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Protection against murine cytomegalovirus infection in aged mice and mice with severe combined immunodeficiency disease with the biological response modifiers polyribosinic-polycytidylic acid stabilized with L-lysine and carboxymethylcellulose, maleic anhydride divinyl ether and colony stimulating factor 1

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Summary

A variety of biological response modifiers (BRMs) have provided antiviral protection to immunocompetent mice, and this prompted us to determine their efficacy against murine cytomegalovirus (MCMV) infection in immunocompromised mice-including the profoundly immunocompromised SCID mice and C57Bl/6 and B_6D_2F1 aged mice. SCID mice showed a marked decrease (>20-fold) in resistance to MCMV, while there was a slight decrease (3-fold) in aged mice. In BRM antiviral protection studies, SCID mice were almost completely protected against MCMV infection by the pleiotropic immunomodulators, MVE-2 and pICLC, but much less by the more selective CSF-1. pICLC-induced IFN and NK cell cytotoxicity were maintained in SCID mice, suggesting that pleiotropic immunomodulatory effects may be required for antiviral protection in such a profoundly immunocompromised model. pICLC also effectively protected aged mice against lethal MCMV infection and

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effectively induced IFN. These results emphasize the potential for BRM treatment in immunocompromised hosts.

SCID mice; Aged mice; MCMV; Biological Response Modifiers pICLC; MVE-2; CSF-1

Introduction

Infection of mice with the herpesvirus murine cytomegalovirus (MCMV), like the human CMV infection for which it is a model, is exacerbated during immunosuppression in a variety of situations, including severe combined immunodeficiency disease (SCID) (Selgrade et al., 1982; Bukowski et al., 1984; Welsh et al., 1991). The usefulness of SCID mice as an immunocompromised viral infection model for the evaluation of viral immunotherapy has not been fully exploited, and few other immunocompromised models have been used in antiviral studies (Okada and Minashima, 1987; DeCastro et al., 1991; Morrey et al., 1992 Smee et al., 1992; Morahan and Pinto, 1992).

Mice with the SCID mutation lack functional B and T lymphocytes (Bosma et al., 1983). This mutation allows the study of the remaining components of host resistance without the influence of B or T cells. Thus, one can investigate whether enhancement of any remaining components of host resistance such as natural killer (NK) cells, macrophages (MΘ), or interferon (IFN) can compensate for the deficiency and protect mice against MCMV infection. For example, it has been reported that NK cells are present (Dorshkind et al., 1985) and are activated during the acute stages of MCMV infection in SCID mice (Welsh et al., 1991), although this natural enhancement of NK cell activity is insufficient to clear the MCMV infection and mortality results. Depletion of NK cells in SCID mice produces earlier mortality (Welsh et al., 1991), supporting the concept of a role for NK cells in the early clearance of MCMV. It is possible that enhancement of NK cells with biological response modifiers (BRMs) might protect the mice. The paired induction of IFN and subsequent NK cell activity as found with pyrimidinones (Brideau and Walcott, 1985) may be useful in MCMV infection in SCID mice.

Aging represents another source of immunosuppression (Rytel, 1987; Murasko and Goonewardene, 1990). It has been well established that the elderly are immunocompromised in various T and B cell functions, but the implications for management of infections are not completely understood (Sigel, 1952; Louria et al., 1982). Successful treatment with anti-infectives in immunocompromised individuals such as those with SCID, aging and AIDS is clearly more difficult to achieve than in immunocompetent individuals; it has not yet been investigated how effectively immunotherapy can augment immunity and increase antimicrobial efficacy.

