JOURNAL OF VIROLOGY, Apr. 1986, p. 152–156
0022-538X/86/040152-05$02.00/0

Vol. 58, No. 1

Genetically Determined Resistance to Murine Cytomegalovirus and Herpes Simplex Virus in Newborn Mice

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Received 8 July 1985/Accepted 18 December 1985

Mice which were infected with the herpesvirus murine cytomegalovirus or herpes simplex virus type 1 on the day of birth exhibited mouse strain-dependent differences in the development of lethal disease. The pattern of resistance among the strains was distinct for each virus and closely resembled that reported in adult mice. However, much lower doses of the viruses were required in newborn mice to reveal these resistance patterns. For murine cytomegalovirus, both *H-2*–associated and non–*H-2* genes conferred resistance, and, as has been shown for adults, there was a 25-fold difference in the dose required to kill 50% of the animals belonging to the most resistant and susceptible strains. The resistance of newborn mice to herpes simplex virus type 1 was conferred by non–*H-2* genes in C57BL/6 mice, as has been reported for adults, and newborn C57BL/6 mice were considerably more resistant than mice of susceptible strains. Resistance was also reflected in the titer of these viruses in the spleen or liver early in infection and, with murine cytomegalovirus, in the survival time of infected mice. The resistance of newborn mice to lethal disease was not conferred postnatally by the mother. This appears to be the first report of genetically determined resistance to herpesviruses in newborn mice. Such autonomous virus-specific resistance may provide a significant barrier to naturally acquired infection in genetically resistant strains. Similar genetically regulated mechanisms may protect the newborns of many species, including humans, against infection with herpesviruses.

The greater susceptibility to virus infection of newborn compared with adult animals has been extensively documented in many species (21). With respect to the herpesviruses cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV-1), newborn mice are much more susceptible than adults (2, 5, 8, 9, 13, 17), and in man pre- or postnatal infection with CMV or HSV-1, respectively, can be severe or even lethal (14, 15, 23), in contrast to the benign form experienced by healthy adults. However, the effectiveness of resistance mechanisms not only is age dependent but is also regulated by genetic factors which are generally virus specific. In adult mice, resistance to murine CMV (MCMV) is regulated by both *H-2*– and non–*H-2*-associated genes (3, 5), and resistance to HSV-1 is independently regulated by non–*H-2* genes (11, 12).

Genetic control of resistance has not been widely studied in newborn animals, and several reports have suggested that resistance genes do not influence infection with MCMV or HSV-1 in the newborn period (5, 24). However, our recent observation of genetically controlled resistance to these viruses in vitro in mouse embryo fibroblasts (7) prompted us to reexamine whether resistance genes influence infection with MCMV or HSV-1 in newborn mice. We report that virus-specific, genetically determined resistance to infection does indeed function in mice infected on the day of birth with these viruses and shows the same mouse strain distribution pattern as in adult mice.

MATERIALS AND METHODS

Animals. Pregnant, specific pathogen-free BALB/c, BALB.B, BALB.K, A/J, C3H/HeJ, CBA/CaH, C57BL/10ScSn, C57BL/6J, B10.BR, and B10.A mice were obtained from the Animal Resources Centre, Murdoch, Western Australia, Australia. They were held under barrier conditions in a minimal disease facility with sterile bedding, food, water, and cages with filter tops. Mice were injected intraperitoneally (i.p.) when less than 1 day old with 50 μl of a freshly prepared dilution of virus in phosphate-buffered saline (pH 7.2) with the osmolality of mouse serum, containing 0.1% fetal calf serum.

Virus. Salivary gland homogenates containing MCMV were prepared from BALB/c mice infected with the Smith strain of MCMV, as described elsewhere (1). Ampoules of a bacterially sterile master stock were stored in liquid nitrogen. The preparation of MCMV from which this stock was derived has been shown to be free of a number of contaminating viruses (6). HSV-1 2931 was kindly supplied by Carlos Lopez, Memorial Sloan-Kettering Institute for Cancer Research, New York, N.Y. It was passaged once in Vero cells at a low multiplicity of infection, the supernatant was clarified at 3,000 × *g*, and the virus was pelleted at 50,000 × *g* for 1 h at 4°C. The resuspended pellet was put in an ampoule and stored in liquid nitrogen. For the estimation of virus content in organs, 10% homogenates were prepared as described elsewhere (1). The number of PFU of HSV-1 or MCMV was counted after incubation for 3 days in Vero cells or 5 days in CD-1 secondary mouse embryo fibroblasts, respectively, using a methyl cellulose overlay (1).

