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Mapping of the Region Critical for the Cytolytic Activity of Vaginolysin, the Main Virulence Factor of *Gardnerella vaginalis*

Milda Pleckaityte*, Gintautas Zvirblis, Rita Lasickiene, Indre Kucinskaite-Kodze, Aurelija Zvirbliene

Institute of Biotechnology, Lithuania

Introduction.

Bacterial vaginosis is characterized by a decrease in lactobacillus colonization and increase in the quantity of vaginal anaerobic bacteria such as *Gardnerella vaginalis*. The main virulence factor of *G.vaginalis* is a protein toxin vaginolysin (VLY) that belongs to the group of cholesterol-dependent cytolysins (CDCs). These toxins bind to cholesterol-rich membranes and form oligomeric transmembrane pores causing eukaryotic cell lysis. VLY is a member of the subgroup of CDCs that recognize human complement regulatory molecule CD59. The interaction of VLY with human CD59 occurs through the domain 4 of VLY. To characterize the structure-function relationship of VLY in more detail, we have generated recombinant VLY, developed a panel of monoclonal antibodies (MAbs) against VLY and mapped the epitope recognized by the neutralizing MAbs.

Methods.

VLY gene was amplified using genomic DNA isolated from *G.vaginalis* clinical isolate. His-tagged VLY was expressed in *E.coli* and purified using affinity chromatography. The MAbs were generated using hybridoma technology. MAb specificity was studied by Western blotting, ELISA and *in vitro* hemolytic assay. For epitope mapping, recombinant truncated VLY variants were expressed in *E.coli*.

Results.

Recombinant VLY was functionally active: it induced lysis of human erythrocytes *in vitro*. Thirteen MAbs against recombinant VLY were generated. Some of them neutralized the hemolytic activity of VLY. One MAb (clone 9B4) showed the most potent neutralizing activity (IC_{50}=6.7x10^{-11}). By using a series of overlapping truncated VLY variants, the epitope for MAb 9B4 was mapped within the region spanning aa 112-165 of VLY. This region is located in the domain D3 of VLY and contains the conserved motif VAARMQYD that is supposed to be inolved in oligomerization.

Conclusions.

Neutralizing MAbs were useful to study the relationship between the structure and the mode of action of VLY. The region critical for the cytolytic activity of VLY was localized between aa 112 and 165.

Keywords: vaginolysin, Gardnerella vaginalis, cytolysin, monoclonal antibodies