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Variable Susceptibility of Mice to Group B Coxsackievirus Infections

LINDA L. MINNICH AND C. GEORGE RAY*

Departments of Pathology and Pediatrics, University of Arizona College of Medicine, Tucson, Arizona 85724*

Laboratory strains of group B coxsackievirus serotypes 1 to 6 were inoculated intraperitoneally into newborn mice of differing genetic backgrounds. Of the four genetic strains investigated, C3H mice appeared to be resistant to all six serotypes, whereas BALB/c mice were most susceptible. Swiss mice and a random-bred Swiss strain (COH) were intermediate in susceptibility. The findings underscore the fact that clinical isolation attempts and experimental studies involving group B coxsackieviruses must take into account both the virus strain used and the genetic background of the host. For clinical isolation of these viruses, the BALB/c mouse may be the most suitable of the strains tested.

Group B coxsackieviruses have been associated with clinical syndromes in humans which range from asymptomatic infections to fatal disseminated disease (1). These viruses have also been employed in various experimental models, including the mouse and the monkey. Group B coxsackieviruses usually produce spastic paralysis in suckling mice when inoculated during the first 48 h of life (1). Older mice demonstrate varying degrees of susceptibility; the heart has been the target organ most extensively investigated (1).

In 1952, Godman et al. described the histopathology of group B coxsackieviruses in suckling mice (3). Since then, numerous investigators have used various mouse strains and different serotypes. In an attempt to determine what effects the six serotypes cause in differing mouse strains, a standardized dose of each was inoculated into suckling mice of varying genetic backgrounds. The observations indicate the need for special consideration when selecting mouse strains for clinical diagnostic or research purposes.

MATERIALS AND METHODS

Virus. Group B coxsackievirus types 1, 2, 3, 4, 5, and 6 were obtained from ill patients and passaged at least 20 times in LLC-MK2 cell cultures (a continuous cell line derived from rhesus monkey kidney). When cells displayed 75 to 100% viral cytopathic effects, the cultures were harvested by scraping the cells into the fluids. Suspensions were centrifuged at 3,000 rpm for 10 min, and the supernatant fluids were dispensed and frozen at −70°C. A sample of each serotype was titered in LLC-MK2 cells and typed with specific antisera obtained from Microbiological Associates (Bethesda, Md.) and the Center for Disease Control, Atlanta, Ga. Virus preparations were thawed and diluted in phosphate-buffered saline immediately before mouse inoculation. Uninfected LLC-MK2 cell cultures were processed in like manner to serve as a tissue culture control.

Mice. BALB/c, Swiss, and C3H mice were obtained from the colony maintained at the Department of Microbiology and Immunology, University of Washington, Seattle. COH mice (a random-bred Swiss strain) were obtained from a colony maintained at Children's Orthopedic Hospital, Seattle, Wash. Mice were allowed free access to water and Purina mouse chow. Inoculated mice were placed in a separate room from the breeding colony to prevent cross-infection.

Procedure. At least 20 newborn mice (less than 12 h of age) of each strain were inoculated intraperitoneally with 5×10^4 50% tissue culture infectious doses (5) of group B coxsackievirus type 1, 2, 3, 4, 5, or 6 in 0.05 ml, or with 0.05 ml of tissue culture control, and immediately returned to their mothers. They were observed at least every 8 h for morbidity and mortality for 2 weeks. Morbidity was defined as spastic paralysis, dehydration, failure to nurse, failure to gain weight, or convulsions. Surviving mice were sacrificed at 2 weeks of age. Immediately after death, heart, brain, liver, and pancreas were removed and placed in Bouin fixative for 24 h, then transferred to buffered Formalin. Tissues were paraffin embedded, sectioned at 5 μm, stained with hematoxylin and eosin, and examined. Whole-organ sections were read under code and scored as follows: 0, no necrosis or inflammation evident; +, mild, focal necrosis, inflammation, or both (less than 10% of section affected); ++, moderate necrosis, inflammation, or both (10 to 20% of section affected); +++, severe necrosis, inflammation, or both (more than 20% of section affected).

RESULTS

Total morbidity and mortality of the four strains of mice infected with group B coxsackievirus serotypes 1 to 6 are summarized in Tables 1 and 2. No effects were noted in mice injected with tissue culture control.

None of the C3H mice injected with any group

B coxsackievirus serotype was affected. Additional C3H mice were inoculated and serially sacrificed at 2-day intervals to observe histopathology. No evidence of myocarditis, pancreatitis, hepatitis, or encephalitis could be found.

BALB/c mice were most uniformly affected, with 100% maximum morbidity with all six serotypes. They also had the highest mortality with group B coxsackievirus types 1, 3, and 4 and intermediate mortality with types 5 and 6.

Swiss mice had 100% maximum morbidity coxsackievirus types B1, B2, B3, B5, and B6 and 94% maximum morbidity with type B4. However, these mice were less clinically ill as demonstrated by their lower mortality rates, ranging from 9% with type B3 to a high of 20% with types B1 and B4.

The COH mice demonstrated the lowest maximum morbidity of the three susceptible mouse strains investigated. Morbidity ranged from 55% with coxsackievirus type B5 to 100% with type B2. The mortality rates ranged from none with type B5 to 63% with type B4.

