Title	Enhancement of Bactericidal Activity of Mouse Peritoneal Macrophages against Staphylococcus aureus by Mouse Interferon Preparations
Author(s)	Imanishi, Jiro; Oishi, Kinzo; Suganuma, Atsushi et al.
Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1982, 25(2), p. 71-77
Version Type	VoR
URL	https://doi.org/10.18910/82486
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

https://ir.library.osaka-u.ac.jp/

The University of Osaka

BIKEN JOURNAL Vol. 25 71–77, 1982

ENHANCEMENT OF BACTERICIDAL ACTIVITY OF MOUSE PERITONEAL MACROPHAGES AGAINST *STAPHYLOCOCCUS AUREUS* BY MOUSE INTERFERON PREPARATIONS

JIRO IMANISHI, KINZO OISHI, ATSUSHI SUGANUMA[1], YOSHITAKE YOKOTA[2] and TSUNATARO KISHIDA

Department of Microbiology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamikyo-ku, Kyoto 602, Japan

(Received June 18, 1981)

SUMMARY The effect of mouse interferon on the bactericidal activity of macrophages against pyogenic cocci was examined. Mouse peritoneal macrophages were cultivated with *Staphylococcus aureus in vitro* and viable Staphylococcus was recovered by treatment of the mixed macrophage-bacteria culture with sodium dodecyl sulphate (SDS) solution. Results showed that *S. aureus* was phagocytized and killed by the macrophages. Mouse L cell interferon enhanced the bactericidal activity of macrophages. A mouse brain interferon preparation also enhanced this activity. However, heat-inactivated L cell interferon and heterologous rabbit RK-13 cell interferon and human leukocyte interferon did not enhance it. This suggests that interferon enhances the bactericidal activity of macrophages against *S. aureus*.

INTRODUCTION

Interferon has many kinds of biological activity besides antiviral activity. In paticular, it is known to influences humoral (Braun and Levy, 1972; Brodeur and Merigan, 1974; Brodeur and Merigan, 1975; Chester et al., 1973) and cellular (De Maeyer et al., 1975) immunities.

Several investigations have shown that interferon influences various functions of macrophages, such as phagocytic activity (Huang et al., 1971; Imanishi et al., 1977), tumoricidal activity (Schultz et al., 1978; Schultz et al., 1977) and spreading of macrophages (Schultz et al., 1978).

Some bacteria such as *Mycobacterium tuberculosis*, *Listeria monocytogens* and *Brucella abortus* can multiply within cultured macrophages, but some pyogenic cocci such as *Staphylococcus aureus* (*S. aureus*) and *Streptococcus haemolyticus* can not multiply within macrophages and are killed by the macrophages. Thus the behaviour of the first type of bacteria within macrophages seems to be different from that of the second type. Mizunoe et al. (Mizunoe et al., 1973) reported that interferon suppresses intracellular multiplication of *M. tuberculosis*

1 Present address: National Hospital of Maizuru, Maizuru, Japan.

2 Present address: Department of Microbiology, Osaka Medical College, Takatsuki, Japan.

of the first type of bacteria. In the present study we examined the growth kinetics of *S. aureus* of the second type of bacteria in cultures of mouse peritoneal macrophages and the effect of mouse interferon on the bactericidal activity of the macrophages.

MATERIALS AND METHODS

1. *Bacterium*

S. aureus MS-D strain was grown in heart infusion broth for 20 h at 37 C, and then centrifuged for 10 min at 3,000 rpm. The precipitated cells were suspended in Eagle's minimum essential medium (MEM) without antibiotics, washed twice by centrifugation, and adjusted to an appropriated concentration with MEM.

2. *Mouse peritoneal macrophages*

Mouse peritoneal macrophages were isolated from untreated normal ICR mice by washing the peritoneal cavity with MEM containing 10% calf serum. They were washed twice with physiological saline with centrifugation at 1,000 rpm for 10 min, and then suspended at 10^5–10^6/ml in the same medium. Viable cells were counted by the nigrosin dye exclusion test. Samples of 300 μl of cell suspension were put into test tubes (1.5×12 cm) and cultivated in a humidified atmosphere of 5% CO_2 in air at 37 C. After 5 h, cells not adhering to the glass were removed by washing with MEM.

