Biochemical Society Transactions (2020) **48** 2029–2037
https://doi.org/10.1042/BST20200194

Review Article

The tryptophan biosynthetic pathway is essential for *Mycobacterium tuberculosis* to cause disease

J. Shaun Lott

Maurice Wilkins Centre for Molecular Biodiscovery and School of Biological Sciences, The University of Auckland, Auckland, New Zealand

Correspondence: J. Shaun Lott (s.lott@auckland.ac.nz)

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is the most significant cause of death from a single infectious agent worldwide. Antibiotic-resistant strains of *M. tuberculosis* represent a threat to effective treatment, and the long duration, toxicity and complexity of current chemotherapy for antibiotic-resistant disease presents a need for new therapeutic approaches with novel modes of action. *M. tuberculosis* is an intracellular pathogen that must survive phagocytosis by macrophages, dendritic cells or neutrophils to establish an infection. The tryptophan biosynthetic pathway is required for bacterial survival in the phagosome, presenting a target for new classes of antitubercular compound. The enzymes responsible for the six catalytic steps that produce tryptophan from chorismate have all been characterised in *M. tuberculosis,* and inhibitors have been described for some of the steps. The innate immune system depletes cellular tryptophan in response to infection in order to inhibit microbial growth, and this effect is likely to be important for the efficacy of tryptophan biosynthesis inhibitors as new antibiotics. Allosteric inhibitors of both the first and final enzymes in the pathway have proven effective, including by a metabolite produced by the gut biota, raising the intriguing possibility that the modulation of tryptophan biosynthesis may be a natural inter-bacterial competition strategy.

Introduction

In many bacteria, the amino acid tryptophan is synthesised *de novo*, starting from chorismate. In contrast, many pathogenic micro-organisms are auxotrophic for tryptophan production, and this biosynthetic deficit is exploited by the mammalian immune system: intracellular pathogens are starved of tryptophan by the action of the degradative enzyme indoleamine 2,3-dioxygenase (IDO). This review will focus on the role of tryptophan biosynthesis in the development of disease by *Mycobacterium tuberculosis*, the causative agent of TB, which is an intracellular pathogen naturally prototrophic for tryptophan. Auxotrophic mutants of *M. tuberculosis* are unable to establish an infection in mice, establishing tryptophan biosynthesis as an essential *in vivo* activity of the bacterium that is required for pathogenesis. Subsequent work has identified many inhibitors of various steps of the biosynthetic pathway, some of which show *in vivo* efficacy in animal models of disease, demonstrating that the tryptophan biosynthetic pathway could potentially be utilised as a target for new antibiotics active against *M. tuberculosis.* However, more experiments will need to be performed to complete the validation of the pathways as a viable target for effective therapy, given the complexity of the human disease.

M. tuberculosis is a globally significant human pathogen for which new therapies are required

The World Health Organization (WHO) rates tuberculosis (TB) as the most significant cause of death from a single infectious agent worldwide, ranking it above the human immunodeficiency virus (HIV) as the deadliest human communicable disease. Approximately 1.45 million people are estimated to have died from TB in 2018, the most recent year for which figures are available [1]. Drug-sensitive

Received: 24 June 2020
Revised: 21 August 2020
Accepted: 26 August 2020

Version of Record published:
11 September 2020

Biochemical Society Transactions (2020) 48 2029–2037
https://doi.org/10.1042/BST20200194

forms of the disease have a high cure-rate: 85% of cases can be treated successfully with the current standard therapeutic regimen, which consists of four antibiotics (isoniazid and ethambutol, which both inhibit cell wall biosynthesis; rifampicin, which inhibits mRNA production; and pyrazinamide, which is thought to inhibit coenzyme A biosynthesis). The overall TB mortality rate has been slowly declining in recent years, but there remains a substantial concern that the continued presence of multidrug-resistant (MDR) strains of *M. tuberculosis*, defined as those resistant to both isoniazid and rifampicin, may reverse this progress. It is estimated that ∼400 000 people were diagnosed with MDR-TB in 2018. MDR-TB is more complicated and slower to treat, and requires the use of second-line antibiotics, many of which have significant side effects, for up to 20 months. The success rate for MDR-TB treatment is only 56%, leading to an estimated total death toll of ∼200 000 people in 2018. Extensively drug-resistant TB (XDR-TB) is defined as MDR-TB plus resistance to two of the second-line drugs; the treatment success rate for these strains drops further to only 39% [1].

The main challenges to the effective current and future treatment of TB include the duration, toxicity and complexity of the required drug regimens, especially for second-line therapies. All of these factors negatively affect patient compliance, which is turn drives the further selection of drug-resistant strains of *M. tuberculosis*. Hence, despite the development of new effective drugs such as the ATP synthase inhibitor bedaquiline and the mycolic acid biosynthesis inhibitor delaminid [2], there remains a pressing need for new drugs that are more effective, more affordable and less toxic, and that require shorter treatment times.

M. tuberculosis as an intracellular pathogen

Person-to-person spread of *M. tuberculosis* occurs via aerosol transmission [3]. Once it has been inhaled into the lower respiratory tract, the bacterium is engulfed by phagocytic cells, primarily by alveolar macrophages but also by dendritic cells and neutrophils [4]. The phagosomes containing the bacteria would normally then undergo a process referred to as phagosome maturation, which includes acidification of the phagocytic vacuole and eventual fusion with the lysosome. However, *M. tuberculosis* has been observed to arrest this process and survive within non-acidified phagosomes [5]. The prevailing paradigm has, therefore, been that the bacterium primarily resides within arrested macrophage phagosomes, and various studies have visualised it in this cellular location using electron microscopy, from the 1970s onwards, e.g. [6]. An alternative view, first put forward in the 1980s [7] is that the bacterium is able to escape into the cytoplasm [8]. The situation is complex and dynamic (reviewed in [4]), but an essential point for understanding the significance of tryptophan biosynthesis to the progression of disease is that the bacterium must survive the harsh intravacuolar environment, and this initial bottleneck plays a critical part in modifying bacterial metabolism [9].