Sufficient enhancement of the nonspecific mediators of the early resistance

(NK cells, MΘ IFN) therefore has potential to provide antiviral protection in immunosuppression due to reduced or absent T and B cell function. It has been well established that enhancement of these mediators by BRMs can provide antiviral protection in immunocompetent mice (Pinto et al., 1988). MVE-2, or pyran copolymer (Carrano et al., 1984), and polyriboinosinic-polycytidylic acid stabilized with L-lysine and carboxymethylcellulose (pICLC) (Levy et al., 1975; Kern et al., 1977) are pleiotropic agents which enhance MΘ and NK cell activity, induce IFN, and induce excellent antiviral protection against HSV-2, MCMV and numerous other viruses in immunocompetent mice (Pinto et al., 1988; Kunder et al., 1992a). Macrophage colony stimulating factor (M-CSF or CSF-1), a selective MΘ enhancing agent and maturation factor, has also demonstrated antiviral activity against MCMV infection in immunocompetent mice (Chong and Piazza, 1989). This efficacy of BRMs in immunocompetent mice prompted us to evaluate the ability of these drugs to protect immunosuppressed SCID and aged mice against MCMV infection. Protection was provided in SCID mice by the drugs with multiple immunomodulatory activities, MVE-2 and pICLC, but not by the more selective CSF-1. Aged mice were protected by pICLC against MCMV infection as well. These results suggest that BRM-mediated protection in severely immunosuppressed individuals against infection with MCMV might be achieved by vigorous enhancement of multiple mediators of host resistance.

Materials and Methods

Mice

Balb/c and SCID 4–6 week old male mice from Balb/c CB17 backgrounds (Taconic, Germantown, NY), and 8–10 week old SCID male and female mice from the colonies of Dr. Sandra Harris and Dr. Mark Stearn (Medical College of Pennsylvania) were used. SCID mice were periodically examined for T cell leakage and found to be profoundly immunodeficient. Barrier raised, specific pathogen-free 6–7 week old CD-1 female mice (Charles River, Montreal, CN or Portage, MI) were used for some experiments. For aging studies, aged and young C57Bl/6 and B_6D_2F1 mice (males, 20–23 months and 6 weeks old) were obtained through the National Institute of Aging (Charles River, Stone Ridge, NY). 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 injected i.p. for the immunomodulator-mediated

resistance studies. MVE-2 (lot XA471-19-4) was obtained from Hercules Co., Wilmington, DE, stored as a powder at room temperature and prepared as required in saline at pH 7 to provide an injection concentration of 50 mg/kg (Carrano et al., 1984). The stock solution of pICLC (lot UI 84-101, gift of Dr. H. Levy, NCI, Frederick, MD) was stored at 4C and prepared in saline to provide an injection concentration of 1 mg/kg (Levy et al., 1975). Highly purified recombinant human macrophage colony-stimulating factor (CSF-1, lot DP-408) (Ladner et al., 1987; Hallenbeck et al., 1989) was provided courtesy of Dr. Lea Aukerman, Cetus Co., Emeryville, CA, and prepared in physiologic saline to provide 3.6 mg/kg (2×10^6 U/mouse). Aliquots were stored at −70C. The antiviral protection of CSF-1 against MCMV infection was determined in immunocompetent CD-1 mice and a dose of 2×10^6 U/mouse was found to protect mice completely (data not shown).

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 −70C. 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.

NK cell cytotoxicity assay

Spleens were removed from mice, weighed and homogenized into single-cell suspensions. Debris was removed by a series of three washes and centrifugations. All manipulations were carried out at 4°. Cytotoxicity was measured in a 4 h [^{51}Cr] release assay using 10^4 ^{51}Cr-labeled YAC-1 target cells and from 5×10^5 to 2×10^6 (effector:target cell ratios from 100:1 to 25:1) spleen cells per well as previously described (Kunder et al., 1993a).

Interferon 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° for assay. Serum samples were assayed for IFN by protection against the cytopathic effect of mouse encephalomyocarditis virus infection on L-929 cell monolayers, relative to protection provided by internal IFN α/β standards, as previously described (Kunder et al., 1993b). In every assay, an internal IFN-α/β standard was assayed simultaneously, and the titers corrected against the NIH mouse IFN α/β standard.

Antiviral protection assays

All immunomodulators were administered 24 h prior to infection, with the

exception of CSF-1, which was injected daily for 4 days prior to infection, with the last injection given 4 h prior to infection. Survival was monitored for 21 days following infection.

