J. gen. Virol. (1985), **66**, 701–709. *Printed in Great Britain*

Key words: *encephalomyocarditis virus/interferon/sex*

Role of Sex and Early Interferon Production in the Susceptibility of Mice to Encephalomyocarditis Virus

By BRUNO POZZETTO† AND ION GRESSER*

Department of Viral Oncology, Institut de Recherches Scientifiques sur le Cancer, 94802 Villejuif Cedex, France

(*Accepted 19 December 1984*)

SUMMARY

Adult female Swiss mice showed a greater resistance to intraperitoneal (i.p.) infection with encephalomyocarditis virus (EMCV) than male mice. This difference was not observed in weanling mice, in castrated adult mice or in adult mice injected intracerebrally. Administration of antibody to mouse interferon α/β enhanced the virulence of EMCV for both sexes and no difference was then observed in susceptibility between male and female mice. Six h after EMCV infection, serum interferon titres were higher in adult female mice than in male mice. There was a close correlation between the early serum interferon titre (at 6 h) and survival of EMCV-infected mice. No differences in serum interferon titres were observed between male or female weanling mice or castrated adult mice. Potent preparations of exogenous interferon provided the same degree of protection against EMCV infection in male and female mice. We conclude that the more marked early interferon response of female mice to i.p. EMCV infection is one of the important factors underlying the differential susceptibility to EMCV. It is possible that the interferon system is also involved in the reported greater prevalence of picornavirus infections of men compared with women.

INTRODUCTION

Males are more susceptible than females to infection with picornaviruses. Despite the same incidence of infection in humans of both sexes with enteroviruses, the clinical manifestations of such diseases are regularly more severe in men than women as exemplified by poliomyelitis (Weinstein, 1957), coxsackievirus myocarditis (Woodruff, 1980), non-polio-enterovirus diseases (Nelson *et al.*, 1979; Morens *et al.*, 1979) and hepatitis A (McCollum, 1976) the causative agent of which is related to enteroviruses (Gust *et al.*, 1983). Several studies have demonstrated that male mice are more susceptible than female mice to experimental infections with coxsackie B1 (Berkovich & Ressel, 1965, 1967) and B3 viruses (Wong *et al.*, 1977; Huber *et al.*, 1981*a*, *b*, 1982). Sex hormones and sex differences in the cytotoxic T-cell responses were implicated, but no satisfactory mechanism of action has been proposed to explain the enhanced susceptibility of males (or the resistance of females).

Encephalomyocarditis virus (EMCV), which, like enteroviruses, belongs to the Picornaviridae family, was also shown to be more virulent in male mice than in female mice (Weinstein & Chang, 1961; Friedman *et al.*, 1972; Boucher *et al.*, 1975). Friedman *et al.* (1972) emphasized the role of sex hormones in this phenomenon, since castration of male mice resulted in a pattern of resistance similar to that of female mice, whereas castration of female mice did not affect their susceptibility to EMCV. Furthermore, in this model, testosterone and oestradiol, but not progesterone, markedly increased the sensitivity of castrated mice of both sexes to EMCV (Friedman *et al.*, 1972).

The results of some recent studies have emphasized the importance of the 'early' production of interferon in resistance of adult mice to herpes simplex virus infection (Engler *et al.*, 1982; Zawatzky *et al.*, 1982*a*, *b*) and the influence of sex in determining the extent of this early

† Present address: Laboratoire de Bactériologie et Virologie, Hôpital Nord, Avenue Albert Raimond, 42277 Saint-Priest-en-Jarez, France.

interferon production in mice infected with herpes simplex virus (Zawatzky *et al.*, 1982*c*; Pedersen *et al.*, 1983) or Newcastle disease virus (DeMaeyer-Guignard *et al.*, 1982). In the present study, we report the influence of various factors on the sex-related sensitivity of Swiss mice to EMCV. Our results suggest that the early interferon response is an important factor underlying the differential susceptibility to EMCV of male and female Swiss mice.

METHODS

Mice. Male and female Swiss, C57BL/6 and DBA/2 mice were obtained from the pathogen-free colonies at the Institut du Cancer, Villejuif, France.

EMCV and assay of antibodies to it. The origin, methods of preparation and assay of the EMCV used in this study have been previously described (Gresser *et al.*, 1968). The titre of the EMCV stock used in all the experiments *in vivo* was $10^{9\cdot3}$ $TCID_{50}$ per 0·2 ml in monolayer cultures of L cells. Mice were injected intraperitoneally (i.p.), or subcutaneously (s.c.) with 0·2 ml, or intracerebrally (i.c.) with 0·03 ml of the given virus stock dilution(s). Although the majority of deaths occurred in the 10 days following EMCV infection, mice were kept 3 to 4 weeks longer. EMCV infectivity in mouse serum was assayed by serial tenfold dilutions in L cell cultures in microplates. Serum antibodies to EMCV were titrated by serum-neutralization tests: serial twofold dilutions were assayed against 100 $TCID_{50}$ of EMCV for inhibition of cytopathic effect in L cells.

