

## Viral antibody studies in pancreatic disease

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**SUMMARY** Viral studies were performed on sera from 54 patients with recent acute pancreatitis, 10 with recurrent acute pancreatitis, seven with chronic pancreatitis, and 10 with pancreatic carcinoma, and on sera from 81 age- and sex-matched controls. In 29 of the acute pancreatitis patients from whom paired sera were obtained no convincing evidence of recent viral infection was found. A higher incidence of raised antibody titres against Coxsackie B<sub>3</sub> and B<sub>4</sub> was observed in the group of acute pancreatitis patients compared with their controls. The possible significance of these observations and their relationship to the aetiology of the pancreatitis and to other immunological findings are discussed.

Accepted factors predisposing to the development of acute pancreatitis include biliary tract disease, alcoholism, trauma or recent abdominal surgery, pancreatic neoplasia, metabolic disorders resulting in hypercalcaemia and hyperlipidaemia, mumps virus infection, corticosteroids and possibly other drugs, while rarely there may be a genetic component (Gambill, 1973). In approximately one-third of cases none of these factors can be incriminated (Trapnell and Duncan, 1975). However, acute pancreatitis may occur occasionally in patients with evidence of other viral infections—for example, congenital rubella (Bunnell and Monif, 1972) and infectious mononucleosis (Myhre and Nesbitt, 1949; Wislocki, 1966; Everett *et al.*, 1969). There may also be an association between infective hepatitis and pancreatitis, reflecting a possible common viral cause (Lisney, 1943; Joske, 1955; Achord, 1968; Gillespie, 1973; Ham and Fitzpatrick, 1973). Pancreatitis has been reported in Coxsackie B infection (Kibrick and Benirschke, 1958; Fechner *et al.*, 1963; Murphy and Simmul, 1964; Ursing, 1973), and in one epidemiological study serum amylase levels were elevated in 31% of patients during an outbreak of Coxsackie B<sub>5</sub> infection (Nakao *et al.*, 1964). Pancreatic damage can be produced in animals by several viruses, including infectious pancreatic necrosis virus in trout (Wood *et al.*, 1955), and Coxsackie B (Pappenheimer *et al.*, 1951; Minkowitz and Berkovich, 1970; Burch *et al.*, 1971; Harrison *et al.*, 1972; Tsui *et al.*, 1972; Ross *et al.*, 1974), encephalomyocarditis (Craighead, 1965; Burch *et al.*, 1974), foot and mouth disease (Platt,

1956), and mouse hepatitis (Hirano and Ruebner, 1968) viruses, in mice.

Evidence that virus infection is commonly responsible for human pancreatitis is lacking and in a recent serological study, significantly elevated antibody titres were not encountered (Leinikki *et al.*, 1973). However, significantly raised antibody titres to *Mycoplasma pneumoniae* occurred in one-third of patients and pancreatitis has been reported as a complication of *Mycoplasma pneumoniae* infection (Mårdh and Ursing, 1974).

We report here further virological studies on a group of patients with pancreatic disease, whose sera have also been tested for the presence of autoantibodies reacting with pancreatic acinar cells (Lendrum and Walker, 1975).

### Methods

#### SUBJECTS WITH PANCREATIC DISEASE

The sera examined in this study had been collected initially as part of another investigation (Lendrum and Walker, 1975) and stored at  $-20^{\circ}\text{C}$ . Sera were available from 54 patients whose clinical picture and serum amylase levels were compatible with acute pancreatitis (27 males, 27 females; ages 19 to 90 years). Paired sera were available from 29 of these patients. In these subjects, serum was taken within seven days of the onset of abdominal pain, and there was an interval of six or more days between acute and convalescent sera. In the remaining 25 patients, single sera only were examined. These had been obtained at varying intervals after the onset of illness (within one week—12 patients; within one month—five patients; between one and four

Associated Conditions	Total Numbers	Patients with paired serum samples available
Alcohol abuse	12	10
Biliary tract disease	13	4
Recent abdominal surgery	2	2
Carcinoma of pancreas	1	—
None	26*	13

Table 1 Associated clinical conditions in 54 patients with acute pancreatitis

\*Two of these patients were receiving oral contraceptive drugs, and two others were receiving frusemide.

months—eight patients). Associated clinical conditions in the pancreatitis patients are shown in Table 1. No causative factors for the pancreatitis were evident in 26 patients, although two of these were receiving oral contraceptive drugs and two others were being treated with frusemide. Both of these drugs have been suggested as possible rare causes of the disease (Bank and Marks, 1970; Davidoff *et al.*, 1973; Jones and Oelbaum, 1975).

