SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY

Scandinavian Journal of Gastroenterology is the scientific periodical for Dansk Gastroenterologisk Selskab, Finsk Gastroenterologisk Förening, Islandsk Gastroenterologisk Forening, Norsk Gastroenterologisk Forening, and Svensk Förening for Gastroenterologi.

SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY (ISSN 0036–5521) is published ten times a year (January, March, April, May, June, August, September, October, November and December) by Norwegian University Press. Supplements are supplied free of charge to subscribers, and copies may also be purchased on application to the publishers. Annual subscription price for 1987 for the Nordic countries is NOK 1600,– and for the rest of the world is USD 267.00, to be paid in advance. Air freight and mailing in the USA by Publications Expediting Inc., 200 Meacham Ave., Elmont, NY 11003. Second-class postage paid at Jamaica, NY 11431. U.S. Postmaster: send address changes to *Scandinavian Journal of Gastroenterology*, c/o Publications Expediting Inc., 200 Meacham Ave., Elmont, NY 11003, USA.

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Typeset by Gardiner-Caldwell Communications Limited, Macclesfield,

CONCEPTS AND CONTROVERSY IN GASTROENTEROLOGY

Proceedings from a satellite symposium
São Paulo, Brazil

10 September 1986

Edited by:
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Autoimmunity to Pancreatic Juice in Crohn's Disease

Results of an Autoantibody Screening in Patients with Chronic Inflammatory Bowel Disease

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Stöcker W, Otte M, Ulrich S, Normann D, Finkbeiner H, Stöcker K, Jantschek G, Scriba PC. Autoimmunity to pancreatic juice in Crohn's disease. Results of an autoantibody screening in patients with chronic inflammatory bowel disease. Scand J Gastroenterol 1987, 22 (suppl 139), 41–52

The sera of 59 patients with Crohn's disease (CD) and of 46 patients with ulcerative colitis (UC) were tested for autoantibodies (Aab) by indirect immunofluorescence with modern histochemical techniques using 19 different human tissues as antigenic substrates. Control collectives consisted of 19 patients with coeliac disease and of 100 healthy subjects. It was possible to demonstrate a specific marker for CD: Aab against exocrine pancreas (Pab) were present in 39% of the CD sera (UC 4%, coeliac disease 0%, healthy controls 3%). High Pab titres were only detectable in CD sera (29%). The CD-related autoantigen was demonstrated to be a component of normal pancreatic juice. Pab in CD were fundamentally different from those sometimes occurring in chronic and acute pancreatitis. It is suggested that CD is caused by autoimmune reactions against a component of pancreatic juice. Pab in CD correspond to Aab against intestinal goblet cells (Gab), which occurred exclusively in UC (28%). Pab and Gab, but obviously none of the other Aab investigated in this study, are of diagnostic value in chronic inflammatory bowel disease.

Key words: Autoantibodies; autoimmunity; chronic inflammatory bowel disease; coeliac disease; Crohn's disease, aetiology; Crohn's disease, serodiagnosis; fluorescent antibody techniques; pancreatic autoantigens; pancreatitis; ulcerative colitis, serodiagnosis

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In chronic inflammatory bowel disease (CIBD), a number of observations support the concept of an autoimmune aetiology. Remarkably, in the sera of some patients with ulcerative colitis (UC) autoantibodies (Aab) can be observed which react with antigens of intestinal goblet cells. These antibodies were first demonstrated by means of hemagglutination and gel diffusion methods (1) and later by indirect immunofluorescence (2–10).

Since in CIBD primarily the bowel is concerned, studies were usually restricted to Aab against intestinal tissue. Despite the frequently occurring extraintestinal manifestations of Crohn's disease (CD), only very few studies have dealt with the prevalence of Aab directed against other than bowel antigens in CIBD, and the number of Aab tested were limited (8, 11–13). To obtain a comprehensive overview on Aab in CIBD, Aab profiles were established in sera from patients with CD and UC. Patients with coeliac disease, as well as healthy subjects without evidence of bowel disease, served for control.

As reported earlier (10), in these experiments the occurrence of Aab to intestinal goblet cells (Gab) in UC could be confirmed. Beyond this, it was possible to demonstrate CD-specific Aab

against an antigen of the exocrine pancreas (Pab). In the present paper, the results of the Aab screening are summarized and further data are provided to evaluate the significance of the established CD-specific autoimmunity.

MATERIALS AND METHODS

Subjects

Serum samples were obtained from patients with Crohn's disease ($n = 59$), ulcerative colitis ($n = 46$), coeliac disease ($n = 19$), and healthy subjects ($n = 100$). Diagnoses of the patients were established by clinical examination and, in each instance, confirmed both by endoscopy and histology.

