

SEVERITY OF LYMPHOCYTIC CHORIOMENINGITIS
VIRUS DISEASE IN DIFFERENT STRAINS OF
SUCKLING MICE CORRELATES WITH INCREASING
AMOUNTS OF ENDOGENOUS INTERFERON*

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Administration of potent mouse interferon preparations to newborn mice induces an acute syndrome characterized by inhibition of growth, delay in the maturation of several organs, extensive liver cell degeneration, and death (1). An identical syndrome is seen in different strains of mice infected at birth with lymphocytic choriomeningitis (LCM)¹ virus (i.e., impaired growth, delay in organ maturation, liver cell degeneration, and death in the first few weeks of life.) We have shown that the induction of endogenous interferon by LCM virus is an important pathogenetic factor, because injection of a potent anti-interferon globulin, which neutralized the virus-induced endogenous interferon, markedly inhibited this acute syndrome (2). In the course of these studies, we noted (in accord with previous observations [3, 4]) that the incidence of mortality in suckling mice infected at birth with LCM virus varies considerably between different strains of mice. Thus, we observed: 100% mortality for C3H and C57BI/6; 94% for CBA; 79% for AKR; 36% for Swiss; 10% for DBA/2; and 0% for BALB/C mice. Because we have suggested that interferon is in large part responsible for the different manifestations of LCM virus disease in suckling mice (2), it seemed of interest to determine whether this varying severity of disease in three different strains of mice (BALB/c, Swiss, and C3H mice) was related either to the amount of endogenous interferon produced after virus infection or to a different sensitivity to this endogenous interferon. The results of these studies are reported herein.

Materials and Methods

Mice. Pathogen-free C3H, CBA, AKR, and C57BI/6 mice were obtained from IFFA CREDO, France. Swiss, C3H, and BALB/c mice were obtained from the Institut de Recherches sur le Cancer, Villejuif, Paris, France.

Virus: LCM virus strain CIPV 76001 of the Pasteur Institute was titered by intracerebral inoculation of 3-wk-old Swiss mice. The titer of LCM virus was 10⁸ median lethal doses (LD₅₀) per gram of brain homogenate. Newborn mice were inoculated in the first 24 h of life in the

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¹ Abbreviations used in this paper: LCM, lymphocytic choriomeningitis; LD₅₀, mean lethal dose.

interscapular region subcutaneously with $10^{4.7}$ LD₅₀ of virus in 0.05 ml. Control mice were injected with an extract of normal brain.

Determination of Virus and Interferon Levels in Plasma and Liver. There were on the average eight suckling mice per litter. In the days after viral inoculation, litters were sacrificed; the plasma from mice in each litter were pooled and titered for virus or interferon as previously described (2).

Virus in the liver (50% wt/vol homogenate) was titered by plaque assay in cultures of L cell monolayers (5). In our experiments 1 plaque-forming unit is approximately the equivalent of 25 mouse LD₅₀.

Preparations and Assay of Mouse Interferon. Mouse C-243 cell interferon was prepared, concentrated, and partially purified as previously described (6). Interferon was assayed as previously described (7) on monolayers of mouse L cells challenged with 100 median tissue culture infective doses (TCID₅₀) of vesicular stomatitis virus. All plasma and liver homogenates were kept at pH 4 for 24 h to inactivate LCM virus before assay. Mouse interferon units quoted are as measured in our laboratory and one of our units equal four mouse interferon reference units.

Histology. Tissues were fixed in Bouin's solution and stained with hematoxylin and eosin.

Results

Disease in BALB/c, Swiss, and C3H Suckling Mice Inoculated at Birth with LCM Virus

GROWTH CURVE. To determine the effect of LCM virus on the growth of suckling mice, litters of BALB/c, Swiss, or C3H mice were left uninoculated, inoculated with normal brain extract, or inoculated with $10^{4.7}$ LD₅₀ of LCM virus. All LCM virus-infected mice grew normally for the 1st wk of life (Fig. 1). Thereafter, a slight inhibition of growth was observed for virus-infected BALB/c mice, a greater inhibition for Swiss mice, whereas virus-infected C3H mice lost weight (Fig. 1).

