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Effect of liposome-encapsulation on immunomodulating and antiviral activities of interferon-γ[1]

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Abstract

The effect of liposome-encapsulation on the immunomodulating and antiviral activities of interferon-γ (IFN-γ) was evaluated in this study. The immunomodulating activity was measured by increases in phagocytic activity and in nitric oxide production by peritoneal macrophages from mice treated with both free and LIP-IFN-γ (4000 U/mouse, intraperitoneal injection). Resident peritoneal macrophages harvested from mice treated with free unencapsulated IFN-γ or muramyl dipeptide showed significant increases in macrophage yield, and enhanced ability to phagocytize zymosan particles. In mice treated with liposome-encapsulated IFN-γ (LIP-IFN-γ), both macrophage yield and phagocytic activity further increased by 2-fold over unencapsulated IFN-γ. In addition, the activation of peritoneal macrophages with LIP-IFN-γ showed enhanced production of NO when the cells were cultured ex vivo. Using a murine respiratory influenza infection model, intranasally administered LIP-IFN-γ conferred protection to 70% in mice challenged intranasally with 10 LD_{50} doses of influenza A/PR/8 virus compared with a 20% survival rate using free IFN-γ. Together these results suggest that liposome-encapsulation increases the immunomodulating and antiviral activities of IFN-γ. Liposome-encapsulation of IFN-γ may provide additional therapeutic advantages by reducing IFN-γ toxicity while prolonging its body retention.

Keywords: Liposomes; Interferon-γ; Influenza infection; Macrophage

1. Introduction

Interferon-γ (IFN-γ) is a multifunctional protein known to possess antiviral, antiprotozoal and immunomodulating activities (Dulbecco and Ginsburg, 1980; Eisen, 1980; Joklik, 1990). Produced mainly by T-lymphocytes, natural killer and virus-infected cells, IFN-γ's antiviral activity is believed to be mediated through binding to specific IFN-γ receptors which in turn induces the production of a number of transient proteins that are known to inhibit virus replication (Joklik, 1990; Pinto et al., 1990). IFN-γ is also an important regulator of a number of immunological functions such as increasing the expression of major histocompatibility antigens and regulating the production of antibody by B-lymphocytes (Joklik, 1990). IFN-γ stimulates macrophage

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function by increasing the macrophage production of interleukin-1 and hydrogen peroxide (Joklik, 1990). Thus, activation of macrophages by IFN-γ may play an important role in their ability to destroy invading microorganisms, particularly infectious viruses or parasites. More recently, interferons have been demonstrated to stimulate the production of reactive nitrogen intermediates, in particular, nitric oxide (NO), which mediates intracellular killing and a wide range of host responses (Fortier et al., 1992; Green and Nacy, 1993; Zhang et al., 1994) including the inhibition of viral replication (Karupiah et al., 1993).

These characteristics make IFN-γ a promising, broad-spectrum therapeutic agent for the prevention and treatment of infectious diseases. However, the therapeutic potential of IFN-γ as an antimicrobial or immunomodulating agent is limited by its partial effectiveness in vivo and its known toxicities to cardiac, renal and CNS functions (Joklik, 1990; Quesada et al., 1986). In addition, as a small protein, IFN-γ administered into the body is likely to be subjected to immediate dilution, rapid clearance from the body and may not reach its target cells (Bino et al., 1982; Gutterman et al., 1984; Kurzrock et al., 1985). These problems can potentially be overcome with the use of a liposome delivery system for IFN-γ.

Liposomes are microscopic lipid vesicles which have a number of characteristics making them an attractive drug delivery system for various antimicrobial and immunomodulating agents (Fidler et al., 1980; Poste et al., 1980; Gregoriadis, 1990; Wong et al., 1992). Therapeutic agents encapsulated within liposomes can be protected from in vivo degradation and dilution (Fidler et al., 1980; Poste et al., 1980; Wong et al., 1994). The encapsulated drugs are readily released from liposomes in a gradual and sustained manner, thus increasing their prophylactic/therapeutic efficacies while reducing their intrinsic toxicities (Fidler et al., 1980; Poste et al., 1980, 1984; Petenazzo et al., 1989). Liposomes are particularly well suited as carriers of macrophage stimulating agents such as IFN-γ since they are readily taken up by the macrophages upon systemic, pulmonary or peritoneal administration and the agents can be delivered to macrophages in concentrated levels (Fidler et al., 1980; Poste et al., 1980, 1984), without adversely affecting healthy, non-target cells.

