Exploring lysis and adaptive resistance to vancomycin in (leaky) *Pseudomonas aeruginosa*

A major challenge is to eliminate the Gram-negative bacterium *Pseudomonas aeruginosa* (PA) from the lungs of infected cystic fibrosis patients. PA is difficult to eradicate owing to many different resistance mechanisms that can develop when it lives in the lung and is exposed to antibiotics. Even the best antibiotics available, the beta-lactams that destroy the bacterial cell wall, are becoming ineffective. Because the cell wall is a perfect target to kill bacteria, we have explored the potential of other antibiotics that blocks its synthesis, including vancomycin (VNC). This antibiotic bypasses two typical resistance pathways for beta-lactams but cannot be used in the clinic to treat PA because it cannot reach the cell wall which is located between the outer and the cytoplasmic membrane. Our results confirm the essential role of the outer membrane (OM) in protecting PA against several non-beta-lactam antibiotics targeting the cell wall but they also show that mutants with defect in the pathways that insert lipid and proteins that form pores (porins) in the OM become sensitive to these antibiotics. We have also determined the response of PA and one of these leaky mutants upon exposure to VNC. The leaky mutant showed a massive response to VNC with more than a third of the genes being up or down regulated at the RNA level, which could be expected since this mutant eventually explode (lyse) in presence of VNC. Surprisingly, the wild-type PA, whose growth is almost unaffected by VNC, also responded to this antibiotic, for instance by up-regulating genes implicated in resistance to colistin another last resort antibiotic targeting the bacterial cell envelop. These results show that PA can sense VNC and raised the question of the potential contribution of this response to its resistance to VNC. Other experiments lead us to hypothesize that minute amount of VNC can cross the OM through porins. We are currently trying to verify these hypotheses. Finally, our results suggest that VNC could potentiate the action of new antibiotics that target the insertion of lipid or porin in the OM, which are currently in development.