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Influence of the Genotype of Mice on the Effect of Interferon on Phagocytic Activity of Macrophages (41604)

MIKLOS DEGRÉ,* KIRSTEN BELSNES,* HALVOR ROLLAG,† SOLVEIG BECK,† AND GERALD SONNENFELD‡

*Department of Virology, National Institute of Public Health, University of Oslo, Oslo 1, Postuttak, Norway, †The Wilhelmsens Institute of Bacteriology, Rikshospitalet, University of Oslo, Oslo 1, Norway, and ‡Department of Microbiology and Immunology, University of Louisville, School of Medicine, Louisville, Kentucky 40292

Abstract. The influence of genotype on interferon effect on phagocytic activity of unstimulated mouse peritoneal macrophages (MPM) was studied in *in vitro* experiments. Treatment of MPM from BALB/c and ICR mice with mouse fibroblast interferon (MuIFN-β) enhanced the ingestion of non-opsonized *Escherichia coli.* This effect was dose-dependent and neutralized by anti-interferon globulin. MPM from C57Bl/6 mice were not stimulated by the same treatment. Treatment of MPM with pH 2-sensitive immune interferon (MuIFN-γ) depressed the ingestion independently on the genotype of mice.

Both antiviral and nonantiviral activities of interferon (IFN) can be influenced by the genotype of the host (1). The BALB/c mouse genotype appears to determine an enhanced sensitivity to the antiviral activity of IFN against encephalomyocarditis virus infection as compared to the C57Bl/6 genotype (2). Correspondingly, sensitization and expression of delayed type hypersensitivity to sheep erythrocytes was inhibited by IFN to a higher degree in BALB/c mice compared to C57Bl/6 mice, and *in vitro* bone-marrow derived macrophage and erythroid precursor cell proliferation was also inhibited to a greater extent by IFN in BALB/c derived than in C57Bl/6 derived cells (3, 4). Type I or IFN-β was used in these experiments.

To further evaluate the influence of genotype on other activities of IFN, we have studied the effect of IFN preparations on phagocytosis by cells from different mouse genotypes. Several groups of investigators, including our own, have shown that moderate concentrations of IFN-α and IFN-β enhance the ingestion of particles by mononuclear and polymorphonuclear phagocytes (5–9). This effect was species-specific, pH 2-resistant, and it was neutralized by specific anti-interferon globulin (8). We have also shown that homologous IFN-γ preparation inhibited phagocytic activity (10). All these experiments were performed using cells from ICR, NMRI, or Swiss mice. The present results show that phagocytosis by peritoneal macrophages (MPM) of BALB/c mice is more sensitive to IFN-β then cells from C57Bl/6 mice, in a similar fashion as the antiviral activity, inhibition of delayed hypersensitivity activity and cell proliferation. On the other hand genotype does not appear to influence sensitivity of the phagocytic activity to the IFN-γ preparation.

Materials and Methods. *Cells.* ICR, C57Bl/6, and BALB/c mice were obtained from Bomholtgaard (Ry, Denmark). Peritoneal macrophages (MPM) were harvested from the peritoneal cavity of unstimulated young adult mice of either sex. Harvesting and processing of the cells was described in detail previously (11). MPM were cultivated in 24-well Costar tissue culture plates, ca. 1×10^6 cells per well in Eagle's minimal essential medium (MEM) supplemented with 20% fetal bovine serum (FBS). At the time of the phagocytosis experiments more than 95% of the cells could be characterized as macrophages by morphological criteria, phagocytic ability, and nonspecific esterase straining.

Interferon. A mouse fibroblast interferon preparation (MuIFN-β), produced by inoculation of L-929 cells with Newcastle disease virus, was obtained from the late Dr. K. Paucker, Philadelphia, Pennsylvania. This preparation was purified to an activity of 1.3×10^8 U/mg protein by ultrafiltration and affinity chromatography on an anti-interferon

