A 013

Preparation and purification of antibodies against bovine β-lactoglobulin

Cow milk allergy is probably the commonest food allergy in infants. Even though several cow milk proteins are suggested to be allergenic, β-lactoglobulin is the predominant one. However, in Sri Lanka a reliable method of diagnosing cow's milk allergy is still not available.

Objective of this study was to prepare specific antibodies against β-lactoglobulin. It will be used to develop an Enzyme Linked Immunosorbent Assay (ELISA) to determine the β-lactoglobulin concentration in the serum of children reported to be allergic to cow milk and that in the formulas based on cow's milk.

Bovine β-loctoglobulin (L 3908, Sigma) was further purified by SDS-PAGE. The β-lactoglobulin band was cut and crushed in liquid nitrogen, suspended in phosphate buffered saline and mixed with Freund's complete adjuvant. It was inoculated sub-cutaneouly in the flank region of rabbits. After primary and secondary booster doses of β lactoglobulin in Freund's incomplete adjuvant, serum was collected and stored at -20 ^{o}C. Immunoglobulins (IgG) were purified by $(NH_4)_2SO_4$ precipitation followed by affinity chromatography on a protein A sepharose CL-4B column (Pharmacia).

Purity of the rabbit IgG preparation was checked by subjecting the purified IgG to SDS-PAGE under reducing and non reducing conditions. Specificity of anti β-lactoglobulin IgG was investigated by Western blotting using purified cow's milk proteins, human milk and human serum as samples. 1:8000 dilution of purified antibody (1 mg/mL) gave clear positive results with β-lactoglobulin but not with other proteins present in cow's milk, human milk or human serum when the antigen concentrations were 1 μg/mL. Studies are in progress to develop an ELISA using this purified IgG to quantitate β-lactoglobulin.