Statistical methods

Statistically significant differences ($P<0.05$) in percent mortality were determined by the chi-square test, using the ABStat program (Anderson-Bell Corp., Arvada, CO). The median survival time (MST) 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, Medical College of Pennsylvania. Differences in natural resistance were determined by the method of Reed and Meunch (1938). The Student's *t*-test was used to determine significance between groups in cytotoxicity and viral plaque assays.

Results

BRM protection against MCMV in the severely immunodeficient SCID mice

In order to evaluate BRMs in SCID mice, experiments were performed to compare the natural resistance to MCMV in immunocompetent and SCID mice and determine biologically equivalent challenge doses of MCMV to use for the immunocompetent and immunodeficient mice (Table 1). The LD_{90-100} dose for SCID mice was about 20-fold less than that in immunocompetent mice, showing the decreased resistance due to lack of functional T and B cells. Interestingly, despite the reduced resistance the median survival time of SCID mice infected with MCMV (MST = 12 days) was consistently greater than that of the immunocompetent CD-1 and Balb/c mice (both strains, MST = 6 days) at biologically equivalent (LD_{90}) MCMV doses.

TABLE 1

Immunodeficient SCID mice are more sensitive to MCMV infection than immunocompetent mice

Strain	MCMV dose[a]	#D/T[b]	% mortality	MST (days)[c]
Balb/c	8×10^5	8/8	100	6
	5×10^4	0/5	0	>21
SCID	5×10^4	7/7	100	12
	1×10^4	6/7	85	12
	5×10^3	1/6	16	>21

[a]Mice of each strain were inoculated i.p. with the indicated viral challenge dose and mice observed for mortality for 21 days. The results in Balb/c mice were similar to those in the immunocompetent outbred CD-1 mice, where a challenge dose of 8×10^5 PFU also caused 100% mortality with a MST of 12 days.

[b]#D/T number dead/total.

[c]MST, median survival time.

