Virchows Archiv A Pathol Anat (1993) 422:405–413

Virchows Archiv A
Pathological Anatomy and Histopathology

Outbred mice infected by an encephalomyocarditis virus variant: a model for studying chronic viral heart disease

Johannes P. Kruppenbacher[1], Georg Arnold[2], Thomas Mertens[1,*], Armin Fischer[1], Janina Zimmermann[2], Hans J. Eggers[1]

[1] Institut für Virologie der Universität zu Köln, Fürst-Pücklerstrasse 56, W-5000 Cologne, Germany
[2] Institut für Pathologie der Universität zu Köln, Joseph-Stelzmannstrasse 9, W-5000 Cologne, Germany

Received July 6, 1992 / Received after revision January 30, 1993 / Accepted February 1, 1993

Abstract. Male 8 to 20-week-old NMRI mice (an outbred strain) infected with the encephalomyocarditis virus (EMCV) plaque variant (PV) 7 consistently develop a distinct myocarditis with a relatively low mortality (21%). Myocarditis occurs in essence independent of the virus dose applied, and other internal organs are not affected. Nevertheless, 3.5-week-old NMRI mice perished within 5 days of virus inoculation and exhibited disseminated myofibrillar degeneration (MFD); this obviously virus-induced myocardial damage was accompanied by scanty inflammatory infiltrates. EMCV PV7 infection of adult male C57Bl/6 and DBA/2 mice causes myocarditis comparable to that seen in NMRI mice. In DBA/2 mice, however, the virus-induced myocardial necrosis is complicated by subtotal calcification. This strain has a genetically determined "spontaneous" calcification of the myocardium, as shown by the study of uninfected controls. EMCV PV7-infected NMRI mice appear a promising model for study of long-term effects of viral myocarditis, possibly including cardiomyopathy. Furthermore, this outbred mouse strain offers the possibility of examining the pathogenesis of direct viral cytolysis and its relation to MFD as well as immunologically mediated cell damage.

Key words: Encephalomyocarditis virus – Virus-induced myocarditis – Myofibrillar degeneration – Myocarditis model – Virus-induced cytolysis

Introduction

Enteroviruses are suspected to be a frequent cause of myocarditis in men. The pathogenesis of myocarditis, however, is poorly understood, and hence, simple animal models appear to be of importance for investigation. Yet in mice most of the myocarditis inducing picornaviruses are highly lethal; encephalomyocarditis virus (EMCV) M strain killed 43% of infected BALB/c mice (Matsumori and Kawai 1982a), 33–67% of Swiss mice (Craighead 1966a, b) and collectively 92% of BALB/c, C3H/He, or DBA/2 mice (Kishimoto et al. 1983). Infection with coxsackie B3 virus (CBV3) caused death in 60% (Woodruff and Woodruff 1974) or 80% (Huber et al. 1982) of BALB/c mice. Several inbred, apparently uninfected mouse strains are affected by "spontaneous" myocardial calcification (DiPaolo et al. 1964; Eaton et al. 1978; Brownstein 1983) resulting from calcium deposition in necrotic myocytes (Ball and Williams 1965). The underlying genetic defect and the pathogenetic mechanisms leading to myocardial necrosis and calcification are unknown. However, viral myocarditis per se may also be followed by calcification of necrotic myocytes. Therefore, study and quantitation of the pathogenetic pathways of virus-induced myocardial damage meet with difficulties in these inbred strains.

Since, high lethality and "spontaneous" calcification hamper the study of virus-induced myocarditis in various inbred mice strains, we looked for an experimental model in which picornaviruses induce myocarditis reliably with low lethality, and in which the mouse strain is not burdened by a genetic defect resulting in "spontaneous" calcifications. In this way long-term effects of viral myocarditis might be investigated as well as the consequences of successive picornavirus infections on heart disease.

Materials and methods

Breeding pairs of NMRI mice were purchased from the Central Institute for Experimental Animals (Zentralinstitut für Versuchstiere) in Hannover, Germany. Colonies derived from these mice were maintained in our departmental animal facilities. DBA/2 and C57Bl/6 mice were provided by Zentralinstitut für Versuchstiere and Charles River Wiga, Sulzfeld, Germany. Some DBA/2 mice, which served as controls, were also obtained from SAVO-Ivanovas,

**Present address:* Institut für Mikrobiologie der Universität Ulm, Abteilung Virologie, Oberer Eselsberg M23, W-7900 Ulm (Donau), Germany

Correspondence to: H.J. Eggers

Kisselegg, Germany, and Bomholtgørd, Denmark, respectively. All mice had access to water and laboratory mouse chow ad libitum. The experiments were conducted with 8- to 12-week-old male mice housed in individual cages. In order to evaluate the influence of age on the pattern of virus myocarditis, experiments were also done with 3.5-, 16-, and 20-week-old NMRI mice.

EMCV plaque variant (PV) 7 was isolated from EMCV D (Yoon et al. 1980) as described previously (Kruppenbacher et al. 1985).

EMCV PV7 stocks were prepared and quantitated in L cell monolayers, a continuous mouse fibroblast cell line obtained from Rolly, Hoechst, Frankfurt, Germany. Cells were maintained in minimum essential medium (MEM) containing 0.1 mg/ml neomycinsulphate and supplemented with 5% fetal calf serum (FCS).

Confluent cell monolayers were inoculated with virus. After adsorption for 1 h at room temperature, MEM supplemented with 2% FCS was added, and cells were incubated at 37° C. When 75–100% of the cells showed cytopathic changes virus was released from cells by freezing-thawing followed by ultrasonication in ice-cooled medium (50 W, 15 s, three times with interruptions of 5 s each). Cell debris were removed by centrifugation at 1,700 *g* for 20 min, and the supernatant was stored in small aliquots at −20° C. For determination of infectivity, plaque assays were performed as previously published (Schürmann and Eggers 1983).

