Differential Sensitivity of Macrophages from Herpes Simplex Virus-resistant and -susceptible Mice to Respiratory Burst Priming by Interferon-α/β

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SUMMARY

Herpes simplex virus primes mouse macrophages for a genetically determined respiratory burst mediated in an autocrine manner by interferon (IFN)-α/β. We have analysed the effect of IFN-α/β on the respiratory burst capacity of mouse peritoneal macrophages by luminol-dependent chemiluminescence using phorbol myristate acetate as trigger. Crude macrophage-produced IFN-α/β as well as purified IFN-α and -β regularly augmented the respiratory burst capacity of peritoneal cells in a concentration-dependent manner. The augmented response was exclusively mediated by macrophages and was manifest after 4 h incubation with IFN-α/β, peaked after 8 h and gradually declined to near background levels after 24 h. The effect of macrophage-produced IFN-α/β was completely abolished by preincubation of IFN with antiserum to IFN-α/β. The data obtained with this antiserum indicated that endogenous IFN, undetectable by a standard cytopathic effect-inhibition assay, was sometimes spontaneously produced by the peritoneal cells. Furthermore, the crude macrophage preparation seemed to contain a macrophage deactivating factor counteracting the effect of IFN-α/β. Genetic analysis of the sensitivity of macrophages for the respiratory burst-priming effect of IFN-α/β revealed that the trait is inherited as a co-dominant autosomal feature. Macrophages from herpes simplex virus-resistant C57BL/6 mice were more sensitive than macrophages from virus-susceptible BALB/c mice and cells from mice of the reciprocal crosses showed an equal sensitivity intermediate between those of the parental strains. A physiological role of differential IFN sensitivity in the context of resistance to virus infections is suggested.

INTRODUCTION

A central feature of macrophage antimicrobial and antitumour activity is the ability of these cells on appropriate activation to react with a respiratory burst forming highly reactive oxygen species like superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (¹O_2) (Babior, 1978; Nathan et al., 1979; Badwey & Karnovsky, 1980; Klebanoff, 1982). Whether these oxygen-dependent cytotoxic mechanisms play any role in the intrinsic (Mogensen, 1979) or extrinsic (Morahan, 1984) antiviral activity of macrophages has only been sporadically addressed (Rager-Zisman et al., 1982).

We have recently shown that herpes simplex virus type 2 (HSV-2) primes mouse macrophages for an increased respiratory burst capacity both after in vivo and in vitro infection (Mogensen et al., 1989). The effect in vitro was shown largely or exclusively to be mediated in an autocrine manner by interferon (IFN)-α/β produced very early by the macrophages in response to the virus. Like resistance to infection (Mogensen, 1977, 1979) the trait was genetically determined in that macrophages from virus-resistant mice showed a higher respiratory burst capacity after infection than those from virus-susceptible mice. This association might indicate a possible role of oxidative metabolism products in genetically determined resistance, which is strongly
influenced by macrophages (Mogensen, 1979). On the other hand the higher respiratory burst capacity of macrophages from resistant mice could simply reflect the observation that macrophages from these mice show higher early IFN-α/β responses on infection with HSV-2 than macrophages from susceptible mice (Ellermann-Eriksen et al., 1986a). We therefore found it of interest to examine the genetics of the respiratory burst-priming effect of IFN-α/β in macrophages from mice resistant or susceptible to HSV-2.

Here we report that macrophages from HSV-2-resistant and -susceptible mice show a differential sensitivity to the respiratory burst-priming effect of IFN-α/β. The phenomenon is inherited as a co-dominant autosomal trait and thus shows the same genetic pattern as the antiviral action of IFN-α/β in mouse fibroblasts (Ellermann-Eriksen et al., 1986b).