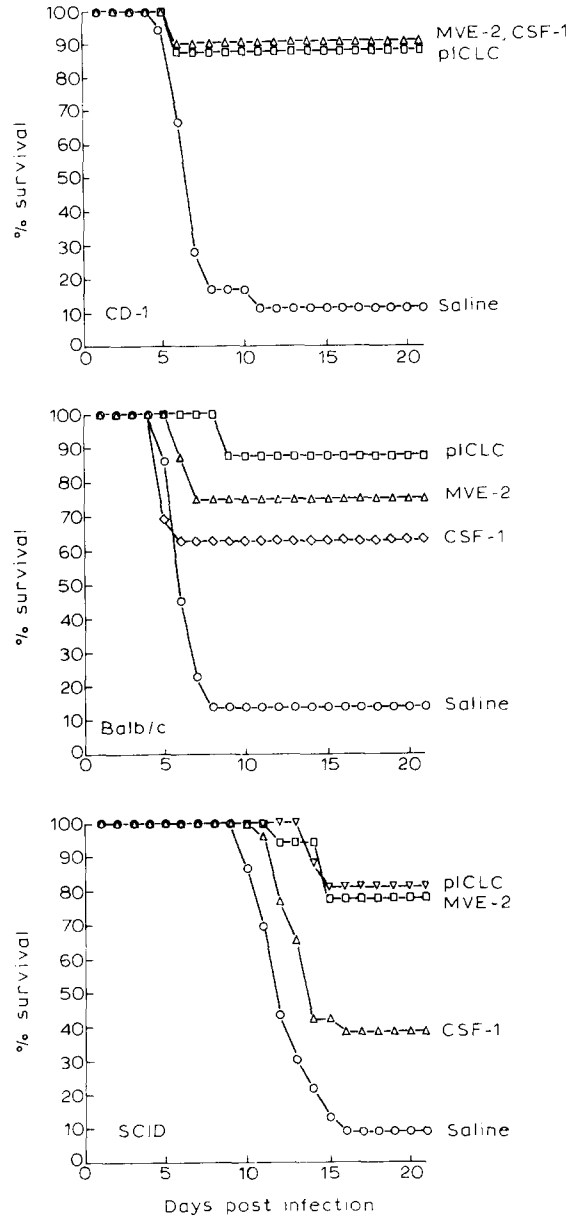

Fig. 1. Comparative immunomodulator protection against MCMV infection in immunocompetent and immunodeficient mice. Immunocompetent CD-1 and Balb/c mice and immunodeficient SCID mice were injected i.p. with pICLC (1 mg/kg) or MVE-2 (50 mg/kg) 24 h prior to MCMV infection or with CSF-1 (2×10^6 U) daily for 4 days prior to infection (which was 4 h after the last injection). CD-1 and Balb/c mice were infected i.p. with 8×10^5 PFU MCMV, while SCID mice were infected i.p. with 5×10^4 PFU MCMV to provide comparable mortality. All mice were monitored for 21 days for mortality. These data depict three combined experiments.

The ability of the BRMs MVE-2, pICLC and CSF-1 to protect SCID mice against mortality from lethal MCMV infection was determined and compared with antiviral activity in immunocompetent mice (Fig. 1). Prophylactic treatment with pICLC and MVE-2 provided significant protection from death to SCID mice as compared with saline-treated controls (81% and 78% survival, respectively). The median survival time was also significantly increased by treatment with either pICLC and MVE-2 (to >21 days MST) as compared with the control group treated with saline (12 days MST). While overall CSF-1 treatment provided significant protection in SCID mice as compared with the saline control group (survival increased from 10% to 38%, P,0.05), there was no significant increase in MST. Moreover, CSF-1 protection in SCID mice against MCMV was variable, with significant protection as evidenced by both increased survival and MST occurring in only one of three experiments.

The ability of MVE-2 and pICLC to protect SCID mice against MCMV infection was also shown by decreased viral titers in treated mice (Table 2). The titers of MCMV were significantly decreased ($P<0.05$) in the liver and spleen following treatment with either MVE-2 or pICLC.

Induction of IFN and NK cell cytotoxicity in SCID mice

To determine whether IFN induction or NK cell activation could be involved in the antiviral protection of the BRMs, pICLC was used as a prototypic strong IFN inducer and persisting NK cell activator (Levy et al., 1975). SCID, CD-1, and Balb/c mice were injected with pICLC and serum was taken 6 h later at the peak of IFN induction with this drug (Kunder et al., 1992b). IFN was induced effectively in all mouse strains, indicating that this nonspecific host resistance mechanism was operative in the severely immunodeficient SCID mice (Table 3).

NK cell cytotoxicity was assayed in uninfected or MCMV infected mice on day +4 following pICLC treatment, which was day +3 after MCMV infection when NK cell activity has been shown to be increased in immunocompetent mice infected with sublethal doses of MCMV (Bukowski et al., 1984). pICLC significantly enhanced splenic NK cytotoxicity in both SCID and Balb/c mice, whether infected or not with MCMV (Table 4). In fact, pICLC reversed the

TABLE 2

Antiviral effect of MVE-2 and pICLC in SCID mice on MCMV replication

Groups	Mean MCMV PFU/gr in Spleen[a]	Liver
Control	$2.3 \times 10^5 \pm 1.3 \times 10^5$	$8.3 \times 10^4 \pm 1.8 \times 10^4$
MVE-2	$<1 \times 10^{3b}$	$<1 \times 10^{3b}$
PICLC	$1.3 \times 10^4 \pm 3.8 \times 10^{3b}$	$3.7 \times 10^4 \pm 1.9 \times 10^3$

[a]Organs were harvested from groups of 3 mice on day +3 following infection, homogenized and assayed individually by plaque formation on 3T3 cells.

[b]$P<0.05$ as compared to respective control group.

TABLE 3

Similar IFN induction by pICLC in SCID, CD-1, and Balb/c mice, and in young and aged B_6D_2F1 mice

Mouse strain	Age (mos.)	pICLC	IFN titer[a]
CD-1	1.5	–	<1, <1
CD-1	1.5	+	>2560, 3840, 4641
Balb/c	1.5	–	<1, <1,
Balb/c	1.5	+	3456
SCID	1.5	–	<1, <1,
SCID	1.5	+	1800, 3456
B_6D_2F1	2	–	<1
		+	36, 482, 6912, 6912
	22	–	<1
		+	100, 200, 3456, 3456

[a]IFN titers were determined in individual mice from serum taken by retroorbital bleeding 6 h after i.p. administration of 1 mg/kg pICLC.

suppression of NK cell cytotoxicity that occurred in Balb/c or SCID mice lethally infected with MCMV. In SCID mice, spleen weights (Table 5) and total numbers of nucleated cells (data not shown) were also significantly reduced as compared with those of Balb/c control groups. The increased splenic NK cell cytotoxicity therefore occurred in both uninfected and infected SCID mice despite the reduction in spleen cells.

