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Sommario	Shigella infections are one of the top causes of moderate and severe diarrhea throughout the world, mainly affecting children younger than 5 years in developing countries. The serotype-specific O-antigen (OAg) moiety of Shigella lipopolysaccharide has been recognized as a key target for protective immunity. Currently no vaccines are available against Shigella, but many OAg based candidates are in development. Recently, the Generalized Modules for Membrane Antigens (GMMA) technology has been proposed as an alternative approach to traditional glycoconjugate vaccines for OAg delivery. GMMA are outer membrane vesicles naturally released from genetically engineered Gram-negative bacteria, able to display the OAg in its natural context. The main aim of my PhD project was to compare GMMA and glycoconjugate technologies for the development of a vaccine against S. flexneri serotype 6. Genetic strategies for GMMA production and conjugation approaches for linkage of the OAg to CRM197 carrier protein have been established. In a head-to-head immunogenicity study in mice, GMMA induced higher anti-OAg IgG than glycoconjugate administered without Alhydrogel. When formulated on Alhydrogel, GMMA and

	glycoconjugate elicited similar levels of persistent anti-OAg IgG with bactericidal activity. Different parameters, such as OAg length, density and structural features like the amount and position of O-acetyl groups, can impact the immune response induced by GMMA. Moreover, mutations introduced in wild type bacteria for GMMA production can also have an impact on OAg features. Within my PhD project I verified that OAg density and length do not have a major impact on the immune response induced in mice by S. sonnei, S. flexneri 6 and S. flexneri 2a GMMA, independently from the OAg repeat structure (zwitterionic, negatevily charged and neutral respectively for S. sonnei, S. flexneri 6 and S. flexneri 2a). Furthermore, even very short OAg chains, when present on GMMA, are able to induce an immunogenic response. However, a minimal epitope can be needed for induction of the immune response. By comparing GMMA in wild type and T-cell knock out mice, it was also verified that GMMA, independently from sugar length and density, are able to induce a mixed T-dependent/T-independent response. In parallel, analytical methods were developed for the complete characterization of GMMA-based vaccines with particular attention to the OAg component. In particular, a novel method based on acid hydrolysis with concomitant use of trifluoroacetic acid (TFA) and hydrochloric acid (HCl) followed by High Performance Anion Exchange Chromatography-Pulsed Amperometric Detection analysis was developed for the quantification of S. sonnei OAg. A Design of Experiment approach was used for the identification of optimal hydrolysis conditions. The method is more sensitive than the methods so far available and it is also more specific. Overall, the results of the research carried out within my PhD project substantially contributed to the design and development of a vaccine against Shigella.
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