashi T, Beck L, Rossetto C, et al. Inhibition of experimental asthma by indoleamine 2,3-dioxygenase. *J Clin Investig*. 2004;114:270-279.
42. Desvignes L, Ernst JD. Interferon-gamma-responsive nonhematopoietic cells regulate the immune response to mycobacterium tuberculosis. *Immunity*. 2009;31:974-985.
43. Gurczynski SJ, Pereira NL, Hrycaj SM, et al. Stem cell transplantation uncovers TDO-AHR regulation of lung dendritic cells in herpesvirus-induced pathology. *JCI Insight*. 2021;6:e139965.
44. de Luca A, Bozza S, Zelante T, et al. Non-hematopoietic cells contribute to protective tolerance to Aspergillus fumigatus via a TRIF pathway converging on IDO. *Cell Mol Immunol*. 2010;7:459-470.
45. Fox JM, Crabtree JM, Sage LK, Tompkins SM, Tripp RA. Interferon lambda upregulates IDO1 expression in respiratory epithelial cells after influenza virus infection. *J Interferon Cytokine Res*. 2015;35:554-562.
46. Zegarra-Moran O, Folli C, Manzari B, et al. Double mechanism for apical tryptophan depletion in polarized human bronchial epithelium. *J Immunol*. 2004;173:542-549.

47. Iannitti RG, Carvalho A, Cunha C, et al. Th17/Treg imbalance in murine cystic fibrosis is linked to indoleamine 2,3-dioxygenase deficiency but corrected by kynurenines. *Am J Respir Crit Care Med*. 2013;187:609-620.
48. Heseler K, Spekker K, Schmidt SK, MacKenzie CR, Däubener W. Antimicrobial and immunoregulatory effects mediated by human lung cells: role of IFN-gamma-induced tryptophan degradation. *FEMS Immunol Med Microbiol*. 2008;52:273-281.
49. Chacko A, Barker CJ, Beagley KW, et al. Increased sensitivity to tryptophan bioavailability is a positive adaptation by the human strains of *Chlamydia pneumoniae*. *Mol Microbiol*. 2014;93:797-813.
50. Nagy BM, Nagaraj C, Meinitzer A, et al. Importance of kynurenine in pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2017;313:L741-L751.
51. Théate I, van Baren N, Pilotte L, et al. Extensive profiling of the expression of the indoleamine 2,3-dioxygenase 1 protein in normal and tumoral human tissues. *Cancer Immunol Res*. 2015;3:161-172.
52. Hansen AM, Driussi C, Turner V, Takikawa O, Hunt NH. Tissue distribution of indoleamine 2,3-dioxygenase in normal and malaria-infected tissue. *Redox Rep*. 2000;5:112-115.
53. Xiao Y, Christou H, Liu L, et al. Endothelial indoleamine 2,3-dioxygenase protects against development of pulmonary hypertension. *Am J Respir Crit Care Med*. 2013;188:482-491.
54. Chilosi M, Poletti V, Ravaglia C, et al. The pathogenic role of epithelial and endothelial cells in early-phase COVID-19 pneumonia: victims and partners in crime. *Mod Pathol*. 2021;34:1444-1455.
55. Doglioni C, Ravaglia C, Chilosi M, et al. Covid-19 interstitial pneumonia: histological and immunohistochemical features on cryobiopsies. *Respiration*. 2021;100:488-498.
56. Chilosi M, Doglioni C, Ravaglia C, et al. Unbalanced IDO1/IDO2 endothelial expression and skewed keynurenine pathway in the pathogenesis of COVID-19 and post-COVID-19 pneumonia. *Biomedicines*. 2022;10:1332.
57. Fallarino F, Grohmann U, Vacca C, et al. T cell apoptosis by tryptophan catabolism. *Cell Death Differ*. 2002;9:1069-1077.
58. Bellanti F, Lo Buglio A, Vendemiale G. Redox homeostasis and immune alterations in coronavirus disease-19. *Biology*. 2022;11:159.
59. Karagianni AE, Kapetanovic R, McGorum BC, Hume DA, Pirie SR. The equine alveolar macrophage: functional and phenotypic comparisons with peritoneal macrophages. *Vet Immunol Immunopathol*. 2013;155:219-228.