Procedure for castration and hormonal treatments. Four-week-old Swiss mice were anaesthetized by i.p. injection of 0·15 to 0·25 ml Imalgene^R/500 (containing 50 mg/ml ketamine; IFFA Mérieux, les Oncins, France). Removal of the ovaries was performed through a single dorsal incision. The testes were removed through a slit in the scrotal sac. Sham-operated mice were anaesthetized and incised like castrated mice but without resection of gonads. Long-acting preparations of sex hormones were diluted with peanut oil and injected intramuscularly once a week in castrated Swiss mice: 0·5 mg/0·1 ml for testosterone hexahydrobenzoate and 0·1 mg/0·1 ml for oestradiol hexahydrobenzoate.

Mouse interferon and sheep anti-mouse interferon globulin. The techniques used in the production, partial purification and assay of mouse C-243 cell interferon (Tovey *et al.*, 1974) or sheep anti-mouse interferon globulin have been previously described (Gresser *et al.*, 1976). Mouse interferon had a titre of $1{\cdot}6 \times 10^6$ laboratory units against vesicular stomatitis virus (VSV) on L cells. The neutralizing titre of the anti-interferon serum was $3{\cdot}2 \times 10^6$ against 4 to 8 laboratory units of interferon. In the experiments, 0·2 ml of these preparations were injected i.p. at 1 : 1, 1 : 10 and 1 : 100 dilutions for mouse interferon and 1 : 20 for anti-interferon globulin.

Induction and assay of endogenous interferon in mice. Interferon was induced by i.p. inoculation of $10^{7\cdot6}$ $TCID_{50}$ of EMCV. At given times, mice were exsanguinated, sera were diluted 1 : 5 in cell culture medium and dialysed for 6 to 8 days against a pH 2 buffer solution at 4 °C. Interferon was assayed by inhibition of cytopathic effect of VSV in monolayer cultures of L cells in plastic microplates (0·2 ml/well) as previously described (Gresser *et al.*, 1969). One of our units is the equivalent of 4 international mouse reference units.

Preparation of EMCV vaccine and vaccination procedure. The method of preparation of an anti-EMCV vaccine, adapted from Frantzen (1948), consisted of treating the supernatant fluid from a culture of L cells infected with EMCV with 1% formalin for 18 h at 4 °C. The vaccine was then dialysed for 3 to 4 days against phosphate-buffered saline, sterilized by filtration through a 0·22 μm filter, tested for infectivity on L cells and stored at −70 °C. After three i.p. injections of 0·2 ml of undiluted vaccine at intervals of 3 days, more than 90% of mice were protected against 10^5 LD_{50} of virulent EMCV.

Statistical analysis. LD_{50} and $TCID_{50}$ were calculated by the method of Reed & Muench (1938) and the standard errors ($S.E._{50}$) were estimated by the method of Pizzi (1950). Other statistical estimations used standard parametric tests: Chi square for comparison of qualitative variables, Student's *t*-test for comparison of means and the Pearson *r* coefficient for correlation analysis.

RESULTS

Factors involved in the susceptibility of male and female mice to EMCV

Table 1 summarizes the results of 12 experiments in which we investigated the role of mouse age, route of infection and mouse strain on the susceptibility of male and female mice to EMCV infection. Swiss adult male mice were consistently more susceptible to i.p. inoculation of EMCV than female mice. It was of interest to note, however, that this difference was not observed in 3-week-old mice. When Swiss mice were injected s.c., this difference in susceptibility between male and female mice was not statistically significant. Likewise, no difference was observed when the virus was injected i.c. (Table 1).

Table 1. *Response of male and female mice to infection with EMCV: roles of mouse strain, age and route of infection*

Strain	Age (weeks)	Route of infection	No. of mice per dilution per sex	LD_{50}* ± S.E.$_{50}$ Males	LD_{50}* ± S.E.$_{50}$ Females	*P* value
Swiss	3	i.p.	10	6·19 ± 0·34†	5·71 ± 0·33	NS‡
Swiss	4	i.p.	15	6·32 ± 0·18	5·67 ± 0·24	<0·03
Swiss	5	i.p.	8	5·88 ± 0·32	4·71 ± 0·29	<0·01
Swiss	8	i.p.	8	5·80 ± 0·28	4·88 ± 0·25	<0·02
Swiss	20	i.p.	10	6·16 ± 0·38	4·82 ± 0·32	<0·01
Swiss	5–6	s.c.	8	6·71 ± 0·29	5·88 ± 0·35	NS
Swiss	7–8	s.c.	15	5·89 ± 0·24	5·33 ± 0·29	NS
Swiss	5–6	i.c.	8	8·80 ± 0·30	8·70 ± 0·27	NS
DBA/2	4–5	i.p.	8	6·67 ± 0·28	6·21 ± 0·47	NS
DBA/2	7–8	i.p.	15	6·00 ± 0·27	5·66 ± 0·26	NS
C57BL/6	4	i.p.	8	6·41 ± 0·27	6·33 ± 0·33	NS
BALB/c	4	i.p.	8	6·38 ± 0·25	6·00 ± 0·37	NS