Eight to 12-week-old male mice were inoculated intraperitoneally (i.p.) with 10^5 plaque forming units (pfu) of virus in 0.5 ml and observed daily (standard conditions). In order to evaluate the influence of virus dose on the severity of myocarditis, 8-week-old NMRI mice were also inoculated i.p. with 10, 10^2, 10^4, 10^7 pfu, respectively. Seven, 14 and 21 days following infection mice were sacrificed by thoracotomy under ether anaesthesia (standard conditions). Animals found moribund were killed and examined, whereas cadavers were discarded. Moribund mice were counted as dead animals. Mortality is indicated as number of deaths/number of infected animals regardless of whether animals were withdrawn and killed for histological examination. Calculation of mortality (%) was performed by the method of Kaplan and Meier (1958).

Hearts were removed quickly, immersed immediately into isotonic potassium chloride solution for 1 min, cut into halves (by turns) transversely or longitudinally, then fixed in a 6% formalin solution for 24 h, dehydrated in a graded series of alcohols and xylene, and embedded in paraffin. Sections 5 µm thick were stained with haematoxylin and eosin. Additional sections were stained with van Gieson's, Ladewig's, or von Kossa's stains, respectively. For detection of myofibrillar degeneration or contraction band necrosis (a rapidly developing form of elective myocyte necrosis), a modified Luxol-fast blue (LFB) stain (Arnold et al. 1985) was used. For assessment of extracardiac pathological changes sections of the brain, lung, liver, spleen, pancreas, kidney, and the small intestine were examined routinely.

All hearts were coded and examined in a blinded fashion. At least three or more adjacent sections of both halves of the heart were examined microscopically for the presence of myocardial lesions (inflammatory infiltration, focal myocytic degeneration and/or necrosis, calcification of necrotic myocytes, and fibrosis). The severity of myocardial damage was graded as slight when only a few small lesions were scattered throughout the myocardium. Myocarditis was regarded as medium, when several larger lesions were found in the sections, consisting of dense infiltrates or small groups of necrotic myocytes or patchy replacement fibrosis. Severe alterations consisted of many lesions, each of them representing a large focus of inflammatory infiltrates and/or necrosis or fibrosis; confluent foci were often detected.

Results

Inoculation with EMCV PV7 reliably provoked a significant myocarditis in the left and right ventricular myocardium in all mice of the tested strains. It should be emphasized that irrespective of the severity of myocarditis the course of morphological alterations showed a consistent pattern during the observation time of 21 days exemplarily described in the following for 8-to 20-week-old NMRI mice.

Seven days post-infection (p.i.) the myocardium contained at least some, and most often multiple, foci of myocytic necrosis interspersed with inflammatory infiltrates. In some foci the necrotic myocytes were completely preserved as hypereosinophilic cells with loss of nuclei. Occasionally, eosinophilic cytoplasmatic remnants of myocytes, sometimes with tiny granular calcifications, were discernible between the surrounding dense cellular infiltrates. Most often, the myocytes had already disappeared due to resorption and were predominantly replaced by compact collections of lymphocytes and histiocytes. Granulocytes and granulocytic nuclear debris were always present in small amounts (Fig. 1). In foci with discernible eosinophilic myocardial necrosis, however, granulocytes made up a major component of the infiltrates.

Fourteen days p.i. remnants of myocytes were visible only in those relatively rare cases in which calcification of necrotic myocytes had occurred. Granulocytes had nearly disappeared. At this time lymphocytes and histiocytes were intermingled with fibroblasts. Small foci were spindle shaped. The density of the infiltrate had significantly decreased in most foci 21 days p.i. At the margins of the spindle-shaped lesions fibroblasts predominated. Usually, a reticular fibrosis had developed and protruded between adjacent viable myocytes (Fig. 2). Small foci were completely collagenized and nearly free of mononuclear infiltrate. Calcified myocytes surrounded by histiocytes and collagenized stroma were present only in a few cases. In control mice calcification of the myocardium was never detected.

The vast majority of male adult NMRI mice suffered from a notable and impressive myocarditis (Table 1): its degree was severe (Fig. 3) in 16 of 34 (47%) and medium (Fig. 4) in 12 of 34 (35%) animals, respectively. Slight myocarditis (Fig. 5) occurred in only 6 of 34 (18%). The mortality in males was 35% (8 of 38, Table 1; product limit estimate). It appeared lower in a separate experiment (15%; 4 of 48) when mice had been infected with different virus doses. In each dose group one mouse died (Table 2). In a further experiment with 29 infected animals mortality was 14% (8-week-old, 10^5 pfu/animal; data not shown). In adult female mice myocarditis was less severe and no mouse (0 of 15) died (Table 1).

All other organs (brain, lung, liver, spleen, pancreas, kidney, and the small intestine; except for striated muscles – see below) of the EMCV PV7-infected male and female NMRI mice showed no pathological alterations in any age group.