**METHODS**

*Mice.* Inbred specific pathogen-free C57BL/6ScCrBOM (C57BL/6) and BALB/cABOM (BALB/c) mice were originally obtained from the G1. Bomholtgaard Animal Breeding and Research Center. The parental mice for experiments were bred locally behind specific pathogen-free barriers as were F1, hybrid mice of reciprocal crosses (BALB/c ♂ × C57BL/6 ♀) F1, and (C57BL/6 ♂ × BALB/c ♀) F1. Mice of each sex were used at an age of 8 to 12 weeks. However, in individual experiments mice were of specified sex and born in the same week as stated in each experiment.

*Peritoneal cells.* Resting cells were harvested by peritoneal lavage by standard procedures. Cell counts were performed in a haemocytometer and when necessary macrophages, lymphocytes, polymorphonuclear leukocytes and mast cells were identified in a fluorescence microscope after cytoplasmic staining with acridine orange. Viability was assessed by trypan blue dye exclusion. Cells were washed twice in RPMI 1640 medium with 10% foetal calf serum (FCS), 1% glutamine and antibiotics [200 international units (IU) of penicillin and 200 μg of streptomycin per ml]. Thereafter 1 × 10^6 to 3 × 10^6 cells were seeded in 0.5 ml of medium in polystyrene cuvettes (51 × 12 mm, Sarstedt) and incubated with or without IFN for indicated time periods at 37°C in a humidified atmosphere with 5% CO₂. Cells harvested from IFN-treated mice [100 IU intraperitoneally (i.p.) for 9 h] were processed immediately without further incubation for chemiluminescence assay.

Separation of peritoneal cells in adherent and non-adherent subsets was performed as previously described by serial adherence (three cycles) to plastic (Ellermann-Eriksen et al., 1986a). The adherent cells were removed by lidocain (Rinehart et al., 1978). By this procedure the resultant adherent cell subset was more than 98% macrophages and the non-adherent subset less than 2% macrophages.

*Interferons and anti-interferon.* Three IFN preparations were used. (i) Crude macrophage-produced IFN (MIF-IFN) was prepared as previously described (Mogensen et al., 1989) by infecting peritoneal macrophage cultures derived from C57BL/6 mice with Newcastle disease virus. After 2 h of virus adsorption cells were washed and cultured in RPMI medium for an additional 22 h. The supernatant was kept at pH 2.0 for 24 h, redjusted to pH 7.4 and stored at −70°C until used. This MIF-IFN was of the α/β type since it was acid-stable and neutralized by the antiseraum against mouse IFN-α/β described below. (ii) Purified mouse IFN-α (sp. act. 1.6 × 10^6 IRU/mg of protein, catalogue no. 22061, lot 85042) and (iii) IFN-β (sp. act. 5.3 × 10^7 IRU/mg of protein, catalogue no. 20171, lot 83005) were purchased from Lee BioMolecular. These purified IFN preparations contain less than 0.5% of the opposite species (manufacturer's information). All IFN preparations were titrated in a microtitre assay in L-929 cells with vesicular stomatitis virus as challenge virus.

Goat antiserum against mouse IFN-α/β was originally provided by E. De Maeyer, Eskaer, Paris, France. It has been produced by immunizing goats with purified IFN-α/β and does not exhibit neutralizing activity against IFN-γ (De Maeyer & De Maeyer-Guignard, 1983). It was used at a concentration of 100 neutralizing units/ml, which can neutralize 1000 IU of IFN.

*Chemiluminescence assay.* The respiratory burst capacity of peritoneal cells was measured by luminol-dependent chemiluminescence with 4b-phorbol-12β-myristate-13α-acetate (PMA) (Sigma) as trigger as previously described (Mogensen et al., 1989). Briefly, cells in the polystyrene cuvettes were washed in phosphate-buffered saline pH 7.4 with 2% FCS to remove phenol red, and resuspended in 0.8 ml of Swim's S-77 medium without phenol red supplemented with 5% FCS. Luminol (5-amino-2,3-dihydro-1,4-phthalazine-1,4-dion, Fluka Chemie) at a final concentration of 8 × 10^-5 M was added. Immediately before the assay the cells were triggered for a respiratory burst with PMA (final concentration 0.4 μg/ml; 6.5 × 10^-7 M). Chemiluminescence was measured in a luminometer (LKB Wallac 1250, LKB Instruments) at intervals as indicated in individual experiments.