TABLE 4

Similar enhancement by pICLC of NK cell cytotoxicity in SCID and Balb/c mice

Treatment[a]		% Lysis by splenic cells from			
	E:T	Balb/c mice		SCID mice	
Drug	ratio	Uninfected	MCMV	Uninfected	MCMV
Saline	100:1	16.4 ± 0.7[b]	0.9 ± 0.9	6.7 ± 1.4	0.0
	50:1	14.5 ± 0.5	0.0	3.0 ± 1.8	0.0
	25:1	14.0 ± 0.8	0.0	2.0 ± 2.0	0.0
pICLC	100:1	21.6 ± 0.1[c]	29.1 ± 7.0[c]	25.5 ± 5.5[c]	53.2 ± 2.3[c,d]
	50:1	23.6 ± 5.5[c]	25.5 ± 1.9[c]	22.2 ± 4.3[c]	44.3 ± 1.3[c,d]
	25:1	19.1 ± 0.1[c]	22.7 ± 2.5[c]	18.5 ± 4.0[c]	38.4 ± 1.9[c,d]

[a]Balb/c and SCID mice were injected i.p. with saline or 1 mg/kg pICLC 24 h prior to i.p. infection with MCMV. Balb/c mice were infected with 8×10^5 PFU and SCID mice were infected with 5×10^4 PFU of MCMV; these biologically equivalent doses provide 90–100% mortality in the respective hosts. Controls were injected i.p. with virus diluent. Spleens were harvested on day +3 following infection or diluent injection.

[b]Cytotoxicity of each group of individual mice ($n=3$) ± S.E. against YAC-1 target cells at various effector:target (E:T) ratios.

[c]Increased ($P < 0.05$) as compared with respective saline control group.

[d]Increased ($P < 0.05$) as compared with corresponding pICLC treated and uninfected group.

TABLE 5

Comparison of spleen weights in Balb/c and SCID mice treated with pICLC and infected with MCMV

Treatment	Spleen weight (gm) in[a]			
	Balb/c mice		SCID mice	
	Uninfected	MCMV	Uninfected	MCMV
Saline	0.15 ± 0.02	0.21 ± 0.01[b]	0.05 ± 0.00[c]	0.04 ± 0.00[c]
pICLC	0.21 ± 0.01[b]	0.23 ± 0.01[b]	0.06 ± 0.01[c]	0.05 ± 0.01[c]

[a]Spleens were weighed prior to NK cell cytotoxicity assay.
[b]$P < 0.05$, increase as compared to uninfected Balb/c saline control.
[c]$P < 0.05$, decrease as compared to uninfected Balb/c saline control.

Natural and pICLC-induced resistance to MCMV infection in aged mice

Young (2 months old) and aged (20–22 months old) C57Bl/6 and B_6D_2F1 mice were infected with MCMV over a range of challenge doses to determine if aging decreased natural host resistance to MCMV. In both strains of aged mice, MCMV infection resulted in slightly increased mortality as compared with that of young mice (Table 6). The LD_{50} was decreased about 0.5 $\log_{10}$ for the C57Bl/6 aged mice and 0.6 $\log_{10}$ for the B_6D_2F1 aged mice (Reed and Meunch, 1938). This was particularly apparent in B_6D_2F1 mice at the middle dose tested ($P < 0.053$). There was no significant change in MST between young and aged mice at any of the viral challenge doses.