Virus dose affected the severity of myocardial damage only slightly (Table 2). However, the higher the virus dose, the more consistent became the development and distribution of the foci of myocardial damage. The lesions produced by 10^4–10^7 pfu tended to progress somewhat more rapidly than lesions induced by smaller virus

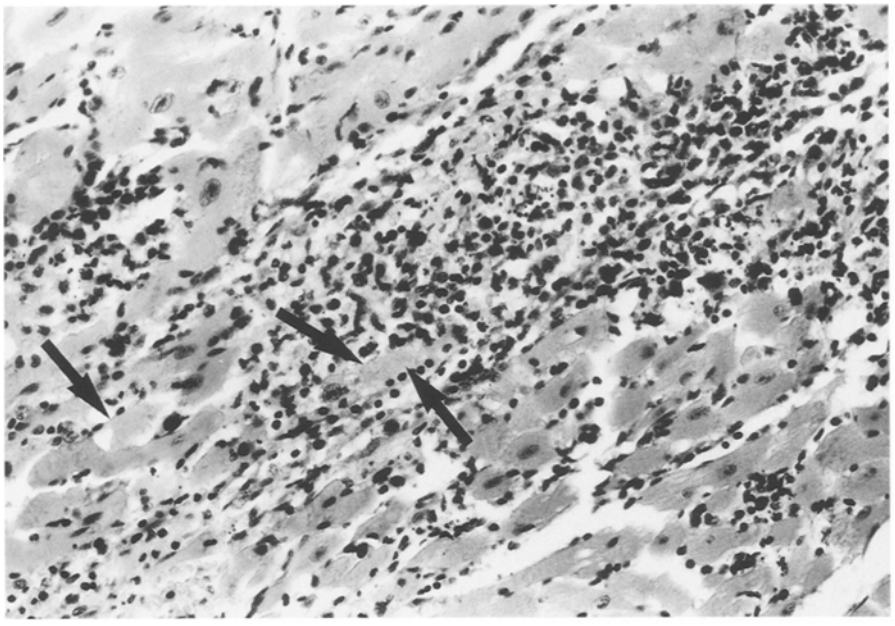

Fig. 1. Myocarditis 7 days after intraperitoneal inoculation of 10^5 plaque forming units of encephalomyocarditis virus plaque variant 7 into an 8-week-old NMRI mouse; dense collection of mononuclear cells interspersed with tiny granular debris of granulocytes in sites of destroyed myocytes; only a few residual necrotic myocytes with loss of nuclei (*arrows*). H&E, ×510

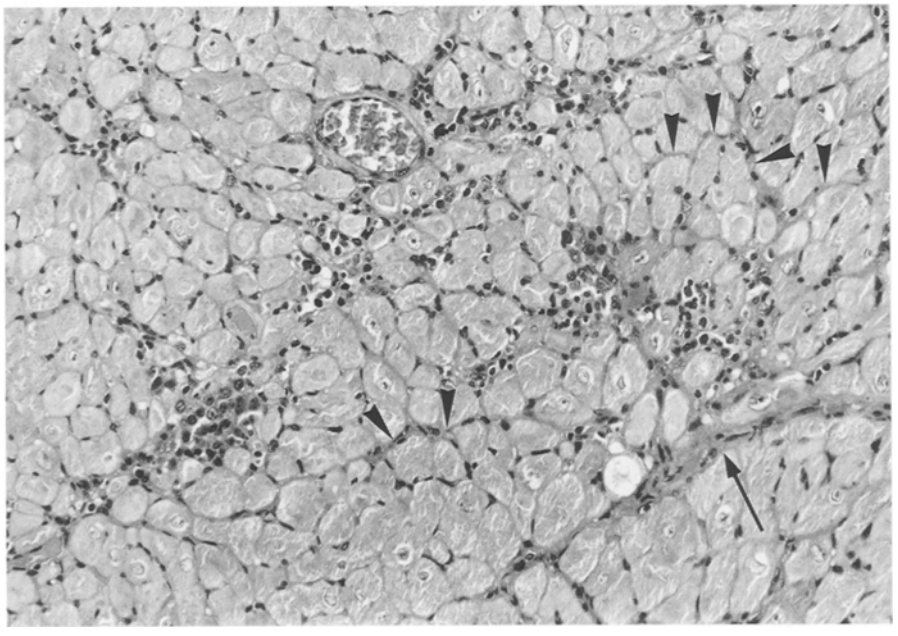

Fig. 2. Myocardium of encephalomyocarditis virus plaque variant 7-infected NMRI mouse 21 days after intraperitoneal inoculation; patchy residual aggregates of histiocytes; occasionally in contact to foci of replacement fibrosis (*arrow*); note reticular fibrosis surrounding individual myocytes (*arrowheads*). van Gieson, ×510

doses. In the groups inoculated with 10^2–10^7 pfu all animals displayed myocarditis, more than 80% (34 of 42) exhibiting medium or severe myocarditis. Even 10 pfu induced medium (25%) or severe (25%) myocarditis in half of the animals.

The effect of age of the infected mice on the outcome of infection became obvious in 3.5-week-old weanling mice (male and female). They all (22 of 22) died 3–5 days p.i. with signs of myocardial damage, irrespective of the route of infection (i.p. or subcutaneous; Table 1). Nevertheless, only 12 of the 86 infected male (8- to 20-week-old; animals in Tables 1 and 2 taken together) and none of the female (9- to 16-week-old) mice (0 to 15) died within 21 days. In adult mice of different age groups, the amount and distribution of myocarditis did not exhibit essential variations.