3. *Interferon preparations*

A mouse L cell interferon preparation was prepared from a L cell culture infected with the Miyadera strain of Newcastle disease virus (NDV). A mouse L cell monolayer was infected with NDV at about 10 multiplicity of infection (moi). After incubation at 37 C for 24 h, the supernatant was separated, adjusted to pH 2.0 with 1 *N* HCl and stood for 4 days to inactivate inducing virus. Then it was neutralized with 1 *N* NaOH, concentrated with zinc acetate (Imanishi, 1975) and stocked at -80 C to prevent inactivation of the interferon. Its specific activity was 1×10^5 IU/mg protein. A mock interferon preparation was prepared in the same way as the L cell interferon preparation except that UV-inactivated NDV instead of active NDV was added to the L cell culture.

A mouse brain interferon preparation was prepared from ICR mouse brain infected with the JaGAr01 strain of Japanese encephalitis virus (JEV). JEV was inoculated intracerebrally into ICR mice, and 3 to 5 days later infected brains were removed and emulsified with Eagle's MEM. The emulsion of brain was acidified to pH 2.0–2.2 with 1 *N* HCl, stood for 4 days to inactivate residual virus and then neutralized with 1 *N* NaOH. The interferon was then concentrated by ammonium sulphate precipitation and purified by CM-sephadex C-25 ion exchange chromatography. The specific activity of the purified preparation was 4.0×10^4 IU/mg protein.

Human leukocyte interferon was prepared from human peripheral leukocytes infected with Sendai virus. It was concentrated by ammonium sulphate and purified first by CM- and DEAE-cellulose chromatographies and then by Sephadex G-100 gel filtration. Its specific activity was 5×10^5 IU/mg protein.

Rabbit interferon was kindly supplied by Dr. M. Iizuka, Basic Research Laboratory, Toray Industries, Inc., Kamakura, Japan (Imanishi et al., 1977). It was prepared in RK-13 cell cultures infected with NDV. The supernatant was acidified to pH 2.0 with 1 *N* HCl, stood for 4 days and then readjusted to pH 6.4 with 1 *N* KOH. The preparation was then concentrated about 100-fold in a rotary evaporator at 60 C and subjected to ultrafiltration (Hollow Fiber HlDp10 apparatus: Amicon Corp.).

4. *Assay of interferon activity*

Mouse L cell interferon and mouse brain interferon were assayed by the microassay method with L cells and vesicular stomatitis virus (VSV) as a challenge virus (Imanishi, 1975). Human leukocyte interferon was assayed by the microassay method and plaque reduction method with FL cells and VSV. Rabbit RK-13 cell interferon was assayed by a radioisotope method with RK-13 cells and VSV (Suzuki et al., 1974). Interferon activities were expressed as international units (IU) calibrated against a standard reference preparation.

5. *Assay of viable bacteria*

Viable bacteria phagocytized by macrophages were counted by the method of Mauel et al. (Mauel et al., 1973), by which macrophages phagocytizing bacteria were destroyed in a solution of sodium dodecyl sulphate (SDS) and the phagocytized viable bacteria were recovered. A sample of 100 μl of bacterial

suspension was added to the macrophage culture in 0.3 ml of MEM and the culture was incubated at 37 C for 2–3 h. Then 0.1 ml of 0.25% SDS solution was added (final concentration of SDS, 0.05%), and the mixture was stirred for 30 sec. Finally 4.5 ml of MEM was added and the mixture was serially diluted with heart infusion broth and seeded on agar plates. After incubation at 37 C for 48 h, bacterial colonies were counted. Almost 100% of the viable bacteria were recovered by this method.

