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# Gene with Quantitative Effect on Circulating Interferon Induced by Newcastle Disease Virus

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Circulating interferon production, induced by Newcastle disease virus, is about seven times higher in $C_{57}$ Black mice than in Balb/c/Gif mice. A Mendelian analysis was carried out and circulating interferon production was measured in reciprocal $F_1$ hybrids, in the $F_2$ generation, in progeny of backcrosses of $F_1$ hybrids to either parent strain, and in second backcross progeny. The results indicate that a single, partly dominant, autosomal factor is responsible for the difference in circulating interferon production between both parent strains.

Large amounts of interferon (6) are released into the bloodstream of animals injected intravenously with viruses (1). This method of inducing what is called circulating or serum interferon is being widely used to obtain information concerning the role and production of interferon during viraemia. In a preliminary communication, we mentioned that, upon intravenous injection of Newcastle disease virus (NDV), mice of the $C_{57}$Black strain produce considerably more circulating interferon than do mice belonging to the $C_3H$/He or Balb/c strains (3). This difference in interferon production merited further attention, particularly in view of the well-documented resistance of the $C_{57}$Black strain to a number of virus infections (2, 5, 8, 10). Therefore, several aspects of the high interferon production by $C_{57}$Black mice are currently under investigation in this laboratory. In this paper, we present the results of a Mendelian analysis of the phenomenon, made by using $C_{57}$Black as high producers and Balb/c as low producers of interferon.

## MATERIALS AND METHODS

**Mice.** $C_{57}$ Black mice were obtained from the Laboratory Animals Centre (M.R.C., Carshalton, Surrey, U.K.). First-generation mice, derived from the Carshalton breeding stock, were used as parents in this study.

Balb/c mice have been maintained in our laboratory by brother-sister mating for five generations. The parents of our subline were received from D. Friedman, Hôpital St. Louis, Paris, who originally obtained his Balb/c line from M. A. Sabourdy, Centre de Selection des Animaux de Laboratoire du C.N.R.S., Gif-sur-Yvette, France.

**Virus strains.** NDV, Kumarov strain, was used to induce circulating interferon. Stock suspensions of the virus were prepared by infecting the chorioallantoic cavity of 10-day-old chick embryos, and harvesting allantoic fluids 2 days later. Collected fluids were clarified by 30-min centrifugation at 5,000 rev/min and kept at −70 C until used for interferon induction. The same batch of NDV was used throughout the experiments reported here; its titer was $10^8$ median egg infective dose ($EID_{50}$) per 0.1 ml of allantoic fluid.

Vesicular stomatitis virus (VSV), Indiana strain, was used as challenge virus in the interferon titrations. Stock suspensions of this virus were prepared in mouse L-cell cultures.

**Tissue culture.** Interferon titrations were carried out in L cells (9). The culture medium was Eagle's minimum essential medium (MEM; reference 4) prepared from powdered medium (Gibco, Long Island, N.Y.). Of inactivated calf serum, 8% was added for growth and 3% for maintenance. Plaque assays were carried out under Eagle's MEM solidified with 7.5% hydrolyzed starch.

**Induction of circulating interferon in mice.** All mice in which interferon production was measured were 7 to 10 weeks old unless indicated otherwise. An 0.2-ml amount of NDV suspension was injected into the right orbital sinus of the animal; 8 hr later, blood was drawn with a plastic 1-ml syringe, also from an orbital sinus. Blood samples were left to coagulate overnight in a refrigerator; the sera were then separated and residual cells were removed by 2-min centrifugation at 15,000 × *g*. Sera were stored at −20 C until interferon titration. The decision to measure the circulating interferon levels 8 hr after injection of the virus was taken as a result of numerous preliminary experiments. It was of utmost importance to obtain the sera when circulating interferon levels were at their highest, since our study was concerned with quantitative differences. Different authors have

reported different times of appearance of peak levels of interferon in the serum after intravenous injection of NDV. It is evident from a survey of the literature that no generalization can be made and that kinetic studies have to be carried out in each laboratory with the particular virus-animal combination under study. Thus, we found that, both in Balb/c and in $C_{57}$Black mice, NDV-induced serum interferon levels reached maximum values between 6 and 9 hr after injection of the virus. During this period, interferon levels usually did not vary much and appeared to have reached a plateau. Nevertheless, to be as sure as possible that we were dealing with maximal interferon levels, many experiments in Balb/c, $C_{57}$Black, and $F_1$ hybrids were run in duplicate. One series of animals was bled 10 or 11 hr after virus injection, and their interferon levels were compared to the levels of mice bled at 8 hr. Interferon levels were in all instances significantly higher in the 8-hr series. This procedure could, of course, not be adopted for the heterogenous backcross and $F_2$ series, and these animals were therefore all routinely bled at 8 hr after virus injection. Thus, we cannot completely exclude the possibility that a certain error has crept into our analysis because peak values were missed in some of the mice. However, we do feel that the greatest care was taken to minimize this error as much as possible.