MORTALITY. The results of several experiments presented in Fig. 2 show that 0/115 (0%) BALB/c mice, 47/129 (36%) Swiss mice, and 87/87 (100%) C3H mice, died in the 1st mo of life after inoculation of $10^{4.7}$ LD₅₀ of LCM virus. C3H mice died sooner than Swiss mice (Fig. 2).

HISTOLOGIC EXAMINATION OF THE LIVER. In accord with our previous observations (2), the only gross and histologic abnormalities in virus-infected suckling mice were noted in the liver. In two experiments LCM virus-infected BALB/c, Swiss, and C3H mice were sacrificed and histologic sections of the liver were examined at different intervals or at a given time (9th d) (Table I). In both experiments the lesions were

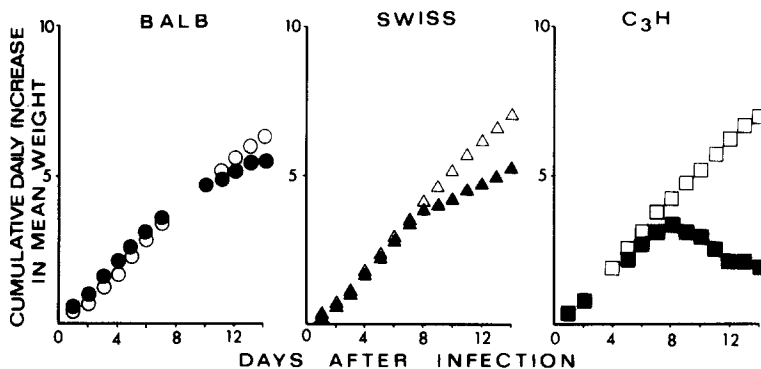


FIG. 1. Cumulative daily increase in mean weight (grams) for uninoculated BALB/c, Swiss, and C3H suckling mice (open symbols) or inoculated with $10^{4.7}$ LD₅₀ of LCM virus (closed symbols).

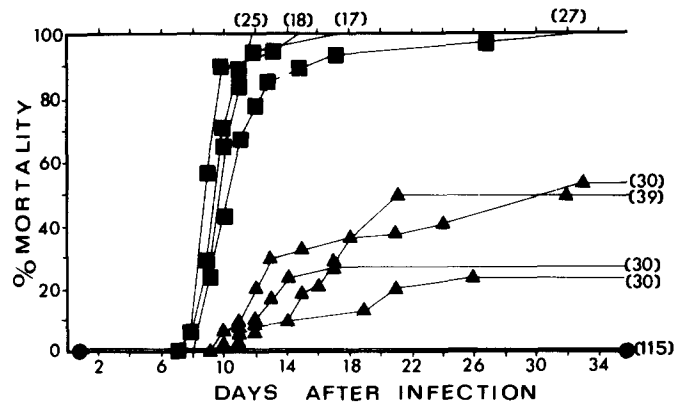


FIG. 2. Mortality in suckling C3H ■, Swiss ▲, and BALB/c ● mice inoculated subcutaneously at birth with $10^{4.7}$ logLD₅₀ of LCM virus. (The figures in parentheses indicate the number of newborn mice per experiment.)

TABLE I
Incidence and Severity of Liver Lesions in LCM Virus-infected Mice Sacrificed at 9 d

Mouse strain	No. of mice with histologic liver lesions/No. of mice inoculated*	Mean liver score‡
BALB/c	12/15	1.1
Swiss	10/12	1.8
C3H He/VF	10/10	3.5
C3H/ICO	10/10	4.0

* There were six sections of liver per mouse examined by two investigators. All slides were coded.

