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The bare essentials of antibiotic target validation

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Abstract

The convergence of competitive fitness experiments and phenotypic screening would seem to be an auspicious beginning for validation of an antibacterial target. IMPDH was already identified an essential protein in *Mycobacterium tuberculosis* when not one, but two, groups discovered inhibitors with promising antitubercular activity. A new target appeared to be born. Surprisingly, the two groups come to completely different conclusions about the vulnerability of IMPDH and its future as a drug target. This Viewpoint discusses these papers and how to resolve this conundrum.

Keywords

Mycobacterium tuberculosis; IMP dehydrogenase; essential genes; target validation

New antibiotics are urgently needed to combat the relentless emergence of drug resistant pathogens. The identification of new drug targets and scaffolds critically relies on screening conditions that accurately reflect the environment of the pathogen during infection. Unfortunately, antibiotic development is frequently upended when actual infections fail to conform to expectations shaped by *in vitro* experiments. This problem has spawned a wave of genome-wide screens to identify the genes required to establish infections in mice. Such competitive fitness experiments consistently find that nucleotide biosynthesis is required for infections *in vivo*. In particular, inosine monophosphate dehydrogenase (IMPDH), the enzyme that catalyzes the first and rate-limiting step of guanine nucleotide biosynthesis (Figure 1)¹, has been deemed essential in every pathogen analyzed to date, including three of the most serious bacterial threats: *Mycobacterium tuberculosis* (*Mtb*), *Staphylococcus aureus* and *Pseudomonas aeruginosa*^{2–4}. These pathogens harbor purine salvage pathways that should bypass IMPDH, so the logical conclusion is that guanine and guanosine, as well as xanthine and xanthosine, are not available in sufficient quantities to support infection.

Given this backdrop, it was perhaps inevitable that phenotypic screening for inhibitors of pathogen growth would eventually yield IMPDH inhibitors, as reported for *Mtb* in two papers in this issue^{5–6}. The two papers are strikingly similar in their initial approach and findings. Both groups identified promising aryl sulfonamide inhibitors of *Mtb* growth with little mammalian cell cytotoxicity. The compounds are bacteriocidal, and display efficacy against bacteria replicating within macrophages. Both groups set out to investigate

Conflict of interest.

The author is also developing IMPDH inhibitors as potential antibiotics.

mechanism of action by selecting resistant mutants, and both find mutations in *guaB2*, the gene that encodes the enzymatically active IMPDH of *Mtb*. High concentrations of guanine (100 μ M) protect *Mtb* from both inhibitors, though no rescue was observed with guanosine and xanthine. These observations further confirm that IMPDH is the target, but also demonstrate that *Mtb* can bypass IMPDH if sufficient guanine is available. Inhibition of IMPDH was confirmed down to x-ray crystal structures of enzyme inhibitor-complexes. The foundation of a serious drug discovery effort appears to be laid. The surprise is that the two groups arrive at the exact opposite conclusions about the availability of guanine *in vivo* and the future of IMPDH as a target for antitubercular drugs.

The compound characterized by Singh et al has some ADMET liabilities, so this group undertook a genetic approach for further target validation. They constructed a conditional *guaB2* knockout strain of *Mtb* and demonstrated that depletion of IMPDH killed bacteria *in vitro*, both in culture and in macrophages. Again, high concentrations of guanine rescued growth. Curiously, under some growth conditions, a subset of *Mtb* cells survived, reactivating when guanine was supplied. Perhaps some IMPDH remains in these cells, permitting survival. Nonetheless, depletion of IMPDH was rapidly bacteriocidal in mice, preventing infection. Therefore Singh et al conclude that guanine concentrations are too low to support infection in mice. Thus *Mtb* is vulnerable to IMPDH inhibitors, and IMPDH is a promising new target for antitubercular drugs.