60. Swanson KA, Zheng Y, Heidler KM, Mizobuchi T, Wilkes DS. CDllc$^+$ *cells modulate pulmonary immune responses* by production of indoleamine 2,3-dioxygenase. *Am J Respir Cell Mol Biol*. 2004;30:311-318.
61. Lee SM, Park HY, Suh YS, et al. Inhibition of acute lethal pulmonary inflammation by the IDO-AhR pathway. *Proc Natl Acad Sci*. 2017;114:E5881-E5890.
62. Cho SJ, Hong KS, Schenck E, et al. Decreased IDO1-dependent tryptophan metabolism in aged lung during influenza. *Eur Respir J*. 2021;57:2000443. doi:10.1183/13993003.00443-2020
63. de Araújo EF, Feriotti C, Galdino NADL, et al. The IDO–AhR axis controls th17/Treg immunity in a pulmonary model of fungal infection. *Front Immunol*. 2017;8:880. doi:10.3389/fimmu.2017.00880
64. Preite NW, Kaminski VDL, Borges BM, Calich VLG, Loures FV. Myeloid-derived suppressor cells are associated with impaired Th1 and Th17 responses and severe pulmonary paracoccidioidomycosis which is reversed by anti-Gr1 therapy. *Front Immunol*. 2023;14:1039244.
65. Ajamian F, Wu Y, Ebeling C, et al. Respiratory syncytial virus induces indoleamine 2,3-dioxygenase activity: a potential novel role in the development of allergic disease. *Clin Exp Allergy*. 2015;45:644-659.
66. Odemuyiwa SO, Ghahary A, Li Y, et al. Cutting edge: human eosinophils regulate T cell subset selection through indoleamine 2,3-dioxygenase. *J Immunol*. 2004;173:5909-5913.
67. Peng K, Monack DM. Indoleamine 2,3-dioxygenase 1 is a lung-specific innate immune defense mechanism that inhibits growth of Francisella tularensis tryptophan auxotrophs. *Infect Immun*. 2010;78:2723-2733.
68. Jacoby DB, Choi AM. Influenza virus induces expression of antioxidant genes in human epithelial cells. *Free Radic Biol Med*. 1994;16:821-824.
69. Christen S, Peterhans E, Stocker R. Antioxidant activities of some tryptophan metabolites: possible implication for inflammatory diseases. *Proc Natl Acad Sci*. 1990;87:2506-2510.
70. González Esquivel D, Ramírez-Ortega D, Pineda B, et al. Kynurenine pathway metabolites and enzymes involved in redox reactions. *Neuropharmacol*. 2017;112:331-345.
71. Barbi J, Pardoll D, Pan F. Metabolic control of the Treg/th17 axis. *Immunol Rev*. 2013;252:52-77.
72. Van der Leek AP, Yanishevsky Y, Kozyrskyj AL. The kynurenine pathway as a novel link between allergy and the gut microbiome. *Front Immunol*. 2017;8:1374.
73. Odemuyiwa SO, Ebeling C, Duta V, et al. Tryptophan catabolites regulate mucosal sensitization to ovalbumin in respiratory airways. *Allergy*. 2009;64:488-492.
74. Hossain FMA, Park SO, Kim HJ, et al. Indoleamine 2,3-dioxygenase in hematopoietic stem cell-derived cells suppresses rhinovirus-induced neutrophilic airway inflammation by regulating th1- and th17-type responses. *Immune Netw*. 2021;21:e26.
75. Paveglio SA, Allard J, Foster Hodgkins SR, et al. Airway epithelial indoleamine 2,3-dioxygenase inhibits CD4$^+$ T cells during *Aspergillus fumigatus* antigen exposure. *Am J Respir Cell Mol Biol*. 2011;44:11-23.
76. Araújo EF, Loures FV, Bazan SB, et al. Indoleamine 2,3-dioxygenase controls fungal loads and immunity in Paracoccidioidomicosis but is more important to susceptible than resistant hosts. *PLoS Negl Trop Dis*. 2014;8:e3330.
77. Sage LK, Fox JM, Mellor AL, Tompkins SM, Tripp RA. Indoleamine 2,3-dioxygenase (IDO) activity during the primary immune response to Influenza infection modifies the memory T cell response to influenza challenge. *Viral Immunol*. 2014;27:112-123.