‡ Mean liver score based on total number of mice. Severity of lesions based on a score of 0-4. 1+ = Steatosis without necrosis; 2+ = steatosis and foci of subcapsular necrosis; 3+ = foci of necrosis extending into body of liver (25-50% of liver parenchyma); 4+ = massive liver cell necrosis involving >75% of liver parenchyma.

discrete in BALB/c mice (Fig. 3 B), of intermediate severity in Swiss mice (Fig. 3 C), and massive in C3H mice (Fig. 3 D and Table I).

TITERS OF VIRUS AND INTERFERON IN PLASMA AND LIVER. The results of the preceding experiments showed that LCM virus disease was mild in BALB/c mice, of intermediate severity in Swiss mice, and most marked in C3H mice (as judged by effects on growth, incidence, and severity of liver lesions and percentage mortality). As shown in Fig. 4 (upper panel) and Fig. 5 this different pattern of disease was not attributable to differences in LCM virus multiplication because plasma and liver virus titers were similar in all three strains of mice, (as high in BALB/c mice as in C3H mice).

A striking difference however was observed in the amount and duration of endogenous blood interferon induced in these strains of mice (lower panel, Fig. 4). BALB/c mice had a very low interferon response limited for the most part to the 3rd d; Swiss mice had greater amounts of interferon on the 3rd and 4th d. In contrast, C3H mice had high levels of plasma interferon on the 3rd to the 6th d. Liver interferon titers were uniformly low and inconsistent (due to cell toxicity of liver

