



NarE, a novel ADPRT from *Neisseria meningitidis* can modulate innate and antigen specific adaptive immune responses

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Introduction

Neisseria meningitidis is a gram negative bacterium that is both a nasopharyngeal commensal and a pathogen exclusive to humans. In its pathogenic form it is a major cause of meningitis (inflammation of the membrane covering the brain and spinal cord) and meningococcal septicaemia (blood poisoning).

The availability of the bacterial genome sequence allowed a process of genomic screening to be carried out for features of a group of known bacterial toxins, the ADP-ribosyltransferases (ADPRTs). Members of this group including cholera toxin are pathogenicity factors and also potent immunomodulators.

A novel ADPRT *Neisseria meningitidis* ADP-ribosylating enzyme (NarE) was identified and cloned (Masignani *et al.* 2003). This study investigated the ability of NarE to modulate innate and adaptive immune responses to *Neisseria meningitidis* outer membrane vesicles (OMV). OMVs are released from the bacterium during infection and are potent activators of the immune system.

Masignani *et al.* (2003) NarE: a novel ADP-ribosyltransferase from *Neisseria meningitidis*. *Molecular Microbiology* 50(3), 1055-1067

NarE does not induce DC cytokine production, but selectively reduces OMV-induced IL-10 and IL-12 production

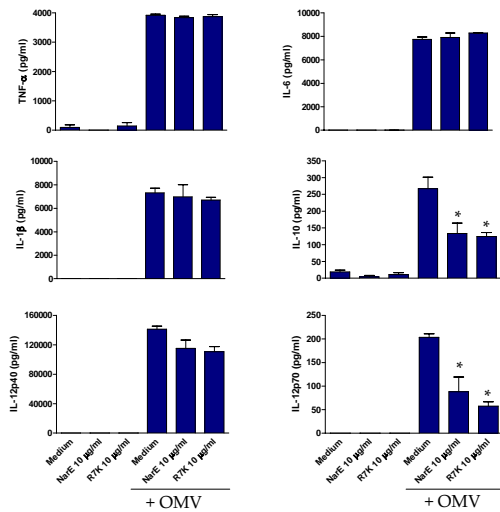


Figure 1. Murine bone marrow-derived dendritic cells (DC, 6.25×10^5 cells/ml), were pre-incubated with NarE or NarE R7K (enzymatically reduced NarE) for 24 h and then stimulated with outer membrane vesicles (OMV, 5 μ g/ml) for a further 24 h. Supernatants were analysed for cytokine concentration by ELISA. * $p < 0.05$

NarE inhibits OMV-induced DC maturation

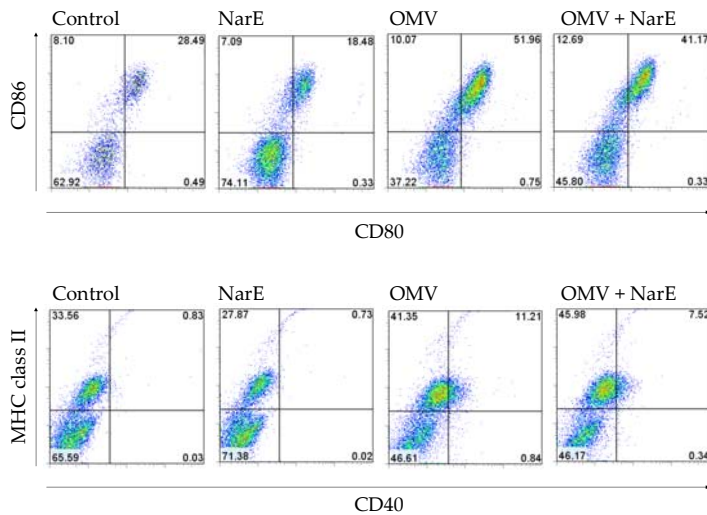


Figure 2. DCs were treated with NarE (10 μ g/ml) for 1 h alone or for 1 h prior to stimulation with OMV (5 μ g/ml) for a further 24 h. DC maturation was determined by measuring surface expression of co-stimulatory molecules CD86, CD80, CD40 and MHC class II.