The tryptophan biosynthetic pathway in *M. tuberculosis*

The bacterial tryptophan biosynthetic pathway comprises six catalytic steps, starting from the key metabolic intermediate chorismite (Figure 1) [10]. The seven enzymes responsible and the genes that encode them are represented by the letters A–G, assigned based on the gene order in *Escherichia coli* [11,12]. The first committed step in the biosynthesis is the conversion of chorismate to anthranilate, which is catalysed by the enzyme anthranilate synthase (AS). Anthranilate synthase is a functional heterodimer composed of AS-I (TrpE), which catalyses the production of anthranilate from chorismate and ammonia, and AS-II (TrpG), a glutamine amidotransferase (GAT) that provides the required ammonia by converting glutamine to glutamate. Anthranilate phosphoribosyl transferase (AnPRT; TrpD) then transfers a 5′-phospho-ribose unit from phosphoribosyl pyrophosphate (PRPP) onto the amino group of anthranilate to produce phosphoribosyl-anthranilate (PRA). The ribose ring of PRA is opened by PRA isomerase (PRAI; TrpF) to produce the isomer 1-carboxyphenylamino-1′-deoxyribulose-5′-phosphate. A ring closure reaction catalysed by indole-3-glycerol phosphate synthase (IGPS; TrpC) forms the characteristic indole heterocyclic ring system. Finally, the heterotetrameric enzyme tryptophan synthase (TrpAB) cleaves the indole ring from the glycerol phosphate backbone, and forms the final amino acid product by condensation of the indole moiety with serine. Allosteric regulation of the pathway is provided by feedback inhibition of tryptophan binding to a regulatory site in the TrpE protein [13,14].

Once the complete genome sequence of *M. tuberculosis* was published in 1998 [15], the open reading frames (ORFs) that encode the enzymes of the tryptophan biosynthetic pathway could be assigned by sequence comparison. However, not all of the enzymes in the pathway could be unambiguously identified from sequence information alone, and unlike the genetic arrangement in *E. coli*, the ORFs are not arranged in a single operon. Consequently, subsequent biochemical confirmation was required to establish which ORFs encode the enzymes in the pathway.

Biochemical Society Transactions (2020) **48** 2029–2037
https://doi.org/10.1042/BST20200194

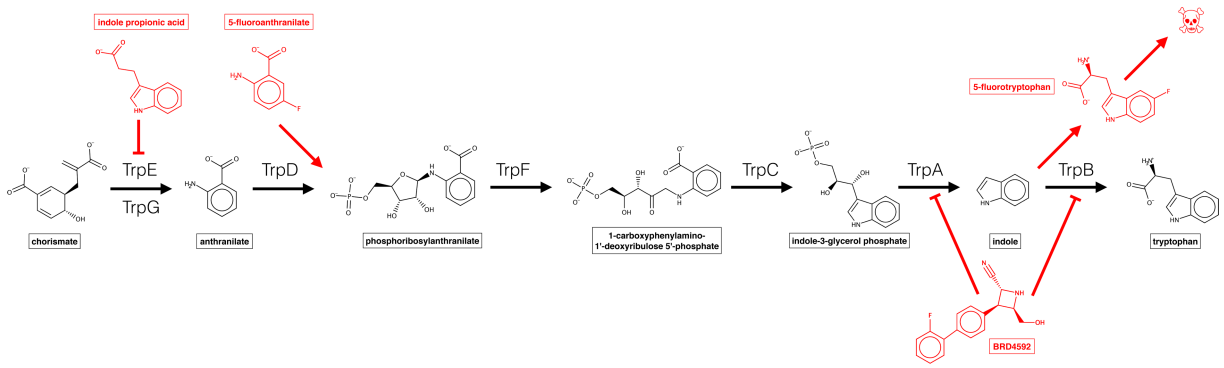

Figure 1. The tryptophan biosynthetic pathway in *M. tuberculosis*. The steps in the biosynthesis of tryptophan are shown in black. The points of action in the pathway of the inhibitors BRD4592 and IPA and the antimetabolite 5-fluoroanthranilate are shown in red.

Two ORFs, Rv1609 and Rv2386c, were initially identified as TrpE candidates, but biochemical and structural analysis showed that Rv2386c in fact encodes a salicylate synthase [16], confirming Rv1609 as TrpE. The identity of TrpG proved more elusive, but of the four potential GATs (Rv0013, Rv0788, Rv1602 and Rv2604c), only Rv0013 forms a functional AS complex with Rv1609 [17]. However, the interaction between the two subunits is too weak for the complex to be crystallised, implying that Rv0013 may promiscuously provide GAT activity to other biosynthetic pathways, as is the case in *Bacillus subtilis*, where a single GAT functions in the synthesis of both tryptophan and *p*-aminobenzoate [18].

Rv1603 encodes a bifunctional protein that has both PRAI and *N*′-[(5′-phosphoribosyl)formimino]-5-aminoimidazole-4-carboxamide ribonucleotide (ProFAR) isomerase activity thus functioning as both TrpF and as HisA in the histidine biosynthetic pathway [19]. The predicted TrpA (Rv1613), TrpB (Rv1612), TrpC (Rv1611) and TrpD (Rv2192c) proteins have all been heterologously expressed and characterised, and demonstrate the expected biochemical activities *in vitro* [20–24], and all the enzymes in the pathway have been structurally characterised apart from TrpG (Table 1).

Tryptophan biosynthesis is essential for host colonisation by *M. tuberculosis*

M. tuberculosis is fully competent for tryptophan biosynthesis, but its critical dependence on this biosynthetic capability during infection was only revealed when auxotrophic knockout strains were investigated for their potential as vaccine candidates [25]. An *M. tuberculosis* strain with a defined mutation in the *trpD* gene

Table 1. The enzymes of the tryptophan biosynthesis pathway in *M. tuberculosis*.

