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Characterization of a murine model of myocarditis induced by a reactivated Coxsackievirus B3

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Summary. A transfection-reactivated Coxsackievirus B3 (rCVB3), from a full-length cDNA clone of Nancy strain, has previously been shown to be as cardiovirulent as the wild-type virus. Myocarditis induced by this genetically defined virus was compared in SWR mice with the traditional Balb/c model. SWR mice inoculated with rCVB3 developed more severe myocarditis but less severe pancreatitis than Balb/c mice. In contrast to the poor general health and frequent mortality of Balb/c mice following CVB3 infection, the body weight of SWR mice was not affected by CVB3 inoculation and no mortality occurred at titres of 10^2–10^7 plaque forming units (PFU). Typical myocarditis developed in SWR mice 7 days post infection. Myocarditic foci consisting of necrotic myocardial fibres and mononuclear cell infiltrates resolved by day 30, similar to that observed in Balb/c. However, SWR mice were more sensitive to rCVB3-induced myocarditis than were Balb/c mice: mild myocarditis was induced (4/4) by as low as 10^2 PFU of the virus (ID_{50} $< 10^{1.5}$ PFU), and more severe myocarditis was seen at higher PFU of virus in a dose-dependent manner.

The SWR model was tested with attenuated variants derived from cardiovirulent rCVB3. The ID_{50} for myocarditis was 10^7 PFU for a large plaque-size attenuant and 10^6 PFU for a minute plaque-size attenuant, indicating loss of cardiovirulence by a factor of more than 10^4–10^5. rCVB3-induced SWR mouse is a sensitive and reliable model for myocarditis. It is useful in assessing the cardiovirulence of different CVB3 variants and evaluating the efficacies of anti-viral therapies. It will allow follow-up study after high dose infection with cardiovirulent rCVB3.

Keywords: mouse model, viral myocarditis, pancreatitis, cardiovirulence, variants

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Coxsackieviruses belong to the enterovirus group of the picornavirus family. They are the aetiological agents of various human diseases including inflammatory muscle disease (Melnick 1990). Because of the high prevalence of Coxsackievirus B1-5 (CVB) in viral myocarditis, most

studies of viral heart disease have been focused on CVB. Experimental animal models have been developed to study the natural history of viral myocarditis with respect to genetic, immunological and other host factors and virus pathogenicity (Rabin *et al.* 1964; Woodruff & Kilbourne 1970; Woodruff & Woodruff 1974; Hoshino *et al.* 1983; Gauntt *et al.* 1984; Huber & Lodge 1986; Reyes *et al.* 1988; Sherry *et al.* 1989; Lawson *et al.* 1990; Matsumori 1992). There are murine models of CVB1-4-induced myocarditis (Woodruff & Kilbourne 1970; Cao *et al.* 1984; Reyes *et al.* 1988), of which CVB3 is the most widely used model.

Woodruff and Kilbourne (1970) developed a CVB3 myocarditis model using inbred Balb/c mice (H-2^d) and a heart-adapted strain (Nancy) of CVB3. It became the most popular model and many advances in the understanding of viral myocarditis have been generated from research carried out on this model. As in man, the disease in the Balb/c model is most severe in males, in neonatal or young adult animals, and in animals undergoing physiological stress due to forced exercise, or malnutrition (Woodruff & Kilbourne 1970; Lerner & Wilson 1973; Cao *et al.* 1984; Lyden *et al.* 1987a, b). The natural history of myocarditis in this model has been elucidated. The virus appears rapidly in the hearts of mice infected intraperitoneally with 10^4–10^5 PFU of virus. Peak virus titres occur 3 days after infection. Virus elimination from the heart proceeds rapidly, and by the 14th day, no virus can be detected. Necrotic changes of myocardium can be seen from day 4–5 until 2 weeks, usually accompanied by an infiltrate, comprising mainly mononuclear and lymphoid cells. The myocarditis is self-limiting and complete recovery is achieved in 3–4 weeks (Woodruff 1980).

The susceptibility of experimental mice to a particular CVB3 strain is also determined by genetic background. Six strains of inbred mice were compared for their susceptibility to Nancy strain CVB3-induced myocarditis. The A.BY/SnJ [H-2^b), A.SW/SnJ (H-2^S), A.CA/SnJ (H-2^f), B10.S/SgSf (H-2^S), B10.PL/SgSf (H-2^U) and C3H.NB/SnJ (H-2^p) strains were found to vary widely in the extent and duration of viraemia, in the time course of appearance and titre of neutralizing antibody, and in the prevalence, severity, and duration of myocardial disease (Wolfgram *et al.* 1986). In another study, Gauntt *et al.* (1984) tested one strain of semi-inbred mouse and 14 strains of inbred mice for their susceptibility to three CVB3 variants. When the less cardiovirulent variant ts1R was inoculated, little to no myocarditis was induced in any of the nine mouse strains examined carrying H-2 haplotypes b, k or d. Distinct differences in ability of cardiovirulent variants CVB3m or ts10R to induce myocarditis were found in different strains of mice. CVB3m was significantly more cardiovirulent than ts10R in 129/J (H-2^b) mice, whereas the ts10R variant was more cardiovirulent than was CVB3m in C57BL/6 (H-2^k) mice. SWR inbred mice (H-2^q) were included in the above study. Viral myocarditis was more severe in this strain than in Balb/c. Nevertheless, no characterization of SWR mice as a model of viral myocarditis has been done.