To determine whether BRM enhancement of host resistance in aged mice would protect against MCMV infection, B_6D_2F1 mice were prophylactically treated with pICLC at a low dose of pICLC that completely protects young

TABLE 6

Effect of age on natural resistance of C57Bl/6 and B_6D_2F1 mice against MCMV infection

Mouse strain[a]	Age (mo.)	Viral challenge (PFU)	Mortality		MST (days)
			#D/T[b]	%	
C57Bl/6	2	1×10^5	0/7	0	>21
		8×10^5	4/7	57	7
		2×10^6	7/7	100	5
	23	1×10^5	1/7	14	>21
		8×10^5	7/7	100	5
		2×10^6	7/7	100	5
B_6D_2F1	2	5×10^4	0/7	0	>21
		1×10^5	0/7	0	>21
		8×10^5	6/7	86	7
	22	5×10^4	0/7	0	>21
		1×10^5	3/7	43	>21
		8×10^5	7/7	100	6

[a]C57Bl/6 and B_6D_2F1 mice were infected i.p. with the indicated MCMV challenge and monitored for mortality for 21 days.
[b]#D/T = number dead/total. MST = median survival time.

TABLE 7

pICLC protection of aged B_6D_2F1 mice against MCMV infection

Group	Drug	Mortality		
		#D/T	(%)	MST (days)
2 mo.	Saline[a]	8/8	100	6
	pICLC	0/8	0[c]	>21[c]
22 mo.	Saline	8/8	100	5
	pICLC	0/4[b]	0[c]	>21[c]

[a]Mice were injected i.p. with saline or 1 mg/kg pICLC 24 h before i.p. infection with 8×10^5 PFU of MCMV. Mice were monitored for mortality for 21 days. #D/T = Number dead/total. MST = median survival time.

[b]Four aged mice died within 24 h after pICLC injection.

[c]$P < 0.05$ as compared with the respective saline control group.

mice. Mortality was significantly reduced in both young and aged mice receiving pICLC (Table 7), although some aged mice died due to drug toxicity, which may reflect different pharmacokinetics in aged hosts (Wallace et al., 1993). pICLC-induced serum TFN was assayed 6 h following treatment and was induced in mice of both age groups (Table 3).

Discussion

The efficacy of prophylactic BRM treatment against MCMV infection in immunocompetent mice (Kunder et al., 1992a) prompted us to determine whether such protection was possible in immunosuppressed mice. Two BRMs with pleiotropic activities, MVE-2 and pICLC, effectively protected the profoundly immunodeficient SCID mice against MCMV infection as demonstrated by increased median survival time, and decreased organ viral titers. Prophylactic treatment with the more selective BRM, the MΘ activating CSF-1, was consistently less effective in SCID mice than in immunocompetent mice (Fig. 1). Combined prophylactic and therapeutic administration of CSF-1 might be required in immunodeficient mice, while prophylactic treatment is sufficient in immunocompetent mice (Chong and Piazza, 1989). The one drug (pICLC) tested in aged mice also provided protection in these immunosuppressed hosts. These results demonstrate the potential of immunotherapy against MCMV infection in the immunosuppressed host. The SCID model of profound natural immunodeficiency (Smee et al., 1992) compliments models involving immunocompetent mice treated with selective depletion methods (Smee et al., 1992; Morahan and Pinto, 1992).

The SCID mouse, with its lack of functional T and B cell immunity (Bosma et al., 1983) also permits a more focused approach for the elucidation of nonspecific immune mechanisms in BRM antiviral activity (Morrey et al., 1992). Because of the effectiveness in SCID mice of pICLC, which is postulated to act through induction of IFN (Levy et al., 1975), we determined whether

IFN induction and enhancement of NK cell cytotoxicity were maintained in the pICLC treated and infected SCID mice or by MCMV infection. Although NK cells do not appear to be essential for pICLC-induced protection in immunocompetent mice (Kunder et al., 1993a), their role in immunodeficient SCID mice has yet to be determined. Splenic NK cell cytotoxicity of both Balb/c and SCID mice control groups was virtually eliminated by MCMV infection, but was restored by pICLC treatment. The loss of activity in infected mice may be due to the greater viral challenge in our experiments as compared to the dose used by Welsh et al. (1991).

