

Review Article

Exopolyphosphatase of *Mycobacterium Tuberculosis* Might Limit the Growth of Bacteria Which Thrive in Inflamed and Injured Lung

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Abstract

Pulmonary tuberculosis resembles cystic fibrosis and other chronic inflammatory lung diseases in its ability to cause chronic tissue destruction, hypoxia, tissue acidosis, and leakage of blood into the surrounding milieu. Destructive and inflammatory changes of the lung in diseases such as cystic fibrosis support the increased growth of the commensal *Prevotella*, a bacterial commensal which requires polyphosphate and thrives under inflammatory conditions. Since the nutritional needs of *Prevotella* are in some ways mirrored by those of the pathogens *Pseudomonas aeruginosa* and *Berkholderia cepacia*, it is possible that *Prevotella* may serve as a marker for these destructive lung pathogens. The biochemical milieu of pulmonary tuberculosis resembles that of other chronic inflammatory lung diseases in its ability to cause inflammation, destruction, tissue acidosis, and bleeding, yet in pulmonary tuberculosis, contrary to expectation, *Prevotella* species are decreased rather than increased. It is hypothesized that the *M. tuberculosis* exopolyphosphatase may serve to reduce polyphosphates required by *Prevotella* species. Since the nutritional needs of *P. aeruginosa* and *B. cepacia* resemble those of *Prevotella*, it is reasonable to hypothesize that addition of exopolyphosphatase might also hamper the growth of these dangerous lung pathogens, as well.

Keywords: Tuberculosis; Cystic fibrosis; *Prevotella*; *Pseudomonas*; *Berkholderia*; Polyphosphate; Exopolyphosphatase

Introduction

In some respects, the process of decline in pulmonary tuberculosis resembles that of cystic fibrosis and other chronic inflammatory conditions of the lung. In such conditions there may be lung tissue destruction, hypoxia, local tissue acidosis, leakage of blood into the airway, or loss of weight. Bacterial pathogens involved in pulmonary inflammatory processes (including *Pseudomonas aeruginosa* and *Berkholderia cepacia* in cystic fibrosis and various causes of inflammation and bronchiectasis; *Mycobacterium tuberculosis* in pulmonary tuberculosis) often have similar nutritional requirements for iron and polyphosphate. Acquisition of polyphosphate depends on the availability of ATP and bacterial polyphosphate kinase, and leakage of blood and heme into the airway supplies the requirement for iron in ample measure. Out of the bacterial pathogens noted above -- *P. aeruginosa*, *B. cepacia*, and *M. tuberculosis* - all utilize heme for iron, and all depend on polyphosphate kinase to synthesize polyphosphate [1-8].

Since the composition of pulmonary microbiota depends on local nutritional, inflammatory, and infectious factors, it should come as no surprise that inflammatory disease processes such as cystic fibrosis, bronchiectasis, and toxic lung exposure select for pulmonary commensals which require iron and polyphosphate for survival and growth and which are capable of thriving in an acidic environment. *Prevotella* species conform to expectations in both respects: they depend on polyphosphate and iron, and they are capable of growing at lower pH than are many other commensals. Accordingly, commensal *Prevotella* species are increased in cystic fibrosis, in toxic insult to the lung, and in other pulmonary infectious/inflammatory conditions [9-16].

Given the proclivities of *Prevotella*, the presence of this commensal may serve as a marker for certain environmental stress conditions within the lung. Since *Prevotella* requires heme iron and polyphosphate for growth and is capable of thriving at low pH, its increased dominance as a commensal in BAL fluid might suggest the increased presence of iron, blood, polyphosphate, and acid within the lung. Similarly, *Prevotella* species might be useful as a marker for increased risk infection by *P. aeruginosa* or *B. cepacia*, lung pathogens whose nutritional needs resemble those of *Prevotella*, and which may promote acidosis of local tissue.

Although many chronic inflammatory conditions of the lung tend to lead to increased growth of *Prevotella*, pulmonary tuberculosis is an exception to the rule. As a pathogen, *M. tuberculosis* would seem to meet all of the presumed criteria for increased growth of *Prevotella* species: *M. tuberculosis* requires both heme iron and polyphosphate, induces leakage of blood into the airway, and is capable of producing an acidic, inflammatory environment within the lung. In such an environment, one might expect that *Prevotella* species, and perhaps also *Pseudomonas* or *Berkholderia* species, would thrive. Instead, the opposite is true. In pulmonary tuberculosis, *Prevotella* species are decreased rather than increased, and superinfection by organisms such as *Pseudomonas* or *Berkholderia* is much rarer in pulmonary tuberculosis than in cystic fibrosis and other causes of bronchiectasis. [6,17-20] Why might this be?