Besides the fact that Balb/c mice do not develop myocarditis as severely as some other strains, there is another disadvantage in using this model for the study of myocarditis. All the mice inoculated with CVB3 develop fulminant pancreatitis (Vuorinen *et al.* 1989; Gomez *et al.* 1991 and our own observation) and a large proportion of mice die of acute infection (Fohlman *et al.* 1990; Gomez *et al.* 1991; Zhang *et al.* 1993). This phenomenon prevents investigators from following up the development and progression of viral heart disease. Molecular biological techniques have shown that a proportion of cases of human dilated cardiomyopathy is associated with the persistence of enteroviral RNA in myocardium and may be a progression from a previous enteroviral myocarditis (Bowles *et al.* 1986; 1989; Kandolf 1988; Archard & Richardson 1990; Tracy *et al.* 1990; Zoll *et al.* 1992; Koide *et al.* 1992), although other studies failed to correlate enteroviruses with dilated cardiomyopathy (Petitjean *et al.* 1992; Liljeqvist *et al.* 1993). The molecular mechanism of virus persistence and its role in progression from acute myocarditis to chronic dilated cardiomyopathy can be investigated in animal models (Kyu *et al.* 1992; Wee *et al.* 1992; Klingel *et al.* 1992). Ideally, experimental animals survive the initial infection with maximal myocarditis and are then followed up for a long period.

We had derived attenuated variants from a cardiovirulent CVB3 reactivated from a cDNA clone (rCVB3; Zhang *et al.* 1993) and were seeking more sensitive murine models for assessment of cardiovirulence of CVB3 variants and the efficacy of anti-viral therapy as well as a candidate model for studies on progression to dilated cardiomyopathy. In this paper, we describe severe myocarditis in SWR mice induced by rCVB3, and compared to Balb/c mice.

Materials and methods

Virus

Cardiovirulent CVB3 was reactivated from a sequenced cDNA clone (Kandolf & Hofscheider 1985; Klump *et al.* 1990) by transfection of Vero cells (African Green Mon-

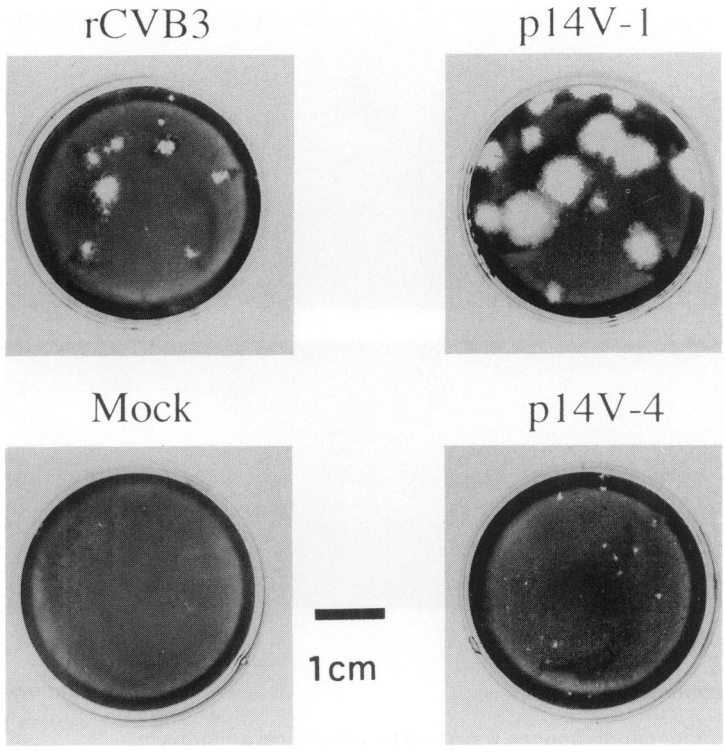


Figure 1. Plaque size phenotype of attenuated variants compared to rCVB3. Vero cell monolayers were inoculated with prototypic rCVB3, p14V-1, p14V-4 or mock-infected. The agar-overlayed cultures were fixed with 10% formalin/PBS and stained with crystal violet 4 days later. p14V-1 and p14V-4 produced larger and minute plaques respectively compared to prototypic virus.

key kidney cell line). This genetically defined virus is as virulent as wild-type CVB3 (Zhang *et al.* 1993). A single virus stock of infectivity titre 5×10^7 plaque forming unit (PFU)/ml was prepared in Vero cells and used throughout this study. Attenuated CVB3 variants were derived from rCVB3 by repeated passage in human dermatofibroblasts (Zhang *et al.* 1993). Both large (p14V-1) and minute (p14V-4) plaque size variants were used in this study (Figure 1).

Maintenance and infection of mice

SWR (H-2^q) and Balb/c (H-2^d) mice were purchased from Harlan OLAC Ltd, UK; some Balb/c mice were purchased from Charles Rivers, UK. Five-week-old male mice were used in this study. Virus-infected mice were housed in a negative-pressure isolator. The condition of animals was checked daily or weekly as appropriate.

The experimental mice were allowed to acclimatize for 1–2 days after arrival. They were distributed randomly into groups for each experiment. Virus stocks were diluted in sterile phosphate-buffered saline (PBS) or cell culture medium. Mice in test groups were injected intraperitoneally with 0.5 ml of virus suspension, whilst mice in control groups received 0·5 ml of diluent as appropriate.

Sample collection and histology

Mice were sacrificed by cervical dislocation at various intervals. The heart and other relevant organs were removed immediately. One-third of the heart was fixed in 10% PBS-buffered formalin and the remainder was snap-frozen in liquid nitrogen. Frozen tissues were stored at −70°C until required.