Interferon was also induced by pICLC effectively in both SCID and Balb/c mice. IFN was previously found to be effectively induced by a lower MCMV challenge in SCID and Balb/c mice (Welsh et al., 1991). Therefore, IFN induction and subsequent NK cell enhancement appear to be potent mechanisms for consideration in selecting BRMs for use against MCMV infection in T and B cell deficient hosts. Non-IFN antiviral mechanisms for pleiotropic BRMs such as pICLC may also be involved, however (Morahan et al., 1991). Smee et al. (1992) reported that therapeutic treatment with either IFNα itself or with bropiramine, which acts via IFN induction (Brideau and Walcott, 1985; Morahan et al., 1991), was ineffective in SCID mice infected with MCMV. It is possible, therefore, that pleiotropic actions are necessary to activate several of the remaining host defense components of the SCID mouse, rather than the selective action of MΘ activation by CSF-1 or selective IFN induction (Smee et al., 1992). More investigation with additional BRMs with very selective and distinct mechanisms of action would help establish whether pleiotropic BRMs are necessary for immunotherapy in immunocompromised mice.

The SCID mouse shows at least a 20-fold decrease in natural resistance to MCMV infection as compared with immunocompetent CD-1 and Balb/c mice (Table 2 and Smee et al., 1992). However, our comparative data clearly show that there is also an increase in resistance early in infection in SCID mice as compared with immunocompetent mice. Mortality due to MCMV infection in SCID mice occurred approximately 12–17 days after infection, while in CD-1 and Balb/c mice death occurred approximately 5–7 days after infection. Our data showing a MST of 12–17 days are comparable to other studies when only SCID mice were infected with MCMV (smee et al., 1992; Welsh et al., 1991). The delayed mortality may be the result of a number of mechanisms. One is compensating nonspecific resistance mechanisms early in the infection that partially contain the lower inoculum of MCMV used. Death follows nevertheless, due to the lack of T lymphocytes which are well established to be required for elimination of the virus (Scott and Kaufmann, 1991).

Although natural resistance was not as depressed as in SCID mice, aging caused a slight decrease (about 0.5 $\log_{10}$) in host resistance against MCMV infection. This decline was not unexpected in view of the existing literature on aging and depressed immunity (Sigel, 1952; Rytel, 1987; Murasko and Goonewardene, 1990). The effective antiviral protection provided by pICLC

against MCMV infection in aged mice provides further support for the use of immunotherapy in such immunosuppressed situations. The clear efficacy of certain BRMs in immunotherapy of viral infection expands the armamentarium of potential treatments available for the immunosuppressed host.

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References

Bosma, G.C., Custer, R.P. and Bosma, M.J. (1983) A severe combined immune immunodeficiency mutation in the mouse. Nature 301, 527–530.

Brideau, R.J. and Walcott, J.A. (1985) Effect of pyrimidinone treatment on lethal and immunosuppressive murine cytomegalovirus infection. Antimicrob. Agents Chemother. 28, 485–488.

Bukowski, J.F., Woda, B.A. and Welsh, R.M. (1984) Pathogenesis of murine cytomegalovirus infection in natural killer cell-depleted mice. J. Virology 52, 119–128.

Carrano, R.A., Iuliucci, J.D., Luce, J.K., Page, J. and Imondi, A.R. (1984) MVE-2: Development of an immunoadjuvant for cancer treatment. In: R.L. Fenichel and M.A. Chirigos (Eds), Immune Modulation Agents and Their Mechanisms, pp. 243–260. Marcel Dekker, NY.

Chong, K.T. and Piazza, D. (1989) The effect of in vivo administration of recombinant human macrophage-stimulating factor (M-CSF) on host resistance to murine cytomegalovirus (MCMV) infection. Blood 74 (suppl 1), 69a.

DeCastro, L.M., Kern, E.M., DeClercq, E., Ghaffar, A., Mayer, E.M., Vogt, P.E. and Gangemi, J.D. (1991) Phosphonyl-methoxyalkyl purine and pyrimidine derivatives for treatment of opportunistic cytomegalovirus and herpes simplex virus infections in murine AIDS. Antiviral Res. 16, 101–114.

