J. Biochem. 124, 697-701 (1998)

Cationic Liposome-Mediated Efficient Induction of Type I Interferons by a Low Dose of Poly I:Poly C in Mouse Cell Lines[1]

Sachiko Okamoto,*,[2] Yoshihiko Watanabe,† Yoshinobu Takakura,* and Mitsuru Hashida*,[3]

*Departments of Drug Delivery Research and †Molecular Microbiology Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501

Received for publication, March 23, 1998

Double-stranded polyriboinosinic acid:polyribocytidylic acid (poly I:poly C) is a powerful inducer of type I interferons (IFNs). However, the dose of poly I:poly C required for efficient IFN induction is so high as occasionally to be cytotoxic. In this work, we examined the IFN-inducibility of poly I:poly C complexed with several cationic reagents in mouse fibroblast L cells and found that Lipofectin and LipofectACE can induce the production of a substantial amount of type I IFNs (mostly β-type) even at a two-order lower dose compared with poly I:poly C alone. Such effects of poly I:poly C were optimal at 0.1 μg/ml for 2-10 μg/ml of Lipofectin and LipofectACE. These conditions caused no significant cytotoxicity in the recipient cells. Furthermore, a short treatment (less than 10 min) with the complexes was sufficient for the maximum induction. This IFN induction method was applicable to other cell types and other species including human. Hence, our observations may pave the way for clinical application of the IFN inducer.

Key words: cationic liposomes, efficient IFN induction, FITC-poly I:poly C, interferon, poly I:poly C.

The interferons (IFNs) are a family of multifunctional cytokines, which are subdivided into two types, type I (IFN-α/β) and type II (IFN-γ), and have many biological activities including antiviral effects, inhibition of cell proliferation, regulation of cell differentiation, and modulation of the immune system (*1, 2*). Thus it is rational that IFNs have been clinically applied as antiviral and antitumor reagents for more than a decade, and more recently IFN gene transfer to tumors has been intensively attempted for therapeutic purposes (see Ref. *3* for a review, *4*). IFN inducers also have the potential for use in clinical treatments. The synthetic double-stranded RNA poly I:poly C is a particularly potent inducer of type I IFNs (*5-7*), and the pretreatment of cells with IFN (priming) can enhance the inducibility of IFN (*8, 9*). Effective doses of poly I:poly C are, however, so high as to be cytotoxic, and primed cells are occasionally more susceptible to the cytotoxicity than unprimed cells (*10-13*). To overcome this disadvantage, mismatched analogues of poly I:poly C, for instance, $r(I)_n:r(C_{12},U)_n$ (Ampligen), were developed which are less cytotoxic, but also less effective for IFN induction, than poly I:poly C (*14-16*). An alternative approach is through more efficient delivery of poly I:poly C into cells without cytotoxicity. This approach has been attempted using phospholipid particles or liposomes:liposome-encapsulation of poly I:poly C augmented cellular uptake of poly I:poly C (*17*) and potentiated its IFN-inducibility (*18-20*), but generally resulted in enhanced cytotoxicity (*17-20*).

Several methods have recently been devised for gene delivery, including the use of mild cationic liposomes (*e.g.*, Lipofectin) (*21*). Complexing of plasmid DNA with cationic liposomes is considered to diminish charge-repulsion by anionic cell surfaces and thereby promote cellular uptake of exogenous plasmid DNA without serious cytotoxicity. This prompted us to examine the effect of modern cationic liposomes on IFN induction by poly I:poly C in various mouse cell lines. Here we present fundamental properties as potent IFN inducers of the complexes of poly I:poly C and cationic reagents, including Lipofectin and LipofectACE, and discuss their clinical potential.

MATERIALS AND METHODS

Cell Lines—Mouse fibroblast L cells were cultured in Eagle's minimal essential medium (MEM) (Nissui Pharmaceutical) supplemented with 6% of fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (GIBCO-BRL) in 5% CO_2 at 37°C. Mouse colon tumor CT-26, bladder tumor MBT-2, macrophage tumor RAW 264, kidney tumor RENCA, fibrosarcoma Meth A cells, and

[1] This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan and the grant of "Basic Research on Drug Innovation" by the Japan Health Sciences Foundation.

