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Study of the Antigenic cross-reactivity between *Neisseria meningitidis* and commensal Neisseria species using dioctadecyldimethylammonium bromide bilayer fragments (DDA-BF) as adjuvant

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OMVs from *N. lactamica* have emerged as an alternative to vaccination against meningococcal disease. Considering that this species has no capsule, OMVs vaccines based on *N. lactamica* are not serogroup or subtype specific, but they have been shown to protect mice against a lethal challenge in a model of meningitis, even in the absence of a bactericidal antibody response. Bilayer fragments of the cationic lipid dioctadecyldimethylammonium bromide (DDA-BF) can be obtained by dispersion of the white powder in an aqueous solution at low ionic strength after sonication at a temperature below 60°C. This low ionic strength allows the compound to remain stable due to the electrostatic repulsion of the molecules. DDA-BF can be used as an adjuvant, and its main advantage is that DDA-BF requires a lipid concentration lower than the concentrations traditionally used in liposomal formulations. Therefore, DDA-BF typically causes less toxicity. Aluminum compounds, which were identified as having immunostimulatory properties more than 70 years ago, remain the only adjuvant that is licensed worldwide. However, this adjuvant exhibits low colloid stability. Complexes of 25 μg of OMVs of *Neisseria lactamica* in 0.1 mM of DDA-BF were colloidally stable, exhibiting a mean diameter and charge optimal for antigen presentation. Immunogenicity tests for these complexes were performed in mice. Because vaccines based on *N. lactamica* OMVs aim to protect individuals against meningococcal infection, the cross-reactivity of the antibodies against *N. meningitidis* was tested by an ELISA, Dot-ELISA and immunoblot. In the present study, the immunogenicity of the OMVs of *N. lactamica* was tested in association with fragments of the lipid bilayer of dioctadecyldimethylammonium bromide (DDA-BF) used as adjuvant. In addition, DDA-BF was compared to alum, which had been implicated in the increased immunogenicity of OMVs vaccines to *N. meningitidis* B. The evaluation of the cross reactivity of the serum of the animals was performed 45 days after the first immunization. By Dot-ELISA they were tested against different meningococcal strains 39 strains of serogroup A, 42 strains of serogroup C (1972 to 1974), 131 strains of serogroup B (1990-2007) and 120 strains of serogroups B, C, W and Y (2011-2012) of Brazil. By Immunoblot we analysed the cross reactivity against OMVs of *N. meningitidis* A, B and C with different serotypes and subtypes and OMVs of Neisseria species. Results demonstrate by Dot ELISA for a total of 335 strains analyzed 93% of strains present reactivity with polyclonal serum from mice immunized with OMVs of *N. Lactamica* and DDA-BF compared with 26% reactivity with aluminum. The cross reactivity of antibodies was also evaluated by immunoblot. Serum from immunized animals specifically recognized antigens of 20 to 130 kDa protein of *N.lactamica* and interestingly, this sera also recognized OMVs in *N. meningitidis* 10 to 85 kDa. In conclusion, OMVs of N. lactamica were effective in generating cross-reactive IgG antibodies to *N. meningitidis*. Interestingly, DDA-BF was superior to alum as an adjuvant for subcutaneous immunization with OMVs, both with measures of humoral and cellular immunity. Future work based on this study intends to examine any protective effects offered by the combination of OMV of *N. meningitidis* B-DDA-BF.