*Statistics.* Statistical analysis was performed on log₁₀ values of the peak chemiluminescence response by the Student's t-test for equal variances, which were tested by Levene's test (Levene, 1960).
IFN-primed respiratory burst of macrophages

RESULTS

Effect of IFN-α/β on the respiratory burst capacity of peritoneal cells

In a series of experiments we analysed the influence of different IFN preparations on the chemiluminescence response of peritoneal cells. It appeared from these experiments that pretreatment of peritoneal cells with IFN-α/β regularly augmented the ability of the cells to react to PMA triggering with a respiratory burst, whereas IFN treatment by itself did not activate the burst. Furthermore, it was evident that the assay exhibited a pronounced day-to-day variation both in the basal respiratory burst capacity and in the response after IFN priming. This is exemplified in the experiments depicted in Fig. 1. In all these experiments peritoneal cells from 10-week-old C57BL/6 mice were primed with increasing concentrations of IFN for 10 h and assayed for chemiluminescence response with PMA.

The three experiments in Fig. 1(a) were performed at intervals of several weeks, whereas the curves in Fig. 1(b) stem from a separate experiment performed on a single pool of cells. Both purified IFN-α and IFN-β as well as crude macrophage-produced IFN-α/β induced an increased capacity of peritoneal cells for a respiratory burst, which was manifest with 10 to 20 IU of IFN and increased in a dose-dependent manner. The only exceptions to this were seen with high concentrations (1600 IU/ml) of macrophage-produced IFN-α/β in the form of undiluted macrophage supernatant, which were less active than diluted preparations and, in the experiment in Fig. 1(a), actually diminished the basal respiratory burst capacity of peritoneal cells.

Since we are particularly interested in the effect of autocrine priming of macrophages for a respiratory burst after virus infection we have chosen to use the macrophage-produced IFN in

![Graph](image)

Fig. 1. Chemiluminescence response of mouse peritoneal cells primed in vitro with three different IFN preparations. Resting peritoneal cells from 10-week-old female (a) and male (b) C57BL/6 mice were incubated for 10 h with various concentrations of purified IFN-α (○), purified IFN-β (●), macrophage-produced IFN-α/β (■) or medium only (C; △). The luminol-dependent chemiluminescence response was measured after PMA triggering. (a) Three separate experiments performed at intervals of several weeks are shown. Each point from experiments with IFN-β and macrophage-produced IFN-α/β represents one single value, whereas points from the experiment with IFN-α represent the mean value from two cultures. (b) Depicts one experiment with all three IFN-preparations. Each point represents the mean value from four cultures. Bars indicate the standard error of the mean.
Fig. 2. Influence of antiserum to IFN-α/β on the respiratory burst-priming of mouse peritoneal cells by macrophage-produced IFN-α/β. Resting peritoneal cells from 10-week-old male C57BL/6 mice were incubated with medium only (unshaded), 100 IU/ml of macrophage-produced IFN-α/β (shaded), 100 neutralizing units/ml of antiserum to IFN-α/β (hatched), or 100 IU/ml of macrophage-produced IFN-α/β and 100 neutralizing units/ml of antiserum to IFN-α/β (cross-hatched) Ten h later the cultures were assayed for a PMA-triggered respiratory burst. Each column represents the mean response of four cultures in the first experiment and five cultures in the second experiment. The bars indicate the standard error of the mean.

Fig. 3. Kinetics of IFN-mediated priming of peritoneal cells for a respiratory burst in vitro. Resting peritoneal cells from 12-week-old C57BL/6 mice were treated with 100 IU/ml of macrophage-produced IFN-α/β for various lengths of time and assayed for a PMA-induced respiratory burst. Each point represents the mean value from four cultures, and the bars indicate the standard error of the mean.