RESULTS

1. *Growth kinetics of S. aureus in macrophage cultures*

Samples of 3.2×10^5/0.1 ml of bacterial suspension were added to macophage cultures in 0.3 ml of MEM or to an equal volume of cell-free medium (ratio of macrophages to bacteria, 2: 1). The cultures were then incubated for 3, 6 and 9 h, and viable bacteria were counted. When bacteria were cultivated with macrophages, the number of viable bacteria in the supernatant decreased with time. On the contrary, in MEM without macrophages the number of bacteria increased with time (Fig. 1). Thus *S. aureus* was phagocytized by mouse peritoneal macrophages. When a mixture of macrophages and bacteria was treated with 0.25% SDS solution (final concentration, 0.05%), the total number of viable bacteria in the supernatant and in the macrophages could be counted. Results showed that growth of *S. aureus* was suppressed in cultures of macrophages, but not in MEM alone (Fig. 1), again indicating that *S. aureus* was phagocytized by macrophages.

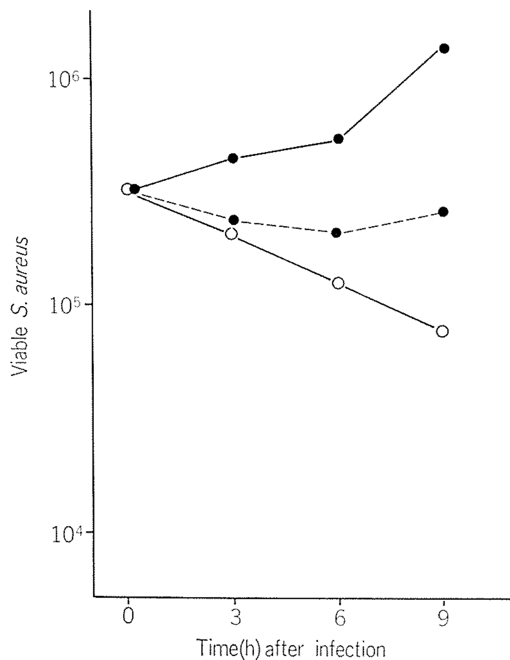

FIGURE 1. Growth kinetics of *Staphylococcus aureus* in macrophage culture.

Mouse peritoneal macrophages were cocultivated with 3.2×10^5 bacteria. After 3, 6 and 9 h, viable bacteria in medium without macrophages (●—●), those in the supernatant of macrophage cultures (○—○) and total viable bacteria in macrophage cultures (●---●) were counted by the pour plate method on agar of heart infusion broth.

2. *Effect of L cell interferon on the growth of Staphylococcus in macrophage cultures*

Mouse peritoneal macrophages were treated with 500 or 200 IU/ml of L cell interferon, mock interferon or MEM alone for 24 h. Then the medium was replaced by fresh medium, 0.1 ml of MEM containing 1.4×10^5 *S. aureus* was added and the macrophage cultures were incubated at 37 C for 18 or 24 h. When the macrophages were treated with 1000 IU/ml of interferon, the total number of viable bacteria recovered with SDS was much less than when the macrophage culture was treated with mock interferon (Fig. 2a). This difference shows that L cell interferon enhanced the bactericidal activity of macrophages. The possibility that interferon suppressed the ingestion of bacteria by macrophages rather than enhancing the bactericidal activity was excluded by demonstrating in smear samples stained with Giemsa's solution that the number of bacteria ingested by macrophages was not affected by treating macrophages with interferon. Interferon at 500 or 200 IU/ml also enhanced the bactericidal activity of macrophages, whereas medium only did not (Fig.