Patients with Crohn's disease (CD). This group includes 22 men (average age, 26.0 years) and 37 women (average age, 28.4 years). At time of blood drawing, 49 patients with CD received salicylazosulfapyridin, 43 patients of them were treated additionally with corticosteroids. Disease activity was estimated according to Best et al. (14; CDAI). In 14 of the CD patients symptoms were recorded which can be regarded as extraintestinal manifestations of the disease. None of the patients with CD had clinically apparent chronic or acute pancreatitis.

Patients with ulcerative colitis (UC). Of 46 patients with UC, 23 men (average age, 40.3 years) and 23 women (average age, 34.5 years), 40 had been treated with salicylazosulfapyridin, 30 also with corticosteroids. The current activity of UC was assessed using a modified CDAI.

Control subjects. Collectives of 19 patients with coeliac disease (6 men, average age 52.2 years, and 13 women, average age 54.8 years) and of 100 healthy volunteers (62 men; average age, 34.3 years; and 38 women; average age, 28.6 years) served for control.

Blood was drawn from all subjects for determination of autoantibody profiles by indirect immunofluorescence. Sera were separated and stored at −80 °C for up to 2 years before testing.

Screening for autoantibodies

Aab were determined by indirect immunofluorescence, employing a broad panel of tissues (blood group O) as antigenic substrates which included human adult pancreas, parotis, liver, lung, striated and smooth muscle, heart, thyroid (unfixed sections plus methanol-fixed sections), parathyroid, adrenal, ovary, placenta, testis, hypophysis, kidney, and sterile human fetal esophagus, stomach, duodenum, ileum, and colon. Normal adult human tissue was obtained at surgery, fetal tissue from a still-born infant at 32 weeks' gestation. Organs were snap-frozen in melting isopentane and stored up to 3 years at −196 °C.

The test procedure was facilitated by new rapid and economic microtechniques described earlier (15, 16). In brief, 4-μm tissue sections were cut with a Leitz-1720 cryotome (Leitz, Wetzlar, FRG) at −22 °C. Sections were applied to chemically activated glass cover slides, thawed, and dried. The cover slides were cut with a glazier's diamond, and broken into pieces (fragmenting technique). Chemical activation (17) was performed, to enable covalent coupling of the tissue sections to the glass surface, thus effectively improving the adhesion of the sections.

Section fragments, carrying relevant organ structures, were sealed in blisters and stored in liquid nitrogen until use. They were glued on reaction areas of a glass plate (sections' support), samples or fluorescein-labelled antisera were pipetted on hydrophilic reaction areas of another, plane, glass plate (reagents' support). The reaction areas were surrounded by a hydrophobic coating, which prevented mixing of neighbouring samples. The sections' support was laid above the reagents' support in such a way that the tissue sections were immersed in the liquid (titerplane technique).

Aab screening was performed by a composite-section technique. Section fragments of 19 different tissues were glued side by side on large reaction areas and co-incubated in the same drop of sample or reagent (200 μl per 19 sections). The single-section technique was used to determine the immunoglobulin classes of established Aab, to identify their ability to fix complement, and to determine Aab concentrations by titration: Section fragments were glued on small reaction areas and incubated in droplets of 10 μl, each section

fragment being immersed individually in a single droplet of the liquids.

Immunofluorescence staining

Composite sections were incubated for 60 min with sera which were usually diluted 1:10 in phosphate-buffered saline. Sections were stained for a further 60 minutes with a polyvalent fluorescein-conjugated rabbit antihuman immunoglobulin, directed against IgA + IgG + IgM + kappa + lambda (DAKO, Hamburg, FRG). The sections were washed for 60 min after each stage in three changes of phosphate-buffered saline, with gentle agitation. Positive sera were titrated in steps of 1:32, 1:100, 1:320, etc., using single sections.

Heavy- and light-chain specificities of Pab and Gab were determined using monospecific fluorescein-conjugated rabbit antihuman IgA, IgD, IgE, IgG, IgM, kappa and lambda (Behringwerke, Marburg, FRG), instead of the polyvalent antiserum.

Each serum sample was tested for complement-fixing Pab and Gab by a three-layer indirect immunofluorescent test. The first layer was composed of 1:10 diluted patient's serum (incubation at room temperature for 1 h), the second of undiluted normal human serum (blood group AB) as complement source (1 h), the third contained fluorescein-conjugated rabbit antihuman C1q, C3c, or C4 (1 h; Behringwerke). Essential controls in each serum included staining with FITC-conjugated rabbit antihuman globulin directed against albumin.

Microscopic examination

Results were read subjectively with a Leitz Dialux-20 microscope by two individuals, independently of each other, without knowledge of the patients' diagnoses. The microscope was fitted with epi-illumination, 50-W HB-50 mercury lamp, filter combination J 2, mirror RKP 510, magnification ×250 (Leitz, Wetzlar, FRG). All results were read double-blind with positive and negative control sera in each batch.