Generally, four serial formalin-fixed, paraffin-embedded sections (4 μm) were cut followed by four more after 40 μm deeper into the tissue. Routinely, sections were stained with haematoxylin and eosin (H & E) and, when appropriate, calcium staining was carried out using the Vonkossa method. Normally two sections and two deeper sections from each mouse were examined by two examiners. Histopathological lesions were counted microscopically (Gauntt *et al.* 1984). Briefly, the number of myocarditic foci in each section was counted at × 100 magnification. Four sections per heart were examined and the mean lesion count was calculated.

Isolation and titration of virus

A portion of myocardium from representative mice was homogenized in PBS and sterilized by filtration (0.22 μm). The homogenate was titrated on Vero cell monolayers by plaque assay and virus isolated by picking single plaque (Crowell & Landau 1979).

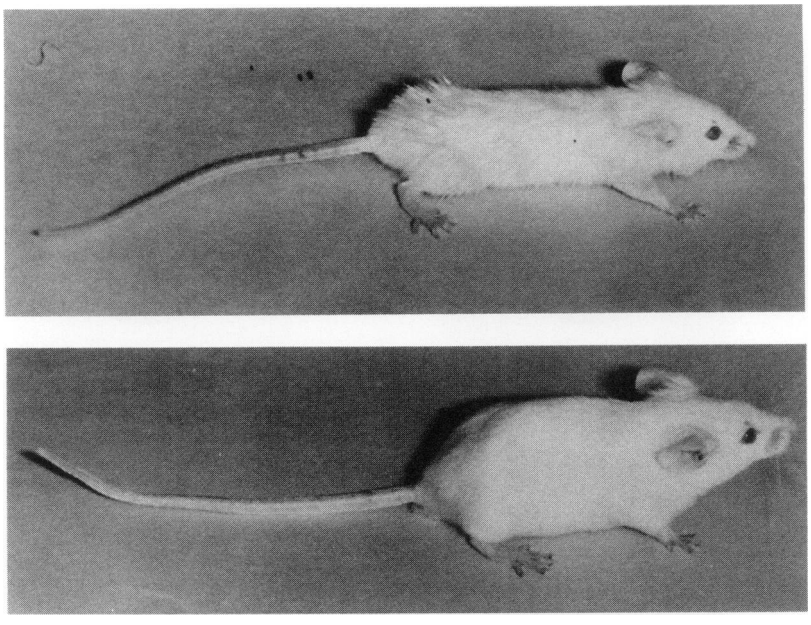


Figure 2. Photographs of infected SWR and Balb/c mice. Experimental mice were inoculated with 10^5 PFU of rCVB3 intraperitoneally. Pictures were taken 6 days p.i. (Top) Balb/c mouse; (Bottom) SWR mouse.

Immunofluorescence

Immunofluorescence was used to detect viral antigens in infected heart. Briefly, 8-μm frozen sections were incubated with monoclonal antibody (MAb) 5-D8/1, specific for VP1 of enterovirus (Yousef *et al.* 1987), in a moist chamber at 37°C for 1 hour. After three washes in PBS, fluorescein isothiocyanate(FITC)-labelled rabbit anti-mouse immunoglobulin (Dako Limited, UK) was added at a dilution of 1:50. The slides were incubated at 37°C for 45 minutes, washed in PBS and counterstained with 0.01% Evans' blue in PBS, and examined by fluorescence microscopy (Zeiss, Germany).

Results

rCVB3-induced myocarditis in SWR mice

SWR mice are similar to Balb/c mice in appearance, colour and size, but more active. In a preliminary experiment, four SWR and four Balb/c mice were inoculated with 10^5 PFU of rCVB3 and two control mice received diluent only. The general condition of the infected SWR mice remained good showing only minimal clinical illness. All gained body weight until sacrifice at day 6 compared with control mice. As with past experience, infected Balb/c mice showed poor general condition and lost body weight dramatically (Figures 2 and 3). Severe myocarditis was observed in each of four SWR mice. The lesions were localized and more frequent than those in Balb/c mice and large diffuse lesions involving several to many myocardial fibres were present. Necrotic myocardial fibres were infiltrated with mainly monocytes and some lymphoid cells (Figure 4). Inflammatory cells were also infiltrating other parts of the heart where no necrosis was observed. Heavy calcium salt deposits in myocarditic lesions were seen in two of four SWR mice but not in Balb/c. Myocarditic lesions were both atrial and ventricular, and occurred on both right and left sides of the heart. Mice receiving only diluent showed normal cardiac histology.

The susceptibility of SWR mice to rCVB3-induced myocarditis was evaluated *in vivo* using 10^2–10^7 PFU of virus. None of the 20 infected mice had died of the viral infection by day 7. They all maintained or gained body weight. All the mice, including those inoculated with only 10^2 PFU of rCVB3, developed myocarditis: the ID_{50} is therefore $< 10^{1.5}$ PFU (Table 1). A correlation of severity of myocarditis with virus dose was observed. A few lesions were present in the sections of the heart tissue from mice inoculated with 10^2 PFU, while extensive myocarditis involving a large area of myocardium of both left and right ventricles and atria developed in mice inoculated with 10^7 PFU. In contrast, the severity of myocarditis in Balb/c mice did not correlate well with virus dose: higher virus inoculum did not induce correspondingly more severe myocarditis (Table 2).