The histopathology observed in the heart, liver, pancreas, and brain is summarized in Table 3. Myocarditis was produced in varying degrees by all six serotypes. Group B coxsackievirus types 3 and 4 most consistently produced myocarditis in the three susceptible mouse strains. No myocarditis could be detected in COH or BALB/c mice inoculated with type B2. In general, the lesions consisted of focal necrosis with lymphocytic and plasma cell infiltrates. Polymorphonuclear cells were observed only in the myocardial sections from mice that died less than 6 days after inoculation.

Like myocarditis, hepatitis was one of the more nearly uniform findings. It was moderate to severe in BALB/c, Swiss, and COH mice inoculated with virus types B1, B2, and B3. When types B4, B5, and B6 produced hepatitis, it was mild and focal. The hepatic lesions consisted of necrotic areas of hepatocytes with mononuclear infiltrates. This inflammation was transient; surviving mice sacrificed at 2 weeks did not show evidence of persistent hepatic damage.

Pancreatitis was produced in Swiss mice only by coxsackievirus type B1. BALB/c mice developed mild to moderate pancreatitis when challenged with types B1, B3, B4, and B6. Types B2, B3, and B4 produced pancreatitis in COH mice. The lesions were primarily acinar, with cell necrosis and infiltrates of polymorphonuclear cells, lymphocytes, a few plasma cells, and eosinophils. Occasional islets also had focal necrosis with mononuclear infiltrates.

BALB/c mice developed encephalitis most frequently, ranging from mild with virus types B1 and B2 to moderate with types B4 and B5. Individual mouse variation was observed with type B3, and no encephalitis was seen with coxsackievirus type B6. The encephalitis consisted of focal neuronal necrosis and occasional perivascular mononuclear cell infiltrates.

COH mice infected with coxsackievirus type B1 developed a persistent polymyositis of the extensor muscles of the hind legs. This was not observed with other serotypes or other mouse strains, and is the subject of a separate report (4).

TABLE 1. *Maximum percent morbidity in four genetic strains of mice infected with group B coxsackieviruses*

Mouse strain	Coxsackievirus serotype						
	B1	B2	B3	B4	B5	B6	TCC[a]
COH	65	100	87	87	55	66	0
Swiss	100	100	100	94	100	100	0
BALB/c	100	100	100	100	100	100	0
C3H	0	0	0	0	0	0	0

[a] Tissue culture control.

DISCUSSION

These studies utilized laboratory strains of group B coxsackieviruses standardized according to passage level and inoculum. The findings indicated that generalizations regarding the behavior of different group B coxsackievirus serotypes in a mouse model must be made cautiously, particularly where the genetic strain of mouse used is concerned.

TABLE 2. *Total percent mortality in four genetic strains of mice infected with group B coxsackieviruses*

Mouse strains	Coxsackievirus serotype						
	B1	B2	B3	B4	B5	B6	TCC[a]
COH	29 (30/105)[b]	29 (8/28)	20 (8/41)	63 (27/43)	0 (0/33)	3 (1/32)	0 (0/23)
Swiss	20 (6/30)	10 (2/20)	9 (2/22)	20 (7/35)	12 (3/26)	17 (5/29)	0 (0/24)
BALB/c	23 (7/31)	0 (0/24)	60 (12/20)	45 (9/20)	4 (1/23)	15 (3/20)	0 (0/26)
C3H	0 (0/26)	0 (0/23)	0 (0/22)	0 (0/22)	0 (0/26)	0 (0/21)	0 (0/30)

[a] Tissue culture control.
[b] Total deaths/total inoculated.

TABLE 3. *Histopathological findings by mouse strain and group B coxsackievirus serotype*

Finding	Mouse strain[a]	Coxsackievirus serotype[b]					
		B1	B2	B3	B4	B5	B6
Myocarditis	BALB/c	+	0	+++	++	+	+-++
	Swiss	+	+	+-+++	+++	+	0-+
	COH	+	0	+	+-+++	++	+
Hepatitis	BALB/c	+-++	+++	++	0-+	0	++
	Swiss	++	++	+++	0-+	+	0
	COH	++-+++	+	+-+++	+	+-++	+
Pancreatitis	BALB/c	+	0	+	++	0	+-++
	Swiss	++	0	0	0	0	0
	COH	0	++	+	++	0	0
Encephalitis	BALB/c	+	+	0-+++	++	++	0
	Swiss	0	0	0	+++	0	0
	COH	+	0	+	+	0	0

[a] No histopathology was noted in C3H mice.
[b] See the text for scoring system.

Another important factor in the production of organ-specific lesions is the strain of virus used. Studies by ourselves (4) and others (2, 6) have shown that there can be a great variability in tissue tropism by different strains within a given group B coxsackievirus serotype. Gauntt et al. have investigated this phenomenon in type B3 variants, and were unable to account for the differences on the basis of defective interfering particle production, interferon induction, or interferon sensitivity (2).

The remarkable resistance of C3H mice to all six virus strains tested remains unexplained and deserves further investigation. Yoon et al. observed a similar resistance by C3H and BALB/c mice when they were challenged with a diabetogenic variant of type B4 virus (6).

Finally, the findings in our limited study of mice with different genetic backgrounds suggest that the strain of mouse should be considered when mice are required for isolation, propagation, and identification of group B coxsackieviruses from clinical specimens.

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