* Each LD_{50} was calculated on the mortality ratios of at least four $\log_{10}$ EMCV dilutions.
† LD_{50} and S.E.$_{50}$ are expressed as $\log_{10}$.
‡ NS, Not significant.

Table 2. *Influence of castration and hormone treatment on the susceptibility of male and female Swiss mice to i.p. infection of EMCV*

Age at EMCV infection (weeks)*	Treatment†	Mortality Males	Mortality Females	*P* value‡
5–6	None	13/16 (81%)‖	7/16 (44%)	<0·03
	Castration	7/12 (58%)	8/11 (73%)	NS¶
7	None	23/26 (88%)	13/24 (54%)	<0·01
	Sham operation	19/26 (73%)	16/26 (62%)	NS
	Castration	14/25 (56%)	21/25 (84%)	<0·04
	Castration + testosterone	19/24 (79%)	22/24 (92%)	NS
	Castration + oestradiol§	22/24 (92%)	20/25 (80%)	NS
8	None	10/10 (100%)	5/12 (42%)	<0·01
	Castration	6/11 (55%)	6/8 (75%)	NS

* Swiss mice were injected at various ages with 0·2 ml i.p. of $10^{4·3}$ $TCID_{50}$ of EMCV.
† Surgery was performed at 4 weeks of age.
‡ *P* values were calculated with the Chi square test.
§ Long-acting preparations of sex hormones were injected once a week for 3 weeks as described in the text. The difference between castrated, and castrated and testosterone-treated mice was significant for male mice ($P < 0·1$) but not for female mice. The difference between castrated, and castrated and oestradiol-treated mice was highly significant for male mice ($P < 0·01$) and not significant for female mice.
‖ Number of dead mice/total number of mice (percentage of deaths).
¶ NS, Not significant.

There was no significant difference in mortality between male and female DBA/2, C57BL/6 and BALB/c mice after i.p. injection with EMCV (Table 1). However, in all these strains, and especially in DBA/2 mice, the evolution of disease was more rapid in male than in female mice.

Effect of castration and hormone treatment on the susceptibility of Swiss mice to EMCV infection

Four-week-old Swiss mice were castrated and 2, 3 or 4 weeks later injected with 0·2 ml i.p. of $10^{4·3}$ $TCID_{50}$ of EMCV. As shown in Table 2, 5- to 8-week-old mice were more susceptible to EMCV than female mice in accord with the results presented above. In each experiment castration rendered male mice more resistant and female mice more susceptible to EMCV than unoperated mice, and in one experiment castrated female mice were even more susceptible than castrated male mice (Table 2). In the same experiment, sham-operated male mice exhibited a susceptibility intermediate between castrated and non-operated male mice (Table 2). After

Table 3. *Effect of anti-interferon globulin on the susceptibility of male and female Swiss mice to EMCV*

Treatment	LD_{50}* ± S.E.$_{50}$ Males	Females	*P* value
None	6·29 ± 0·40‡	5·12 ± 0·41	<0·05
Anti-interferon globulin†	8·10 ± 0·38	8·50 ± 0·25	NS§

* LD_{50} were calculated on the mortality ratios in mice injected with five $\log_{10}$ EMCV dilutions. There were eight mice per group per sex.

† Five-week-old Swiss mice were injected i.p. with 0·2 ml of a 1:10 dilution of anti-interferon globulin 3 days before injection of virus.

‡ LD_{50} and S.E.$_{50}$ are expressed as $\log_{10}$.

§ NS, Not significant.

sacrifice of surviving mice, we noted that the testes has ascended in sham-operated mice. Although the testes appeared grossly normal it is possible that the production or secretion of hormones was affected by the sham operation.

It was considered of interest to determine the effect of injection of testosterone or oestradiol on the susceptibility of castrated male and female mice to EMCV. Accordingly, 7-week-old castrated mice were injected intramuscularly 3 times with long-acting preparations of either hormone 10 and 3 days prior and 4 days after i.p. inoculation of EMCV. Both treatments increased the susceptibility of castrated male mice to EMCV and exerted no protective effect in castrated females whose susceptibility to EMCV was increased compared with normal female mice (Table 2).