[2] Present address: Biomedical Group, Takara-Shuzo Co., Ltd., Norocho, Kusatsu, Shiga 525-0055.

[3] To whom correspondence should be addressed. Tel: +81-75-753-4525, Fax: +81-75-753-4575, E-mail: hashidam@pharm.kyoto-u.ac.jp

Abbreviations: DC-chol liposome, 3β-(*N*-(*N'*,*N'*-dimethylaminoethane) carbamoyl) cholesterol liposome; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; IFN, interferon; MEM, Eagle's minimal essential medium; PBS, phosphate buffered saline; poly I:poly C, polyriboinosinic acid:polyribocytidylic acid.

squamous cell carcinoma Pam-T cells, a variant of Pam 212 (*22, 23*), were grown in RPMI1640 (Nissui Pharmaceutical) supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Induction of IFN by Poly I:Poly C-Cationic Reagents Complexes—L cells (5×10^4/well) were inoculated in a 24-well tissue culture plate and cultured for 24 h. The cells were thoroughly washed with serum-free medium and treated with mixtures of various concentrations of poly I:poly C (about 200–1800 bp) (Pharmacia Biotech) and Lipofectin (GIBCO-BRL), LipofectACE (GIBCO BRL), LipofectAMINE (GIBCO BRL), 3β-(*N*-(*N'*,*N'*-dimethylaminoethane) carbamoyl) cholesterol (DC-chol liposome), synthesized as described (*24*), or DEAE-dextran (Promega) in serum-free medium. After 6 h of incubation with the complexes, the medium was replaced with fresh growth medium and the plates were incubated for a further 18 h. The culture fluids were then assayed for IFN (see below).

IFN Bioassay—The IFN activity was measured by the reduction of the cytopathic effect of vesicular stomatitis virus (VSV) on mouse L cells as previously described (*25, 26*). Subtypes of IFNs were determined by using a combination of monoclonal rat antibodies against mouse IFN-α (4E-A1) and -β (7F-D3) (*27*) in bioassay.

MTT Assay—L cells (3×10^4/well) grown in 0.1 ml of growth medium in a 96-well tissue culture plate were treated with poly I:poly C-cationic reagent complexes for 6 h, and then the medium containing the complexes was replaced with 100 μl of MTT (0.5 mg/ml) solution. After 4 h of incubation, 100 μl of 10% SDS solution was added to each well, and cells were incubated overnight at 37°C. Absorbance of the solution in each well was measured with a two-wavelength microplate photometer (Model 450 microplate reader, BIO-RAD) at test and reference wavelengths of 570 and 660 nm, respectively (*28*).

Time Course of IFN Production of L Cells and Northern Blot Analysis—L cells (5×10^4/well) were inoculated in a 24-well culture plate and cultured for 24 h. The cells were treated with poly I:poly C (0.1 μg/ml)-Lipofectin (8 μg/ml) complexes for 3 h, then the medium was replaced with 0.5 ml of fresh medium. Culture fluids were harvested at the indicated times thereafter and assayed for IFN activity. For Northern analysis, 4×10^6 L cells were cultured in each 10-cm dish and treated with poly I:poly C (1 μg/ml)-Lipofectin (8 μg/ml) complexes. At the indicated times, total cellular RNAs were prepared using ISOGEN (Nippon Gene). Northern blotting was performed as described previously (*29*). For detection of mouse IFN-β gene and mouse β-actin gene transcripts, the *Xho*I fragment from pCMV-Muβ (*30*) and the *Pst*I fragment from pAL41 (*31*) were used, respectively, as probes. The hybridized membranes were imaged with BAS 2000 (FUJI Photo Film).

Intracellular Uptake of FITC-Labeled Poly I:Poly C—Poly I:poly C from the same lot as used for IFN induction experiments was labeled with FITC using a FasttagFL Labeling kit (VECTOR laboratories). L cells (1×10^5/plate) were grown on glass-bottomed plates, washed with phosphate-buffered saline (PBS), and incubated with serum-free medium containing 3 μg/ml FITC-labeled poly I:poly C and Lipofectin (8 μg/ml) for 6 h at 37°C. The cells were washed with serum-free medium, and then fixed with 10% neutral formalin for 30 min at 4°C. After being washed with PBS, the cells were scanned with a confocal laser microscope (ACAS 570 interactive laser cytometer, Meridian Instruments).