Indeed, liposome-encapsulated IFN-γ has been shown by a number of investigators to be more effective than free IFN-γ for the treatment of a number of experimental infections including bacterial (*Listeria monocytogenes*, Melissen et al., 1993; *Klebsiella pneumoniae*, ten Hagen et al., 1995) and protozoal (*Leishmania donovani*, Hockertz et al., 1991). Its potential efficacy against viral infections, however, remains unexploited. In addition, the effect of liposome-encapsulation on the intrinsic immunomodulation effect of IFN-γ has not been studied extensively. The purpose of the present study is to evaluate the effectiveness of a liposome delivery system in potentiating the antiviral as well as immunomodulatory activities of IFN-γ. The specific modulation of cell-mediated immunity by free and liposome-encapsulated IFN-γ (LIP-IFN-γ) was determined by increase in macrophage activation, measured by increased phagocytic activity and production of NO. The antiviral activity was assessed in vivo for the efficacy of IFN-γ and LIP-IFN-γ to protect mice against a lethal respiratory influenza A infection. The approach to use a delivery system for IFN-γ may provide a useful means to enhance the host's defence resistance to respiratory virus infections, and has the potential to decrease the inherent toxicity associated with the use of interferons.

2. Materials and methods

2.1. Animals

Six-week-old female BALB/c mice were purchased from Charles River Canada (St. Constant, Que.). Mice were acclimated for 1 week in the vivarium before use. Care and handling of the animals followed the guidelines set out by the Canadian Council on Animal Care.

2.2. Reagents

Recombinant mouse IFN-γ was purchased from Boehringer Mannheim Canada (Dorval, Que.). Phosphatidylcholine, phosphatidylserine

and cholesterol used for the preparation of the liposomes were from Avanti Polar Lipids (Alabaster, AL).

2.3. Liposomes

Liposomes used in this study for the encapsulation of IFN-γ were prepared using a modification of the freeze-drying method described by Kirby and Gregoriadis (1984). Negatively-charged liposomes were prepared using phosphatidylcholine–cholesterol–phosphatidylserine in a molar ratio of 7:2:1. Briefly, a total of 20 μmoles of the lipids in chloroform–methanol (2:1, v/v) were dried by heating at 45°C. Throughout this procedure, the contents of the tube was purged with a gentle stream of dry nitrogen. The lipids were further dried for 30 min in a vacuum oven to remove residual organic solvent. The lipids were then rehydrated with 1 ml of 100 mM HEPES buffer in normal saline, pH 6.7 (HEPES buffer). IFN-γ was then added to the lipid mixture and freeze-dried overnight (total volume of 400 μl, 100 000 U/ml). The mixture was reconstituted in 100 μl of HEPES buffer and vortexed for 2–3 min. The reconstituted liposomes were then left for rehydration for 2 h at room temperature and then were washed twice with 8 ml of HEPES buffer and ultracentrifuged at 125 000 $\times$ g for 30 min at 4°C after incubating at room temperature for 2 h. After ultracentrifugation, the liposome pellets were resuspended in 1 ml HEPES buffer prior to use. The liposomes were negatively stained with 2% sodium phosphotungstate (pH 7.4) and the morphology and vesicle size distribution were analyzed by electron microscopy. The multilamellar vesicles (MLV) prepared using this method were found to be heterogenous in size with the vesicle diameters ranging from approximately 300 nm to 2 μm.

2.4. Macrophages

BALB/c female mice were intraperitoneally (i.p.) injected with a single dose of 500 μl muramyl dipeptide (MDP, 1 mg/ml in PBS) (Sigma Chemical Co., St. Louis, MO), free IFN-γ (200 μl, 20 000 U/ml), or LIP-IFN-γ (200 μl, 1 μmole equivalent lipid containing 4000 U IFN-γ). The control mice were injected with 200 μl PBS (i.p.). At 24, 48 and 72 h post-injection, macrophages were harvested from these mice using a modification of the peritoneal lavage procedure described by Mischell and Shiigi (1980). Lavage fluid used in peritoneal washes (100 ml) contained 90 ml RPMI 1640, 10 ml fetal bovine serum (FBS) (Flow Laboratories, Mississauga, Ont.) and 0.0738 g sodium chloride. Harvested cells were washed in RPMI 1640 with 10% FBS. These solutions were filter sterilized with a 0.45 μm Nalgene filter (Nalge Company, Rochester, NY) and stored at 4°C.

