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Biological response modifier-mediated resistance to herpesvirus infections requires induction of α/β interferon

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Summary

The role of interferon α/β (IFN) induction by the immunomodulators pICLC and CL246,738 was investigated in CD-1 mice infected with murine cytomegalovirus (MCMV) or herpes simplex virus type 2 (HSV-2). Mice were treated with either normal sheep serum or anti-α/β IFN antiserum, inoculated with the immunomodulators, and infected with virus. Because anti-IFN treatment also decreased natural resistance to HSV-2 and MCMV, two viral challenge doses were used to ensure that the mice with control serum or anti-IFN antiserum received biologically equivalent infections. Antiviral protection of pICLC and CL246,738 against HSV-2 infection was completely abrogated by treatment with anti-α/β interferon antiserum. Mice treated with pICLC also lost antiviral protection against MCMV when interferon α/β was depleted. These results indicate that induction of interferon α/β appears to be a major mechanism for both natural resistance and pICLC-induced antiviral protection against MCMV and HSV-2 herpesvirus infections.

Interferon; Biological response modifier; MCMV; HSV-2; pICLC; CL246,738

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Introduction

Interferon (IFN) induction appears to play a protective role in natural host resistance to herpesvirus infections (Gresser et al., 1976; Engler et al., 1982; Allen and Shellem, 1985; Chong et al., 1983; Wrzos et al., 1986), which may be strain specific in murine cytomegalovirus (MCMV) infection (Grundy (Chalmer) et al., 1982; Quinnan and Manischewitz, 1987). The in vivo mechanism of action of IFN induced by herpes viral infections, however, is unclear. IFN may exert direct antiviral activity or indirect action through the activation of effector cells such as macrophages (Mϕ) and/or natural killer (NK) cells. For example, NK cells have been shown to provide protection in natural resistance to murine cytomegalovirus (MCMV) infection, and IFN-induced enhancement of NK cells may be involved (Welsh, 1986).

Induction of IFN has been demonstrated by numerous biological response modifiers (BRMs) that have antiviral effects. It has been difficult, however, to show unequivocally that the BRM-induced antiviral protection is due to the IFN induced. The kinetic correlation of the IFN induction has not consistently coincided with antiviral protection provided by these BRMs (Morahan et al., 1991; Morahan and Pinto, 1992). Moreover, most BRMs are noted for exerting pleiotropic immunomodulatory effects.

A few recent studies have directly investigated the requirement for IFN induction in the antiviral activity of several BRMs against the RNA arboviruses, Semliki forest virus (SFV) and Banzi virus. The BRMs studied have included polyriboinosinic-cytidylic acid (pIC), pIC complexed with lysine carboxymethyl-cellulose (pICLC), 3,6-*bis*(2-piperidinoethoxy)acridine trihydochloride (CL246,738), and 7-*thia*-8-oxoguanosine (Sarzotti et al., 1989; Smee et al., 1990; Morahan et al., 1991; Barnhart et al., 1992). There have been no comparable studies with the herpesviruses. Protection against the two RNA arboviruses by CL246,738, pIC and 7-*thia*-8-oxoguanosine was reported to be completely mediated by induction of IFN α/β, as determined by the almost complete abrogation of antiviral protection by treatment of mice with anti-α/β IFN antiserum (Sarzotti et al., 1989; Smee et al., 1990; Morahan et al., 1991; Barnhart et al., 1992). The protection of pICLC against SFV infection, however, was not lost by treatment with high doses of anti-IFN α/β antiserum that decreased natural resistance to SFV (Morahan et al., 1991). Thus, the role of IFN as a common mechanism in BRM protection remains unclear. This prompted our study using pICLC and CL246,738 to determine whether IFN is a necessary mediator for these two BRMs against infection of mice with the herpesviruses MCMV and herpes simplex virus type-2 (HSV-2).