Single serum samples were also examined from 10 patients with relapsing acute pancreatitis (one to five previous attacks), within five weeks of the most recent episode. Single sera from seven patients with chronic pancreatitis diagnosed radiologically or on biopsy, and from 10 patients with pancreatic carcinoma confirmed at laparotomy or at necropsy, were also studied.

#### CONTROLS

Sera from the patients with pancreatic disease were matched with stored sera which previously had been investigated for possible virus infections. None of these control sera was from patients with known pancreatic disease. The sera were matched for age and sex, and the date of their collection was matched to within one month to counteract any bias due to seasonal incidence of viral infections. Sera fulfilling these criteria were chosen without regard to previous virological findings. All sera, both from patients and from controls, were collected from patients living in West London.

#### VIROLOGICAL METHODS

Antibody testing by complement fixation (Bradstreet and Taylor, 1962) was performed using the following antigens: measles, mumps soluble and viral antigens, influenza A and B, parainfluenza 1 and 3, respiratory syncytial virus, adenovirus, varicella-zoster, cytomegalovirus, herpes simplex, *Rickettsia burneti*, psittacosis/LGV, and *Mycoplasma pneumoniae*. The sera were screened initially at a dilution of 1:20, and any which were positive were titrated in serial doubling dilutions. Rubella virus antibodies

were determined by the haemagglutination-inhibition test (Stewart *et al.*, 1967) using sera which had been pre-treated with heparin and manganese chloride (Feldman, 1968). Antibodies to Coxsackie B virus types 1-6 were determined by a conventional neutralization test in Vero cells, using 100 TCID<sub>50</sub> virus challenge. The serum/virus mixtures were incubated at 37°C for 90 minutes before inoculation, and results read after three days. All sera were tested for the presence of Hepatitis B antigen (HB<sub>s</sub>Ag) by the turkey cell haemagglutination test (Wellcome Reagents, Ltd), and for evidence of infectious mononucleosis by a slide absorption test (Mercia Diagnostics, Ltd).

#### Results

Among the 29 patients with acute pancreatitis in whom paired sera were tested, two showed four-fold rises in antibody titres against herpes simplex; one of these two also showed a four-fold rise against varicella zoster. No other significant increases in antibody titres were observed. One of the 54 patients with acute pancreatitis had a high antibody titre against the psittacosis/LGV group antigen (1:1280) on a single serum specimen; there was no clinical evidence of infection with these organisms. No other patient had antibody levels suggestive of recent infection with any of the agents tested.

Sera from the 54 patients with acute pancreatitis were compared with their controls. The convalescent serum from the 29 patients with paired sera was used for this comparison. There was a significantly greater incidence of antibodies to Coxsackie B<sub>3</sub> and B<sub>4</sub> viruses among the patients with acute pancreatitis (B<sub>3</sub>, 59%; B<sub>4</sub>, 59%) compared with

Virus	Acute pancreatitis		Relapsing acute pancreatitis	
	Patients (54)	Controls (54)	Patients (10)	Controls (10)
Adenovirus	8	20*	0	5
Mumps S	3	4	0	0
Mumps V	9	12	3	4
Rubella	44	38	8	5
Coxsackie				
B <sub>1</sub>	10	11	4	4
B <sub>2</sub>	24	24	4	3
B <sub>3</sub>	32†	13	7	2
B <sub>4</sub>	32†	18	5	4
B <sub>5</sub>	4	3	3	0
B <sub>6</sub>	1	0	0	0

Table 2 Numbers of patients with pancreatitis, and of controls, who showed antibody titres  $\geq 1:20$  against different viruses

\*Significantly greater number of controls,  $\chi^2 = 5.8$ ,  $P < .025$

†Significantly greater number of patients; B<sub>3</sub>  $\chi^2 = 12.3$ ,  $P < .005$   
B<sub>4</sub>  $\chi^2 = 6.3$ ,  $P < .025$

Associated conditions	Total numbers*	Antibodies to Coxsackie virus $\geq 1:20$			
		$B_3$ alone	$B_4$ alone	$B_3$ and $B_4$	Neither
None	26 (7)	4 (1)	7 (2)	9 (4)	6
Biliary disease	13 (3)	3 (1)	—	6 (2)	4
Alcohol abuse	12 (7)	3 (1)	4 (1)	4 (4)	1 (1)
Recent abdominal surgery	2 (0)	1	—	1	—
Carcinoma of pancreas	1 (1)	—	—	1 (1)	—
Totals	54 (18)	11 (3)	11 (3)	21 (11)	11 (1)

Table 3 Numbers of acute pancreatitis patients with Coxsackie  $B_3$  or  $B_4$  antibody titres  $\geq 1:20$ , related to associated clinical conditions

\*Figures in parentheses represent patients with autoantibodies to acinar cells, demonstrable by immunofluorescence.

controls ( $B_3$ , 24%;  $B_4$ , 33%) when the sera were screened at 1:20 dilution. There were no other differences in antibody levels between the two groups apart from a significantly higher incidence of antibodies to adenovirus in the control group (Table 2).

