Prof. Goodlett

LIPID A AS A THERAPEUTIC AND DIAGNOSTIC TARGET

ABSTRACT. Lipid A is the membrane anchor for Gram-negative bacteria that holds the much larger lipopolysaccharide (LPS) molecule in place in the outer membrane. Importantly in mammals, Toll receptor 4 (TLR4) recognizes lipid A the result of which can be activation of a cytokine cascade that can aid the host in clearing the infection. Depending on the configuration of the lipid A structure (i.e. the presence or absence of specific length fatty acids or phosphate groups), altered endotoxic activity is observed that can range from antagonistic to agonistic. Through an effort with the Ernst laboratory, with which we have collaborated for nearly twenty years, we have characterized numerous lipid A structures by mass spectrometry for the purpose of developing it both as diagnostic and therapeutic agents. As a model organism, we have focused on the Gram-negative bacterium, Francisella tularensis subspecies novicida (Fn), which is a murine pathogen with lipid A structures that poorly activated the TLR4 complex thereby delaying activation of the innate immune cascade resulting in lethality.

In order to better understand the structure function relationship of lipid A we are currently developing a lipid A structure-activity relationship (SAR) library (Scott). Having a better understanding of the lipid A SAR will allow us to design novel lipid A structures/functions that are antagonistic or agonistic toward the MD2/TLR4 receptor complex. At the heart of this work is structure determination by mass spectrometry. This is made difficult as lipid A extracts consist of complex mixtures of molecules closely related in structure that are not water soluble. For example, over 100 structural variants of Fn lipid A were characterized using electrospray ionization (ESI) with a linear ion trap (IT) Fourier transform ion cyclotron resonance (FT-ICR) hybrid mass spectrometer (Shaffer; Ting). These results were generated using hierarchical tandem mass spectrometry (HiTMS) by combination of manual and automated data analysis (Ting). Recently, we investigated more efficient ways to characterize lipid A species using quadrupole-CID (q-CID) on a SYNAPT G2 Q-TOF-MS (Yoon), as opposed to our traditional ion trap method (Li), as well as using surface acoustic wave nebulization (SAWN) that does not suffer from the clogging issues of ESI and is less energetic than ESI or MALDI (Huang). Recently, we have begun to explore top-down fragmentation of LPS to characterize simultaneously the lipid A moiety as well as the immunogenic carbohydrate moiety that can encompass as much as 80% of the mass of LPS. Finally, we will review development of lipid A and related compounds from Gram-positive bacteria and fungi to identify microbes in a manner analogous to protein extracts on the Bruker Biotyper (Leung), but with a number of advantages.

References:

Huang Y, Yoon SH, Heron SR, Masselon CD, Edgar JS, Tureček F, Goodlett DR. J Am Soc Mass Spectrom. 2012.

Li Y, Yoon SH, Wang X, Ernst RK, Goodlett DR. Rapid Commun Mass Spectrom. 2016.

Leung LM, Fondrie WE, Doi Y, Johnson JK, Strickland DK, Ernst RK, Goodlett DR. Scientific Reports, 2017, in review.

Scott AJ, Oyler BL, Goodlett DR, Ernst RK. Biochim Biophys Acta. 2017.

Shaffer SA, Harvey MD, Goodlett DR, Ernst RK. J Am Soc Mass Spectrom. 2007

Ting YS, Shaffer SA, Jones JW, Ng WV, Ernst RK, Goodlett DR. J Am Soc Mass Spectrom. 2011.

Yoon SH, Liang T, Schneider T, Oyler BL, Chandler CE, Ernst RK, Yen GS, Huang Y, Nilsson E, Goodlett DR. Rapid Commun Mass Spectrom. 2016.

BIOGRAPHY. Prof Goodlett has spent his career using mass spectrometry to solve biomedical problems via novel technology and software developments. His PhD training was with Richard B van Breemen at N.C. State University and his postdoctoral study with Richard D. Smith of Pacific Northwest National Laboratory. After this he spent five years in the pharmaceutical industry before returning to academics. He was first Director of Proteomics at the Institute for Systems Biology and then Professor at the University of Washington. He is now a Professor at the University of Maryland. He has been active in a variety of fields including medicine, oceanography, pharmacy, microbiology, proteomics, lipidomics, and protein & glycolipid structure-function relationships publishing over 230 papers and given as many invited lectures in the US, the EU and Asia. He has an iCite of 543 with an h-index of 71 and recently received two NIH funded MPI RO1s with longtime microbiology collaborator R.K Ernst that have provided biological drivers for his laboratory. This research revolves around use of bacterial glyclolipids to identify bacteria direct from specimen and development of therapeutics around the lipid A scaffold for use as vaccine adjuvants and anti-septic prophylactics. He has two startup companies: Deurion in Seattle WA which makes SAWN ion sources for mass spectrometry and Pataigin in Baltimore MD which is commercializing a novel method of rapid bacterial identification.