Is detection of islet cell antibodies (ICA) by a novel enzyme-linked immunosorbent assay (ELISA) more sensitive in comparison with indirect immunofluorescence in type 1 diabetes (T1D) diagnostics?

Dirk Roggenbuck*, Christina Fritz*, Bernd Bierwolf*, Dirk Reinhold**

** Institute of Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg

Introduction

Type 1 diabetes, also known as insulin-dependent diabetes mellitus, results from a chronic autoimmune process which destroys the insulin-secreting pancreatic beta cells. Before the onset of disease T1D patients may demonstrate autoantibodies against different autoantigens of islet cells. These autoantibodies have been shown to be important markers for the identification of individuals with an increased risk to develop T1D at a time when all metabolic tests available still show normal results. Islet cell autoantibodies detected by indirect immunofluorescence (IIF) have been the first serological markers described for T1D. This technique using tissue sections from human or primate pancreas, however, still reveals methodological problems, especially with regard to inter-laboratory standardisation. After discovery of the two main protein antigens of ICA - the enzyme glutamic acid decarboxylase (GAD65) and the islet cell protein IA2 - it has been possible to measure these specific autoantibodies by radioligand assays and recently by sensitive ELISA.

Material, Methods & Experimental Design

**ICA screen ELISA**

ICA screen ELISA (MEDIPAN, Germany) is an enzyme immunoassay for the semi-quantitative determination of islet cell autoantibodies (ICA) in human serum. The assay system uses the ability of ICA to bind to their corresponding antigens bivalently, that is, to form a bridge between autoantigens coated onto the plate and biotinylated autoantigens in liquid phase. Briefly, in the first incubation step ICA of the patient sample bind to autoantigens coated on the microtiter plate. In a second step biotinylated autoantibodies bind to this complex which correlates with the amount of ICA in the patient sample. Non-bound biotinylated autoantibodies are removed by washing. Subsequently, the bound autoantigen-biotin is quantified by adding streptavidin-peroxidase and a substrate (TMB) followed by reading the optical density (OD) at 450 nm. Binding index (BI) is then calculated by dividing ODsample/ODcut-off control.

**Anti-GAD and anti-IA2 radioimmunoassays (RIA)**

Commercial radioligand assays (MEDIPAN, Germany) employing highly purified recombinant 125I-labeled GAD65 and IA2 were used. Briefly, autoantibodies of patient samples bind to their specific antigen (GAD65 and/or IA2) in liquid phase. After addition of protein A (Staphylococcus aureus) immune complexes are formed. The bound fraction is separated from non-bonded autoantibodies by centrifugation. Radioactivity (cpm) is measured in the precipitate and the radioactive signal is proportional to the autoantibody concentration in the patient sample.

**Indirect immunofluorescence (IIF)**

Two different assays were performed: Assay 1 was a home made test using human pancreas sections as antigen source. Assay 2 was a commercial assay (Euroimmun, Germany) using primate pancreas sections. Briefly, in the first incubation step ICA of the patient sample react with islet cell autoantigens of pancreas tissue. Subsequently, secondary anti-human-IgG antibodies labeled with FITC react with the bound autoantibodies. Slides are read out by a fluorescence microscope. Positive samples show a specific fluorescence pattern of the cytoplasm of Langerhans’ islets.

**Results**

**Experiment 1**

Sera of 20 healthy blood donors and 61 patients with type 1 diabetes were analysed with the ICA screen ELISA and with a commercial anti-GAD and anti-IA2 RIA. Two different assays were performed: Assay 1 was a home made test using human pancreas sections as antigen source. Assay 2 was a commercial assay (Euroimmun, Germany) using primate pancreas sections. Discrepant samples were analysed with commercial anti-GAD and anti-IA2 RIA.

**Experiment 2**

Sera of 20 healthy blood donors and 61 patients with type 1 diabetes were analysed with the ICA screen ELISA and with a commercial immunofluorescence assay using primate pancreas sections. Discrepant samples were again analysed with commercial anti-GAD and anti-IA2 RIA.

**Conclusion**

The ICA screen ELISA is a robust and sensitive diagnostic tool for the detection of ICA. In comparison with established methods for the detection of ICA this assay showed superior sensitivity. These data strongly support further investigation to confirm the better sensitivity of the ICA screen ELISA.