Materials and Methods

Mice

Barrier raised, specific pathogen-free 6–7 week old CD-1 (Charles River,

Montreal, CN or Portage, MI) female mice were used in all studies. Mice were maintained in microisolator cages in a barrier facility with 100% High Efficiency Particulate Filtered Air and were fed sterilized food and water ad libitum. All manipulations and procedures were carried out in laminar flow hoods within the barrier facility (ALAAC accredited) to decrease risk of inapparent infections that could influence results (Dempsey et al., 1986). Animals were maintained in accordance with guidelines established by the US Department of Health and Human Services (1985).

Immunomodulators

The following agents were employed in immunomodulator-mediated resistance studies. CL246,738 (Wang et al., 1986) (lot # PC 0594, courtesy of Dr. Fred Durr, Lederle Laboratories, Pearl River, NY) was stored as a powder at room temperature and prepared as needed to provide an injection concentration of 100 mg/kg for p.os administration. The stock solution of pICLC (lot # UI 84-101, gift of Dr. H. Levy, NCI, Frederick, MD) (Levy et al., 1975) was stored at 4°C and prepared in saline to provide an injection concentration of 1 mg/kg for i.p. administration. These doses were selected from previous studies as the lowest doses which provided antiviral protection against MCMV or HSV-2 infections (Kunder et al., 1992; Pinto et al., 1988).

Virus preparation and titration

The Smith strain of MCMV (ATCC VR-194) was passaged by i.p. infection ($10^{4.5}$ PFU/mouse) in 4–6 week old female CD-1 mice. Salivary glands were harvested 17–19 days after infection, homogenates (10%, w/v) were prepared in gelatin lactalbumin broth media, and aliquots were frozen at −70°C. The virus was titrated for plaque forming units (PFU) on monolayers of 3T3 mouse embryo fibroblasts (ATCC CCL163) in 35 mm diameter wells. Plaques were counted after 5 days of incubation and the titers expressed as PFU/ml.

The MS strain of HSV-2 (ATCC VR-540) was grown in Vero cells or secondary rabbit kidney fibroblasts by infecting cells with a low multiplicity of infection and harvesting when more than 75% of the cells showed cytopathic effect. The virus was titrated on monolayers of Vero cells by assay for PFU. Simultaneous plaque titrations were generally performed for both viruses each time viral dilutions were prepared.

Anti-α/β IFN antiserum

The antiserum used for these studies was prepared in sheep by immunization with highly purified mouse IFN α/β that was induced in L929 cells by Newcastle disease virus (kind gift of Dr. Donna Murasko, Medical College of Pennsylvania) (Dalton and Paucker, 1981). The final preparation of antiserum had a neutralizing titer of 1×10^6 units/ml. This anti-IFN antiserum has been used in several previous studies, including assessment of the role of IFN in BRM mediated antiviral protection (Morahan et al., 1991). Normal sheep serum (NSS) was used as a control for antiserum. An i.p. injection volume of

0.2 ml was used for either antisera or NSS.

IFN assay

Mouse serum was assayed for IFN by a modification of the microplate method of Havell and Vilcek (1972). Briefly, mice were anesthetized with ether vapor and bled through the retroorbital sinus. Serum was collected by centrifugation and samples stored at −20°C for assay. Several dilutions of serum samples were assayed for IFN by protection against the cytopathic effect of mouse encephalomyocarditis virus (EMC) infection on L-929 cell monolayers, relative to protection provided by internal IFN α/β standards. Cells and IFN were incubated for 24 h, and then challenged with EMC. After 24 h, plates were examined for cytopathic effect. The IFN titer of samples was recorded as the reciprocal of the highest dilution at which 50% cytopathic effect was observed. In every assay, an internal IFN-α/β standard was assayed simultaneously, and the titers corrected against the NIH mouse IFN α/β standard (kind gift of Dr. Donna Murasko, Medical College of Pennsylvania).

