Analysis of the isotype specificity of three platypus immunoglobulin Fc receptors

Popular Science Summary

SRINIVAS AKULA

The host defense mechanism against disease causing substances like microbes and macromolecules is called immunity. A complex set of cells and molecules involved in host immunity are collectively called the immune system. The immune system is broadly divided into early non-specific responses to microbes, innate immunity. Later responses to disease are termed adaptive immunity. In adaptive immunity, antibodies which are known as immunoglobulins play a vital role. Immunoglobulins are Y shaped proteins made of four polypeptide chains: two identical heavy chains and two identical light chains. Digestion with trypsin cleaves immunoglobulins into two fragments: a fragment for antigen binding (Fab) and fragment crystalline (Fc). The complexity of immunoglobulins has been gradually increased during vertebrate evolution.

Immune cells like mast cells, basophils, eosinophils, monocytes, macrophages, NK cells and dendritic cells have membrane receptors that interact with the Fc region of immunoglobulins. These receptors are called Fc receptors and they have three structural parts: a ligand binding extracellular part, consisting of several domains, a transmembrane region and a cytoplasmic tail. The cytoplasmic tail consists of ITAM (Immuno tyrosine activation motif) and ITIM (Immuno tyrosine inhibition motif) that are involved in signal transduction. Interaction of the Fc receptors to immunoglobulins regulate various immune reactions such as antibody dependent cytotoxicity, mast cell degranulation and phagocytosis. The aim of this project was to study the specificity of three platypus Fc receptors FcRA, FcRB, and FcRC towards different isotypes of the platypus immunoglobulins IgG1, IgG2I, gA1, IgA2 and IgE.

To study the isotype specificity of three platypus Fc receptors, recombinant clones of Fc receptors were constructed and transfected into HEK 293 cells. Expressed proteins from the HEK 293 cells were purified by affinity chromatography and analyzed by SDS-PAGE. Unfortunately this was unsuccessful in our study. The possible reasons could be low efficiency of transfection, the sheer complexity of the mammalian expression system or low expression levels of Fc receptors. If we can express the desired protein in HEK 293 cells, we can study the interaction of these proteins with immunoglobulins using ELISA.

Degree project in molecular biotechnology, 2012
Examensarbete i molekylär bioteknik 45 hp till masterexamen, 2012
Biology Education Centre and Molecular Immunology, Department of Cell and Molecular Biology, Uppsala University
Supervisor: Lars Hellman