78. Cheung MB, Sampayo-Escobar V, Green R, et al. Respiratory syncytial virus-infected mesenchymal stem cells regulate immunity via interferon beta and indoleamine-2,3-dioxygenase. *PLoS One*. 2016;11:e0163709.
79. Jasiewicz M, Moniuszko M, Pawlak D, et al. Activity of the kynurenine pathway and its interplay with immunity in patients with pulmonary arterial hypertension. *Heart*. 2016;102:230-237.
80. Felonato M, Pina A, de Araujo EF, et al. Anti-CD25 treatment depletes Treg cells and decreases disease severity in susceptible and resistant mice infected with Paracoccidioides brasiliensis. *PLoS One*. 2012;7:e51071.
81. Araújo EFD, Medeiros DH, Galdino NADL, et al. Tolerogenic plasmacytoid dendritic cells control Paracoccidioides brasiliensis infection by inducting regulatory T cells in an IDO-Dependent manner. *PLoS Pathog*. 2016;12:e1006115.
82. Yoshida R, Urade Y, Tokuda M, Hayaishi O. Induction of indoleamine 2,3-dioxygenase in mouse lung during virus infection. *Proc Natl Acad Sci*. 1979;76:4084-4086.
83. Choi AM, Knobil K, Otterbein SL, Eastman DA, Jacoby DB. Oxidant stress responses in influenza virus pneumonia: gene expression and transcription factor activation. *Am J Physiol Lung Cell Mol Physiol*. 1996;271:L383-L391.
84. Fox JM, Sage LK, Huang L, et al. Inhibition of indoleamine 2,3-dioxygenase enhances the T-cell response to influenza virus infection. *J Gen Virol*. 2013;94:1451-1461. Published online 2013. doi:10.1099/vir.0.053124-0
85. Fox JM, Sage LK, Poore S, et al. Drug analog inhibition of indoleamine 2,3-dioxygenase (IDO) activity modifies pattern recognition receptor expression and proinflammatory cytokine responses early during influenza virus infection. *J Leukoc Biol*. 2014;96:447-452.
86. Huang L, Li L, Klonowski KD, et al. Induction and role of indoleamine 2,3 dioxygenase in mouse models of influenza A virus infection. *PLoS One*. 2013;8:e66546.
87. Cai Z, Tian S, Klein T, et al. Kynurenine metabolites predict survival in pulmonary arterial hypertension: a role for IL-6/IL-6Rα. *Sci Rep*. 2022;12:12326.
88. Gaelings L, Söderholm S, Bugai A, et al. Regulation of kynurenine biosynthesis during influenza virus infection. *FEBS J*. 2017;284:222-236.
89. Bortolotti P, Hennart B, Thieffry C, et al. Tryptophan catabolism in pseudomonas aeruginosa and potential for inter-kingdom relationship. *BMC Microbiol*. 2016;16:137.
90. Napolioni V, Pariano M, Borghi M, et al. Genetic polymorphisms affecting IDO1 or IDO2 activity differently associate with aspergillosis in humans. *Front Immunol*. 2019;10:890.
91. Choera T, Zelante T, Romani L, Keller NP. A multifaceted role of tryptophan metabolism and indoleamine 2,3-dioxygenase activity in Aspergillus fumigatus-Host interactions. *Front Immunol*. 2017;8:1996.
92. Zelante T, Choera T, Beauvais A, et al. Aspergillus fumigatus tryptophan metabolic route differently affects host immunity. *Cell Rep*. 2021;34:108673.
93. Cao T, Dai G, Chu H, et al. Single-nucleotide polymorphisms and activities of indoleamine 2,3-dioxygenase isoforms, IDO1 and IDO2, in tuberculosis patients. *Hereditas*. 2022;159:5.
94. Warraki SE, El-Gammal MY, El-Asmar MF, Wahba N. Serum kynurenine in bronchial asthma and chronic bronchitis. *Chest*. 1970;57:148-150.
95. Kucharewicz I, Kasacka I, Pawlak D, et al. The concentration of kynurenine in rat model of asthma. *Folia Histochem Cytobiol*. 2008;46:199-203.
96. van der Sluijs KF, van de Pol MA, Kulik W, et al. Systemic tryptophan and kynurenine catabolite levels relate to severity of rhinovirus-induced asthma exacerbation: a prospective study with a parallel-group design. *Thorax*. 2013;68:1122-1130.