Neutralization tests

The CD-related autoantigen was detected by a Pab neutralization technique (18): Diluted Pab-containing CD sera were incubated for one hour with equal volumes of different diluted organ homogenates or different liquids:

Supernatants of homogenized human liver, heart, pancreas (homogenization was accomplished by producing 5-μm-thick frozen sections with the cryotome; to the slices nine volumes of buffered saline were added);

Human duodenal juice;

Human pancreatic juice (collected by endoscopic cannulation of the main pancreatic duct);

Fluids of pancreatic pseudocysts (obtained from patients with chronic pancreatitis for diagnostic purposes); or

FPLC column effluents (fast protein liquid chromatography).

After incubation, titres of free Pab in the mixtures were determined by the indirect fluorescent antibody test using frozen sections of human adult pancreas, as described above. The capacity of these substances to extinguish specific immunofluorescence was recorded.

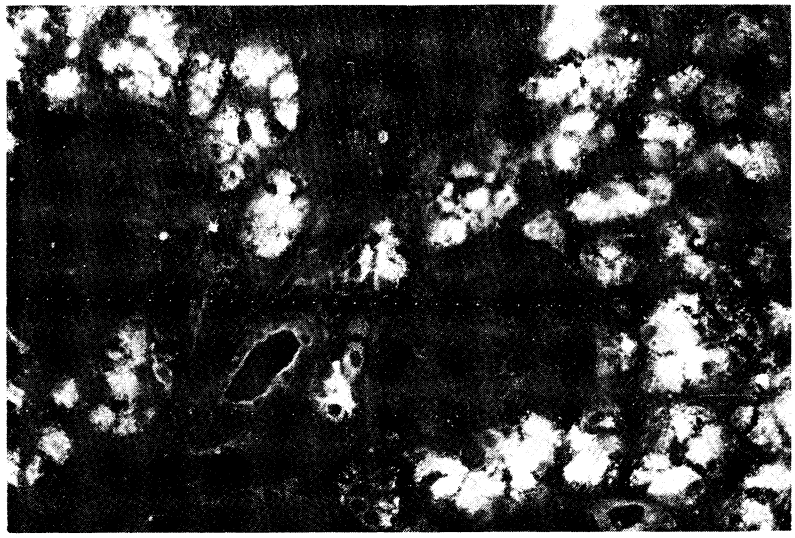
Chromatography

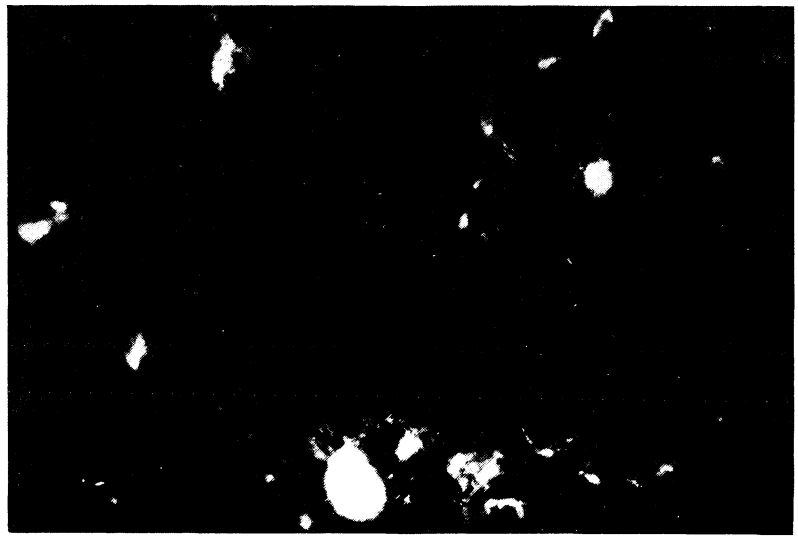
Human pancreatic juice and fluids of pancreatic pseudocysts were analyzed by gel filtration chromatography FPLC (Superose 6, Pharmacia Fine Chemicals, Freiburg, FRG). The antigen-containing fractions were identified by monitoring the capacity of the column effluents to neutralize Pab.

RESULTS

Autoantibody profiles

The prevalence of 22 different autoantibodies (Aab) was determined in Crohn's disease (CD) and in ulcerative colitis (UC), as well as in the control collectives, which consisted of patients with coeliac disease and of healthy persons. In CD, Aab against exocrine pancreas (Pab) turned out to be very frequent, and they occurred in high concentrations (Figs. 1 and 2). The Aab screening confirmed the prevalence of Aab against intestinal goblet cells (Gab) in UC (Fig. 3).