**Interferon assay.** Interferon was measured by a plaque reduction method (11). Monolayers of L cells, in plastic petri dishes, were incubated overnight with fivefold dilutions of each serum, starting at 1/500, in 1.6 ml of maintenance medium; usually, two plates per dilution were assayed. The medium also contained 1% (v/v) of hyperimmune rabbit anti-NDV serum. This concentration of antiserum was chosen because it was sufficient to neutralize 500 $EID_{50}$ of NDV, the maximal amount of NDV remaining in 1 ml of undiluted mouse serum. Since the lowest mouse serum dilution in the interferon assay was 1/500, practically no infectious NDV remained even at this dilution, and the presence of antiserum provided an extra safety margin. After overnight incubation of the cells, the medium was aspirated and about 100 plaque-forming units of VSV, suspended in 1 ml of medium, was added. Unadsorbed virus was removed 1 hr later, and 4 ml of a starch overlay was added. The assay plates were then incubated for 48 hr at 37 C in a $CO_2$ incubator; they were then stained with May-Grünwald solution (Merck A. G., Darmstadt, Germany). Plates were left on the bench, and the plaques were counted any time between 6 and 96 hr after staining. Interferon titers were expressed as units, one unit corresponding to the amount of inhibitor necessary to reduce the number of challenge plaques by 50% (7). A reference interferon preparation, made in mouse L cells infected with NDV, was included each time to evaluate the relative sensitivity of each assay. This allowed the correction of the serum interferon titers, if necessary, so that values obtained in assays carried out on different days could be compared.

**Criteria for attributing antiviral activity to interferon.** A number of criteria were applied before attributing the antiviral activity, appearing in mouse sera after intravenous injection of NDV, to interferon(s). This procedure was not followed with each individual mouse serum, but only with representative pools of sera from both parent strains, $F_1$ hybrids, and high- and low-producing $F_2$ and backcross animals.

(i) Species specificity. The sera had no antiviral activity when assayed against VSV in primary cultures of chick embryo cells, in a continuous line of rabbit kidney cells (RK 13), and in a continuous line of primate cells (MA 104). Both of these cell lines are susceptible to interferon of the corresponding species (J. Coppey, *personal communication*).

(ii) Lack of virus specificity. When tested in L cells, the sera were active not only against VSV but also against two other viruses tested as challenge, namely Semliki Forest and vaccinia viruses.

(iii) Effect of acidity. Dialysis against glycine buffer at *p*H 2 for 24 hr at 4 C did not affect significantly the antiviral activity.

(iv) Macromolecular nature. The interfering activity was nondialyzable. Exposure to trypsin (0.05% for 30 min at 37 C and *p*H 7.4) reduced the antiviral activity to less than 10% of the original activity.

## RESULTS

**Parent strains.** Circulating interferon levels were measured in individual male and female Balb/c and $C_{57}$Black mice. The individual titers (Table 1) confirmed the previously obtained results with pooled sera (3). Furthermore, no difference was observed between males and females of the same strain.

**Reciprocal $F_1$ hybrids.** Circulating interferon levels induced in mice derived from crosses between both parent strains are given in Table 2. For each cross, males and females are listed together because of the absence of any difference due to sex in the parent strains and because of the absence of maternal or paternal effect in the $F_1$ hybrids. The arithmetic and logarithmic averages of the titers of these $F_1$ hybrids were definitely higher than the average titer of the Balb/c parents but much lower than the average titer of the $C_{57}$Black parents. Statistical analysis of the data was carried out by using the $\log_{10}$ values of the interferon titers instead of their arithmetic value. This decision was taken because of the apparent normal distribution of the titers when plotted on a $\log_{10}$ scale (Fig. 1). Comparison of both mean arithmetic and mean logarithmic interferon titers between parent strains and reciprocal $F_1$ hybrids showed that the difference was highly significant between $F_1$ animals and Balb/c and $C_{57}$Black parents, but not between the two reciprocal $F_1$ series (Table 2).

