RABBIT DILATED CARDIOMYOPATHY

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INTRODUCTION

Viruses have long been recognized as important etiologic agents of heart disease in man and experimental animals¹. Epidemiologic evidence suggests that between 2-5% of a virus-infected population experiences some degree of cardiac involvement². Virus infection may result in degeneration and necrosis of myocytes by direct cytotoxicity and cause myocarditis, or inflammation of the heart muscle³. Myocarditis may progress to arrhythmias, conduction disturbances, circulatory collapse and/or acute congestive (dilated) cardiomyopathy⁴. Clinically, dilated cardiomyopathy is diagnosed by an increase in the heart weight, heart weight/body weight ratios, enlargement of the left and/or right ventricular cavities, thinning/hypertrophy of the ventricular walls, and low ejection fractions. In humans particularly ominous signs of dilated cardiomyopathy include the presence of pulmonary edema, pleural effusion, and congestion of the lungs and liver^{4,5}. The incidence of idiopathic dilated cardiomyopathy is estimated between 3-5 cases/100,000 population/year and accounts for about 30% of all heart disease related deaths in some areas of the world⁶. The mechanism(s) by which virus infection progresses to myocarditis and dilated cardiomyopathy is unclear.

Animal model systems provide considerable insight into the mechanisms of virus-induced heart disease. Coxsackie B virus (CBV) and encephalomyocarditis virus (EMC) infection in mice results in and/or dilated cardiomyopathy^{7,8,9}. CBV and EMC-induced myocarditis is primarily immune-mediated rather than caused by direct virus cytotoxicity to myocytes. The mechanism(s) by which other viruses cause myocarditis and dilated cardiomyopathy is unknown.

Recently, a new model system for virus-induced heart disease was described in rabbits. Rabbit cardiomyopathy is characterized by: (1) multifocal to diffuse myocardial degeneration and necrosis, (2) myocarditis, (3) and death in 60-70% of the infected animals¹¹. In this article, we demonstrate that RbCV infection is associated with increases in heart weight, heart weight/body weight ratios, dilation of the right and left ventricles, and alternations in a variety of parameters consistent with the development of dilated cardiomyopathy.

MATERIALS AND METHODS

Animals and Virus

Rabbit coronavirus (RbCV) was originally obtained from Dr. James D. Small (Glaxo Inc., Research Triangle Park, NC). Virus stocks were diluted to 10^4 - 10^5 rabbit infectious dose (RID) and stored at -145°C . Male New Zealand white rabbits (2.5-3.0Kg) were inoculated intravenously via the marginal ear vein with 0.2ml of virus. Daily records were kept on body weight, temperature, and the animals observed for clinical signs of infection. Clinical signs of infection included severe congestion of the conjunctivae, temperatures in excess of 40°C , and weight loss.

Pathology and Measurement Studies

Moribund animals were euthanized by intravenous injection with 60mg/Kg sodium pentobarbital (nembutal) and weighed. Necropsies were performed as previously described^{11, 12}, and the heart excised, trimmed of fat and excess connective tissue, and weighted. The heart was immersed in 10% buffered formalin and sectioned transversely at the midpoint of the ventricles. Sections were stained with hemotoxylin-eosin or Masson's Trichrome and mounted for measurement studies and histology. Heart tissue was also stained for the presence of calcification with the von Kossa stain, or the presence of monocytes with DE histochemical staining techniques^{13,14,15}. A computerized Zeiss Videoplan was used to calculate the dimensions of the cardiac walls and cavities as well as determining the area within each ventricular section. Mean values were averaged between animals which had died during the acute and subacute phases of infection and reported as means $\pm$ SD. Student's test for unpaired observations were used to evaluate differences in the cardiac dimensions.

RESULTS

Clinical Course of RbCV Infection

New Zealand white rabbits were inoculated with approximately 10^3 RID and examined daily for clinical signs of infection. Mortality rates peaked at 4 days post-infection, decreased, and then increased again by day 7 or 8. No animals died after 12 days post-infection, and the overall mortality rate was about 60% (Figure 1). Heart from animals dying between days 3-5 were enlarged and characterized by the presence of right ventricular dilation. Rabbits dying between days 9-12 contained pleural effusion, pulmonary edema, ascites, enlarged hearts and congestion of the lungs and liver. The cause of death appeared to be congestive heart failure. From these data, we have divided RbCV infection into an acute(2-5) and subacute(6-12) phases based upon day of death and pathologic findings in infected animals. These findings are similar to previous studies of RbCV and PEDV infection in rabbits and EMC-induced myocarditis in mice^{15,7,8,11}.