Figs. 1 and 2. Autoantibodies against exocrine pancreas in Crohn's disease. Determination by indirect immunofluorescence. Unfixed frozen sections of human adult pancreas were incubated in the first step with 1:10 diluted sera of patients with CD, in the second step with a polyvalent fluorescein-conjugated rabbit antihuman immunoglobulin. Fig. 1 (*top*). Serum of CD patient 22; reticulogranular pattern. Fig. 2 (*bottom*). Serum of CD patient 27; droplet-like pattern.

Results of the Aab screening are summarized in Table I. In addition to Pab and Gab, in patients with CD and UC the following Aab occurred with a higher frequency than in coeliac disease and in healthy subjects: Aab to gastric mucosa (preference to CD), Aab to 'single cells' of connective tissue and to cell nuclei (preference to UC), and Aab to enterocytes and nerve tissue (elevated in both). None of these Aab correlated with Pab and Gab. Their bearing on the pathogenesis of CIBD is not yet understood.

Aab to the other tissues employed in this study have not been more frequent in CIBD than in healthy control subjects.

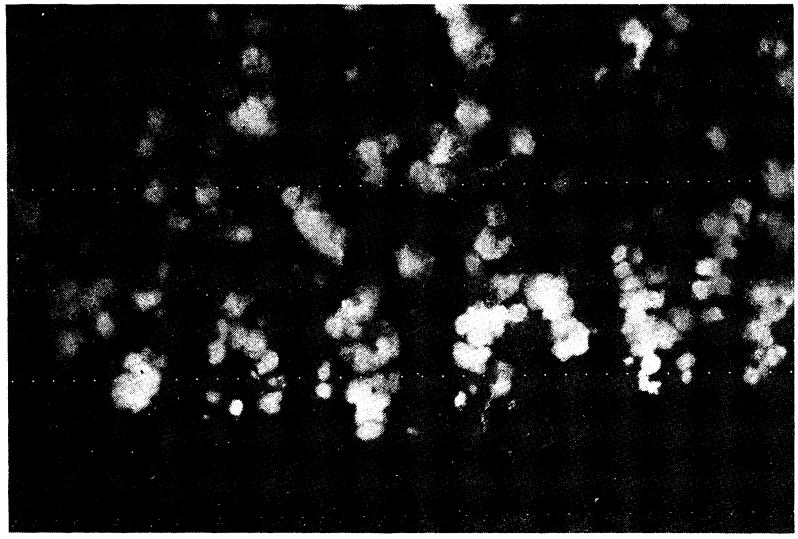


Fig. 3. Autoantibodies against intestinal goblet cells in ulcerative colitis. Determination by indirect immunofluorescence. An unfixed frozen section of human fetal duodenum was incubated in the first step with the 1 : 10 diluted serum of UC patient 38, in the second step with a polyvalent fluorescein-conjugated rabbit anti-human immunoglobulin.

Autoantibodies to exocrine pancreas (Pab)

Aab to the exocrine pancreas (Figs. 1 and 2) were discernible by a striking reticulogranular fluorescence in the cytoplasm of the pancreatic acinar cells, with an increasing intensity in the direction of the acinar centre. With the most positive sera fluorescent droplets became visible in the centre of the acini. Pancreatic islets were not stained by Pab.

Pab could be observed in 23 out of 59 patients with Crohn's disease (39%). In 17 patients (29%) the titre of Pab was 1 : 100 or higher. Such high concentrations have been found neither in UC, nor in coeliac disease and healthy controls.

Six of the 23 Pab fixed complement. In these cases, the CDAI was twice as high (225.3 ± 139.4) than in 17 patients with non-complement-fixing Pab (99.7 ± 85.2).

In patients whose CD had been present for less than 2.5 years the prevalence of Pab was determined as being only 25%. If CD existed longer than 2.5 years, patients exhibited Pab in 46%. In CD patients having extraintestinal manifestations of the disease, the prevalence of Pab was 50%, in patients without extraintestinal manifestations only 33%.

The prevalence of Pab was dependent neither on sex, old age or blood group (A, B, AB, O) of the patients, nor on the intestinal localization of the disease or on a therapy with salicylazosulfapyridin. Corticosteroids seemed to reduce the prevalence of Pab (15 of 43 patients = 35%), patients without corticosteroids showed Pab in 8 of 16 cases (50%).

Pab were also found in 2 out of 46 patients with the diagnosis of UC (4%; titres 1 : 10 and 1 : 32). Three sera of 100 control subjects exhibited Pab in titres of 1 : 10.