Further experiments. Such an IFN has been shown to consist predominantly of IFN-β and contains only 7% IFN-α (Brehm & Kirchner, 1986). As seen from Fig. 2 the respiratory burst priming activity of this IFN-α/β preparation (100 IU for 10 h) was completely abolished after preincubation for 1 h with an antiserum to IFN-α/β. In the first experiment depicted in Fig. 2 the antiserum alone actually diminished the respiratory burst capacity to below control values (P < 0.001); a similar effect was seen in a further three experiments (data not shown) which might indicate occasional spontaneous IFN production in the macrophage cultures. In all the experiments the combination of Mφ-IFN-α/β and antiserum yielded respiratory burst values below those obtained with antiserum alone, suggesting that the suppressive effect observed with undiluted macrophage supernatant in Fig. 1 is also seen, although to a lesser extent, with supernatant diluted 1:16 and unmasked by anti-IFN-α/β.

Separation of peritoneal cells by serial adherence in adherent (> 98% macrophages) and non-adherent (generally < 2% macrophages, 90 to 95% lymphocytes, 2 to 4% polymorphonuclear leukocytes and 2 to 4% mast cells) subsets revealed that all of the respiratory burst activity after 10 h of Mφ-IFN priming was confined to the adherent (macrophage) subset (Table 1). These experiments also illustrate the day-to-day variation in comparable experiments.

Finally we analysed the kinetics of IFN-α/β priming for a respiratory burst (Fig. 3). Cultures of peritoneal cells from C57BL/6 mice were stimulated with 100 IU/ml of macrophage-produced

Fig. 4. Influence of antiserum to IFN-α/β on the respiratory burst-priming of mouse peritoneal cells by macrophage-produced IFN-α/β. Resting peritoneal cells from 10-week-old male C57BL/6 mice were incubated with medium only (unshaded), 100 IU/ml of macrophage-produced IFN-α/β (shaded), 100 neutralizing units/ml of antiserum to IFN-α/β (hatched), or 100 IU/ml of macrophage-produced IFN-α/β and 100 neutralizing units/ml of antiserum to IFN-α/β (cross-hatched) Ten h later the cultures were assayed for a PMA-triggered respiratory burst. Each column represents the mean response of four cultures in the first experiment and five cultures in the second experiment. The bars indicate the standard error of the mean.

Fig. 5. Kinetics of IFN-mediated priming of peritoneal cells for a respiratory burst in vitro. Resting peritoneal cells from 12-week-old C57BL/6 mice were treated with 100 IU/ml of macrophage-produced IFN-α/β for various lengths of time and assayed for a PMA-induced respiratory burst. Each point represents the mean value from four cultures, and the bars indicate the standard error of the mean.
Table 1. Chemiluminescence response of in vitro IFN-α/β-treated peritoneal cell subsets from C57BL/6 mice

<table>
<thead>
<tr>
<th>IFN (IU/ml)†</th>
<th>Peak chemiluminescence response (mV/3 × 10^6 cells)*</th>
<th>Non-separated</th>
<th>Adherent‡</th>
<th>Non-adherent§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1</td>
<td>160 MCF-IFN</td>
<td>22.3 ± 3.6 (3)</td>
<td>12.7 ± 1.6 (3)</td>
<td>0.6 ± 0.1 (3)</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>64 MCF-IFN</td>
<td>ND</td>
<td>13.8 ± 1.4 (3)</td>
<td>0.5 ± 0.1 (4)</td>
</tr>
<tr>
<td>Expt. 3</td>
<td>64 MCF-IFN</td>
<td>4.2 ± 0.5 (4)</td>
<td>9.6 ± 0.8 (3)</td>
<td>0.4 ± 0.1 (4)</td>
</tr>
<tr>
<td>Expt. 4</td>
<td>64 MCF-IFN</td>
<td>4.5 ± 0.2 (3)</td>
<td>8.2 ± 2.0 (3)</td>
<td>0.5 ± 0.2 (3)</td>
</tr>
<tr>
<td>Expt. 5</td>
<td>500 IFN-α</td>
<td>30.2 ± 2.7 (3)</td>
<td>44.7 ± 7.3 (4)</td>
<td>0.7 ± 0.2 (3)</td>
</tr>
<tr>
<td>Expt. 6</td>
<td>500 IFN-α</td>
<td>66.5 ± 2.8 (2)</td>
<td>116.4 ± 1.2 (2)</td>
<td>1.2 ± 0.2 (3)</td>
</tr>
</tbody>
</table>