The average interferon titers of the $F_1$ hybrids were fortunately much closer to the Balb/c value than to the $C_{57}$Black values. It was there-

TABLE 1. *Serum interferon titers in both parent strains*

| BALB/c[a] | | | | | $C_{57}BL$[a] | | | |
|---|---|---|---|---|---|---|---|---|
| ♀ | | ♂ | | | ♀ | | ♂ | |
| 1,250 | 3,500 | 650 | 2,000 | 3,250 | 10,100 | 20,400 | 10,000 | 21,000 |
| 1,450 | 3,640 | 780 | 2,000 | 3,640 | 13,000 | 20,400 | 12,000 | 22,000 |
| 1,550 | 3,640 | 815 | 2,200 | 3,700 | 13,000 | 20,800 | 13,000 | 22,000 |
| 1,550 | 3,640 | 960 | 2,300 | 3,725 | 13,000 | 21,000 | 14,000 | 22,000 |
| 1,800 | 3,800 | 1,100 | 2,500 | 4,000 | 14,500 | 21,400 | 14,000 | 23,000 |
| 2,000 | 4,000 | 1,100 | 2,500 | 4,000 | 14,500 | 22,400 | 14,600 | 24,000 |
| 2,200 | 4,600 | 1,200 | 2,640 | 4,000 | 16,000 | 22,400 | 16,500 | 24,000 |
| 2,300 | 4,600 | 1,260 | 2,800 | 4,000 | 16,000 | 23,000 | 16,700 | 24,000 |
| 2,900 | 4,800 | 1,540 | 2,850 | 4,250 | 16,000 | 24,600 | 17,000 | 24,000 |
| 2,900 | 4,800 | 1,600 | 2,900 | 4,550 | 16,000 | 25,000 | 18,000 | 26,000 |
| 2,900 | 4,800 | 1,600 | 2,900 | 5,250 | 16,000 | 25,600 | 19,000 | 28,000 |
| 2,900 | 5,000 | 1,660 | 2,960 | 5,800 | 16,000 | 27,000 | 19,000 | 29,000 |
| 2,900 | 5,520 | 1,700 | 2,960 | 6,300 | 16,200 | 27,200 | 19,500 | 30,000 |
| 3,000 | 7,250 | 1,830 | 3,125 | 7,900 | 18,000 | 29,800 | 20,000 | 31,000 |
| | | 1,900 | 3,130 | 8,250 | 18,700 | 31,000 | 21,000 | 33,000 |
| | | 1,900 | 3,160 | | 19,500 | | 21,000 | 34,000 |
| No. of animals: 28 | | No. of animals: 47 | | | No. of animals: 31 | | No. of animals: 32 | |
| Mean titer (arithmetic): 3,399 | | Mean titer (arithmetic): 2,917 | | | Mean titer (arithmetic): 19,629 | | Mean titer (arithmetic): 21,615 | |
| Mean $\log_{10}$ value[b]: 3.491 | | Mean $\log_{10}$ value[b]: 3.393 | | | Mean $\log_{10}$ value[b]: 4.277 | | Mean $\log_{10}$ value[b]: 4.315 | |
| SD: 0.195 | | SD: 0.258 | | | SD: 0.119 | | SD: 0.132 | |

[a] Result of *t* test: difference between means of ♀ and ♂ not significant ($P > 0.05$).
[b] Obtained by adding the decimal logarithm of all individual interferon titers and dividing the sum by the number of animals.

TABLE 2. *Serum interferon titers of reciprocal $F_1$ hybrids*[a]

| Determination | $C_{57}BL$ ♀ × BALB/c ♂ | BALB/c ♀ × $C_{57}BL$ ♂ |
|---|---|---|
| No. of animals | 43 | 69 |
| Range of individual titers | 550–16,000 | 330–10,800 |
| Mean titer (arithmetic) | 5,691 | 4,662 |
| Mean $\log_{10}$ value | 3.636 | 3.608 |
| SD | 0.355 | 0.252 |

[a] Result of *t* tests: difference between means not significant ($P > 0.05$); compared to Balb/c (combined ♀ + ♂), difference highly significant ($P < 0.001$); compared to $C_{57}B1$ (combined ♀ + ♂), difference highly significant ($P < 0.001$).

fore possible, in view of the interpretation of results to be obtained in further crosses, to put the cutoff value between $F_1$ and $C_{57}$Black genotype at 11,000, all interferon titers of 11,000 or lower being considered to belong to either $F_1$ or Balb/c genotype. Of the total of 75 Balb/c animals, none had a titer higher than 8,250. Out of a total of 64 $C_{57}$Black animals, only 2 (3.1%) had a titer lower than 11,500. Out of a total of 112 $F_1$ hybrids, only 5 (4.5%) had an interferon titer higher than 11,000.