In weanling mice, the mortality and the pattern of myocardial damage differed completely from that of adults. Myocardial lesions in weanling mice consisted of disseminated myofibrillar degeneration (MFD), a special form of elective heart muscle necrosis (also called contraction band necrosis). The extent of this lesion in the left and right ventricular myocardium was best demonstrated in a modified LFB stain (Arnold et al. 1985) (Fig. 6). The nuclei of the damaged myocytes were pyknotic or lost. Many of the necrotic cells exhibited slight granular calcification; severe calcification was rarely encountered. These extended myocytic changes – in con-

Table 1. Encephalomyocarditis virus, plaque variant 7-infected NMRI and C57Bl/6 mice: age and sex dependence of myocarditis and mortality

Strain	Age (weeks)	Sex	Mortality	Myocardial damage[a]				Number not examined
				n	slight	medium	severe	
Infected groups								
NMRI	3.5	Male	22/22[b]	0	2	8	4	(8)
	8–9	Male	4/15	0	4	4	6	(1)
	12	Male	1/12	0	2	4	6	
	20	Male	3/11	0	0	4	4	(3)
Total of	8–20	Male	8/38[c]	0	6	12	16	(4)
NMRI	3.5	Female	10/10	0	3	4	0	(3)
	9	Female	0/5	0	2	3	0	
	16	Female	0/10	0	2	8	0	
Total of	9–16	Female	0/15	0	4	11	0	
C57Bl/6	10	Male	1/8	0	2	2	3	(1)
Control groups								
NMRI	3.5	Male	0/10	10	0	0	0	
NMRI	8–20	Male	1/38	38	0	0	0	
NMRI	3.5	Female	0/10	10	0	0	0	
NMRI	9–16	Female	1/15	14	0	0	0	
C57Bl/6	10–12	Male	0/14	14	0	0	0	

[a] For definition, see Methods
[b] Moribund animals were added to the perished ones and hearts were examined histologically; six animals were inoculated subcutaneously
[c] Two, 11, 9 and 8 apparently healthy mice were examined on days 5, 7, 14 and 21 respectively; additionally 2 moribund (on days 8 and 15) and 2 dead animals (on days 6 and 9) were examined

Fig. 3. Severe myocarditis in encephalomyocarditis virus plaque variant 7-infected NMRI mouse with areas of confluent foci of dense mononuclear infiltrates replacing necrotic myocytes; focal calcification at the lower right (black in the reproduction). H&E, ×318

trast to adult mice – were not usually accompanied by inflammatory infiltrates: lymphocytes, histiocytes and granulocytes were encountered only rarely and focally, mostly in the vicinity of calcified myocytes. Skeletal and diaphragmatic muscles also exhibited severe necrosis which, however, was accompanied by a more pronounced inflammatory infiltrate (muscular tissue other than myocardium was examined in 3.5-week-old mice only). The lack of other organ lesions suggests that the extensive amount of disseminated myocardial necrosis is responsible for death (see Discussion).

C57Bl/6 mice exhibited the same morphological de-

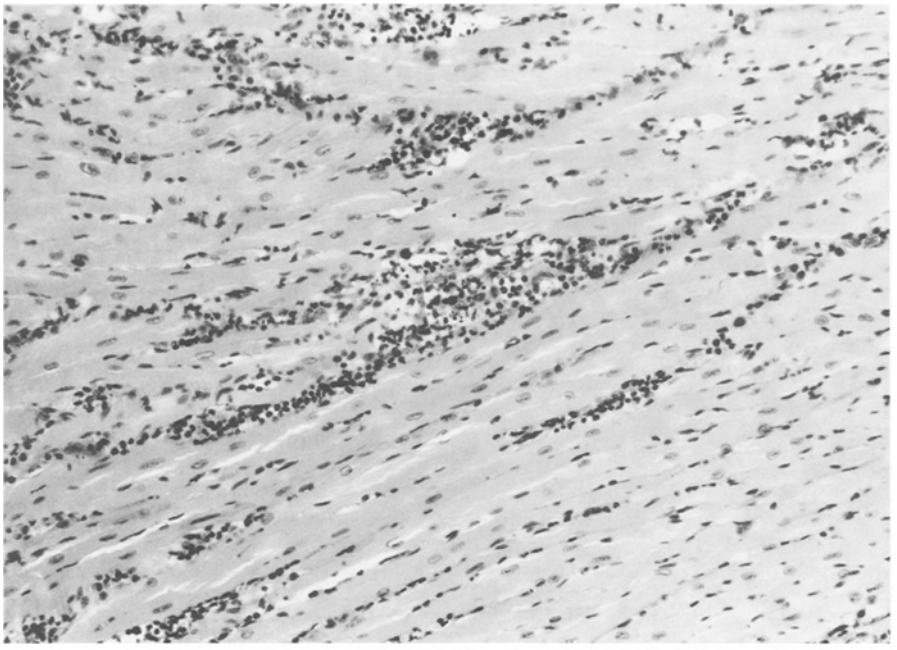

Fig. 4. Medium myocarditis in encephalomyocarditis virus plaque variant 7-infected NMRI mouse with several neighbouring dense infiltrates of varying size. H&E, ×318