Autoantibodies to intestinal goblet cells (Gab)

Autoantibodies to intestinal goblet cells (Gab) (Fig. 3) were a distinguishing mark of UC. Gab were discernible in the epithelium of the mucosa as hazy confined, cloudy spots. They could be detected with each segment of the bowel used as antigenic substrate from duodenum to the rectum. But positive and negative staining could be distinguished better with small intestine than with

Table I. Autoantibody profiles in Crohn's disease, ulcerative colitis, coeliac disease, and healthy subjects. Prevalence of clinically significant Aab titres

Prevalence (%) of autoantibodies to	Crohn's disease ($n = 59$)	Ulcerative colitis ($n = 46$)	Coeliac disease ($n = 19$)	Healthy controls ($n = 100$)
Exocrine pancreas (Pab), titre 1:100–1:1000	29	0	0	0
Exocrine pancreas (Pab), titre 1:10–1:1000	39	4	0	3
Intestinal goblet cells (Gab), titre 1:10–1:1000	0	28	0	0
Gastric mucosa, (granular pattern)	41	11	5	6
'Single cells' of connective tissue	2	17	0	0
Cell nuclei	8	26	5	6
Enterocytes	39	33	10	14
Nerve tissue	22	26	5	2
Mitochondria	0	0	10	0
Smooth muscle	3	2	0	5
Thyroid microsomes	0	7	5	6
Parietal cells	0	7	0	9
Sinusoids of the liver	0	0	20	0

The Aab screening was carried out employing the following human tissues as antigenic substrates: Adult pancreas, parotis, liver, lung, striated and smooth muscle, heart, thyroid, parathyroid, adrenal, ovary, placenta, testis, hypophysis, kidney, and sterile human fetal esophagus, stomach, duodenum, ileum, and colon. In addition to the Aab listed in the table, the control group exhibited Aab to thyroglobulin (5%), striated muscle (1%), and pancreatic islets (1%). Further Aab were not detectable in any of the four groups.

colon, since in the small intestine goblet cells are separated from each other and in the colon goblet cells appear as a coalescing cloudy mass.

Gab were present in 13 of 46 patients with UC (28%), but in no case of CD and coeliac disease and in none of the healthy control persons. Serum concentrations varied from patient to patient, titres were determined between 1:10 and 1:1000.

The (UC-adapted) activity index in patients with Gab was 136 (±93). In patients without Gab, disease activity was 85 (±75).

None of the established Gab fixed complement. The occurrence of Gab correlated neither with the patients' old age, nor with the duration of the disease or the therapy.

A preponderance of Gab was observed in males: Gab occurred in 10 of 23 male UC patients (43%), but only in 3 out of 23 female patients (13%). Additionally, the distribution of Gab in patients with different blood groups was not even: In blood group A the prevalence was 38% (6 of 16), in group B 40% (2 of 5), in group AB 66% (2 of 3), in group O only 16% (3 of 19).

Immunoglobulin classes of Pab and Gab

Pab mainly belonged to IgG and IgA, one patient exhibited IgD and one patient IgM. Pab of class IgE were not observed (Table II).

In Gab, only immunoglobulin classes IgA and IgG could be observed (Table II). IgM were not detected.

Light chains of Pab and Gab

To find out whether Pab and Gab were of oligoclonal or of polyclonal origin, their light chains were analyzed by indirect immuno-

Table II. Immunoglobulin classes of Pab and Gab, and their ability to bind complement factor C3c. Pab contained in two cases additionally IgD, but never IgE. Gab consisted exclusively of IgA and/or IgG

	Total	IgA alone	IgG alone	IgA + IgG	IgM alone	Reaction with C3c
Pab in CD	23	2	8	12	1	6
Gab in UC	13	1	3	9	0	0

fluorescence: kappa and lambda were evenly distributed in 11 of 23 Pab; in 7 cases kappa overbalanced lambda, and in 5 cases lambda overbalanced kappa (Table III). Similar data were obtained in Gab: kappa was equal to lambda in 9 of 13 cases, kappa predominated in 3 cases, and lambda in 1 case.

Other autoantibodies

Aab to gastric mucosa gave a granular staining in single, up to now not identified cells of the gastric glands. They were present in only very low concentrations (to detect these Aab, the patients' sera had to be used undiluted in the first step of the indirect fluorescent antibody test). Of the CD patients 41% exhibited this antibody (UC, 11%; coeliac disease, 5%; healthy controls, 6%), which correlated neither with Pab nor with parietal cell antibodies.

Aab to 'single cells' of connective tissue: In the sera of some patients with UC an Aab was found which reacted with the cytoplasm of large, sporadically distributed cells in the connective tissue of nearly every organ including pancreas, liver, kidney, and thyroid. The target cells have not yet been identified. Serum titres between 1:10 and 1:320 were observed (UC, 17%; CD, 2%; coeliac disease, 0%; controls, 0%). Clinical symptoms could not be detected, which would explain the implication of these Aab.