* Mean ± S.D., number of cultures in parentheses.
† Peritoneal cell subsets treated with IFN for 10 h.
‡ >98% macrophages.
§ <2% macrophages.

IFN for the indicated time periods and assayed for PMA-triggered chemiluminescence. A significant augmentation was seen after 4 h of incubation and the maximal response was obtained after 8 h. Thereafter the respiratory burst capacity gradually declined to reach almost background levels after 24 h. Similar kinetics were observed in cells from BALB/c mice (data not shown).

Genetics of the IFN-α/β-induced priming of peritoneal cells for a respiratory burst

The genetics of the sensitivity of macrophages for the respiratory burst-priming effect of IFN-α/β was analysed in cultures prepared from individual 10- to 12-week-old male C57BL/6, BALB/c and F_1 hybrid mice of reciprocal crosses between the parental mouse strains. The cultures were primed for 12 h with 100 IU/ml of macrophage-produced IFN-α/β. In all of four experiments, each comprising cultures from three to four individual mice from each group, cells from C57BL/6 mice showed a higher respiratory burst capacity after IFN-α/β stimulation than cells from BALB/c mice. Both reciprocal crosses yielded equal responses intermediate between those of the parental strains. In Fig. 4 the data from these four experiments are pooled so that each curve represents 13 to 15 mice. Due to day-to-day variation in the respiratory burst of normal and IFN-α/β-primed macrophages it was necessary to normalize the data from separate experiments. This was done essentially as described by Vogel & Rosenstreich (1979) by multiplying all values in a given experiment by a factor derived from the ratio between the mean of the control values of all four experiments and the mean of the control values of the particular experiment. It thus appears that high and low sensitivity of macrophages to the respiratory burst priming effect of IFN-α/β are inherited as co-dominant autosomal characters with no signs of X-linkage of the traits [BALB/c versus C57BL/6, P < 0.0001; (BALB/c ♀ × C57BL/6 ♂) F_1, versus (C57BL/6 ♀ × BALB/c ♂) F_1, P = 0.93].

The sensitivity of macrophages for in vivo priming with IFN-α/β was examined by inoculating 12-week-old female C57BL/6 and BALB/c mice with 100 IU of macrophage-produced IFN-α/β i.p. After 9 h peritoneal cells were harvested and processed immediately for chemiluminescence. Again virus-resistant mice yielded macrophages with the higher respiratory burst capacity (representative experiment depicted in Fig. 5).

DISCUSSION

The reciprocal interplay between macrophages and interferons has been suggested to play an important role in resistance against virus infections (reviewed by Mogensen & Virelizier, 1987). In our model of genetically determined resistance of inbred mice to generalized infection with HSV-2 we have previously shown that resistance is strongly influenced by the function of macrophages with IFN-α/β as a possible mediator. Thus, resistance of the animal to focal necrotizing hepatitis and subsequent death, macrophage restriction of virus replication and
Fig. 4. Genetics of IFN-mediated priming of mouse peritoneal cells in vitro for a respiratory burst. Resting peritoneal cells from individual male 10- to 12-week-old C57BL/6 (○), BALB/c (○), and F₁ hybrid mice of reciprocal crosses (C57BL/6 × BALB/c) F₁ (■) and (BALB/c × C57BL/6) F₁ (□), were incubated in vitro with 100 IU/ml of macrophage-produced IFN-α/β. After 12 h of incubation they were assayed for a PMA-triggered respiratory burst. The data stem from four individual experiments and the values from each experiment were normalized as described in the Results section. Each point represents the mean result from 13 to 15 mice [C57BL/6, 13; BALB/c, 14; (C57BL/6 × BALB/c) F₁, 15; (BALB/c × C57BL/6) F₁, 15], and the bars indicate the standard error of the mean.

