Mechanism of action of new synthetic Toll-Like receptor 4 agonists for the development of innovative vaccine adjuvants

Nicole Gotri[1], Fabio Alessandro Facchini[1], Alessio Romerio[1], Andrea Luraghi[1], Francesco Peri[1]

E-mail: n.gotri@campus.unimib.it

[1] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza Della Scienza, 2; 20126, Milan, Italy

Keywords: TLR4, synthetic molecules, vaccine adjuvants, immunostimulators

Abstract:

For 70 years aluminum salts have been the only adjuvant approved for human use. Recently, with the introduction of much safer vaccines, some antigens need the presence of stronger adjuvants that are essential for enhancing vaccine potency by improving humoral and cell-mediated immune responses. Given the recent advances in understanding the receptor/ligand interactions of innate immunity, TLR ligands have been introduced as adjuvants in modern vaccine and the only TLR4 agonist approved by the FDA is MPLA (3'-O-deacylated monophosphoryl lipid A) *(Didierlaurent et al. 2009)*. MPLA is a purified, detoxified derivative of LPS, the natural ligand of TLR4, isolated from Gram-negative bacterium *Salmonella Minnesota* R595 strain and adopted for hepatitis B vaccine (*FENDrix*™) and human papillomavirus cervical cancer vaccine (*Cervarix*™) *(Garçon 2010)*. However, despite its success, MPLA production requires expensive and laborious procedures due to the chemical transformations of Gram-negative bacteria precursors *(Ji et al. 2020)*. For these reasons, our research group has synthetized new TLR4 agonists compounds based on structure-activity information. These molecules, named FP11 and FP18, have a glucosamine core with one phosphate group in C1 and three fatty acid chains of 14 and 12 carbon atoms, respectively. In this study, we investigate the capacity of these molecules to activate TLR4 pathways and the cytokines profile induced. TLR4 stimulation contributes to the activation of the innate immune response by activating two important adaptor proteins, MyD88 and TRIF. MyD88 pathway triggers the activation of NF-κB and MAPK that induce the production of proinflammatory cytokines, such as TNF-α, IL-6 and the IL-1β precursor that in turn enhance the adaptive immune response, while TRIF pathway activates IRF-3 triggering IFN-α and IFN-β production. Moreover, TLR4 pathways are related with another intracellular complex named NLRP3-inflammasome which is activated by different stimuli, including particulate adjuvants (such as Aluminum salts) *(Li et al. 2008)*, triggering the activation of caspase-1 and the subsequent release of IL-1β mature form. Although MPLA does not activate inflammasome, we also investigated the ability of our compounds to activate this complex. We can conclude that these two new synthetic agonists selectively activate TLR4 and only FP18 induces IL-1β production in a NLRP3-dependent fashion. Further studies must be conducted to investigate their adjuvant activity, firstly on PBMCs and then *in vivo,* to understand how these molecules lead up from innate to adaptive immunity.