The pancreases of some infected mice were also examined. Approximately half of the exocrine acinar epithelium was necrotic with moderate inflammatory infiltrate in SWR mice receiving the lowest virus inoculum. When the mice were inoculated with a higher titre of virus, a large number of the acini were necrotic with

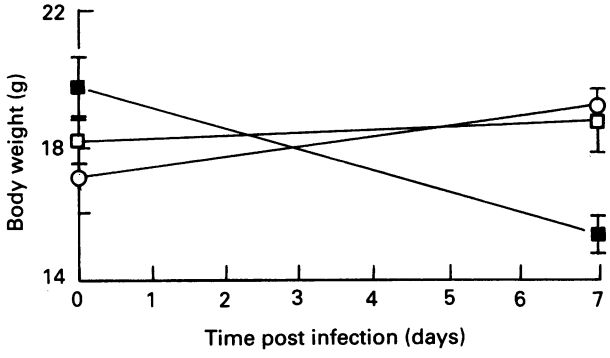


Figure 3. Representative body weight curves of infected SWR mice. □, Four mice were infected with 10^7 PFU of rCVB3 and ○, two mice inoculated with PBS. ■, Four Balb/c mice were infected with 10^5 PFU of rCVB3 for comparison. Mice were weighed before inoculation (day 0) and 7 days after inoculation. Data are represented as mean value ± SEM.

extensive infiltration. Nevertheless, a small number of exocrine acini remained intact. In contrast, fulminant pancreatitis affecting the entire exocrine pancreas was observed in Balb/c mice (Figure 5). Endocrine islets of Langerhans were not affected in either model.

Natural history of rCVB3 myocarditis in SWR

SWR mice were inoculated with 10^5 PFU of virus, sacrificed on scheduled days and the histopathological changes were examined. The first appearance of myocarditis lesions was on day 5 post infection (p.i.) although scattered inflammatory cells were seen on day 3. In this early stage of myocarditis, there were a few necrotic foci each involving one or two myocardial fibres and surrounded by a few infiltrating cells. Myocarditis became progressively prominent, and by day 6 or 7 p.i. large lesions involving 5–10 or more myocardial fibres were seen. The number of infiltrating cells also increased and calcification of the lesions was seen in some mice. Severe myocarditis continued up to day 16, when large necrotic lesions with monocytes and increased number of lymphoids were still present; repair was in progress and fibrosis could be seen in and around the lesions. Acute myocarditis was resolved by day 30 p.i., but two small foci of calcification or fibrosis were present in one of three mice. Small residual lesions were also seen in one of three mice on day 60 p.i. (Table 3 and Figure 4). A similar natural history of rCVB3 myocarditis was observed in Balb/c mice.

Table 1. Myocarditis morbidity (ID_{50}) in SWR mice by rCVB3, p14V-1 and p14V-4*

Virus dose (PFU)	Morbidity of myocarditis		
	rCVB3	p14V-1	p14V-4
10^2	4/4	ND†	ND
10^3	4/4	ND	ND
10^4	4/4	ND	ND
10^5	4/4	0/4	0/4
10^6	ND	0/3	2/4
10^7	4/4	0/2	ND
ID_{50}	$<10^{1.5}$	$>10^7$	10^6

* Mice were sacrificed 7 days post infection.
† Not done.

Table 2. Comparison of myocarditis severity between Balb/c and SWR mice*

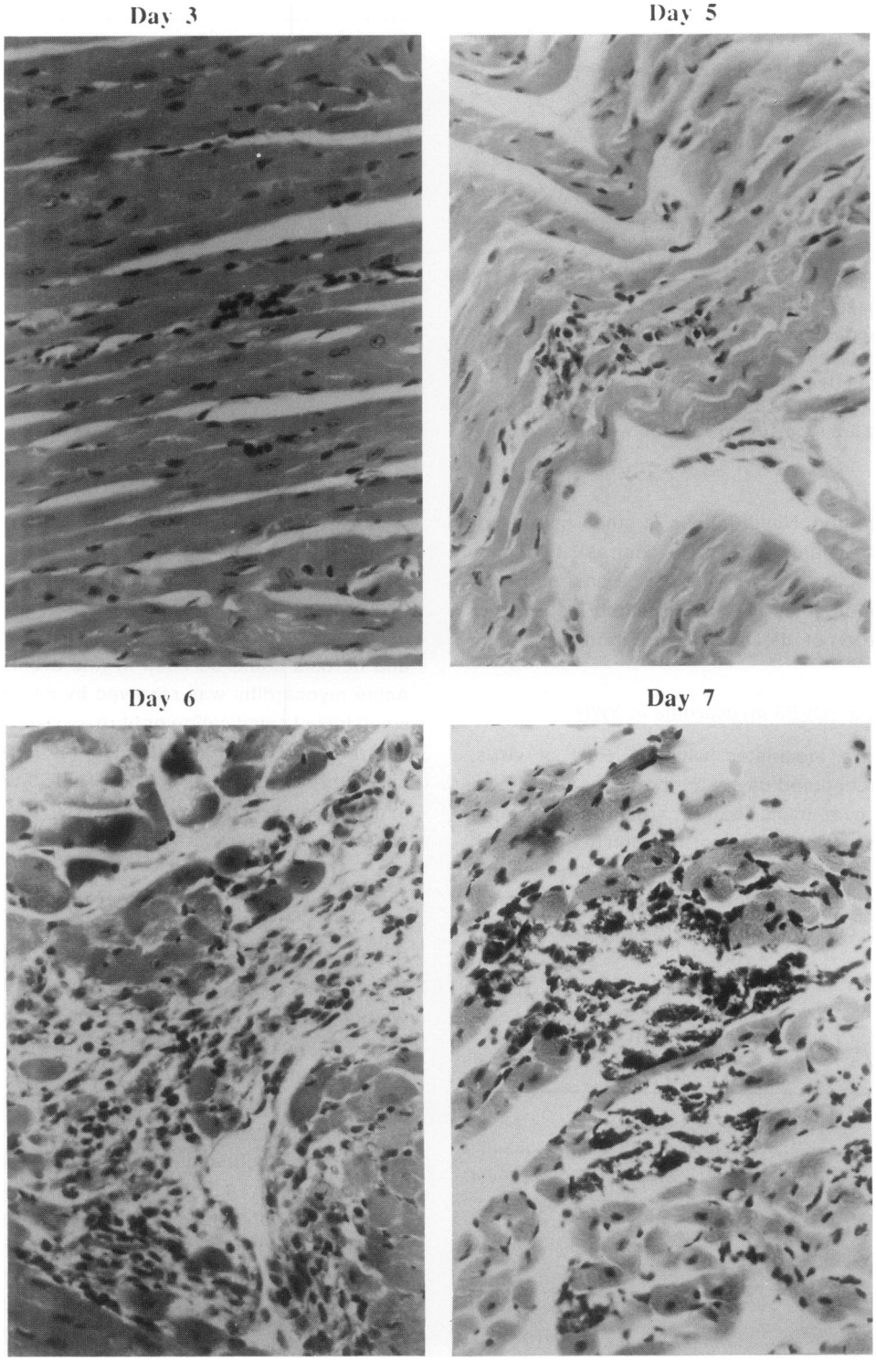
Virus dose (PFU)	Balb/c		SWR	
	Morbidity	Lesion no. (mean ± s.e.m.)	Morbidity	Lesion no. (mean ± s.e.m.)
10^3	3/4	4.7 ± 2.7	4/4	23.8 ± 8.0
10^5	4/4	5.4 ± 0.7	4/4	47.5 ± 3.5
10^7	4/4	9.0 ± 1.0	4/4	73.1 ± 20.0