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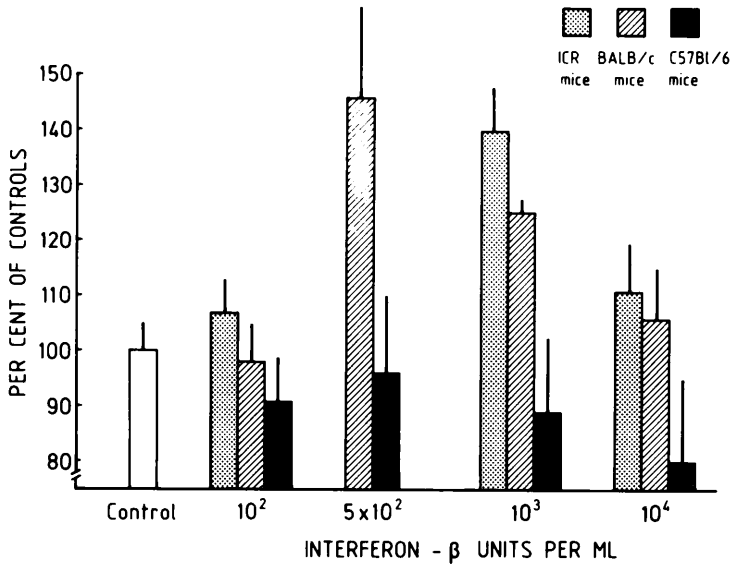

FIG. 1. Phagocytosis of *E. coli* by MPM obtained from different mouse strains. MPM were grown on solid surfaces, and treated with various concentrations of MuIFN-β overnight. After a phagocytosis period of 90 min, the uptake of ^{32}P-labeled *E. coli* was determined by measuring cell-associated radioactivity, and expressed as counts per milligram protein per minute (CPMM). The columns represent the relative activity compared to controls, the mean of five determinations, and the bars indicate the range of individual results.

column. Mock IFN was prepared by the same procedure except for virus inoculation which was omitted.

Immune interferon (MuIFN-γ) was induced in mice by tuberculin challenge of mice previously sensitized with *Mycobacterium bovis*, strain BCG. Gamma-interferon activity was in the serum (12). The mock preparation was produced by tuberculin inoculation of nonsensitized mice.

Sheep anti-β-interferon immunoglobulin was obtained from Dr A. D. Inglot, Wroczlaw, Poland. Partially purified MuIFN-β was used as the antigen for the production of this antiserum. After adsorption and concentration a 1:10,000 dilution of this preparation had the capacity to neutralize 10 units of MuIFN-β in a tube assay with 2×10^5 L-cells and vesicular stomatitis virus (VSV) as the challenge virus.

Interferon activity was tested by means of an infectivity inhibition microtest (13) using VSV and L-929 cells. The highest dilution of the sample giving 50% inhibition of cytopathogenic effect after 72 hr incubation, when control cells were completely destroyed, was considered as the titer. One unit of the international reference standard preparation (G-002-904-511), obtained from the National Institute of Allergy and Infectious Diseases, titrated 1 unit in our system.

Phagocytosis. Preparation of ^{32}P-labeled *Escherichia coli* suspension and the performance of phagocytosis experiments has been described in detail previously (11). In brief, cells were incubated with IFN or mock preparation overnight. The medium was then removed and fresh medium containing the prewarmed bacterial suspension was added. After the phagocytosis period of 90 min, the suspension was decanted and the cells were thoroughly washed to remove nonmacrophage-associated radioactivity. Macrophages were dissolved by addition of 0.1 *N* NaOH. The radioactivity and the protein content of the solution were determined individually for each well and the uptake was expressed as counts per milligram protein per minute (CPMM).

Four or five parallel wells were tested for each sample. The results are given as mean $\pm$ SD. The Wilcoxon's two sample rank test was applied to test statistical differences.

Results. In a series of experiments MPM

from different strains of mice were treated overnight with increasing concentrations of MuIFN-β or mock preparation and ingestion of ^{32}P-labeled non-opsonized *E. coli* was then measured (Fig. 1). MPM from ICR mice were stimulated in a dose-dependent manner by interferon as previously reported (8). A similar dose-dependent stimulation was found in MPM from BALB/c mice with a maximum at ca. 500 U/ml MuIFN-β. On the other hand ingestion of bacteria by MPM from C57Bl/6 mice was not influenced by the same concentrations of interferon in any of the five experiments performed.

