Synthesis of *Alcaligenes faecalis* Lipid A Conjugates with Tumor-Associated Carbohydrate Tn Antigen Towards the Development of Self-Adjuvanting Vaccine

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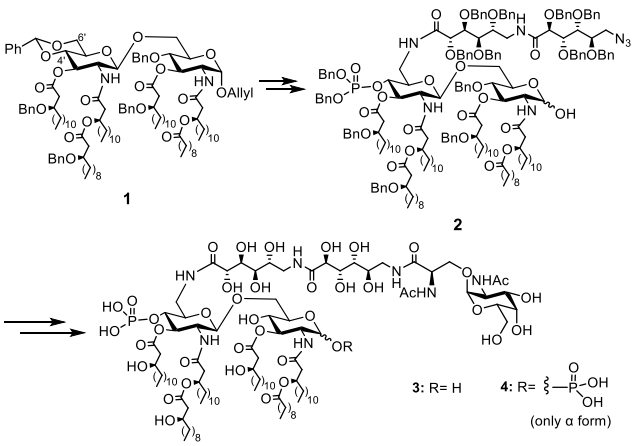
Lipopolysaccharide (LPS) and its active center, lipid A, of Gram-negative bacterial outer membrane are representative innate immunostimulants which have potential to function as vaccine adjuvants. However, canonical *E. coli* LPS and lipid A induce lethal toxicity due to excessive inflammatory effects hence not safe for adjuvant utilization. We have revealed that symbiotic *Alcaligenes faecalis* LPS and synthetic *A. faecalis* lipid A (AfLA)[1] induce effective antigen-specific IgA production without toxicity[2] hence promising adjuvant candidates.

Meanwhile, self-adjuvanting vaccine strategy, in which antigen and adjuvant are covalently linked has recently been well studied especially in the development of carbohydrate-based vaccines[3,4]. The strategy enhances active and simultaneous uptake of antigen with the conjugated innate immune ligand (adjuvant) by same immune cell thereby promoting efficient antigen-specific immune responses.



On the other hand, there are only few reports of lipid A-based self-adjuvanting vaccines[4,5] because structural modifications often inactivate lipid A. Thus a simple and universal conjugation method that can retain significant lipid A activity is required. Here we conjugated *A faecalis* lipid As with tumor-associated carbohydrate Tn antigen by employing a strategy which mimics natural LPS structure; linking 6'-position of lipid A to the antigen via hydrophilic sugar-chain mimic linker based on D-mannitol. The synthetic process involved azidation of 6'-position of **1** followed by 4'-phosphorlyation and then condensation of the linker and allyl group cleavage to yield **2**. Thereafter, the condensation of Tn-antigen to **2** was followed by global deprotection to obtain conjugate **3**. Meanwhile, condensation of the Tn antigen to **2** was followed by anomeric phosphorylation and subsequently, global deprotection was performed to synthesize conjugate **4**. Both conjugates **3** and **4** showed significant IL-6 cytokine induction at the same level as the unmodified lipid As.

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