P1PK blood group antigens in birds: role of Gb3/CD77 synthase

Enterohemorrhagic strains of *Escherichia coli,* especially ones producing Shiga toxins (STEC) that may cause bloody diarrhea and hemolytic uremic syndrome, pose a major threat to the public health. The main virulence factor of STEC strains are Shiga toxins (Stx), and their main receptors are Gb3 (P^k) and P1 glycosphingolipids, which belong to the human P1PK histo-blood group system. The common feature of these receptors is the terminal structure Galα1→4Gal, synthesized by the Gb3/CD77 synthase (α1,4-galactosyltransferase). It has recently been shown that the same enzyme can synthesize N-glycans with Galα1→4Galβ1→4GlcNAc structures (called P1 glycotopes). The major reservoirs for enterohaemorrhagic strains of *E. coli* are ruminants (mainly cattle), but recently birds also began to be considered as spillover hosts. The molecular mechanism of birds' resistance to Stx has never been studied before and remains unclear.

In this study, it was shown that human P1 and P^k antigens are present on the erythrocytes of avian species belonging to the parvclass of modern birds, but not on the erythrocytes of ratites. In the evaluated sera, no antibodies recognizing P1PK blood group antigens were identified, while antibodies reactive with the human blood group antigen A were found. Two paralogs of Gb3/CD77 synthase were identified in pigeons (*Columba livia*) and named M and P enzymes. Both enzymes can add terminal Gal creating Galα1→4Gal structures, but the Gb3/CD77 synthase P is specific only for glycoprotein acceptors, while Gb3/CD77 synthase M can transfer galactose residues also to glycosphingolipids. The cells of human 2102Ep cell line transfected with the vectors encoding the pigeon Gb3/CD77 synthase P and its M paralog exhibited increased sensitivity to the Stx1 holotoxin. This may suggest that glycosphingolipids and glycoproteins are functional receptors for Shiga toxins. In contrast, the pigeon endothelial cells, which have P^k and P1 antigens on their surface, were found to be insensitive to the Stx1 holotoxin. This may imply that the N-linked glycans containing P1 glycotopes may serve as decoy receptors for toxins: they bind toxin molecules, but do not internalize them.