Antiviral Research, 15 (1991) 241–254

ADONIS 016635429100006R

AVR 00474

Varying role of alpha/beta interferon in the antiviral efficacy of synthetic immunomodulators against Semliki Forest virus infection

Page S. Morahan[1], Angelo Pinto[1], Deneen Stewart[1], Donna M. Murasko[1] and Margo A. Brinton[2,*]

[1]*Department of Microbiology and Immunology, The Medical College of Pennsylvania, Philadelphia, Pennsylvania, U.S.A. and* [2]*Wistar Institute, Philadelphia, Pennsylvania, U.S.A.*

(Received 17 September 1990; revision accepted 6 December 1990)

Summary

The question of whether interferon alpha/beta is the common mechanism of antiviral action of synthetic immunomodulators was investigated in B6C3F1 mice infected with Semliki Forest virus. Mice were treated with various concentrations of normal sheep serum or potent anti-alpha/beta interferon antiserum, inoculated with the immunomodulators, and infected 24 hours later with virus. Three patterns emerged. The antiviral action of the pyrimidinone (ABMP) and the oral interferon inducer (CL246,738) appeared to be mediated primarily by interferon alpha/beta; their protective ability was almost completely abrogated by treatment with low levels of anti-alpha/beta interferon antiserum. The antiviral action of two other immunomodulators, a mismatched polyribonucleotide (Ampligen) and a polyanionic copolymer (MVE-2) at least partially involved interferon. Activity of these compounds was reduced, but not consistently eliminated by treatments with high doses of antiserum. The antiviral activity of another polyribonucleotide, polyriboinosinic-cytidylic acid complexed with lysine carboxymethylcellulose (poly ICLC), was not affected by treatment with even the highest amount of antiserum (two injections of 100000 neutralizing units each). Almost complete protection by poly ICLC was observed despite the fact that this high concentration of antiserum, when given alone, caused a decrease in natural resistance to Semliki Forest virus infection. Taken together, these results indicate that induction of interferon alpha/beta does

**Present address:* Dep. of Biology, Georgia State University, Atlanta, GA 30303, U.S.A.

Correspondence to: Page Morahan, Dept. of Microbiology and Immunology, The Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129, U.S.A.

not appear to be the major common mechanism of antiviral activity among these diverse synthetic immunomodulators.

Interferon; MVE-2; Ampligen; CL246,738; Poly ICLC; ABMP; Semliki Forest virus; Anti-interferon serum

Introduction

It is well established that treatment with interferons (IFN) or various biological and synthetic immunomodulators provide broad-spectrum, nonspecific protection against virus infections in vivo (Finter, 1973; Pinto et al., 1990a; Breinig and Morahan, 1980). The question, thus, has been raised whether IFN is the common mechanism for antiviral resistance induced by various immunomodulators.

Correlative kinetic data have not been able to definitively prove whether immunomodulator-induced IFN has a cause-and-effect relationship with subsequent decreases in virus titers and increased survival of animals, or whether immunomodulators might induce a low level of protective IFN that is localized at the critical sites of infection and is undetectable in the circulation. IFN titers have been demonstrated to increase after inoculation of certain immunomodulators (Finter, 1973). However, kinetic studies have also shown the converse, that some very effective immunomodulators induce no detectable or very low levels of circulating IFN, suggesting that antiviral activity may be independent of IFN (Giron et al., 1980; Morahan, 1980; Morahan et al., 1972).

Another experimental approach has been to assess the antiviral effect of immunomodulators in animals that are selectively depleted of effector cells which are important in resistance. Depletion of natural killer (NK) cells has shown that normal levels of NK cells are not required for the antiviral activity of 7-thia-8-oxoguanosine against Semliki Forest virus (SFV) (Smee et al., 1990). Depletion in mice of peritoneal macrophages (Morahan et al., 1977), spleen and liver macrophages (Pinto et al., 1990b), T lymphocytes (Morahan and McCord, 1975), or circulating monocytes, granulocytes and NK cells (Morahan et al., 1986) has revealed that normal levels of these nonspecific effector cells are not required for the antiviral activity of pyran/MVE-2 against herpes simplex virus. Data such as these emphasize the usefulness of immunomodulators in immunosuppressed hosts (Ikeda et al., 1987). The results, however, cannot distinguish whether the antiviral activity results from small numbers of cells remaining in immunosuppressed hosts that are sufficient for immunomodulatory activity, or whether the drugs act through cells other than those affected. In either case, the antiviral action could be mediated directly through cells, or indirectly through humoral antiviral factors such as IFN or other cytokines.