Park et al chose the small molecule inhibitor route for *in vivo* target validation. Two inhibitors were tested in mouse models of tuberculosis, and both compounds failed to display efficacy. This disappointment can easily be attributed to insufficient plasma concentrations. A new tuberculous drug could conceivably result from a sustained medicinal chemistry effort. However, concerned about the ability of guanine to rescue IMPDH inhibition, Park et al also measured guanine concentrations in lungs. Little guanine was observed in mouse lung, in keeping with the observations of Singh et al., but stunningly high guanine concentrations were found in infected rabbit and human lungs, 200–800 μ M in granulomas and as high as 2 mM in uninvolved lung. These values are well above the solubility limit of guanine at neutral pH (~100 μ M). It is worth noting that metabolite concentrations are tricky to measure because concentrations can change rapidly after cells are ruptured (and here rabbit/human samples were homogenized prior to freezing, whereas the mice samples were frozen immediately)⁷, so it is prudent to consider that the differences in guanine concentrations may derive from sample handling rather than real differences in species metabolism. Further verification of guanine levels in normal and infected lung is clearly desired. Still, if indeed guanine is abundant in patient lungs, *Mtb* will not be susceptible to IMPDH inhibitors. These findings cast a dark shadow on the potential of IMPDH as a target for antitubercular drugs.

Setting aside questions of inhibitor quality and guanine availability, there are several other reasons a small molecule inhibitor might not elicit the same response as a gene knockout. First, a knockout removes all enzymatic activity, whereas 100% inhibition is difficult to achieve, much less maintain, with a small molecule. Moreover, a knockout removes all protein functions. In the case of IMPDH, the enzyme is a square planar homotetramer, with each monomer comprised of a catalytic domain and regulatory (CBS) subdomain of unclear

function (Figure 2). The CBS subdomains of bacterial IMPDHs bind MgATP, regulating the formation of higher order structures such as octamers and filaments⁸. In *Escherichia coli*, the CBS subdomain controls the balance of the adenine and guanine nucleotide pools via an unknown mechanism⁹. IMPDH also binds oligonucleotides and is a transcription factor in yeast^{1, 10}, and these functions also involve the CBS subdomain. IMPDH Thus it is quite possible that the essentiality of IMPDH derives from the moonlighting functions of the CBS subdomain rather than enzymatic activity per se, and thus would not be susceptible to enzyme inhibition.

More generally, an inhibitor confronts an established infection, whereas the conditional knockout of Singh et al, and competitive fitness experiments in general, assess the ability to initiate infection. Inflammation and bacterial factors can cause host cell rupture, releasing purines at the site of infection. Gene expression changes in the host, and new salvage pathways may become operational. Thus it is not hard to imagine that IMPDH may be required during the initial stages of an infection, but dispensable for maintenance. The conditional silencing of *guaB2* expression in an established infection would answer this question for IMPDH, and Singh et al appear positioned to do this experiment. This self-same question looms large in the validation of other so-called essential genes as well as virulence factors as drug targets. The difference between establishing and maintaining infection may be the difference in developing prophylaxis and therapy.

Acknowledgments

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Figure 1.

Guanine nucleotide biosynthesis and salvage. IMPDH is shown in red, other enzymes in blue. IMP is the product of *de novo* purine biosynthesis as well as the salvage of hypoxanthine (Hyp) and inosine (Ino). IMPDH catalyzes the conversion of IMP into XMP with the concomitant reduction of NAD⁺. XMP can also be produced by the salvage of xanthine (Xan) and xanthosine (Xao). GMP synthetase (GMPS) converts XMP into GMP, and GMP is also produced by the salvage of guanine or guanosine. The salvage of nucleobases typically involves a phosphoribosyltransferase (PRT) reaction, whereas nucleoside salvage can occur via a nucleoside kinase (NK), or via initial conversion to the nucleobase by a nucleoside hydrolase (NH) or nucleoside phosphorylase (NP) followed by phosphoribosylation. Nucleobases can also be interconverted by the actions of xanthine oxidase (XO) and guanine deaminase (GD). PRPP, phosphoribosyl pyrophosphate; P_i, phosphate; PP_i, pyrophosphate.

The success of IMPDH inhibitors *in vivo* is critically dependent on a pathogen's ability to salvage guanine or xanthine (or their nucleosides) and thus bypass IMPDH. Such salvage depends on the availability of purines and the presence of the appropriate transporters and salvage enzymes. The specificity of transporters and salvage enzymes can be difficult to predict from sequence alone, and the competition between purines for these proteins can determine salvage efficiency.

Figure 2. Structure of IMPDH. A. *Streptococcus pyogenes* IMPDH: a tetramer, with individual monomers shown in different colors, IMP is shown in spacefill to identify the catalytic domain (PDB 1ZFJ). B. *Pseudomonas aeruginosa* IMPDH: an octamer comprised of a dimer of tetramers. One monomer from each tetramer is shown in ribbons, MnATP is shown in spacefill to highlight how the CBS subdomains interact to form the octamer (PDB 4DQW).