RESULTS

Comparison of the Effects of Various Cationic Reagents on IFN Induction by Poly I:Poly C in L Cells—We first examined IFN inducibility in L cells with relatively low doses of 0.1 and 1 μg/ml of poly I:poly C mixed with Lipofectin, LipofectACE, LipofectAMINE, DC-chol liposome, and DEAE-dextran. As tabulated in Table I, poly I:poly C alone showed no detectable induction of IFN, whereas the same doses of poly I:poly C mixed with some of the cationic liposomes caused dramatic IFN-induction. With 0.1 μg/ml poly I:poly C, Lipofectin and LipofectACE at either 4 or 8 μg/ml were the most potent inducers, yielding respectively 560 and 550 IU/ml/day of IFN. DEAE-dextran at 100 or 300 μg/ml admixed with 1 μg/ml poly I:poly C exhibited a comparable potency (520 IU/ml/day), but brought about severe cell damage (data not shown). Next, the optimal doses of poly I:poly C were examined at constant concentrations of 8 μg/ml of Lipofectin and LipofectACE, and examined IFN production was found with 0.1 and 0.03–0.1 μg/ml poly I:poly C, respectively (Fig. 1A). Poly I:poly C alone even at 30 μg/ml did not induce detectable IFN activity (data not shown). Next, the most efficient concentrations of the two liposomes were examined at the optimal doses of poly I:poly C. Figure 1B shows that the values ranged from 2 to 10 μg/ml for Lipofectin and from 2 to 16 μg/ml for LipofectACE. The IFN activity induced in L cells was a mixture of the major β type (>95%) and the minor α type (<5%) as determined by use of neutralizing monoclonal antibodies specific for IFN-α and -β in the IFN bioassay (data not shown).

IFN Induction by Poly I:Poly C-Lipofectin Complexes in Several Mouse Cell Lines—The optimal conditions for IFN induction by poly I:poly C complexed with 8 μg/ml Lipo-

TABLE I. **Comparison of the effects of various cationic reagents on inducibility of IFN by poly I:poly C in mouse L cells.**

Poly I:poly C (μg/ml)	Cationic reagent (μg/ml)		IFN yield[a] (IU/ml)
0.1	None		<3
	Lipofectin	4	560
		8	560
	LipofectACE	4	550
		8	550
	LipofectAMINE	4	<3
		8	110
	DC-chol liposome	4	43
		8	43
	DEAE-dextran	100	97
		300	150
1	None		<3
	Lipofectin	4	160
		8	160
	LipofectACE	4	540
		8	560
	LipofectAMINE	4	12
		8	210
	DC-chol liposome	4	69
		8	57
	DEAE-dextran	100	520
		300	520

[a]The values represent the mean of duplicate determinations (SD <20%).