There have been surprisingly few studies directly addressing this potential role of IFN. Treatment of mice with antibody that neutralizes IFN (anti-IFN) can clearly decrease natural resistance to viruses, but this does not necessarily mean that

IFN is involved in the antiviral activity of immunomodulators. Early studies, using antibody prepared against relatively impure IFN, showed no major effect on pyran-induced antiviral resistance (Giron et al., 1980; Morahan, 1980) or polyribonucleotide-induced antitumor resistance (Gresser et al., 1978); it was concluded that IFN was not required for the protective ability of these immunomodulators. The recent use of anti-IFN antibody preparations that are more potent and specific has revealed that IFN is required for NK cell activation by pyrimidinones (Lotzova et al., 1986). It has also recently been reported that the antiviral activity of the oral IFN inducer, CL246,738, against SFV infection is mediated by the independent induction of alpha and beta IFN (Sarzotti et al., 1989), and that IFN alpha is required for the antiviral activity of 7-thia-8-oxoguanosine against SFV infection (Smee et al., 1990). The present study was undertaken to determine whether IFN is a common mediator (either directly or indirectly through activation of effector cells) for immunomodulators that exhibit broad-spectrum nonspecific antiviral resistance; this is a critical issue for the development of immunotherapy for viral infections.

Materials and Methods

Mice

Virus-free, barrier-raised, 6-week old female B6C3F1 mice (Taconic Farms, Germantown, NY) were housed in autoclaved microisolator cages (MCP) or isolator chambers (Wistar). To ensure that no intercurrent viral infections had occurred (Dempsey et al., 1988), mouse sera were periodically tested for seroconversion to mouse hepatitis virus and Sendai virus (Biocon Labs, Rockville, MD).

Semliki Forest virus (SFV)

A mouse brain pool of SFV, strain L10, was prepared in newborn CD-1 mice as we have previously described (Pinto et al., 1988). SFV was titrated by plaque forming units (PFU) on BHK21 cells; the titer was about 6.8×10^7 PFU/ml. Mice were injected with 3–10 LD_{50} doses of virus.

Immunomodulators

All immunomodulators were administered prophylactically, 24 h before i.p. injection of SFV. All drugs were administered i.p. except for CL246,738, which was administered by oral gavage. The polyanionic maleic anhydride divinyl ether copolymer (MVE-2) (Hercules, Inc., Wilmington, DE) was dissolved in phosphate buffered saline to a final inoculation dose of 50 mg/kg. Ampligen, a mismatched polyriboinosinic-cytidylic acid polyanionic polynucleotide (USAMRIID, Frederick, MD) was dissolved in physiological saline to a final inoculation dose of 4 mg/kg, heated at 67°C for 16 h and then at 37°C for 1 h prior to injection. The poly-

riboinosinic-cytidylic acid polynucleotide complexed with lysine carboxymethylcellulase (poly ICLC, courtesy of Dr. Hilton Levy, National Cancer Institute, Bethesda, MD) was diluted in physiological saline to a final inoculation dose of 1 mg/kg. 3,6-Bis(2-piperidinoethoxy)acridine (CL246,738, courtesy of Dr. Fred Durr, Lederle, Pearl River, NY) was prepared to an injection dose of 50 mg/kg in distilled water. The pyrimidinone, 2-NH_2-5-Br-6-methyl-4-[^{3}H]pyrimidinone (ABMP, courtesy of Dr. Harold Renis, Upjohn Co., Kalamazoo, MI) was suspended in 1% carboxymethylcellulose to a final injection dose of 200 mg/kg. To ensure a uniform suspension, it was vortexed vigorously just prior to injection. Recombinant human alpha-IFN A/D (rHu IFN-α A/D, courtesy of Drs. Michael Brunda and Peter Sorter, Hoffman-LaRoche, Nutley, NJ) was diluted in phosphate buffered saline containing 0.2% bovine serum albumin to an injection dose of 4000–10000 IU/mouse. This IFN was retitered the week prior to assay, in order to ensure that the appropriate dose was administered.

Anti-alpha/beta interferon antiserum (anti-IFN)

The antiserum used for these studies was prepared in sheep by immunizations with highly purified mouse IFN that was induced in L929 cells by Newcastle disease virus. This IFN is composed of approximately 70% IFN-β and 30% IFN-α. The antiserum was then exhaustively adsorbed with normal cell and viral products (Dalton and Paucker, 1981). The final preparation of antiserum had a neutralizing titer of 2×10^6 units/ml. This adsorbed anti-IFN antibody preparation has been successfully used in previous studies investigating the role of IFN in NK activity (Korngold et al., 1983), natural resistance to Friend leukemia virus (Blank and Murasko, 1981) and poly IC-induced inhibition of antigen-specific macrophage-dependent T cell proliferation (Blank et al., 1985). For most experiments, the anti-IFN was inoculated i.p. 4 h prior to administration of the immunomodulator to allow antisera dissemination for facilitation of the interaction between IFN induced by the drug and the anti-IFN antibody. As a control, mice were injected with normal sheep serum at a similar protein concentration.

Interferon assays

Mouse plasma, serum, or peritoneal lavage fluids were obtained from mice after various treatments. These were diluted serially, and assayed for their ability to protect L929 cells from cytopathic effect produced by infection with encephalomyocarditis virus, using a modification of the microplate method of Havell and Vilcek (1972). In every assay, an internal IFN-α/β standard was assayed simultaneously, and the titers were corrected against the NIH mouse α/β reference standard. The assay is sufficiently sensitive to measure consistently 1 IU/ml, and sometimes as low as 0.2 IU/ml.