Table III. Reactions of Pab and Gab with fluorescein-labelled anti-kappa or anti-lambda. Titre 1:3.2 and 1:10 = +; 1:32 and 1:100 = ++; 1:320 and 1:1000 = +++

		Lambda			
	Kappa	0	+	++	+++
Antibodies to exocrine pancreas in CD (23 positive sera)	0	–	2	0	0
	+	4	7	2	0
	++	1	2	1	1
	+++	0	0	0	3
Antibodies to intestinal goblet cells in UC (13 positive sera)	0	–	0	1	0
	+	2	9	0	0
	++	0	0	0	0
	+++	1	0	0	0

Significant concentrations of Aab to cell nuclei (titre 1:100 or higher) have been stated with a significantly higher frequency than in control subjects in UC and with a lower extent in CD (UC 26%; CD, 8%; coeliac disease, 5%; healthy controls, 6%).

Aab to enterocytes were common in both CD (39%) and UC (33%), but also in coeliac disease (10%) and in healthy controls (14%; Table I). They reacted with the cytoplasm of the entire population of intestinal epithelial cells including the goblet cells. These Aab exhibited the same affinity to each of the different bowel segments. Aab to enterocytes were different from Pab and Gab, they occurred independently from these Aab in CD and UC.

Aab to nerve tissue reacted with nerves and with the cytoplasm of plexus cells in many organs. They were especially well discernible with human fetal tissue of the gastrointestinal tract (plexus Auerbachi et Meissneri). Their prevalence in CD was 22% and in UC 26% (coeliac disease, 5%; healthy controls, 2%).

Detection of the CD-related autoantigen in pancreatic juice

Neutralization experiments have been performed with different pancreas-derived substances to evaluate the possible role of the exocrine pancreas in the pathogenesis of CD.

The neutralizing efficacy of crude duodenal juice or homogenized pancreas could not be

Table IV. Neutralization of autoantibodies with different pancreas-derived substances, whose capacity to extinguish specific immunofluorescence was estimated by titration. Liquids were used undiluted; supernatants of organ homogenates were diluted 1 : 10 in phosphate-buffered saline. In the table, the reciprocal titres are listed

	Neutralization of autoantibodies with							
$(Titre)^{-1}$ of autoantibodies to	Phosphate-buffered saline	Cystic fluid 1	Cystic fluid 2	Pancreatic juice	Column effluent fract. 14	Parotic saliva	Homog. liver	Homog. heart
Exocrine pancreas (serum 11)	100	3.2	100	3.2	10	100	100	100
Exocrine pancreas (serum 13)	1000	32	1000	10	32	1000	1000	1000
Pancreatic islet cells	100	100	100	100	100	100	100	100
Intestinal goblet cells	320	320	320	320	320	320	320	320
Cell nuclei	1000	1000	1000	1000	1000	1000	10	100
Mitochondria	1000	1000	1000	1000	1000	1000	100	3.2

evaluated. Their inherent proteinases became activated and digested the tissue sections, thus it was not possible to interpret the results of the fluorescent antibody test.

Well-interpretable results were obtained, however, when pancreatic juice or fluids of pancreatic pseudocysts were used for neutralization. In both cases positive Pab reactions were abolished or titres were reduced by at least 4 double dilution steps in each of the 23 Pab-positive sera studied. In contrast, pancreatic juice and fluids of pancreatic pseudocysts did not neutralize autoantibodies against cell nuclei, mitochondria, smooth muscle, gastric parietal cells, intestinal goblet cells, thyroid microsomes, and pancreatic islets (Table IV).

Separation of the CD-related autoantigen: The hypothetical CD-related autoantigen was contained in one single peak and behaved chromatographically as a macromolecule of about 10^6 dalton. It was different from functionally active amylase, lipase, trypsin, and chymotrypsin, which were identified by biochemical standard procedures in separate peaks (18).

With the pancreatic autoantigen containing fraction, each of the positive Pab reactions in the 23 CD sera studied could be abolished in the neutralization experiments. Thus, the antigenic specificity of Pab seems to be uniform.

DISCUSSION

Autoantibody profiles were established in chronic inflammatory bowel disease (CIBD) by indirect immunofluorescence with modern histochemical techniques. Results of this study confirm that significant autoimmune reactions do exist in CIBD. In Crohn's disease (CD) as well as in ulcerative colitis (UC), disease-specific Aab could be demonstrated, which were directed against exocrine pancreas in CD (10, 19) and against intestinal goblet cells in UC. With regard to serum concentration and disease specificity, Pab and Gab were as conspicuous as other autoantibodies in proven autoimmune diseases.