Fig. 5. Genetics of in vivo priming of peritoneal cells for a respiratory burst by IFN-α/β. C57BL/6 (○) and BALB/c (○) mice (12-week-old females) were injected i.p. with 100 IU of macrophage-produced IFN-α/β. After 9 h the peritoneal cells were harvested and immediately assayed for a PMA-induced respiratory burst.

early IFN-α/β (5 h) production in vivo and by macrophages in vitro after infection with HSV-2 are all dominant features influenced by an X-linked gene (Mogensen, 1977, 1979; Pedersen et al., 1983; Ellermann-Eriksen et al., 1986a). Recently we have shown that early macrophage activation, as measured by priming of macrophages for a respiratory burst capacity after HSV-2 infection in vivo and in vitro, is also genetically determined and that macrophages from virus-resistant C57BL/6 mice are more readily primed for a respiratory burst by the virus infection than are macrophages from more HSV-2-susceptible BALB/c mice. Furthermore, this feature is largely or exclusively mediated in an autocrine manner by IFN-α/β produced during the infection (Mogensen et al., 1989).

We have analysed in more detail the ability of IFN-α/β to prime mouse macrophages for a respiratory burst. This was of interest primarily as a means of analysing the genetics of the sensitivity of macrophages to IFN-α/β, since a direct antiviral activity of IFN towards HSV-2 in freshly explanted macrophage cultures is not readily measurable due to the inherent non-permissivity of such cells for HSV-2 replication. Second, a virus-induced, IFN-mediated respiratory burst potential of macrophages could be directly involved in resistance to infection, either through inactivation of extracellular or intracellular virus by reactive oxygen species (Belding et al., 1970; Rager-Zisman et al., 1982) or through direct or antibody-dependent macrophage-mediated cytotoxicity towards HSV-infected cells (Kohl et al., 1979; Stanwick et al., 1980).
IFN-primed respiratory burst of macrophages

Both crude macrophage-produced IFN-α/β and purified IFN-α and IFN-β were able to activate mouse macrophages for a PMA-triggered respiratory burst in a dose-dependent manner with an optimal response obtained after 8 h. This is essentially in agreement with results obtained with IFN-α by Ito et al. (1985). Previously, Boraschi et al. (1982, 1983) have described a reduction in zymosan-induced O$_2^-$ and H$_2$O$_2$ generation of mouse macrophages incubated for 20 h with IFN-β. However, it should be recalled that the chemiluminescence assay used by Ito et al. (1985) and by us, is measuring the net generation of reactive oxygen metabolites, which may include diminished, unchanged or increased generation of individual species. Actually, Ito et al. (1985) found in their study that the major effect of IFN-α seemed to be an enhanced generation of OH•, whereas H$_2$O$_2$ and O$_2^-$ levels were not changed significantly. IFNs can, however, augment the H$_2$O$_2$-releasing capacity of mononuclear phagocytes. Thus Nathan et al. (1983) found that partially purified native IFN-γ and pure recombinant IFN-γ induced a slow but prolonged H$_2$O$_2$ secretory capacity to human macrophages peaking after 2 to 4 days of incubation and lasting for at least 6 days. This delayed reactivity of cells treated with IFN-γ as compared with IFN-α/β is also known from the antiviral activity of these cytokines (Dianzani et al., 1978).