Antiviral protection assays

Mice (10 per group) were treated with anti-IFN α/β serum or NSS at −25 and −21 h before infection with MCMV or HSV-2. The BRMs were administered at −24 h. Mice were monitored daily for 21 days for signs of clinical illness and for mortality. Each experiment was replicated at least once. Statistically significant differences ($P < 0.05$) in percent mortality were determined by the chi-square test, using the ABStat program (Anderson-Bell, Arvada, CO). The median survival time was calculated, and the survival distribution data were analyzed with the Lee-Desu method (Lee and Desu, 1972) using the Survival Analysis computer program prepared by Dr. Edward Gracely, Department of Community and Preventative Medicine, MCP.

Results

Treatment of mice with anti-IFN α/β antiserum completely eliminates

TABLE 1

Selection of the schedule of anti-IFN serum to eliminate circulating IFN levels produced by pICLC

Serum[a]	Injection relative to pICLC (h)	IFN Titer/ml at hours after pICLC					
		0	3	6	9	12	24
NSS	−6, −1, +3	<1	3840	3840	3840	1440	982
Anti-IFN	−1		<1	<1	<1	<1	
Anti-IFN	−1, +3				<1	<1	<1
Anti-IFN	−6, −1 +3				<1	<1	<1

[a]Mice (2–3/group/time-point) were inoculated with 0.2 ml of either normal sheep serum (NSS) or anti-IFN serum (100 000 neutralizing units) at the schedules indicated in relation to i.p. inoculation of 1 mg/kg pICLC.

TABLE 2

Anti-IFN serum effectively depletes circulating IFN levels in HSV-2 infected mice treated prophylactically with pICLC

Serum[a]	IFN titer/ml after pICLC (h)			
	3	24	48	72
NSS	>2560	933	<1	<1
Anti-IFN	<1	<1	<1	<1

[a]Mice (2/group/time-point) were inoculated with 0.2 ml of either normal sheep serum (NSS) or anti-IFN serum (100 000 neutralizing units) at −1 and +3 h relative to i.p. injection of 1 mg/kg pICLC. Mice were inoculated with HSV-2 (1.3×10^4 PFU) at +24 h following pICLC treatment. Parallel groups of mice were observed for antiviral efficacy against HSV-2 infection.

circulating IFN produced by PICLC. The in vivo neutralizing efficiency of the anti-IFN α/β antiserum was measured by inoculating mice with 100 000 neutralizing units of antiserum or NSS by several administration schedules, then administering pICLC followed by measurement of serum IFN levels at various intervals (Table 1). All schedules of antiserum administration tested completely abolished serum IFN levels to <1 unit/ml at all times tested after pICLC treatment. A schedule using two injections of antiserum at −1 h and +3 h relative to BRM administration was selected for future experiments to ensure all circulating IFN would be eliminated. IFN measurements were made concurrently with each antiviral protection study. Serum IFN levels from a representative HSV-2 protection experiment are shown in Table 2. The antiserum completely abolished circulating IFN levels induced by pICLC for at least 72 h.

Effects of IFN α/β neutralization on natural resistance and BRM-mediated protection against HSV-2 infection. Natural resistance to HSV-2 was reduced significantly as shown by a decrease in median survival time of the saline control group from 9 to 5 days by treatment of mice with anti-IFN antiserum. We compared pICLC and CL246,738 for their requirement for IFN in protecting mice against HSV-2 infection (Fig. 1). Protection induced by either drug was lost when IFN induction was eliminated by neutralization of IFN.

Because of the decreased natural resistance when IFN was neutralized, it was possible that the BRMs exhibited less protection due to an overwhelming viral challenge dose. The HSV-2 inoculum was therefore decreased in order to obtain a more biologically equivalent HSV-2 dose in the anti-IFN antiserum treated group. At this lower HSV-2 inoculum (Fig. 2B), pICLC-induced protection was still totally abrogated by anti-IFN α/β antiserum, as it was at the higher HSV-2 challenge dose (Fig. 2A), There was complete protection in the control group (Fig. 2A).