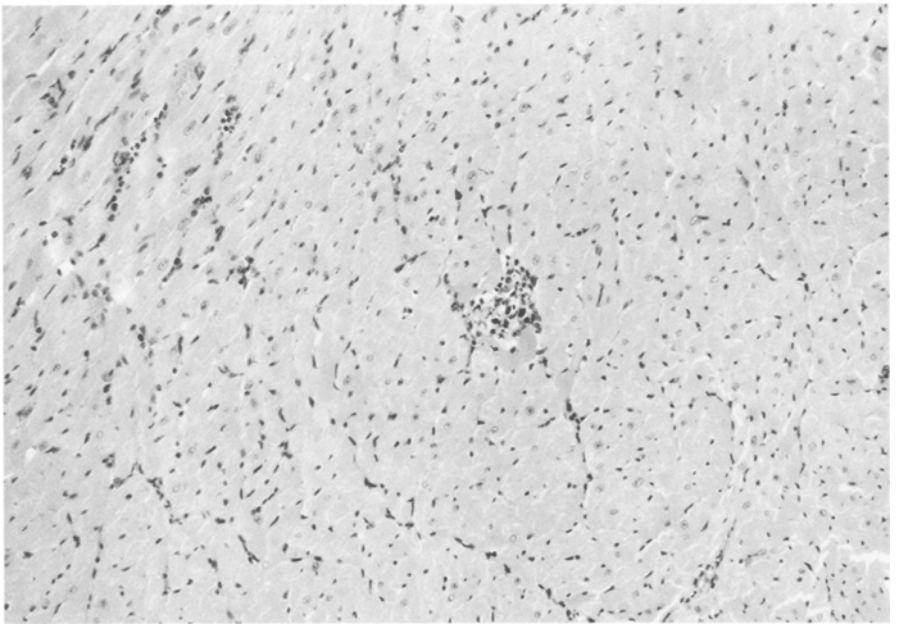

Fig. 5. Disseminated myocarditis in encephalomyocarditis virus plaque variant 7-infected NMRI mouse of slight degree; only a few small lesions are scattered throughout the myocardium (center and upper left). H&E, ×318

Table 2. Effect of virus dose on myocarditis and mortality in encephalomyocarditis virus plaque variant 7-infected NMRI mice

Virus dose (plaque forming units/animal)	Mortality	Myocardial damage				Number not examined
		n	slight	medium	severe	
10^1	1/12	2	4	3	3	
10^2	1/12	0	3	6	2	(1)
10^4	1/12	0	2	6	3	(1)
10^5	1/10[a]	0	3	3	3	(1)
10^7	1/12	0	0	7	4	(1)

[a] Animals included in group of 8- to 9-week-old males in Table 1

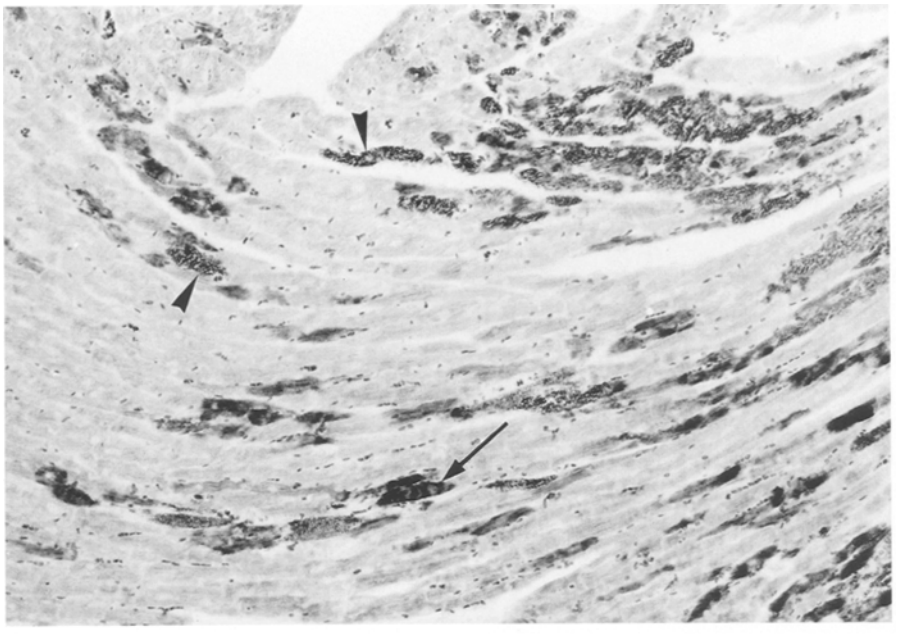

Fig. 6. Myocardial damage in a 3.5-week-old NMRI mouse dying 4 days after intraperitoneal inoculation of 10^5 plaque forming units of encephalomyocarditis virus plaque variant 7; disseminated myofibrillar degeneration (black), sometimes exhibiting prominent cross bands (*arrow*); note absence of mononuclear infiltrates; fine granulation of damaged myocytes (*arrowheads*) is due to calcification. Modified Luxol-fast blue stain, ×318

Fig. 7. Encephalomyocarditis virus plaque variant 7-induced myocarditis in an 8-week-old DBA/2 mouse, 7 days after intraperitoneal inoculation; scanty to moderate mononuclear infiltration; extensive granular and patchy calcification of necrotic myocytes (black in the reproduction). H&E, ×255

velopment of EMCV PV7-induced myocarditis as described for NMRI mice. The severity of myocarditis ranged from slight to severe (Table 1). No calcification was seen in infected or in control animals.

EMCV PV7-infected DBA/2 mice have been used as a model for virus-induced myocardial damage (Matsumori and Kawai 1982b), although "genetically predisposed" mineralization – mainly of the epicardium – is well known (DiPaolo et al. 1964; Ball and Williams 1965; Nabors and Ball 1969; Rings and Wagner 1972; Eaton et al. 1978; Brownstein 1983), and has also been observed in our laboratory. To examine whether "spontaneous" calcification may interfere with the course of virus-induced myocarditis ten male and ten female 8-week-old DBA/2 mice were infected with EMCV PV7. Three of the ten male animals perished before day 17. EMCV PV7 infection resulted in severe myocardial damage characterized by a subtotal calcification of clusters of necrotic myocytes (Fig. 7). The moderate to severe inflammatory infiltrate at the sites of necrosis consisted predominantly of lymphocytes and histiocytes, as seen in NMRI mice. The calcifications persisted after subsiding of the myocarditis and made up large confluent intramyocardial deposits subdivided and surrounded by fibrosis in animals of either sex examined on day 108 p.i.