Heart Weight, Heart Weight/Body Weight Ratio's during RbCV Infection

During EMC-induced dilated cardiomyopathy, heart weight, heart weight/body weight ratio's increase following infection⁷⁸. Since RbCV infection results in a similar clinical course of infection and pathology in the heart muscle, we examined body weight, heart weight, and heart weight/body weight ratios during the acute and subacute stages of infection. Body weights slowly decreased during the course of infection and were notably decreased ($\sim 0.5\text{kg}$) during the subacute phase of the disease (data not shown).

Uninfected controls sacrificed between days 3-5 or 6-12 had heart weights of $6.1\pm 0.3\text{g}$ and $6.1\pm 0.5\text{g}$ between days 3-5 and 6-12, respectively.

FIGURE 1. Clinical Course of RbCV Infection in Rabbits.

Following RbCV infection, heart weights were significantly increased during the acute ($8.4 \pm 1.4\text{g}$; $p < 0.001$) and the subacute phase of infection ($8.7 \pm 1.6\text{g}$; $p < 0.001$) (Table 1). Heart weight/body weight ratios were also increased during the acute and subacute phases of the infection, and were $3.07 \pm 0.33 \times 10^{-3}$ ($p < 0.001$) and $3.46 \pm 0.61 \times 10^{-3}$ ($p < 0.001$), respectively. Conversely, the heart weight/body weight ratios of uninfected controls were $2.19 \pm 0.23 \times 10^{-3}$ (3-5 days) and $2.16 \pm 0.20 \times 10^{-3}$ (6-12 days), respectively (Figure 2b). Chronological changes in the size of the heart and dimensions of the ventricles were evident through day 36 and are illustrated in Figure 2. These data suggest that right ventricular dilation is prominent during the acute and subacute phase of infection and that dilation of the left ventricle increases progressively throughout the subacute phase of infection.

Dimensions of the Cardiac Walls and Cavities during RbCV Infection

The dimensions of the ventricular walls were measured through the coronal axis at the midpoint of the ventricles. During the acute phase of infection, the dimensions of the right ventricular wall were significantly decreased from $2.037 \pm 0.292 \times 10^3 \text{ um}$ (uninfected) to $1.545 \pm 0.248 \times 10^3 \text{ um}$; $p < 0.001$ (infected). Thinning of the right ventricular wall continued through the subacute stage of infection and was decreased from $2.065 \pm 0.300 \times 10^3 \text{ um}$ (uninfected) to $1.295 \pm 0.319 \times 10^3 \text{ um}$; $p < 0.001$ (infected) (Table 1). The dimensions of the intraventricular septum were not significantly altered during the acute stage of infection between the uninfected ($4.470 \pm 0.676 \times 10^3 \text{ um}$) and infected animals ($4.419 \pm 0.740 \times 10^3 \text{ um}$; $p < 0.87$). However, some thinning of the intraventricular septum was noted during the subacute phase of the disease (uninfected- $4.471 \pm 0.503 \times 10^3 \text{ um}$; infected- $3.579 \pm 0.604 \times 10^3 \text{ um}$; $p < 0.002$) (Table 1). The dimensions of the left ventricular wall were also similar during the acute phase of the disease (uninfected- $4.188 \pm 0.547 \times 10^3 \text{ um}$; infected- $4.369 \pm 0.512 \times 10^3 \text{ um}$; $p < 0.442$), and were reduced slightly during the subacute phase of infection (uninfected- $4.410 \pm 0.617 \times 10^3 \text{ um}$; infected- $3.776 \pm 0.718 \times 10^3 \text{ um}$; $p < 0.044$) (Table 1). Dilated cardiomyopathy is also characterized by increases in the dimensions of the left and/or right ventricular cavity. Consequently, we measured the widest portion of the right and left ventricular cavity in animals dying during the acute or subacute stage of infection (Table 1). During the acute phase, the dimensions of the right ventricular cavity were significantly increased as compared to the uninfected controls (uninfected- $3.571 \pm 1.07 \times 10^3 \text{ um}$; infected- $7.334 \pm 1.707 \times 10^3 \text{ um}$; $p < 0.001$), and markedly increased during the subacute stage of infection

FIGURE 2. Progression of Dilated Cardiomyopathy during RbCV Infection.

Transverse sections through the midpoint of the ventricles were prepared and stained with hemotoxylin-Eosin or Massons Trichrome. Representative sections are shown from days 4-36 post-infection.

(uninfected- $3.127 \pm 1.167 \times 10^3 \mu\text{m}$; infected- $8.669 \pm 2.084 \times 10^3 \mu\text{m}$; $p < 0.001$). Dimensions of the left ventricular cavity were not significantly altered during the acute stage (uninfected- $8.537 \pm 0.996 \times 10^3 \mu\text{m}$; infected- $9.361 \pm 1.427 \times 10^3 \mu\text{m}$; $p < 0.115$), and significantly dilated during the subacute stage (uninfected- $8.488 \pm 1.143 \times 10^3 \mu\text{m}$; infected- $10.027 \pm 0.986 \times 10^3 \mu\text{m}$; $p < 0.004$) (Table 1).

