COXSACKIEVIRUS INFECTION OF MICE. II. VIRAL KINETICS AND HISTOPATHOLOGICAL CHANGES IN MICE EXPERIMENTALLY INFECTED WITH COXSACKIEVIRUS B3 BY INTRAPERITONEAL ROUTE

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Summary. – The study was focused on kinetics of Coxsackievirus B3 serotype (CVB3) in different organs of Swiss albino mice following intraperitoneal (i.p.) infection. The results indicated that the virus replicated in the heart, spleen, thymus, pancreas, small and large intestines in the acute stage of the infection. Infectious virus was present in the spleen till day 35 post infection (p.i.). Histopathology of the hearts showed mild foci of infiltration of mononuclear cells in the acute stage of infection and massive inflammation of exocrine pancreas on day 5 p.i. These results, when compared to those of our previous study (Bopegamage *et al*., 2003), suggest that the pathogenesis of the disease may be influenced by the route of virus administration into the host.

Key words: Coxsackievirus; B3 serotype; Swiss albino mice; intraperitoneal infection; histopathology

Introduction

Coxsackievirus (CVB) are members of the *Picornaviridae* family, the *Enterovirus* genus (van Regenmortel *et al*., 2000). They comprise of 6 serotypes (B1 to B6). Different mechanisms of the pathogenesis of CVB infection have been suggested: direct lysis of beta cells of the pancreas (Szopa *et al*., 1990) or the myocytes (Chow *et al*., 1992) and molecular mimicry, which result in insulin-dependent diabetes mellitus (reviewed by Atkinson and Maclaren, 1994; Vreugdenhil *et al*., 1998) or myocarditis (Neumann *et al*., 1994; reviewed by Gauntt *et al*., 1995). Involvement of the T-cell response (reviewed by Oldstone, 1998; Huber, 2001) and persistence of the virus genome have also been regarded as mechanisms responsible for the pathogenesis of CVB infection; however, different views exist on the viral persistence (reviewed by Melchers *et al*., 1994; Muir and Archard, 1994). Most of the studies on the mechanisms of the pathogenesis have used the CVB3-mouse model. In this model age of the mice, the mouse strain and the CVB3 strain employed define the disease (reviewed by Gauntt *et al*., 1993). Differences in the ability of a CVB strain to induce myocarditis depend also on the mouse strain (Wolfgram *et al*., 1986; reviewed by Gauntt *et al*., 1993). Individual nucleotide substitutions in non-coding and coding regions of the viral genome determine the virulence (reviewed by Chapman *et al*., 1990). The results of our previous study in which peroral infection of mice was employed revealed a prolonged presence of infectious virus in the spleen and the small intestine. The histopathology showed mild necrosis in the heart and absence of inflammatory changes in the pancreas.

To analyze whether these observations were related to the particular virus strain or route of infection, we undertook the present study on the same model – CVB3 Nancy strain and Swiss albino mouse – but using a different, i.p. route of infection.

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Abbreviations: CVB = Coxsackievirus B; CVB3 = CVB3 serotype; CPE = cytopathic effect; i.p. = intraperitoneal; p.i = post infection; PBS = phosphate-buffered saline

Table 1. Antibody titers in sera of mice infected i.p. with CVB3

Day p.i.	Antibody titer
0	<4
3	<4
5	8
7	256
10	128
14	256
21	128
28	64
35	64
49	128
63	32
98	32

Materials and Methods

Virus. CVB3 Nancy strain not adapted to mouse organs investigated in this study was employed.

Mice. Swiss albino (ICR) outbred mice were employed.

Infection of mice. Mice were infected i.p. with 2 x 10^3 $TCID_{50}$ of the virus in 0.2 ml. Mock-infected control mice were given 0.2 ml phosphate-buffered saline (PBS). The mice were sacrificed daily from day 0 to day 10 p.i. and then at weekly intervals from day 14 to day 63 p.i. and at day 98 p.i. The blood was taken by cardiac puncture aseptically, and portions of the heart, pancreas, thymus, spleen, and small and large intestines were washed in PBS and either snap-frozen in liquid nitrogen and stored at -80°C or fixed in 10% formaldehyde for histopathology.

Cells, virus isolation, titration of infectious virus in organs, titration of virus neutralizing antibodies, and histolopathology were described previously (Bopegamage *et al*., 2003).

Statistical analysis. Group size (n) of 3 and a 95% confidence interval were used.

Results and Discussion

Antibody response to infection

As shown on Table 1, a neutralizing antibody titer (8) in the serum of infected mice was first detectable on day 5 p.i., reached a maximum (256) on day 7 p.i., and could be detected throughout the study period. Mock-infected mice showed no antibody response.