Antiviral protection studies

For antiviral protection experiments, there were generally 10 mice in each experimental group and 15 mice in each placebo control group. A small LD_{50} dose response assay was included in each experiment to ensure that the appropriate LD_{50} dose was achieved. Virus-infected mice were monitored daily for signs of clinical illness and for mortality. At the end of the test period (usually 14 days), the percent mortality and median survival times of all control and experimental groups were calculated.

Statistical analysis

Statistically significant differences ($P < 0.05$) in percent mortality were determined with an Apple IIe microcomputer using the Chi square test included in the Applestat statistical package. The median survival time was calculated and the survival distribution data analyzed using the Lee-Desu method of group comparison included in the SPSSX statistical package on the VAX. This procedure allows the most appropriate analysis of survival data with censored observations (i.e. mice still alive at the end of the observation period) (Lee and Desu, 1972).

Results

Effects of anti-IFNα/β on IFN in vitro and in vivo

The ability of the anti-IFN antibody to neutralize mouse alpha/beta IFN in vitro was confirmed (Table 1). The anti-IFN antibody very efficiently neutralized both the NDV-induced L929 cell α/β IFN and the poly ICLC-induced plasma IFN. The level of neutralization achieved demonstrated the accuracy of the titers and the specificity of the antiserum. As expected, the anti-mouse IFN antibody did not efficiently neutralize recombinant human alpha IFN. This recombinant molecule has the biological activity of mouse IFN, but maintains the antigenicity of the two parent human A and D IFN alpha molecules.

TABLE 1

In vitro neutralization of interferon with anti-interferon serum

Interferon	Interferon titer (IU/ml) after addition of[a]		
	NSS	5000 U antibody	50 U antibody
rHuIFN-α A/D	346	346	364
MuIFN-α/β	346	<7	230
Poly ICLC plasma[b]	86	<7	43

[a]One concentration of interferon was mixed with either normal sheep serum (NSS) or anti-interferon sheep serum diluted to contain 5000 or 50 neutralizing U/ml.

[b]Plasma was obtained from mice from the retro-orbital sinus 6 h after administration of 1 mg/kg of poly ICLC. This plasma was diluted to obtain about 100 IU of IFN/ml.

TABLE 2

In vivo effects of anti-interferon serum on plasma interferon levels induced by administration of poly ICLC

Treatment	Geometric mean titers of serum interferon (IU/ml at				
	3 h	6 h	12 h	24 h	48 h
NSS	1871	4641	332	263	<2
Anti-IFN	<2	297	<2	9	<2

B6C3F1 female mice were inoculated i.p. with normal sheep serum or 100000 neutralizing units of anti-interferon serum 1 h prior to i.p. injection of poly ICLC (1 mg/kg), and were reinjected with normal sheep serum or antibody 6 h after injection of poly ICLC. Most groups contained 3 mice. The IFN level in mice receiving PBS alone was <2 IU/ml.

Of the immunomodulators, only poly ICLC and Ampligen induced substantial levels of plasma IFN in the B6C3F1 mice. After injection of poly ICLC, there was a peak of 4641 IU/ml at 6 h, and a decline to 263 IU/ml at 20–24 h (Table 2). The mismatched polyribonucleotide, Ampligen, induced 135 IU/ml at 3–4 h, declining to <45 IU by 6 h. The other immunomodulators tested induced no detectable or minimal levels (50 IU or less) of plasma IFN at all times that were tested including the times expected for peaks of IFN (3–4, 6, and 24 h). Since the virus was injected i.p. and the initial stages of infection occurred in the peritoneum, IFN titers induced by the immunodulators in the peritoneal fluid were also measured. IFN titers were detectable in peritoneal fluid only after administration of poly ICLC or rHuIFN-α A/D. Poly ICLC induced 22 and 17 IU/ml, while rHuIFN-α A/D showed 65 and <45 IU/ml respectively at 6 and 24 h after drug administration.

Based on these data and the in vitro neutralizing activity of the anti-IFN antibody, it was estimated that administration of 10000 units of antibody should be sufficient to neutralize the poly ICLC-induced IFN, while much less should be effective against the other immunomodulators. The in vivo neutralizing efficiency of the anti-IFN antibody was measured directly by inoculating mice with normal sheep serum or anti-IFN antibody, then administering poly ICLC and measuring plasma IFN levels at 4 or 20 h. Administration of 50000 neutralizing units did not significantly change the 3–4 h titer of IFN, while administration of 100000 units decreased the titer by about 50% (data not shown). Higher doses of anti-IFN antibody did neutralize poly ICLC-induced antiviral activity. Two injections of 100000 units were administered 1 h prior to and 6 h after inoculation of poly ICLC (Table 2). Plasma levels of IFN were reduced to 10 IU/ml or less at all times assayed except for 6 h after poly ICLC when the titer was reduced ($P < 0.05$) from 4691 to 297 IU/ml.