* All infected mice were sacrificed 7 days post infection except for four Balb/c mice inoculated with 10^3 PFU of rCVB3, which were sacrificed 10 days post infection.

Detection of virus in the myocardium

SWR mice developed more severe myocarditis than Balb/c and responded to virus dose. To investigate whether this was due to a difference in virus replication in the myocardium between SWR and Balb/c, heart tissue taken 7 days p.i. from either SWR or Balb/c mice was homogenized and inoculated onto Vero cell monolayers. Virus was isolated from all four SWR and four Balb/c mice inoculated with 10^5 PFU of rCVB3. The mean titre was $\log_{10}$ 4.57 ± 0.32 PFU/100 mg tissue and 3.89 ± 0.46 PFU/100 mg tissue, respectively. The difference between the two is not significant ($P > 0.5$).

Expression of viral antigen in the myocardium of infected SWR mice was also evaluated by immunofluorescence, using enterovirus-specific MAb 5-D8/1. Viral antigens were detcted in frozen sections of the myocardium 7 days p.i. from the mice inoculated with 10^5 PFU of rCVB3. Frozen sections of the myocardium from mice 30 days p.i. did not show any viral antigen.


Day 3
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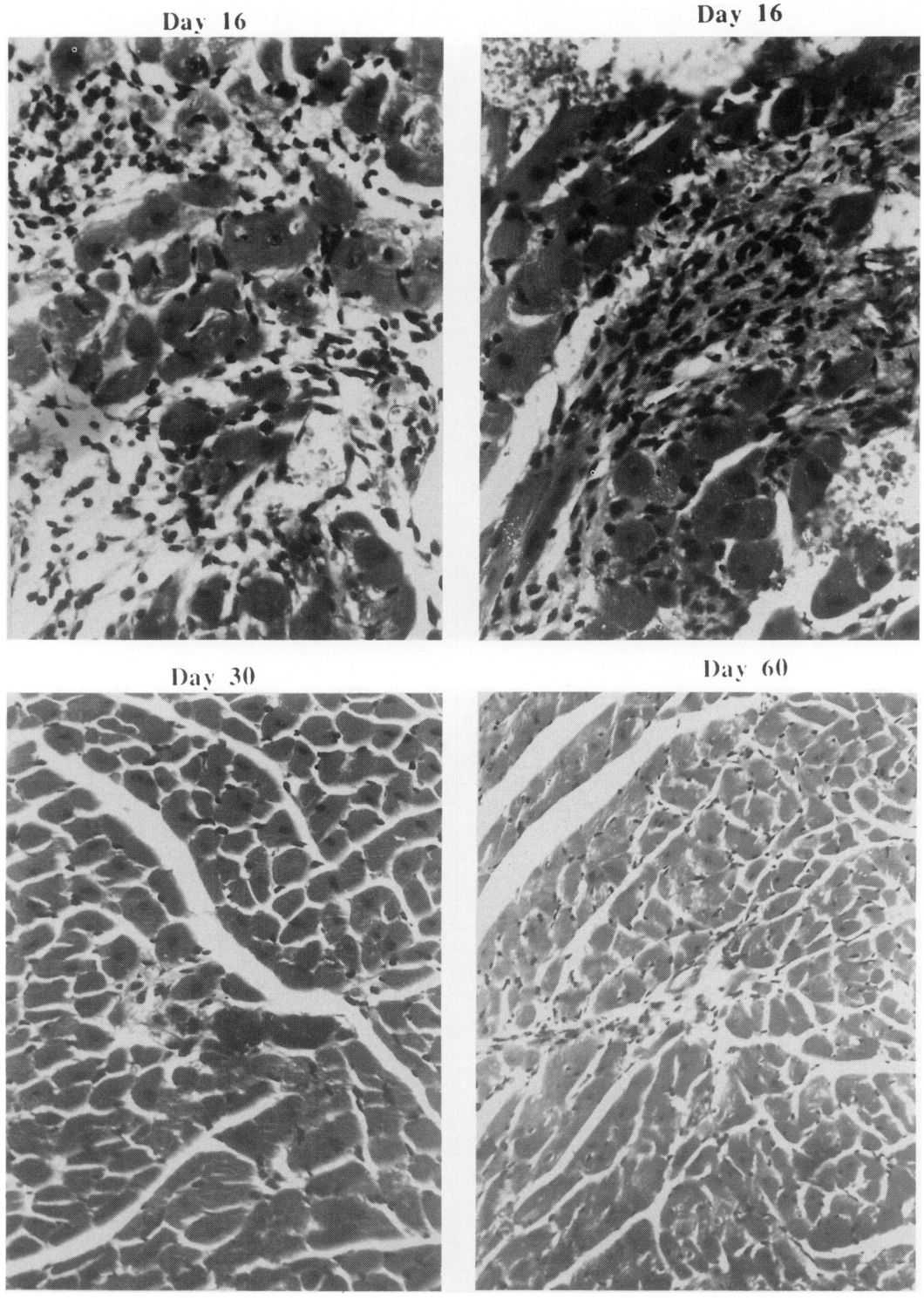
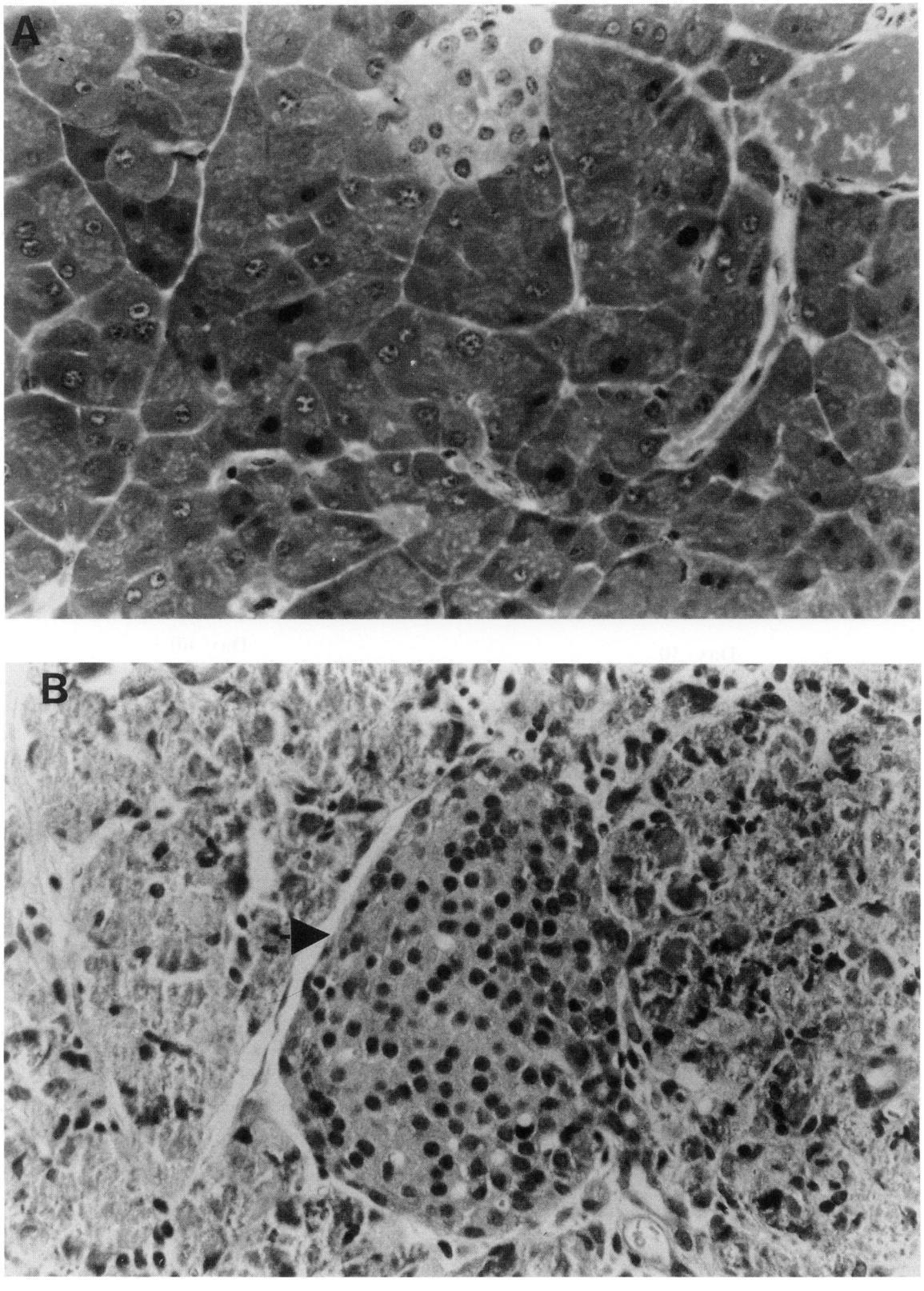