Similar experiments were done using the MuIFN-γ preparation. The concentrations were used on the basis of our earlier experiences (10). The uptake of *E. coli* was inhibited in a dose-dependent manner, regardless of the source of the macrophages (Fig. 2).

To ensure that the effects were due to interferon activity the MuIFN-β preparation was preincubated with anti-interferon globulin. Both the antiviral activity and the effect on ingestion of bacteria by MPM were neutralized by this treatment (Table I). The MuIFN-γ preparation was subjected to pH 2 treatment for 2 days, which eliminated both its antiviral activity and the phagocytosis-depressing effect (Table I).

Discussion. The present data indicate that the ability of β-interferon to enhance the phagocytic activity of macrophages is genetically regulated. This was not entirely unexpected, since it has been shown by others that sensitivity to other interferon activities, including antiviral activity (2) and several non-antiviral activities (3, 4) were also controlled by the genetic make-up of the mouse strain. It is attractive to speculate that all these activities are determined by the same gene(s), but a complete genetic analysis is still required. A multigenic control influenced by genes linked to If-1 has been suggested (2). These genes have been shown to be distinct from the If-rec gene which probably codes for interferon receptors (14) and the Mx gene governing interferon sensitivity of influenza virus (15).

The effect of γ-interferon on phagocytic cells seems to be different in several respects. First, our present data confirm the earlier report (10) that even low concentrations of the γ-IFN preparation depress phagocytic activity, in contrast to similar concentrations of α and β interferons. Second, this activity does

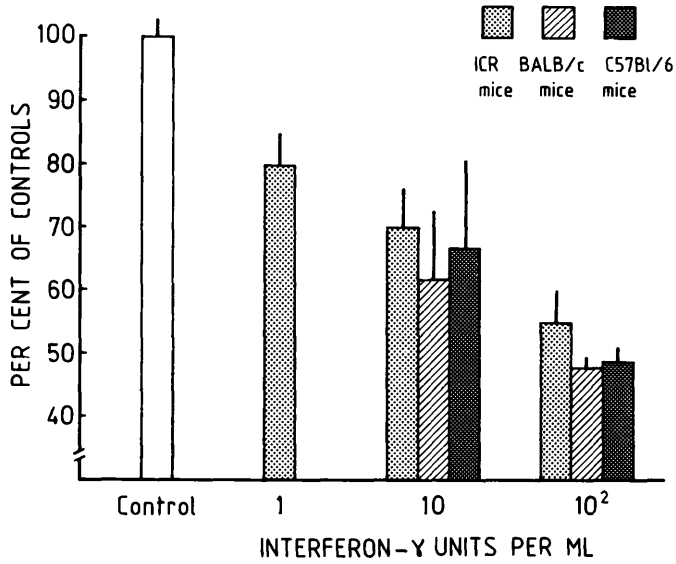

FIG. 2. Phagocytosis of *E. coli* by MPM obtained from different mouse strains. MPM were grown on solid surfaces, and treated with various concentrations of MuIFN-γ overnight. Experimental details as in Fig. 1.

TABLE I. SPECIFICITY OF INTERFERON EFFECT ON PHAGOCYTOSIS BY MACROPHAGES FROM DIFFERENT MOUSE STRAINS

Interferon		Uptake of *E. coli* by MPM from		
Preparation	Activity (units/ml)	ICR	BALB/c	C57Bl/6
Experiment 1				
Mock IFN	<10	70.5 ± 3.2[a]	58.0 ± 6.8	63.0 ± 5.1
Mu-IFN-β	500	106.0 ± 5.1 (150)[b]	102.5 ± 11.0 (176)[b]	70.0 ± 5.9 (111)[c]
Mu-IFN-β + anti-IFN-globulin	<10	59.5 ± 2.8 (85)[d]	65.0 ± 5.2 (110)[d]	55.5 ± 8.8 (90)[e]
Experiment 2				
Mock IFN	<10	84.0 ± 4.5		
Mu-IFN-γ	10	64.0 ± 5.5 (76)[b]		
Mu-IFN-γ (10 u/ml) pH 2-treated	<10	83.0 ± 9.1 (98)[d]		

[a] CPMM, mean of four cultures ± SD (percentage of controls in parenthesis).
[b-e] Significance of difference: [b] $P < 0.05$ from mock IFN; [c] $P > 0.1$ from mock IFN; [d] $P < 0.05$ from IFN; [e] $P > 0.05$ from IFN.

not appear to be determined by the same genetic factors as the β-interferon, phagocytosis-enhancing activity. This is, to our knowledge, the first demonstration of genetic differences between sensitivity to α and β interferons on one side compared to γ-interferon. At the present time we have no data concerning the mechanism of the diverging activities. One possibility is presence of different receptors on the cell surfaces, since it has been shown recently that all type I human interferon (both α and β) were competing for the same receptors while HuIFN-γ did not (16).