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

Dorshkind, K., Pollack, S.B., Bosma, M.J. and Phillips, R.A. (1985) Natural killer (NK) cells are present in mice with severe combined immunodeficiency (SCID). J. Immunol. 134, 3798–3801.

Hallenbeck, R., Kawasaki, E., Wrin, J. and Koths, K. (1989) Renaturation and purification of biologically active recombinant human macrophage colony-stimulating factor expressed in *E. coli*. Bio/Technol. 7, 710–715.

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

Ho, M. (1991) Cytomegalovirus: Biology and Infection. 2nd edit, Plenum Medical Book Co, New York, NY.

Kern, E.R., Olsen, G.A., Overall, J.C. and Glasgow, L.A. (1977) Treatment of a murine cytomegalovirus infection with exogenous interferon, polyinosinic-polycytidylic acid, and polyinosinic-polycytidylic acid poly-L-lysine complex. Antimicrob. Agents Chemotherapy 13, 344–3.

Kunder, S.C., Wu, L. and Morahan, P.S. (1993a) Role of NK cells in immunomodulator-mediated resistance to herpesvirus infection. Antiviral Res., in press.

Kunder, S.C., Kelly, K.M. and Morahan, P.S. (1993b) Biological response modifier mediated resistance to herpesvirus infections requires induction of α/β interferon. Antiviral Res., in press.

Ladner, M.B., Martin, G.A., Noble, J.A., Nikoloff, D.M., Tal, R. Kawasaki, E.S. and White, T.J. (1987) Human CSF-1: Gene structure and alternative splicing of mRNA precursors. EMBO J. 6, 2693–2698.

Lee, E and Desu, M. (1972) A computer program for comparing k samples with wright-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.

Louria, D.B., Purnendu, S. and Buse, M. (1982) Age-dependent differences in outcome of infections with special reference to experiments in mice. J. Amer. Geriat. Soc. 30, 769–773.

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, pp. 243–251. Plenum, NY.

Morrey, J.D., Mead, J.R., Warren, R.P., Okleberry, K.M., Burger, R.A. and Sidwell, R.W. (1992) Elucidation of mode of retroviral-inhibitory effects of imexon through use of immune competent and severe combined immune deficiency (SCID) mice. Antiviral Res. 17, 223–233.

Murasko, D.M. and Goonewardene, I.M (1990) T cell function in aging: mechanisms of decline. In: V.J. Cristafalo (Ed) Ann. Rev. Gerentol. Geriatrics. 10, 71–96.

Okada, M. and Minashima, Y. (1987) The efficacy of biological response modifiers against murine cytomegalovirus infection in normal and immunodeficient mice. Microbiol. Immunol. 31, 45–57.

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.

Reed, L.J. and Meunch, H. (1938) A simple method of estimating fifty percent endpoints. Amer. J. Hyg. 27, 493–497.

Rytel, M.W. (1987) Effect of age on viral infections: possible role of interferon. J Amer Geriatric Soc 35, 1092–1099.

Scott, P. and Kaufmann, S.H. (1991) The role of T-cell subsets and cytokines in the regulation of infection. Immunol. Today 12, 346–348.

Selgrade, M.K. and Osborn, J.E. (1974) Role of macrophages in resistance to murine cytomegalovirus. Infect. Immun. 10, 1383–1390.

Sigel, M.M. (1952) Influence of age on susceptibility to virus infections with special reference to laboratory animals. Ann. Rev. Microbiol. 6, 247–280.

Wallace, P.K., Pinto, A.J., Murasko, D.M. and Morahan, P.S. (1993) Effects of age on tumor resistance, immunotherapy and macrophage ($M\Theta$) function. Abstract, Second International Congress on Biological Response Modifiers, San Diego, California.

Welsh, R.M., Brubaker, J.O., Vargas-Cortes, M. and O'Donnell, C.L. (1991) Natural killer (NK) cell response in mice with severe combined immunodeficiency. The stimulation of NK cells and the NK cell-dependent control of virus infections occur independently of T and B cell function. J. Exp. Med. 173, 1053–1063.