**Results in $F_2$ hybrids.** Because of the lack of maternal or paternal effect in the $F_1$ hybrids, all further studies were carried out with ($C_{57}$Black ♀ × Balb/c ♂) $F_1$ hybrids as parents. Out of 134 $F_2$ hybrids analyzed for circulating interferon determination, 35 (26%) had interferon titers higher than 11,000, the average titer of these high producers being very close to and not significantly different from the average titer of the $C_{57}$Black animals (Table 3). The average titer of the 99 "low" producers was situated between the Balb/c and the $F_1$ averages. These results suggested that a limited number of genes, and possibly only one, were responsible for the high production of $C_{57}$Black mice. This possibility was further explored by measuring interferon production of backcross progeny.

**Backcross to $C_{57}$Black.** In all backcross experiments ($C_{57}$Black ♀ × Balb/c ♂) hybrids were used as $F_1$ parents. Tables 4 and 5 summarize the results of two backcross experiments, one with $C_{57}$Black mice as mothers and the other with $C_{57}$Black as fathers. Out of 84 mice derived

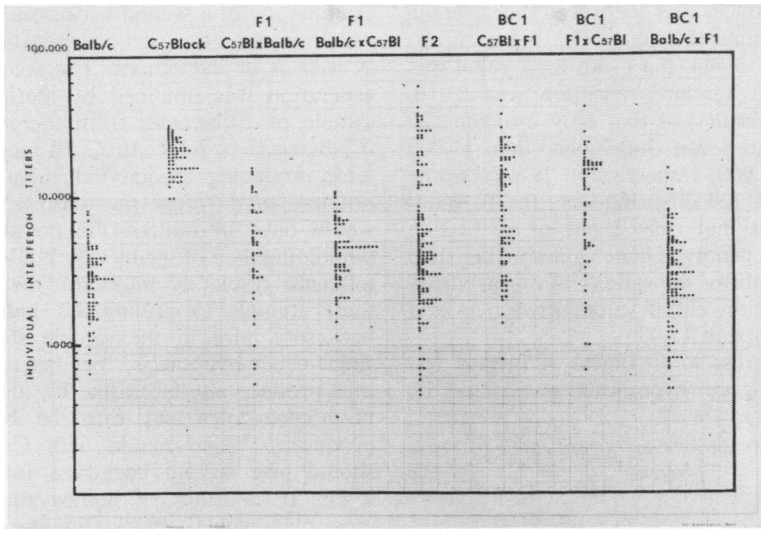


FIG. 1. *Interferon determinations in both parent strains and their different hybrid combinations. Graphic presentation of results given in Table 1–5.*

TABLE 3. *Serum interferon titers of $F_2$ hybrids*[a]

| Determination | (C57BL × BALB/c) ♀ × (C57BL × BALB/c) ♂ | |
|---|---|---|
| | Low producers (≤ 11,000) | High producers |
| No. of animals | 99 | 35 |
| Range of individual titers | 460–8,000 | 11,500–50,000 |
| Mean titer (arithmetic) | 3,501 | 23,308 |
| Mean $\log_{10}$ value | 3.464 | 4.329 |
| SD | 0.284 | 0.182 |

[a] $X^2$ analysis for single-gene hypothesis: low producers observed, 99; expected, 100; high producers observed, 35; expected, 34; $X^2$, 0.039; $\alpha$, 0.90.