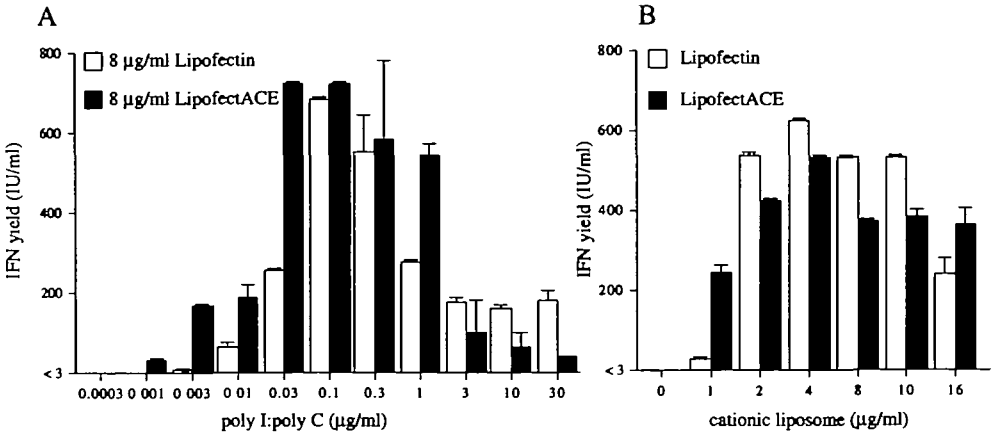

Fig. 1. **IFN induction by poly I:poly C-cationic reagent complexes in L cells.** L cells (5×10^4/well) grown in 24-well tissue culture plates were treated with (A) complexes of various amounts of poly I:poly C with 8 μg/ml Lipofectin (open bar) or 8 μg/ml LipofectACE (closed bar), or (B) complexes of various concentrations of Lipofectin (open bar) and LipofectACE (closed bar) with 0.1 and 0.03 μg/ml poly I:poly C, respectively, and incubated for 6 h. After washing, the cells were incubated for a further 18 h, and the IFN activity in the culture fluids was measured.

TABLE II. **Optimal doses of poly I:poly C complexed with Lipofectin for IFN induction in various mouse cell lines.**[a]

Cell line	Cell type	Optimal dose of poly I:poly C (μg/ml)	IFN yield (IU/ml)
CT-26	Colon tumor	1-3	830
MBT-2	Bladder tumor	1-3	250
Pam-T	Skin tumor	1	3,800
RAW 264	Macrophage tumor	1	100
RENCA	Kidney tumor	1	420
Meth A	Fibrosarcoma	10	2,100
L	Fibroblast	0.1	720

[a]Experiments were performed at 8 μg/ml Lipofectin as in the case of L cells in Fig. 1. The data represent the average of duplicate determinations (SD < 30%). The data on L cells in Fig. 1 are included for comparison.

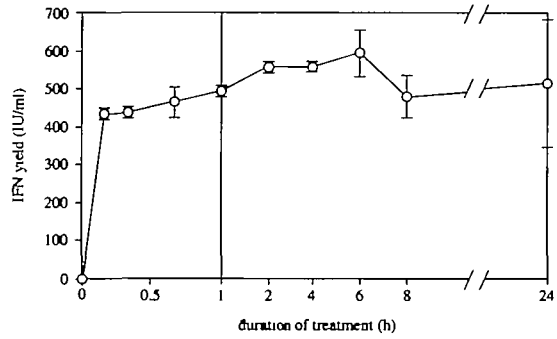

Fig. 2. **Effects of treatment duration of poly I:poly C-Lipofectin complexes on IFN induction in L cells.** L cells (5×10^4) grown in a 24-well tissue culture plate were treated with poly I:poly C (0.1 μg/ml)-Lipofectin (8 μg/ml) complex for times from 10 min to 24 h. After the treatment, the cells were washed and incubated with fresh growth medium until 24 h after the complex addition. The culture fluids were then subjected to IFN bioassay.

fectin were examined in several other cell lines. As shown in Table II, this complex worked as an efficient IFN inducer in all the cell lines examined. The optimal doses of poly I:poly C ranged from 0.1 to 3 μg/ml in all cells except Meth A, where it was 10 μg/ml. Although it is unclear why the IFN yield differed from one cell line to another, the major portion of the IFN activity was generally of β-type, as in L cells (data not shown).

Cytotoxicity of Poly I:Poly C-Cationic Reagents Complexes—We analyzed the cytotoxicity of 1 μg/ml poly I:poly C complexed with 8 μg/ml Lipofectin or 8 μg/ml LipofectACE in L cells by MTT assay. The results showed that the treatment with either complex brought about no significant cell damage (data not shown).

Duration of Treatment with Poly I:Poly C-Lipofectin Complexes for Maximal IFN Induction—As shown in Fig. 2, the maximal IFN production was reached after a short treatment (10 min) with poly I:poly C (0.1 μg/ml)-Lipofectin (8 μg/ml) complexes in L cells; longer treatment did not significantly increase the IFN production. It seems that poly I:poly C complexed with Lipofectin binds rapidly to the cell surface and enters the cells, where it induces IFN production.