The minimum i.p. dose which killed 50% of the animals (LD_{50}) was calculated by using the Kaerber equation from the data obtained by injecting newborn mice with one of a series of 2-fold dilutions of MCMV or 10-fold dilutions of HSV-1 which covered the range of 0 to 100% mortality. Three to five litters were used for each dilution. Only litters containing four to seven offspring were used, because the most reproducible results are obtained with litters of this size. Litters were observed for deaths until the mice were 6 weeks old. A single batch of each virus was used in all newborn mice.

Statistics. Chi-square analysis was used for comparing differences in the dose response of the various mouse strains over the same range of virus dilutions. Differences in the

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TABLE 1. Genetically determined resistance to herpesviruses in newborn mice and comparison with the resistance of adult mice

Strain	H-2 haplotype	MCMV				HSV-1		
		Newborn mice		Adult mice		Newborn mice		Adult mice
		Rel. LD_{50}[a]	Dose for 1 LD_{50} (PFU)	Rel. LD_{50}	Dose for 1 LD_{50} (PFU)	Rel. LD_{50}[b]	Dose for 1 LD_{50} (PFU)	Dose for 1 LD_{50} (PFU)[c]
BALB/c	*d*	1.0	≅10	1.0	5×10^4	1	≅1	$10^{2.34}$
BAAB.B	*b*	1.0	≅10	1.0		3	≅4	ND[d]
BALB.K	*k*	22.0	224	10–12		1	≅1	ND
C57BL/10	*b*	6.1	62	2–4		ND	ND	ND
C57BL/6	*b*	ND	ND	2–4		362	520	$>10^6$
B10.A	*a*	8.8	89	2–4		362	520	$>10^6$
B10.BR	*k*	24.6	250	30–40	1.6×10^6	157	226	$>10^6$
A/J	*a*	<1.2	<12	0.5		1	≅1	$10^{1.33}$
C3H	*k*	12.0	122	20–30		5	≅7	ND
CBA	*k*	20.3	206	20–30		5	≅7	$10^{2.34}$

[a] Rel. LD_{50}, Relative LD_{50}; the LD_{50} value for a given mouse strain compared with the value for BALB/c mice of the same age was arbitrarily set at 1.0^{50} unit. Relative LD_{50} values in adults were previously determined in our laboratory (1,3,5), using 8- to 10-week-old female mice.

[b] Value relative to the LD_{50} in HSV-1–infected newborn BALB/c mice, set at 1.0 unit.

[c] Taken from Lopez (11,12), who used the same strain of HSV-1 (2931), and confirmed for BALB/c, C57BL/6, CBA, and A/J mice in this laboratory (G. R. Shellam, unpublished observation).

[d] ND, Not done.

mean time to death of the various strains were analyzed by Student's *t* test.

RESULTS

Genetically determined resistance to MCMV in newborn mice. When LD_{50} titrations were performed with MCMV in mice which were infected on the day of birth, mouse strain-dependent variations in the LD_{50} were observed (Table 1). These variations were reproducible, and the differences between certain strains were statistically significant. Furthermore, the variations closely resembled those which have been established in this laboratory for adult mice of the same strains (1, 3, 5; Table 1). Newborn BALB/c (*H-2*^{d}), BALB.B (*H-2*^{b}), and A/J (*H-2*^{a}) mice were the most susceptible. Their LD_{50} corresponded to about 10 PFU, and the mortality curves obtained for these strains over the same range of virus dilution were not significantly different ($P > 0.1$). In contrast, the LD_{50} in BALB/c mice differed from that in all other strains, and the difference between the mortality curves of BALB/c and these strains was highly significant ($P < 0.001$). Intermediate resistance was exhibited by C57BL/10 (*H-2*^{b}) and B10.A (*H-2*^{a}) mice, whereas B10.BR (*H-2*^{k}), CBA (*H-2*^{k}), BALB.K (*H-2*^{k}), and C3H (*H-2*^{k}) were the most resistant strains. The *H-2*^{k} haplotype was protective (cf. BALB.K and BALB/c, $P < 0.001$; cf. B10.BR and C57BL/10, $P < 0.001$), as were non–*H-2* genes in the C57BL/10 strain (cf. C57BL/10 and BALB.B, $P < 0.001$; cf. B10.A and A/J, $P < 0.001$), as previously described in adult mice (1, 3, 5). This experiment was repeated in BALB/c, C57BL/10, CBA, and C3H mice with very similar results. However, newborn mice were considerably more susceptible than their adult counterparts (Table 1), confirming previous studies with MCMV (5, 13, 17) and experience with other viruses (21). One LD_{50} in newborn BALB/c mice