Effects of IFN α/β neutralization on natural resistance and pICLC-mediated protection against MCMV infection. Protection against MCMV infection provided by pICLC also required IFN induction. Using either an MCMV

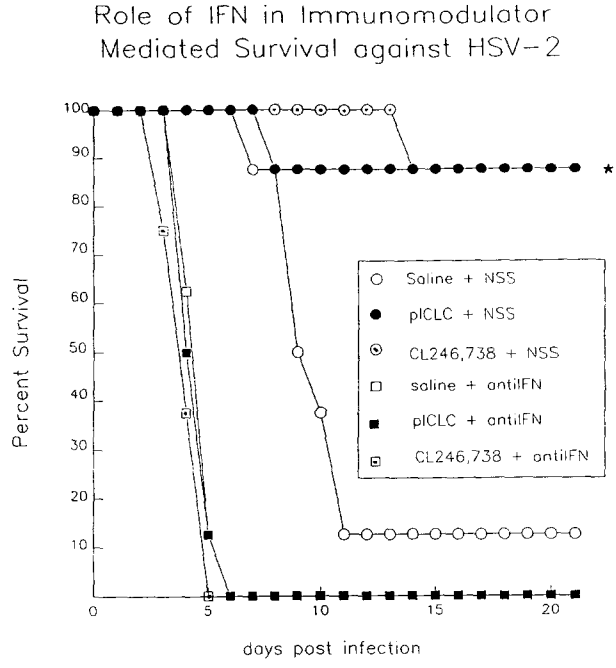

Fig. 1. Role of IFN in immunomodulator-mediated protection against HSV-2. CD-1 female mice (5–7 weeks old) were treated with sheep-anti-mouse IFN α/β (100 000 units) or normal sheep serum at −25 and −21 h and infected with 8.8×10^3 PFU HSV-2 at 0 h. Saline and pICLC were inoculated i.p. and CL246,738 p.os at −24 h. Survival was monitored for 21 days following infection. * = Significant decrease ($P < 0.05$) in mortality and/or increase in median survival time as compared to corresponding control group.

inoculum of 8×10^5 PFU that caused 100% mortality in the NSS treated controls (Fig. 3) or 5×10^4 PFU that caused 10% mortality in the NSS treated controls (Fig. 4), a significant decrease in pICLC protection was observed in the mice treated with anti-IFN antiserum. In contrast with HSV-2 infection, the higher challenge dose of MCMV, no difference in median survival time was observed in control mice depleted of IFN α/β. At the lower dose, as with HSV-2, depletion of IFN reduced natural resistance to MCMV as shown by both an increase in mortality from 10% to 100% and a decrease in median survival time from >21 days in the NSS treated group to 6 days in the anti-IFN treated group (Fig. 4).

