Variations in the Interferogenic Responses of Two Strains of Mice (36873)

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It has been reported that the interferogenic response of mice is quantitatively related to their genetic composition (1, 2). In these studies, it was found that circulating interferon production, induced by ultraviolet irradiated Newcastle disease virus (NDV), was markedly higher in $C_{57}Bl$ mice when compared with Balb/c/Gif mice. Mendelian analyses indicated that a single, partially dominant, autosomal factor was responsible for this difference. Recently, Rice *et al.* (3), have extended these observations to include eight-inbred and one random-bred strains of mice. In this study, poly (I)·poly (C) and poly-D-lysine, poly (I)·poly (C) complexes were used as interferogens. Marked differences between strains were observed in the interferogenic responses. In contrast to previous work, it was found that $C_{57}Bl$ mice produced less interferon than Balb/c mice. Furthermore, the response of random-bred Swiss mice was thirty times greater than that of inbred $C_{57}Bl$ mice.

Since much of the studies on interferogens is dependent upon detecting circulating interferon in mice, it was felt that these observations on strain differences in the interferogenic response should be confirmed and extended to include other interferon inducers. In view of this, the following experiments were undertaken, first, to verify the results with NDV and $C_{57}Bl$ mice, and second, to determine if the strain differences in the interferogenic response are seen with other interferon inducers or if it is limited to viral and polynucleotide interferogens.

Materials and Methods. Inbred $C_{57}Bl/Jac$ (Sprague–Dawley, Madison, Wis.) and random-bred Swiss mice (Charles River Breeding Labs, Wilmington, Mass.) 20 to 25 g, were housed in groups of 25.

Mouse L-cells were grown on 32 oz glass prescription bottles in Eagle's minimal medium (MEM) supplemented with 10% heat-inactivated (56° for 30 min) fetal-calf serum. Monolayer cultures for interferon assays were prepared by adding 2×10^6 cells in 6 ml of MEM to 25 cm^2 plastic tissue culture flasks (Falcon Plastics) 36 hr before use.

Stocks of Newcastle disease virus (NDV), Hertz strain, were prepared by infecting the chorioallantoic cavity of 10-day old chicken embryos. The allantoic fluids of infected embryos were harvested 24 hr later, pooled, and clarified by centrifugation (1,000*g* for 30 min). Vesicular stomatitis virus (VSV), Indiana strain, was prepared in mouse L-cell cultures (4). All virus stocks were stored at −70° until used.

Tilorone hydrochloride (a gift of Merrell National Laboratories, Cincinnati, Oh.), Statolon (a gift of Eli Lilly and Co., Indianapolis, Ind.), divinyl ether-maleic anhydride copolymer (a gift of Hercules, Inc., Wilmington, Del.), endotoxin (*E. coli* lipopolysaccharide B, purchased from Difco Labs, Detroit, Mich.), and NDV were used as interferon inducers. The inducers were administered intraperitoneally as aqueous solutions. Serum for interferon assay was obtained from blood collected from the brachial artery of mice under deep ether anesthesia. Sera from five animals were pooled from each time interval, and the pooled, undiluted sera stored at −20° until titrated.

Samples were assayed for interferon activity on monolayers of mouse L-cells. Serial dilutions of each sample were made in NCTC 199 plus 1% heat-inactivated fetal-calf serum. Cell cultures were incubated with interferon dilutions (4 ml per culture) for 18 hr. Interferon titer was determined by the 50% plaque reduction technique (5) using

TABLE I. Comparison of the Interferogenic Response of $C_{57}Bl$ and Swiss Mice to NDV.

Time post-induction (hr)	Serum interferon titer	
	$C_{57}Bl$	Swiss
2	180	220
4	11,000	5600
6	40,000	9000
8	30,000	15,000
12	23,000	4500
24	1350	400

VSV as the challenge virus.

Results. To determine if a difference exists in the interferogenic responses of $C_{57}Bl$ and Swiss mice to a viral interferogen, one group of ten Swiss mice and a similar group of $C_{57}Bl$ mice were inoculated with ultraviolet irradiated NDV. Each mouse received 10^9 virus particles intraperitoneally. Fourteen hours later, by which time substantial interferon titers should have been reached, the mice were bled. In agreement with previous reports (1, 2) it was found that the $C_{57}Bl$ mice produced higher titers of interferon than did the Swiss mice (4800 units for the $C_{57}Bl$ vs 980 units for the Swiss mice). To rule out the possibility that the higher interferon titers seen with the $C_{57}Bl$ mice were simply due to differences in the kinetics of interferon production in the two strains, mice of each strain were inoculated as above with NDV. At 2, 4, 6, 8, 12, and 24 hr post-inoculation, animals were sacrificed and the sera obtained titered for interferon content. As can be seen in Table I, little strain difference was seen in the kinetics of interferon production, although the $C_{57}Bl$ mice responded with markedly higher titers of interferon than did the Swiss mice.

To determine if the strain difference in the interferogenic response is seen with nonviral interferogens, replicate groups of Swiss and $C_{57}Bl$ mice were inoculated intraperitoneally with either tilorone hydrochloride, Statolon, divinyl ether-maleic anhydride copolymer, or bacterial indotoxin. At appropriate time intervals, the animals were sacrificed and their sera titered for interferon content. The results of such an experiment are presented in Table II. Although little difference between the two murine strains was seen in the kinetics of the interferogenic responses, marked differences were consistently seen in the amounts of interferon produced. $C_{57}Bl$ mice produced more interferon in response to endotoxin than did Swiss mice. However, when tilorone hydrochloride, divinyl ether-maleic anhydride copolymer, or Statolon were used as interferogens, the Swiss mice responded with greater interferon production than did the $C_{57}Bl$ mice.