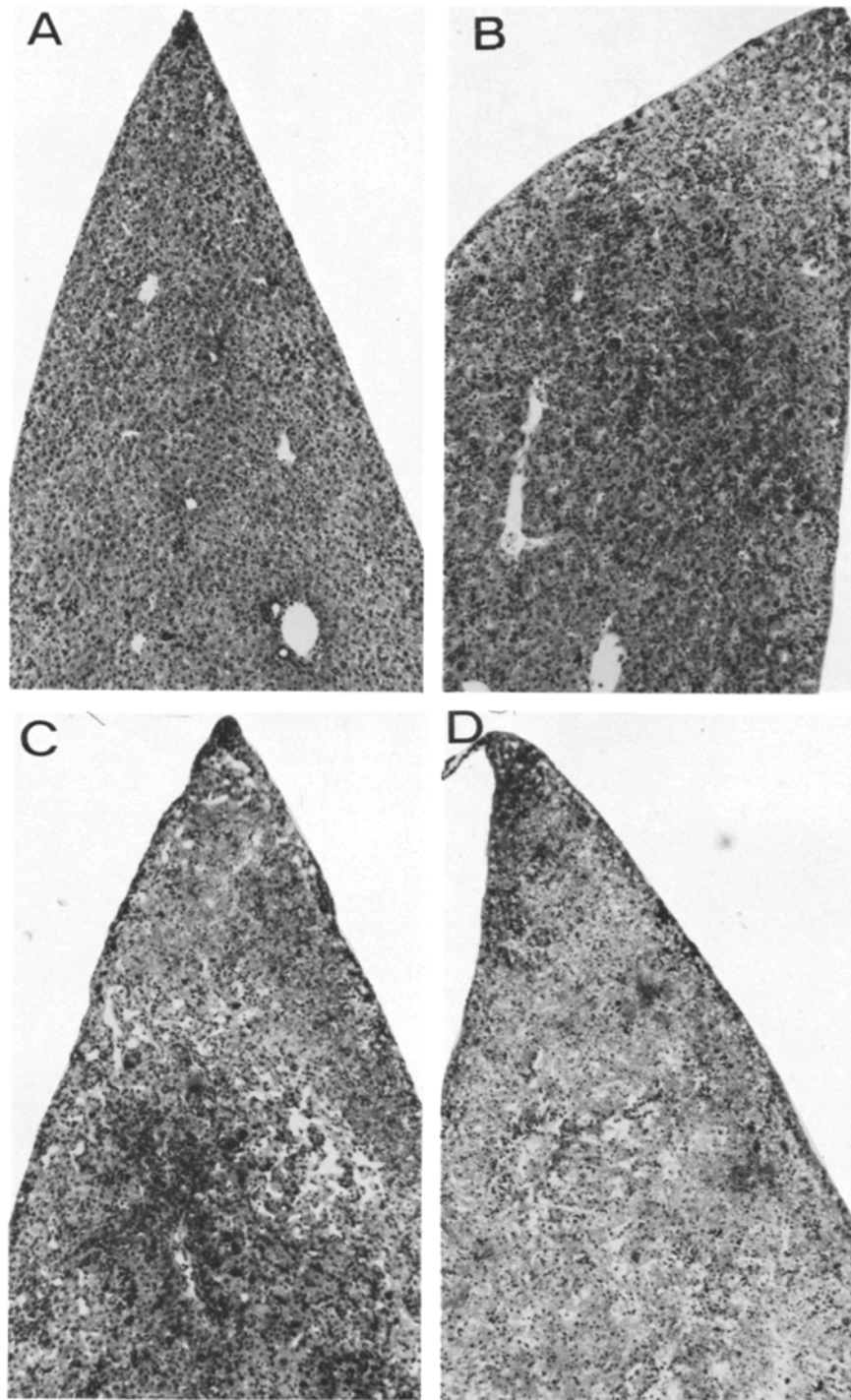


FIG. 3. Sections of liver from BALB/c, Swiss, and C3H mice injected at birth with $10^{4.7}$ LD₅₀ of LCM virus and sacrificed on the 9th d. Stained with hematoxylin and eosin ($\times 63$). (A) From uninoculated BALB/c mouse. (B) From virus-infected BALB/c mouse. Note subcapsular necrosis (2+ lesion). (C) From virus-infected Swiss mouse. Note extensive necrosis but large areas of parenchyma are spared (3+ lesion). (D) From virus-infected C3H mouse. Note massive necrosis (4+ lesion). (For lesion scores see legend in Table I.)

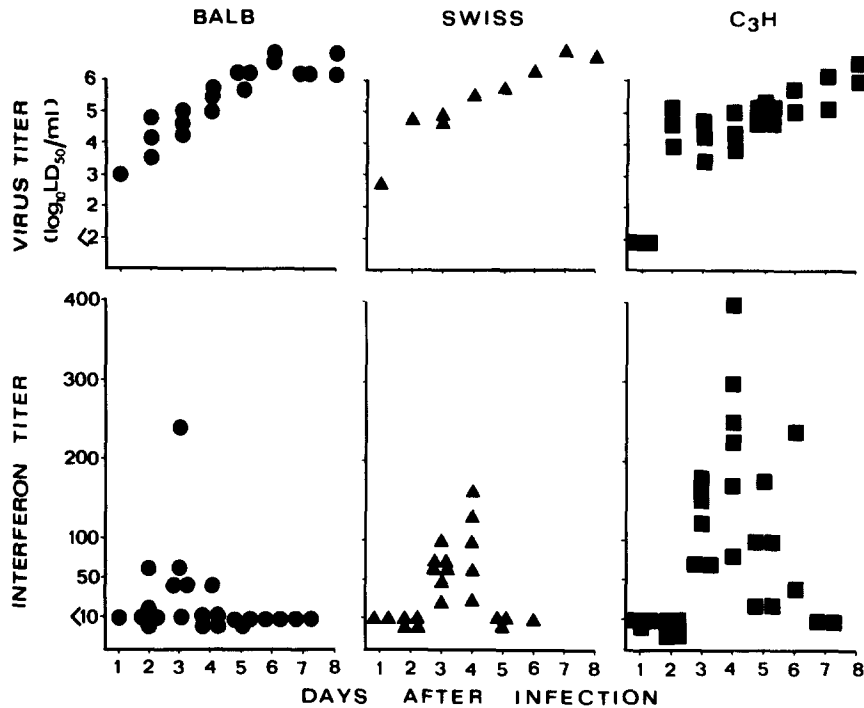


FIG. 4. Plasma titers of virus and interferon in BALB/c, Swiss, and C3H mice infected at birth with $10^{4.7}$ LD₅₀ of LCM virus. Each symbol indicates a pool of plasma from a litter of suckling mice (average, 10 mice).