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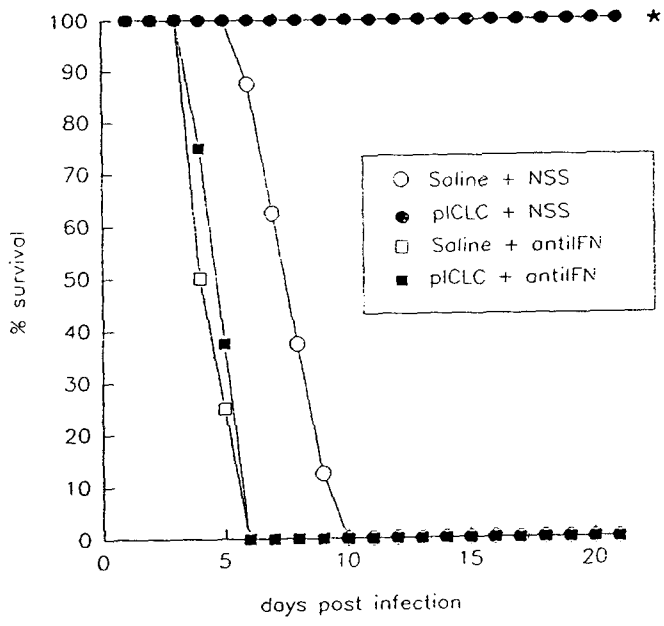
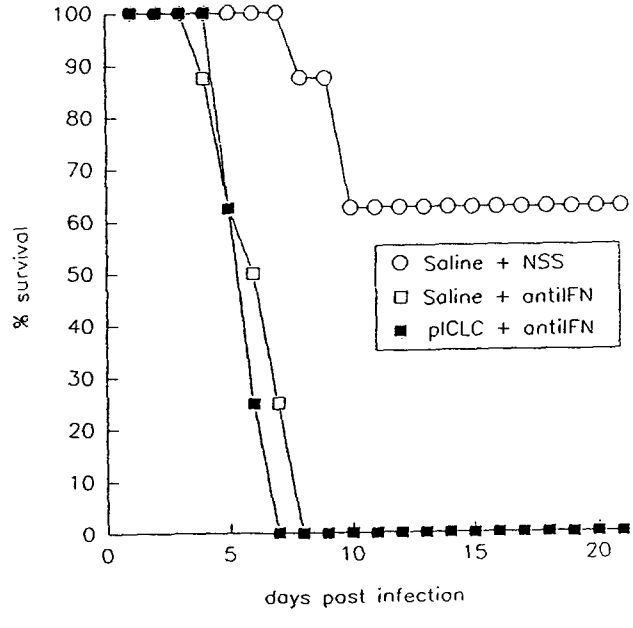
Fig. 2. Effect of different HSV-2 doses on IFN requirement for pICLC protection. Female CD-1 mice (5–7 weeks old) were treated with sheep-anti-mouse IFN α/β (100 000 units) or normal sheep serum (NSS) at −25 and −21 h and infected with either 1.3×10^4 PFU (panel A.) or with 0.4×10^4 PFU (panel B.) HSV-2 at 0 h. Mice were treated with either saline or pICLC (1 mg/kg, i.p.) at −24 h. Survival was monitored for 21 days following infection. * = Significant decrease ($P < 0.05$) in mortality and/or increase in median survival time as compared to corresponding control group.

A. HSV-2, 1.3×10^4 PFU/mouse



B. HSV-2, 0.3×10^4 PFU/mouse



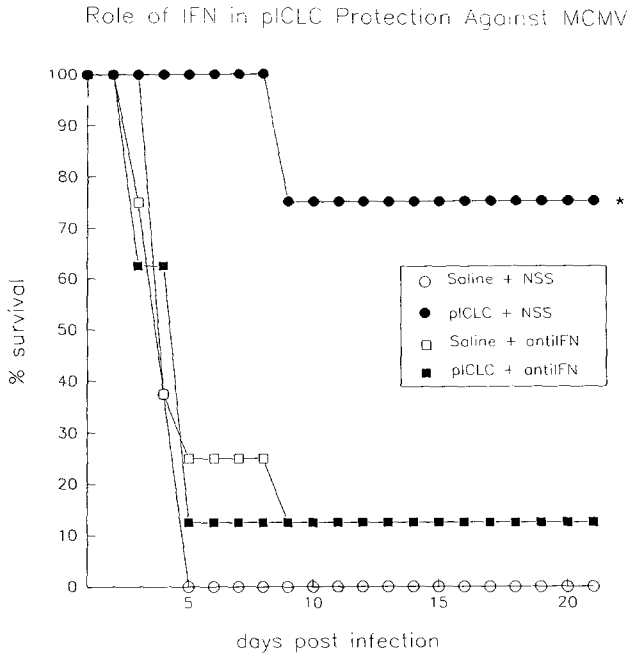


Fig. 3. Role of IFN in pICLC protection against MCMV infection. Female CD-1 mice (5–7 weeks old) were treated with sheep-anti-mouse IFN α/β (100 000 units) or normal sheep serum (NSS) at -25 and -21 h and infected with 9.0×10^5 PFU MCMV at 0 h. Mice were treated with either saline or pICLC (1 mg/kg, i.p.) at -24 h. Survival was monitored for 21 days following infection. * = Significant decrease ($P < 0.05$) in mortality and/or increase in median survival time as compared to corresponding control group.

