

VIRUS-SPECIFIC INTERFERON ACTION

Protection of Newborn *Mx* Carriers against
Lethal Infection with Influenza Virus*

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Interferon, originally detected by its capacity to inhibit viral replication *in vitro* (1), has long been suspected to play a role in viral infections *in vivo* (2). Thus, it was noted that interferon appeared at the right time and in the right place to limit viral growth (3). Nevertheless, a simple correlation between the amount of interferon and the severity of a viral disease seems insufficient to make a strong case for interferon as a defense mechanism. With the availability of potent anti-interferon antisera, it became possible to demonstrate more convincingly that interferon may determine viral pathogenicity (4–6).

An interesting illustration for this is influenza virus infection in mice carrying the dominant allele *Mx*. Adult animals with this gene resist the lethal effect of large doses of mouse-adapted influenza viruses, titers of virus grown in their organs remain low, and interferon is hardly detectable (7, 8). Nevertheless, injection of potent anti-interferon antibodies renders *Mx*-bearing mice as susceptible to lethal influenza virus infection as non-*Mx*-bearing mice (8). That interferon has a protective role *in vivo*, however, has so far not been demonstrated directly. Because newborn *Mx*-bearing mice are virtually as susceptible to orthomyxoviruses as their *Mx*-negative counterparts (9), we were able to determine the efficacy of interferon in protecting newborn mice differing at the *Mx* locus.

Materials and Methods

Mice. A/J and CBA/J mice were obtained from G. L. Bomholtgård, Ry, Denmark. Inbred A2G mice, homozygous for the dominant resistance allele *Mx* (7) were bred locally. Timed pregnancies of crosses (A2G × CBA/J)_{F1}, heterozygous for *Mx*, and (A/J × CBA/J)_{F1}, devoid of *Mx*, were arranged in our laboratory.

Interferon Treatment. Interferon was induced by Newcastle disease virus in mouse C-243 cells and was partially purified to 1×10^7 reference units per mg protein, as previously described (10). Mock interferon was prepared in the same way except that the interferon inducer was omitted. Newborn mice were marked and injected subcutaneously in the interscapular region with 0.05 ml of interferon on days 1, 2, and 3 after birth to give the total amounts of reference units per mouse indicated in the text. Marked controls within each litter were similarly inoculated with mock interferon.

Virus Challenge. Animals were infected intraperitoneally on day 2 after birth. Lethal doses

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of the following viruses were used: TURH, a hepatotropic influenza A variant, was derived from A/Turkey/England/63 (Hav1, Nav3) (11). Encephalomyocarditis virus (EMC) was passaged in monolayer cultures of L₉₂₉ cells as described (12). Vesicular stomatitis virus (VSV), serotype Indiana, was grown and stored as previously described (12).

Results and Discussion

Newborn mice of crosses (A/J × CBA/J)F₁ and (A2G × CBA/J)F₁ were used. A/J and CBA/J mice are devoid of *Mx*, whereas A2G mice are homozygous for *Mx*. Because A/J and A2G mice are related strains sharing, for example, the major histocompatibility locus (13), there is a considerable overall similarity between the two sets of crosses. Within each litter, some mice were treated with interferon, and others were similarly treated with control material (mock interferon). The challenge viruses used were TURH, to which adult *Mx*-bearing animals are highly resistant (11), and EMC and VSV, both of similar virulence for mice bearing *Mx* or devoid of it (14).

From the data in Table I, it appears that interferon, even at the highest dosage used (8×10^5 interferon U/mouse), did not protect (A/J × CBA/J)F₁ mice from death upon challenge with the influenza virus TURH. This confirms earlier observations on the negligible role played by interferon in influenza virus infection of ordinary mice (5). In contrast, all (A2G × CBA/J)F₁ mice treated with the highest dose of interferon survived, and 10 times less interferon protected almost half of them. With 100 times less interferon, both *Mx* and non-*Mx* bearers died, with the difference that death was significantly delayed in presence of *Mx* ($P < 0.001$). When no interferon was given, *Mx* bearers died also somewhat later than their non-*Mx* counterparts, but this was barely significant. This slight delay of death may have been due to the production of traces of interferon in response to infection. Tables II and III show that the lethal effects of EMC and VSV were inhibited to a similar extent in both sets of mice.