TABLE 4. *Serum interferon titers of backcross progeny*[a]

| Determination | C57BL ♀ × F1 ♂ | |
|---|---|---|
| | Low producers (≤ 11,000) | High producers |
| No. of animals | 45 | 39 |
| Range of individual titers | 800–10,000 | 11,500–43,500 |
| Mean titer (arithmetic) | 4,311 | 18,653 |
| Mean $\log_{10}$ value | 3.582 | 4.246 |
| SD | 0.227 | 0.141 |

[a] $X^2$ analysis for single-gene hypothesis: low producers observed, 45; expected, 42; high producers observed, 39; expected, 42; $X^2$, 0.42; $\alpha$, 0.50.

from the backcross with $C_{57}$Black as mothers, 39 (46.4%) were high producers and 45 (53.5%) had an average interferon titer not significantly different from the $F_1$ genotype. Of the 60 mice derived from the backcross with $C_{57}$Black as fathers, 31 (51.6%) were high producers and 29 (48.4%) had an interferon production not significantly different from the $F_1$ genotype. These results showed that the offspring of the backcross matings clearly fell into two groups, one apparently homozygous for the $C_{57}$Black allele with high interferon production, and one heterozygous, with interferon production like $F_1$ animals. Of the combined backcross progeny, 70 of 144 animals were high producers and 74 produced interferon like the $F_1$ This agrees well with the 1:1 ratio one would expect if one gene were responsible for the difference (*see* Table 4 and 5).

**Backcross to Balb/c.** If one major gene were responsible for the difference between Balb/c and $C_{57}$Black mice, the progeny of a backcross between $F_1$ and Balb/c would be equally divided in homozygotes for the Balb/c genotype and in heterozygotes. However, because of the extensive overlapping of $F_1$ and Balb/c interferon titers, one would not expect to be able to distinguish between the groups. This is borne out by the results of a backcross experiment with Balb/c mice as mothers and ($C_{57}$Black × Balb/c) $F_1$ as fathers, in which the range of individual titers was 500 to 15,200, i.e., the average titer of a

total of 120 mice was between the average Balb/c and average $F_1$ titers. The mean (arithmetic) titer was 3,544, the mean $\log_{10}$ value was 3.448, and the standard deviation was 0.310. An important result was that only 2 of the 120 mice had an interferon titer higher than 11,000 (13,000 and 15,000, respectively). If we assume that 60 of these 120 offspring have the $F_1$ genotype, it appears that only 2 out of 60 (3.3%) animals with $F_1$ genotype have values higher than 11,000. This confirms the validity of our arbitrary decision to put the cutoff value between $F_1$ and $C_{57}$Black genotype at 11,000.

**Second backcross to $C_{57}$Black.** A further test of the single gene hypothesis was made by examination of a second backcross generation, in which one would expect a segregation of families as well as of individuals. The second backcross generation was obtained by mating a random sample of 20 females from the first backcross ($C_{57}$Black ♀ × $F_1$ ♂: to $C_{57}$Bl males. The interferon-producing capacity of these females was not measured before the matings, because this would have introduced the possibility of contaminating their offspring with NDV, and perhaps influence results of interferon measurements in these animals. Approximately half of the first backcross mice in the sample should, on the single-gene hypothesis, be heterozygotes and thus produce interferon like $F_1$, and half should be homozygotes and thus be high-interferon producers. When mated with $C_{57}$Black, these should give second backcross families of two kinds. (i) Matings of homozygous first backcross mice with $C_{57}$Black should give only high-producing offspring. (ii) Matings of heterozygous first backcross mice with $C_{57}$Black should give equal numbers of high- and low ($F_1$ type)-producing offspring.

Of the 20 first backcross females mated, 18 had offspring; the circulating interferon production of these offspring was measured when the mice were about 2 months old. The circulating interferon production of the mothers was also measured at that time (these animals were then about 5 months old). The results of the interferon determinations are given in Table 6 and Fig. 2. Of the 18 mothers, 8 had interferon titers higher than 11,000; they were therefore classified as

TABLE 5. *Serum interferon titers of backcross progeny*[a]

| Determination | $F_1$ ♀ × $C_{57}$BL ♂ | |
|---|---|---|
| | Low producers (≤ 11,000) | High producers |
| No. of animals | 29 | 31 |
| Range of individual titers | 680–11,000 | 11,500–40,000 |
| Mean titer (arithmetic) | 4,995 | 17,420 |
| Mean $\log_{10}$ value | 3.604 | 4.225 |
| SD | 0.319 | 0.108 |

[a] $X^2$ analysis for single-gene hypothesis: low producers observed, 29; expected, 30; high producers observed, 31; expected, 30; $X^2$, 0.066; $\alpha$, 0.50.