Discussion

We have shown that antiviral protection by the BRM pICLC against infection of mice with the herpesviruses MCMV and HSV-2 requires induction of IFN α/β. These experiments are the first to demonstrate that IFN plays a critical role in BRM-mediated protection against MCMV and HSV-2 infections. These results were obtained at challenge doses of MCMV and HSV-2 that were biologically equivalent in the intact and IFN-depleted mice, indicating that the loss of antiviral activity was not merely due to an overwhelming viral challenge. The few previous BRM antiviral protection studies against arboviruses with IFN depletion did not examine lower viral doses to determine if the viral challenge doses used in anti-IFN treated mice were such as to overwhelm the protection of the BRM (Sarzotti et al., 1989; Smee et al., 1990; Morahan et al., 1991). The present data, demonstrating that CL246,738 required IFN induction for antiviral protection against HSV-2, are similar to our previous results and those of Sarzotti et al. using the RNA arbovirus, SFV infection (Morahan et al., 1991; Sarzotti et al., 1989). Thus, IFN induction appears to be the common mechanism of antiviral activity of this small orally active BRM. Whether IFN is a common mechanism for the

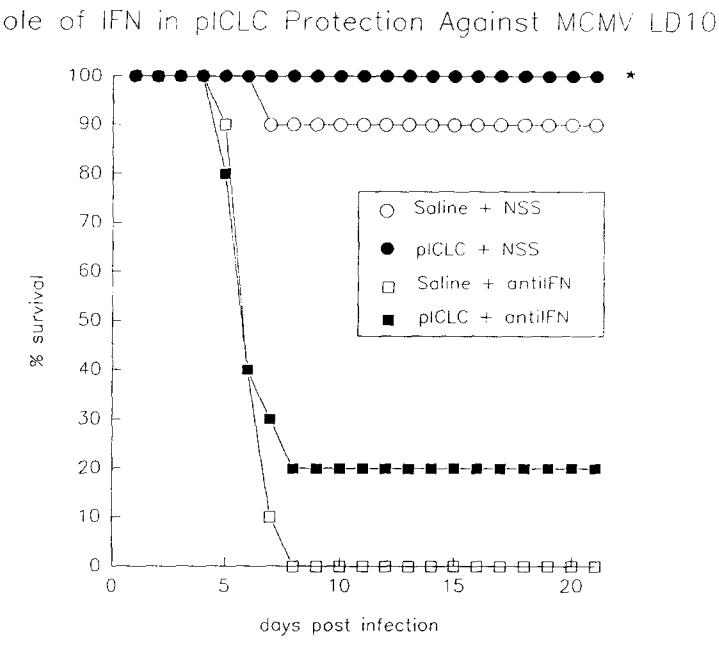


Fig. 4. Effect of decreased MCMV dose on IFN requirement for pICLC protection. Female CD-1 mice (5 7 weeks old) were treated with sheep-anti-mouse IFN α/β (100 000 units) or normal sheep serum (NSS) at -25 and -21 h and infected with 5×10^4 PFU MCMV at 0 h. Mice were treated with either saline or pICLC (1 mg/kg, i.p.) at -24 h. Survival was monitored for 21 days following infection. * = Significant decrease ($P < 0.05$) in mortality and/or increase in median survival time as compared to corresponding control group.

antiviral activity of pICLC is not as clear. At the viral challenge used, IFN was found to be required for BRM-induced protection against SFV infection by 7-*thia*-8-oxoguanosine, CL246,738 and ABMP, to be partially required by MVE-2 and Ampligen, and not required by pICLC (Smee et al., 1990; Sarzotti et al., 1989; Morahan et al., 1991). These results suggest that other mechanisms of BRM action, independent of IFN induction (Li et al., 1990), may be able to protect against some viral infections.