The recovered lavage fluid (3 $\times$ 5 ml lavages) for each mouse was pooled and centrifuged at 160 $\times$ g for 7 min. The resulting cell pellet was washed in RPMI 1640 plus 10% FBS (Flow Laboratories, Rockville, MD) and resuspended in a final volume of 3 ml of the same buffer. The cell suspension was then layered onto 3 ml of Histopaque 1083 (Sigma) and centrifuged at 700 $\times$ g for 30 min. The macrophages were collected by aspiration of the cells at the density gradient interface using a pasteur pipet. The cells were suspended in RPMI 1640 to a final volume of 13 ml and centrifuged at 450 $\times$ g for 10 min. Red blood cells were removed from the pellet by lysis with deionized water for 1 min. and the cells were suspended in RPMI 1640 and centrifuged as above. The pellet was resuspended in 1–1.5 ml of RPMI 1640 and viable cells were counted by trypan blue dye exclusion. The isolated cells were visually identified as macrophages. Slides were prepared by centrifuging the 0.5 ml of purified sample onto glass slides using a Cytospin 2 centrifuge (Shandon Southern Products Ltd. Astmoor, UK). After the slides were left to dry overnight, the preparation was stained with STAT STAIN (VWR Scientific Inc., Brisbane, CA). The samples were examined on a microscope using oil immersion (1000 $\times$ magnification).

2.5. Chemiluminescence assay

The phagocytic activity of the peritoneal macrophages was determined by chemiluminescence whereby phagocytosis of zymosan particles

by the macrophages in the presence of lucigenin results in the emission of light, which is measured by a luminometer. In this assay, a macrophage concentration of 2×10^6 cells/ml of RPMI 1640 was used to assay phagocytic activity by chemiluminescence. Luminometer cuvettes were filled with 400 μl of cells plus 20 μl of 0.002 M lucigenin (Sigma) and placed in an LKB 1251 luminometer (LKB, Stockholm, Sweden) at 37°C. The luminometer-controlled dispensers delivered 200 μl of RPMI 1640, or human serum activated zymosan to each sample at time zero of the assay. The total chemiluminescence (measured in mV) was recorded over a 15 s period at 2–3 min intervals for each sample for up to 2 h.

2.6. Nitrite assay

Macrophage activation was also measured as a function of nitric oxide release from peritoneal macrophages measured in the form of its oxidized product, NO_2^- or nitrite ions. Groups of five mice were administered two daily doses (1000 U) of free or LIP-IFN-γ. At 4 days post-administration, peritoneal macrophages from treated and control mice were harvested by peritoneal lavage as described before and were incubated for an additional 3 days in phenol red-free medium. The phenol red-free medium used was RPMI 1640 (Sigma), supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin G (100 U/ml), streptomycin sulphate (100 μg/ml) and amphotericin B (0.25 μg/ml) (Gibco Laboratories, Grand Island, NY) and 0.075% sodium bicarbonate (Gibco). The resident peritoneal cells were resuspended at 10^6 cells per ml (0.5 ml) in phenol red-free RPMI and incubated at 37.6°C for 72 h in a 5% CO_2 atmosphere. Aliquots (100 μl) of the culture supernatant are assayed for nitric oxide release as a function of nitrite concentration in solution as previously reported (Green et al., 1982; Ding et al., 1988).