TABLE 6. *Serum interferon titers of second backcross progeny*

($F_1$ × $C_{57}$ BL) ♀ × $C_{57}$ BL ♂

| Progeny of low-producer mothers | | | | Progeny of high-producer mothers | | | |
|---|---|---|---|---|---|---|---|
| Interferon titer of mother | No. of offspring | Low producers (≤ 11,000)[a] | High producers[a] | Interferon titer of mother | No. of offspring | Low producers (≤ 11,000)[b] | High producers[b] |
| 7,600 | 8 | 2 | 6 | 19,600 | 7 | 0 | 7 |
| 3,800 | 6 | 6 | 0 | 18,000 | 8 | 1 | 7 |
| 6,000 | 10 | 8 | 2 | 17,250 | 8 | 0 | 8 |
| 8,000 | 9 | 1 | 8 | 13,200 | 7 | 0 | 7 |
| 8,900 | 11 | 6 | 5 | 20,000 | 7 | 0 | 7 |
| 5,000 | 7 | 5 | 2 | 26,000 | 9 | 0 | 9 |
| 3,600 | 11 | 3 | 8 | 21,000 | 6 | 0 | 6 |
| 7,125 | 8 | 2 | 6 | 15,100 | 8 | 0 | 8 |
| 7,625 | 7 | 5 | 2 | | | | |
| 7,050 | 9 | 8 | 1 | | | | |

[a] Total number of low producers, 46; high producers, 40; arithmetic average of titers of low producers, 4,484; high producers, 19,625.

[b] Total number of low producers, 1; high producers, 59; arithmetic average of titer of high producers, 20,908.

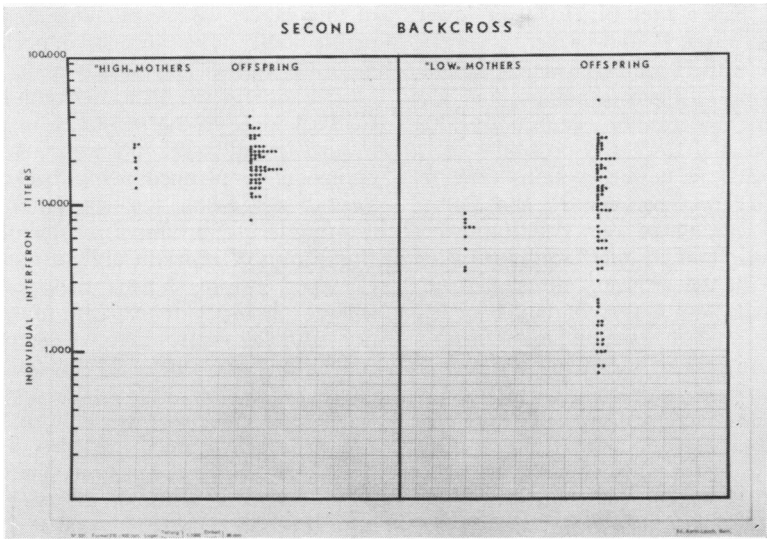


FIG. 2. *Serum interferon titers of second backcross progeny. Graphic presentation of results summarized in Table 6. Each point represents an individual interferon titer.*

high producers, probably homozygous. All progeny of these eight mothers, with the exception of one animal, were high producers. The one offspring with an interferon titer lower than 11,000 had a titer of 8,000. The progeny of each of the mothers with $F_1$ type interferon production consisted of high producers and low producers, with the exception of one mother who had only low-producing offspring; this could have been due to chance variation alone. The ratio for the combined progeny from these mothers was 46 low producers and 40 high producers. This agrees well with the expected ratio of 1:1 ($\chi^2 = 0.42$; $\alpha = 0.5$).

## DISCUSSION

Although stimulation of circulating interferon production through intravenous injection of viral and nonviral inducers is now widely used, little attention has been given to the possible effect of genetic factors of the host on quantitative or qualitative aspects of interferon production. The only study we are aware of was recently undertaken by Glasgow, who found that a random-bred strain of mice, CD-1, produced more interferon than did Balb/c mice when infected with Rauscher virus, whereas $F_1$ hybrids produced intermediate amounts (L. A. Glasgow, *personal communication*). Therefore, in view of our observation concerning the high interferon production of the $C_{57}$Black strain (3), it appeared worthwhile to attempt a Mendelian analysis of this phenomenon. The pronounced difference in interferon production according to animal genotype was interesting enough in itself to merit further analysis; furthermore, $C_{57}$Black mice are highly resistant towards a number of virus infections (2, 5, 8, 10), so that it seemed relevant to obtain more information about their high interferon production.