When IFN levels were measured at 6, 12, 18, 24 and 48 h after SFV infection, no detectable levels of IFN were found in either the plasma or peritoneal fluids. There was also no significant change in LD_{50} when mice were treated 24 h prior to infection with normal sheep serum or one dose of anti-IFN antibody ranging from 2000 to 100000 neutralizing units, although the mice generally died a few hours earlier (Tables 3 and 4). Therefore, antiviral efficacy could be tested reliably at one chal-

TABLE 3

Effect of single administration of 2000 or 10000 neutralizing units of anti-interferon serum and immunomodulators on Semliki forest virus infection[a]

Drug	Dose		Mortality		Survival
	Drug (mg/kg)	Anti-IFN antibody	Dead/total	(%)	MST
Controls					
Normal sheep serum (NSS)	–	–	8/10	(80%)	5.8
Anti-interferon (anti-IFN)	–	10000	15/15	(100%)	4.8
Anti-interferon (anti-IFN)	–	2000	15/15	(100%)	5.2
Experimental groups					
Ampligen	4	–	1/10	(10%)*	>14.0*
	4	2000	0/10	(0%)*	>14.0*
MVE-2	50	–	0/10	(0%)*	>14.0*
	50	2000	0/10	(0%)*	>14.0*
Poly ICLC	1	–	0/10	(0%)*	>14.0*
	1	10000	0/10	(0%)*	>14.0*
ABMP	200	–	4/10	(40%)	>14.0
	200	2000	8/9	(89%)	5.1

[a]B6C3F1 female mice, aged 5 weeks, were treated i.p. with the indicated dose of anti-IFN serum or NSS 4 h prior to i.p. injection of the immunomodulator, and infected 24 h later with 4.5 PFU (4 LD_{50} doses) of Semliki Forest virus.

*Statistically significant ($P<0.05$) as compared with the corresponding placebo group.

lenge level of virus, whether mice were treated with normal sheep serum or the anti-IFN antibody.

Effects of single administration of anti-IFN antibody on antiviral protection against SFV infection

When mice were treated with 2000 (against ABMP, MVE-2 or Ampligen) or 10000 (against poly ICLC) neutralizing units of anti-IFN antibody 4 h prior to inoculation of immunomodulators, the antiviral efficacy of ABMP was markedly reduced, with no effect on the activity of the other immunomodulators (Table 3). When the dose of antibody was increased to 50000 neutralizing units, the antiviral activity of Ampligen was significantly reduced, while the activity of CL246,738 remained unchanged (Table 4). Increasing the dose of antibody to 100000 neutralizing units resulted in abrogation of the antiviral activity of CL246,738, MVE-2, and Ampligen (Table 4). Treatment with 50000 or 100000 neutralizing units of antibody did not affect the protective effect of rHuIFN-α A/D (ca. 10000 IU, data

TABLE 4

Effect of single administration of 50000 or 100000 neutralizing units of anti-interferon antibody and immunomodulators on Semliki Forest virus infection[a]

		Treatment with 50000 units			Treatment with 100000 units		
		Mortality		Survival	Mortality		Survival
	Anti-IFN serum	Dead/ total	(%)	MST	Dead/ total	(%)	MST
Normal sheep serum (NSS)	–	9/10	(90%)	6.6	8/10	(80%)	5.8
Anti-interferon (Anti-IFN)	+	10/10	(100%)	5.1	8/10	(80%)	4.8
Poly ICLC	–	0/10	(0%)*	>13.0*	1/6	(17%)*	>13.0*
	+	3/10	(30%)*	>13.0*	2/10	(20%)*	>13.0*
Ampligen	–	0/10	(0%)*	>13.0*	0/10	(0%)*	>13.0*
	+	5/10	(50%)	6.0*	5/5	(100%)	5.2
CL246,736	–	2/10	(20%)*	>13.0*	2/10	(20%)*	>13.0*
	+	3/10	(30%)*	>13.0*	10/10	(100%)	4.6
MVE-2	–	ND	ND	ND	2/10	(20%)*	>13.0*
	+	ND	ND	ND	9/10	(90%)	5.5

[a]B6C3F1 female mice, aged 5 weeks, were treated with anti-IFN or NSS 4 h before i.p. injection of immunomodulators, and infected 24 h later with 4.5 PFU (5 LD_{50} doses) of Semliki Forest virus. The drugs were administered at the doses shown in Table 3. ND = not done.
*Statistically significant ($P<0.05$) as compared with the corresponding placebo group.

not shown), as expected from the data showing lack of neutralization of this human IFN (Table 1).

Effects of two administrations of anti-IFN antibody on antiviral protection against SFV infection

Since two inoculations of 100000 neutralizing units of anti-IFN antibody were needed to significantly reduce circulating levels of poly ICLC-induced IFN, the amount of anti-IFN antibody administered was increased. Two doses of 100000 units were administered, one shortly before administration of the immunomodulator, and a second dose a few hours later. After this anti-IFN antibody treatment, the antiviral activity of poly ICLC remained intact, the protective ability of Ampligen and MVE-2 were reduced substantially but not completely, while the protective ability of ABMP and CL246,738 was completely abrogated (Fig. 1). When mice were treated with two doses of anti-IFN antibody at 20000 neutralizing units, the antiviral activity of ABMP and CL246,738 was also lost completely, the antiviral activity of MVE-2 was reduced but there was still a significant increase in the median survival time, and the antiviral activity of Ampligen and poly ICLC was not affected (data not shown). The protective ability of poly ICLC was particularly striking, since two treatments with the 100000 neutralizing units of anti-IFN anti-

