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# Strain Differences in Production of Murine Interferons (40908)

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*Abstract.* Studies were conducted comparing interferon production of various mouse strains. Methods used included the production of interferon *in vivo* and *in vitro* using spleen cell cultures. Strain differences observed *in vivo* were generally consistent with the results obtained using cell culture methods. In general, high-producing strains *in vivo* were also high producers using *in vitro* methods. Strain differences *in vivo* were greatest using BCG with old Tuberculin (OT) as a challenge. In general, $BDF_1$ and C57BL/6J mice were high producers while BALB/cByJ and NZB/BLNJ were consistently low responders to both type I and type II interferon inducers. C3H/He and HA/ICR mice could be classified as intermediate interferon producers.

A number of studies have suggested that differences exist between certain strains of mice in their ability to produce interferon. C57BL mice have been reported to produce seven times as much interferon as BALB/c mice following intravenous injection of Newcastle disease virus (NDV) (1). Differences between the two strains were also noted when poly I:C was used as the inducer.

Glasgow and Friedman (2) compared BALB/c to random-bred CD-1 mice for their ability to produce interferon in response to a number of viruses. Both Sindbis virus and NDV induced similar levels in both strains, however, Rauscher leukemia virus (RLV) induced fourfold more interferon in CD-1 mice than in the BALB/c strain. CD-1 mice are also resistant to leukemia following RLV infection, suggesting a possible relationship between interferon production and susceptibility to this virus. Other investigators (3) have also shown differences in interferon production between various strains of mice exposed to RLV.

A recent report in the literature compares the ability of various strains of mice to produce immune interferon (4). In their experiments, $BDF_1$, C57BL/6, and BALB/c mice were sensitized to herpes simplex virus (HSV). Spleen cells were removed from these animals and exposed to HSV antigen. All of the strains exhibited maximum interferon titers at approximately the same time after challenge, however, the levels produced by the individual strains were different. These differences paralleled the *in vivo* susceptibility to infection by HSV, i.e., resistant strains made more interferon than the susceptible.

Differences in interferon production following injection of *Mycobacterium bovis* strain BCG has also been shown between various strains of mice (5). These investigators noted that C57BL/ksJ responded with the production of interferon while C57BL/6 did not release detectable amounts unless pretreated with complete Freund's adjuvant. Delayed-type hypersensitivity to BCG also varies between strains (6). The SWM/Ms strain appears to be a "high responder", whereas the C3H/He strain is a "low responder." Tokunaga *et al.* (7) showed that BCG has an immunotherapeutic effect on 3-methylcholanthrene-induced tumors in SWM/Ms mice but not in C3H/He mice. Additional evidence for strain differences comes from the report of Allen *et al.* (8), who observed that C57BL/6 mice responded in a dose-dependent fashion to killed BCG by marked enlargement of the spleen and lung. Neither CBA nor C3H mice, on the other hand, responded to such treatment.

For the purpose of this study, the term immune interferon is synonymous with type II. It is sensitive to pH 2 and not inactivated by antibodies to L cell interferon. Type I interferon is characterized by its stability at pH 2 and its susceptibility to anti-L cell interferon. In these studies,



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temperature was not an effective discriminator between the various interferon types since all interferon preparations were readily inactivated after 15 min exposure at 56°. Differences in stability of interferons at this temperature have been reported previously (9).

*Materials and methods. Animals.* The following strains of mice were purchased from Jackson Laboratories (Bar Harbor, Maine): C57BL/6J, $BDF_1$ (c57BL/6J females × DBA/2 males), NZB/BLNJ, HA/ICR, C3H/He, and BALB/cByJ. Unless otherwise noted, all animals were males, 7–14 weeks old, and weighed 28–37 g. Mice were housed five to six per cage and maintained on commercial rodent chow and tap water *ad libitum*.

*Chemicals.* BCG cell walls were generously supplied by Dr. Edgar Ribi of the Rocky Mountain Laboratory (Hamilton, Mont.). The BCG cell walls were prepared as an injectable vaccine by the procedure of Salvin *et al.* (10). The final product contained a total of 300 μg of cell wall/0.2 ml injection. Old tuberculin (OT) was donated by the American Cynamid Company (Pearl River, N.Y.). Concanavalin A was obtained from Difco Laboratories (Detroit, Mich.). The lyophilized form of poly I:C was purchased from P-L Biochemicals, Inc. (Milwaukee, Wis.). RPMI 1640 medium was obtained from Gibco (Grand Island, N.Y.) and supplemented with 10% heat-inactivated fetal calf sera, 100 units/ml of penicillin G and 100 μg/ml of streptomycin sulfate. In addition, 0.025 *M* Hepes buffer (Gibco) was added to the medium. The pH was adjusted to 7.2 with $NaHCO_3$.