In addition to calcification, apparently directly related to EMCV PV7 infection we found subepicardial mineralization of the right ventricular myocardium which was embedded in a fibrous stroma and lacked pro-

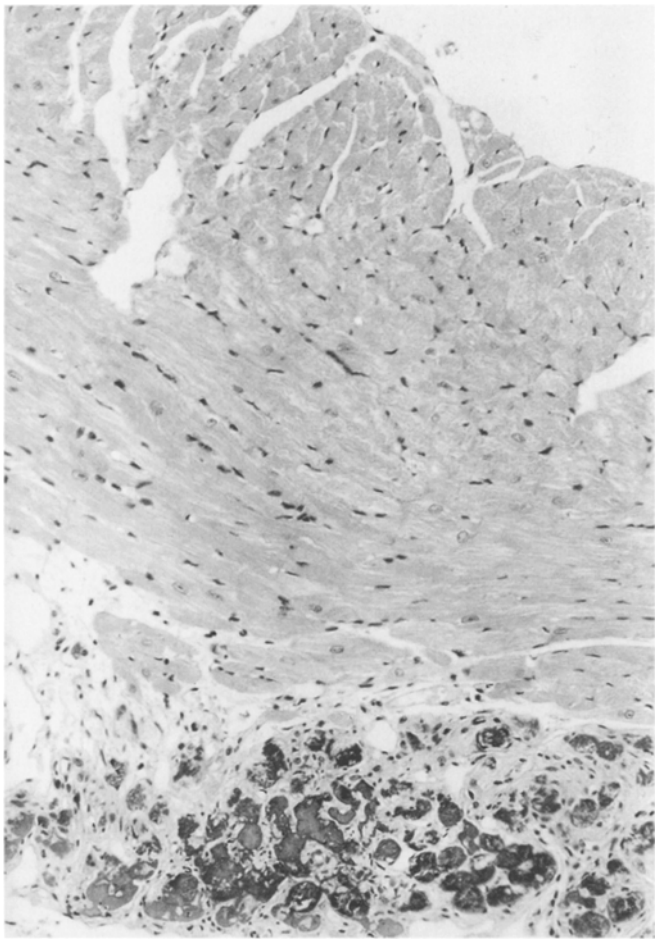

Fig. 8. Subepicardial rim of right ventricular calcification in DBA/2 non-infected control mouse of 8 weeks; fibrosis and sparse mononuclear infiltrates between the foci of calcification. H&E, × 382

nounced cellular infiltrates. This change occurred to varying degrees in noninfected control (Fig. 8) and in infected DBA/2 mice aged 8 weeks or older; control animals purchased from different breeding farms consistently showed the same degree of myocardial calcinosis. Most of the uninfected controls also exhibited calcification in the inner layers of the left myocardium but these were restricted to scattered single cell calcification.

Discussion

A significant result of our work is that EMCV PV7-infected adult (≥8-week-old) male NMRI mice consistently develop a distinct myocarditis. Well-defined stages of myocarditis are seen up to 21 days post virus inoculation. An exceptionally important fact is that in this model no other organs are damaged as determined by histopathological examination. Additional data are evident from the results on virus replication, which confirm the histological observations. Five days p.i. virus titres in the heart ($10^{5.4}$ pfu/ml 10% organ suspension) exceed those in the pancreas ($10^{2.5}$) and brain ($10^{2.9}$) significantly (data not shown).

Relatively low mortality (21%; 16 of 115) in adult male NMRI mice is a further advantage of the EMCV PV7-induced myocarditis model, since EMCV-M caused death in 33–67% of 12-week-old Swiss mice (Craighead 1966a, b), in 58–100% of 4-week-old DBA/2 mice (Matsumori and Kawai 1982b; Matsumori et al. 1985) and, taken together, in 92% of BALB/c, C3H/He and DBA/2 mice (Kishimoto et al. 1983). Other inbred mouse models used for studies of entero- and cardiovirus-induced myocarditis are also burdened by high mortality (43%, Matsumori and Kawai 1982a; 60%, Woodruff and Woodruff 1974; 80%, Huber et al. 1982), or low incidence of myocarditic damage (7 of 40 animals examined up to 22 days p.i.; Wilson et al. 1969).

In C57Bl/6 mice EMCV PV7 infection caused a similar low mortality to that in NMRI mice. Because of the general advantages of outbred mouse strains over inbred ones, all further experiments to study factors which might influence the incidence and severity of myocarditis were done with NMRI mice.

EMCV PV7 infection in female mice causes a less severe disease than in males: no mouse exhibited severe myocarditis and all mice survived (Table 1). Less severe infection in females has also been reported with other virus and mouse strains (Huber et al. 1981). According to Huber et al. (1982) mortality, cardiac necrosis score, and virus titre in the heart of CBV3-infected 6- to 8-week-old BALB/c mice account for 95%, score 2.6, and $10^{5.5}$ TCD_{50}/organ in males, and 9%, score 0.5, and $10^{3.8}$ TCD_{50} in females, respectively.

Myocardial lesions are present regardless of virus dose (Table 2), but with increasing doses myocarditis was more evenly distributed in each individual mouse. This observation was also reported for EMCV M-infected Swiss mice (Craighead 1966a).