The difference in peak interferon titers between Balb/c and $C_{57}$Black genotype was large enough to make an attempt at analysis worthwhile, there being no overlapping at all between the two genotypes. The decision to base the statistical analysis of the data on the decimal logarithm of the titers was taken because of the apparently normal distribution of the values when plotted on a $\log_{10}$ scale. The decision to lump together males and females for the anaysis of the different crosses was justified by the absence of any significant differences between interferon titers of males and females of both parental strains and by the absence of any significant difference between interferon titers of their reciprocal $F_1$ hybrids. The average interferon titers of the $F_1$ hybrids were situated between the values obtained for both parental strains; we therefore conclude that both alleles are semidominant. However, the $F_1$ titers were much closer to, although significantly different from, the Balb/c titers. As a result of this it became possible, in view of the interpretation of $F_2$ and backcross results, to distinguish between $C_{57}$Black and $F_1$ genotypes by putting the cutoff value at 11,000. A comparison of the totality of $F_1$ hybrids showed that only 5 out of 112 (4.5%) had an interferon titer higher than 11,000 and, out of 64 $C_{57}$Black

mice, 2 (3.1%) had a titer of 11,000 or lower. This means that, in the $F_2$ and backcross generation, some mice with $F_1$ genotype were probably counted as having $C_{57}$Black genotype, and vice versa. However, this cannot significantly alter the results, especially since the possibility of a small error existed in both directions. The results of the backcross between $F_1$ and Balb/c confirmed the validity of our decision, since only 2 of 120 mice had an interferon titer higher than 11,000. With the arbitrary decision to assume that animals with a titer of 11,000 or less belong to the $F_1$ or Balb/c genotype, the data of the $F_2$ generation and of the first backcross to $C_{57}$Black are in line with the postulate of a single Mendelian factor responsible for the difference between Balb/c and $C_{57}$Black mice. This postulate was also supported by the result of the second backcross in which, of eight $C_{57}$Black type mothers, all progeny except one were of the high-producing type and, of 10 $F_1$-type mothers, approximately half of the progeny were high producers and half were low producers. The possible error introduced into the segregation analysis of the data, by considering 11,000 as the cutoff value, would seem negligible because the values are rather well-grouped around their means, and a $\chi^2$ analysis of the results of $F_2$ and backcrosses to $C_{57}$Black based upon a cutoff value of 10,000 or 12,000 is still in agreement with the single-gene hypothesis (Table 7).

Several hypotheses can be envisaged to explain the mode of action of the gene responsible for the high interferon production of the $C_{57}$Black strain, but an enumeration of these different possibilities would be irrelevant to the contents of this paper; we are pursuing this problem experimentally. The second important question meriting further investigation is the possible contribution of the high interferon production of $C_{57}$Black mice to the resistance of these animals toward several viral infections. A series of experiments is planned using backcross and $F_2$ mice to see whether the segregation of high- and low-interferon producers coincides with the segregation of resistant and susceptible animals. However, before starting these experiments, it will be necessary to examine interferon induction with the viruses involved, since our present conclusions are obviously limited to NDV as interferon inducer. Preliminary experiments with Sendai and influenza virus indicate that $C_{57}$Black and $C_{57}$Black/6 mice are also high-interferon producers for these agents, compared to Balb/c or $C_3H$ mice. Therefore, the conclusion of the present report is probably valid at least for viruses belonging to the myxovirus group.

TABLE 7. $X^2$ *analysis for single-gene hypothesis of $F_2$ and backcross results for alternate cutoff values*

| Hybrid | Expected | Observed | |
|---|---|---|---|
| | | Cutoff value 10,000 (high ≧ 10,000) | Cutoff value 12,100 (high ≧ 12,100) |
| $F_2$ | | | |
| Low producers... | 100 | 99 | 103 |
| High producers.. | 34 | 35 | 31 |
| | | $X^2$: 0.039 | $X^2$: 0.35 |
| | | $\alpha$: 0.90 | $\alpha$: 0.50 |
| Backcrosses to $C_{57}$Bl (combined) | | | |
| Low producers... | 72 | 71 | 82 |
| High producers.. | 72 | 73 | 62 |
| | | $X^2$: 0.027 | $X^2$: 2.77 |
| | | $\alpha$: 0.90 | $\alpha$: 0.05 |

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