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ABSTRACTS OF SUBMITTED PRESENTATIONS

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An inactivated whole-virus influenza vaccine given intranasally induced protective levels of serum antibodies in humans
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Objective: To study immune responses to influenza virus after intranasal administration of adjuvanted and unadjuvanted influenza vaccine.

Materials and methods: Three groups of healthy adults (altogether 28) were immunized intranasally with whole influenza virus (A/New Caledonia/H1N1) inactivated with β -propiolacton, either alone in saline, or combined with killed whole *Bordetella pertussis* cells or a thixotropic substance based on cellulose. Twenty-two vaccinees received the whole series of four doses, each corresponding to 250 μ g virus protein, given as nasal drops at weekly intervals, whereas six of them received one to three doses. Samples of nasal secretions, saliva and blood were collected before, and 5 and 9 weeks from start. Nasal secretions and saliva were analyzed for IgA antibodies (ELISA). Serum was tested for IgG (ELISA) and hemagglutination inhibition (HI) antibodies. Cell-mediated immune responses were measured by an antigen specific T-cell proliferation assay. The vaccinees were asked to record any local or systemic reactions following vaccination.

Results: Twenty-one (75%) of the 28 vaccinees developed HI antibody titer increases from ≤ 10 (before vaccination) to ≥ 40 , considered to be protective against influenza infection. Significant, but modest increases of antibody concentrations, as measured by ELISA, were found in secretions and serum, whereas T-cell proliferative responses were substantial. There was no positive effect of adding *B. pertussis* as a mucosal adjuvant, or of the thixotropic substance intended to increase the adhesion of the vaccine onto mucosal surfaces. Only mild and transient reactions, mostly stuffy, irritated or runny nose, were reported by the vaccinees.

Conclusion: Our results indicate that an inactivated nasal influenza vaccine may be effective. The discrepancy between HI and ELISA antibody responses suggests that the serum antibodies were of high functional quality. More studies are needed, however, to optimize the vaccine formulation and administration.

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***Bordetella pertussis* may act as adjuvant as well as inhibitor of immune responses to nasal vaccines.**

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Objective: To study the effects of whole killed *Bordetella pertussis* (Bp) bacteria on antibody responses to an influenza virus (INV) vaccine and soluble ovalbumin (OVA) given intranasally.

Materials and methods: Groups of mice were immunized intranasally with formalin-inactivated whole INV (A/H3N2) or OVA, either alone or simply mixed with Bp or cholera toxin (CT). Separate groups were given Bp intranasally one day before or one day after the INV vaccine. Four vaccine doses were given at weekly intervals (primary immunization), and repeated two months later (secondary immunization). IgA and IgG antibody levels in saliva and serum, respectively, were measured by ELISA.

Results: Bp augmented the antibody responses to INV, as well as to OVA, when given together with the antigens in solution. This effect on antibodies in both serum and saliva was more marked after primary than after secondary immunizations, but equal to that of CT. When Bp was given one day ahead of the INV vaccine, however, the antibody responses to INV in both saliva and serum were nearly abolished, even after secondary immunizations. The responses to INV were also markedly reduced by giving Bp one day after the INV vaccine, but this inhibitory effect was largely overcome by increasing the amount of INV or number of doses.

Discussion and conclusions: Our results show that Bp, similarly to CT, can act as a mucosal adjuvant for both particulate and soluble antigens, possibly by facilitating the antigen uptake and transport through the mucosal membrane. The inhibitory effects of Bp when given one day before the INV vaccine, may thus be explained by Bp blocking the antigen uptake by M cells. The negative effect of Bp on antibody responses to INV, when given one day after the INV vaccine, cannot be explained by Bp acting on the mucosal surface or epithelial transport mechanisms. The influence of Bp on antigens may therefore include activities underneath the epithelium.

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Supra-Molecular Biovector™ and non toxic E.Coli LTK63 mutant for intra-nasal immunisation with meningococcal C conjugated vaccine.
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Supra-Molecular Biovector™ (SMBV™), LTK63 and their combination were evaluated for intranasal immunization of mice with the group C meningococcal conjugated vaccine in comparison with injected antigen with alum.

SMBV™ are nanoparticles made of cationic cross-linked polysaccharides surrounded by a lipidic bi-layer and are under development as vaccine delivery systems. LTK63 is a non toxic mutant of *Escherichia coli* enterotoxin. Group C meningococcal vaccine consists of capsular oligosaccharide conjugated to the non toxic mutant of diphtheria toxin, CRM197 (CRM-MenC).

Immunisations at D0, D21 and D35, in 20 μ l volume, were given intranasally on unanesthetised mice. Control groups received the CRM-MenC vaccine given subcutaneously with aluminium hydroxide (alum).

SMBV™ and LTK63 alone had comparable adjuvanticity in inducing serum anti-MenC IgG and IgA antibodies after intranasal immunization. IgG titers were similar to those obtained after subcutaneous immunization with the CRM-MenC vaccine given with alum. Only groups immunised intra-nasally had detectable mucosal IgA antibodies. Moreover SMBV™ or SMBV™ + LTK63 group exhibited a very high mucosal IgA response.

These data show that LT mutants and SMBV™ are very promising candidates for the preparation of vaccines to be given intranasally. Strong MenC-specific antibody responses were induced by both systems.

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