Gene name	ORF number*	Enzyme activity	PDB code(s)
TrpA	Rv1613	Tryptophan synthase α subunit	TrpA/TrpB complexes: 5ocw, 5tcj, 5tch, 5tci, 5tcf, 5tcg, 6uap, 6ub9, 6e9p, 6dwe
TrpB	Rv1612	Tryptophan synthase β subunit	TrpB: 2o2e, 2o2j
TrpC	Rv1611	Indole-3-glycerol phosphate synthase	3qja, 3t40, 3t44, 3t55, 3t78, 4fb7
TrpD	Rv2192c	Anthranilate phosphoribosyl transferase	1zvw, 2bpq, 3r88, 3twp, 3uu1, 4ij1, 4giu, 4gkm, 4m0r, 4n5v, 4n8q, 4n93, 4owu, 4owv, 4owm, 4own, 4owo, 4owq, 4ows, 5byt, 5bne, 5c1r, 5c2l, 5c7s
TrpE	Rv1609	Anthranilate synthase	5cwa
TrpF	Rv1603	Phosphoribosyl-anthranilate isomerase	2y85, 2y88, 2y89, 3zs4
TrpG	Rv0013	Glutamine amidotransferase	

*'Rv' numbers are used to designate ORFs in the *M. tuberculosis* reference genome, as the first sequenced genome was from the virulent H37Rv laboratory strain of the bacterium; Rv numbers suffixed with 'c' indicate that the ORF is encoded on the complementary strand of the genome, relative to the designated start point for ORF numbering, which is the gene *dnaA*.

Biochemical Society Transactions (2020) **48** 2029–2037
https://doi.org/10.1042/BST20200194

(Rv2192c), which effectively disrupts tryptophan biosynthesis, failed to cause disease in mice. Tryptophan biosynthesis, therefore, appears to be required for TB disease formation.

The essentiality of biosynthetic pathways for some other amino acids, for example, leucine [26], arginine [27], lysine [28] and methionine [29], for *in vivo* growth and disease formation by *M. tuberculosis* has also been demonstrated. This implies that the bacterium is required to survive in niches with limited access to amino acids during the establishment of an infection, presumably including the phagosomes of macrophages in the lungs.

The tryptophan auxotrophic strain of *M. tuberculosis* made by knocking out the *trpD* gene was strikingly avirulent, even in severe, combined immune-deficient (SCID) mice, which lack a functional adaptive immune system. Not only did these mice survive infection, they also cleared the auxotrophic bacteria from their lungs to the limit of detection. This implies that suitable inhibitors of tryptophan biosynthesis may act as effective antibiotics, at least during the initiation of infection. However, it remained unproven from these data whether an established infection by biosynthetically intact wild-type bacteria could be effectively killed via this approach, as although tryptophan biosynthesis is required to establish infection, it may not be critical to maintain an existing or ongoing infection.

The role of indoleamine 2,3-dioxygenase (IDO-1) in infection by *M. tuberculosis* and other intracellular pathogens

One of the innate immune system's responses to microbial infection is an interferon-γ (IFN-γ)-induced depletion of cellular tryptophan [30]. IFN-γ induces an isoform of the host enzyme indoleamine 2,3-dioxygenase (IDO-1) that converts tryptophan to *N*-formylkynurenine, which is further metabolised to kynurenine. This degradative process is known as the kynurenine pathway, is the major route used for tryptophan breakdown in mammals [31], and when strongly induced by IFN-γ, is capable of depleting all of the detectable tryptophan in the lung in a tissue-specific manner [32,33]. Many intracellular pathogens are sensitive to tryptophan depletion, including *Chlamydophila psittaci* [34], *Chlamydia trachomatis* [35], *Streptococcus agalactiae* [36] and *Leishmania donovani* [37], which are all natural auxotrophs for tryptophan biosynthesis. Additionally, an auxotrophic mutant of *Francisella novicida* is sensitive to tryptophan depleted by IFN-γ-induced IDO-1 expression in the lung, but is able to cause disease in a IDO-1 knockout mouse [38].

Although tryptophan depletion can be an effective defence, kynurenines are potent negative regulators of inflammation and T-cell activity. Dendritic cells expressing IDO-1 produce kynurenine that inhibits the proliferation of active T-cells, and induces the expansion of regulatory T-cells, resulting in an immune tolerance that may allow chronic bacterial infection [39,40].

Patients with pulmonary TB show increased IDO-1 activity, decreased tryptophan concentration and increased kynurenine concentration in both serum [41] and pleural fluid [42], suggesting that infection with *M. tuberculosis* also triggers IFN-γ-induced tryptophan depletion, possibly in a lung-specific fashion. This implies that if the *M. tuberculosis* tryptophan biosynthetic pathway were to be inhibited, the increased activity of IDO-1 would cause *M. tuberculosis* cell death through tryptophan starvation, as was seen for *F. novicida*. However, as *M. tuberculosis* is prototrophic for tryptophan, elevated IDO-1 activity has little direct effect on the ability of the bacterium to replicate and cause disease. Indeed, IDO-1 activity itself has been used a predictor of death from *M. tuberculosis* infection [41]. It is possible then that the immunomodulatory effects of kynurenine may actually serve to increase the severity of pulmonary infection. It makes sense, therefore, to consider IDO-1 as a potential target for host-directed therapy against *M. tuberculosis*. Gautam *et al.* [43] used the IDO inhibitor D-1-methyl-tryptophan to test this idea in a non-human primate model of TB disease. They found that inhibition of IDO-1 reduced the bacterial burden and severity of disease and increased the proliferation of $CD4^+$ and $CD8^+$ memory T cells. Additionally, IDO-1 inhibition allowed remodelling of granulomas to occur, giving $CD4^+$ T cells greater access to the necrotic, bacteria-rich central region of the granuloma, thus significantly increasing immune-mediated bacterial cell death.