2.7. Influenza A protection

Protection against influenza A virus infection was evaluated using intranasal administration of free or liposome-encapsulated IFN-γ. Influenza A/PR/8 virus was adapted in mice by four blind passages using egg-propagated virus as described previously by Wong et al. (1994). The volume for all intranasal administrations was 50 μl. For intranasal administration, mice were anesthetized with sodium pentobarbitol (50 mg/kg, i.p.). When the animals were unconscious, they were carefully supported by hands with their nose up, and the material to be administered was gently applied with a micro-pipetor into the nostrils. The applied volume was naturally inhaled into the lungs. Groups of sodium pentobarbitol-anesthetized mice (10 mice per group) were inoculated intranasally with either 50 μl of IFN-γ (1000 U per mouse), LIP-IFN-γ (1 μmol total lipid containing 1000 U IFN-γ per mouse), or with sham liposomes (sham LIP, 1 μmole total lipid per mouse, no IFN-γ). The mice were pre-treated at 24-h intervals beginning at either 1, 2, 3 or 4 days before intranasal challenge with 10 LD_{50} of mouse-adapted influenza A/PR/8 virus. At day 14 post-virus challenge, the number of mice surviving the virus challenge was recorded.

Statistical analysis of the data was carried out using Student's t-test. The analysis was performed using SigmaPlot software program from Jandel Scientific (San Rafael, CA).

3. Results

3.1. Macrophage yield and phagocytic activity

The average yield of macrophages from control mice and those treated with MDP, IFN-γ or LIP-IFN-γ is shown in Table 1. Treatment with MDP or IFN-γ increased the macrophage yield by 3-fold compared to that of the control untreated group. The highest macrophage yield was found in mice treated with LIP-IFN-γ with a 5-fold increase in yield compared to that of the control group. Phagocytic activity of peritoneal macrophage was measured by the chemiluminescence assay. Macrophages isolated from mice treated with a single dose of IFN-γ demonstrated increased phagocytic activity as early as 18 h post-IFN-γ administration. The phagocytic activity was highest at 42 h post-administration, but at

Table 1
The average number of macrophages harvested per mouse

Test group[a]	Cells harvested per mouse (10^6) ($\pm$ S.E.)
PBS control	1.1 $\pm$ 0.6
MDP	3.7 $\pm$ 1.6*
Free IFN-γ	2.5 $\pm$ 1.4
Sham LIP	3.7 $\pm$ 1.6*
LIP-IFN-γ	5.3 $\pm$ 2.0*

*$P < 0.05$ vs. PBS control. The macrophages were harvested at 24 h post-treatment. This experiment was carried out four times.[a]Doses used per mouse were MDP (500 μg), free IFN-γ (4000 U), Sham LIP (1 μmole lipid equivalent of empty liposomes) and LIP-IFN-γ (4000 U IFN-γ in 1 μmole lipid equivalent of liposomes).

72 h, it decreased to near the level at 18 h (Fig. 1). Phagocytic activity of macrophages, as measured by chemiluminescence, was found to be highest in mice treated with LIP-IFN-γ (Fig. 2). The peak level of phagocytic activity in the LIP-IFN-γ treated group was found to be approximately 10-fold higher than that of the PBS control group, 4-fold higher than the sham liposomes group, and 2-fold higher than the IFN-γ group (all statistical significant at $P < 0.001$).

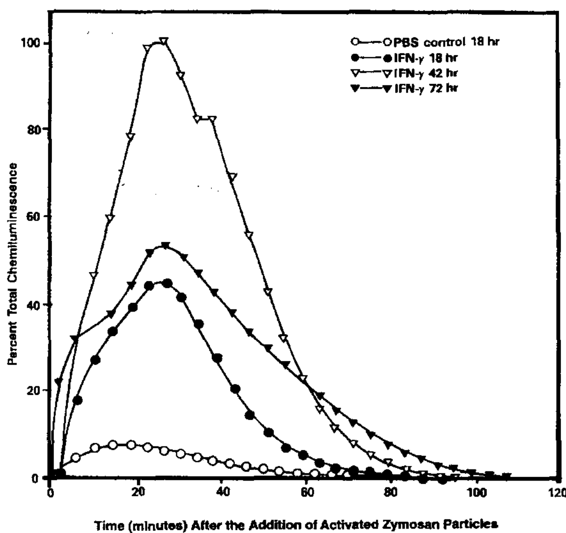

Fig. 1. Phagocytic activity of mouse macrophages from mice treated with free IFN-γ as determined by chemiluminescence assay. The cell concentrations used in the assay for the PBS control at 18 h (open circles), IFN-γ at 18 h (closed circles), IFN-γ at 42 h (open triangles) and IFN-γ at 62 h (closed triangles) were 2.15 $\times$ 10^6 cells/ml, 2.3 $\times$ 10^6 cells/ml, 2.67 $\times$ 10^6 cells/ml and 2.73 $\times$ 10^6 cells/ml, respectively. This experiment was carried out three times. The dose of IFN-γ used in this experiment was 4000 U per mouse.