97. Kositz C, Schroecksnadel K, Grander G, et al. High serum tryptophan concentration in pollinosis patients is associated with unresponsiveness to pollen extract therapy. *Int Arch Allergy Immunol*. 2008;147:35-40.
98. Ciprandi G, De Amici M, Tosca M, Fuchs D. Tryptophan metabolism in allergic rhinitis: the effect of pollen allergen exposure. *Hum Immunol*. 2010;71:911-915.
99. Licari A, Fuchs D, Marseglia G, Ciprandi G. Tryptophan metabolic pathway and neopterin in asthmatic children in clinical practice. *Ital J Pediatr*. 2019;45:114.
100. Kofler H, Kurz K, Grander G, Fuchs D. Specific immunotherapy normalizes tryptophan concentrations in patients with allergic rhinitis. *Int Arch Allergy Immunol*. 2012;159:416-421.

101. Maneechotesuwan K, Supawita S, Kasetsinsombat K, Wongkajornsilp A, Barnes PJ. Sputum indoleamine-2, 3-dioxygenase activity is increased in asthmatic airways by using inhaled corticosteroids. *J Allergy Clin Immunol*. 2008;121:43-50.
102. Maneechotesuwan K, Ekjiratrakul W, Kasetsinsombat K, Wongkajornsilp A, Barnes PJ. Statins enhance the anti-inflammatory effects of inhaled corticosteroids in asthmatic patients through increased induction of indoleamine 2, 3-dioxygenase. *J Allergy Clin Immunol*. 2010;126:754-762.e1.
103. Maneechotesuwan K, Kasetsinsombat K, Wongkajornsilp A, Barnes PJ. Decreased indoleamine 2,3-dioxygenase activity and IL-10/IL-17A ratio in patients with COPD. *Thorax*. 2013;68:330-337.
104. Maneechotesuwan K, Wongkajornsilp A, Adcock IM, Barnes PJ. Simvastatin suppresses airway IL-17 and upregulates IL-10 in patients with stable COPD. *Chest*. 2015;148:1164-1176.
105. Badawy AA, Guillemin G. The plasma [kynurenine]/[tryptophan] ratio and indoleamine 2,3-dioxygenase: time for appraisal. *Int J Tryptophan Res*. 2019;12:1178646919868978.
106. Tornheim JA, Paradkar M, Zhao H, et al. The kynurenine/tryptophan ratio is a sensitive biomarker for the diagnosis of pediatric tuberculosis among Indian children. *Front Immunol*. 2021;12:774043.
107. Adu-Gyamfi CG, Snyman T, Makhathini L, et al. Diagnostic accuracy of plasma kynurenine/tryptophan ratio, measured by enzyme-linked immunosorbent assay, for pulmonary tuberculosis. *Int J Infect Dis*. 2020;99:441-448.
108. Weiner J, Parida S, Maertzdorf J, et al. Biomarkers of inflammation, immunosuppression and stress are revealed by metabolomic profiling of tuberculosis patients. *PLoS One*. 2012;7:e40221.
109. Collins JM, Siddiqa A, Jones DP, et al. Tryptophan catabolism reflects disease activity in human tuberculosis. *JCI Insight*. 2020;5:137131.
110. Gough M, Singh DK, Moodley C, et al. Peripheral blood markers correlate with the progression of active tuberculosis relative to latent control of mycobacterium tuberculosis infection in macaques. *Pathogens*. 2022;11:544.
111. Shi W, Wu J, Tan Q, et al. Plasma indoleamine 2,3-dioxygenase activity as a potential biomarker for early diagnosis of multidrug-resistant tuberculosis in tuberculosis patients. *Infect Drug Resist*. 2019;12:1265-1276.
112. Pett SL, Kunisaki KM, Wentworth D, et al. Increased indoleamine-2,3-dioxygenase activity is associated with poor clinical outcome in adults hospitalized with influenza in the INSIGHT FLU003Plus study. *Open Forum Infect Dis*. 2018;5:ofx228.
113. Arshad H, Siokis A, Franke R, et al. Reprogramming of amino acid metabolism differs between community-acquired pneumonia and infection-associated exacerbation of chronic obstructive pulmonary disease. *Cells*. 2022;11:2283.
114. Zahed H, Johansson M, Ueland PM, et al. Epidemiology of 40 blood biomarkers of one-carbon metabolism, vitamin status, inflammation, and renal and endothelial function among cancer-free older adults. *Sci Rep*. 2021;11:13805.
