Antibodies to pancreatic duct cells in Sjögren's syndrome and rheumatoid arthritis

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SUMMARY In the present investigation the occurrence of humoral immunity to pancreatic duct cells (PDC) was studied in 12 patients with Sjögren's syndrome (SS), 31 patients with rheumatoid arthritis (RA), and 64 controls. Four sera of patients with SS and eight of patients with RA produced diffuse cytoplasmic fluorescence of intra- as well as of interlobular PDC of human and rhesus monkey origin. All sera positive with PDC antigens gave also positive staining reaction with parotid, sub-mandibular, and lacrimal duct cells. In absorption studies antibody activity to PDC and salivary duct cells could be absorbed equally well with human or monkey parotid gland or pancreas with almost identical antigen concentrations. These findings point to the presence of common antigenic determinants in the organs studied. Human thyroid microsomes and rat liver homogenate did not reduce antibody activity. The demonstration of antibodies to PDC in addition to the reported mononuclear cell infiltration of the pancreas point to the involvement of autoimmune mechanisms in pathogenesis of the commonly observed subclinical exocrine insufficiency in SS and in some cases of RA.

Sjögren's syndrome (Sjögren, 1933) consists of the triad of keratoconjunctivitis sicca, xerostomia, and rheumatoid arthritis or other connective tissue diseases (Bloch et al., 1965). The lymphocytic infiltration of the lacrimal and salivary gland and the metaplastic changes in the epithelium of striated ducts followed by destruction and fibrosis are the characteristic features of this syndrome. However, in a histological examination (Waterhouse and Doniach, 1966) lymphocytic sialadenitis was found in almost all rheumatoid arthritis patients studied. This suggests that a subclinical form of Sjögren's syndrome (SS) might occasionally occur in rheumatoid arthritis. Other excretory glands-for example, those of the respiratory, gastrointestinal, and genital tracts-are reported (Whaley et al., 1973) to be often histologically and functionally involved in SS. Although systematic investigations of pancreatic function have not been undertaken in patients with SS, there seems to be increasing evidence (Whaley et al., 1973) that subclinical exocrine pancreatic disease may be present in many patients. As antibodies to salivary duct epithelial cells can be found in a considerable number of patients with SS and less

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frequently in those with rheumatoid arthriti^S (Bertram and Halberg, 1964; MacSween *et al.*, 1967 Feltkamp and Rossum, 1968) it seemed worthwhile to look for the occurrence of antibodies to pancreatic duct epithelial cells in these disorders. The sporadic reports about diffuse mononuclear cell infiltrations of exocrine pancreas (Ellman *et al.*, 1951; Bucher and Reid, 1959) were an additional stimulus to look for a possible autosensitisation to pancreatic antigens in Sjögren's disease.

Methods

PATIENTS

Forty-three patients, 12 of whom presented with SS and 31 with rheumatoid arthritis, were studied. In all patients with SS the three major components of the syndrome described by Bloch and others (1965) were present. In these patients characteristic results of the Schirmer-test (I, II), Rose Bengal test, and parotis Tc-uptake (Havlik, Scherak, Bergmann and Kolarz, 1976) were observed. The diagnosis of rheumatoid arthritis was established in 31 patients according to the criteria of the American Rheumatism Association (Ropes *et al.*, 1958). Mean age of patients with SS was $65 \cdot 4 \pm 5$ and of rheumatoid arthritis patients 53 ± 4 years; mean duration of the disease was 7.3 years in the former and 8.2 years in the latter group of patients.

CONTROLS

In our outpatient clinic 64 sera were selected from patients with no signs and symptoms of arthritis or sicca syndrome. These were tested as controls.

IMMUNOFLUORESCENCE STUDIES

Human parotid, submandibular, and lacrimal glands were obtained from several kidney donors shortly after termination of artificial perfusion. Salivary glands from one kidney donor with blood group 0, which were found most suitable, were used in all further studies. The same organs were also obtained from Maccacus rhesus monkeys, quick frozen in liquid nitrogen, and processed with standard techniques for immunofluorescence tests. All sera were stored at -20° C until tested on unfixed 5 μ cryostat tissue sections. When staining reactions were positive further titrations were performed. Commercially available FITC labelled goat antisera to human IgA, batch 461 D.; IgG, batch 572; and IgM, batch 440 A (Behring Comp., Marburg, Germany) were used. Monospecific reactivity of these conjugates was tested on plasma cell preparations of selected monoclonal bone marrows.