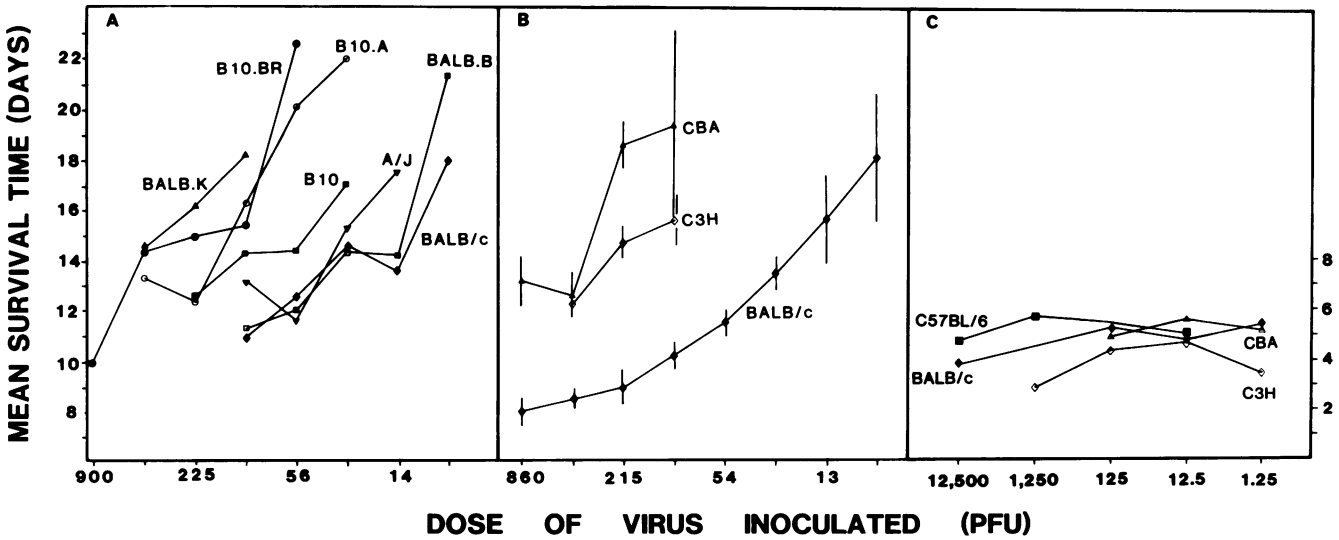

FIG. 1. Strain-dependent variations in survival time after MCMV or HSV-1 infection. Mice were inoculated i.p. when <1 day old with one of a range of doses of these viruses. Three to five litters were used for each virus dilution. Panels A and B detail two separate experiments with MCMV. In panel C, HSV-1 was used.

TABLE 2. Survival time of mice infected at birth with lethal doses of MCMV

Strain	Mean time to death (no. of days ± SE) with	
	1.22×10^4 PFU	7.63×10^2 PFU
BALB/c	5.1 ± 0.1[a]	6.8 ± 0.2[b]
CBA	9.6 ± 0.9	12.9 ± 0.6
(BALB/c × CBA)F_1	7.7 ± 0.5	10.5 ± 0.6
C3H	9.7 ± 0.3	10.9 ± 0.5
C57BL/6	7.9 ± 0.4	11.0 ± 0.2

[a] For comparison with all other groups, $P < 0.0005$. There were between 8 and 14 mice (two to three litters) per group.
[b] For comparison with all other groups, $P < 0.0005$. There were between 22 and 34 mice (four to seven litters) per group.

corresponded to about 10 PFU of MCMV, whereas the LD_{50} in adult BALB/c mice was 5×10^4 PFU with the same batch of virus. Similarly, in B10.BR mice, 1 LD_{50} corresponded to 250 PFU in newborns, compared with 1.6×10^6 PFU in adult mice.