Effect of injection of anti-interferon globulin on the susceptibility of male and female mice to EMCV

As we had previously demonstrated the important role of interferon on the evolution of EMCV infection in mice by the use of antibody to mouse interferon α/β (Gresser *et al.*, 1976), it was of interest to compare its effect in virus-infected male and female mice. As previously observed (Gresser *et al.*, 1976), injection of anti-interferon globulin considerably enhanced the susceptibility of both male and female mice to EMCV (Table 3), resulting in a LD_{50} close to that observed in mice injected i.c. (see Table 1). However, injection of anti-interferon globulin abolished the difference in susceptibility between male and female mice (Table 3).

Presence of endogenous interferon in male and female mice after infection with EMCV

The finding that injection of anti-interferon globulin abrogated the difference in susceptibility between male and female mice suggested that interferon might be one of the factors underlying the different response of male and female Swiss mice to i.p. infection with EMCV. As shown in Fig. 1, the overall serum interferon titres in male and female mice infected with EMCV were similar. However, 6 h after virus inoculation, the mean serum interferon titres in female mice were significantly greater ($P < 0·05$ by Student's *t*-test). Prior to 6 h only trace amounts of interferon were present in the sera and after 7 h a significant difference was no longer observed between male and female mice. Other experiments were performed to verify the difference in early interferon levels. The sera of a total of 22 mice of each sex were assayed at 6 h after EMCV infection. The mean serum interferon titres were significantly greater in female mice (4·67 ± 1·54 $\log_2$ IFN units versus 3·70 ± 1·25 in male mice; Student's *t*-test = 2·33, $P < 0·05$).

In view of these results it was of interest that serum EMCV titres were higher in male than in female mice at 12 and 18 h after virus infection (Fig. 2).

Influence of age and sex on the serum interferon titre

As a significant difference in the serum interferon titre was observed between male and female mice 6 h after EMCV infection, we examined in more detail the effect of age, sex and castration on the serum interferon titre at this time. At 3 weeks of age, no difference in

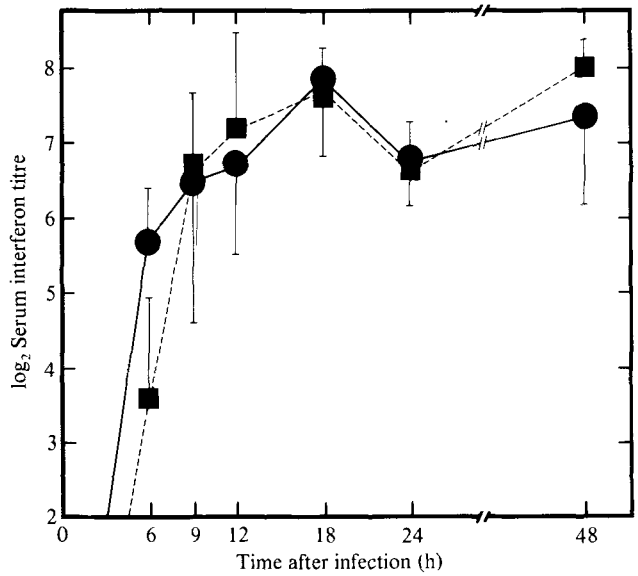

Fig. 1. Serum interferon titres in male and female Swiss mice after infection with EMCV. Four-week-old Swiss mice were injected i.p. with $10^{7.6}$ $TCID_{50}$ of EMCV. Each point represents the mean interferon titre for six male (■) and six female (●) mice.