Intracellular Uptake of FITC-Poly I:Poly C—To verify the incorporation of the complexes, we examined poly I:poly C uptake of L cells using FITC-labeled poly I:poly C. Figure 3 demonstrates that FITC-labeled poly I:poly C (3 μg/ml) complexed with Lipofectin (8 μg/ml) was detected inside L cells at 6 h after the treatment, whereas FITC-poly I:poly C alone was not. It is thus supposed that the complexing of negatively charged poly I:poly C with positively charged Lipofectin facilitates its attachment to and efficient uptake by the cells.

Time Course of IFN Induction by Poly I:Poly C-Lipofectin Complexes—Finally we examined the time course of IFN induction of L cells stimulated by poly I:poly C (0.1 μg/ml)-Lipofectin (8 μg/ml) complexes in terms of the IFN activity and IFN-β mRNA. The cumulative IFN activity became detectable at 3 h after the treatment and gradually increased to reach a plateau at 24 h. Consistent with this, the IFN production maintained a peak at 6 to 24 h post-treatment and thereafter decreased drastically, ceasing at 48 h (Fig. 4A). Also, on Northern blot analysis, that IFN-β mRNA expression became detectable at 6 h, reached a peak at 10 to 12 h, then diminished at 24 h, while the β-actin mRNA level was apparently constant (Fig. 4B).

DISCUSSION

Cationic liposomes have been devised for efficient gene delivery (*32, 33*). These cationic reagents form complexes

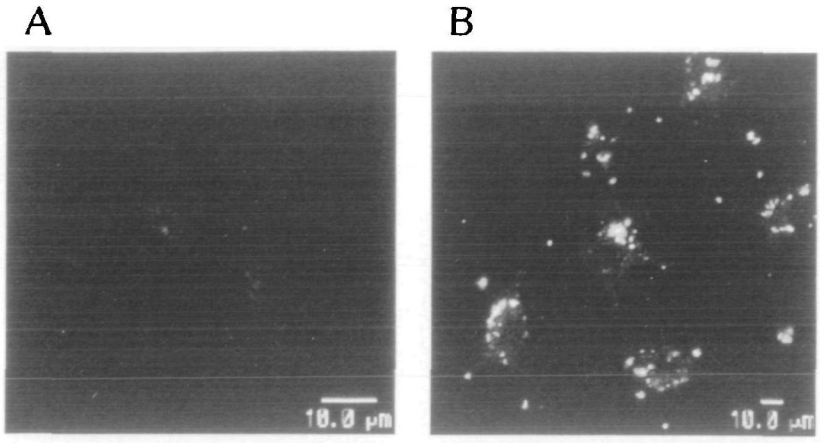

Fig. 3. **Intracellular uptake of FITC-labeled poly I:poly C in L cells.** FITC-labeled poly I:poly C (3 μg/ml) alone (A) or its complex with 8 μg/ml Lipofectin (B) was added to L cells (1×10^5/plate) on a glass-bottomed plate. The panels are the scanned data of the cells treated for 6 h.

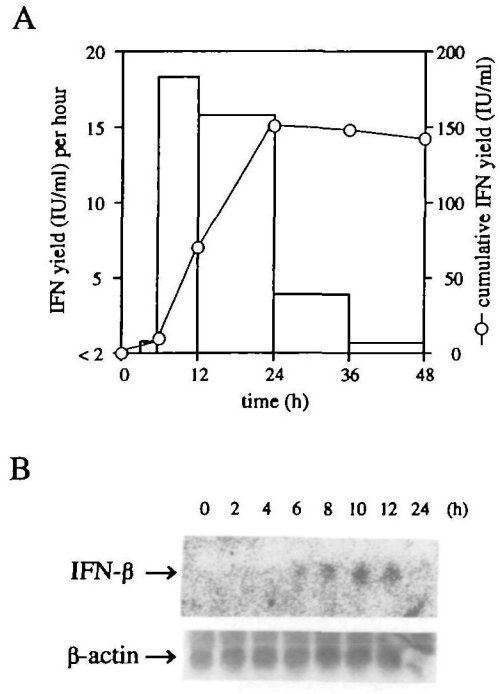

Fig. 4. **Time-course of IFN production and IFN-β mRNA expression in L cells treated with poly I:poly C-Lipofectin complexes.** In A, L cells (5×10^4/well) in 24-well tissue culture plates were treated with a mixture of poly I:poly C (0.1 μg/ml) and Lipofectin (8 μg/ml) for 3 h. IFN yields per hour (open bar) and cumulative IFN yields (solid line, open circle) in the medium are shown. B shows the Northern analysis of mouse IFN-β gene and β-actin gene transcripts at the indicated times after stimulation, performed as described in "MATERIALS AND METHODS." Each lane was loaded with 20 μg total cellular RNA.

