Microbial Biochemistry and Pathogenesis Research Group

Miguel A. Valvano, Mw D. Professor of Microbiology and Infectious Diseases

Centre for Infection and Immunity, Queen's University Belfast, Belfast. United Kingdom

http://publish.uwo.ca/~mvalvano/index.html m.valvano@qub.ac.uk

Foto de grupo. Al frente, de izquierda a derecha,Yasmine Fathy, Kina Patel, Janet Torres, Gonzalo Pradenas, Amy Ford, Cristobal Mujica, Sudeepta Chakravarty, Laura Burns, Bernadette Kevin, Miguel Valvano, Fondo: Tina Schmidt, Lucie Kalferstova, Anna Hanuszkiewicz, Seamus O'Brien, Chris Connolly.

WHAT DO WE DO?

Our research involves an interdisciplinary approach using molecular genetics, biochemistry, cell biology, and structural biology to understand the pathogenesis of Gram-negative bacteria at the molecular level. *Burkholderia cenocepacia*, *Escherichia coli*, *Salmonella enterica*, and *Shigella flexneri* are the model organisms we use in different aspects of our research program.

WHY IS IT IMPORTANT?

Opportunistic infections pose a significant threat to human health, especially to those patients who benefit most from advancements in the treatment of genetic diseases, cancer, and organ transplantation, but who become immunosuppressed. *Burkholderia cepacia* complex bacteria (Bcc) and *Burkholderia cenocepacia* in particular are our main model organisms to study the pathogenicity of opportunistic bacteria. Bcc bacteria are a major health risk for children and young adults with genetic conditions like cystic fibrosis and chronic granulomatous disease. These patients commonly suffer from lung and airways infections by these microorganisms, which are very difficult to treat given the extraordinary resistance of these bacteria to clinically useful antimicrobials. Therefore, throughout research we hope to find novel ways to prevent or ameliorate the effect of these infections in susceptible patients.

Enteric bacteria such as *Escherichia coli*, *Salmonella*, and *Shigella* are also important human pathogens, which are particularly endemic in certain parts of the world. Lipopolysaccharide (LPS) is a complex glycolipid molecule located on the surface of Gram-negative bacteria that is also a critical structural component of the bacterial outer membrane. Bacteria with defects in the LPS molecule are more sensitive to a variety of antibiotics and they can be easily killed by host defensive mechanisms such as the serum complement

and antimicrobial peptides. Therefore, by understanding how the LPS is made and assembled on the bacterial cell surface we hope to design inhibitors that will interfere with this process, which may be useful as novel antimicrobials. Also, we are looking at ways to alter LPS biosynthesis at various levels to increase the overall permeability of the outer membrane to antibiotics and antimicrobial peptides. What we learn in *Escherichia coli*, *Salmonella enterica*, and *Shigella flexneri* is also applied to *Burkholderia cenocepacia*.

EXAMPLES OF KEY QUESTIONS WE CURRENTLY INVESTIGATE

We discovered that genetic ablation of LPS synthesis or chemical inhibitors of certain LPS biosynthesis enzymes can «weaken» the bacterial outer membrane and facilitate the entry of conventional antibiotics and antimicrobial peptides, especially in *B. cenocepacia* and other Bcc bacteria. We have also conducted detailed studies on proteins required for the synthesis of core oligosaccharide and O antigen moieties of the LPS molecules. These components are crucial for the virulence of *E. coli*, *Salmonella*, and *Shigella*. Now we want to learn:

How LPS components are synthesized?
What are the mechanistic bases of the translocation of O-antigen LPS biosynthesis intermediates across the plasma membrane?
How Bcc bacteria can survive the attack of antimicrobial cationic peptides and other antibiotics?

We discovered that Bcc bacteria survive intracellularly in free-living amoebae and macrophages by altering the maturation of the phagosome, and this research has led to the hypothesis that these cells become a reservoir for the persistence and dissemination of these bacteria in the host. We have also discovered a novel secretory system in *B. cenocepacia* (type VI) that appears to alter the cytoskeleton of infected macrophages and is required for infection in an animal model of chronic lung infection. Now we want to learn:

How does intracellular B. cenocepacia delay phagosome maturation and phagosomal acidification?
What is the role of a novel type 6-secretion system in B. cenocepacia intracellular survival?
How does intracellular B. cenocepacia affect the assembly of the macrophage NADPH oxidase?