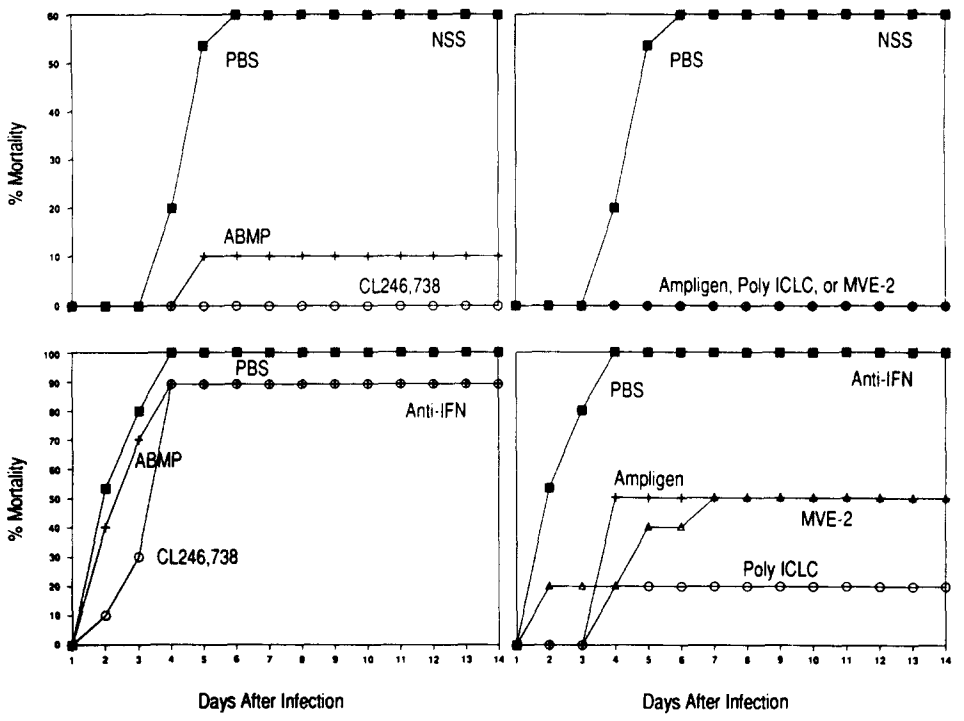

Fig. 1. Effect of two treatments each with 100000 neutralizing units of anti-IFN antibody or normal sheep serum on antiviral efficacy of various immunomodulators against SFV infection. B6C3F1 female mice were treated with the immunomodulators at the doses and routes indicated in Materials and Methods, 24 h prior to i.p. challenge with SFV. The first treatment of antibody was given i.p. 1 h prior to administration of all the immunomodulators. The second treatment of antibody was given i.p. 6 h after administration of Ampligen and poly ICLC, or 20 h after administration of MVE-2, ABMP and CL246,738.

body alone significantly reduced the natural resistance of the mice to SFV infection, as shown by a reduction in the median survival time ($P < 0.05$) as compared with mice treated with normal sheep serum (Fig. 1).

Discussion

These results are the first to demonstrate directly, using high titered anti-IFN antibody, that IFN plays varying roles in the antiviral resistance induced by prophylactic treatment with five synthetic immunomodulators of diverse chemical structure. There was no correlation between the ability of the immunomodulators to induce circulating IFN and the degree of antiviral activity against SFV infection, and the use of the anti-IFN serum indicated that IFN was probably not the only mechanism of antiviral activity of the immunomodulators.

IFN-α/β was clearly the major factor responsible for prophylactic antiviral

activity against SFV infection for two of the immunomodulators: the ABMP pyrimidinone, 2-NH_2-5-Br-6-methyl-4-[^{3}H]pyrimidinone (Li et al., 1989) and the CL246,738, a heterocyclic of the acridine class (Litton et al., 1990)). Both of these immunomodulators have been shown to induce IFN, activate NK cells and macrophages, induce broad spectrum antiviral, antibacterial and antitumor protection, as well as exhibit other immune enhancing properties (Li et al., 1985; Lotzova et al. 1983; Morahan et al., 1987; Skulnick et al., 1985; Wang et al., 1985, 1987). Our finding undetectable circulating levels of IFN after administration of ABMP and CL246,738 was unexpected; the results may reflect response differences in mouse strain or be due to differences in the route of administration. The present studies establish that, despite undetectable circulating IFN, IFN plays a central role in the antiviral action of ABMP and CL246,738, at least against SFV. The data with CL246,738 confirm the data of Sarazotti et al. (1989), who showed that the antiviral action of CL246,738 against SFV infection was primarily mediated by IFN. Whether the broad spectrum antiviral action of CL246,738 or ABMP is directly mediated by IFN, or indirectly through IFN activation of NK cells and macrophages or induction of other cytokines, remains to be established.