This host gene-dependent difference of interferon action selective for a given virus, demonstrated here in vivo, mirrors faithfully the features observed in vitro. Isolated *Mx*-bearing cells, such as macrophages (12), liver parenchymal cells (16), or embryo fibroblasts (H. Arnheiter and O. Haller, unpublished observations) are much more sensitive than non-*Mx*-bearing cells to protection by interferon against influenza

TABLE I
*Protection of Newborn Mice by Interferon against Lethal Intraperitoneal Infection with Influenza Virus TURH**

Interferon U/mouse	(A/J × CBA/J)F ₁ (genotypes +/+)		(A2G × CBA/J)F ₁ (genotype <i>Mx</i> /+)		Significance of difference in survival time‡
	Surviving/total number infected	Median survival time <i>d</i>	Surviving/total number infected	Median survival time <i>d</i>	
8×10^5	0/20	3.7	19/19	>42	<0.001
8×10^4	0/13	3.4	13/30	8.5	<0.001
8×10^3	0/10	2.9	0/18	4.4	<0.001
—	0/37	2.5	0/44	3.3	0.03

* Newborn mice were treated with mouse interferon as described in Materials and Methods. Controls within the same litter were inoculated with a mock interferon preparation. Animals were infected intraperitoneally on day 2 after birth with 100 LD₅₀ (as titrated in susceptible adult mice) of hepatotropic influenza A/TUR/Eng/63 (Hav1, Nav3) (11). Deaths were recorded daily.

‡ Probability *P* that the observed differences between *Mx* bearers and +/+ mice were due to chance was calculated by the ranking test of Wilcoxon-Mann-Whitney (15).

TABLE II
Protection of Newborn Mice by Interferon against Lethal Intraperitoneal Infection with the Picornavirus EMC

Interferon U/mouse	(A/J × CBA/J)F ₁ (genotype +/+)		(A2G × CBA/J)F ₁ (genotype Mx/+)		Significance of difference in survival time‡
	Surviving/total number infected	Median survival time	Surviving/total number infected	Median survival time	
		<i>d</i>		<i>d</i>	<i>P</i>
8 × 10 ⁵	2/5	7.0	4/8	>7.0	>0.05
8 × 10 ⁴	0/13	5.0	0/10	4.1	>0.02
—	0/16	2.3	0/11	2.5	>0.05

* Newborn mice were given mouse interferon as indicated in Materials and Methods. They were infected at day 2 after birth with 0.05 ml of virus dilution containing 10⁵ mean tissue culture infective doses (as titrated on L₉₂₉ cells) per mouse of EMC (12). Deaths were recorded daily.

‡ Probability *P* as indicated in Table I.

TABLE III
Protection of Newborn Mice by Interferon against Lethal Intraperitoneal Infection with the Rhabdovirus VSV

Interferon U/mouse	(A/J × CBA/J)F ₁ (genotype +/+)		(A2G × CBA/J)F ₁ (genotype Mx/+)		Significance of difference in survival time‡
	Surviving/total number infected	Median survival time	Surviving/total number infected	Median survival time	
		<i>d</i>		<i>d</i>	<i>P</i>
8 × 10 ⁴	7/14	>9.0	9/14	>9.0	>0.05
8 × 10 ³	2/20	5.7	3/14	5.5	>0.05
—	0/20	3.3	0/23	3.3	>0.05

* Newborn mice were treated with interferon as indicated in Materials and Methods. They were given 0.05 ml of a dilution of VSV containing 250 mean tissue culture infective doses (as titrated in L₉₂₉ cells) (12) on day 2 after birth. Deaths were recorded daily.

‡ Probability *P* as indicated in Table I.

viruses. When unrelated viruses are used, no influence of *Mx* on the antiviral activity of interferon is seen.

The abrogation of resistance of adult *Mx*-bearing mice by anti-interferon antibodies and the enhanced potency of exogenous interferon toward orthomyxoviruses as observed both in *Mx*-bearing cells and in intact animals, taken together, make up a coherent picture of the role of interferon in this case of inborn resistance: upon infection with influenza virus in mature hosts, a first round of replication generates small amounts of interferon, sufficient to prevent viral spread in *Mx* bearers at the level of potential target cells, whereas the same or even larger amounts of interferon eventually formed in mice devoid of *Mx* only marginally influence the final outcome (17). The susceptibility of immature *Mx* bearers may be related to their inability to make adequate amounts of interferon, rather than to their inability to respond to it. The effect of interferon toward other viruses is independent of *Mx*. How this seemingly specific action of interferon is brought about we do not know. Several antiviral mechanisms seem to be activated in interferon-treated cells (18). It is therefore possible that different viruses are inhibited by different interferon mechanisms that may or may not be elicited in a given host cell. Observations by Nilsen et al. (19), for example, indicate that interferon induces a selective antiviral state in embryonal carcinoma cells. It may therefore be appropriate to postulate a variety of antiviral states, each affecting the replication pathway of defined groups of viruses and each governed by certain host genes. The present data would be compatible with such a view.

Our findings have practical implications. Had we used newborn mice to evaluate

the protective activity of interferon toward influenza virus, the same preparation judged as showing antiviral activity in *Mx*-bearing mice would not exhibit any such activity in mice of a different genotype. Furthermore, it is likely that similar interactions between interferon and host genes are important in the expression of non-antiviral effects of interferon, such as effects on delayed type hypersensitivity (20) and cell proliferation (21). It may be that, in man, host genes affecting interferon action will also prove to be important in determining sensitivity to viral infections and response to interferon therapy.