A Leitz Ortholux fluorescence microscope with epüllumination (Ploemopak II) was used. Filters: 2 KP 490 + K 455, TK 510, K 515 + S 525.

ABSORPTION STUDIES

Human and monkey parotid and pancreas glands, human thyroid microsomes (Wellcome, Beckenham, England), and rat liver tissue were homogenised in saline. Cell detritus was separated from the homogenate by centrifugation (four times at 700 g). The retained crude extract was diluted to 25 mg, 2.5 mg, 0.5 mg, and 0.025 mg tissue/ml saline. Equal amounts of tissue homogenates were incubated for one hour at 37°C, overnight at 4°C with 1:10 dilution of serum of patient M.O. This serum was used for all absorption experiments, because it showed the highest titre (1:60) with parotid and salivary duct cells. After incubation, the mixtures were centrifuged (700 g) and the absorbed supernatants tested for antibody activity to salivary and pancreatic duct cells.

Results

Antibodies to salivary duct epithelial cells were found in five of the 12 patients with SS and in 11 of the 31 patients with rheumatoid arthritis (Table 1). These antibodies stained with almost equal intensity parotid, submandibular, and lacrimal gland duct cells of human and of monkey origin. Cytoplasmic fluorescence of human parotid duct cells is shown in Fig. 1. Antibody activity ranged from undiluted serum to 1:60, although 10 of the sera showed only relatively low titres (< 1:8).

Four sera of the five patients with SS and eight of the 11 patients with rheumatoid arthritis positive for salivary duct cell antibodies produced also staining reaction with pancreatic duct cells (PDC). Specific fluorescence, again in a diffuse cytoplasmic staining pattern, was observed with interlobular (Fig. 2) as well as with intralobular (Fig. 3) duct cells. The staining intensity of serum was slightly lower with PDC antigens than with salivary duct cell (SDC) antigens. These antibodies were found to be complement fixing and to belong entirely to the IgG

 Table 1
 Frequency of salivary and pancreatic duct cell antibodies in patients and controls

Subjects studied (No.)	Antibodi duct cell	ies to salivary Is	Antibodies to pancreatic duct cells		
	(No.)	(%)	(No.)	(%)	
Sjögren's syndrome $(n = 12)$	5	41.7	4	33-3	
Rheumatoid arthritis $(n = 31)$	11	35-4	8	25.8	
Controls $(n = 64)$	1	1.6	0		

Table 2 Absorption experiments with salivary and pancreatic duct cell antibody positive serum MO and different tissue homogenates (absorption of antibody activity with human and monkey parotid and pancreas at 25 mg tissue homogenate/ml 1:10 serum dilution)

Tissue homogenates %	25 mg/ml		5 mg/ml		2.5 mg/ml		0·5 mg/ml	
	Parot. DC	Pancr. DC	Parot. DC	Pancr. DC	Parot. DC	Pancr. DC	Parot. DC	Pancr. DC
Human parotid Monkey parotid Human pancreas Monkey pancreas Rat liver Human thyroid microsomes PBS	- - - ++ ++	- - - ++ ++		+ - + - + - + - + + + + ++ ++	+ + + + ++ ++ ++ ++	++ ++ + - + - ++ ++ ++	++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++



Fig. 1 Salivary duct cell antibodies staining interlobular duct cells of human parotid gland.



Fig. 2 Serum of patient with SS showing cytoplasmic fluorescence of human pancreatic duct.



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Fig. 3 Cytoplasmic staining of intralobular human pancreatic ducts obtained with patients sera also positive for salivary duct cells.

class, immunofluorescence being negative with anti-IgA and anti-IgM conjugates. In the sera of two patients weak ribosomal antibodies and in one serum antibodies to exocrine pancreatic antigens could be found, although antibody activity to ductular cells was not observed in these sera. All sera negative with human and monkey salivary and lacrimal duct cells were also negative with pancreatic duct cells of the respective species. One serum of the control group showed weak reactivity with SDC, but did not produce staining with PDC.