The requirement for IFN by pICLC against MCMV and HSV-2 infections may reflect a number of direct and indirect antiviral mechanisms. It is possible that MCMV and HSV-2 may be more sensitive to the in vivo antiviral effects of IFN than SFV (Martinotti et al., 1990; Morahan et al., 1991). Natural resistance against MCMV, HSV-2 and SFV infections appears to require IFN, and all three viruses have been shown to be sensitive to IFN in vitro (Stewart et al., 1969; Allan and Shellam, 1985; Martinotti et al., 1990). Comparisons of the in vitro and in vivo sensitivities of these viruses to IFN, however, have not been performed. Direct IFN-mediated antiviral effects have been proposed for pIC-induced protection against Banzi virus, because the antiviral effects of pIC against Banzi virus infection were abolished by IFN depletion, while protection did not apparently require NK cells or Mϕ (Barnhart et al., 1992). In this

regard, although pICLC produces prolonged activation of NK cells, we have found that NK cells are also not required for BRM-induced antiviral protection against MCMV and HSV-2 infection (Kunder et al., 1992). Whether indirect effects of pICLC-induced IFN on Mϕ are involved remains a possibility. It is well established that IFN and Mϕ have an antiviral 'alliance' (Mogensen and Virelizier, 1987). Pyo et al. have reported that IFN-β, induced by pIC, activated Mϕ for antiviral activity against HSV-1 (Pyo et al., 1991). Other cytokines secreted by IFN-activated Mϕ, such as tumor necrosis factor, may also directly lyse HSV-infected cells and account for part of the antiviral protection (Koff and Fann, 1986).

The present results clearly demonstrate that IFN is required for antiviral activity of pICLC against both HSV-2 and MCMV infections. It is likely that the in vivo mechanism of IFN activity includes a combination of direct and indirect effects. These are likely to vary depending on the critical natural resistance mechanisms in particular viral infections, and may be also related to different viral pathogenesis and replication strategies. Further selective effector cell depletions and in vitro studies will be helpful in elucidating the relative importance of direct and indirect IFN-induced antiviral mechanisms for pICLC-induced protection. These are important considerations so that the mechanisms of action of BRMs may serve as a basis for their selection in rational immunotherapy.

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References

Allen, J.E. and Shellam, G.R. (1985) Characterization of interferon induction in mice of resistant and susceptible strains during murine cytomegalovirus infection. J. Gen. Virol. 66, 1105–1112.

Barnhart, D.C., Mayer, E.P., Ghaffer, A. and Gangemi, J.D. (1992) Cellular and humoral factors associated with polyriboinosinic-polyribocytidylic acid (polyI:C) induced resistance to Banzi virus encephalitis. Antiviral Res. 17 (suppl. 1), 152.

Chong, K.T., Gresser, I. and Mims, C.A. (1983) Interferon as a defense mechanism in mouse cytomegalovirus infection. J. Gen. Virol. 64, 1–4.

Dalton, B.J. and Paucker, 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 hepatitis virus infection on macrophages and host resistance. J. Leuk. Biol. 39, 559–565.

Engler, H., Zawatzky, R., Kirchner, H. and Armmerding, D. (1982) Experimental infection of inbred mice with herpes simplex virus. IV. Comparison of interferon production and natural killer cell activity in susceptible and resistant adult mice. Arch. Virology 74, 239–247.

