

Self-adjutant and immune-stimulating activity of the anti Meningococcus B vaccine candidate *Neisseria meningitidis* Adhesin A as a soluble recombinant antigen (NadA₃₅₁₋₄₀₅) or as part of bacterial outer membrane vesicles

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Abstract

The gram⁻ bacterium *Neisseria meningitidis*, found in the upper respiratory tract of a large part of non-symptomatic people, can gain access to the bloodstream where it proliferates inducing fatal septic shock or meningitidis, especially in young patients. Infection with serogroup B strains, a principal cause of meningococcal casualties in developed countries, could not be prevented with vaccines based on capsular polysaccharide-protein conjugates, successfully applied to other serotypes, because their capsule is formed by the human self antigen poly-syalic acid. Consequently, a reverse vaccinology approach led to the identification of serogroup B new vaccine candidates, among which the adhesin NadA. The soluble recombinant form NadA_{Δ351-405}, missing the outer membrane anchor sequence, is a good immunogen included in a five-components universal anti-meningococcal vaccine at present under trial.

Functionally, NadA is an interesting molecule that likely contributes to *N. meningitidis* parasite cycle and pathogenicity. In fact, the expression of this adhesin is linked to hypervirulence and has been proposed to improve epithelial cell colonization and invasion. Moreover, functional data suggest that NadA expression on hypervirulent *Men B* strains contributes to adhesion/invasion of respiratory epithelial cells, thanks to the presence of specific binding sites on these cells.

Interestingly, not only epithelial cells but also human dendritic cells, monocytes and macrophages express specific receptors for NadA and are activated by this meningococcal adhesin. In dendritic cells NadA_{Δ351-405} up-regulates molecules involved in antigen presentation and the secretion of cytokines in a IFN- γ -dependent way. In monocytes and macrophages, NadA_{Δ351-405} stimulates cytokine and chemokine release, with a certain tendency for chemokine production at the expenses of shock-related cytokines like TNF- α and IL-1. NadA is also a survival and differentiation signal for monocytes: after 3 days in the presence of the meningococcal adhesin they show the characteristic of a special macrophage type up-regulating CD80, HLA-DR, the LPS co-receptor CD14 and the FcRIII IgG low affinity receptors CD16.

Another approach to develop anti meningococcal vaccines is based on the use of outer membrane vesicles (OMVs) preparations. A limit of such vaccines is that they are effective against the same strains from which OMVs are obtained and therefore fail to protect from other strains able to infect humans. OMVs are spontaneously and massively released during meningococcal sepsis and, similarly to NadA, are immunogens active on antigen presenting cells and other defensive cells. Interestingly, the over expression of membrane full-length NadA in outer membrane vesicles of meningococcus B strains, but not of *E. coli* strains, determined a IFN γ -independent up regulation of cytokine secretion and of protein linked to antigen presentation in macrophages, but not in monocytes: in fact the expression of NadA on *N. meningitidis* OMVs turned out to selectively enhance macrophage functions, while it is far less relevant for monocyte functions, which are normally equally influenced by adhesin negative outer membranes.

This suggests that NadA expression is with no effect on the intrinsic efficacy of OMVs to stimulate shock-related cytokines by circulating monocytes but on the contrary may improve antigen presenting activity of tissue macrophages. The up-modulation of molecules necessary for antigen presentation by NadA in OMVs is predicted to enhance macrophage *N. meningitidis* phagocytosis and killing by favouring the adjuvant action of CD4⁺ T Th1 lymphocytes. Our data reinforce the hypothesis NadA when inside the body as part of OMVs can be recognised by innate immune cells, like macrophages. Finally, our experiments suggest that full-length wt NadA may increase the efficacy of vaccine formulations based on *N. meningitidis* outer membranes. Taken together, these data suggest that NadA may be considered an active self-adjuvant antigen in vaccine formulation based both on purified proteins and on OMVs.