In contrast to the reproducible, but non-lethal myocarditis in adults, EMCV PV7 was lethal in younger animals. This fact corresponds with the observation that in enterovirus-infected human newborns myocarditis has been most severe and accompanied by high death rate (Nagington et al. 1978). In our experiments all 3.5-week-old EMCV PV7-infected NMRI mice perished within 5 days (Table 1). The only damage demonstrable in these animals (except for necrosis of striated muscles, which may not be responsible for death) consists of disseminated MFD, a special, but pluricausal form of elective heart muscle cell necrosis (Fig. 6). This type of myocardial necrosis is well known, for instance, to be caused by endo- or exogenous catecholamine excess (Karch and Billingham 1986), cardiovascular shock (Reichenbach and Benditt 1968; Arnold et al. 1985), or different hypoxic or ischaemic myocardial conditions (Reichenbach and Benditt 1970; Arnold et al. 1985). In all these pathophysiological settings MFD is mediated by cellular calcium overload due to cell membrane damage (Fleckenstein et al. 1971; Karch and Billingham 1986). If disseminated MFD is extensive, it may cause death due to heart failure.

In 3.5-week-old EMCV PV7-infected mice as well as in all other age groups no lesions in organs were found apart from striated muscles (see above). Other possible

causes of death or the development of MFD-like cardiovascular shock due to severe organ necrosis (Kruppenbacher et al., in preparation) – were excluded. Our findings strongly suggest that the occurrence of MFD in EMCV PV7-infected weanling mice, with little inflammatory infiltrate, may be the consequence of a direct cytolytic virus effect. The mechanism by which virus-induced cell damage is connected with the calcium-linked pathophysiological pathway of MFD is unknown. The detection of calcium deposits in mitochondria of 3-week-old NMRI mice 24 h after EMCV infection (Meessen et al. 1975) may also point to a possible role of calcium disturbances in virus-induced cell death. This view is further supported by the tiny granular calcifications in cells with MFD in the infected weanling NMRI. EMCV PV7 infection in weanling NMRI mice could be an important model to examine the molecular biological pathomechanism of virus-induced cytolysis and its relationship to MFD.

The extensive calcification of EMCV PV7-induced myocardial necrosis in DBA/2 mice may indicate a possible connection of the cytolytic effects to calcium-mediated necrosis. DBA/2 mice consistently developed a pronounced myocarditis with low mortality comparable to that observed in NMRI mice. In contrast with NMRI mice, which only rarely exhibited patchy calcification of necrotic myocytes, DBA/2 mice constantly developed a subtotal calcification of the necrotic myocytes. Uninfected DBA/2 controls often exhibit globular calcium depositions in a band-like distribution in the subepicardial region of the right ventricular myocardium (Fig. 8) and calcified myocytes scattered in the left ventricle, whereas NMRI controls were free of any calcification. The extensive calcification of myocytes in EMCV PV7-infected DBA/2 mice appears to depend, at least partially, on a genetic defect of the DBA/2 strain, but it is uncertain how this genetically determined lesion may interfere with or aggravate virus-determined alterations. In contrast to Matsumori and Kawai (1982b) we do not believe that DBA/2 mice are suitable for study of the pathomechanisms of direct picornavirus-induced myocarditis and its long-term effects.

To our knowledge experimental myocarditis with consistent and considerable histologically verified lesions, no damage to other organs, and low mortality such as EMCV PV7 in adult NMRI mice has not been reported. Our present examinations indicate that the incidence, degree and nature of myocardial damage depend on properties of the murine host such as the H-2 haplotype (Reyes and Lerner 1985), age and sex, and on the infecting virus, since other EMCV mutants show quite a different pathological picture such as EMCV PV2 (Kruppenbacher et al. 1985) and EMCV B (Yoon et al. 1980) which are highly diabetogenic; EMCV PV21 (Kruppenbacher, unpublished data) and EMCV E (Craighead 1966a) which are lethal due to generalized infection. Even subtle changes of the picornaviral RNA may alter pathogenicity in a dramatic way, as has been shown for polioviruses (Evans et al. 1985; Eggers and Mertens 1987; Eggers 1991). This apparently is also true of EMCV (Kruppenbacher et al. 1985; Duke et al. 1990). The possible role of intraserotypic variants of picornaviruses for human myocarditis has not previously been studied.

In conclusion, we have established a picornavirus-outbred mouse strain (NMRI) model which achieves viral myocarditis reproducibly and with low mortality in adult animals. NMRI mice are not burdened by a genetic background which predisposes to spontaneous calcification, which occur in certain inbred mouse strains. The histological type of myocarditis and mortality are age-dependent; this offers the opportunity to study the basic determinants for virus-induced direct cytolysis as well as immunologically mediated cell damage. Further investigation of morphological and molecular biological features is feasible, for example the effects of sequential virus infections (Beck et al. 1990; Okada et al. 1992). Long-term changes analogous to those observed in human cardiomyopathy may also be explored.

Acknowledgement. This work was supported in part by the Deutsche Forschungsgemeinschaft (grant: IIB6-Eg 10/7-1).

References

Arnold G, Kaiser C, Fischer R (1985) Myofibrillar degeneration: a common type of myocardial lesion and its selective identification by a modified luxol fast blue stain. Pathol Res Pract 180:405–415

Ball CR, Williams WL (1965) Spontaneous and dietary-induced cardiovascular lesions in DBA mice. Anat Rec 152:199–203

Beck MA, Chapman NM, McManus BM, Mullican JC, Tracy S (1990) Enterovirus infection in the murine model of myocarditis: pathologic and immunologic aspects. Am J Pathol 136:669–681

Brownstein DG (1983) Genetics of dystrophic epicardial mineralization in DBA/2 mice. Lab Anim Sci 33:247–248

Craighead JE (1966a) Pathogenicity of the M and E variants of the encephalomyocarditis (EMC) virus. I. Myocardiotropic and neurotropic properties. Am J Pathol 48:333–345