*Interferon induction.* The six strains of mice were compared for their ability to produce interferon in four induction systems. These four systems consisted of two for the production of type I interferon and two for the production of type II interferon. Each of these was represented by both *in vivo* and *in vitro* systems. At least five mice from each strain were employed for each experiment, and their sera or their spleen cell products were assayed individually for interferon. These experiments were repeated at least one time to minimize variability.

For the *in vivo* production of type I interferon, mice were inoculated ip using 2 mg/kg poly I:C. After 6 hr, the mice were exsanguinated by cardiac puncture and their sera individually assayed for interferon. In the *in vitro* experiments, spleens were removed from each mouse and their cells suspended at 5 × 10/ml of RMPI 1640 (complete). The cell suspensions were placed in 2-ml aliquots in tissue culture tubes, and 20 μg of poly I:C was added per milliliter. After 4 to 24 hr incubation at 37°, the cells were centrifuged and the supernatants assayed for interferon activity. Type II interferon was also prepared using mice from each of the six strains. The mice were inoculated iv with 20 mg/kg BCG cell walls. After 4 weeks, the mice were challenged iv with OT at 3.0 mg/kg. Four hours after challenge, the mice were exsanguinated by cardiac puncture. The experiment was repeated as described previously.

*Interferon assay.* Interferon-containing sera or supernatants from cell cultures were assayed using a plaque reduction assay (11). The L-929 strain of mouse fibroblasts and the Indiana serotype of vesicular stomatitis virus (VSV) were employed. Interferon titers were expressed as a reciprocal of the dilution producing a 50% reduction in plaque number. Each sample was assayed in duplicate. Statistical analysis of data was carried out using Student's *t* test.

*Results.* Differences in interferon production were noted between strains of mice injected with poly I:C. C3H/He and C57BL/6J mice made higher levels of interferon than the other four strains tested (Fig. 1). HA/ICR, NZB/BLNJ, and BALB/cByJ mice did not differ significantly from

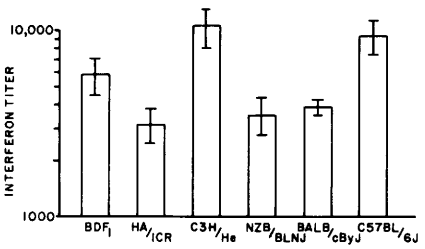


FIG. 1. Strain differences in production of poly I:C-induced Interferon (*in vivo*). Each mean ± SE represents five mice.

TABLE I. STRAIN DIFFERENCES IN PRODUCTION OF POLY I:C-INDUCED INTERFERON (*IN VITRO*)[a]

| Strain | Time (hr) | | | |
|---|---|---|---|---|
| | 4 | 8 | 12 | 24 |
| NZB/BLNJ | 16 ± 2[b] | 21 ± 2 | 21 ± 2 | 9 ± 0 |
| HA/ICR | 36 ± 9 | 112 ± 39 | 78 ± 22 | 21 ± 3 |
| C57BL/6J | 138 ± 34 | 211 ± 24 | 116 ± 29 | 101 ± 25 |
| C3H/He | 55 ± 7 | 132 ± 26 | 47 ± 3 | 28 ± 7 |
| BALB/cByJ | 14 ± 1 | 25 ± 4 | 17 ± 4 | 12 ± 0 |
| $BDF_1$ | 146 ± 4 | 242 ± 21 | 178 ± 11 | 38 ± 7 |

[a] 20 μg poly I:C/5 × $10^6$ spleen cells/ml of complete RPMI 1640.
[b] Interferon titer; each mean ± SE represents triplicate cultures.

one another in their interferon production. $BDF_1$ mice were intermediate in their response. Differences were also observed following poly I:C induction of interferon in spleen cells (Table 1). In these studies, $BDF_1$ and C57BL/6J mice made the highest levels of interferon, while the HA/ICR and C3H/He mice were intermediate in their response with the NZB/BLNJ and the BALB/cByJ mice producing the least amount of interferon. In all of these studies, interferon titers peaked after 8 hr incubation and dropped thereafter.

Results using Con A as an inducer were similar to that seen with the use of poly I:C. Table 2 illustrates that maximum interferon titers were obtained after 48–72 hr. The highest producers in the system were the $BDF_1$ mice, but their observed interferon titers were not significantly greater than those of the C3H/He and the C57BL/6J. HA/ICR mice were intermediate while the NZB/BLNJ and BALB/cByJ were the lowest. Differences in production of interferon were most evident following the injection of BCG/OT (Fig. 2). $BDF_1$ and C57BL/6J mice were the most efficient producers of interferon. Interferon titers produced by these mice were significantly greater ($P < 0.01$) than the other four strains. C3H/He gave an intermediate response which was greater than HA/ICR, NZB/BLNJ, or BALB/cByJ. The data reported here represent the results from individual experiments. All experiments were repeated with similar results.