Kinetics of CVB3 load in various mouse organs following infection

The results of this experiment are shown in Table 2. In the heart, the virus was detectable from day 3 to day 14 p.i., with a maximum on day 7 p.i. Virus titrations were done also on days 21, 28, 35, 49, 56, 63 and 98, but, their results were negative.

In the pancreas, the virus was first detectable on day 3 p.i. and then it rose steeply to a maximum on day 5 p.i. The subsequent decrease in the titer was again steep; the virus was undetectable starting with day 14 p.i.

In the thymus, the kinetics of the virus titer was similar to that in the pancreas; the virus titer was detected between days 3 and 7 p.i. with a maximum on day 5 p.i.

In the spleen, the first appearance of the virus and the time when the virus titer reached maximum were similar to those in the pancreas and thymus (days 3 and 5 p.i.,

Table 2. Viral load (log_{10} $TCID_{50}$/ml) in organs of mice infected i.p. with CVB3

Organ	Day p.i.								
	0	3	5	7	10	14	21	28	35
Heart	–	+	1.68 (0.5696)	2.6833 (0.5696)	1.2333 (0.7275)	1.7833 (1.3583)	–	–	–
Pancreas	–	1.8667 (0.8661)	4.0167 (0.2613)	3.2 (0.5880)	+++	–	–	–	–
Thymus	–	1.6333 (0.8698)	2.555 (0.3970)	1.7333 (1.7071)	–	–	–	–	–
Spleen	–	2.3417 (0.4004)	3.0167 (0.2613)	2.55 (0.3920)	2.3167 (0.3920)	2.3167 (0.5397)	+++	+	+
S. intestine	–	2.8167 (0.1819)	5.1667 (0.3267)	4.625 (0.4321)	3.1667 (0.5889)	–	–	–	–
L. intestine	–	+	1.0833 (0.49)	–	–	–	–	–	–

(–) = undetectable virus titer.

(+) = undetectable virus titer but positive virus isolation in the second passage.

(+), (++), and (+++) = viral titer detectable in pool No. 1, No. 2 and No. 3, respectively. Each pool consisted of parts of three organs.

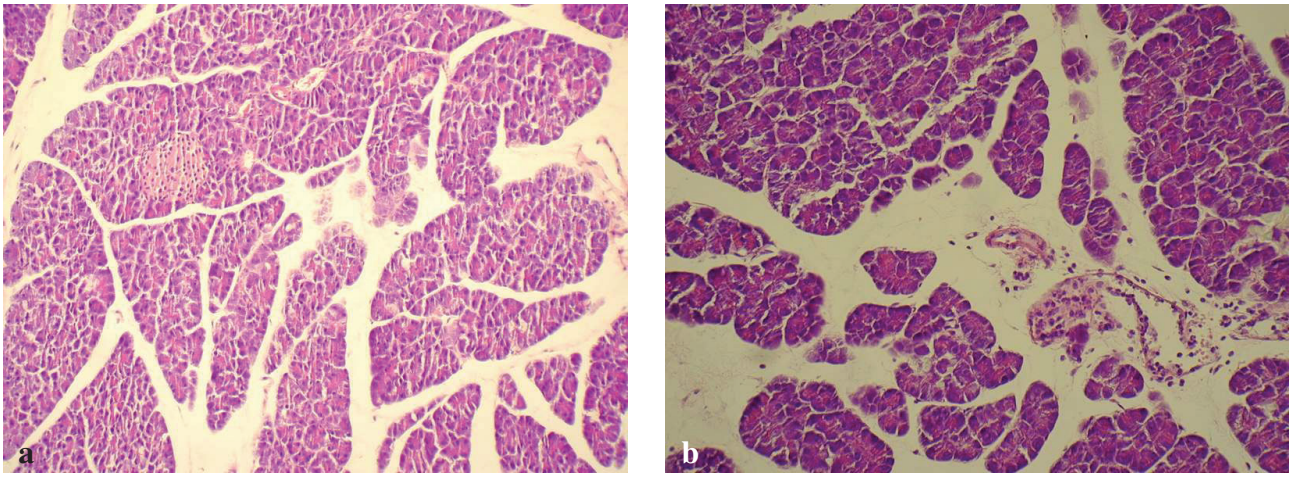

Fig. 1
Sections of pancreas stained by hematoxylin-eosin
Mock-infected mouse (a); CVB3-infected mouse, day 5 p.i. (b). Magnification 200x.

respectively), but the titers declined more slowly, namely till day 35 p.i.

In the small intestine, the virus kinetics concerning the start and maximum resembled again the patterns of the preceding three organs. Regarding the period of decrease, the small intestine resembled that of the heart.

On the other hand, the large intestine showed a unique behavior. The virus was detectable on days 3 to 5 p.i. only.