with negatively charged DNA, thereby reducing the electrostatic repulsion of negatively charged cell surfaces and promoting the efficient incorporation and expression of the DNA by the cells. A similar approach was examined here for enhancing the potency of an IFN-inducer, poly I:poly C, which is also negatively charged. Our results showed that a small amount of poly I:poly C complexed with certain cationic liposomes was able to induce a relatively high titer of IFN (about 500-4,000 IU/ml/day) in several mouse cell lines. Among the cationic reagents examined in this work, Lipofectin and LipofectACE were the most potent in L cells: the optimal doses were 0.1 μg/ml poly I:poly C for 2-10 μg/ml Lipofectin, and 0.03-0.1 μg/ml poly I:poly C for 2-16 μg/ml LipofectACE. The IFN yield was approximately 550-600 IU/ml/day in both cases. These doses of poly I:poly C are two to three orders lower than those usually used for IFN-induction. Similar low optimal doses were confirmed in other mouse cell lines (Table II) and also some human cell lines (data not shown). The IFN-induction potency of these complexes with cationic reagents appears to be attributable to the efficiency of cellular interaction and subsequent intracellular delivery of poly I:poly C, which would depend on the physicochemical properties of the complexes, such as size and electric charge. Although data on these properties are not available, the net charges of the liposomes, which increase in the order of LipofectACE, Lipofectin, and LipofectAMINE, may be reflected, at least in part, in the induction of IFN production. The IFN activity induced with the poly I:poly C-Lipofectin complexes consisted predominantly of the β-type (>95%) with a small amount of the α-type (<5%) in all the cell lines examined in this work.

In previous reports, pretreatment of cells with IFN, so-called priming, enhanced IFN production by 20-100 times compared with unprimed cells (*9*). In our experiments with L cells, however, priming did not significantly enhance IFN induction by poly I:poly C-Lipofectin complexes (data not shown), although the reason for this is unclear.

The augmentation of IFN induction by poly I:poly C is due to increased intracellular incorporation of poly I:poly C, as revealed by a confocal microscopical observation using FITC-poly I:poly C. The interaction of poly I:poly C-Lipofectin complexes with cells was so rapid that incubation for 10 min was sufficient for maximal IFN-induction. In gene delivery, preincubation of plasmid DNA with cationic liposomes such as Lipofectin before cell treatment is important for efficient gene transfection, and preincubation with serum tends to prevent efficient transfection. In contrast with this, poly I:poly C does not require preincubation with Lipofectin or LipofectACE for efficient IFN-induction (data not shown). Furthermore, serum-containing medium did not significantly reduce the efficient IFN-induction (data not shown), provided that the complexes were prepared with serum-free medium.

The time course of the IFN production of L cells treated with poly I:poly C-Lipofectin complexes was consistent with the previous report (7); the IFN yield became detectable in the culture fluids at 3 h post-treatment, and the cumulative production steeply increased to reach a plateau at 24 h. The production was transient and largely ceased at 36-48 h. This transient kinetics was also confirmed at the transcription level (Fig. 4B). The cells were thereafter not refractory to IFN-induction, but capable of responding to retreatment of poly I:poly C-Lipofectin complexes (data not shown).