Figure 4. Photomicrographs of progress of rCVB3 myocarditis in SWR mice. SWR mice inoculated with 10^5 PFU of rCVB3. Infected mice were sacrificed on designated days for histopathological examination. H & E ×400.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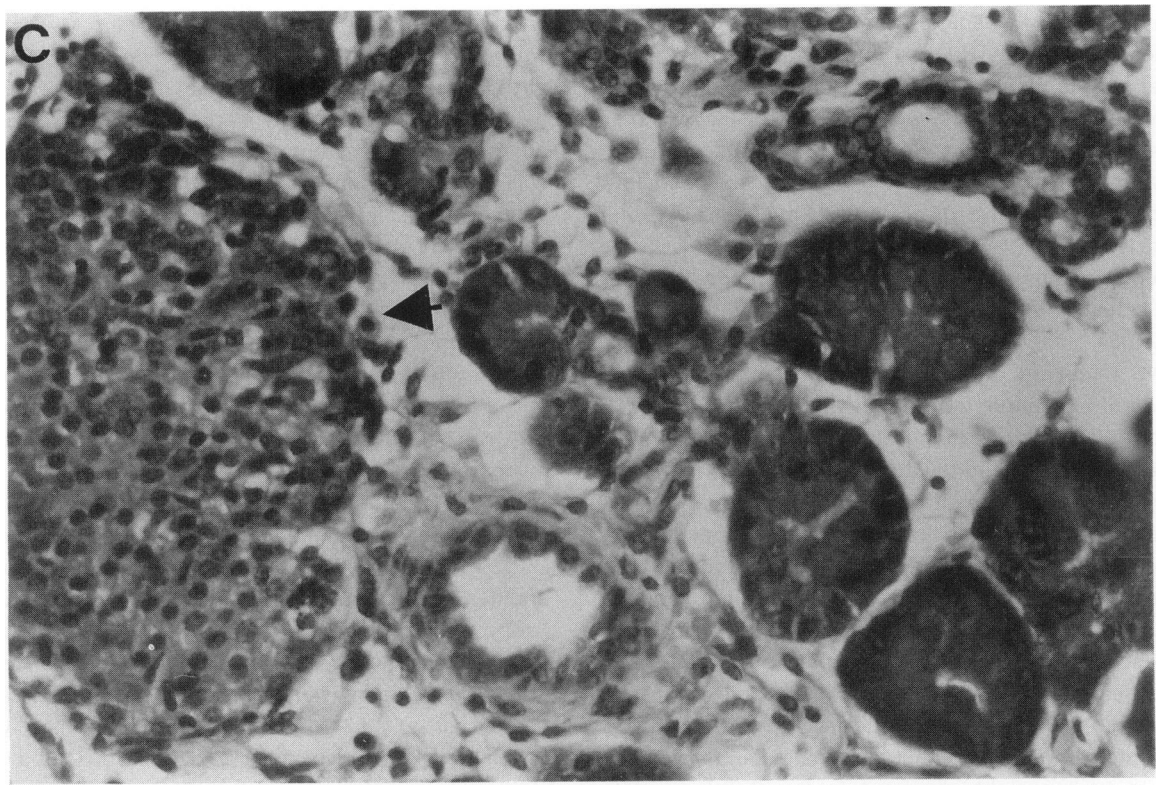

Figure 5. Photomicrographs of rCVB3 pancreatitis in SWR and Balb/c mice. Experimental animals were sacrificed 7 days after infection with 10^5 PFU of rCVB3. A, normal histology of mouse pancreas; B, a section of infected pancreas from Balb/c. Severe pancreatitis affects the entire exocrine acini; C, a section of pancreas from SWR showing moderate pancreatitis and functioning acini. Arrows indicate unaffected endocrine islets of Langerhans. H & E × 400.

Assessment of attenuated CVB3 variants in SWR model

Attenuated variants derived from cardiovirulent rCVB3 show different plaque-size phenotypes. A large and a minute plaque-size variant (p14V-1 and p14V-4) were used to test the SWR model (Figure 1). As expected, myocarditic lesions were not observed in SWR mice inoculated with either 10^5–10^7 PFU of p14V-1, or 10^5 PFU of p14V-4 on day 7 p.i. When four mice were inoculated with 10^6 PFU of p14V-4, a few small myocarditic lesions were seen in two of them (Table 1). It is clear, in comparison to rCVB3, variants p14V-1 and p14V-4 had lost cardiovirulence by a factor of more than 10^4–10^5.

Table 3. Natural history of myocarditis in SWR mice*

Days post infection	Morbidity	Lesion no. (mean ± s.e.m.)
1	0/2	0
3	0/2	0
5	2/2	3.5 ± 0.5
6	3/3	29.5 ± 4.1
16	4/4	84.8 ± 25.7
30	1/3	2.0†
60	1/3	3.0†

* Mice were inoculated with 10^5 PFU.
† Residual lesions, i.e. calcification or scar (fibrosis).

Discussion

Many studies on viral myocarditis have been carried out in murine models. Among them, the CVB3-induced myocarditis model is of greatest interest. This is because CVB3 is one of the most common agents associated with viral myocarditis, and this human enterovirus is readily adapted to experimental mice. In fact, CVB3 isolated from the heart tissue of a patient with myocarditis was cardiovirulent in mice (Tracy *et al.* 1991). Furthermore, murine myocarditis induced by CVB3 is histologically and clinically similar to human disease, implying that they should share a similar pathogenesis, and therefore any findings in murine models should be relevant to human beings. A unique advantage of murine models is

the availability of a panel of inbred strains. Employment of these genetically defined mice can minimize individual variation so that the best reproducibility of experiments can be achieved. When host factors are investigated, a panel of inbred mouse strains each carrying a particular H-2 haplotype can be used for comparison against a given virus (Gauntt *et al.* 1984; Wolfgram *et al.* 1986; Lawson *et al.* 1990); if virus virulence is to be studied, a given inbred mouse can be used to test different variants (Trousdale *et al.* 1979; Cao *et al.* 1984; Huber & Lodge 1986; Zhang *et al.* 1993).