Mice of susceptible strains also died sooner than mice of resistant strains when given the same dose of MCMV (Fig. 1A and 1B; Table 2), and a 10- to 20-fold-larger dose was required to kill CBA and C3H mice at the same time after infection as mice of the susceptible BALB/c strain (Fig. 1B). Even with a dose of 1.22×10^4 PFU, which was equivalent to about 60 LD_{50}s in newborn CBA mice, C3H, CBA, and C57BL/6 mice survived significantly longer than BALB/c mice ($P < 0.005$; Table 2), and these differences were larger when 7.63×10^2 PFU was used. Both virus doses were uniformly lethal in all strains (Table 2). (BALB/c x CBA)F_1 mice died later than BALB/c mice ($P < 0.005$) and had a resistance between those of the parental strains (Table 2).

Resistance to HSV-1. Newborn mice infected with HSV-1 were also much more susceptible than adults, and a dose estimated to be about 1 PFU was sufficient to kill 50% of the BALB/c, BALB.K, and A/J mice (Table 1). However, genetically controlled resistance was apparent (Table 1), with a mouse strain distribution pattern similar to that reported for the same strain of HSV-1 in 12- to 16-week-old male mice (11, 12). Thus, C57BL/6 (*H-2*^{b}), B10.A (*H-2*^{a}), and B10.BR (*H-2*^{k}) mice were much more resistant than CBA (*H-2*^{k}), C3H (*H-2*^{k}), BALB/c (*H-2*^{d}), BALB.B (*H-2*^{b}), BALB.K (*H-2*^{k}), and A/J (*H-2*^{a}) mice (cf. C57BL/6, B10.A, and B10.BR with BALB/c, $P < 0.001$; cf. BALB/c and either BALB.B, BALB.K, A/J, CBA, or C3H, $P > 0.1$). Resistance appeared to be conferred by non–*H-2* genes in the C57BL strain (cf. C57BL/6 and BALB.B, $P < 0.001$; cf. B10.A and A/J, $P < 0.001$; cf. B10.BR and BALB.K, $P < 0.001$). This experiment was repeated in BALB/c, CBA, and C57BL/6 mice with very similar results. However, although genetically controlled resistance exhibited the same mouse strain distribution pattern in newborn and adult mice, it should be noted that the difference between the LD_{50}s in the most susceptible and resistant strains was greater in adult than newborn mice. Thus, in adult mice there was an approximately 100,000-fold difference in dose compared with about 500-fold in newborn animals.

Virus specificity and the role of maternal factors. For each virus the distribution of the resistance phenotype among the mouse strains was different (Table 1). Thus, for MCMV, C57BL mice were moderately susceptible and CBA, C3H, and BALB.K mice were resistant compared with BALB/c mice. In contrast, for HSV-1, C57BL mice were resistant and CBA, C3H, and BALB.K mice were susceptible, as were BALB/c mice. Another difference between the viruses was in the survival time of infected mice. Infection with HSV-1 resulted in earlier deaths than with MCMV, and in contrast to MCMV there was no correlation between survival time and resistance status. Over a 10,000-fold HSV-1 dose range, deaths usually occurred between day 3 and 6 in C57BL/6, BALB/c, C3H, and CBA mice (Fig. 1C) and in all the other strains in which HSV-1 was used (data not shown), whereas over a similar range of MCMV doses, deaths occurred between day 5 and 22 (Fig. 1A and 1B; Table 2). At about 1 LD_{50} of HSV-1 appropriate for each strain, BALB/c, C57BL/6, and CBA mice died at 5.4, 5.7, and 4.7 days, respectively, whereas at close to 1 LD_{50} of MCMV for each of these strains, deaths occurred at a mean of 15.6, 14.4, and 18.6 days, respectively.