115. Theofylaktopoulou D, Midttun Ø, Ulvik A, et al. A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland Health Study. *Clin Exp Immunol*. 2013;173:121-130.
116. Zinellu A, Fois AG, Zinellu E, et al. Increased kynurenine plasma concentrations and kynurenine-tryptophan ratio in mild-to-moderate chronic obstructive pulmonary disease patients. *Biomark Med*. 2018;12:229-237.
117. Naz S, Bhat M, Ståhl S, et al. Dysregulation of the tryptophan pathway evidences gender differences in COPD. *Metabolites*. 2019;9:212.
118. Kim DJ, Oh JY, Rhee CK, et al. Metabolic fingerprinting uncovers the distinction between the phenotypes of tuberculosis associated COPD and smoking-induced COPD. *Front Med*. 2021;8:619077.
119. Hodgson S, Griffin TJ, Reilly C, et al. Plasma sphingolipids in HIV-associated chronic obstructive pulmonary disease. *BMJ Open Respir Res*. 2017;4:e000180.
120. Gulcev M, Reilly C, Griffin TJ, et al. Tryptophan catabolism in acute exacerbations of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2016;11:2435-2446.
121. Luukkainen A, Karjalainen J, Hurme M, et al. Relationships of indoleamine 2,3-dioxygenase activity and cofactors with asthma and nasal polyps. *Am J Rhinol Allergy*. 2014;28:e5-e10.
122. Hu Y, Chen Z, Jin L, Wang M, Liao W. Decreased expression of indolamine 2,3-dioxygenase in childhood allergic asthma and its inverse correlation with fractional concentration of exhaled nitric oxide. *Ann Allergy Asthma Immunol*. 2017;119:429-434.
123. Ünüvar S, Erge D, Kılıçarslan B, et al. Neopterin levels and indoleamine 2,3-dioxygenase activity as biomarkers of immune system activation and childhood allergic diseases. *Ann Lab Med*. 2019;39:284-290.
124. Muhlebach MS, Sha W, MacIntosh B, Kelley TJ, Muenzer J. Metabonomics reveals altered metabolites related to inflammation and energy utilization at recovery of cystic fibrosis lung exacerbation. *Metab Open*. 2019;3:100010.
125. Suzuki Y, Suda T, Asada K, et al. Serum indoleamine 2,3-dioxygenase activity predicts prognosis of pulmonary tuberculosis. *Clin Vaccine Immunol*. 2012;19:436-442.
126. Suzuki Y, Miwa S, Akamatsu T, et al. Indoleamine 2,3-dioxygenase in the pathogenesis of tuberculous pleurisy. *Int J Tuberc Lung Dis*. 2013;17:1501-1506.
127. Zinellu A, Zinellu E, Sotgiu E, et al. Systemic transsulfuration pathway thiol concentrations in chronic obstructive pulmonary disease patients. *Eur J Clin Invest*. 2020;50:e13267.
128. Taher YA, Piavaux BJA, Gras R, et al. Indoleamine 2,3-dioxygenase–dependent tryptophan metabolites contribute to tolerance induction during allergen immunotherapy in a mouse model. *J Allergy Clin Immunol*. 2008;121:983-991.e2.
129. Blumenthal A, Nagalingam G, Huch JH, et al. M. tuberculosis induces potent activation of IDO-1, but this is not essential for the immunological control of infection. *PLoS One*. 2012;7:e37314.
130. Mehra S, Alvarez X, Didier PJ, et al. Granuloma correlates of protection against tuberculosis and mechanisms of immune modulation by Mycobacterium tuberculosis. *J Infect Dis*. 2013;207:1115-1127.
131. Gautam US, Foreman TW, Bucsan AN, et al. In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of *Mycobacterium tuberculosis*. *Proc Natl Acad Sci*. 2018;115:E62-E71.
132. Singh B, Moodley C, Singh DK, et al. Inhibition of indoleamine dioxygenase leads to better control of tuberculosis adjunctive to chemotherapy. *JCI Insight*. 2023;8:e163101. doi:10.1172/jci.insight.163101
133. Hage CA, Horan DJ, Durkin M, et al. *Histoplasma capsulatum* preferentially induces IDO in the lung. *Med Mycol*. 2013;51:270-279.