Understanding and targeting cholesterol catabolism in *Mycobacterium tuberculosis*.

Mycobacterium tuberculosis (*Mtb),* the causative agent of tuberculosis, kills over 1 million people annually. Due to the emergence of drug resistant strains such as MDRh and XDRh TB, *Mtb* remains a global threat for which novel therapeutics are urgently needed.

Mtb utilizes hosth derived cholesterol during infection. Based on a combination of transposon mutagenesis, deep sequencing and chemical genetics, cholesterol catabolic enzymes have been identified as potential therapeutic targets. Deletion of many of these genes results in avirulent or attenuated strains, and small molecules selected for their ability to inhibit growth of *Mtb* in macrophages include many that specifically inhibit cholesterol catabolism. One of the most intriguing potential drug targets is IpdAB, a predicted CoA transferase that is essential for growth of *Mtb* on cholesterol. Deletion of the *ipdA* gene in the related horse pathogen, *Rhodococcus equi*, yielded a mutant that has been patented as a live vaccine. However, the function of IpdAB remains unknown.

IpdAB is involved in the degradation of the last two rings of cholesterol. Degradation of the last half of the cholesterol molecule is poorly understood, but is now known to involve coenzyme A (CoA) thioesters. Due to their large size and charge, CoA thioester metabolites do not cross the cell membrane. Recently, the Eltis Lab has developed some novel approaches to monitor the intracellular CoA metabolome of cholesterolh catabolizing bacteria. In brief, specific gene deletion mutants are incubated with cholesterol and then lysed. The extracted metabolites are enriched for CoA thioesters using a solid phase extraction technique. The CoA metabolome is then characterized using liquid chromatography coupled mass spectrometry (LCh MS) to separate CoA thioesters. Since CoA thioesters fragment in a highly predictable manner, multiple reaction monitoring (MRM) can be used to confirm the presence and identity of each CoA metabolite.

The project will focus on characterizing the function of IpdAB using three approaches. First, the aboveh described metabolomics approach will be used on an *ipdAB* deletion mutant incubated with cholesterol to identify which unique CoA thioesters accumulate. Second, heterologously produced IpdAB will be incubated with the purified CoA metabolome of the *ipdAB* mutant. Changes in the CoA metabolome of the *ipdAB* mutant will be monitored by LCh MS and used to assign function to IpdAB. Lastly, once function is assigned, a highh throughput assay to monitor IpdAB activity will be developed to enable the screening of small molecule libraries for inhibitors of IpdAB. Such inhibitors are potential lead compounds for novel therapeutics. Volunteering in Dr. Eltis' lab over the past year has enabled me to learn a variety of skills that are relevant skills to this project, including running an HPLC and molecular biology.

Overall, understanding the function of IpdAB will provide important insight into how *Mtb* uses host cholesterol as a nutrient during infection and should contribute to developing urgently needed therapeutics against this pathogen.