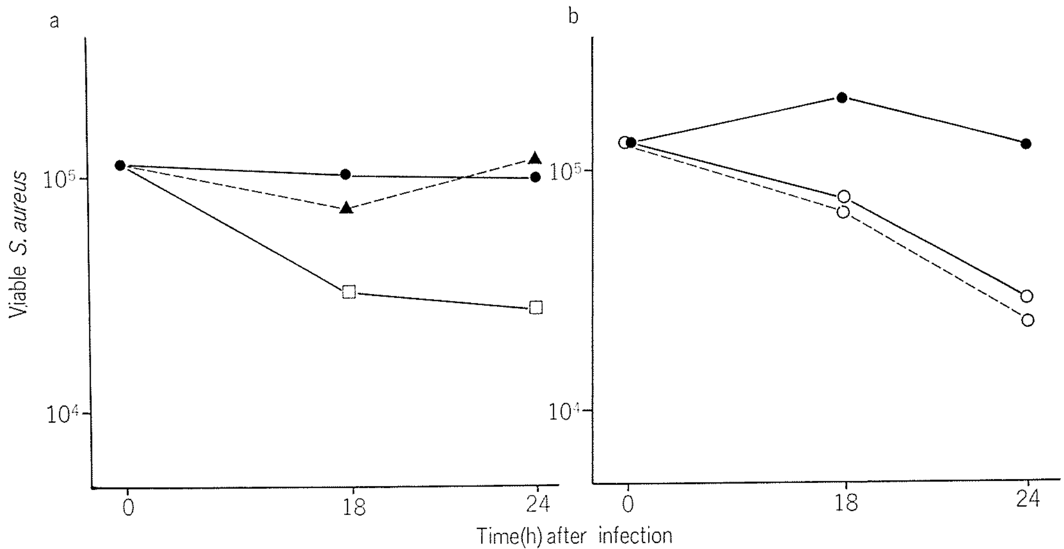

FIGURE 2. Effect of L cell interferon and mock interferon on the bactericidal activity of macrophages.
Mouse peritoneal macrophages were treated with 200 IU/ml (○—○) or 500 IU/ml (○---○) (2b) or with 1000 IU/ml (□—□) (2a) of L cell interferon, mock interferon (▲---▲) (2a) or control medium (●—●) for 24 h at 37 C. After 18 and 24 h, total viable bacteria recovered with SDS solution were counted. The ratio of the number of macrophages to that of bacteria was 4: 1.

TABLE 1. *Effect of mouse brain interferon and heterologous rabbit RK-13 cell interferon and human leukocyte interferon on the bactericidal activity of mouse peritoneal macrophages*[a]

	Expt. I			Expt. II		
time (h) after infection	0	24	48	0	24	48
	viable	*S. aureus*	($\times 10^4$)	viable	*S. aureus*	($\times 10^4$)
control (untreated control)	410	162	128	558	68	159
mouse brain IFN[b]						
100 IU/ml			1.6			4.9
500 IU/ml			1.5			0.1
Rabbit RK-13 cell IFN						
100 IU/ml			178			149
500 IU/ml			167			118
human leukocyte IFN						
100 IU/ml			169			134
500 IU/ml			193			157

[a] Mouse peritoneal macrophages isolated from ICR mice were treated with interferons for 24 h and inoculated with *Staphylococcus aureus*. After 48 h, total viable bacteria recovered in the supernatant and by SDS treatment of the macrophage culture were counted by the pour plate method on agar of heart infusion medium. The ratio of macrophages to bacteria was 6/1.
[b] IFN: interferon.

2b).

3. *Effect of mouse brain interferon on the bactericidal activity of macrophages*

Mouse peritoneal macrophages were treated with 500 or 100 IU/ml of interferon for 24 h, and then 410×10^4 viable bacteria were added to the macrophage cultures. Viable bacteria decreased more in interferon-treated cultures than in untreated cultures. Thus mouse brain interferon also enhanced the bactericidal activity of macrophages (Table 1).

4. *Effect of heat-inactivated L cell interferon on the bactericidal activity of macrophages*

Next L cell interferon was heated at 60 C and at 80 C for 30 min to determine whether the enhancement was due to interferon itself or to substances contained in the interferon preparation. During heat-treatment the interferon activity decreased from 200 IU/ml to 24 and 4 IU/ml, respectively. These inactivated interferons did not enhance the bactericidal activity of macrophages against *S. aureus*, while untreated L cell interferon (200 IU/ml) enhanced it in 24 h (Fig. 3).

