An Investigation of the Binding Affinities of Recombinant Domain Mutants of the Human Polymeric Immnuoglobulin Receptor (pIgR) for IgM.

E. Prinsloo, RJ Naude′ and V. Oosthuizen

Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, PO Box 1600, Port Elizabeth, South Africa

The Polymeric Immunoglobulin Receptor (pIgR), a membrane bound glycoprotein (105kDa), is the primary transport molecule of polymeric immunoglobulins (i.e. IgA and IgM) across epithelial cells via transcytosis in order to establish immunity at mucosal surfaces. During this process pIgR binds (via five homologous immunoglobulin-like domains of the ectodomain) to the polymeric immunoglobulin. Binding is covalent (to IgA) and non-covalent (to IgM). The ectodomain of pIgR is cleaved at Arginine-585 and released bound to the polymeric immunoglobulin. It is thereafter referred to as Secretory Component and is responsible for protection of the mucosal surfaces against microorganisms (reviewed in 1). It is known that domain I of pIgR is the primary domain involved in the interaction with polymeric immunoglobulins. Binding of pIgR domain I to IgA and IgM has been characterised by ELISA and is believed to be the major contributor to total immunoglobulin binding (2). This study aimed to characterise the binding of recombinant human pIgR domain mutants to polymeric IgM using evanescent wave biosensor analysis on BIAcore X, allowing greater insight into the contribution of each of the five ectodomains towards ligand binding through analysis of association and dissociation rates. Recombinant domain mutants of human pIgR were amplified, cloned and expressed in *Escherichia coli* BL21 (DE3). Mutants were refolded (*in vitro*) and purified to homogeneity and the binding was analysed using BIAcore X at varying flowrates and ligand concentrations. Provisional results show the contribution of the individual domains to total binding is attributed to reduced dissociation rates.

1. Areoti B, Casanova J, Okamoto C, Cardone M, Pollack A, Tang K and Mostov K (1992) *Polymeric Immunoglobulin Receptor.* Int. Rev. Cytology 137 B: 157-168.

2. Bakos M, Widen SG and Goldblum RM (1994) *Expression and purification of biologically active domain I of the human pIgR.* Mol. Immunol. 31 (2): 165-168.