The above data indicate that there is a marked genetic influence on the quantitative response of different strains of mice to a particular interferogen. However, this reponse appears to be tempered by the nature of the inducer. An alternative to this is that the amount of interferon produced by the mouse strains is similar, but interferon antagonists (6, 7, 8) are concomitantly released in response to the different interferogens. The presence of such antagonists in the sera of

TABLE II. Comparison of the Interferogenic Responses of $C_{57}Bl$ and Swiss Mice to Nonviral Interferogens.

Interferogen	Dose (mg/kg)	Time post-inoculation (hr)	Interferon $C_{57}Bl$	Titer Swiss
Tilorone HCl	80	6	73	31
		12	358	1408
		18	334	1932
		24	65	30
Divinyl ether	50	6	<5	<5
		12	<5	110
		18	19	255
		24	35	71
		48	<5	30
Statolon	10	4	<5	660
		8	300	2700
		10	720	2200
		12	680	720
		16	220	280
Endotoxin	20	1	<5	<5
		2	225	58
		3	200	<5
		4	59	24
		5	<5	39

TABLE III. Effects of Mixing Sera from $C_{57}Bl$ and Swiss Mice on Interferon Titer.

Serum		Interferon titer
A	$C_{57}Bl$ NDV induced	3800
B	Swiss NDV induced	2300
C	$C_{57}Bl$ NDV induced	4800
D	Swiss normal	<5
E	$C_{57}Bl$ normal	<5
A + B[a]		2000
C + D[a]		4450
C + E[a]		4300
C + saline[a,b]		

[a] 0.5 ml of each was mixed and treated as described in text.

[b] Needed to determine dilution effects.

test animals would result in an apparent lowering of the serum interferon levels of these animals. Therefore, the following experiment was conducted to test for the presence of such inhibitors. Various combinations of equal amounts of low-titer interferon sera from NDV-induced Swiss and $C_{57}Bl$ mice and/or normal sera were mixed, incubated for 1 hr at 37°, and titered on mouse L-cell cultures. NDV-induced mice were used since this interferogen produced the greatest difference between strains in the interferogenic response. If interferon antagonists were present, the apparent interferon titer of the $C_{57}Bl$ sera should be lowered when mixed with ND-induced Swiss sera. Table III summarizes this experiment. Although there was a slight depression of interferon titer when sera from NDV-induced $C_{57}Bl$ and Swiss mice were mixed, no clear evidence was obtained for the presence of interferon antagonists in the sera of Swiss mice.

Discussion. The results of this study support and extend previous observations that the interferogenic response of mice is quantitatively related to their genetic composition (1–3). We have found that when a variety of interferogens were examined, marked quantitative differences were observed in the interferon responses of the two strains of mice used in this study. Inbred $C_{57}Bl$ mice exhibited a higher interferogenic response when induced with NDV or bacterial endotoxin than did random-bred Swiss mice. However, the interferogenic responses of Swiss mice were consistently higher than that of $C_{57}Bl$ mice when tilorone hydrochloride, divinyl ether-maleic anhydride copolymer or Statolon were used as interferogens. These results indicate differences in the abilities of the two strains of mice to respond to various interferogens. When the kinetics of the interferogenic responses following induction with these various inducers were examined, it was found that although there were strain-related differences in the absolute amounts of interferon produced, the kinetics of interferon production were characteristic of the particular interferogen used. Clearly the magnitude of the interferon response is not only related to the genetic composition of the test animal, but is also tempered by specific inducers.

It has been reported (4, 11, 12) that various interferogens induce the syntheses of different molecular species of interferon. Recent work has shown that these molecular species of interferon probably represent oligomeric forms of a basic interferon subunit (9, 10). It is possible that the strain differences described here are related to the ability of a given murine strain to preferentially synthesize a particular molecular species of interferon. Interferogens which induce high molecular-weight interferon would then stimulate the production of more interferon in those strains of mice which are genetically able to produce greater amounts of high molecular-weight interferon than in those strains which cannot. Conversely, interferogens which induce low molecular-weight interferon would induce comparatively more interferon in those mouse strains which preferentially respond with the production of low molecular-weight interferon. It has been reported that the interferon induced by endotoxin and Statolon are of the same molecular size (4). If the mechanism described above were operative one would expect these interferogens to be highly interferogenic in the same strain of mouse. As shown in Table II, $C_{57}Bl$ mice responded with more interferon than Swiss mice following induction with endotoxin. However, when induced with Statolon, Swiss mice responded with more interferon than

$C_{57}Bl$ mice. Strain-related variations in the molecular size of the interferon induced by NDV have been reported (13). Whether such variations are seen with other interferogens is yet to be determined.

Summary. Marked quantitative differences have been observed in the interferogenic responses of two murine strains. Inbred $C_{57}Bl$ mice exhibited a higher interferogenic response when induced with NDV or bacterial endotoxin than did random-bred Swiss mice, although the kinetics of interferon induction were essentially the same in both strains. When induced with tilorone hydrochloride, divinyl ether-maleic anhydride copolymer, or Statolon, random-bred Swiss mice consistently responded with greater interferon production than did $C_{57}Bl$ mice. While the studies reported here do not allow discrimination among several hypotheses that can be envisaged to account for these strain-related differences in the interferogenic responses, it is apparent that the magnitude of the interferon response is not only related to the genetic composition of the test animal, but is also tempered by the nature of the interferogen.

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