Structural differences aside, one answer is suggested by the unique ability of *M. tuberculosis* to adjust its metabolism in order to adapt to starvation conditions, such as that of the oxygen and inorganic phosphate-limited environment of the pulmonary granuloma. This

metabolic adaptation, or “stringent response,” is controlled by the enzyme Rel_{Mtb}. Inorganic phosphate starvation, hypoxia, and polyphosphate accumulation activate transcription of Rel_{Mtb}, which upregulates synthesis of the small molecules involved in the stringent response: guanosine 5'-diphosphate 3'-diphosphate (ppGpp) and guanosine 5'-triphosphate 3'-diphosphate (pppGpp), together denoted (p)ppGpp. (p)ppGpp, in turn, serves as a second messenger for the stress response [21]. The result is decreased growth and decreased biofilm formation.

An important aspect of the stringent stress response in *M. tuberculosis* is that it is triggered both by inorganic phosphate deficit and also by polyphosphate excess. In *M. tuberculosis*, polyphosphate is synthesized via the action of polyphosphate kinase and hydrolysed via the action of exopolyphosphatase 2 (ppx2). When inorganic phosphate is scarce in the local environment, the stringent response regulates growth. On the other hand, when polyphosphate reaches a critical threshold, once again, the stringent response regulates growth [22,23].

Chuang et. al. (2015) found that, in *M. tuberculosis*, in a ppx2 knockdown strain, excess accumulation of polyphosphate resulted in growth restriction and reduced biofilm formation [22,23]. The resultant phenotype is one of growth regulation on both ends of the spectrum. *M. tuberculosis* differs from pathogens such as *P. aeruginosa* and *B. cepacia* in its ability to persist for many years in a latent or semi-latent state within the lung. It is possible that the regulation of growth by *M. tuberculosis* during times of plenty might contribute to the ability of *M. tuberculosis* to evade host immunity, thereby persisting over extended periods of time in a latent or semi-latent state. [24].

In pulmonary infection by *M. tuberculosis*, polyphosphate overproduction triggers the stringent response to restrict bacterial growth, which in turn reduces subsequent polyphosphate production by the organism. Ongoing bacterial exopolyphosphatase activity hydrolyzes polyphosphate, which enables growth, but growth, in turn, requires further production of polyphosphate. The effect is a tightly regulated system in which less polyphosphate may be available in the surrounding milieu.

This polyphosphate regulating behavior of *M. tuberculosis* could have implications for predisposition to superinfection, as well as for growth of associated microbiota such as *Prevotella*. In pulmonary tuberculosis, a commensal such as *Prevotella* would not be expected to lack for heme iron and would not be hampered by the inflammatory milieu. Despite conditions which might seem to support the growth of *Prevotella*, the ongoing activity of mycobacterial exopolyphosphatase and to ongoing growth restriction during times of excess polyphosphate production appear to suppress the growth of *Prevotella* instead. It is tempting to associate the observed growth suppression of *Prevotella* with exopolyphosphatase-induced scarcity of readily available polyphosphate.

In contrast to the observed suppression of *Prevotella* in the context of *M. tuberculosis*, in cystic fibrosis and in chronic traumatic/toxic lung damage, the situation is different. With cystic fibrosis and with traumatic lung damage, the underlying cause of lung damage is genetic/metabolic or traumatic rather than infectious; regulatory processes and exopolyphosphatases of *M. tuberculosis* do not play a role in limiting environmental polyphosphate; and *Prevotella* species thrive within the lung.

The most interesting aspect of this hypothesis may relate to its application to bacterial pathogens of the lung which resemble *Prevotella* with respect to their nutritional needs and environmental tolerances. For what can be said for *Prevotella* might also be said for pathogens such as *P. aeruginosa* and *B. cepacia*. If *M. tuberculosis* exopolyphosphatase can control the growth of *Prevotella*, perhaps it can limit the growth of *Pseudomonas* and *Berkholderia* species, as well. This trait may hold potential for use of exopolyphosphatases in control of pulmonary infection in cystic fibrosis and in other chronic bronchiectasis producing conditions.

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Conflicts of interest

The author declares no conflicts of interest.

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