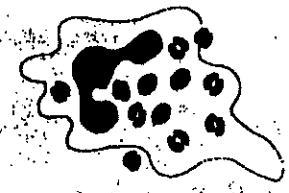

# Abstracts of the Tenth International Pathogenic Neisseria Conference

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## *Neisseria meningitidis* LOS micelle-based vaccine

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Endotoxin (LPS/LOS) is a glycolipid considered as one of the most significant antigens expressed by Gram negative bacteria and responsible for the toxic effects leading from endotoxicosis to septic shock. Toxicity is associated with the induction of endogenous cytokines like tumor necrosis factor (TNF) through the interaction of the conserved lipid A moiety of LPS with cell receptor proteins like CD14. We have recently elucidated the features needed by peptide structures to competitively bind and detoxify the conserved lipid A structure (1). These studies, using synthetic anti-endotoxin peptides (SAEP) to inhibit the toxicity of LPS systemically and in organ tissues, have revealed that a fundamental factor related to the full expression of the biological toxicity of LPS resides in its micellar architecture (2). Studies investigating the molecular architecture of LPS by Nuclear Magnetic Resonance (NMR) have come to similar conclusions (3). Lipid A is responsible for the micellar configuration of LPS and any approach which tends to chemically modify or eliminate the lipid A structure leads to the lack of the supramolecular architecture of LPS with the consequent reduction or elimination of toxicity. There is very limited but significant information about the importance that such supramolecular architecture may also have for optimal expression of antigenic and immunogenic activity (4,5). Since meningococcal group B LOS can be considered an important vaccine candidate, we have studied a vaccine formulation which is based on the use of purified LOS micelles detoxified by complex formation (1) with an appropriate amount of SAEP in order to reach the necessary level of safety, for investigation of the role of anti-LOS antibodies in conferring protection against bacteremia and endotoxemia. LOS purified from N. meningitidis A1, which is cross-reactive with Group B LOS but that does not contain the lacto-N-neotetraose structure similar to human glycolipids (5), has been therefore reacted with a synthetic cyclic peptide (SAEP-2). In selected experiments, after complex formation, the bound peptide has been "locked" into the lipid A binding site by covalent cross-linking with tailored bifunctional spacers in order to achieve its irreversible binding to the lipid A moiety. For either model of vaccine, the non-covalent or covalent complex, detoxification was ascertained in a variety of assays which included inhibition of LPS-induced LAL clotting, systemic and local TNF release by LPS challenge in mice, inhibition of LPS-induced local hemorragic dermonecrosis in rabbits. The vaccines and a control of purified LOS were then injected subcutaneously in SW mice at the dose of 5 ug/mouse in various schedules of treatment in order to follow the kinetic of the induced antibodies.

Sera were obtained from the animals on weekly basis and the anti-LOS antibodies induced were quantitated by ELISA and characterized for isotype (IgG and IgM) and sub-isotype (IgG1, IgG2a, IgG2b, IgG3). The antibody response induced in all groups of animals contained essentially IgG antibodies which peaked after three injections with end-point titers in the range of dilution $10^{-4}$-$10^{-5}$. The sub-isotype present within the IgG population of the animal groups were mainly IgG2 (44 %, equally distributed between 2a and 2b) followed by IgG1 (36 %) and IgG3 (20 %). Interestingly, the sub-isotype IgG distribution induced by either the vaccines or native LOS in mice was similar to that reported in febrile patients affected by typhoid to LPS of Salmonella typhi (6). The murine anti-LOS antibodies induced were biologically functional in fixing and activating guinea pig complement thus resulting in the lytic activity on N. meningitidis A1 LPS-coated sheep erythrocytes at serum dilution of 1:200. Analysis of immunochemical specificity of the induced antibodies for different antigenic regions of A1 LOS, performed by inhibition ELISA, revealed that all antibodies were directed against the carbohydrate region and none of them recognized the lipid A moiety. Accordingly, no cross-reactivity of the anti-A1 LOS antibodies was detected against heterologous LPS purified from P. aeruginosa, S. typhosa, S. enteritidis, S. flexneri, H. influenzae and B. pertussis. In contrast, the induced antibodies were cross-reactive with purified N. meningitidis group A and B LOS as well as with three bacterial strains (Group A, strain A1; Group B, strain BB431; Group B, strain 44/76) sharing the immunotype L8 determinant (5).

These results show that peptide-detoxified LOS represents a novel new method for safely administering LOS/LPS in micellar configuration which induces an immunogenic response in mice comparable to that qualitatively and quantitatively induced by native (toxic) LOS, in contrast to lipid A-deprived LOS conjugated to carrier proteins that have shown a lower level of immunogenicity when compared to native LPS (5). Studies are in progress for investigating in appropriate animal models the efficacy of the anti-LOS antibodies for prevention of meningococcal bacteremia and endotoxemia.

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