

## **Characterization of a Unique, Naturally-occurring Immunomodulatory Lipopeptide: 1-peptidyl-2,3-diacylglyceride (PDAG)**

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To limit disease caused by emerging antibiotic-resistant pathogens, or genetically engineered agents of bioterrorism, there is a constant quest to develop novel therapeutic agents and adjuncts to antimicrobials. A screen of the low-molecular weight (< 10 kDa) inflammatory proteome in search of immunoactive agents uncovered a new lipopeptide that was characterized as 1-peptidyl-2-arachidonoyl-3-stearoyl-*sn*-glyceride (“PDAG”). We determined the unique amino acid sequence of the peptidyl moiety – acetylALYDKGYTSKEQKDCVGI – which is 100% identical to a stretch of 17 amino acids of the third extracellular loop of TRPC1. We have synthesized the complete lipopeptide and it mimics the biological activity of natural PDAG, *in vitro*. Natural PDAG stimulates the innate immune response resulting in activation of tissue resident immune cells, recruitment of phagocytic cells, and clearance of pathogens. Serum-derived, *i.e.*, natural PDAG stimulates significant release from whole human blood of proinflammatory cytokines IL-6, IL-8, MCP-1, MIP-1 $\alpha$  and MIP-1 $\beta$ . The cytokine profile induced by PDAG differs from that induced by LPS, indicating a lack of endotoxin contamination of the natural product, and a different mechanism of action. Similarly, PDAG stimulates isolated human macrophages, neutrophils and THP-1 macrophage-like cells to release IL-6 and IL-8 in a dose and time dependent manner. Interestingly, PDAG also stimulates cultured human fibroblasts and keratinocytes to secrete IL-6 and IL-8. We determined whether PDAG activity was due to the entire molecule, the PDAG peptide or DAG alone. Neither DAG nor synthetic PDAG peptide alone, appear to induce cytokine release from whole human blood or isolated white cells. Thus, the whole molecule is required for activity. We continue to study additional cell types responsive to PDAG and to investigate the source(s) and mechanism of action of PDAG activity. PDAG is an exciting new drug-like molecule that has many possible clinical uses, including the modulation of inflammation or immediate boosting of cellular and humoral antimicrobial responses. PDAG could be used as an adjunct to antimicrobials, immunotherapeutic agents and vaccines to help control infectious diseases, including those caused by select agents. This work was funded by Drexel University College of Medicine and the Institute for Hepatitis and Virus Research.