Inhibitors targeting tryptophan biosynthesis in *M. tuberculosis*

Zhang *et al.* [44] used transposon insertion mutagenesis to confirm that an intact tryptophan biosynthetic pathway is necessary for *M. tuberculosis* to survive in immunocompetent mice, but not in mice lacking $CD4^+$ T-cells. They subsequently showed that a tryptophan auxotrophic strain of *M. tuberculosis* (produced by a deletion of the *trpE* gene) was susceptible to tryptophan starvation, both *in vitro* when grown in a defined medium

and also by IFN-γ-induced, IDO-mediated tryptophan depletion in stimulated macrophages. The *in vivo* essentiality of tryptophan biosynthesis to *M. tuberculosis* thus makes the pathway a potential target for new antibiotics with a novel mode of action. Also in its favour is the lack of the equivalent biosynthetic pathway in mammals, which makes host toxicity less likely. These qualities have inspired a range of investigations into identifying inhibitors of the pathway that could potentially function as new antibiotics.

Anthranilate synthase is a member of the structurally conserved chorismate-utilising enzyme (CUE) family. Many inhibitors have been designed against CUE family members, and inhibitors designed against one family member often function against other family members [45], as the active sites of the enzymes are similar. However, almost none of them have achieved better than micromolar potency against their targets [46]. Consistent with this, isochorismate-like inhibitors designed to act against *M. tuberculosis* salycilate synthase [47–49] were also active against *M. tuberculosis* anthranilate synthase, albeit with reduced potency [17].

A series of substrate mimetic inhibitors of *M. tuberculosis* AnPRT have been synthesised [50–53]. AnPRT has a surprisingly complex substrate-binding pattern, with PRPP binding in the base of the active site cleft, and two molecules of anthanilate binding nearer the mouth [24,52]. The most effective of the inhibitors mimic the ability of the enzyme to bind anthranilate in multiple places in the active site cleft, but still only exhibit micromolar K_i values and show only modest activity against mycobacterial cells [50].

Two derivatised anthranilates, fluorinated at the 5- or 6-positions on the ring, were shown to be effective against *M. tuberculosis*, both in axenic culture and, with more modest effect, when administered intravenously in a mouse model of TB [44]. The genome of an escape mutant strain showed that a mutation in the *trpE* gene results in a hypermorphic version of the enzyme that has lost its usual allosteric feedback inhibition [17], confirming tryptophan biosynthesis as the cellular target of these compounds. However, none of the four possible fluorinated anthanilate isomers act as inhibitors of *M. tuberculosis* AnPRT *in vitro*; rather they act as substrates, producing fluorinated versions of PRA, which are more stable than the parental compound. The fluorinated PRAs are substrates for *E. coli* PRAI and IGPS *in vitro*, resulting in the production of fluoro-indole-3-glycerol phosphate [51]. A subsequent metabolic tracing study [54] showed that anthanilates fluorinated at the 4-, 5- or 6-position are all metabolised by *M. tuberculosis* to the cognate fluorinated isomers of tryptophan, which subsequently cause cell killing when incorporated into proteins. This generally cytotoxic cellular effect of fluorinated tryptophan has previously been demonstrated in *E. coli* [55]. Hence, the fluoroanthranilates can be thought of as pro-drugs.

Two phenotypic screens of high-diversity chemical libraries independently identified inhibitors of *M. tuberculosis* tryptophan synthase that are active against the bacterium in culture (MIC_{50} values in the 0.5–3 μM range) and also show moderate *in vivo* efficacy in both zebrafish and mouse models of TB [20,21,56]. Tryptophan synthase is a remarkable and complex enzyme. The α-subunit (TrpA) cleaves indole-3-glycerol phosphate (I3GP) into indole and glyceraldehyde 3-phosphate. The indole is then transported through a 25 Å-long channel to the active site of the β-subunit (TrpB), where it displaces the hydroxyl group of serine using pyridoxal 5′-phosphate (PLP) as cofactor. The identified inhibitors bind in the channel, at the interface between the two subunits, resulting in a complex and potent inhibition of the enzyme. The best characterised of the compounds discovered, BRD4592, was shown to be an uncompetitive inhibitor with respect to I3GP and to be a mainly non-competitive inhibitor with respect to serine. Additionally, BRD4592 promotes a slow allosteric change in the enzyme that traps tryptophan in the active site, effectively inducing product inhibition.

A phenotypic screen of a chemical fragment library identified indole propionic acid (IPA) as active against *M. tuberculosis* in culture (MIC_{50} = 68 μM), and also as having modest effect against the bacterium in a mouse model of disease [57]. Intriguingly, IPA is a metabolite produced by several species of bacteria found in the normal gut biota, including species of *Clostridium* and *Peptostreptococcus* [58], that is detectable at a concentration of ∼80 μM in the peripheral blood of mice whose gut flora contain these species [58]. IPA is an allosteric inhibitor of *M. tuberculosis* anthranilate synthase, and exerts its antibacterial effect by down-regulating the biosynthetic pathway and preventing the bacterium from making tryptophan [59]. (Interestingly, 5-fluorotryptophan, the metabolic end-product of 5-fluoranthranilate, is also an allosteric inhibitor of anthranilate synthase of similar potency.) The identification of IPA as an inhibitor of mycobacterial tryptophan synthesis is interesting in two respects: first as a starting point for the synthesis of synthetic allosteric inhibitors of the pathway, and secondly as an indication that the inhibition of the pathway may be a natural inter-bacterial competition strategy.

