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Protective immunity induced by an intranasal multivalent vaccine consisting of 10 *Lactococcus lactis* strains expressing highly prevalent M-protein antigens derived from Group A *Streptococcus*

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Streptococcus pyogenes (group A *Streptococcus*) causes infections ranging from mild pharyngitis to severe toxic shock syndrome and necrotizing fasciitis. M-protein elicits protective antibodies and is a potential vaccine target. However, M-proteins are hypervariable and there are more than 200 different M types. M-protein subunit vaccines have been developed for parenteral administration and primarily elicit systemic antibodies. Intranasal immunization has the potential of eliciting both mucosal and systemic immunity, which would theoretically provide protection against mucosal and invasive infections. We are developing an intranasal live bacterial vaccine comprised of 10 strains of *Lactococcus lactis*, each expressing one M-protein designed to confer protection against streptococcal pharyngitis and also severe invasive infections. The evaluation of individual vaccine types (M1, M2, M3, M4, M6, M9, M12, M22, M28 and M77) showed that most of the vaccines protected mice against challenge with virulent *S. pyogenes*. All of the 10 strains combined in a 10-valent vaccine (Mx10) induced serum and BAL IgG titers that ranged from 3 to 10-fold those of unimmunized mice. Mice immunized with Mx10 and challenged with M28 *Streptococci* were significantly less colonized with *S. pyogenes* in oropharyngeal washes two days after intranasal challenge compared to unimmunized mice. In contrast, mice immunized with Mx10 and challenged with *S. pyogenes* M75 (M type not included in the vaccine) were still colonized in their oropharynx two days after challenge. Our results suggest that *L. lactis*-based vaccines may provide a low-cost solution to the development of broadly protective group A streptococcal vaccines.

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