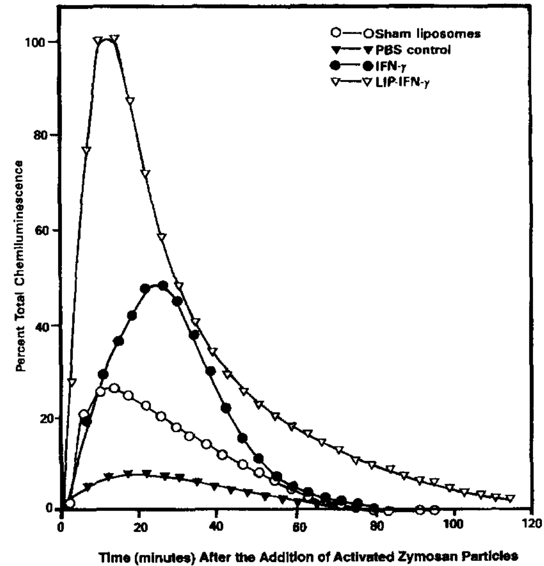

Fig. 2. Phagocytic activity of mouse macrophages from mice treated with LIP-IFN-γ as determined by chemiluminescence assay. The cell concentrations used in the assay for the Sham LIP (open circles), IFN-γ (closed circles) and LIP-IFN-γ (open triangles) were 2.3 $\times$ 10^6 cells/ml in each assay. Macrophage phagocytic activity was assayed 18 h post-treatment. This experiment was carried out three times. The dose of IFN-γ used was 4000 U per mouse for both free and liposome-encapsulated IFN-γ.

3.2. Nitrite release

To assess the effect of IFN-γ and LIP-IFN-γ on the production of NO by these activated macrophages, tissue culture supernatants in which activated macrophages were incubated for 3 days were assayed for presence of NO_2^- or nitrite ions. The data from Fig. 3 demonstrate that there is small but not statistical significant increase in nitrite levels from macrophages from IFN-γ group compared to the PBS control group ($P > 0.05$). In contrast, in tissue culture supernatants from macrophages treated with LIP-IFN-γ, levels of nitrite were found to be significantly increased compared to that of either the IFN-γ or the PBS control group ($P < 0.01$).

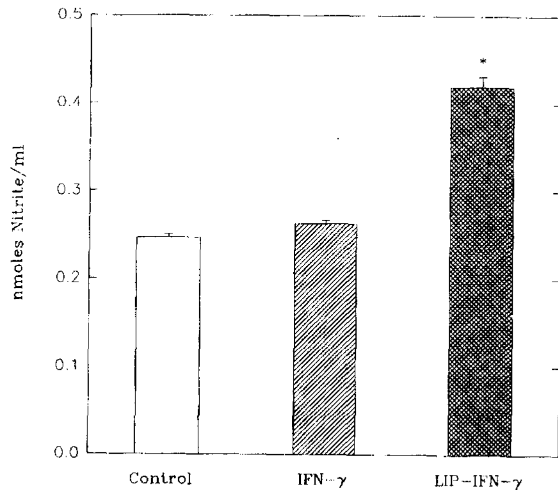

Fig. 3. Nitrite release from peritoneal cells treated in vivo with liposome encapsulated IFN-γ. Mice were given two daily administrations of Sham LIP, IFN-γ (1000 U) or LIP-IFN-γ (1000 U). Peritoneal macrophages were harvested and suspended as described in Section 2 and incubated at 37°C overnight. Nitrite release into the cell supernatant was measured using the Griess reagent. * = $P < 0.01$. This experiment was carried out two times.