Gresser, I., Tovey, M.G., Maury, C. and Bandu, M-T. (1976) Role of interferon in the pathogenesis of virus disease in mice as demonstrated by the use of anti-interferon serum. J. Exper. Med. 144,

1316–1323.
Grundy (Chalmer), J.E., Trapman, J., Allan, J.E., Shellam, G.R. and Melief, C.J.M. (1982) Evidence for a protective role of interferon in resistance to murine cytomegalovirus and its control by non-H2-linked genes. Infection Immunity 37, 143–150.
Havell, E. and Vilcek, J. (1972) Production of high titered interferon in cultures of human diploid cells. Antimicrob. Agents Chemother. 2, 476–484.
Koff, W.C. and Fann, A.V. (1986) Human tumor necrosis factor-α kills herpesvirus-infected but not normal cells. Lymphokine Res. 5, 215–221.
Kunder, S.C., Wu, L. and Morahan, P.S. (1993) Role of NK cells in immunomodulator-mediated resistance to herpesvirus infection. Antiviral Res. 21, 103–118.
Lee, E. and Desu, M. (1972) A computer program for comparing k samples with right-censored data. Computer Programs Biomed. 12, 315–321.
Levy, H.B., Baer, G., Baron, S., Buckler, C.E., Gibbs, C.G., Iadorola, M.T., 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, S.W., Moskow, J.J. and Suhadolnik, R.J. (1990) 8-azido double-stranded RNA photoaffinity probes. J. Biol. Chem. 265, 5470–5474.
Martinotti, M.G., Gariglio, M., Cofano, F., Cavallo, G. and Landolfo, S. (1990) The role of interferons in the resistance to murine cytomegalovirus. Microbiologica 13, 305–309.
Mogensen, S.C. and Virelizier, J-L. (1987) The interferon-macrophage alliance. Interferon 8, 55–84.
Morahan, P.S., Pinto, A.J., Stewart, D., Murasko, D.M. and Brinton, M.A. (1991) Varying role of alpha/beta interferon in the antiviral efficacy of synthetic immunomodulators against Semliki Forest virus infection. Antiviral Res. 15, 241–254.
Morahan, P.S. and Pinto, A.J. (1992) Biologic response modifiers as antivirals in immunosuppressed hosts. In: H. Friedman and T.W. Klein (Eds), Microbial Infections: Role of Biological Response Modifiers. Plenum, 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. Immunopharmac. 10, 197–209.
Pyo, S., Gangemi, J.D., Ghaffar, A. and Mayer, E.P. (1991) PolyI:C-induced anti-herpes simplex type I activity in inflammatory macrophages is mediated by induction of interferon-β. J. Leuk. Biol. 50, 479–487.
Quinnan, G.V. and Manischewitz, J.F. (1987) Genetically determined resistance to lethal murine cytomegalovirus infection is mediated by interferon-dependent and -independent restriction of virus replication. J. Virology 61, 1875–1881.
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 interferon-α and interferon-β. J. Interferon Res. 9, 265–274.
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 activity of 7-*thia*-8-oxoguanosine against Semliki Forest virus infections in mice. Antiviral Res. 13, 91–102.
Stewart, W.E., Scott, W.D. and Sulkin, S.E. (1969) Relative sensitivities of viruses to different species of interferon. J. Virol. 4, 147–153.
Wang, B.S., Lumanglas, A.L., Ruszala-Mallon, V.M. and Durr, F.E. (1986) The mechanism of action of 3,6-*bis*(2-piperidinoethoxy)acridine trihidrochloride (CL246,738) in the potentiation of natural killer cells. J. Immunol. 1, 2640–2645.
Welsh, R.M (1986) Regulation of virus infection by natural killer cells. Nat. Immun. Cell Growth Regul. 5, 169–199.
Wrzos, H., Murasko, D.M. and Rapp, F. (1986) Effect of antibody to interferon on genital herpesvirus infection in mice. Microbial Path. 1, 71–78.