In spite of different virus load kinetics in different organs, there are the following common features. (i) The virus appears first on day 3 p. i. in the organ regardless its type. (ii) The virus reaches its maximum titer on day 5 p.i. regardless its type. In contrast the period of decline lasts different length of time as measured from the day of maximum titer; it is none (the l. intestine), 5 days (the thymus), 9 days (the pancreas, s. intestine), and 44 days (the spleen).

Histopathological observations

The hearts of i.p. infected mice revealed on day 10 p.i. small foci of beginning necrosis of myocardial cells along with a moderate increase in the number of mononuclear cells similar to that in the perorally infected mice as described previously (Bopegamage *et al*., 2003). No pathological changes were observed in the hearts at day 98 p.i. Cellular infiltration and necrosis were absent in the heart of control mice.

The infected mice showed beginning of inflammation of the exocrine pancreas on day 3 p.i. in two mice. This inflammation increased on day 5 p.i., one mouse showing massive inflammation of the exocrine pancreas with dystrophic changes and infiltration of mononuclear cells (Fig. 1b). These changes were totally absent in control mice (Fig. 1a). On day 7 p.i. the inflammation decreased in all mice though one mouse showed more extensive inflammation than the other two. The inflammation subsequently subsided by day 21 p.i. in all mice. In the small intestines a few enlarged Peyer's patches were observed, but changes in the villi were not found.

The mouse is of prime choice for modelling human diseases. Over 450 inbred strains of mice have been described, providing a wealth of different genotypes and phenotypes for genetic and other studies (reviewed by Beck *et al*., 2000). Availability of the inbred and knockout transgenic strains of mice which are genetically defined, has lead to common use of these mice in studying particular host and genetic factors, though the outbred model imitates the natural variations in the human populations.

The dose of virus used for i.p. infection depends on the virus strain, and also on the strain and age of the mouse as host. Among the inbred mouse-CVB3 models, immunocompetent BALB/c mice have been extensively studied, and these mice are the genetically nearest to Swiss albino mice (reviewed by Gauntt *et al*., 1993). The dose commonly used for i.p. infection of these mice ranges from 1 x 10^3 $TCID_{50}$ to 1 x 10^8 $TCID_{50}$ depending on the age of the mice. The present study was undertaken to create some analogy with our previous study of oral infection. Kaplan and Melnick (1951) and Loria *et al*. (1974) have suggested that a reduction by 4 to 5 log units in virus load occurs on meeting the gut associated lymphoid tissue during peroral infection. Considering this fact, a dose of 2 x 10^3 $TCID_{50}$ of CV B3 (Nancy strain) in 0.2 ml was employed in the i.p. challenge.

The presence of the virus within lymphoid organs probably represents association of viruses with the immune system (Notkins *et al*., 1970), suggesting that the nature of the interaction of viruses with immune organs and immune cells *in vivo* determines the outcome of such events. To evaluate the association of CVB3 with splenocytes the temporal load of infectious virus in the spleen of A/J and C57/BL/6J mice during the early stage of CVB3 infection and *in vitro* replication in the splenocytes from uninfected mice has been studied by Anderson *et al*. (1996). CVB replicates to high titers in the mouse pancreas. Localization studies identified virus in acinar cells but not in islets by Mena *et al*. (2000), who have shown a contribution of the perforin-mediated lysis to CVB3-induced pancreatic disease. Our study showed histological changes in the pancreas of i.p. infected mice (day 5 p.i.), namely a massive inflammation of the exocrine pancreas with dystrophic changes and infiltration with mononuclear cells. An extensive inflammatory cell infiltration in acinar tissue of the pancreas in DBA/2 mice infected i.p. with 1.8 x 10^5 PFU was found by Blay *et al*. (1989) on day 7 p.i. Tracy *et al*. (2000) have shown replication of different serotypes of CVB3 in the pancreas until day 8 p.i. They have also observed that cardiovirulent virus strains tend to replicate to higher titers and persist longer in the serum, pancreatic and cardiac tissues than the non-cardiovirulent strains and the virus replication in pancreatic tissue is not an indicator of a pancreato- or cardio-virulent viral phenotype.

Even within one serotype, different strains may show difference in virulence. The i.p. route of infection resulted in a prolonged presence (up to day 35 p.i.) of the virus in the spleen. This may be a peculiarity of this virus and mouse strain model. A prolonged infection was not observed in the small intestine or other organs of the i.p infected mice. The pathology of the pancreas differed from that of the perorally infected mice as demonstrated in our previous study.

From this study the following question arises: would the mortality, viral kinetics and histopathological features such as the increased involvement of the heart muscle differ if higher doses (such as that used for peroral infection) are employed for i.p. infection of CVB3 Nancy strain and the same mouse model? Such a study is underway.

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