Abstract

Neisseria meningitidis, a major cause of bacterial meningitis and septicemia, secretes multiple virulence factors to the extracellular environment, including the adhesion and penetration protein (App) and the meningococcal serine protease A (MspA). Both proteins belong to the serine protease autotransporter family and have the capacity to adhere to host epithelial and endothelial cells. Besides, both proteins have been previously shown to be internalized and translocated to the nuclei of human brain microvascular endothelial cells and dendritic cells (DCs), where they modulate gene expression. However, host cell receptors, endocytic pathways, nuclear translocation mechanisms, and gene expression modulation associated with App or MspA are poorly defined.

In an effort to address this knowledge gap, recombinant forms of the passenger domains of both proteins were purified under native conditions using cold shock expression technology. The purified proteins, designated rpdApp and rpdMspA, were used to raise polyclonal, affinity-purified guinea pig antisera. These antibodies facilitated the investigation of interactions between both bacterial proteins and host cell molecules. One of these interactions highlighted in the present study is the entry of rpdApp and rpdMspA into human monocyte-derived immature DCs via receptor-mediated endocytosis. *In vitro* internalization and blocking assays showed that mannose receptor (MR) and transferrin receptor 1 are involved in the uptake of both proteins into DCs. Besides, two different approaches revealed that the binding of rpdApp and rpdMspA to MR is mediated specifically by the C-type lectin-like carbohydrate recognition domains 4-7 (CTLD4-7). Additionally, the recognition of both bacterial proteins by these domains was shown to be calcium dependent, in addition to being inhibitable by D-mannose or L-fucose, but not D-galactose.

Using gel overlay (Far-Western) analysis, rpdApp and rpdMspA were shown to interact with two nucleocytoplasmic shuttling proteins, namely heat shock protein 70 (Hsp70) and galectin-3 (Gal-3), with some data suggesting that interactions with Gal-3 may be mediated by its carbohydrate-recognition domain. Hsp70, which also interacts with the bacterial proteins, is characterized by possessing lectinic activity towards *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc).

Given the binding of App and MspA to different lectins, this study sought to examine the glycosylation status of both proteins, with a focus on *O*-GlcNAc modifications and their possible interplay with phosphorylation. Using *in silico* prediction methods, multiple sites within each protein were identified as potential sites for phosphorylation, *O*-GlcNAc modification, and alternative phosphorylation/*O*-GlcNAc modification. These predictions were supported by a series of Western blot analyses, which reproducibly showed that several meningococcal secreted proteins, including those of the expected size of App or MspA (115-120 kDa), are modified by both *O*-GlcNAc and tyrosine phosphorylation. The identity of the 115- to 120-kDa proteins was confirmed by stripping and reprobing the blots with antibodies against rpdApp and rpdMspA. However, attempts to detect these post-translational modifications in a sample of meningococcal secreted proteins tagged with TAMRA-alkyne by tandem mass spectrometry were unsuccessful. This may be attributed to the suppressive effect of the attached tag on trypsin cleavage and/or ionization efficiency.

Finally, the results of Far-Western and ELISA assays clearly demonstrated the interactions of rpdApp or rpdMspA with various histone subunits. Additionally, both proteins were shown to have trypsin-like serine protease activity and to be capable of cleaving recombinant histone H3.1 *in vitro*. The proteolytic activity of both proteins on H3.1 was shown to be specifically abolished by pre-incubation with serine protease inhibitors.

Collectively, these results extend our understanding of the virulence potential of App and MspA beyond the adhesive or invasive functions, and reveal their capacity to participate in multiple interactions with host molecules. Besides, the current findings provide the basis for future studies to explore the epigenetic alterations induced by both proteins. Hopefully, the information gained will provide new insights into meningococcal pathogenesis and will help in the development of improved therapeutic approaches against the disease.