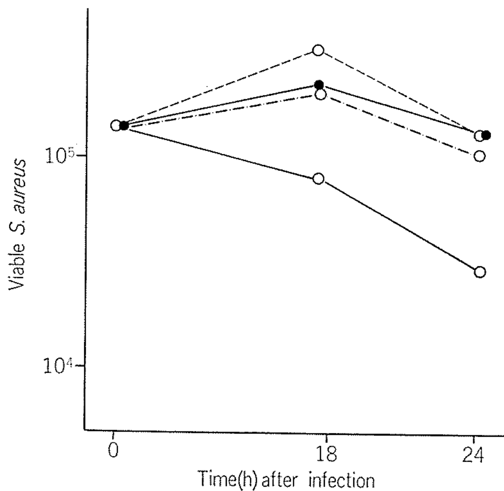

FIGURE 3. Effect of heat-inactivated L cell interferon on the bactericidal activity of macrophages. The antiviral activity of the L cell interferon preparation heated at 80 C and 60 C for 30 min decreased from 200 IU/ml to 4 and 24 IU/ml, respectively.

Mouse peritoneal macrophages were treated with untreated L cell interferon (○—○), L cell interferon heated at 80 C (○---○) or 60 C (○-·-○) for 30 min or control medium (●—●). After 18 and 24 h, total viable bacteria were counted.

5. *Effect of heterologous interferons on the bactericidal activity of macrophages*

The antiviral action of interferon is species-dependent. Therefore we examined the species-dependency of the enhancing effect of interferon on the bactericidal activity of macrophages. Mouse peritoneal macrophages were treated with 500 and 100 IU/ml of rabbit RK-13 interferon and human leukocyte interferon, respectively. Table 1 shows that neither preparation enhanced the bactericidal activity of macrophages (Table 1).

All the above experiments were repeated at least three times and reproducible results were obtained.

DISCUSSION

Macrophages are important in nonspecific host-resistance to viral and bacterial infections as well as in immunologically specific host resistance. Macrophages can cause nonspecific host-resistance by phagocytizing and killing various microbes.

Interferon can activate macrophages or monocytes and enhance their phagocytic capacity. Huang et al. (Huang et al., 1971) showed that mouse interferon enhanced the phagocytosis of carbon particles by mouse peritoneal macrophages. Imanishi et al. (Imanishi et al., 1977) showed that human leukocyte interferon also augmented the phagocytic activity of human peripheral monocytes on latex particles. Furthermore, Schultz et al. reported that mouse interferon and interferon inducer enhanced the tumoricidal activity of mouse peritoneal macrophages against murine leukemic cells (Mizunoe et al., 1975; Schultz et al., 1978). The authors (Kishida et al., 1973) found that mouse interferon enhanced

the adherence of macrophages to murine mammary carcinoma cells (FM_3A). Mizunoe et al. (Mizunoe et al., 1975) reported that interferon suppressed the multiplication of *Mycobacterium tuberculosis* in mouse peritoneal macrophages. Thus, interferon influences various actions of macrophages.

The present study showed that macrophages phagocytized and killed *S. aureus* slowly in vitro. This finding is consistent with the suggestion of Baughn and Bonventre (Baughn and Bonventre, 1975) that *S. aureus* is not a typical extracellular parasite. The present study also showed that mouse L cell and brain interferons enhanced the bactericidal activity of mouse peritoneal macrophages. This enhancing effect seemed to be due to interferon itself, because mouse L cell interferon and mouse brain interferon enhanced the bactericidal activity of macrophages, but heat-inactivated mouse interferon and heterologous rabbit and human interferons did not. Interferon may suppress staphylococcal infection in vivo with the help of interferon-activated macrophages, because Weinstein et al. (Weinstein et al., 1970) reported that polyriboinosinic-polyribocytidylic acid (poly I: C), which is a potent interferon inducer, induced resistance to staphylococcal infection in mice. We plan to examine the enhancing effect of interferon on the bactericidal activity of macrophages in vivo.