Table 1. Alternations in cardiac sturcutres during RbCV infection.

	<u>Acute</u>		<u>Subacute</u>	
	<u>Uninfected</u>	<u>Infected</u>	<u>Uninfected</u>	<u>Infected</u>
Body Weight (kg)				
Heart Weight (g)	$6.1 \pm 0.3\text{g}$	8.4 ± 1.4	$6.1 \pm 0.5\text{g}$	8.7 ± 1.6
Heart Wt/Body Wt. ($\times 10^{-3}$)	2.19 ± 0.23	3.07 ± 0.33	2.16 ± 0.20	3.46 ± 0.61
Right Vent. Wall ($\mu\text{m} \times 10^3$)	2.04 ± 0.30	1.55 ± 0.25	2.06 ± 0.30	1.30 ± 0.32
Intravent. Septum ($\mu\text{m} \times 10^3$)	4.47 ± 0.70	4.42 ± 0.74	4.47 ± 0.50	3.58 ± 0.60
Left Vent. Wall ($\mu\text{m} \times 10^3$)	4.20 ± 0.55	4.37 ± 0.50	4.41 ± 0.62	3.77 ± 0.70
Right Vent. Cavity ($\mu\text{m} \times 10^3$)	3.57 ± 1.07	7.33 ± 1.70	3.13 ± 1.17	8.67 ± 2.08
Left Vent. Cavity ($\mu\text{m} \times 10^3$)	8.54 ± 1.0	9.36 ± 1.43	8.48 ± 1.14	$10,027 \pm 1.0$
Right Vent. Area ($\mu\text{m}^2 \times 10^6$)	38.82 ± 11.8	100 ± 39.74	41.23 ± 13.1	146.2 ± 51.8
Left Ventr. Area ($\mu\text{m}^2 \times 10^6$)	57.56 ± 6.1	66.18 ± 25.0	59.95 ± 11.0	77.56 ± 20.3

To obtain a more accurate estimate of the dilation of the right and left ventricular cavities during infection, we also examined the effects of virus infection on right and left ventricular area. In the uninfected controls, the area within the right ventricular cavity was $38.82 \pm 11.76 \times 10^6 \mu\text{m}^2$ and $41.23 \pm 13.11 \times 10^6 \mu\text{m}^2$ between days 3-5 and 6-12, respectively. Following RbCV infection, area in the right ventricular cavity increased

dramatically during the acute ($100 \pm 39.74 \times 10^6 \mu\text{m}^2$; $p < 0.001$), and during the subacute phases of infection ($146.20 \pm 51.79 \times 10^6 \mu\text{m}^2$; $p < 0.001$) (Table 1). Area within the left ventricular cavity was similar between the uninfected control animals sacrificed between days 3-5 or 6-12 (uninfected acute- $57.56 \pm 6.09 \times 10^6 \mu\text{m}^2$; uninfected subacute- $59.95 \pm 11.03 \times 10^6 \mu\text{m}^2$). Viral infection did not result in significant increases in the area within the left ventricular cavity during the acute phase ($66.18 \pm 25.04 \times 10^6 \mu\text{m}^2$; $p < 0.29$), but significant increase were evident during the subacute phase of infection ($77.56 \pm 20.29 \times 10^6 \mu\text{m}^2$; $p < 0.025$) (Table 1).

Pathologic Findings during RbCV Infection

Yellowish-white patches were seen on the surface of the ventricles of infected rabbits after 3-4 days post-infection. Histological changes in the heart consisted of degeneration and necrosis of myocytes and interstitial edema through day 12 post-infection. Little if any myocarditis was detected through day 8 or 9 post-infection consistent with previous findings by our group¹¹. However, animals examined after day 9 had increasing amounts of myocarditis in the heart muscle (Figure 3a). Calcification was rarely present, but occasionally detected in animals dying late in the subacute phase of the disease (Data not shown). Congestion of the lungs and liver was common in about 12% of the animals dying during infection especially in the latter phase of the subacute stage of infection (Figure 3b). Myocarditis was present in 100% of the survivors through 65 days post-infection and a significant percentage (~30%) had evidence of right and/or left-sided dilated cardiomyopathy.

FIGURE 3. Pathologic Examination of RbCV-Infected Animals
Panel A: Myocarditis 12 days post-injection; Panel B:
congestion of the liver.