Seven of the 10 patients with relapsing acute pancreatitis had antibody titres  $\geq 1:20$  against Coxsackie  $B_3$ , compared with only two of their controls. No differences between patients and controls were found among the smaller numbers of chronic pancreatitis or carcinoma subjects.

The raised antibody titres against Coxsackie B viruses did not correlate in any way with the presence or absence of other non-viral factors associated with the pancreatitis (Table 3). Included among the 54 acute pancreatitis patients were 18 who had been found to have serum autoantibodies reacting in indirect immunofluorescent tests with the acinar cells of normal pancreas (Lendrum and Walker, 1975) from patients of blood group O. Seventeen of these had antibody titres  $\geq 1:20$  against Coxsackie  $B_3$  or  $B_4$ . Twenty-six of the 36 patients without detectable acinar-cell autoantibodies had Coxsackie  $B_3$  or  $B_4$  antibodies at this level. There was no statistically significant difference in incidence of Coxsackie  $B_3$  and  $B_4$  antibodies between those with and without acinar-cell autoantibodies ( $\chi^2 = 2.4$ ,  $P > 0.1$ ).

No rises in antibody titres against *Mycoplasma pneumoniae* were observed in the paired sera, and titres  $\geq 1:20$  against *Mycoplasma pneumoniae* were seen in only four of all those with acute pancreatitis, and in four controls. No evidence of recent mumps infection was found in acute pancreatitis patients or in any of those with other pancreatic diseases. Similarly, all patients and controls were HB<sub>s</sub>Ag negative, and were negative in the infectious mononucleosis screening test.

## Discussion

No evidence was found in the present study that

pancreatic disease was caused by acute viral infection, although two acute pancreatitis patients in whom paired sera were studied showed small increases in antibody titres against herpes simplex, and one of these also had a rise in titre against the varicella-zoster antigen. In both patients, however, the acute pancreatitis had followed immediately upon an episode of excessive alcohol intake. These minor changes in titres are felt to be aetiologically insignificant. Even in those acute pancreatitis patients where known predisposing factors—for example, alcohol abuse, biliary disease—were absent, no four-fold rises in antibody titre were found. Of the patients where only single serum samples were tested, one had a high titre of antibody to the psittacosis/LGV antigen, the significance of which was not clear. No other patient with acute or chronic pancreatic disease had viral antibody titres suggestive of recent infection.

The finding of a greater incidence of antibody titres  $\geq 1:20$  against Coxsackie  $B_3$  and  $B_4$  viruses among patients with acute pancreatitis, irrespective of cause, compared with controls, requires explanation. The difference is unlikely to have arisen because of the particular sera used as controls, since these had been obtained from patients with suspected viral disease and might have been expected therefore to have shown increased viral antibody titres. This may explain the increased incidence of adenovirus antibodies in the control group. The control and pancreatitis sera were matched within one month for time of collection, and came from patients living in the same area of London, so that seasonal and geographical factors are unlikely to be of importance. The raised titres in the pancreatitis patients may have resulted from a selective anamnestic response brought about by the pancreatitis itself, irrespective of its aetiology. Alternatively, persistent low-grade infection with Coxsackie B viruses may perhaps be present in this group of patients. Again, it is possible that pancreatic antigens released by the inflammatory process share common determinants with

Coxsackie antigens, thus giving rise to cross-reacting antibodies. Although no significant correlation was found between autoantibodies against acinar cells in fluorescent tests and raised Coxsackie antibody titres, it is quite possible that other cross-reacting antibodies are present.

These findings are similar to those in a study of insulin-dependent diabetics who showed a greater incidence of neutralizing antibody titres  $\geq 1:20$  against Coxsackie B<sub>4</sub> compared with matched controls (Gamble *et al.*, 1969). One possible common explanation for these similar observations in diabetes and in pancreatitis might be a histocompatibility-linked immune response as is known to occur in animals (Benacerraf and McDevitt, 1972). Further studies of the inter-relationships between viral antibodies, autoantibodies and HL-A antigens would be of interest.

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