The antiviral efficacy of two other synthetic immunomodulators, the MVE-2 polyanionic co-polymer and Ampligen, a mismatched polyribonucleotide, appeared to at least partially involve IFN. Activity was significantly reduced by treatment with 100000 neutralizing units of anti-IFN antibody, but was not completely abrogated when mice were treated with the 200000 units of anti-IFN, even though this dose of anti-IFN significantly reduced natural resistance to SFV. Whether alpha or beta IFN is the active mediator involved in the antiviral effects of MVE-2 or the polyribonucleotide Ampligen remains to be determined. Recently alpha IFN has been shown to be essential for the antiviral protection against Banzi flavivirus that is induced by another polyribonucleotide, poly IC (Barnhart, Gangemi, Mayer and Ghaffar, personal communication). Antiviral activity was eliminated when mice were treated with antibody to alpha/beta IFN, but not by treatment with antibody to beta IFN. The present data, demonstrating no detectable IFN-inducing ability of MVE-2 in contrast with the efficient IFN induction by Ampligen, are similar to published reports (Morahan et al., 1972; Green et al., 1978). The lack of correlative kinetics between IFN induction and antiviral activity of pyran/MVE-2, coupled with the inability of low-titered anti-IFN serum to abrogate antiviral activity (Morahan, 1980; Giron et al., 1980), led to the hypothesis that the antiviral activity of this immunomodulator was independent of IFN. The present data with higher titered anti-IFN serum indicate that IFN appears to be responsible for some of the antiviral activity of pyran/MVE-2; additional cells and cytokines that are involved remain to be elucidated.

The mechanisms involved in the antiviral activity of Ampligen, in addition to IFN, also remain to be defined. In several systems, the antiviral and antiproliferative activity of Ampligen have been shown to synergize with IFN (Montefiori et al., 1989; Dick and Hubbell, 1987; Hubbell et al., 1987; Montefiori and Mitchell, 1987). These data suggest that some of the biologic actions of Ampligen are mediated by mechanisms independent of IFN induction.

Our observations, that the antiviral activity of poly ICLC was not altered after treatment with sufficient anti-IFN antibody to decrease natural resistance to SFV, were unexpected. The results suggest that the ability of poly ICLC to induce high levels of circulating IFN (Levy et al., 1975) may not play a prominent role in its antiviral efficacy against SFV infection. While levels of circulating IFN have been correlated with antiviral activity of poly ICLC, the exact antiviral mechanism has not yet been established (Crane et al., 1984; Kende et al., 1987; T'so et al., 1976; Lesnick and Derbyshire, 1988). For example, there is prolonged activation of NK cells after administration of poly ICLC, but similar activation occurs after administration of polyadenosinic-polyuridylic acid which is not an effective antiviral agent (Twilley et al., 1987; Morahan et al., 1972). A recent report noted that the cell growth-inhibitory properties of poly ICLC are associated with degradation of rRNA, and are independent of the action of IFN (Chapekar et al., 1988). To further exclude IFN as a mediator of the antiviral action of poly ICLC, it will be useful to establish the effects of treatment with anti-IFN serum by schedules that completely prevent induction of circulating IFN at all times after drug administration, and to determine the effects of antibody treatment on varying doses of poly ICLC. We also plan to determine the effect of anti-IFN antibody treatment on the protective ability of poly ICLC against Caraparu virus, a virus that we have shown is not sensitive to IFN alpha/beta, but is sensitive to poly ICLC (Pinto et al., 1990a).

Considering all the present data, there are several possibilities for the antiviral activity of poly ICLC, Ampligen and MVE-2:
(1) cells, that are not affected by the selective depletion method used, can function as antiviral effector cells because of the pleiotropic activating effects of the immunomodulator and the protective redundancy of the nonspecific immune system;
(2) small numbers of cells, remaining after depletion, are all that are required to start an amplifying cascade of cytokines such as IFN that directly exert antiviral activity;
(3) endogenous IFN may be induced at critical sites, and be consumed rapidly by local cells so that this IFN is not available to be neutralized by anti-IFN. Low levels of IFN may be particularly important against a viral infection such as SFV, which is very sensitive to IFN.

To determine which of these possibilities is operative, it will be useful to investigate drug efficacy in animals receiving combinations of selective cell and cytokine depletions. By documenting the effect of such selective immunosuppression on antiviral efficacy, together with characterizing the types of effector cells and cytokines that are present locally and early in infection, it should be possible to establish definitively what components of the nonspecific immune system are instrumental in immunomodulator-induced inhibition of viral pathogenesis.

Acknowledgements

We thank Dr. Edward Gracely for his assistance in statistical analysis of the data, Edith Gavin for technical assistance with animal experiments, and Michael

Goonewardene for assay of IFN activities. This work was supported by Army contract DMAD 17-86-6-6117 and NIH grant AI25751.

References

Blank, K.J. and Murasko, D.M. (1981) Effect of anti-interferon serum on Friend virus infection and FV-1 mediated resistance. J. Interferon Res. 1, 437–442.

Blank, K.J., McKernan, L.N. and Murasko, D.M. (1985) Poly I:C or IFN-α/β treatments inhibit macrophage induced T cell proliferation. J. Interferon Res. 5, 215–221.

Breinig, M.C. and Morahan, P.S. (1980) Interferon inducers: polyanions and others. In: D.A. Stringfellow (Ed.), Interferon and Interferon Inducers: Clinical Applications. pp. 239–261. Marcel Dekker, Inc., New York.

Chapekar, M.S., Knode, M.C. and Glazer, R.I. (1988) The epidermal growth factor and interferon independent effects of double stranded RNA in A431 cells. Mol. Pharmacol. 34, 461–465.