Biochemical Society Transactions (2020) **48** 2029–2037
https://doi.org/10.1042/BST20200194

Future challenges to utilising tryptophan biosynthesis as a drug target in *M. tuberculosis*

There is now a clear body of data to show that tryptophan auxotroph strains of *M. tuberculosis* fail to establish infections [20,25,44], and that inhibitors of tryptophan synthesis show *in vivo* efficacy against mycobacterial infections in both zebrafish and mice [20,21]. Unlike many other bacteria, tryptophan synthesis is not controlled transcriptionally in mycobacteria [60], and they constitutively express the tryptophan biosynthetic genes, even in the presence of exogenous tryptophan [61]. However, tryptophan inhibits its own biosynthesis via allosteric feedback inhibition of anthranilate synthase: it has been variously estimated that the enzyme is 50% inhibited by a tryptophan concentration of 200 nM [44], 1.5 μM [59] or 6.3 μM [17]. Either way, this seems at odds with the reported 20 μM K_m of mammalian IDO-1 [62]; if there is likely to be sufficient residual tryptophan available to inhibit its own synthesis, then the precise mechanism of the observed growth inhibition by exogenous compounds *in vivo* remains unclear. Given the diverse range of microenvironments encountered by *M. tuberculosis* during infection [63], the complete validation of tryptophan biosynthesis as a viable target for effective antibacterial compounds requires further experiments. It will be especially helpful to establish the true *in vivo* concentration of host tryptophan to which *M. tuberculosis* is exposed, the rate of uptake of tryptophan by the bacterial cell, and the relative affinities of transporter(s) involved. An additional challenge in designing drugs that inhibit bacterial tryptophan biosynthesis is the potentially complex results of interfering with tryptophan metabolism in the human gut microbiome, which has recently been recognised to have an important influence on the gut-brain axis, with an associated possible impact on a range of neurological conditions [64].

Perspectives

- **Highlight the importance of the field:** It has been recognised for several years that widespread antibiotic resistance has the potential to undermine modern medicine as we know it. TB is the world's leading cause of death due to a single bacterial infection and the rise of multidrug resistance necessitates the development of new effective treatments that have novel mechanisms of action. Most antibiotics, including those in clinical use and those currently in development, redundantly target a limited range of cellular activities. Hence, the discovery that biosynthetic pathways for key cellular chemicals such as tryptophan are druggable targets, provides fresh avenues for the development of new classes of antibiotic.

- **Summary of the current thinking:** Genetic knockout strains of *M. tuberculosis* have confirmed that the pathway is essential for the bacterium to survive *in vivo* and cause disease in animal models. Inhibitors have been identified for several of the enzymes in the pathway, the most successful of which show good activity against *M. tuberculosis* cells and moderate activity against the bacterium in animal infection models. The most active compounds identified so far have been allosteric inhibitors of the first and final steps in the pathway.

- **Comment on future directions:** Much still needs to be learned about the biological availability of host tryptophan to *M. tuberculosis* at the various stages of disease progression, and whether the bacterium is able to circumvent inhibition of tryptophan biosynthesis by using environmental sources of the amino acid. This is made especially challenging by the range of both intracellular and extracellular microenvironments to which different bacterial subpopulations are exposed during the course of the disease.

Competing Interests

The author declares that there are no competing interests associated with the manuscript.

Open Access

Open access for this article was enabled by the participation of University of Auckland in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with CAUL.

Abbreviations

AnPRT, anthranilate phosphoribosyl transferase; AS, anthranilate synthase; CUE, chorismate-utilising enzyme; HIV, human immunodeficiency virus; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-γ; IGPS, indole-3-gycerol phosphate synthase; IPA, indole propionic acid; MDR, multidrug-resistant; MIC_{50}, minimum inhibitory concentration required to inhibit the growth of 50% of bacteria; ORF, open reading frame; PLP, pyridoxal 5′-phosphate; PRA, phosporibosyl-anthranilate; PRAI, phosporibosyl-anthranilate isomerase; ProFAR, *N*′-[(5′-phosphoribosyl)formimino]-5-aminoimidazole-4-carboxamide ribonucleotide; PRPP, phosporibosyl pyrophosphate; SCID, severe, combined immune-deficient; TB, tuberculosis; WHO, World Health Organisation; XDR, extensively drug-resistant.