The data obtained with the IFN-γ preparation should also be qualified, because of the possible presence of biologically active impurities. The results should be confirmed with homogenous preparations of IFN-γ before they are accepted as an established fact.

The above results suggest that there may be some genetic control of the effects of interferon on phagocytosis. Future studies in our laboratory will explore more deeply the nature of the genetic control. Further studies should also clarify whether the genetic control of this interferon activity may have any significance on the host defense.

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1. Stewart WE II. The Interferon System. London/New York, Springer-Verlag, 2nd ed, p184, 1981.
2. Dandoy F, De Maeyer-Guignard J, De Maeyer E. Mouse genotype influences the antiviral activity of interferon. In: De Maeyer E, Galasso G, Schellekens H, eds. The Biology of the Interferon System. Amsterdam, Elsevier/North-Holland, p197, 1981.
3. Gallien-Lartigue O, Carrez D, De Maeyer E, De Maeyer-Guignard J. Strain dependence of the antiproliferative action of interferon on mouse erythroid precursors. Science **209**:292–294, 1980.
4. Dandoy F, De Maeyer E, De Maeyer-Guignard J. Antiproliferative action of interferon on murine bone-marrow derived macrophages is influenced by the genotype of the marrow donor. J. Interferon Res **1**:263–270, 1981.
5. Huang KY, Donahoe RM, Gordon FB, Dressler HR. Enhancement of phagocytosis by interferon containing preparations. Infect Immun **4**:581–588, 1971.
6. Donahoe RM, Huang KY. Interferon preparations enhance phagocytosis in vivo. Infect Immun **13**:1250–1253, 1976.
7. Imanishi J, Yokota T, Kishida T, Mukainaka T, Matsuo A. Phagocytosis-enhancing effect of human leukocyte interferon preparation on human peripheral monocytes in vitro. Acta Virol **19**:52–55, 1975.
8. Rollag H, Degré M. Effect of interferon preparations on the uptake of non-opsonized *Escherichia coli* by mouse peritoneal macrophages. Acta Pathol Microbiol Scand Sect B **89**:153–159, 1981.
9. Melby K, Midtvedt T, Degré M. Effect of leukocyte interferon on phagocytic activity of polymorphonuclear leukocytes. Acta Pathol Microbiol Scand Sect B **90**:181–184, 1982.

10. Degré M, Sonnenfeld G, Rollag H, Mörland B. Effect of gamma interferon preparations on in vitro phagocytosis and degradation of *Escherichia coli* by mouse peritoneal macrophages. J. Interferon Res **1**:505–512, 1981.
11. Rollag H. Uptake of non-opsonized *E. coli.* by unstimulated mouse peritoneal macrophages. Acta Pathol Microbiol Scand Sect C **87**:99–105, 1979.
12. Youngner JS, Salvin SB. Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. J Immunol **111**:1914–1922, 1973.
13. Dahl H, Degré M. A microassay for mouse and human interferon. Acta Pathol Microbiol Scand Sect B **80**:863–870, 1972.
14. Cox DR, Epstein LB, Epstein CJ. Genes coding for sensitivity to interferon (IFRec) and soluble superoxide dismutase (SOD-1) are linked in mouse and man and map to mouse chromosome 16. Proc Nat Acad Sci USA **77**:2168–2172, 1980.
15. Haller O, Arnheiter H, Lindenman J, Gresser I. Host gene influences sensitivity to interferon action selectively for influenza virus. Nature (London) **283**:660–661, 1980.
16. Branca AA, Baglioni C. Evidence that types I and II interferons have different receptors. Nature (London) **294**:768–770, 1981.

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