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Subject Index

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K.11 A fluorescent lectin-agarose bead immunoassay for a pancreatic autoantigen involved in Crohn's disease

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Pancreatic autoantigen (PAg) which is recognized by many sera of patients with Crohn's disease (CD) could up to now only be identified by a neutralization technique, in which the capacity of samples to extinguish a specific immunofluorescence reaction of CD-sera with human pancreatic tissue sections was recorded. However, the neutralizing efficacy of crude duodenal juice or homogenized pancreas could not be evaluated, since activated proteinases digested the tissue sections. Solid phase immunotests, based on direct binding of PAg to plastic surfaces gave inconsistant results, possibly depending on the low affinity of PAg to the materials used. We have found out that PAg binds to soybean agglutinin (SBA) which is known to react with N-acetylgalactosamine. Thus, it was possible to develop a new assay for the detection of PAg: Samples were diluted in microtiter plates (50 µl per well) and incubated for 15 min with 10 µl of a 50 % suspension of SBA-coated agarose beads (Camon, Wiesbaden). 50 µl of 1 % ovalbumine were added. After washing in PBS, beads were further incubated with 50 µl of HL-1 (monoclonal antibody against PAg; for 30 min, washed again and stained for 30 min with FITC-labelled goat anti-mouse IgG. Results were read with a fluorescence microscope. Positive beads exhibited a bright fluorescence. Of 200 samples which were identified to contain PAg as determined by the neutralization test, each was clearly positive in the lectin agarose bead immunoassay. Semiquantification was possible by titration of samples in twofold dilutions, which resulted in a clear cut end point. PAg could easily be detected in homogenized pancreas and contents of the bowel. The new assay for PAg was simple to perform, easy to read and seems to be more widely applicable than the neutralization test.