DISCUSSION

Heart disease is associated with a variety of viral infections in man and experimental animals. Viral infections may cause degeneration and necrosis of myocytes, myocarditis, and/or progress to dilated cardiomyopathy^{1,2,3}. Dilated cardiomyopathy is probably a common end product of a variety of cardiac disorders including viral infections, alcohol and other conditions^{1,4}. Few model systems for virus-induced myocarditis and dilated cardiomyopathy are amenable to experimental analysis and have been limited to picornavirus infection in mice^{7,8,9}. In this article, we describe a new model system for virus-induced myocarditis and dilated cardiomyopathy in rabbits. The etiologic agent is probably an enveloped RNA virus antigenically related to the group II human and porcine coronaviruses¹¹. RbCV is also antigenically related to the etiologic agent responsible for pleural effusion disease in rabbits (PEDV) and both viruses produce similar diseases in vivo¹².

Rabbit coronavirus infection produces a similar disease as EMC infection in mice. EMC infection in BALB-C mice results in myocarditis, extensive calcification, dilation of the heart and ventricles and about 15% of the animals die from congestive heart failure. EMC-infected DBA-2 mice develop dilation and hypertrophy of the heart muscle, myocarditis, extensive calcification and fibrosis by 3 months post-infection. This model represents the best studied system for virus-induced dilated cardiomyopathy. In the rabbit model, animals dying during the acute stage of infection are characterized by right-sided degeneration and necrosis of myocytes and dilation of the right ventricle. The left ventricle is also infected but to a lesser extent. The most logical explanation for the increase in heart weight, heart weight/body weight ratios during the acute stage of infection is due to interstitial edema. Little or no myocarditis was evident through day 8 post-infection. During the subacute stage of the disease, a more global involvement of the heart muscle is noted, and interstitial edema is probably responsible for the increases in heart weight, heart weight/body weight ratios. Myocarditis peaks by 12-13 days post-infection after exposure to the virus. Calcification is rarely detected during infection and is only present in a small percentage of animals dying late in the subacute phase of the disease. Dilation of the ventricles, pleural effusion, ascites, pulmonary edema, and congestion of the lungs, liver, heart, and spleen are common findings in animals that die late in the subacute stage of infection (9 days). Death is probably due to congestive heart failure. Myocarditis (2 to ~15 foci) and fibrosis are detected in all survivors examined through 64 days post-infection. Roughly 30% of these animals have evidence of dilated right and/or left hearts. In contrast to findings in EMC infected animals, no calcification is detected in survivors. In this regard, pathologic findings in the rabbit are more typical of findings in humans suffering from dilated cardiomyopathy and consist of few inflammatory foci, dilated hearts, and little, if any, calcification. It is unclear whether dilation and hypertrophy of the heart muscle persists beyond 64 days post-infection.

Virus-induced congestive heart failure has been reported accompanying chicken pox, mumps, CBV, poliomyelitis, dengue and chikungunya fever viruses and influenza virus infection^{1,2,3,4}. A mouse model system for CBV-induced dilated cardiomyopathy has been described in which infected mice are forced to swim during infection. Cardiac damage is concentrated in the left ventricle and intraventricular septum, particularly at the atrioventricular junction¹⁰. The mechanism by which EMC induces myocarditis and dilated cardiomyopathy in mice is probably immune-mediated rather than due to direct virus cytotoxicity in myocytes⁴. In the rabbit model, significant cardiac damage is occurring prior to immune induction suggesting that direct virus cytotoxicity to

myocytes is primarily responsible for the disease. This is supported by recent findings in our laboratory that cortisone-immunosuppressed animals are not protected from infection and are characterized by necrosis and degeneration of myocytes and right ventricular dilation (Edwards, et al, manuscript in preparation).

Dilated cardiomyopathy is normally recognized by ventricular dilation, hypertrophy of the heart muscle, and low ejection fractions^{1,2,3,4}. In most patients, the left ventricle is more severely effected. However, a variety of right-sided disorders have been reported in man and experimental animals. Right-sided perimyocarditis has been described previously during CBV3 infection of BALB-C mice²⁰, and idiopathic right ventricular myocardial hypoplasia in association with right ventricular dilation (Uhl's anomaly) is well documented in children²¹. Right ventricular dilated cardiomyopathy occurs in humans and is characterized by a male preponderance, syncope, ventricular tachycardia and right heart failure. Clinically, right-sided heart failure has a poor prognosis in humans²². The mechanism by which RbCV produces a predominantly right-sided disease is unclear. This model will provide new insight into the mechanism by which viruses cause heart disease and right-sided dilated cardiomyopathy in rabbits. This model is particularly important since rabbits are amenable to a variety of experimental techniques not available in mice and will allow us to measure hemodynamic alterations and muscle contractibility at different times post-infection.

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