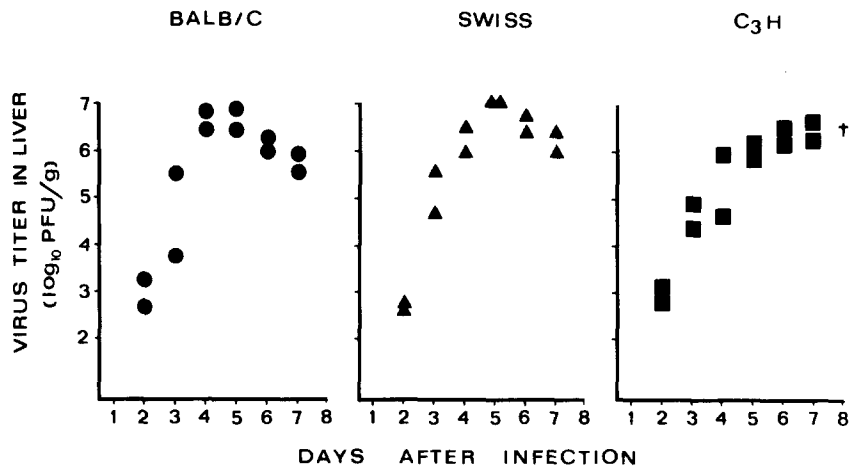


FIG. 5. Titer of virus in the livers of BALB/c, Swiss, and C3H mice infected at birth with $10^{4.7}$ LD₅₀ of LCM virus. Each symbol indicates the virus titer from an individual mouse. (+) C3H mice died between the 7th and 9th d; Swiss mice died after the 10th d. All BALB/c mice survived.

extracts, low titers were difficult to interpret). No significant difference was observed between the different strains.

DECREASED MORTALITY IN LCM VIRUS-INFECTED SUCKLING C3H MICE INOCULATED WITH ANTI-INTERFERON GLOBULIN. Because administration of anti-interferon globulin had

previously been shown to decrease the incidence of mortality in virus-infected Swiss mice (2) we undertook the same type of experiment in virus-infected C3H mice.

Newborn C3H mice inoculated with 10^5 LD₅₀ of LCM virus were injected on day 0 and day 3 with potent sheep anti-mouse interferon globulin (titer 8×10^{-5}) (8). Whereas 100% of virus-infected control C3H mice died, (9th to 12th d), only 6/28 (21%) of anti-mouse interferon globulin-treated mice died. These six mice died between the 15th and the 27th d.

Exogenous Interferon Can Induce Disease in BALB/c Mice. Although the minimal disease observed in LCM virus-infected BALB/c mice appeared to be related to their minimal interferon response, it was of interest to determine the sensitivity of BALB/c mice to daily administration of interferon. Accordingly, in five litters 11 newborn BALB/c mice were injected daily for 7 d with 0.05 ml of a mouse C-243 cell interferon preparation (6) having a titer of 1.6×10^{-6} . Eight litter mates were inoculated with a control preparation.

Interferon treatment inhibited the growth of suckling BALB/c mice. Thus, the mean weights (in grams) on the 8th d of life for interferon-treated suckling mice compared with control inoculated litter mates for the five litters were: interferon treated/control: 4/6.1; 2.3/4.4; 2.3/4.0; 3.4/5.3; and 2.2/3.0. Extensive liver cell necrosis was seen in interferon-treated mice sacrificed on the 8th d.

Discussion

The severity of disease observed in suckling mice injected at birth with LCM virus depends on the mouse strain (4). In our studies BALB/c mice were relatively unaffected: they grew almost as well as uninfected mice, they had only minimal liver lesions and none died. In contrast, virus-infected C3H mice grew poorly, they had extensive liver cell degeneration, and all mice died. An intermediate pattern was observed in Swiss mice. In referring to the variation in mortality of LCM virus-infected suckling mice of different strains Von Boehmer and co-workers stated "we know of no cause which satisfactorily explains the differences between mouse strains or the fluctuation within strains" (4). In agreement with the findings of Von Boehmer et al. (4) we found no difference in virus titers in plasma or liver between the three strains of mice (Figs. 4 and 5).