Crane, L., Milne, D., Sunstrum, J. and Lerner, M. (1984) Comparative activities of selected combinations of acyclovir, vidarabine, arabinosyl hypoxanthine, interferon and polyriboinosinic acid-polyribocytidylic acid complex against herpes simplex virus type 2 in tissue culture and intravaginally inoculated mice. Antimicrob. Agents Chemother. 26, 557–562.

Dalton, B.J. and Pauker, K. (1981) Preparation and absorption of antiserum against mouse L cell interferon. Methods Enzymol. 79, 582–584.

Dempsey, W.L, Smith, A.L. and Morahan, P.S. (1986) Effect of inapparent murine hepatitits virus infection on macrophages and host resistance. J. Leukoc. Biol. 39, 559–565.

Dick, R.S. and Hubbell, H.R. (1987) Sensitivities of human glioma cell lines to interferons and double stranded RNAs individually and in synergistic combinations. J. Neurooncol. 5, 331–337.

Finter, N.B. (1973) Antiviral effects in experimental animals. In: N.B. Finter (Ed.), Interferons and Interferon Inducers. pp. 295–301. North Holland Publishing Company, Amsterdam.

Giron, D.J., Liu, R.Y., Hemphill, F.E., Pindak, F.F. and Schmidt, J.P. (1980) Role of interferon in the antiviral state elicited by selected interferon inducers. Proc. Soc. Exp. Biol. Med. 163, 146–150.

Green, J.J., Alderfer, J.L., Tazawa, I., Tazawa, S., Ts'o, P.O.P., O'Malley, J.A. and Carter, W.A. (1978) Interferon induction and its dependence on the primary and secondary structure of poly(inosinic acid)-poly(cytidylic acid). Biochemistry 17, 4214–4220.

Gresser, I., Maury, G., Bandu, M., Tovey, M. and Maunorry, M.T. (1978) Role of endogenous interferon in the antitumor effect of poly I:C and statalon as demonstrated by the use of anti-mouse interferon serum. Int. J. Cancer 21, 72–77.

Havell, E. and Vilcek, J. (1972) Production of high titered interferon in cultures of human diploid cells. Antimicrob. Agents Chemother. 2, 476–484.

Hubbell, H.R., Peqignot, E.C., Todd, J., Reymond, L.C., Mayberry, S.D., Carter, W.A. and Strayer, D.R. (1987) Augmented antitumor effect of combined human natural interferon-alpha and mismatched double-stranded RNA treatment against a human malignant melanoma xenograft. J. Biol. Response Mod. 6, 525–532.

Ikeda, S., Sai, K., Nishimura, C. and Yamamoto, A. (1987) Antiherpes activity of the immunomodulator OK-432, a streptococcal preparation, in immunosuppressed mice. Antiviral Res. 10, 299–304.

Kende, M., Lupton, H.W., Rill, W.L., Gibbs, P., Levy, H.B. and Canonico, P.G. (1987) Ranking of prophylactic efficacy of poly (ICLC) against Rift valley fever infection in mice by incremental relative risk of death. Antimicrob. Agents Chemother. 31, 1194–1198.

Korngold, R., Blank, K.J. and Murasko, D.M. (1983) Effect of interferon on thoracic duct lymphocyte output: induction with either poly I: poly C or vaccinia virus. J. Immunol. 130, 2236–2240.

Lee, E. and Desu, M. (1972) A computer program for comparing *k* samples with right-censored data. Comput. Methods Programs Biomed. 12, 315–321.

Lesnick, C.E. and Derbyshire, J.B. (1988) Activation of natural killer cells in newborn piglets by interferon induction. Vet. Immunol. Immunopathol. 18, 109–112.

Levy, H.B., Baer, G., Baron, S., Buckler, C.E., Gibbs, C.G., Iadorola, M.J., London, W.T. and Rice, J.

(1975) A modified polyriboinosinic-polycytidylic acid complex that induces interferon in primates. J. Infect. Dis. 132, 434–439.

Li, L.H., Wallace, T.L., Richard, K.A. and Tracey, D.E. (1985) Mechanism of antitumor action of pyrimidinones in the treatment of B16 melanoma and P388 leukemia. Cancer Res. 45, 532–539.

Li, L.H., Wallace, T.L., Wierenga, W., Skulnick, H.I. and Dekoning, T.F. (1989) Antitumor activity of pyrimidinones, a class of small molecule biologic response modifiers. J. Biol. Response Mod. 6, 44–55.

Litton, G.J., Hong, R., Grossberg, S.E., Vechlekar, D., Goodavish, C.N. and Borden, E.C. (1990) Biological and clinical effects of the oral immunomodulator 3,6-bis(2-piperidinoethoxy)acridine trihydrochloride in patients with malignancy. J. Biol. Response Mod. 9, 61–70.

Lotzova, E., Savary, G.A. and Stringfellow, D.A. (1983) 5-Halo-6-phenyl pyrimidinones: new molecules with cancer therapeutic potential and interferon inducing capacity are strong inducers of murine natural killer cells. J. Immunol. 130, 963–969.