Several reports have shown the effects of poly I:poly C and $r(I)_n$:$r(C_{12},U)_n$ (Ampligen) in model experiments of cancer therapy; cancer progression was inhibited to some extent (*34, 35*). However, the doses required are so high that physiological conditions deteriorate: for instance, diminution of hematopoietic stem cells, embryo-toxicity, and destruction of endothelial cells (*10, 36*). Our procedures will provide one approach to surmount these problems: lower doses will be less toxic. Actually, our poly I:poly C-cationic liposome complexes apparently caused no cell damage (data not shown). Thus our procedures shed further light on poly I:poly C for clinical applications; the repeatability of the complex treatment may promise further efficacy.

REFERENCES

1. DeMaeyer, E. and DeMaeyer-Guignard, J. (1988) *Interferons and Other Regulatory Cytokines*, p. 448, John Wiley, New York
2. Gresser, I. and Tovey, M.G. (1978) Antitumor effects of interferon. *Biochim. Biophys. Acta* **516**, 231-247
3. Watanabe, Y. (1994) Tumorigenicity and immunogenicity of IFN-γ producing tumor cells in *Cytokine-Induced Tumor Immunogenicity* (Forni, G., Foa, R., Santoni, A., and Frati, L., eds.) pp. 233-247, Academic Press, London
4. Abdel-Wahab, Z., Dar, M., Osanto, S., Fong, T., Vervaert, C.E., Hester, D., Jolly, D., and Seigler, H.F. (1997) Eradication of melanoma pulmonary metastases by immunotherapy with tumor cells engineered to secrete interleukin-2 or gamma interferon. *Cancer Gene Ther.* **4**, 33-41
5. Colby, C. and Morgan, M.J. (1971) Interferon induction and action. *Annu. Rev. Microbiol.* **25**, 333-360
6. Kleinschmidt, W.J. (1972) Biochemistry of interferon and its inducers. *Annu. Rev. Biochem.* **41**, 517-542
7. Kelley, K.A. and Pitha, P.M. (1985) Differential effect poly rI·rC and Newcastle disease virus on the expression of interferon and cellular genes in mouse cells. *Virology* **147**, 382-393
8. Stewart, II, W.E., Gosser, L.B., and Lockart, R.Z., Jr. (1971) Priming: a nonantiviral function of interferon. *J. Virol.* **7**, 792-801
9. Dron, M., Lacasa, M., and Tovey, M.G. (1990) Priming affects the activity of a specific region of the promoter of the human beta interferon gene. *Mol. Cell. Biol.* **10**, 854-858
10. Rodgers, R. and Merigan, T.C. (1972) Interferon and its inducers: antiviral and other effects. *Crit. Rev. Clin. Lab. Sci.* **3**, 131-162
11. Carter, W.A. and DeClercq, E. (1974) Viral infection and host defense. *Science* **186**, 1172-1178
12. Harary, R., Gonsky, R., Itamar, D., and Kaempfer, R. (1990) Relief of cytotoxicity and enhancement of interferon inducer activity of double-stranded RNA by eukaryotic initiation factor 2. *Virology* **174**, 494-503
13. Stewart, II, W.E., DeClercq, E., Billiau, A., Desmyter, J., and DeSomer, P. (1972) Increased susceptibility of cells treated with interferon to the toxicity of polyriboinosinic·polyribocytidylic acid. *Proc. Nat. Acad. Sci. USA* **7**, 1851-1854
14. Carter, W.A., Pitha, P.M., Marshall, L.W., Tazawa, S., and Ts'o, P.O.P. (1972) Structural requirements of the $r(I)_n$·$r(C)_n$ complex for induction of human interferon. *J. Mol. Biol.* **70**, 567-587
15. Ts'o, P.O.P., Alderfer, J.L., and Levy, J. (1976) An integrated and comparative study of the antiviral effects and other biological properties of the polyinosinic acid-polycytidylic acid and its mismatched analogues. *Mol. Pharmacol.* **12**, 299-312
16. Hubbell, H.R. (1986) Synergistic antiproliferative effect of human interferons in combination with mismatched double-stranded RNA on human tumor cells. *Int. J. Cancer* **37**, 359-365
