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PATHOGENICITY OF NEWLY ISOLATED COXSACKIEVIRUS B4 FOR MOUSE PANCREAS

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Summary. — Coxsackievirus B4 fresh isolates from patients with upper respiratory illness and aseptic meningitis were studied for their pathogenicity in the pancreas of SJL/J mice. Out of 12 virus isolates, 7 induced hypoglycaemia in mice 2 to 4 days after virus inoculation. All 3 isolates from faeces of patients induced hypoglycaemia in contrast to 3 viruses isolated from the cerebrospinal fluid which did not. Six isolates from throat swabs were either pathogenic (4 isolates) or non-pathogenic (2 isolates). It is concluded that at least two biologically distinct coxsackieviruses B4 prevail among humans.

Key words: coxsackievirus; pathogenicity; mouse pancreas; hypoglycaemia

Introduction

It has been well documented that pancreatic disease in humans, pancreatitis and juvenile-onset type diabetes, is caused by viral infections. However, evidence of viral aetiology of these diseases was proven mainly indirectly in animal experiments. Among various viruses, coxsackievirus B has been extensively studied (Gamble and Taylor, 1969; Gamble *et al.*, 1973; Hierholzer and Farris, 1974; Nelson *et al.*, 1975; Schmidt *et al.*, 1978; Ray *et al.*, 1980; Barnett *et al.*, 1981; Craighead, 1981; Palmer *et al.*, 1982). Coxsackievirus B was reported for the aetiological agent of pancreatitis by Dalldorf *et al.* (1952) and by Pappenheimer *et al.* (1951). Yoon *et al.* (1978) proved coxsackievirus B4 to cause the diabetic ketoacidosis in a 10-year-old child. When investigating the human disease of viral aetiology, it is necessary to consider both viral pathogenicity and host factors which determine susceptibility to the virus. In this report, we present the evidence that, in terms of pathogenicity for mouse pancreas, two groups of coxsackie virus B4 strains are freshly isolated from specimens of human origin can be distinguished.

Materials and Methods

Mouse. Colony-bred SJL/J 5 to 6 week-old male mice originally imported from Jackson Laboratories (Bar Harbor, Maine) were used throughout the experiment. The mice were fed with NMF mouse food (Oriental Yeast Co., LTD., Tokyo).

Virus. Coxsackieviruses type B4 were isolated in Shimane Prefecture from human patients with the clinical diagnoses listed in Table 1. The virus isolations were performed on African green monkey kidney cells (AG-1). Viruses used for animal experiments were in the 3rd in vitro

Table 1. The virus isolates used

Virus isolate	Patients from whom viruses were isolated		Clinical diagnosis
	age	sex	
370	4 years	female	herpangina
509	6	male	herpangina
637	3	female	AM
688	8	male	herpangina
877	7	female	acute URI
974	2	male	AM
976	2	male	AM
982	1	male	AM
1180	5	female	AM
1211	5	female	AM
3106	2	male	acute URI
3107	2	male	acute URI

AM: aseptic meningitis. URI: upper respiratory illness. 974 and 976 were isolated from the same patient.

passage; they were diluted in sterile 0.01 mol/l phosphate buffered saline (PBS) pH 7.2 so that 10^5 plaque forming units (PFU) in 1.0 ml volumes were injected intraperitoneally unless otherwise stated.

Determination of the blood glucose level. Glucose levels in the fed animals were determined by the glucose oxidase method employing New Glucostat (Fujisawa Medical Supply, Osaka) before and 2, 4, 6, 10, 14, 16 and 20 days after injection of the virus. Blood specimens from the tail vein were taken at a fixed time in the morning.

Morphological examinations of the pancreas. Pancreases were fixed by 10% formalin and stained with haematoxylin-eosin (HE) or aldehyde-fuchsin (AF) for microscopical examinations. Ultrathin sections of pancreases for electron microscopy were prepared by common techniques.

Results and Discussion

The blood glucose levels were followed for 20 days after inoculation of the viruses into mice. The variation of the levels up to 6 days are summarized in Figs 1-I and 1-II. As control, 1.0 ml of the diluent was injected to each mouse. Newly isolated coksackievirus B4 strains could be divided into two groups according to induction of hypoglycaemia 2 to 4 days after virus injection. As follows from Figures, strains 370, 509, 974, 982 and 1180 were "non-pathogenic", i.e. unable to induce hypoglycaemia, while 637, 688, 877, 976, 1211, 3106 and 3107 were "pathogenic", i.e. inducing significant decrease in the blood glucose level on days 2 and 4 post-infection. The source of virus isolation and the passage levels of the viruses in vitro are listed in Table 2 in relation to their pathogenicity for mouse pancreas as judged by the ability to induce hypoglycaemia in mice. Although the total number of the virus isolates was small, all 3 strains isolated from the cerebrospinal fluid were not pathogenic in contrast to the 3 strains isolated from faeces, which were pathogenic. Though strains 974 and 976 were isolated from the same patient on the same day after onset of aseptic meningitis, they differed in their pathogenicity for mice. Viruses isolated from throat swabs were classified into both "pathogenic" and "non-pathogenic" groups. The extent of hypoglycaemia

was quite concordant with pathological changes in pancreases. The main pathological changes were degeneration of acinar cells and, secondary, coagulation necrosis of islets and loss of the granules of β - cells. At 20 days after infection, compensatory hypertrophy of islets was found. At the acute stage, 2 to 4 days after infection, virus particles were observed mainly in acinar cells.