References

1 World Health Organization (2019) Global Tuberculosis Report 2019
2 Li, Y., Sun, F. and Zhang, W. (2018) Bedaquiline and delamanid in the treatment of multidrug-resistant tuberculosis: promising but challenging. *Drug. Dev. Res.* **49**, 1700387 https://doi.org/10.1002/ddr.21498
3 Fennelly, K.P. and Jones-López, E.C. (2015) Quantity and quality of inhaled dose predicts immunopathology in tuberculosis. *Front. Immunol.* **6**, 313
4 Bussi, C. and Gutierrez, M.G. (2019) *Mycobacterium tuberculosis* infection of host cells in space and time. *FEMS Microbiol. Rev.* **43**, 341–361 https://doi.org/10.1093/femsre/fuz006
5 Mwandumba, H.C., Russell, D.G., Nyirenda, M.H., Anderson, J., White, S.A., Molyneux, M.E. et al. (2004) *Mycobacterium tuberculosis* resides in nonacidified vacuoles in endocytically competent alveolar macrophages from patients with tuberculosis and HIV infection. *J. Immunol.* **172**, 4592–4598 https://doi.org/10.4049/jimmunol.172.7.4592
6 Armstrong, J.A. and D'arcy Hart, P. (1971) Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *J. Exp. Med.* **134**, 713–740 https://doi.org/10.1084/jem.134.3.713
7 Leake, E.S., Myrvik, Q.N. and Wright, M.J. (1984) Phagosomal membranes of *Mycobacterium bovis* BCG-immune alveolar macrophages are resistant to disruption by *Mycobacterium tuberculosis* H37Rv. *Infect. Immun.* **45**, 443–446 https://doi.org/10.1128/IAI.45.2.443-446.1984
8 van der Wel, N., Hava, D., Houben, D., Fluitsma, D., van Zon, M., Pierson, J. et al. (2007) *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell* **129**, 1287–1298 https://doi.org/10.1016/j.cell.2007.05.059
9 Russell, D.G. (2016) The ins and outs of the *Mycobacterium tuberculosis*-containing vacuole. *Cell Microbiol.* **18**, 1065–1069 https://doi.org/10.1111/cmi.12623
10 Parker, E.J. (2017) The biosynthesis of tryptophan. In *The Handbook of Microbial Metabolism of Amino Acids* and (D'Mello, J.P.F., ed.), pp. 256–266, CAB International. ISBN-13: 978 1 78064 723 4
11 Crawford, I.P. (1975) Gene rearrangements in the evolution of the tryptophan synthetic pathway. *Bacteriol. Rev.* **39**, 87–120 https://doi.org/10.1128/MMBR.39.2.87-120.1975
12 Crawford, I.P. (1989) Evolution of a biosynthetic pathway: the tryptophan paradigm. *Annu. Rev. Microbiol.* **43**, 567–600 https://doi.org/10.1146/annurev.mi.43.100189.003031
13 Spraggon, G., Kim, C., Nguyen-Huu, X., Yee, M.C., Yanofsky, C. and Mills, S.E. (2001) The structures of anthranilate synthase of *Serratia marcescens* crystallized in the presence of (i) its substrates, chorismate and glutamine, and a product glutamate, and (ii) its end-product inhibitor, L-tryptophan. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6021–6026 https://doi.org/10.1073/pnas.111150298
14 Morollo, A.A. and Eck, M.J. (2001) Structure of the cooperative allosteric anthranilate synthase from *Salmonella typhimurium*. *Nat. Struct. Mol. Biol.* **8**, 243–247 https://doi.org/10.1038/84988
15 Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D. et al. (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**, 537–544 https://doi.org/10.1038/31159
16 Harrison, A.J., Yu, M., Gårdenborg, T., Middleditch, M., Ramsay, R.J., Baker, E.N. et al. (2006) The structure of MbtI from *Mycobacterium tuberculosis*, the first enzyme in the biosynthesis of the siderophore mycobactin, reveals it to be a salicylate synthase. *J. Bacteriol.* **188**, 6081–6091 https://doi.org/10.1128/JB.00338-06
17 Bashiri, G., Johnston, J.M., Evans, G.L., Bulloch, E.M.M., Goldstone, D.C., Jirgis, E.N.M. et al. (2015) Structure and inhibition of subunit I of the anthranilate synthase complex of *Mycobacterium tuberculosis* and expression of the active complex. *Acta. Crystallogr. D Biol. Crystallogr.* **71**, 2297–2308 https://doi.org/10.1107/S1399004715017216
18 Slock, J., Stahly, D.P., Han, C.Y., Six, E.W. and Crawford, I.P. (1990) An apparent *Bacillus subtilis* folic acid biosynthetic operon containing *pab*, an amphibolic trpG gene, a third gene required for synthesis of *para*-aminobenzoic acid, and the dihydropteroate synthase gene. *J. Bacteriol.* **172**, 7211–7226 https://doi.org/10.1128/JB.172.12.7211-7226.1990
19 Due, A.V., Kuper, J., Geerlof, A., von Kries, J.P. and Wilmanns, M. (2011) Bisubstrate specificity in histidine/tryptophan biosynthesis isomerase from *Mycobacterium tuberculosis* by active site metamorphosis. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 3554–3559 https://doi.org/10.1073/pnas.1015996108
20 Wellington, S., Nag, P.P., Michalska, K., Johnston, S.E., Jedrzejczak, R.P., Kaushik, V.K. et al. (2017) A small-molecule allosteric inhibitor of *Mycobacterium tuberculosis* tryptophan synthase. *Nat. Chem. Biol.* **266**, 1605–1612 https://doi.org/10.1038/nchembio.2420
21 Abrahams, K.A., Cox, J.A.G., Fütterer, K., Rullás, J., Ortega-Muro, F., Loman, N.J. et al. (2017) Inhibiting mycobacterial tryptophan synthase by targeting the inter-subunit interface. *Sci. Rep.* **7**, 9430 https://doi.org/10.1038/s41598-017-09642-y