The possibility that maternal factors contributed to the strain-related variation in the effect of MCMV or HSV-1 was studied by fostering infected mice on the day of birth with mothers of different resistance status. Because the survival time of MCMV-infected mice reflected their resistance status (Fig. 1A and 1B; Table 2), survival times of fostered and normally reared mice were compared. Susceptible BALB/c mothers did not influence the survival time of MCMV-infected CBA mice, and CBA mothers did not influence the survival time of infected BALB/c mice (Table 3), indicating that there was no postnatal maternal contribution to resistance status.

However, with HSV-1, deaths occurred at about the same time after infection regardless of genetic resistance status (Fig. 1C). Thus, the effect of foster mothers of different genetic status was studied by comparing the proportion of infected mice which survived infection in litters which were reared by natural or foster mothers. It was found that foster mothers did not affect the proportion of survivors (Table 3); C57BL/6 mothers did not make BALB/c newborns more resistant to HSV-1, and BALB/c mothers did not confer greater susceptibility on newborn C57BL/6 mice. Foster mothers also did not affect the survival time of HSV-1–infected mice (data not shown).

The differences in resistance to MCMV or HSV-1 of newborn mice of the various strains were also reflected in

TABLE 3. Effect of fostering newborn mice on mothers of different resistance status[a]

Newborn mouse strain	Foster mother strain	No. of days (mean ± SE) with MCMV at 7.6×10^2 PFU[b]	No. of mice alive/no. inoculated with HSV-1 at:	
			2.5×10^2 PFU	2.5×10^1 PFU
CBA		13.0 ± 0.6		
CBA	BALB/c	14.0 ± 1.7		
BALB/c		6.8 ± 0.2[c]		
BALB/c	CBA	6.4 ± 0.6[c]		
BALB/c				2/20 (10.0)[d]
BALB/c	C57BL/6			3/21 (14.3)
C57BL/6			12/19 (63.2)	
C57BL/6	BALB/c		17/32 (53.1)	

[a] Newborn mice were either left with their natural mothers or were fostered when <1 day old as indicated. For each virus, all mice were inoculated i.p. on the same occasion when <1 day old.
[b] One litter, consisting of five mice, of each strain was used. The MCMV dose used was lethal to all the mice.
[c] For comparison with the groups of newborn CBA mice inoculated with MCMV, $P < 0.005$. For the comparison of CBA mice reared by a natural or BALB/c mother, $P > 0.1$.
[d] The numbers in parentheses are the percentages of survivors.

TABLE 4. Virus titers in mice injected at birth with MCMV or HSV-1[a]

Virus	Mouse strain	Resistance status[b]	Organ	Dose (PFU)	No. of days, pi	PFU per organ ± SE	PFU/g of tissue
MCMV	BALB/c	Susceptible	Spleen	100	4	1,783 ± 293	57,516
			Liver	100	4	471 ± 14	4,309
	CBA	Resistant	Spleen	100	4	<10	<633
			Liver	100	4	21 ± 16	223
	B10.BR	Resistant	Spleen	100	4	<10	<606
			Liver	100	4	28 ± 9	328
HSV-1	A/J	Susceptible	Liver	1,250	3	5,456 ± 626	82,667
	B10.A	Resistant	Liver	1,250	3	84 ± 20	764

[a] Organs were pooled from two to three litters (9 to 17 mice) of each strain. Titers are the means of three determinations.
[b] See Table 1 for details.

the titer of virus in the spleen or liver 3 or 4 days after infection. After the inoculation of newborn mice with 100 PFU of MCMV, the virus titer in these organs was higher in BALB/c than in CBA or B10.BR mice (Table 4). However, when BALB/c and CBA mice received MCMV doses of 10 or 200 PFU, respectively, which approximated the LD_{50} for each strain, titers in the liver were not significantly different between the two strains (data not shown). With HSV-1, mice of the genetially susceptible A/J strain had higher virus titers in the liver by day 3 after infection than resistant B10.A mice given the same virus dose (Table 4).

DISCUSSION

This study describes the existence of genetically regulated mechanisms of resistance to the development of lethal disease induced by the herpesviruses MCMV and HSV-1 in newborn mice. Resistance was specific for each virus, and differences in virus titers in the spleen or liver were evident in resistant and susceptible strains by day 3 or 4 of infection. Resistance appeared to be an autonomous property of the newborn animal and was not influenced postnatally by maternal factors.