These findings suggest the difference in pathogenicity of coxsackievirus B4 isolated freshly from humans. Strains 974 and 976 were isolated from one patient on the same day, from cerebrospinal fluid and faeces, respectively. As observed, strain 976 commonly induced hypoglycaemia, while strain 974 did not cause any changes with mouse pancreas. It is suggested that coxsackievirus B4 which is prevalent among humans is composed of at least two populations with different pathogenicity. This is supported also by the fact that clones of strain 3106, which was originally isolated from the throat swab of

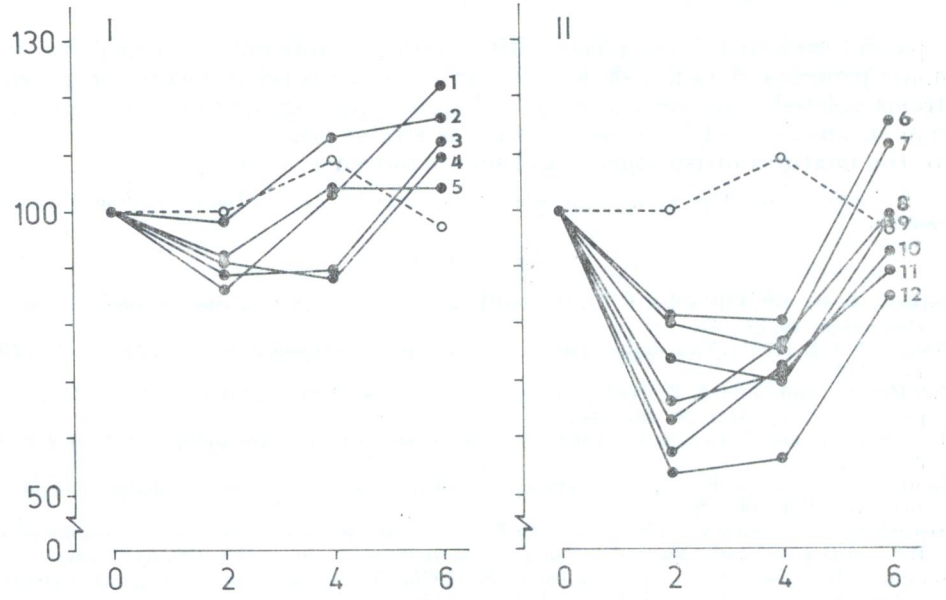

Fig. 1.

Variation of the blood glucose levels in mice after intraperitoneal infection with Coxsackievirus B4

Coxsackievirus B4 was injected intraperitoneally to each mouse (5×10^5 PFU/ml/mouse). On days 2, 4 and 6 post-infection, blood glucose levels were estimated as indicated in Materials and Methods. Figure 1-I shows the variation profiles of blood glucose levels of mice injected with "non-pathogenic" viruses. Virus isolate shown in Fig. 1-II induced significant decrease of the blood glucose level on days 2 and 4. Numbers in the Figure denote virus isolates (1 — isolate No. 370, 2 — 1180, 3— 509, 4 — 974, 5 — 982, 6 — 3107, 7 — 1211, 8 — 688, 9 — 877, 10 — 637, 11 — 3106, 12 — 976). As controls (o), mock infected mice were used.

Abscissa: days post-infection; ordinate: relative blood glucose (in per cent; 100% corresponds to 0.01 mol/l glucose level).

Table 2. Pathogenicity of Coxsackievirus B4

Virus isolate	Specimen used for isolation of the virus	In vitro passage level	Induction of hypoglycaemia
370	TS	2	—
509	TS	2	—
637	F	2	+
688	TS	2	+
877	TS	3	+
974	CSF	2	—
976	F	3	+
982	CSF	2	—
1180	CSF	3	—
1211	TS	2	+
3106	TS	2	+
3107	F	2	+

TS: throat swab, F: faeces, CSF: cerebrospinal fluid.

a patient suffering from pharyngitis, exhibited different pathogenicity for mouse pancreas (data not shown). As far as the virus isolates examined, virus strains isolated from cerebrospinal fluid are less pathogenic to mouse pancreas than strains isolated from faeces, whereas strains isolated from throat swabs are the mixtures of pathogenic and non-pathogenic viruses.

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