22 Shen, H., Yang, Y., Wang, F., Zhang, Y., Ye, N., Xu, S. et al. (2009) Characterization of the putative tryptophan synthase β-subunit from *Mycobacterium tuberculosis*. *Acta Biochim. Biophys. Sin.* **41**, 379–388 https://doi.org/10.1093/abbs/gmp017
23 Czekster, C.M., Neto, B.A.D., Lapis, A.A.M., Dupont, J., Santos, D.S. and Basso, L.A. (2009) Steady-state kinetics of indole-3-glycerol phosphate synthase from *Mycobacterium tuberculosis*. *Arch. Biochem. Biophys.* **486**, 19–26 https://doi.org/10.1016/j.abb.2009.04.001
24 Lee, C.E., Goodfellow, C., Javid-Majd, F., Baker, E.N. and Lott, J.S. (2006) The crystal structure of TrpD, a metabolic enzyme essential for lung colonization by *Mycobacterium tuberculosis*, in complex with its substrate phosphoribosylpyrophosphate. *J. Mol. Biol.* **355**, 784–797 https://doi.org/10.1016/j.jmb.2005.11.016
25 Smith, D.A., Parish, T., Stoker, N.G. and Bancroft, G.J. (2001) Characterization of auxotrophic mutants of *Mycobacterium tuberculosis* and their potential as vaccine candidates. *Infect. Immun.* **69**, 1142–1150 https://doi.org/10.1128/IAI.69.2.1442-1150.2001
26 Hondalus, M.K., Bardarov, S., Russell, R. and Chan, J. (2000) Attenuation of and protection induced by a leucine auxotroph of *Mycobacterium tuberculosis*. *Infect. Immun.* **68**, 2888–2898 https://doi.org/10.1128/IAI.68.5.2888-2898.2000
27 Tiwari, S., van Tonder, A.J., Vilchèze, C., Mendes, V., Thomas, S.E., Malek, A. et al. (2018) Arginine-deprivation–induced oxidative damage sterilizes *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 9779–9784 https://doi.org/10.1073/pnas.1808874115
28 Pavelka, M.S., Chen, B., Kelley, C.L., Collins, F.M. and Jacobs, W.R. (2003) Vaccine efficacy of a lysine auxotroph of *Mycobacterium tuberculosis*. *Infect. Immun.* **71**, 4190–4192 https://doi.org/10.1128/IAI.71.7.4190-4192.2003
29 Berney, M., Berney-Meyer, L., Wong, K.-W., Chen, B., Chen, M., Kim, J. et al. (2015) Essential roles of methionine and S-adenosylmethionine in the autarkic lifestyle of *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 10008–10013 https://doi.org/10.1073/pnas.1513033112
30 Pfefferkorn, E.R. and Guyre, P.M. (1984) Inhibition of growth of *Toxoplasma gondii* in cultured fibroblasts by human recombinant gamma interferon. *Infect. Immun.* **44**, 211–216 https://doi.org/10.1128/IAI.44.2.211-216.1984
31 Cervenka, I., Agudelo, L.Z. and Ruas, J.L. (2017) Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. *Science* **357**, eaaf9794 https://doi.org/10.1126/science.aaf9794
32 Fujigaki, S., Saito, K., Takemura, M., Maekawa, N., Yamada, Y., Wada, H. et al. (2002) L-tryptophan-L-kynurenine pathway metabolism accelerated by *Toxoplasma gondii* infection is abolished in gamma interferon-gene-deficient mice: cross-regulation between inducible nitric oxide synthase and indoleamine-2,3-dioxygenase. *Infect. Immun.* **70**, 779–786 https://doi.org/10.1128/IAI.70.2.779-786.2002
33 Silva, N.M., Rodrigues, C.V., Santoro, M.M., Reis, L.F.L., Alvarez-Leite, J.I. and Gazzinelli, R.T. (2002) Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during *in vivo* infection with *Toxoplasma gondii*: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. *Infect. Immun.* **70**, 859–868 https://doi.org/10.1128/IAI.70.2.859-868.2002
34 Byrne, G.I., Lehmann, L.K. and Landry, G.J. (1986) Induction of tryptophan catabolism is the mechanism for gamma-interferon-mediated inhibition of intracellular *Chlamydia psittaci* replication in T24 cells. *Infect. Immun.* **53**, 347–351 https://doi.org/10.1128/IAI.53.2.347-351.1986
35 Thomas, S.M., Garrity, L.F., Brandt, C.R., Schobert, C.S., Feng, G.S., Taylor, M.W. et al. (1993) IFN-gamma-mediated antimicrobial response. Indoleamine 2, 3-dioxygenase-deficient mutant host cells no longer inhibit intracellular *Chlamydia* spp. or *Toxoplasma* growth. *J. Immunol.* **150**, 5529–5534 PMID: 8515074
36 MacKenzie, C.R., Hadding, U. and Däubener, W. (1998) Interferon-γ-induced activation of indoleamine 2, 3-dioxygenase in cord blood monocyte-derived macrophages inhibits the growth of group B streptococci. *J. Infect. Dis.* **178**, 875–878 https://doi.org/10.1086/515347
37 Murray, H.W., Szuro-Sudol, A. and Wellner, D. (1989) Role of tryptophan degradation in respiratory burst-independent antimicrobial activity of gamma interferon-stimulated human macrophages. *Infect. Immun.* **57**, 845–849 https://doi.org/10.1128/IAI.57.3.845-849.1989
38 Peng, K. and Monack, D.M. (2010) Indoleamine 2,3-Dioxygenase 1 is a lung-specific innate immune defense mechanism that inhibits growth of *Francisella tularensis* tryptophan auxotrophs. *Infect. Immun.* **78**, 2723–2733 https://doi.org/10.1128/IAI.00008-10
39 Mellor, A. (2005) Indoleamine 2,3 dioxygenase and regulation of T cell immunity. *Biochem. Biophys. Res. Commun.* **338**, 20–24 https://doi.org/10.1016/j.bbrc.2005.08.232
40 Zelante, T., Fallarino, F., Bistoni, F., Puccetti, P. and Romani, L. (2009) Indoleamine 2,3-dioxygenase in infection: the paradox of an evasive strategy that benefits the host. *Microbes Infect.* **11**, 133–141 https://doi.org/10.1016/j.micinf.2008.10.007
41 Suzuki, Y., Suda, T., Asada, K., Miwa, S., Suzuki, M., Fujie, M. et al. (2012) Serum indoleamine 2,3-dioxygenase activity predicts prognosis of pulmonary tuberculosis. *Clin. Vaccine Immunol.* **19**, 436–442 https://doi.org/10.1128/CVI.05402-11
42 Suzuki, Y., Miwa, S., Akamatsu, T., Suzuki, M., Fujie, M., Nakamura, Y. et al. (2013) Indoleamine 2,3-dioxygenase in the pathogenesis of tuberculous pleurisy. *Int. J. Tuberc. Lung Dis.* **17**, 1501–1506 https://doi.org/10.5588/ijtld.13.0082
43 Gautam, U.S., Foreman, T.W., Bucsan, A.N., Veatch, A.V., Alvarez, X., Adekambi, T. et al. (2018) *In vivo* inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E62–E71 https://doi.org/10.1073/pnas.1711373114
44 Zhang, Y.J., Reddy, M.C., Ioerger, T.R., Rothchild, A.C., Dartois, V., Schuster, B.M. et al. (2013) Tryptophan biosynthesis protects mycobacteria from CD4 T-cell-mediated killing. *Cell* **155**, 1296–1308 https://doi.org/10.1016/j.cell.2013.10.045
45 Kozlowski, M., Tom, N., Seto, C., Sefler, A. and Bartlett, P. (1995) Chorismate-utilizing enzymes isochorismate synthase, anthranilate synthase, and *p*-aminobenzoate synthase: mechanistic insight through inhibitor design. *J. Am. Chem. Soc.* **117**, 2128–2140 https://doi.org/10.1021/ja00113a002
46 Zhang, X.-K., Liu, F., Fiers, W.D., Sun, W.-M., Guo, J., Liu, Z. et al. (2017) Synthesis of transition-state inhibitors of chorismate utilizing enzymes from bromobenzene *cis*-1,2-dihydrodiol. *J. Org. Chem.* **82**, 3432–3440 https://doi.org/10.1021/acs.joc.6b02801
47 Chi, G., Manos-Turvey, A., O'Connor, P.D., Johnston, J.M., Evans, G.L., Baker, E.N. et al. (2012) Implications of binding mode and active site flexibility for inhibitor potency against the salicylate synthase from *Mycobacterium tuberculosis*. *Biochemistry* **51**, 4868–4879 https://doi.org/10.1021/bi3002067
48 Manos-Turvey, A., Bulloch, E., Rutledge, P., Baker, E., Lott, J. and Payne, R. (2010) Inhibition studies of *Mycobacterium tuberculosis* salicylate synthase (Mbtl). *ChemMedChem* **5**, 1067–1079 https://doi.org/10.1002/cmdc.201000137
49 Manos-Turvey, A., Cergol, K.M., Salam, N.K., Bulloch, E.M.M., Chi, G., Pang, A. et al. (2012) Synthesis and evaluation of *M. tuberculosis* salicylate synthase (Mbtl) inhibitors designed to probe plasticity in the active site. *Org. Biomol. Chem.* **10**, 9223–9236 https://doi.org/10.1039/c2ob26736e
50 Evans, G.L., Gamage, S.A., Bulloch, E.M.M., Baker, E.N., Denny, W.A. and Lott, J.S. (2014) Repurposing the chemical scaffold of the anti-arthritic drug lobenzarit to target tryptophan biosynthesis in *Mycobacterium tuberculosis*. *ChemBioChem* **15**, 852–864 https://doi.org/10.1002/cbic.201300628