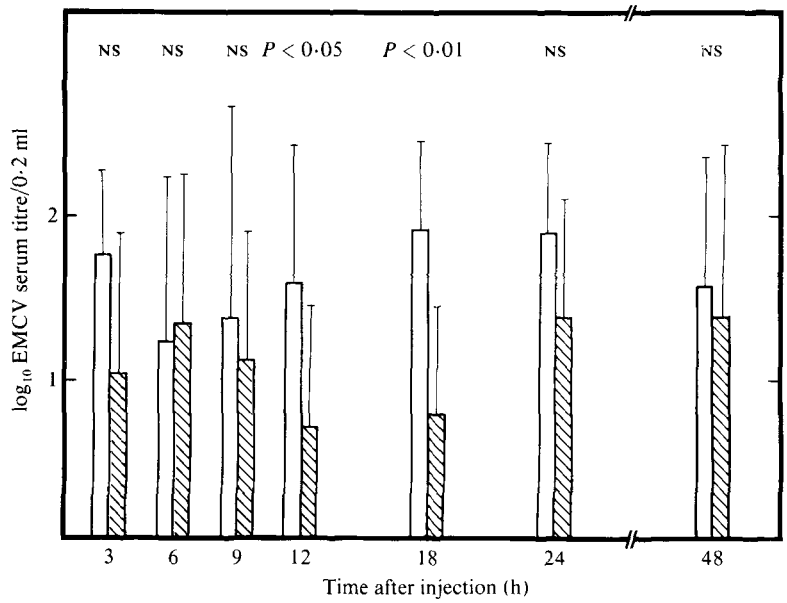

Fig. 2. Titres of EMCV in sera of male and female Swiss mice. Experimental conditions are as in the legend to Fig. 1. Each column indicates the mean and standard deviation of EMCV titres from 6 to 11 male (□) or female (▨) mice. Student's *t*-test was used for statistical analysis. NS, Not significant.

susceptibility to EMCV was observed between male and female mice, and there was no difference in the serum interferon titre between 3-week-old male and female mice 6 h after EMCV infection (Table 4). In contrast, older male mice (4 to 12 weeks) were consistently more susceptible to EMCV than female mice and the serum interferon titre at 6 h was consistently lower in male mice than in female mice (Table 4).

Table 4. *Influence of age and sex on the 'early' serum interferon titre in Swiss mice*

Age at EMCV infection (weeks)*	Sex	Treatment†	Number of mice	Mean IFN titre (log_2 ± S.D.)‡	*P* value§
3	M	None	12	4·57 ± 1·57	NS‖
	F	None	12	4·83 ± 0·97	
4–5	M	None	22	3·70 ± 1·25	<0·05
	F	None	22	4·67 ± 1·54	
7	M	None	12	3·92 ± 0·83	<0·05
	F	None	12	4·58 ± 0·75	
8	M	None	12	3·65 ± 0·96	
	M	Castration	12	4·57 ± 1·25	<0·001
	F	None	12	5·20 ± 0·83	NS
	F	Castration	12	4·32 ± 0·92	
12	M	None	11	3·65 ± 0·84	
	M	Castration	11	4·21 ± 1·31	<0·01
	F	None	9	4·83 ± 0·41	NS
	F	Castration	11	4·46 ± 1·06	

* Swiss mice were injected i.p. with 0·2 ml of $10^{7·6}$ $TCID_{50}$ of EMCV.
† Castration was performed at 4 weeks of age.
‡ Interferon was assayed in the serum 6 h after inoculation of EMCV.
§ Student's *t*-test was used for statistical analysis.
‖ NS, Not significant.

Castration which had abrogated the sex difference in susceptibility also abrogated the difference in the serum interferon titres between male and female mice (Table 4).

In summary, the analysis of 12 series of values from different experiments showed that there was a highly significant correlation ($r = 0·84$, $P < 0·01$) between the serum interferon titres at 6 h (see Table 4) and the percentage of mice surviving an i.p. injection of $10^{4·3}$ $TCID_{50}$ of EMCV (see Table 2).

Antiviral effect of exogenous interferon in male and female mice infected with EMCV

As the results described above suggested that there was a correlation between the higher serum interferon titres early after EMCV infection in adult female mice and their greater resistance to EMCV infection, it was of interest to determine the efficacy of exogenous interferon in protecting male and female mice injected with EMCV.

Accordingly, 4-week-old male and female Swiss mice were injected i.p. with 0·2 ml of partially purified mouse interferon (three groups of mice, receiving $1·6 \times 10^6$, $1·6 \times 10^5$ and $1·6 \times 10^4$ units of interferon), and 3 h later, mice were injected i.p. with approximately 100 LD_{50} of EMCV (i.e. $10^{5·3}$ $TCID_{50}$ for males and $10^{6·3}$ $TCID_{50}$ for females). Exogenous interferon conferred a similar protection to mice of both sexes (data not shown), demonstrating that sex did not affect the efficacy of interferon in protecting mice against EMCV infection.

Influence of sex on the antibody response to anti-EMCV vaccine

Four-week-old male and female mice were immunized with a formalin-inactivated EMCV vaccine. Serum anti-EMCV antibody titres were determined 6 days after the third injection of vaccine. The results summarized in Table 5 demonstrate that after vaccination female mice developed higher titres of neutralizing anti-EMCV antibodies than male mice. However, when these vaccinated mice were subsequently injected with live EMCV, no difference was observed in the post-infection antibody response between male and female mice.

EMCV vaccine did not induce the production of interferon even when the vaccine was injected intravenously. This finding suggests that the increased primary humoral antibody response to EMCV vaccine in female mice compared with male mice may have been independent of the interferon system.