The ability in some experiments of antiserum to IFN-α/β to diminish the respiratory burst capacity of peritoneal cells to below control values could indicate that IFN-α/β may have been spontaneously produced during culture. We have never been able to detect such activity in our cultures (data not shown), but there is indirect evidence from other laboratories that mouse macrophages indeed spontaneously produce IFN-α/β in culture (Ito et al., 1981; Proietti et al., 1986). In the light of our findings of diminished responses with undiluted macrophage supernatant and diluted macrophage supernatant treated with antiserum to IFN-α/β, an alternative or supplementary explanation could be that the crude preparation contains factor(s), which suppress macrophage oxidative metabolism. Such factors have been demonstrated in medium conditioned by different cell types (Szuro-Sudol & Nathan, 1982; Tsunawaki & Nathan, 1986). Also trace levels of bacterial lipopolysaccharide have been shown to interfere with the cytokine-enhanced respiratory burst capacity of macrophages (Ding & Nathan, 1987).

Our finding of a genetically determined difference in the sensitivity of mouse macrophages for respiratory burst priming in vitro by IFN-α/β demonstrates that the differential ability of HSV-2 to activate macrophages for a respiratory burst capacity through autocrine IFN-α/β secretion (Mogensen et al., 1989) is not merely reflecting differences in IFN levels obtained during the infection. Relatively HSV-2-resistant C57BL/6 mice (high IFN-α/β producers) are more sensitive than more susceptible BALB/c mice, and high and low sensitivity are inherited as co-dominant autosomal traits, and are thus genetically different from IFN production after HSV-2 infection, which has been shown to be influenced by X-linked gene(s) (Ellermann-Eriksen et al., 1986a). This supports our previous results that sensitivity of mouse embryonic fibroblasts for the antiviral action of IFN-α/β with HSV-2 as challenge virus is inherited as a co-dominant feature (Ellermann-Eriksen et al., 1986b). In that study we could not exclude the possibility that X-linked loci were involved in differential IFN-α/β sensitivity, but this turned out not to be the case when we later examined fibroblast cultures derived exclusively from male embryos of reciprocal crosses between C57BL/6 (high sensitivity) and BALB/c (low sensitivity) mice (S. Ellermann-Eriksen & S. C. Mogensen, unpublished data). IFN-α/β sensitivity of mouse fibroblasts and macrophages thus shows the same genetics when different IFN activities are measured. In man sensitivity to the antiviral activity of IFN-α/β as well as to a number of non-antiviral IFN-α/β activities, has been mapped to chromosome 21 with dosage effects of the chromosome (Tan et al., 1974; Stewart, 1979).

Dandoy et al. (1982) have previously analysed the genetics of the antiviral effect of IFN-α/β in embryo fibroblasts after infection with encephalomyocarditis virus using the same inbred mouse strains as in our study. In agreement with our previous findings (Ellermann-Eriksen et al., 1986b) their data clearly demonstrate a two- to three-fold higher IFN sensitivity of C57BL/6 fibroblasts as compared with BALB/c fibroblasts (sign test: 2p = 0.002). However, in vivo BALB/c mice were more readily protected by a high dose of IFN (6 × 10$^5$ U) against a lethal infection with the virus than were C57BL/6 mice. Furthermore, the same group has previously...
established that the BALB/c genotype confers higher sensitivity to IFN-α/β-mediated inhibition of delayed type hypersensitivity (De Maeyer & De Maeyer-Guignard, 1979, 1983) and proliferation inhibition of erythroid (Gallien-Lartigue et al., 1980) and macrophage precursors (Dandoy et al., 1981).

These results which show BALB/c mice to be more sensitive to IFN-α/β stem from studies of bone marrow-dependent functions. This might indicate that the mechanisms of IFN sensitivity of bone-marrow stem cells in mice are different from those of differentiated cells like fibroblasts and resting peritoneal macrophages. This difference might have important physiological implications for the role of IFN in early resistance against virus infections. As pointed out by Dandoy et al. (1981) high IFN producers, exemplified by C57BL/6 mice, would be relatively protected against the untoward bone marrow-depressive actions of IFN during viral infections. Our results supplement this notion by showing that the beneficial effects of genetically determined high IFN production, like direct antiviral activity (Ellermann-Eriksen et al., 1986b) and activation of resident macrophages, are further strengthened by a concomitant high sensitivity of target cells for these IFN actions.

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