NarE is poorly immunogenic and does not enhance antigen-specific antibody responses

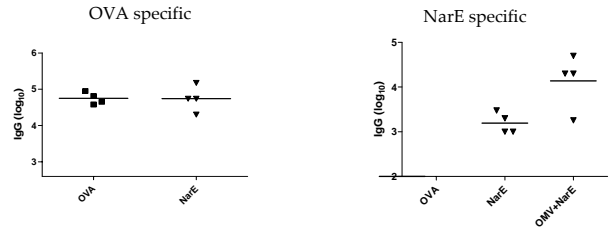


Figure 3. Mice were injected subcutaneously on day 0 and day 14 with OVA, NarE plus OVA or NarE, OVA and OMV. Mice were sacrificed on day 21 and blood was collected by cardiac puncture. Serum IgG anti-NarE and anti-OVA titre was determined by ELISA.

NarE can enhance OMV antigen specific IFN- γ and IL-17 secretion by splenocytes

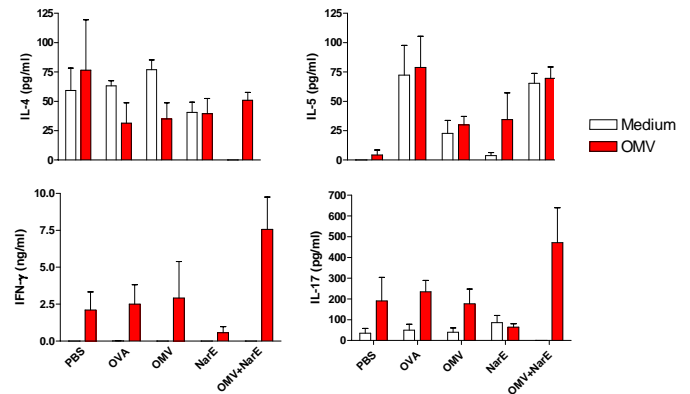


Figure 4. Mice were injected subcutaneously on day 0 and day 14 with OVA (50 μ g/ml), OVA plus NarE (10 μ g/ml) or OVA plus NarE and OMV (20 μ g/ml). Mice were sacrificed on day 21 and spleens were removed and splenocytes isolated. Cells were restimulated with OMV (5 μ g/ml) *in vitro* to determine the antigen specific response. Supernatants were analysed by ELISA for cytokine concentrations.

NarE inhibits antigen-specific Th2 responses

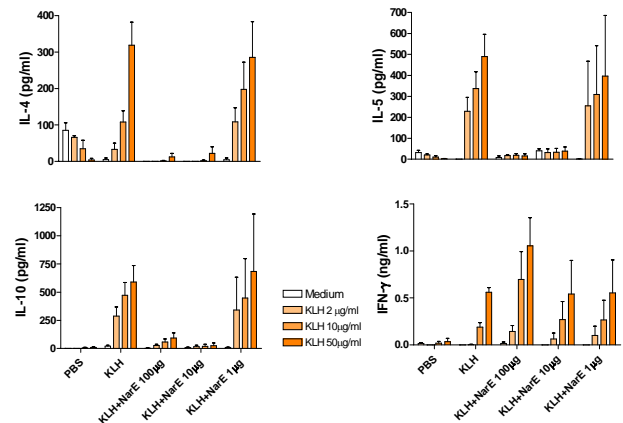


Figure 5. Mice were injected subcutaneously on day 0 and day 14 with varying concentrations of NarE and KLH (20 μ g) as a bystander antigen. Spleens were removed on day 21 and splenocytes isolated. Cells were restimulated with KLH *in vitro* to determine antigen specific responses. Supernatants were analysed by ELISA.

Conclusions

- NarE does not induce DC cytokine secretion and can reduce OMV-induced IL-10 and IL-12 cytokine secretion
- NarE does not promote DC maturation, but reduces OMV-induced expression of maturation markers
- NarE is poorly immunogenic *in vivo* but its response can be enhanced by OMVs
- NarE does not enhance the antibody response to a bystander antigen
- NarE can enhance OMV-specific Th1 type cytokine responses, and reduce Th2 type cytokine responses to a bystander antigen

Acknowledgements

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