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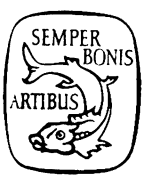

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K.10 Monoclonal antibodies against the Crohn's disease-involved pancreatic autoantigen

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In previous studies, it was possible to show that patients with Crohn's disease (CD) exhibit an autoimmunity against a macromolecular antigen contained in pancreatic juice (1). Some observations suggest that autoimmunity to exocrine pancreas is of essential importance in the pathogenesis of CD. To get means for further investigation, monoclonal antibodies were produced against the CD-involved autoantigen.

To enrich the autoantigen, corresponding to the CD-patients' pancreatic autoantibodies, human pancreatic tissue was homogenized and centrifuged (30,000 × g). The fat-free supernatant was subjected to size exclusion chromatography. The antigen-containing fraction was identified by a neutralization test combined with the immunofluorescent procedure used for detection of pancreatic autoantibodies (2). The antigen was precipitated by 25/50 percent ammoniumsulfate, resolved in phosphate buffered saline to appropriate concentration and used for immunization of female BALB/c mice according to (3). Splenocytes of immunized mice were fused with myeloma cell line P3-X63-Ag8-U1 in a relation of 5:1 in the presence of

40 percent polyethylenglycol (Merck, Darmstadt) and 15 percent dimethyl sulfoxide, following standard techniques. Hybrids were raised in HAT medium containing 20 percent FCS and repeatedly cloned by limited dilution. Positive clones were identified by indirect immunofluorescence, using frozen sections of human pancreas which were incubated with undiluted supernatants and, in the second step, with FITC-labelled antimouse IgG.

Out of 540 clones 12 were selected which produced an antibody reacting with exocrine pancreas and exhibiting a similar pattern as human autoantibodies of patients with CD. Two of these (MA A-17/4-1, MA F-21/2-10) remained stable in producing antibodies which gave a strong reaction with exocrine pancreas. Further characteristics of these monoclonal antibodies are described in detail.

The established monoclonal antibodies against the CD-involved autoantigen might be useful as reagents to purify and identify the CD-involved autoantigen or to localize it in biopsy tissue of patients with chronic inflammatory bowel disease thus enabling a better diagnosis of CD.

1. STÖCKER, W., M. OTTE, S. ULRICH, D. NORMAN, H. FINKBEINER, K. STÖCKER, G. JANTSCHEK, and P. C. SCRIBA. 1987. Scand. J. Gastroenterol. 22, Suppl. **139:** 41–52.
2. FINKBEINER, H., S. BOCK, U. BURMESTER, D. GRAGE, U. REDDIG, D. STRUVE, M. OTTE, and W. STÖCKER. 1985. Immunobiol. **170:** 20–21.
3. STÄHLI, C., T. STAEHLIN, V. MIGGIANO, J. SCHMIDT, and P. HÄRING. 1980. J. Immunol. Meth. **32:** 297–304.