ACKNOWLEDGMENTS

The authors are indebted to Dr. A. Matsuo, Central Research Institute, Green Cross Corp., Osaka, Japan, for human leukocyte interferon, and Dr. M. Iizuka, Basic Research Laboratory of Toray Industries, Inc., Kamakura, Japan, for rabbit RK-13 interferon.

REFERENCES

Baughn, R. E., Bonventre, P. E. 1975. Phagocytosis and intracellular killing of *Staphylococcus aureus* by normal mouse peritoneal macrophages. Infect. Immun. 12: 346–352.

Braun, W., Levy, H. B. 1972. Interferon preparations as modifiers of immune responses. Proc. Soc. Exp. Biol. Med. 142: 769–773.

Brodeur, B. R., Merigan, T. C. 1974. Suppressive effect of interferon on the humoral immune response to sheep red blood cells in mice. J. Immunol. 113: 1319–1325.

Brodeur, B. R., Merigan, T. C. 1975. Mechanism of the suppressive effect of interferon on antibody synthesis in vivo. J. Immunol. 114: 1323–1328.

Chester, T. J., Paucker, K., Merigan, T. C. 1973. Suppression of mouse antibody producing spleen cells by various interferon preparations. Nature 246: 92–94.

De Maeyer, E., De Maeyer-Guignard, J., Vandeputte, M. 1975. Inhibition by interferon of delayed-type hypersensitivity in the mouse. Proc. Natl. Acad. Sci. USA 72: 1753–1757.

Huang, K., Donahoe, R. M., Gordon, G. B., Dressler, H. R. 1971. Enhancement of phagocytosis by interferon containing preparation. Infect. Immun. 4: 581–588.

Imanishi, J. 1975. Inhibition of macrophage migration by virus-induced interferon preparations. Jpn. J. Microbiol. 19: 337–342.

Imanishi, J., Oishi, K., Kishida, T., Negoro, Y., Iizuka, M. 1977. Effects of interferon preparations on rabbit corneal xenograft. Arch. Virol. 53: 157–161.

Imanishi, J., Yokota, Y., Kishida, T., Mukainaka, T., Matsuo, A. 1977. Phagocytosis-enhancing effect of human leukocyte interferon preparation on human peripheral monocytes in vitro. Acta Virol. (Praha) 157: 52–58.

Kishida, T., Morikawa, K., Ito, H., Yokota, Y. 1973. Influence de l'interféron sur l'inhibition par les macrophages, de la multiplication in vitro de la cellule maligne murine (FM_3A). C. R. Soc. Biol. (Paris) 167: 1502–1505.

Mauel, J., Behin, R., Biroum-Noerjasin. 1973. Quantitative release of live microorganisms from infected macrophages by sodium dodecyl sulphate. Nature New Biol. 244: 93–94.

Mizunoe, K., Hiraki, M., Nagano, Y., Maehara, N. 1975. Suppression of intracellular multiplication of *Mycobacterium tuberculosis* by virus-inhibiting factor or interferon. Jpn. J. Microbiol. 19: 235–

236.
Schultz, R. M., Chirigos, M. A., Heine, V. 1978. Functional and morphologic characteristics of interferon-treated macrophages. Cell. Immunol. 35: 84–91.
Schultz, R. M., Papamatheakis, J. D., Chirigos, M. A. 1977. Interferon: An inducer of macrophage activation by polyanions. Science 197: 674–675.
Suzuki, J., Akaboshi, T., Kobayashi, S. 1974. A rapid and simple method for assaying interferon. Jpn. J. Microbiol. 18: 449–456.
Weinstein, M. J., Waitz, J. A., Came, P. E. 1970. Induction of resistance to bacterial infections of mice with poly C. Nature 226: 170.