Summary

The efficacy of interferon in antiviral protection of newborn mice differing at the *Mx* locus was investigated. Adult mice bearing the allele *Mx* exhibit a high degree of specific resistance toward lethal challenge with influenza viruses. In contrast, newborn *Mx* carriers are virtually as susceptible to influenza viruses as newborn mice devoid of *Mx*. Resistance can be abrogated by treating adult animals with anti-interferon serum. Here, we provide direct evidence of a virus-specific effect of interferon in vivo: newborn mice carrying the resistance gene *Mx* could be protected against lethal influenza virus infection with doses of interferon that were not protective in the absence of *Mx*. The efficacy of interferon towards a picornavirus (encephalomyocarditis virus) and a rhabdovirus (vesicular stomatitis virus) was independent of *Mx*.

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References

1. Isaacs, A., and J. Lindenmann. 1957. Virus interference. I. The interferon. *Proc. R. Soc. Lond. B Biol. Sci.* **147**:258.
2. Baron, S., and A. Isaacs. 1962. Absence of interferon in lungs from fatal cases of influenza. *Br. Med. J.* **56**:18.
3. Isaacs, A., and G. Hitchcock. 1960. Role of interferon in recovery from virus infections. *Lancet.* **II**:69.
4. Gresser, I., M. G. Tovey, M. T. Bandu, C. Maury, and D. Brouty-Boyé. 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. *J. Exp. Med.* **144**:1305.
5. Gresser, I., M. G. Tovey, C. Maury, and M. T. Bandu. 1976. Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Moloney sarcoma, vesicular stomatitis, Newcastle disease, and influenza viruses. *J. Exp. Med.* **144**:1316.
6. Virelizier, J. L., and I. Gresser. 1978. Role of interferon in the pathogenesis of viral diseases of mice as demonstrated by the use of anti-interferon serum. *J. Immunol.* **120**:1616.
7. Lindenmann, J., C. A. Lane, and D. Hobson. 1963. The resistance of A2G mice to myxoviruses. *J. Immunol.* **90**:942.
8. Haller, O., H. Arnheiter, I. Gresser, and J. Lindenmann. 1979. Genetically determined, interferon-dependent resistance to influenza virus in mice. *J. Exp. Med.* **149**:601.
9. Lindenmann, J. 1964. Inheritance of resistance to influenza virus in mice. *Proc. Soc. Exp. Biol. Med.* **116**:506.
10. Tovey, M. G., J. Begon-Lours, and I. Gresser. 1974. A method for the largescale production of potent interferon preparations. *Proc. Soc. Exp. Biol. Med.* **146**:809.

11. Haller, O., H. Arnheiter, and J. Lindenmann. 1976. Genetically determined resistance to infection by hepatotropic influenza A virus in mice: effect of immunosuppression. *Infect. Immun.* **13**:844.
12. Haller, O., H. Arnheiter, J. Lindenmann, and I. Gresser. 1980. Host gene influences sensitivity to interferon action selectively for influenza virus. *Nature (Lond.)*. **283**:660.
13. Klein, J. 1975. *Biology of the Mouse Histocompatibility-2 Complex*. Springer-Verlag, New York. 120.
14. Lindenmann, J., and P. A. Klein. 1966. Further studies on the resistance of mice to myxoviruses. *Arch. Gesamte Virusforsch.* **19**:1.
15. Snedecor, G. W., and W. G. Cochran. 1955. *Statistical Methods*. 6th edition. The Iowa State University Press, Ames, Iowa. 593 pp.
16. Arnheiter, H., O. Haller, and J. Lindenmann. 1980. Host gene influence on interferon action in adult mouse hepatocytes: specificity for influenza virus. *Virology*. **103**:11.
17. McLaren, C., and C. W. Potter. 1973. The relationship between interferon and virus virulence in influenza virus infections of the mouse. *J. Med. Microbiol.* **6**:21.
18. Stewart, W. E., II. 1979. *The Interferon System*. Springer-Verlag, New York.
19. Nilsen, T. W., D. L. Wood, and C. Baglioni. 1980. Virus-specific effects of interferon in embryonal carcinoma cells. *Nature (Lond.)*. **286**:178.
20. De Maeyer, E., and J. De Maeyer-Guignard. 1980. Host genotype influences immunomodulation by interferon. *Nature (Lond.)*. **284**:173.
21. Gallien-Lartigue, O., D. Carrez, E. De Maeyer, and J. De Maeyer-Guignard. 1980. Strain dependence of the antiproliferative action of interferon on murine erythroid precursors. *Science (Wash. D. C.)*. **209**:292.