Table 5. *Antibody response to EMCV in vaccinated 4-week-old male and female mice*

Expt.	Treatment	Sex	No. of mice	Neutralizing antibody titre* Mean	Confidence interval	*P* value†
1	Anti-EMCV vaccine‡	M	32	142·0	41·6–484·4	<0·001
		F	32	382·7	140·0–1045·5	
2	Anti-EMCV vaccine followed by virulent EMCV§	M	15	1418·4	442·6–4544·8	NS‖
		F	15	1652·0	560·3–4871·0	

* Anti-EMCV neutralizing antibodies were assayed 5 days after the last injection of vaccine (expt. 1) or 6 days after challenge with virulent EMCV (expt. 2).

† Student's *t*-test was used for statistical analysis.

‡ Mice received three injections i.p. of 0·2 ml of undiluted EMCV vaccine at intervals of 3 days.

§ Mice were vaccinated as in expt. 1. Ten days after the last injection of vaccine, mice received 0·2 ml i.p. of $10^{8\cdot3}$ $TCID_{50}$ of virulent EMCV.

‖ NS, Not significant.

DISCUSSION

It had previously been shown that male mice were more susceptible than female mice to EMCV injected i.p. (Weinstein & Chang, 1961; Friedman *et al.*, 1972; Boucher *et al.*, 1975), although the reasons for this difference had not been elucidated. The first question to pose concerns the role of sex hormones. The results of Friedman *et al.* (1972) and our results (Table 2) clearly show the protective effect of castration in male mice, and the abolition of this effect by testosterone replacement therapy. The results are less clear-cut in female mice. We found that castration rendered female mice more susceptible to infection by EMCV, whereas Friedman *et al.* (1972) found no difference. This difference in results may be related to the strain of mouse used [CD1 mice in the study of Friedman *et al.* (1972) and Swiss mice in our study]. Previous studies on the effect of castration of female mice on the susceptibility to coxsackievirus B1 also showed differences depending on the mouse strain used (Berkovich & Ressel, 1965, 1967). In the study of Friedman *et al.* (1972) as in ours, administration of oestradiol increased the susceptibility to EMCV in castrated mice of both sexes.

The major question, however, concerns the mechanisms responsible for the difference in susceptibility to EMCV between male and female mice. The finding that the sex difference was no longer observed when EMCV was injected i.c. suggested that the host factors responsible underlying the sex difference acted prior to involvement of the central nervous system.

The interferon response seemed to be one important host factor. Thus, injection of potent sheep antibody to mouse interferon α/β abolished the difference between male and female mice in the response to EMCV infection (Table 3) (although it enhanced the lethality of EMCV for both sexes). It was unlikely that male and female mice differed in their sensitivity to interferon as administration of potent interferon preparations afforded an equal protection to EMCV-infected male and female mice. We then examined the kinetics of interferon production in EMCV-infected male and female mice. Recent findings by Engler *et al.* (1982) and Zawatzky *et al.* (1982*a*, *b*) have emphasized the importance of this 'early' interferon response in herpes simplex virus infection in mice. Higher serum interferon levels were in fact detected in female mice 6 h after inoculation of EMCV (Fig. 1).

Several findings in the work presented herein suggested that this difference in early interferon response (6 h) was an important factor underlying the sex difference in susceptibility to EMCV. (i) No difference in susceptibility to EMCV was observed in suckling (3-week-old) male and female mice, and no significant difference was observed in their serum interferon titres at any time. (ii) There was a highly significant correlation ($r = 0{\cdot}84$, $P < 0{\cdot}01$) between the serum interferon titre at 6 h and survival of EMCV-infected mice. (iii) Increased serum interferon titres were observed in female mice at 6 h and lower levels of circulating EMCV were detected in female mice (compared with male mice) at 12 and 18 h (Fig. 2). (It seems reasonable to suggest

that these findings were causally related.) (iv) Castration of male mice conferred a significant degree of protection (Table 2) and was also associated with increased serum early interferon titres (6 h) (Table 4).

There have been several reports of the effects of sex hormones on the production of interferon in cell culture (for review, see Stewart, 1979) and *in vivo* (Kilbourne *et al.*, 1961; Giron *et al.*, 1971*a*, *b*; Talas & Stoger, 1972). More recently, it has been shown that an X-linked locus influences the amount of circulating interferon in mice infected with Newcastle disease (DeMaeyer-Guignard *et al.*, 1982) or herpes simplex viruses (Zawatzky *et al.*, 1982*c*; Pedersen *et al.*, 1983). Our results suggest that both genetic and hormonal factors influence the kinetics of production of circulating interferon in weaned Swiss mice infected i.p. with EMCV. We believe the enhanced 'early' interferon response in female mice is related to their enhanced degree of resistance to EMCV.