3.3. *Protection against influenza A infection*

The ability of IFN-γ and LIP-IFN-γ to protect mice against influenza A virus infection was compared to determine if liposome encapsulation increased the efficacy of IFN-γ's antiviral activity. Groups of mice were pretreated with either PBS, sham liposomes, free IFN-γ or LIP-IFN-γ at various intervals prior to intranasal infection with 10 LD_{50} of influenza A. As summarized in Table 2, groups of 10 mice pretreated with either PBS or sham liposomes were completely unprotected against infection with the influenza virus (mean death time of 7.2 days). When treated with unencapsulated IFN-γ, there was a small survival rate of 20% in the groups of mice only after 3–4 repeated doses prior to viral inoculation. Mice pretreated with LIP-IFN-γ had the highest survival rate. A single dose of LIP-IFN-γ was as effective as 3–4 doses of IFN-γ. Repeated intranasal doses of LIP-IFN-γ 2–4 days prior to infection with influenza virus resulted in survival rates of 50 to 70%. LIP-IFN-γ was most effective when administered in three doses at 24-h intervals prior to infection with influenza A resulted in a 70% survival rate (Table 2), with mean death time of 10.5 days. Increasing the dose of IFN-γ in liposomes from 1000 U to 4000 U per dose did not result in significant increases in survival rates (results not shown).

4. Discussion

The efficacy of interferons to prevent respiratory virus infections has been evaluated against rhinovirus and coronavirus (Hayden and Gwaltney, 1983; Higgins et al., 1983; Douglas et al.,

Table 2
Efficacies of intranasally administered IFN-γ and LIP-IFN-γ in protecting mice against infection with 10 LD_{50} of mouse-adapted influenza A virus

Group[a]	Days before infection	No. of survivors	% Survival	Mean day to death
PBS	3, 2, 1	0/10	0	7.2
Sham LIP	3, 2, 1	0/10	0	7.5
IFN-γ	1	0/10	0	7.1
	2, 1	0/10	0	7.5
	3, 2, 1	2/10	20	7.5
	4, 3, 2, 1	2/10	20	8.2
LIP-IFN-γ	1	2/10	20	7.5
	2, 1	5/10	50*	9.5*
	3, 2, 1	7/10	70*	10.5*
	4, 3, 2, 1	6/10	60	10.5*

*$P < 0.05$ (P vs IFN-γ group). This experiment was carried out twice.[a]Doses used per mouse were Sham LIP (1 μmole lipid equivalent of liposomes), IFN-γ (1000 U) and LIP-IFN-γ (1 μmole lipid equivalent of liposomes containing 1000 U IFN-γ).

1985; Monto et al., 1986). IFN-γ may be considered useful in the prevention of respiratory influenza infection since it provokes both an antiviral and immunomodulating response in the susceptible host. However, one of the major drawbacks of interferon therapy is that interferons are toxic molecules which can cause severe toxicities to the central nervous system, renal and cardiac functions (Quesada et al., 1986; Joklik, 1990). Although the effect of liposome-encapsulation on IFN-γ toxicities was not determined in this present study, Hockertz et al. (1991) had demonstrated that toxic effects seen in mice injected with IFN-γ, such as necrotic lesions, loss of body weight and MHC class II expression, were absent in mice treated with the same dose of INF-γ encapsulated in liposomes. Therefore, liposome-encapsulation may prove extremely valuable in interferon immunotherapy because liposomes have the potential to significantly reduce interferon toxicities through their slow release characteristics. In addition, the targeting of IFN-γ by liposomes to specific infection sites may therefore avoid causing toxic effects in non-target cells and organs (Hockertz et al., 1991; Melissen et al., 1993).

The liposomes used in this study for the encapsulation of IFN-γ were negatively-charged multilamellar vesicles. This liposome type has been shown by a number of investigators to be more efficiently taken up by macrophages than positively-charged or unilamellar vesicles (Fidler et al., 1980; Poste et al., 1980, 1984). The freeze-dry method was used for the production of liposomes because it is a relatively mild method that does not require IFN-γ to be exposed to organic solvent, sonication or detergent and this method has been previously demonstrated to be suitable for the encapsulation of a number of biologically active compounds (Fidler et al., 1980; Poste et al., 1980; Wong et al., 1992, 1994). LIP-IFN-γ administered to mice appears to act as chemotactic agent for phagocytic cells resulting in a 5-fold increase in accumulated peritoneal macrophages in comparison with placebo-treated controls. The chemotactic nature observed with LIP-IFN-γ may be entirely or in part due to liposome effect since sham liposomes also resulted in an increased accumulation of peritoneal macrophages, although this increase of macrophage accumulation due to sham liposomes did not result in an increased protection against viral infection in vivo. The chemotactic nature of LIP-IFN-γ presents an effective mechanism of delivery whereby the encapsulated IFN-γ may be released gradually from the liposomes into close proximity of the congregating macrophages. Multilamellar liposomes have been shown to be naturally taken up by pulmonary macrophages via phagocytosis (Sone et al., 1980; Fidler et al., 1980; Fidler, 1988). The uptake of LIP-INF-γ by macrophages has been reported to allow the IFN-γ to bind to intracellular receptors of macrophages (Fidler et al., 1985). In addition, IFN may exert its antiviral effect on the target cells in the lungs via a receptor-independent delivery of IFN provided by liposome delivery (Killion and Fidler, 1993).