SWR mice are of Swiss mouse origin from A. de Coulon of Lausanne, inbred by Lynch from about 1926 (Lynch 1969). They were introduced to Jackson Laboratory (USA) in 1947 and to OLAC (UK) in 1978. When the SWR model was compared to the traditional Balb/c model, our attention was first drawn to the fact that none of the SWR mice died of rCVB3 infection, even at the highest virus dose (10^7 PFU). In the Balb/c model, inoculation of 10^5 PFU of rCVB3 caused some deaths, the earliest occurring on the day 4 p.i., when myocarditis had not developed fully (Zhang *et al.* 1993), and when the virus dose was increased further, all mice died of the virus infection. The fulminant pancreatitis and dramatic loss of body weight observed in the Balb/c model suggests that these mice died of digestive failure rather than heart dysfunction. Similar mortality in this model has been reported by other research groups. Following inoculation with 10^5 PFU of CVB3, about 60% of mice on day 7 and 95% on day 12 died (Fohlman *et al.* 1990). Weaning Balb/c mice inoculated with 2×10^2 PFU of CVB3 Nancy strain, experienced over 90% mortality by day 7 p.i. (Gomez *et al.* 1991), although contradictory data were also reported (Vuorinen *et al.* 1989). When mice die in early acute phase (1–5 days) of viral infection, the histopathology of myocarditis cannot be accurately assessed because apoptosis and bacteria-related myocardial damage may occur within several hours after death. Even if the heart is processed soon after death on the 4th–5th day p.i., the myocarditic lesions may not have developed fully enough to be seen by light microscopy. The mortality obviously limits opportunities to follow up the progress of heart muscle disease. Furthermore, since the general condition of infected SWR mice in this study remained good, with a maintenance or even gain of body weight compared to Balb/c mice (Figure 3), the model allows the full development of acute myocarditis and follow-up study of its progression to chronic cardiac disease. Another important feature of the SWR model is that these mice develop more severe myocarditis than Balb/c after viral infection. They are very sensitive to CVB3 infection. All the mice inoculated with 100 PFU of cardiovirulent CVB3 developed myocarditis. Myocarditis was also observed in SWR mice receiving only 10 PFU of virus (our unpublished observation). A strong correlation between the severity of myocarditis and virus dose was observed. In other words, the myocarditis in SWR mice was dose dependent. This property is particularly valuable in assessment of changes in cardiovirulence of variants and efficacy of antiviral drugs. It is expected that minor differences in cardiovirulence of various CVB3 isolates or variants could be distinguished. In this study, the SWR model was applied to test the cardiovirulence of two attentuated variants, p14V-1 and p14V-4. The results showed that either virus had an attenuated phenotype with a 10^4–10^5 times reduction in their ability to induce myocarditis.

Severe CVB3-induced myocarditis in SWR mice was previously observed in a study of the cardiovirulence of CVB3 variants in 14 inbred mouse strains. SWR mice and another mouse strain, 129/J bearing a different H-2 allele ($H\text{-}2^b$), showed more severe myocarditis than Balb/c mice ($H\text{-}2^d$). There were no significant differences in virus titres in heart tissue among SWR, 129/J and Balb/c (Gauntt *et al.* 1984). When we examined the virus infectivity of the myocardium, rCVB3 replicated to a similar titre in SWR as in Balb/c. This suggests that the more severe myocarditis observed in SWR model is not due to higher cardiotropism or more active replication of the virus, but rather to some differences in genetic background or host response between SWR and Balb/c. Differences in genetic susceptibility to viral disease may be related to a number of factors such as the presence of viral receptors on specific cell populations, the ability to produce interferons and the immunlogical response to viral antigens (Beisel & Rose 1983). Major histocompatibility complex (MHC) haplotypes were suggested to influence the susceptibility of different inbred mouse strains to CVB3-induced myocarditis. However, the susceptibility of inbred mice to viral myocarditis is not based solely on MHC haplotype. By comparing different congeneic mice carrying the same H-2 haplotype, it was found that it was also influenced by non-MHC genes (Wolfgram *et al.* 1986). When mouse cytomegalovirus-induced myocarditis was investigated in H-2 congeneic and recombinant inbred mouse strains (C57BL/10 and Balb/c backgrounds), it was also found that both H-2 complex-related genes and non-H-2 genes influenced the pathogenesis of myocarditis: genes linked to the H-2 complex influenced susceptibility to peak levels of myocarditis in the acute phase; non-H-2 genes were important in determining the severity of myocarditis (Lawson *et al.* 1990). The different susceptibilities of SWR and Balb/c mice are most probably controlled by their

different H-2-associated genes and other genetic predisposition factors. Immunology of the infected mice was not studied in this project, but it could be predicted that SWR and Balb/c mice should have different immune responses to rCVB3 infection.

We and other researchers reported that viruses derived from a cDNA clone of CVB3 Nancy strain by transfection were as cardiovirulent as wild-type virus (Kandolf & Hofscheider 1985; Tracy *et al.* 1992; Zhang *et al.* 1993). There are several advantages of using a cDNA-derived virus: (1) prototypic rCVB3 are genetically homogeneous and can be used as a standard stock. This plus the use of inbred mice allows the best reproducibility of animal experiments. (2) Extensive passage of virus, which results in undesired accumulation of mutations, can be avoided. (3) The full-length cDNA clone can be stored dried or in ethanol for a long time and easily distributed to other researchers. This can avoid the hazard of distributing infectious live virus. Whenever required, homogeneous virus can be reactivated by standard transfection method. This is why rCVB3 was used in this study to characterize the inbred SWR model. Experimental data derived from this model of rCVB3-induced myocarditis are reproducible and should be consistent among independent laboratories.

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