51 Cookson, T., Castell, A., Bulloch, E., Evans, G., Short, F., Baker, E. et al. (2014) Alternative substrates reveal catalytic cycle and key binding events in the reaction catalysed by anthranilate phosphoribosyltransferase from *Mycobacterium tuberculosis*. *Biochem. J.* **461**, 87–98 https://doi.org/10.1042/BJ20140209
52 Castell, A., Short, F.L., Evans, G.L., Cookson, T.V.M., Bulloch, E.M.M., Joseph, D.D.A. et al. (2013) The substrate capture mechanism of *Mycobacterium tuberculosis* anthranilate phosphoribosyltransferase provides a mode for inhibition. *Biochemistry* **52**, 1776–1787 https://doi.org/10.1021/bi301387m
53 Cookson, T.V.M., Evans, G.L., Castell, A., Baker, E.N., Lott, J.S. and Parker, E.J. (2015) Structures of *Mycobacterium tuberculosis* anthranilate phosphoribosyltransferase variants reveal the conformational changes that facilitate delivery of the substrate to the active site. *Biochemistry* **54**, 6082–6092 https://doi.org/10.1021/acs.biochem.5b00612
54 Islam, M.N., Hitchings, R., Kumar, S., Fontes, F.L., Lott, J.S., Kruh-Garcia, N.A. et al. (2019) Mechanism of fluorinated anthranilate-induced growth inhibition in *Mycobacterium tuberculosis*. *ACS Infect. Dis.* **5**, 55–62 https://doi.org/10.1021/acsinfecdis.8b00092
55 Browne, D.T., Kenyon, G.L. and Hegeman, G.D. (1970) Incorporation of monofluorotryptophans into protein during the growth of *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **39**, 13–19 https://doi.org/10.1016/0006-291X(70)90750-3
56 Michalska, K., Chang, C., Maltseva, N.I., Jedrzejczak, R., Robertson, G.T., Gusovsky, F. et al. (2020) Allosteric inhibitors of *Mycobacterium tuberculosis* tryptophan synthase. *Protein Sci.* **29**, 779–788 https://doi.org/10.1002/pro.3825
57 Negatu, D.A., Liu, J.J.J., Zimmerman, M., Kaya, F., Dartois, V., Aldrich, C.C. et al. (2018) Whole-cell screen of fragment library identifies gut microbiota metabolite indole propionic acid as antitubercular. *Antimicrob. Agents Chemother.* **62**, e01571-17 https://doi.org/10.1128/AAC.01571-17
58 Dodd, D., Spitzer, M.H., Van Treuren, W., Merrill, B.D., Hryckowian, A.J., Higginbottom, S.K. et al. (2017) A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. *Nature* **551**, 648–652 https://doi.org/10.1038/nature24661
59 Negatu, D.A., Yamada, Y., Xi, Y., Go, M.L., Zimmerman, M., Ganapathy, U. et al. (2019) Gut microbiota metabolite indole propionic acid targets tryptophan biosynthesis in *Mycobacterium tuberculosis*. *mBio* **10**, 1402 https://doi.org/10.1128/mBio.02781-18
60 Parish, T. (2003) Starvation survival response of *Mycobacterium tuberculosis*. *J. Bacteriol.* **185**, 6702–6706 https://doi.org/10.1128/JB.185.22.6702-6706.2003
61 Zhang, Y.J. and Rubin, E.J. (2013) Feast or famine: the host-pathogen battle over amino acids. *Cell Microbiol.* **15**, 1079–1087 https://doi.org/10.1111/cmi.12140
62 Yuasa, H.J. (2016) High l-Trp affinity of indoleamine 2,3-dioxygenase 1 is attributed to two residues located in the distal heme pocket. *FEBS J.* **283**, 3651–3661 https://doi.org/10.1111/febs.13834
63 Lenaerts, A., Barry, C.E. and Dartois, V. (2015) Heterogeneity in tuberculosis pathology, microenvironments and therapeutic responses. *Immunol. Rev.* **264**, 288–307 https://doi.org/10.1111/imr.12252
64 Kaur, H., Bose, C. and Mande, S.S. (2019) Tryptophan metabolism by gut microbiome and gut-brain-axis: an *in silico* analysis. *Front. Neurosci.* **13**, 1365 https://doi.org/10.3389/fnins.2019.01365