17. Mayhew, E. and Papahadjopuolos, D. (1977) Cellular uptake and protection against virus infection by polyinosinic-polycytidilic acid entrapped within phospholipid vesicles. *Mol. Pharmacol.* **13**, 488-495
18. Straub, S.X., Garry, R.F., and Magee, W.E. (1974) Interferon induction by poly(I):poly(C) enclosed in phospholipid particles. *Infect. Immun.* **10**, 783-792
19. Magee, W.E. (1978) Potentiation of interferon production and stimulation of lympocytes by polyribonucleotides entrapped in liposomes. *Ann. N.Y. Acad. Sci.* **308**, 308-324
20. Milhaud, P.G., Compagnon, B., Bienvenüe, A., and Philippot, J.R. (1992) Interferon production of L929 and HeLa cells enhanced by polyriboinosinic acid-polyribocytidylic acid pH-sensitive liposomes. *Bioconjugate Chem.* **3**, 402-407
21. Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M., Northrop, J.P., Ringold, G.M., and Danielsen, M. (1987) Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. USA* **84**, 7413-7417
22. Yuspa, S.H., Hawley-Nelson, P., Koehler, B., and Stanley, J.R. (1980) A survey of transformation markers in differentiating epidermal cell lines in culture. *Cancer Res.* **40**, 4694-4703
23. Maruguchi, Y., Toda, K.I., Fujii, K., Imamura, S., and Watanabe, Y. (1991) Survival period of tumor-bearing mice is prolonged after the interferon-γ-producing gene transfer. *Cancer Lett.* **60**, 41-49
24. Gao, X. and Huang, L. (1991) A novel cationic liposome reagent for efficient transfection of mammalian cells. *Biochem. Biophys. Res. Commun.* **179**, 280-285
25. Watanabe, Y. and Kawade, Y. (1987) Induction, production and purification of natural mouse IFN-α and IFN-β in *Lymphokines and Interferons: a Practical Approach* (Clemens, M.J., Morris, A.G., and Gearing, J. eds.) pp. 1-14, IRL, Oxford
26. Watanabe, Y., Kuribayashi, K., Miyatake, S., Nishihara, K., Nakayama, E., Taniyama, T., and Sakata, T. (1989) Exogenous expression of mouse interferon γ cDNA in mouse neuroblastoma C 1300 cells results in reduced tumorigenicity by augmented anti-tumor immunity. *Proc. Natl. Acad. Sci. USA* **86**, 9456-9760
27. Kawade, Y. and Watanabe, Y. (1987) Characterization of rat monoclonal antibodies to mouse interferon-α and -β in *The Biology of the Interferon System 1986* (Cantell, K. and Schellekens, H., eds.) pp. 197-201, Martinus Nijhoff, Dordrecht
28. Mosmann, T. (1983) Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55-63
29. Watanabe, Y. and Sakata, T. (1988) Highly efficient action of autocrine mouse interferon-γ expressed via a retroviral vector. *Eur. J. Immunol.* **18**, 1627-1630
30. Kawabata, K., Kondo, M., Watanabe, Y., Takakura, Y., and Hashida, M. (1997) Non-polarized secretion of mouse interferon-β from gene-transferred human intestinal Caco-2 cells. *Pharm. Res.* **14**, 483-485
31. Minty, A.J., Alonso, S., Guenet, J.L., and Buckingham, M.E. (1983) Number and organization of actin related sequences in the mouse genome. *J. Mol. Biol.* **167**, 77-101
32. Ledley, F.D. (1995) Nonviral gene therapy: the promise of genes as pharmaceutical products. *Hum. Gene Ther.* **6**, 1129-1144
33. Gao, X. and Huang, L. (1995) Cationic liposome-mediated gene transfer. *Gene Ther.* **2**, 710-722
34. Droller, M.J. and Gomolka, D. (1982) Inhibition of tumor growth in association with modification of in vivo immune response by indomethacin and polyinosinic:polycytidylic acid. *Cancer Res.* **42**, 5038-5045
35. Hubbell, H.R., Kvalnes-Krick, K., Carter, W.A., and Strayer, D.R. (1985) Antiproliferative and immunomodulatory actions of β-interferon and double-stranded RNA, individually and in combination, on human bladder tumor xenografts in nude mice. *Cancer Res.* **45**, 2481-2486
36. Carter, W.A. and DeClercq, E. (1974) Viral infection and host defense. *Science* **186**, 1172-1178