Craighead JE (1966b) Pathogenicity of the M and E variants of the encephalomyocarditis (EMC) virus. II. Lesions of the pancreas, parotid, and lacrimal glands. Am J Pathol 48:375–386

DiPaolo JA, Strong LC, Moore GE (1964) Calcareous pericarditis in mice of several genetically related strains. Proc Soc Exp Biol Med 115:496–497

Duke GM, Osorio JE, Palmenberg AC (1990) Attenuation of Mengo virus through genetic engenering of the 5′ noncoding poly(C) tract. Nature 343:474–476

Eaton GJ, Custer RP, Johnson FN, Stabenow KT (1978) Dystrophic cardiac calcinosis in mice: genetic, hormonal, and dietary influences. Am J Pathol 90:173–186

Eggers HJ (1991) Studien zur Pathogenese von Picornavirus-Infektionen. Nova Acta Leopold NF 66 279:49–60

Eggers HJ, Mertens T (1987) Viruses and myocardium: notes of a virologist. Eur Heart J 8 [Suppl]:129–133

Evans DMA, Dunn G, Minor PD, Schild GC, Cann AJ, Stanway G, Almond JW, Currey K, Maizel JV (1985) Increased neurovirulence associated with a single nucleotide change in a noncoding region of the Sabin type 3 poliovaccine genome. Nature 314:548–550

Fleckenstein A, Janke J, Döring HJ, Leder O (1971) Die intracelluläre Überladung mit Kalzium als entscheidender Kausalfaktor bei der Entstehung nicht-coronarogener Myokard-Nekrosen. Verh Dtsch Ges Kreislaufforsch 37:345–353

Huber SA, Job LP, Woodruff JF (1981) Sex-related differences in the pattern of coxsackievirus B-3-induced immune spleen

cell cytotoxicity against virus-infected myofibers. Infect Immun 32:68–73

Huber SA, Job LP, Auld KR (1982) Influence of sex hormones on coxsackie B-3 virus infection in Balb/c mice. Cell Immunol 67:173–189

Kaplan EL, Meier P (1958) Nonparametric estimation of incomplete observations. Am Stat Assoc 53:457–481

Karch SB, Billingham ME (1986) Myocardial contraction bands revisited. Hum Pathol 17:9–13

Kishimoto C, Matsumori A, Kawai C (1983) Electrocardiographic findings in experimental murine myocarditis: arrhythmias in the chronic stage. Jpn Circ J 47:1317–1321

Kruppenbacher JP, Mertens T, Müntefering H, Eggers HJ (1985) Encephalomyocarditis virus and diabetes mellitus: studies on virus mutants in susceptible and non-susceptible mice. J Gen Virol 66:727–732

Matsumori A, Kawai C (1982a) An experimental model for congestive heart failure after encephalomyocarditis virus myocarditis in mice. Circulation 65:1230–1235

Matsumori A, Kawai C (1982b) An animal model of congestive (dilated) cardiomyopathy: dilatation and hypertrophy of the heart in the chronic stage in DBA/2 mice with myocarditis caused by encephalomyocarditis virus. Circulation 66:355–360

Matsumori A, Wang H, Abelmann WH, Crumpacker CS (1985) Treatment of viral myocarditis with ribavirin in an animal preparation. Circulation 71:834–839

Meessen H, Müntefering H, Schmidt WAK, Müller-Ruchholtz ER, Kieker W-R (1975) Virus-induced damage of the myocardial cell. Recent advances in studies on cardiac structure and metabolism, vol 6. In: Fleckenstein A, Rona G (eds) Pathophysiology and morphology of myocardial cell alteration. University Park Press, Baltimore, pp 525–533

Nabors CE, Ball CR (1969) Spontaneous calcification in hearts of DBA mice. Anat Rec 164:153–162

Nagington J, Wreghitt TG, Gandy G, Roberton NRC, Berry PJ (1978) Fatal echovirus 11 infections in outbreak in a special-care baby unit. Lancet II:725

Okada I, Matsumori A, Tomioka N, Kawai C (1992) Successive infections of coxsackievirus B3 and encephalomyocarditis virus: an animal model of chronic myocarditis. J Pathol 167:341–347

Reichenbach DD, Benditt EP (1968) Myofibrillar degeneration. A response of the myocardial cell to injury. Arch Pathol 85:189–199

Reichenbach DD, Benditt EP (1970) Catecholamine and cardiomyopathy: the pathogenesis and potential importance of myofibrillar degeneration. Hum Pathol 1:125–150

Reyes MP, Lerner AM (1985) Coxsackievirus myocarditis: with special reference to acute and chronic effects. Prog Cardiovasc Dis 27:373–394

Rings RW, Wagner JE (1972) Incidence of cardiac and other soft tissue mineralization lesions in DBA/2 mice. Lab Anim Sci 22:344–352

Schürmann W, Eggers HJ (1983) Antiviral activity of an alcoholic hand disinfectant. Comparison of the in vitro suspension test, in vivo experiments on hands, and on individual fingertips. Antiviral Res 3:25–41

Wilson FM, Miranda QR, Chason JL, Lerner AM (1969) Residual pathologic changes following murine coxsackie A and B myocarditis. Am J Pathol 55:253–256

Woodruff JF, Woodruff JJ (1974) Involvement of T lymphocytes in the pathogenesis of coxsackievirus B3 heart disease. J Immunol 113:1726–1734

Yoon JW, McClintock PR, Onodera T, Notkins AL (1980) Virus-induced diabetes mellitus. XVIII. Inhibition by a nondiabetogenic variant of encephalomyocarditis virus. J Exp Med 152:878–892