It has been reported that bone-marrow-derived macrophages exposed to IFN-γ in the presence of a phagocytic stimulus such as protozoan parasite or latex beads were found to produce elevated levels of nitrite, whereas when the stimulus is absent, no nitrite was produced (Corradin et al., 1991). Our findings that IFN-γ alone did not induce nitrite production in peritoneal macrophages appear to be consistent with this observation. However, when IFN-γ was administered in its liposomal form, significant levels of nitrite were produced. Liposomes may, therefore, provide the same type of phagocytic stimulus previously observed to stimulate NO production. Since liposomes are readily taken up by macrophages, IFN-γ may be directly released from the liposomes into the intracellular compartment. However, this phagocytic route of entry may also result in the lysosomal degradation of the liposomes as well as IFN-γ. To overcome this potential problem, we are investigating the feasibility of developing 'fusogenic' liposomes to be capable of fusing directly with the membrane of the target cells, thus permitting content of the liposomes to be delivered intracellularly bypassing the degradative phagocytic process (Nayar and Schroit, 1989).

The present study has demonstrated that the efficacy of IFN-γ for the protection against infec-

tion by influenza A virus can be significantly enhanced by liposome-encapsulation. Protection against influenza mortality could be observed in mice pretreated with free and LIP-IFN-γ. The major advantage of this approach is a single dose of LIP-IFN-γ provided the same level of protection to mice as 3 doses of IFN-γ. Three days of pretreatment with LIP-IFN-γ resulted in up to 70% survival of infected mice, compared to 20% for IFN-γ treated mice. This increase in IFN-γ efficacy may also to be the result of the increased retention of IFN-γ in the lungs afforded by liposome encapsulation. Indeed, radiotracer studies using ^{125}I-IgG indicated that lung retention of liposome-encapsulated IgG was 3-fold more than unencapsulated IgG even at 24 h post-administration (Wong et al., 1994). The observed decrease in survival after 4 days of LIP-IFN-γ pretreatment may reflect a relatively narrow optimum dose range for IFN-γ (Merigan, 1987).

Administration of LIP-IFN-γ to the host significantly delays the onset of the morbidity and mortality associated with the viral disease. Liposome-encapsulation of IFN-γ results in increased non-specific cell-mediated immunity and increased survival against lethal doses of influenza A. A major benefit of this form of treatment is that the gradual release of IFN-γ from liposomes allows the host immune system time to elicit a natural specific immune response to fight the infection. In addition, the ability of LIP-IFN-γ to augment the production of NO by mouse macrophages may play an important role in the further enhancing the body's defence mechanisms to fight viral infections. NO is an effector molecule produced by various mammalian cell types including macrophages. NO and its oxidized intermediates, nitrite (NO_2^-) and nitrate (NO_3^-), are known to play a important role in the cell-mediated killing of a wide range of pathogenic microorganisms, including viruses (Karupiah et al., 1993)

This present study demonstrates that liposome-encapsulation can significantly affect the antiviral and immunomodulating activities of IFN-γ. Liposome delivery was found to enhance the non-specific activation of macrophages, resulting in increased phagocytic activity and NO production. The efficacy of IFN-γ to protect mice against lethal respiratory infection by influenza A virus was also significantly improved by liposome-encapsulation. These immunological and therapeutic advantages, in combination with the potential of liposome delivery to significantly reduce IFN-γ's intrinsic toxicity, may therefore provide a valuable and beneficial approach for the clinical use of IFN-γ for antiviral and immunotherapy.

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