The strongest serum (titre 1:60) containing antibodies to SDC and PDC was used for absorption experiments with different tissue homogenates (Table 2). Antibody activity to SDC and PDC was absorbed with human and monkey parotid homogenate in a concentration of 25 mg tissue/ml 1:10 serum dilution. Similar results were observed with human and monkey pancreas, although human pancreas absorbed antibody activity already at slightly lower concentrations. Incubations with rat liver and human thyroid microsome preparations did not influence antibody activity.

Discussion

The occurrence of antibodies to SDC was first described by Bertram and Halberg (1964). Subsequently MacSween et al. (1967) and Feltkamp and Rossum (1968) found these antibodies with varying frequencies (between 53% and 64%) in patients with SS and in 22% respectively 26% of rheumatoid arthritis patients. The former group of investigators was able to show the autoantibody nature of SDC antibodies, whereas organ specificity of these serum factors was proved by the latter group. In the present investigation a somewhat lower frequency (41%) of SDC antibodies could be found in patients with SS and a slightly increased frequency (33%)in rheumatoid arthritis patients. These results might be influenced by the long mean duration (7.3 years) and the relatively high mean age (65.4 years) of our SS patients. Histological studies (Waterhouse and Doniach, 1966) have shown that mononuclear cell infiltration of salivary gland is a common feature in rheumatoid arthritis. In these patients clinical symptoms or signs of SS are either missing or subclinical, which led Waterhouse and Doniach to assume that a 'Sjögren syndrome en miniature' occurs in a considerable number of rheumatoid arthritis patients. It cannot be excluded that these facts may play a role in our group of patients.

Almost an identical antibody reactivity was observed with human and rhesus monkey duct cells of parotid, submandibular, and lacrimal glands. Thus, it was shown that SDC antibodies do react with duct cells of these glands, although a considerable variation in mononuclear infiltration of the respective tissues could be observed in individual patients. As several reports (Ellman et al., 1951; Lambling and Dejours, 1951) point to an involvement of exocrine pancreas in SS and as pancreatic duct cells are morphologically closely related to duct cells of salivary glands, it seemed obvious to look for antibody activity to these antigens in SS. Antibodies to PDC were found in four of the 12 SS patients and in eight of the 31 rheumatoid arthritis patients, but in none of the 64 healthy controls. Similar staining of intra- and interlobular duct cells was observed. which could be clearly differentiated from the minimal non-specific reaction with interlobular ducts of some normal sera. A specific staining of the cytoplasm of acinar cells-presumably a marker of an inflammatory process in the exocrine pancreas (Lendrum and Walker, 1975) was noted only in one of the RA patients.

In absorption studies antibody activity to SDC and PDC could be absorbed equally well with human and monkey parotid gland or pancreas with almost identical antigen concentrations. These results show the common antigenic nature of duct cells of parotid and pancreatic tissue. Thus, antibody activity to PDC might be due to cross-antigenicity. Rat liver homogenate and suspensions of thyroid microsomes in equal concentrations did not show any effect on antibody activity to SDC and PDC.

The observed mononuclear infiltrations of the exocrine pancreas (Ellman et al., 1951), the reported pancreatic exocrine insufficiency (Lambling et al., 1951), the impaired response to secretin (Gordon and Shanbrom, 1958; Fenster et al., 1964; Bloch et al., 1965) and to pancreocymin (Hradský et al., 1967) as a feature of subclinical exocrine pancreatic insufficiency in SS might point to an almost regular involvement of this organ in SS. Humoral and cellular immunity seems to be an important factor in pathogenesis of salivary gland destruction. The demonstrated antibody activity to PDC and the reported lymphocytic infiltration indicates that similar mechanisms might have a part in pathogenesis of the commonly observed subclinical exocrine insufficiency in SS and in some cases of rheumatoid arthritis.

Systematic refined investigations of excretory pancreatic function and of cellular and humoral anti-exocrine pancreatic immunity seem to be essential in order to clarify the clinical importance of these findings,

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