

Finding antigen culprits in an occupational disease

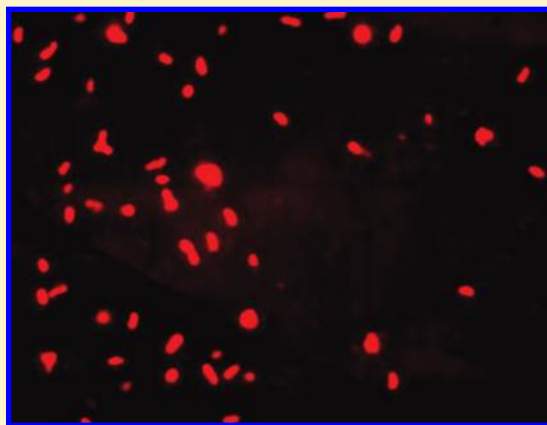
Workers in automotive plants and in other metal-cutting industries are exposed to many occupational hazards such as heavy machinery, razor-sharp saws, extreme heat, and caustic chemicals. But in addition to these obvious hazards, an invisible danger can lurk in the air that the workers breathe. In 2000, the newly recognized species *Mycobacterium immunogenum* was linked to outbreaks of hypersensitivity pneumonitis (HP) in machinists exposed to aerosolized, contaminated metalworking fluid (MWF). To explore how *M. immunogenum* causes the symptoms of HP, Jagjit Yadav and colleagues at the University of Cincinnati identified protein antigens of the mycobacterium with a proteomics approach. Their results are published in *JPR* (DOI 10.1021/pr8009462).

HP is an immune-mediated respiratory illness caused by the inhalation of microbial antigens, which trigger an immune reaction. Symptoms of HP include cough, shortness of breath, chills, fatigue, and headache. *M. immunogenum*, a nontuberculous mycobacterium, was found to colonize MWFs at HP outbreak sites. "MWFs, which are stored in large tanks and circulated through the machines, are used for cooling and grinding metals," says Yadav. "Because of the heat and speed of the machines, the fluids become aerosolized, and those aerosols are inhaled by the workers."

According to Yadav, >1 million machinists in the U.S. are exposed to MWFs. "These fluids invariably have bacteria in them, and some might have mycobacteria," he says. To better understand *M. immunogenum* virulence factors and HP etiology, Yadav and coworkers identified antigenic proteins in this little studied bacterium. "Because

HP is an immunological disorder, we wanted to know which antigens in this particular species of mycobacterium can trigger an immune response in the host," explains Yadav.

First, the researchers prepared different subcellular (cell wall, cell membrane, and cytosolic) fractions of an HP-linked *M. immunogenum* isolate by differential centrifugation. In addition,



Workplace problem-causer? *M. immunogenum* (stained here with a red fluorescent dye) has been linked to outbreaks of the respiratory illness hypersensitivity pneumonitis in machinists.

secreted proteins were isolated by filtration and trichloroacetic acid precipitation of the bacterial culture supernatant. Then, the subcellular and secretory fractions were resolved on separate 2DE gels. Immunoblotting with rabbit-raised polyclonal antibodies against *M. immunogenum* revealed immunoreactive protein spots. The antigenic protein spots in the cell wall, cell membrane, and cytosolic fractions were eluted from their respective gels and identified by MALDI TOFMS. Because the secretome fraction migrated on the 2DE gel primarily as one large, immunoreactive protein spot, the investigators eluted the entire spot, which contained a mixture of secretory proteins, for MALDI TOFMS analysis.

A total of 33 immunoreactive proteins were identified from the four *M. immunogenum* fractions: 6 cell wall, 11 cell membrane, 12 cytosolic, and 4 secretory proteins. Eight of the immu-

noreactive proteins were homologs of known mycobacterial antigens, such as the heat shock protein GroEL and the fibronectin-binding antigen 85A. Interestingly, 25 of the 33 proteins had not been characterized as antigens in other mycobacterial species.

To study the role of secreted proteins in HP, Yadav and colleagues tested the ability of the secretome eluate to induce an immune response in murine alveolar macrophages, cells that mount an early defense against inhaled mycobacteria. Treatment of alveolar macrophages with the secretome eluate caused up-regulation of the proinflammatory cytokines TNF- α , IL-6, IL-1 β , and IL-18 and down-regulation of the anti-inflammatory cytokine IL-10. These results suggest that secreted antigens contribute to lung inflammation in HP.

"We're definitely intrigued by the secretory antigens because these cell-free antigens are not detected in the existing exposure assessment approach, which is based on total mycobacterial count, and thus secretory antigens contribute to a higher actual antigen exposure dose in the aerosolized fluid," says Yadav. "Also, the most common method to control mycobacteria in MWFs is to add biocides, but even if they kill the mycobacteria, the fluids still have secretory antigens in them."

The identification of species-specific antigens for *M. immunogenum* will assist the development of workplace exposure assessment methods and diagnostic tools. Yadav says, "The idea is to improve the exposure assessment methods so that we know early on that these MWFs are contaminated with mycobacteria." Moreover, the newly identified *M. immunogenum* antigens could serve as targets for HP drug therapies or vaccines.

—Laura Cassidy