However, despite similar titers of virus in the plasma and liver, clear-cut differences in the amount of endogenous interferon produced and the duration of interferonemia was observed for the three strains of mice. Thus BALB/c mice produced small amounts of interferon detectable in the plasma mostly on the 3rd d; Swiss mice produced more interferon detectable on the 3rd and 4th d, whereas C3H mice produced larger amounts of interferon and interferonemia lasted between the 3rd and 6th d (Fig. 4). Our results suggest therefore that the amplitude of the interferon response in C3H mice is in large part responsible for the severity of LCM disease. This interpretation is further supported by experiments showing a marked decrease in the incidence of mortality in virus-infected C3H mice when they were injected with a potent anti-mouse interferon globulin (Results). Furthermore, the relative resistance of BALB/c mice was not caused by insensitivity to interferon action because exogenous interferon did inhibit their growth and did induce comparable liver lesions. We suggest therefore that the minimal disease occurring in BALB/c mice was related to their minimal interferon response.

These experiments also suggest that there is a genetic control for the production of interferon in suckling mice infected with LCM virus. De Maeyer and his associates have described four loci which determine the interferon response in mice after injection of Newcastle disease (9), mouse mammary (10), and Sendai (11) viruses. Work is in progress to determine whether the gene(s) controlling the production of interferon after infection with LCM virus is related to these previously described loci.

There is no question that interferon plays a beneficial role in most viral infections, and when administered, can confer considerable protection against viral infection. Our work emphasizes however, that potent biologic substances such as interferon can also be associated with untoward effects. Thus, in the study presented herein, it is the host response in C3H mice manifested as production of large amounts of interferon that proves injurious (100% mortality), whereas the minimal interferon response of BALB/c mice is associated with 100% survival. In LCM virus disease of adult mice, it is also the host response in the form of sensitized lymphocytes that appears to be responsible for disease (12-14). Thus, in both suckling and adult mice, LCM viral multiplication itself is apparently well tolerated (15), but it is the host response that is not tolerated. Recently, Hooks et al. (16) have found elevated levels of interferon in the sera of patients with severe systemic lupus erythematosus, and suggested the possibility that this interferon may play a pathogenetic role. Ida and co-workers (17) have suggested that the interferon produced in the course of respiratory infections may be responsible for some of the "allergic" manifestations. If we may extrapolate the results of our study and the extensive studies of De Maeyer et al. (9-11), it seems possible that there is a genetic control for the production of interferon to different viruses in man. Individuals may vary in the extent of their interferon response to a given viral infection and in their sensitivity to the effects of interferon. In most instances this interferon response will prove beneficial, but possibly there are some instances where too much interferon will prove injurious in a given individual.

Summary

A marked difference was observed in the severity of disease in lymphocytic choriomeningitis (LCM) virus-infected suckling BALB/c, Swiss, and C3H mice. BALB/c mice had minimal liver lesions and none died, whereas C3H mice had extensive liver lesions and all mice died. An intermediate pattern was observed for Swiss mice (36% mortality). Although there was no difference in the titers of LCM virus in the plasma or liver between these three strains of mice, there was a marked difference in the amount of interferon produced and the duration of interferonemia. C3H mice produced more interferon than Swiss mice which produced more interferon than BALB/c mice, indicating a direct correlation between the amount of interferon induced by LCM virus and the extent of disease. Inoculation of a potent anti-mouse interferon globulin markedly reduced the incidence of mortality in virus-infected C3H mice. BALB/c mice were as sensitive to the effects of interferon as C3H mice because daily administration of potent interferon preparations did induce disease in this strain. This ensemble of results supports our contention that endogenous interferon is in large part responsible for the manifestations of acute LCM virus disease in suckling mice.

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