Title:

Identification, Characterization, and Cloning of Polypeptide N-acetylgalactosaminyl Transferase 4 of Cryptosporidium parvum

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Abstract:

Cryptosporidium is an apicomplexan parasite of global importance that causes diarrheal disease in humans and animals. Two main species account for 90% of all human cases of cryptosporidiosis, C. hominis (Ch) affecting humans and the zoonotic C. parvum (Cp) affecting both humans and other mammals. Immunocompromised individuals such as AIDS patients and malnourished children experience the most severe disease, but to date, there are no effective therapies or vaccines that exist to treat or prevent cryptosporidiosis in these patients. Nitazoxanide, a drug with wide antiparasitic properties is the only drug approved for treatment in immunocompetent individuals. Several proteins involved in attachment and invasion are mucin-like O-glycoproteins, which are surface proteins or are secreted from the apical complex of the sporozoite during gliding motility. Gp40, a glycoprotein that our lab has studied, has been shown to elicit an immune response in animal models and infected humans. A monoclonal antibody 4E9 as well as IgG from infected patients recognize αGalNAc glycopeptides derived from gp40, but not their non-glycosylated equivalents. In addition, αGalNAc has been shown to be indispensible for the function of the glycoproteins that contain this glycan as αGalNAc specific lectins and antibodies block attachment and disrupt sporozoite infectivity.

My project is focused on the family of enzymes responsible for catalyzing the first step of mucin-like O-glycosylation, the polypeptide α-N-acetylgalactosaminyl transferases (ppGalNAc-Ts). ppGalNAc-Ts are found in many organisms from Drosophila and C. elegans to mice and humans and have largely conserved domains and motifs. There are twenty ppGalNAc-Ts that have been identified in humans, and to date, the only ppGalNAc-Ts that have been identified in protozoa are in Apicomplexans including Toxoplasma, Cryptosporidium, Eimeria, and Neospora. Previously we have cloned, expressed and characterized Cp-ppGalNAc-T1.

Ongoing work is focused on characterizing Cp-ppGalNAc-T4 and expressing the recombinant protein in a mammalian expression system. The recombinant protein will be used to study enzymatic activity, identify endogenous substrates, and further understand the role of Cp-ppGalNAc-T4 in infection.

Our long-term goal is to determine if effective interventions can be developed, by targeting the enzymes that are responsible for glycosylating the crucial attachment and invasion glycoproteins. The overall objective of my project is to characterize Cp-ppGalNAc-T4 and identify specific inhibitors of Cp-ppGalNAc-T1 and T4 that can inhibit both enzymatic activity and O-glycosylation and subsequently block Cryptosporidium infection in vitro. Thus far, I have identified the complete sequence of Cp-ppGalNAc T4 and have characterized the protein in silico.