These observations contrast with an earlier report (5) in which mouse strain differences in resistance to MCMV were not detected in the early postnatal period, although small differences in mortality were noted in MCMV-infected 4- to 6-day-old C57BL/6 and CBA mice (17). There is no obvious explanation for the failure of other investigators to demonstrate mouse strain variation in mortality caused by MCMV in newborn mice. However, perhaps in previous studies the range of virus doses was not sufficiently wide, and no account may have been taken of the late deaths which occur with small doses of MCMV. The occurrence of late deaths marks a difference with normal adult mice, in which deaths usually occur between 2 and 7 days after infection (3), although adult nude animals, which are killed by smaller doses of MCMV than their normal littermates, die after prolonged infection (6). The late deaths in newborn mice and adult nude animals may be due to the time required for the low-dose inocula to replicate to levels sufficient to cause death. The association of MCMV with late deaths compared with the early deaths which result from HSV-1 infection of newborn mice has its parallel in man. Infants infected at birth with HSV-1 invariably develop symptoms 2 to 10 days later (4), whereas perinatal CMV infections do not become established for 3 to 12 weeks (16).

For HSV-1, previous studies reported the greater susceptibility of newborn compared with adult mice (2, 8, 9, 24). Our observation that differences in resistance to HSV-1 also exist between newborns of various mouse strains may in part reflect the use of HSV-1 2931. The WAL and ANG strains of this virus appear to be too virulent in newborn mice for the detection of mouse strain-related differences when they are used; an LD_{50} of 20 PFU has been reported for these viruses in newborn, genetically resistant C57BL/6 mice (10, 24). In contrast, for strain 2931, used in our study, the LD_{50} was 520 PFU in newborn C57BL/6 mice.

Our observation that genetic resistance to MCMV and HSV-1 is present in newborn mice and in cultured fibroblasts derived from 15- to 17-day-old embryos (7) suggests that resistance to these viruses exists in the fetus. Although it is not known whether genetic resistance to CMV or HSV-1 occurs in man, it is interesting that not all infants who are congenitally infected following a primary CMV infection of the mother during pregnancy exhibit disease. In a recent study, 85% of such congenitally infected infants were asymptomatic at birth, although some exhibit sequelae subsequently (22). Thus, it is possible that the extent of CMV infection in the human fetus in part depends on intrinsic, genetically determined resistance mechanisms.

Although in this study we did not investigate the mechanisms of resistance to these viruses in newborn mice, the data suggest that resistance is not conferred postnatally by maternal factors and is an autonomous property of these animals which is not dependent on functionally mature leukocytes. The observation of genetically determined resistance to these viruses in homogeneous cultures of long-term-passage embryo fibroblasts from resistant strains or in organ cultures of tracheae from adult mice (7) supports this contention. In contrast to these naturally occurring mechanisms, maternal antibodies can passively protect suckling mice if their mothers are hyperimmune to MCMV (13).

It has been proposed that genetically controlled innate mechanisms of resistance are also important in protecting adult mice against MCMV (20). The fact that the difference in MCMV LD_{50}s between strains is the same in adult and newborn mice suggests that resistance genes modulate the activity of similar protective mechanisms in mice of all ages, although the effectiveness of these mechanisms is greatly increased in adult life. For HSV-1, although the same strain distribution pattern of resistance was observed in newborn and adult mice, the difference in the LD_{50} between resistant and susceptible strains was much greater in adult animals. Perhaps there is a greater increase in the efficiency of resistance mechanisms with age in resistant strains, or these strains may acquire additional mechanisms of protection against HSV-1. However, leukocyte-mediated responses are also very important for protection against MCMV and

HSV-1 in older animals, because a variety of immunosuppressive procedures increase susceptibility to these viruses (5, 8, 17), and the nude (6) and beige mutations (18, 19) also increase susceptibility to MCMV infection. Studies are under way to determine the mechanism of genetically controlled resistance to MCMV in newborn mice and the relative importance in adult mice of innate and adaptive mechanisms of protection against this virus.

ACKNOWLEDGMENTS

We thank Jillean Winter for her excellent technical assistance and Ursula Kees for reading the manuscript.

This research was supported by grants from the National Health and Medical Research Council of Australia, the TVW Telethon Foundation of Western Australia, and the Cancer Foundation of Western Australia.

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