A number of further Aab were found which occurred in CIBD with a higher frequency than in control collectives, but were of less significance than Pab and Gab: Aab to enterocytes and to cell nuclei (neither specific for CD nor for UC), Aab to gastric mucosa (occurred in very low concentrations), and Aab to 'single cells' of connective tissue (low prevalence). The other Aab determined in this study were as rare in CIBD as in healthy persons and in patients with coeliac disease.

The results made evident that Crohn's disease is associated with potent autoimmune reactions against pancreatic juice: In CD, autoantibodies to

exocrine pancreas were frequent and they usually occurred in considerably high concentrations in the patients' sera. High titres of Pab could be recorded neither in patients with UC or coeliac disease nor in healthy subjects.

In rare cases of chronic and acute pancreatitis Aab can be found, which also react with acinar cells of the pancreas (20–22). One may ask whether there exist immunological differences between the two conditions. Hellwig et al. (22) have characterized serum Aab against exocrine pancreas in pancreatitis: The prevalence of significant concentrations was as low as 6.5% in chronic and acute pancreatitis (5 of 77 cases) and, if these antibodies were present, titres did not exceed 1:32. Pab in pancreatitis consisted only of IgA, did not fix complement and could not be neutralized by the CD-related autoantigen (Table V). On the other hand, Pab were frequent in CD, they usually occurred in high concentrations, contained IgG in most cases, sometimes fixed complement and could be neutralized by the CD-related autoantigen in each case. Thus, Pab in pancreatitis differed fundamentally from those in CD.

In patients with pancreatitis it is conceivable that antigens released from the injured pancreas induce a secondary immunization without important pathogenetical implications. If pancreatic immunity were also an epiphenomenon in CD, immunoreactions should be of comparable dimensions. But in CD, humoral immunity against exocrine pancreas exceeds by far that found in pancreatitis, thus it is suggested that autoimmunity to pancreatic antigens is essential in CD.

The CD-related autoantigens were distributed like secretory proteins in the exocrine pancreas, as could be observed in the indirect fluorescent antibody test. Are these antigens secreted into the bowel lumen? In this study it was documented by neutralization experiments that the CD-related autoantigens really are contained in normal pancreatic juice. Therefore, it is imaginable that the exocrine pancreas is implicated in the pathogenesis of CD:

Crohn's disease may be caused by autoimmune reactions against the exocrine pancreas. The bowel has developed a state of hypersensitivity against a component of pancreatic juice. The autoantigens are synthesized in the pancreas and secreted into the bowel. In the bowel wall they react with locally present autoantibodies and form immunocomplexes which induce activation of complement and inflammation. It is also conceivable that the autoantigens bind unspecifically to tissue structures and transform them to targets of immunoattacks.

The in vitro observed autoimmunity to pancreatic juice in CD corresponds to a number of histological, clinical, and epidemiological phenomena:

A striking augmentation of plasma cells in the intestinal CD lesions (23, 24) reflects a response to hitherto unknown antigens.

Diversion of the fecal stream by an ileo- or colostoma usually results in an immediate clinical improvement of acute Crohn colitis. In UC, this

Table V. Characteristics of autoantibodies to exocrine pancreas in CD and in pancreatitis

	Crohn's disease	Acute and chronic pancreatitis
Prevalence of Pab	39%	5%
Concentration of Pab	Usually high (titre 1:100 or higher in 3 of 4 positive cases)	Low (titre max. 1:10)
Immunoglobulin class of Pab	Usually IgG or IgG plus IgA	IgA only
Complement fixation of Pab	Sometimes observed	Not observed
Pab neutralize the CD-related autoantigen	In all cases observed	Not observed

treatment is without positive effects (25). Furthermore, an intestinal lavage with physiologic saline reduces the activity of the disease. This indicates that toxic (antigenic?) substances are contained in the bowel lumen (26).

The beneficial effect in CD of parenteral nutrition (27) or a low-fat elemental diet (28, 29), which reduce pancreatic secretion to levels near those observed during fasting (30–33), might also indicate an impaired tolerance to pancreatic juice in CD.

Patients suffering from CD develop a predilection for refined sugar. They consume significantly more carbohydrates than UC patients and healthy control persons (34–37). This has been considered to be a causative factor in CD, but another interpretation seems also to be conceivable: sugar and starch do not stimulate protein synthesis in the exocrine pancreas, and glucose even inhibits the secretin/pancreocymin-stimulated pancreatic secretion (38, 39). Patients with CD unconsciously realize the reduced tolerance to pancreatic juice, and they try to minimize the pancreatic-juice-mediated injury to the bowel.

The increasing incidence of CD in wealthy countries might be attributed to the nutrition, which is rich in protein and fat. The over-consumption of food leads to a strong and long-lasting stimulation of the exocrine pancreas. The gut-associated immune system comes permanently into contact with large amounts of the (hypothetical) autoantigen, and predestinated subjects develop autoimmune reactions to pancreatic juice and CD.

