Journal of Crohn's and Colitis (2013) 7, 780–790

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Autoantibodies against exocrine pancreas in Crohn's disease are directed against two antigens: The glycoproteins CUZD1 and GP2

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Received 19 September 2012; received in revised form 15 October 2012; accepted 17 October 2012

KEYWORDS
Crohn's disease;
Autoantibodies;
Exocrine pancreas;
CUZD1;
GP2

Abstract

Background: Autoantibodies against exocrine pancreas (PAb) have been reported to be pathognomonic markers of Crohn's disease (CD). Recently, the glycoprotein GP2 has been proposed as the exclusive target for PAb but two equally prevalent binding patterns can be observed in the indirect immunofluorescence test (IIFT) using cryosections of human pancreas: a reticulogranular and a droplet pattern.
Aim: To identify autoantigens corresponding to the staining patterns.
Methods: Different lectins were screened for their ability to immobilize PAb-reactive glycoproteins from cell free human pancreas. The glycoproteins were then purified via UEA-I affinity chromatography and identified by mass spectrometry. The two candidate autoantigens were separately expressed in HEK293 cells, and the recombinant cells applied as substrates in IIFT to analyze sera from 96 patients with CD, 89 controls and hybridoma supernatants during the generation of murine monoclonal antibodies.
Results: The UEA-I eluate was able to neutralize PAb reactivity of both patterns in IIFT. It contained two major constituents which were identified as the glycoproteins CUZD1 and GP2. With the recombinant cells, 35.4% of the CD patients exhibited positive reactions

Abbreviations: IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; MALDI-TOF, matrix-assisted laser desorption/ionization trap-time of flight mass spectrometry; QIT, quadrupole ion trap; ROC, receiver-operating characteristics; AUC, area under the curve; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate; IIFT, indirect immunofluorescence test; HRP, horse radish peroxidase; AP, alkaline phosphatase; GPI, glycosylphosphatidylinositol; UEA-I, *Ulex europaeus* agglutinin I.

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1873-9946/$ - see front matter
http://dx.doi.org/10.1016/j.crohns.2012.10.011

(CUZD1 alone 19.8%, GP2 alone 9.4%, and both antigens 6.2%). The reaction with the CUZD1 expressing cells was strictly correlated to the reticulogranular pattern, whereas the antibodies causing the droplet pattern stained the GP2 expressing cells. Antigen-capture ELISA using the newly generated monoclonal antibodies against CUZD1 and GP2 verified this relationship.
Conclusions: The concordant reactivities of the different platforms can be regarded as a proof for the authenticity of the two identified autoantigens.

1. Introduction

Crohn's disease (CD), one form of chronic inflammatory bowel disease (CIBD), affects up to 0.15% of the population in industrialized countries.[1–3] The cause of the disease is unknown, immune mechanisms may be involved in the pathogenesis.

Research on the humoral immunity in CD revealed the presence of autoantibodies against acinar cells of the exocrine pancreas (PAb) in the sera of CD patients.[2,4–8] PAb have been reported to be pathognomonic markers with a prevalence of 39% in the indirect immunofluorescence test[2,4,8] and can be used to differentiate CD from ulcerative colitis (UC; prevalence 2%). Two different staining patterns were observed: a reticulo-granular and a droplet pattern. Apart from PAb, other serological CIBD markers are established: A) antibodies against the cell wall mannan of *Saccharomyces cerevisiae* (ASCA) with 67% prevalence in CD (immunoglobulin classes IgA and IgG together), which are rarely found in UC, but also in 25% of patients with celiac disease[9,10]; B) autoantibodies against the cytoplasm of neutrophil granulocytes (perinuclear pattern, P-ANCA), which appear in 67% of UC but in only 7% of CD patients and are mainly directed to DNA-bound lactoferrin[11]; and C) autoantibodies against intestinal goblet cells, which exclusively appear in 28% of UC patients.[2,12,13]

Next to their high value as CD-specific yet non-invasive markers in the diagnostic work-up of CIBD, particularly in the differential diagnosis of CD and UC,[14] PAb have also been proposed as markers for clinical presentations of the disease, e.g. microbial load, severity of penetration, site of inflammation, age at disease onset, duration of the disease, co-appearance of other diseases etc.[4,15–17] Nevertheless, none of these links have so far been confirmed by independent studies such that they cannot be exploited for the clinical practice, yet.

More recently, the pancreatic major glycoprotein GP2 of the zymogen granule membrane has been described independently by us and another group as a target for the associated autoantibodies.[18–22] Anti-GP2 represent, however, only a proportion of CD associated PAb.[22–24] In this work, it was possible to identify the two CD-relevant target antigens of PAb, combining lectin-based affinity chromatography with mass spectrometry. For verification, the antigens were recombinantly expressed in a human cell line and used in a cell-based immunofluorescence test for the serological diagnosis of CD.

2. Materials and methods

2.1. IBD patients and healthy controls

Serum samples were obtained consecutively from 96 patients with CD (65 female, median age 40, range 18–73 years) and 39 patients with UC (24 female, median age 42, range 17–83 years) attending the Medical Clinic I of the University of Lübeck between August 2005 and March 2007. Most of them were established patients and had already started therapies of different regimens. They met internationally accepted clinical, endoscopical, radiological and histological criteria compatible with either CD or UC.[25,26] Patients with unclear diagnosis and with features suggestive of other coexistent intestinal diseases were excluded. In case of multiple patient appearances, only the sample from the first admission was selected. Presence of clinical signs and symptoms and all relevant information were recorded in electronic databases. Relationships between clinical features and the appearance of autoantibodies against exocrine pancreas (PAb) were, however, not evaluated because A) the study was focussed on the definition of PAb target antigen, B) the number of sera containing PAb was too low for the generation of statistically significant results, and C) the heterogeneity of the patient cohorts with respect to treatment regimens.

A control collective consisted of 50 healthy blood donors (32 females, median age 26, range 18–52 years). The study was designed in December 2004 when the prototypic immunoassays (see below) were established for autoantibody testing, making this a retrospective cohort study.

The experiments conducted were institutionally approved by the ethics committee at the medical faculties of the University of Lübeck (institutional board projects 05-112). In adherence to the Helsinki principles, informed consent was obtained from all patients whose material was used in this study.

2.2. Indirect immunofluorescence test

Autoantibodies in human sera were determined by IIFT using frozen sections of unfixed human pancreas according to the manufacturer's instructions (Euroimmun) as well as wild-type or genetically modified HEK293 cells (generated as stated below). In some cases, monoclonal murine antibodies were used in the first step of IIFT, followed by incubation with Cy2 or Cy3 anti-mouse IgG (Jackson Research Europe, United Kingdom). In neutralization experiments, samples with potential antigen content were mixed