Lotzova, E., Savary, C.A., Lowlachi, M. and Murasko, D.M. (1986) Cytotoxic and morphological profile of endogenous and pyrimidinone-activated murine NK cells. J. Immunol. 136, 732–740.

Montefiori, D.C. and Mitchell, W.M. (1987) Antiviral activity of mismatched double-stranded RNA against human immunodeficiency virus in vitro. Proc. Natl. Acad. Sci. USA 84, 2985–2991.

Montefiori, D.C., Robinson, W.E. and Mitchell, W.M. (1989) In vitro evaluation of mismatched double-stranded RNA (Ampligen) for combination therapy in the treatment of acquired immunodeficiency syndrome. AIDS Res. Hum. Retroviruses 5, 193–202.

Morahan, P.S. (1980) Anionic polymers and polysaccharides: overview of interferon inducing ability, antitumor activity and mechanism of action. In: E.M. Hersh (Ed.), Augmenting Agents in Cancer Therapy. pp. 185–192. Raven Press, New York.

Morahan, P.S. and McCord, R.S. (1975) Resistance to herpes simplex virus type 2 virus induced by an immunomodulator (pyran) in immunosuppressed mice. J. Immunol. 115, 311–313.

Morahan, P.S., Regelson, W. and Munson, A.E. (1972) Pyran and polyribonucleotides: differences in biological activities. Antimicrob. Agents Chemother. 2, 16–22.

Morahan, P.S., Kern, E.R. and Glasgow, L.A. (1977) Immunomodulator induced resistance against herpes simplex virus. Proc. Soc. Exp. Biol. Med. 154, 615–619.

Morahan, P.S., Dempsey, W.L., Volkman, A. and Connor, J. (1986) Antimicrobial activity of various immunomodulators: independence from normal levels of circulating monocytes and NK cells. Infect. Immun. 51, 87–93.

Morahan, P.S., Leake, E.R., Tenney, D.J. and Sit, M. (1987) Comparative analysis of modulators of nonspecific resistance against microbial infections. In: J. Maijde (Ed), Immunopharmacology of Infectious Diseases: Vaccine Adjuvants and Modulators of Nonspecific Resistance. pp. 313–324. Alan B. Liss, Inc., NY.

Pinto, A.J., Morahan, P.S. and Brinton, M.A. (1988) Comparative study of various immunomodulators for macrophage and natural killer cell activation and antiviral efficacy against exotic RNA viruses. Int. J. Immunopharmacol. 10, 197–209.

Pinto, A.J., Morahan, P.S., Brinton, M., Stewart, D. and Gavin E. (1990a) Comparative therapeutic efficacy of recombinant alpha, beta and gamma interferons against alphatogavirus, bunyavirus, flavivirus and herpesvirus infections. J. Interferon Res. 10, 293–298.

Pinto, A.J., Stewart, D., van Rooien, N. and Morahan, P.S. (1990b) Selective depletion of liver and splenic macrophages with liposomes encapsulating the toxin dichloromethylene diphosphonate: effects on host antiviral resistance. J. Leukoc. Biol. (in press).

Sarzotti, M., Coppenhaver, D.H., Singh, I.P., Poast, J. and Baron, S. (1989) The in vivo antiviral effect of CL246,738 is mediated by the independent induction of IFN-A and IFN-B. J. Interferon. Res. 9, 265–274.

Skulnick, H.I., Weed, S.D., Eidson, E.E., Renis, H.E., Wierenga, W. and Stringfellow, D.A. (1985) Pyrimidinones. 1. 2-amino-5-halo-6-aryl-4-[^{3}H]pyrimidinones. Interferon inducing antiviral agents. J. Med. Chem. 28, 1864–1869.

Smee, D.F., Alaghamandan, H.A., Jin, A., Sharma, B.S. and Jolley, W.B. (1990) Roles of interferon and natural killer cells in the antiviral activity of 7-thia-8-oxoguanosine against Semliki Forest virus infections in mice. Antiviral Res. 13, 19–28.

T'so, P.O.P., Alderfer, J.L., Levey, J., Marshall, L.W., O'Malley, J., Horoszewicz, J.S. and Carter, W.A. (1976) An integrated and comparative study of the antiviral effects and other biological properties of polyinosinic acid-polycytidylic acid and its mismatched analogues. Mol. Pharmacol. 12, 299–312.

Twilley, T.A., Mason, L., Talmadge, J.E. and Wiltrout, R.H. (1987) Increase in liver associated natural killer cell activity by polyribonucleotides. Nat. Immun. Cell Growth Regul. 6, 279–289.

Wang, B.A., Lumanglas, A.L., Ruszala-Mallon, V.M. and Durr, F.E. (1985) Induction of tumor-inhibitory macrophages with a novel synthetic immunomodulator, 3,6-bis(2-piperidimethoxy)acridine trihydrochloride (CL246,738). J. Immunol. 135, 679–690.

Wang, B.A., Lumanglas, A.L., Ruszala-Mallon, V.M. and Durr, F.E.(1986) The mechanism of action of 3,6-bis(2-piperidinoethoxy)acridine trihydrochloride (CL246,738) in the potentiation of natural killer cells. J. Immunol. 137, 2640–2645.