Molecular characterisation of a novel ADP-ribosylating putative toxin of *Neisseria meningitidis*

VEGGIi D, *BALDUCCI E, MASIGNANI V, DI MARCELLO F, SAVINO S, ARICO' B, COMANDUCCI M, PIZZA M, RAPPUOLI R
IRIS, Chiron SpA, Via Fiorentina 1, 53100 Siena Italy; *Dipartimento Scienze morfologiche e Biochimiche Comparate, Università degli Studi di Camerino, Camerino, Italy

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Introduction: By computer analysis on the *Neisseria meningitidis* (serogroup B, MC 58 strain) genome sequence, a protein with a feature similar to known bacterial ADP-ribosylating toxins (CT produced by V*ibrio cholerae*, LT by *Escherichia coli* and PT by *Bordetella pertussis*) has been identified. Enzymatic assay has shown that this protein (NM-ADPRT) possesses both NAD glycohydrolase and ADP-ribosyltransferase activity. In this study we describe the identification of the putative catalytic residues, their site-directed mutagenesis, and the resulting activity of the mutants.

Materials and methods: The novel NM-ADPRT and the correspondent mutants, were expressed in *E. coli* as C-terminus His-tag protein fusions. Site-directed mutagenesis was performed using the Multi Site-Directed Mutagenesis Kit (QuikChange). Recombinant NM-ADPRT forms were purified from *E. coli* in their soluble form by metal chelate affinity chromatography.
Both the wild-type and the mutants were assayed for their ADP-ribosylation and NAD-glycohydolase activites, using [adenine –U-^{14}C] NAD and agmatine as ADP-ribose acceptor. Antisera against NM-ADPRT and the mutant derivatives were obtained by immunization of CD1 mice. 20μg of each recombinant protein were given i.p. together with CFA for the first dose and IFA for the second (day 21) and the third (day 35) booster doses. Blood sample were taken on days 34 and 49. Immune sera were used in western blot and tested in a bactericidal assay.

Results and discussion: On the basis of sequence homology of NM-ADPRT with LT, CT and PT we have identified the putative residues involved in enzymatic activity. These residues have been changed by site-directed mutagenesis and the purified mutant toxins have been tested for both ADP-ribosylating and NAD-glycohydrolase activities. Interestingly, some of the mutants show reduced or abolished enzymatic activity indicating that the identified residues play a role in catalysis. Antisera against the wild-type and mutant toxins have bactericidal activity. The titers induced by two mutants were higher than those induced by the wild-type form. These data suggest that the mutations introduced could influence not only the enzymatic activity but also the *in vivo* stability of the toxin.

Conclusion: A novel ADP-ribosyltransferase has been identified in meningococcus B. Catalytic residues have been predicted by sequence homology and their role in catalysis has been confirmed by site-directed mutagenesis. These molecules are also able to induce a bactericidal response.