Extraintestinal manifestations in many patients with CD might be caused by deposition of circulating immunocomplexes (consisting of Pab and the corresponding autoantigen) or by immunoreactions against the CD-related autoantigen which are contained in the affected tissues and presented to the immunosystem more effectively than in the pancreas. In spite of the established pancreatic immunity, pancreatitis is not predominant in CD since the bulk of autoantigens comes into contact with the immune system only outside of the pancreas.

The autoimmunity against exocrine pancreas in CD is a counterpart to autoimmune phenomena in UC. Aab against intestinal goblet cells in UC are well established and may have a pathogenetical implication since Gab are specific for UC and goblet cells show macroscopically and microscopically the same localization as the disease: They are rare in the small intestine, but arranged closely side by side in the colon, increasing in the direction to the rectum. Furthermore, the number of goblet cells is the highest in the crypts. Accordingly, in UC, the small intestine is not affected, but the rectum nearly in each case, and cryptitis is a typical feature of UC.

Instead of adult tissue, fetal intestine was employed as antigenic substrate for determination of Gab by indirect immunofluorescence, to avoid unspecific reactions caused by bacterial or food antigens. Furthermore, it is important to use human tissue: antibodies, determined with rat colon (12, 40–42), do not correlate with Gab (9) and exhibit positive reactions also in CD and in healthy subjects. These 'heterophile' antibodies often consist of IgM (42), in contrary to Aab against human goblet cells, which belonged only to the immunoglobulin classes IgG and IgA.

The occurrence of Gab in UC has been discussed to be a corollary of an immunization against goblet cell antigens shedded from damaged colonic mucosa and thus to be a secondary phenomenon (e.g. Ref. 6). But the strict absence of Gab in patients with CD of the colon is inconsistent with this explanation. On the other hand, one might also speculate that Pab result from a secondary immunization in the gut. This should take place in the small intestine, since high concentrated Pab essentially could not be detected in the colon-restricted UC. But significant Pab-titres also occurred in a number of patients with CD confined to the colon. Thus also the pancreas-specific autoimmunity in CD seems to be a phenomenon of primary significance.

Furthermore, a secondary immunization against pancreatic and goblet cell antigens would probably induce a polyclonal antibody response, and kappa and lambda light chains would be evenly distributed in each Pab or Gab. But the high frequency of antibodies with only one type of light-chain detectable gives evidence of an oli-

gioclonal response: Both Pab and Gab are produced by a very small number of plasma cell clones. This result was confirmed with a higher number of sera (43) and might correspond to the finding of monoclonal lymphocyte populations in the peripheral blood of 12 out of 20 patients with CD or UC (44). The established oligoclonality of Pab and Gab speaks in favour of autoimmunity as being causatively involved in the aetiology of CD and UC.

Pab and Gab seem to be disease-specific, and they do not yet indicate a disposition for CD or UC. This was shown by Döscher et al. (45) and Kosegarten et al. (46), who determined the prevalence of Pab and Gab in an up to now limited number of healthy appearing first-degree relatives of patients with CIBD. These persons carry an elevated risk for developing CIBD. One hundred and fourteen relatives of 46 UC patients and 71 relatives of 52 CD patients did, however, not exhibit Pab or Gab in the sera in significant concentrations. Thus, subjects with a disposition for developing CD or UC obviously cannot be identified by determination of Pab and Gab.

Diagnostic value of Pab and Gab: As with other autoimmune diseases, the cause of sensitivization cannot yet be explained, either in CD or in UC, and further investigation is required to completely reveal the mystery of CIBD. But the knowledge of these autoimmune phenomena can be used with advantage for diagnostic purposes: High titres of Pab are specific markers of CD; they cannot be observed in UC, coeliac disease, control persons, and not even in pancreatitis and subjects with a disposition for CD. On the other hand, Gab are exclusively present in patients with UC. In one third of cases with CIBD, the determination of Pab and Gab is sufficient to establish the diagnosis of CD or UC.

The low frequency of Pab and Gab in CIBD should not give rise to scepticism about their implication in the pathogenesis of CD and UC: It is possible that Aab are neutralized by the corresponding circulating autoantigens or that patients without Aab only exhibit cellular immunoreactions. Or the occurrence of Aab is confined to the intestine in some patients. In most of the disorders with proven autoimmune aetiology, the corresponding disease-specific Aab do not reach a prevalence of 100% either.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Dr. A. Gropp (deceased), Prof. Dr. D. Sellin (Lübeck), and Prof. Dr. P. Schmitz-Moormann (Marburg) for the histological examination of the patients' biopsies, and technical assistants D. Grage, A. Hagedorn, U. Reddig, A. Stolzenberg, and D. Struve for their skillful help.

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