We discovered several master regulators that respond to different types of stress. Mutations in the genes encoding these regulators impair the ability of *B. cenocepacia* to survive within macrophages and also in the animal model of chronic lung infection. We have observed that antioxidant bacterial defenses are critical for intracellular survival. Now we want to learn:

How does B. cenocepacia adapt to various environments including macrophage and epithelial cells?
What are the roles of stress regulators in intracellular survival and in bacterial survival in the rat model of chronic lung infection?
How do bacterial antioxidant mechanisms protect essential bacterial metabolic pathways that are deemed to be critical for adaptation to different environments, including the lung and airways?

Sometimes genetic tools are not available for our specific research needs. Therefore, we place special effort in developing new tools and reagents or modifying those available to our own needs. In particular, our lab has contributed novel molecular tools to handle Bcc bacteria.

BIBLIOGRAFÍA RECIENTE

Andrade A y Valvano MA. (2014). A *Burkholderia cenocepacia* gene encoding a non-functional tyrosine phosphatase is required for the delayed maturation of the bacteria-containing vacuoles in macrophages. Microbiology 160:1332-1345.

Aubert DF, Hamad MA y Valvano MA. (2014). A markerless deletion method for genetic manipulation of *Burkholderia cenocepacia* and other multi antibiotic resistant Gram-negative bacteria. In Host-bacteria interactions: Methods and Protocols, pp. 311-327. Edited by Vergunst A y Callaghan D O: Human Press.

El-Halfawy OM y Valvano MA. (2014). Antimicrobial heteroresistance: an emerging field in need of clarity. Clin Microbiol Rev (in press).

Hanuszkiewicz A, Pittock P, Humphries F, Moll H, Rosales AR, Molinaro A, Moynagh PN, Lajoie GA y Valvano MA. (2014). Identification of the flagellin glycosylation system in *Burkholderia cenocepacia* and the contribution of glycosylated flagellin to evasion of human innate immune responses. J Biol Chem 289:19231-19244.

Mohamed YF y Valvano MA. (2014). A *Burkholderia cenocepacia* MurJ (MviN) homolog is essential for cell wall peptidoglycan synthesis and bacterial viability. Glycobiology 24:564-576.

Schmerk CL, Welander PV, Hamad MA, Bain KL, Bernards MA, Summons RE y Valvano MA. (2014). Elucidation of the *Burkholderia cenocepacia* hopanoid biosynthesis pathway uncovers functions for conserved proteins in hopanoid-producing bacteria. Environ Microbiol doi: 10.1111/1462-2920.12509.

Aubert DF, O'Grady EP, Hamad MA, Sokol PA y Valvano MA. (2013). The *Burkholderia cenocepacia* sensor kinase hybrid AtsR is a global regulator modulating quorum-sensing signalling. Environ Microbiol 15:372-385.

Khodai-Kalaki M, Aubert DF y Valvano MA. (2013). Characterization of the AtsR hybrid sensor kinase phosphorelay pathway and identification of its response regulator in *Burkholderia cenocepacia*. J Biol Chem 288:30473-30484.

Hamad MA, Di Lorenzo F, Molinaro A y Valvano MA. (2012). Aminoarabinose is essential for lipopolysaccharide export and intrinsic antimicrobial peptide resistance in *Burkholderia cenocepacia*. Mol Microbiol 85:962-974.

Patel KB, Toh E, Fernandez XB, Hanuszkiewicz A, Hardy GG, Brun YV, Bernards MA y Valvano MA. (2012). Functional characterization of UDP-Glucose:Undecaprenyl-Phosphate Glucose-1-Phosphate Transferases of *Escherichia coli* and *Caulobacter crescentus*. J Bacteriol 194:2646-2657.

Rosales-Reyes R, Skeldon AM, Aubert DF y Valvano MA. (2012). The Type VI secretion system of *Burkholderia cenocepacia* targets multiple Rho family GTPases disrupting the actin cytoskeleton and the assembly of NADPH oxidase complex in macrophages. Cell Microbiol 14:255-273.

Marolda CL, Li B, Lung M, Yang M, Hanuszkiewicz A, Roa Rosales A y Valvano MA. (2010). Membrane topology and identification of critical amino acid residues in the Wzx O-antigen translocase from *Escherichia coli* O157:H4. J Bacteriol 192:6160-6171.