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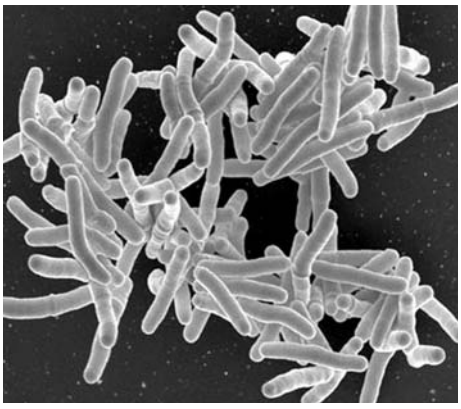

***Mycobacterium tuberculosis;* image kindly provided by NIAID**

New drugs must travel a long pathway to development, from basic science to clinical trials. Rubin's work takes that first step. He and his group have genetically mapped out the organism's aerobic respiratory chain – how electrons build the force necessary to create ATP. They turned their attention to the first enzyme in that chain, NADH:menaquinone oxidoreductase. After screening over 50 phenothiazine compounds, they identified several that were tuberculocidal *in vitro* and inhibited pathogenesis in a mouse model.

Good target

Rubin is under no illusions about the long road to new therapeutic drugs. 'To be honest, these compounds aren't going to become active anti-TB agents. But this enzyme is a good target, and this class of drugs is a good candidate.' Christine Sizemore of the National Institute of Allergy and Infectious Diseases (NIAID) agrees. 'The targets they're going after represent a new direction: oxygen metabolism.' She describes the work as 'an important biochemistry paper,' with the compounds' activity as 'a little bit of a carrot at the end.' Even if they never become drug candidates, she adds, they might have immense utility as tools to understand the inner working of the microbe.

Rubin characterizes the experiments 'as a proof of principle that this enzyme, and even the whole chain, is a good target.' Work from another laboratory [2] has also honed in on this oxidative machinery – specifically ATP synthase. 'They targeted the last enzyme of the pathway, and now we've targeted the front end, the first step that sends electrons down the chain.'

The Holy Grail

The success of *M. tuberculosis* as a pathogen arises from its tenacity. Even deprived of oxygen and nutrients, it can survive for years in the body of its host by entering a dormant state. When resources become available, the bug awakens with devastating effects for its host. But the transition between these states remains mysterious.

Although there's no evidence yet that the reductase plays a direct role in entering or leaving dormancy, Rubin and his team 'believe in our heart of hearts that it might.' Understanding how Mtb survives on lower oxygen levels is critical to learning how to kill the bug. The answer may lie in the oxidative enzymes' high affinity for oxygen. 'We believe that how the bug up- or down-regulates these enzymes is key to whether it stays dormant.'

Sizemore says, 'The Holy Grail is to find drugs that will kill the bug dead rather than force it into its dormant state – to find something that will kill it in any state.' She cautions that, as with development of any drug, 'the realities of economy play into TB treatment.' Next-generation drugs might not be around the next bend, but hopefully we won't have to wait another 40 years for them. And with new targets, hard work, and a bit of luck, maybe we won't have to.

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Looking for new sepsis targets

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A nitric oxide synthase inhibitor that showed initial promise for the treatment of sepsis was found to be associated with increased mortality in a large clinical trial last year. Now scientists are looking at the many pathways involved in the disease for clues that might lead to new targets.

Rising incidence

The incidence of sepsis, the body's response to a bacterial infection, has increased during the past decade from about 80 per 100,000 population to 250 per 100,000 population and is expected to continue rising as the population ages. The disease, which has a mortality rate of about 25%, costs US hospitals alone nearly $17 billion annually [1].

'One of the principal causes of death is severe hypotension, and much of the therapy is based on reversing this extremely low blood pressure that leads to fluid extravasation, inadequate tissue and organ perfusion, and ultimately organ failure and death,' explains Adrian J. Hobbs, Wellcome Trust Senior Research Fellow, Wolfson Institute for Biomedical Research, University College London.

Although pharmaceutical companies have been funding research in this area for years, Xigris, a recombinant human Activated Protein C marketed by Eli Lilly and Company, is the only therapy approved for treating high-risk severe sepsis.

Nitric oxide levels

NG-methyl-L-arginine held promise as a therapeutic for sepsis due to its ability to inhibit the overproduction of nitric oxide, a vasodilator that at sustained high levels can cause systemic hypotension. But, a multiple-center clinical trial found the nonspecific nitric oxide synthase inhibitor increased mortality in patients with septic shock and was prematurely halted [2].

There are, in fact, three isoforms of nitric oxide synthase: inducible (iNOS), endothelial (eNOS), and neuronal (nNOS). It's iNOS that has been long linked to the increased nitric oxide levels triggering septic shock, and research has focused on inhibiting its expression.

However, recent work led by Hobb shows that mice lacking eNOS, an enzyme found predominantly in endothelial cells lining the

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internal surface of blood vessels that plays an important role in cardiovascular homeostasis, have a markedly reduced iNOS response during endotoxemia [3]. These knock-out animals also have lower mortality than wild-type mice in response to bacterial endotoxin.

Because of its protective physiological functions, eNOS isn't considered a feasible therapeutic target. The nitric oxide it synthesizes is responsible for dilating blood vessels, governing fluid exchange and controlling the growth of vascular smooth muscle cells. 'Nonetheless, continued study in this area should give rise to a more detailed understanding of the interactions between eNOS and iNOS and perhaps allow re-evaluation of NOS inhibitors as a potential treatment,' Hobbs says.

Interacting, complex pathways

Given the failure of the NG-methyl-L-arginine trial, some sepsis experts argue against revisiting nitric oxide synthase inhibitors as a therapy and instead propose looking for multiple targets within the broader spectrum of the disease. 'While the nitric oxide pathway is very important, it's one of many interacting, complex pathways,' says Yoram Vodovotz, Director, Center for Inflammation and Regenerative Modeling, University of Pittsburgh. 'Sepsis is a dynamically changing state. It's difficult to come up with a single drug that hits a single mediator.'

Currently, he is using mathematical models and computer algorithms to recreate the inflammatory response induced by sepsis and trauma. 'We are on a five-year project to put all the rules together and to try to find a [therapeutic] strategy,' says Vodovotz.

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