Endogenous Fatty Acids Regulation of Bacterial Lipid A Innate Immune Function

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Innate immunity is activated by either pathogen-associated molecular patterns (PAMPs) derived from bacteria, viruses, fungi, etc. or by damage-associated molecular patterns (DAMPs) released from damaged and dying cells. Representative PAMPs are lipopolysaccharides from Gram-negative bacteria and its active glycolipid center lipid A, which are recognized by host's TLR4/MD-2 receptor.

Lipid A has a tendency to form aggregate in aqueous environment due to its amphiphilic nature. Previous studies conducted by our group in collaboration with a German group have reported that the aggregate formation of lipid A as well as the state and composition of mixed aggregate between lipid A and other amphiphilic lipids can significantly affect its activity[1]. In this study, the effect of fatty acids on lipid A innate immune function was investigated using canonical *E. coli* lipid A **1** (Figure 1), saturated fatty acids (C18:0; C16:0; C14:0 and C12:0), and unsaturated fatty acids (C18:1, C18:2, C18:3, C22:6 and E-C18:1). Fatty acids, which are neither PAMPs or DAMPs, were selected because they are endogenous TLR4 ligands with simple amphipilic structures.

Mixed aggregates of lipid A **1** and fatty acids were prepared using two different methods: simple mixing method (SMM) and homogenized mixing method (HMM). SMM samples tend to form aggregates composed of single components, whereas HMM samples tend to produce homogenous aggregates composed of both lipid A **1** and fatty acid molecules. Immunological assay for SMM samples between *E. coli* lipid A **1** and saturated fatty acids or unsaturated fatty acids showed no effect on lipid A immune function. In contrast, HMM samples prepared using saturated fatty acids showed a concentration-dependent attenuation effect on lipid A innate immune activity (Figure 1). Attenuation effect decreased as fatty acid chain length decreased. Meanwhile, HMM samples containing unsaturated fatty acids showed a concentration-dependent attenuation effect at higher fatty acid concentrations, but a boosting effect was observed at lower fatty acid concentrations (Figure 1). Attenuation effect decreased as fatty acid degree of unsaturation increased.

The ability to activate host immunity makes lipid A a promising adjuvant candidate, and liposomes containing lipid A analogues (AS01B, GSK) have been practically used as vaccine adjuvants. The results of this research provide further insights for the development of liposomes and aggregates containing lipid A as adjuvants.

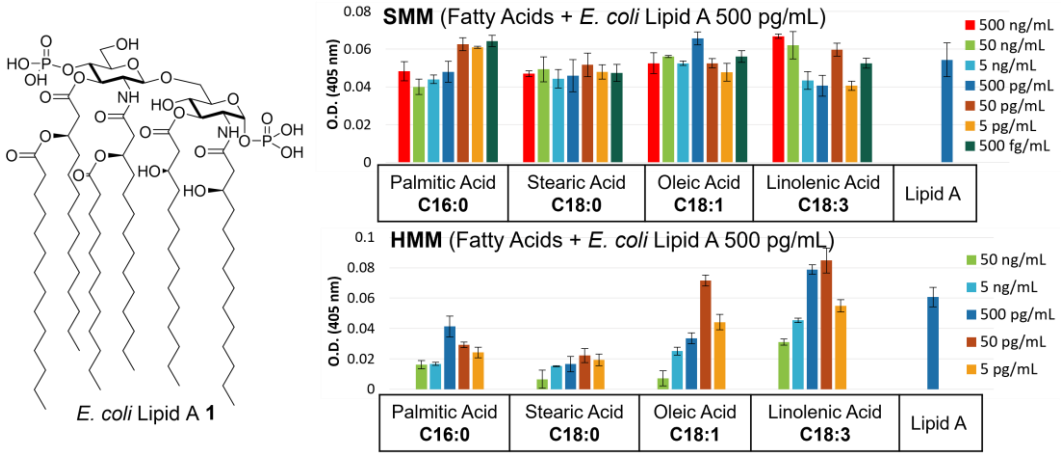

Figure 1. Left: *E. coli* lipid A structure; Right: Effect of fatty acids on lipid A immune activity

References

[1] Muelle, M.; Lindner, B.; Kusumoto, S.; Fukase, K.; Schromm, A. B.; Seydel, U. *J. Biol. Chem.* **2004**, *279*, 26307-26313