*Discussion.* The relative ability of various strains of mice to respond to interferon inducers was investigated. The present studies provide additional information on the relative responses of several strains of mice that have not been previously compared, using Con A and BCG/OT as inducers. The results obtained by the current investigators agree with those of DeMaeyer *et al.* (1), i.e., C57BL mice produced more type I interferon than BALB/c mice which also occurs when spleen cells from these strains are cultured *in vitro*. DeMaeyer *et al.* (1) employed peripheral blood cells for *in vitro* interferon production, whereas spleen cells were used in the present study.

TABLE II. STRAIN DIFFERENCES IN PRODUCTION OF CON A-INDUCED INTERFERON (*IN VITRO*)[a]

| Strain | Time (hr) | | | |
|---|---|---|---|---|
| | 24 | 48 | 72 | 96 |
| NZB/BLNJ | 56 ± 5[b] | 77 ± 20 | 90 ± 22 | 44 ± 9 |
| HA/ICR | 144 ± 18 | 188 ± 19 | 156 ± 11 | 118 ± 14 |
| C57BL/6J | 40 ± 4 | 139 ± 33 | 378 ± 102 | 52 ± 4 |
| C3H/He | 124 ± 24 | 186 ± 5 | 383 ± 101 | 208 ± 12 |
| BALB/cByJ | 44 ± 11 | 79 ± 14 | 68 ± 7 | 60 ± 2 |
| $BDF_1$ | 208 ± 15 | 283 ± 72 | 463 ± 31 | 106 ± 22 |

[a] 10 μg Con A/5 × $10^6$ spleen cells/ml of complete RPMI 1640.
[b] Interferon titer; each mean ± SE represents triplicate cultures.

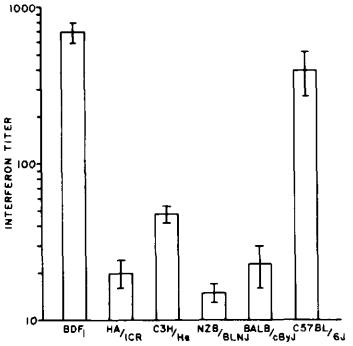


FIG. 2. Strain differences in production of BCG/OT-induced interferon (*in vivo*). Each mean ± SE represents five mice.

It should be noted that even though the spleen is reported to be one of the major organs responding to poly I:C (12), other organs including liver, lungs, thymus, etc., are capable of producing major quantities of interferons. It is conceivable that there are differences in receptors for poly I:C between cells from different organs which in turn could explain differences between *in vivo* and *in vitro* studies.

It is clear that high or low production of interferons is not an absolute phenomenon, but is important in reference to a particular inducer. This was noted with BALB/c mice which produced three times more interferon in response to RLV than did NZB mice (3). Differences using poly I:C were not significantly different in these studies. There are, however, several reports in the literature which compare interferon production of certain mouse strains induced with Con A (13, 14). In the present study, the relative abilities of mouse strains to respond to Con A were in general similar to their responses to poly I:C. As was the case with poly I:C *in vitro*, the poorest responding strains to Con A were NZB/BLNJ and BALB/cByJ which did not differ significantly from one another. HA/ICR mice were intermediate responders and $BDF_1$ and C57BL/6J were the best responders.

Major differences obtained between strains were noted following injection of BCG/OT. Although strain differences have been reported in response to BCG (6–8) there is only one study in which interferon production was the principal parameter measured (5). In their study, the C57BL/6 mouse did not respond, whereas in the present study, this was one of the best producers of interferon. This can be partially explained by differences in sensitizing abilities of different lots of BCG cell wall preparations. Differences between the two highest responding strains, $BDF_1$ and C57BL/6J and the other four strains were highly significant ($P < 0.01$). The relative responses of these two strains and that of BALB/cByJ mice were similar to that reported by Kirchner *et al.* using HSV antigen for the production of type II interferon (4). $BDF_1$ mice were among the best producers of interferons while the BALB/cByJ mice produced less than 10% of that observed in the former strain. C3H/He mice were poor responders to BCG/OT. This might have been predicted from the observations of Tokunaga *et al.* (7), since this strain was a poor responder to the immunotherapeutic effects of BCG on carcinogen-induced tumors. NZB/BLNJ mice were also low responders in all of the systems tested. This is not surprising because these mice experience an age-related loss of T cells (15).

Data presented here have relevance on the selection of a mouse strain for interferon production. This seems especially true when BCG/OT is used as significant differences occur between strains. Further studies are needed to determine if other parameters, such as resistance to viral diseases, are directly related to the capacity to produce interferon.

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