One other aspect of host resistance should however be mentioned: the role of humoral immunity. EMCV multiplies rapidly and mice die 4 to 5 days after infection. It was not feasible therefore to determine with any accuracy whether there was any sex difference in the antibody response to virulent EMCV. However, we did find a greater primary antibody response to formalin-inactivated EMCV in female mice than in male mice (Table 5). Higher specific humoral responses in female mice have already been reported in other picornavirus infections, i.e. mice infected with coxsackievirus B3 (Wong *et al.*, 1977; Huber *et al.*, 1982). The enhanced antibody response to inactivated EMCV appeared to be independent of the interferon response as inactivated EMCV did not induce detectable levels of circulating interferon. Nevertheless, it seems possible that there is some underlying mechanism common to both the accelerated interferon response to virulent EMCV and the increased antibody response to inactivated EMCV in female mice.

Our results considered with the previous investigations mentioned above suggest that the enhanced resistance to EMCV (and other viruses, e.g. herpes simplex virus) of adult female mice compared with male mice is related to interferon production (either kinetics or total amount produced). Clearly, several other factors may enhance or abrogate this sex difference and the difference in the interferon response: strain and age of the mouse, route of infection, etc. It would be of interest, nevertheless, to determine whether a difference in the early interferon response also underlies the increased frequency of enterovirus infections in men compared with women.

We thank Ms Chantal Maury for excellent technical assistance. This work was supported in part by grants from the Richard Lounsbery Foundation, Simone et Cino del Duca Foundation, INSERM (contract 12 70 12), D.R.E.T. (contract 82-420) and the Association pour le Développement de la Recherche sur le Cancer à Villejuif. One of us (B.P.) is grateful to the Foundation pour la Recherche Médicale for support.

REFERENCES

BERKOVICH, S. & RESSEL, M. (1965). Effect of gonadectomy on susceptibility of the adult mouse to Coxsackie B-1 virus infection. *Proceedings of the Society for Experimental Biology and Medicine* **119**, 690–694.

BERKOVICH, S. & RESSEL, M. (1967). Effect of sex on susceptibility of adult mice to Coxsackie B-1 virus infection. *Archiv für die gesamte Virusforschung* **22**, 246–251.

BOUCHER, D. W., HAYASHI, K., ROSENTHAL, J. & NOTKINS, A. L. (1975). Virus-induced diabetes mellitus. III. Influence of the sex and strain of the host. *Journal of Infectious Diseases* **131**, 462–466.

DEMAEYER-GUIGNARD, J., ZAWATZKY, R., DANDOY, F. & DEMAEYER, E. (1982). An X-linked locus influences the amount of circulating interferon levels in the mouse. *Journal of Infectious Diseases* **3**, 241–252.

ENGLER, H., ZAWATZKY, R., KIRCHNER, H. & ARMERDING, D. (1982). Experimental infection of inbred mice with herpes simplex virus. IV. Comparison of interferon production and natural killer cell activity in susceptible and resistant adult mice. *Archives of Virology* **74**, 239–247.

FRANTZEN, A. (1948). On the preparation of a formalinized aluminium hydroxide vaccine with the murine S.K. poliomyelitis strain (Jungeblut & Sanders). *Acta pathologica et microbiologica scandinavica* **25**, 351–355.

FRIEDMAN, S. B., GROTA, L. J. & GLASGOW, L. A. (1972). Differential susceptibility of male and female mice to encephalomyocarditis virus: effects of castration, adrenalectomy and the administration of sex hormones. *Infection and Immunity* **5**, 637–644.

GIRON, D. J., ALLEN, P. T., PINDAK, F. F. & SCHMIDT, J. P. (1971*a*). Inhibition by estrone of the antiviral protection and interferon elicited by interferon inducers in mice. *Infection and Immunity* **3**, 318–322.

GIRON, D. J., SCHMIDT, J. P. & PINDAK, F. F. (1971*b*). Effect of progesterone and testosterone on interferon production and on the viral infection-enhancing activity of estrone and hydrocortisone. *Infection and Immunity* **4**, 537–540.

GRESSER, I., BOURALI, C., THOMAS, M.-T. & FALCOFF, E. (1968). Effect of repeated inoculation of interferon preparations on infection of mice with encephalomyocarditis virus. *Proceedings of the Society for Experimental Biology and Medicine* **127**, 491–496.

GRESSER, I., FONTAINE-BROUTY-BOYE, D., BOURALI, C. & THOMAS, M.-T. (1969). A comparison of the efficacy of endogenous, exogenous and combined endogenous–exogenous interferon in the treatment of mice infected with encephalomyocarditis virus. *Proceedings of the Society for Experimental Biology and Medicine* **130**, 236–242.

GRESSER, I., TOVEY, M. G., BANDU, M.-T., MAURY, C. & BROUTY-BOYE, D. (1976). Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. I. Rapid evolution of encephalomyocarditis virus infection. *Journal of Experimental Medicine* **144**, 1305–1315.

GUST, I. D., COULEPIS, A. G., FEINSTONE, S. M., LOCARNINI, S. A., MORITSUGU, Y., NAJERA, R. & SIEGL, G. (1983). Taxonomic classification of hepatitis A virus. *Intervirology* **20**, 1–7.

HUBER, S. A., JOB, L. P., AULD, K. R. & WOODRUFF, J. F. (1981*a*). Sex-related differences in the rapid production of cytotoxic spleen cells active against uninfected myofibers during Coxsackie virus B-3 infection. *Journal of Immunology* **126**, 1336–1340.

HUBER, S. A., JOB, L. P. & WOODRUFF, J. F. (1981*b*). Sex-related differences in the pattern of Coxsackie virus B-3 induced immune spleen cell cytotoxicity against virus-infected myofibers. *Infection and Immunity* **32**, 68–73.

HUBER, S. A., JOB, L. P. & AULD, K. R. (1982). Influence of sex hormones on Coxsackie B-3 virus infection in Balb/c mice. *Cellular Immunology* **67**, 173–189.

KILBOURNE, E. D., SMART, K. M. & POKORNY, B. A. (1961). Inhibition by cortisone of the synthesis and action of interferon. *Nature, London* **190**, 650–651.

McCOLLUM, R. W. (1976). Viral hepatitis. In *Viral Infections of Humans*, pp. 235–252. Edited by A. S. Evans. London: John Wiley & Sons.

MORENS, D. M., ZWEIGHAFT, R. M. & BRYAN, J. M. (1979). Non polio enterovirus disease in the United States. *International Journal of Epidemiology* **8**, 49–54.

NELSON, D., HIEMSTRA, H., MINOR, T. & D'ALESSIO, D. (1979). Non polio enterovirus activity in Wisconsin based on a 20-year experiment in a diagnostic virology laboratory. *American Journal of Epidemiology* **109**, 352–361.

PEDERSEN, E. B., HAAHR, S. & MOGENSEN, S. C. (1983). X-linked resistance of mice to high doses of herpes simplex virus type 2 correlates with early interferon production. *Infection and Immunity* **142**, 740–746.

PIZZI, M. (1950). Sampling variations of the fifty percent end point determined by the Reed-Muench (Behrens) method. *Human Biology* **22**, 151–190.

REED, L. J. & MUENCH, H. (1938). A simple method of estimating fifty per cent end points. *American Journal of Hygiene* **27**, 493–497.

STEWART, W. E. II (1979). The genetics of interferon production. In *The Interferon System*, pp. 85–87. Edited by W. E. Stewart II. Wien & New York: Springer-Verlag.

TALAS, M. & STOGER, I. (1972). Hormone pretreatment and interferon production in mice. *Acta virologica* **16**, 211–216.

TOVEY, M. G., BEGON-LOURS, J. & GRESSER, I. (1974). A method for the large scale production of potent interferon preparations. *Proceedings of the Society for Experimental Biology and Medicine* **146**, 809–815.

WEINSTEIN, L. (1957). Influence of age and sex on susceptibility and clinical manifestations in poliomyelitis. *New England Journal of Medicine* **257**, 47–52.

WEINSTEIN, L. & CHANG, T. W. (1961). Effect of 'sex' of tissue cultures on growth of encephalomyocarditis virus. *Proceedings of the Society for Experimental Biology and Medicine* **108**, 471–474.

WONG, C. Y., WOODRUFF, J. J. & WOODRUFF, J. F. (1977). Generation of cytotoxic T lymphocytes during Coxsackie virus B-3 infection. III. Role of sex. *Journal of Immunology* **119**, 591–597.

WOODRUFF, J. F. (1980). Viral myocarditis. *American Journal of Pathology* **101**, 426–483.

ZAWATZKY, R., ENGLER, H. & KIRCHNER, H. (1982*a*). Experimental infection of inbred mice with herpes simplex virus. III. Comparison between newborn and adult C57BL/6 mice. *Journal of General Virology* **60**, 25–29.

ZAWATZKY, R., GRESSER, I., DE MAEYER, E. & KIRCHNER, H. (1982*b*). The role of interferon in the resistance of C57Bl/6 mice to various doses of herpes simplex virus type 1. *Journal of Infectious Diseases* **146**, 405–410.

ZAWATZKY, R., KIRCHNER, H., DEMAEYER-GUIGNARD, J. & DEMAEYER, E. (1982*c*). An X-linked locus influences the amount of circulating interferon induced in the mouse by herpes simplex virus type 1. *Journal of General Virology* **63**, 325–332.

(*Received 25 July 1984*)