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Role of NK cells in immunomodulator-mediated resistance to herpesvirus infection

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Summary

Seven chemically diverse biological response modifiers (BRM) were compared for antiviral activity in intact and NK cell-depleted CD-1 mice. Both spontaneous and BRM-induced splenic NK cell cytotoxicity were depleted for at least 5 days following treatment with the monoclonal antibody NK1.1. Antiviral protection of standard doses of MVE-2, pIC, pICLC, rmIFN-τ and CL246,738 against lethal MCMV or HSV-2 infections was not abrogated by NK cell depletion, demonstrating that NK cells are not required for BRM-induced antiviral activity against these herpesviruses. When mice were treated with 100 000 U of rHuIFN-α B/D, NK cells were not required for activity against MCMV, while at a dose of 25 000 U, NK cells appeared to be partially required against MCMV. At lower doses, the activity of rHuIFN-α B/D against MCMV appeared dependent upon the presence of NK cells. A similar dose-related requirement for NK cells was observed for activity of OK-432. Thus, at higher doses of rHuIFN-α B/D and OK-432, elements of the natural immune system in addition to or other than NK cells are apparently involved, while at lower doses NK cells appear to play a more important role in antiviral protection against MCMV infection.

Biological response modifier; MCMV; HSV-2; NK cells

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Introduction

The herpesviruses, which include cytomegalovirus (CMV) and herpes simplex viruses (HSV-1 and HSV-2) are frequent sources of serious disease in immunocompromised humans (Ho, 1991; Osborn,1982). The enhancement of host resistance to herpesvirus infections by biological response modifiers (BRMs), either alone or in combination with antiviral chemotherapy, is one promising approach to treatment (Morahan and Pinto, 1992. For successful BRM treatment of these infections, however, a greater understanding is needed regarding the cells and/or mediators essential to therapy (Pinto et al., 1988).

Murine cytomegalovirus (MCMV) has served as a model system for the study of human CMV because the resulting infections and immune responses are similar (Ho, 1991; Osborn, 1982). HSV-1 and HSV-2 infections in mice have also proved to be useful models for investigating protective immune responses and antiviral therapy for human HSV infections (Harada et al., 1989; Pinto et al., 1988). The major early host resistance mechanisms protecting against MCMV have been demonstrated to be natural killer (NK) cells (Bukowski et al., 1983; Welsh, 1986; Welsh et al., 1990), interferon (IFN) (Bukowski et al., 1987; Chong et al., 1983), and macrophages (Mϕ) (Selgrade and Osborn, 1974; Mims and Gould, 1978). Depletion of any of these arms of host resistance against MCMV infection generally results in increased viral organ titers and often also increased mortality. For example, NK cell depletion with the monoclonal antibody (mab) NK1.1 markedly enhanced the replication of MCMV (Welsh et al., 1990). The use of this mab (Koo and Peppard, 1984; Seaman et al., 1987; Lipscomb et al., 1987) provides a profound and specific depletion of NK cells, without effects on lymphocytes or macrophages. In contrast to the results with MCMV, NK cells appear to be less critical in natural resistance to HSV in mice (Bukowski and Welsh, 1986; Habu et al., 1984). However, a recent clinical report showed a severe HSV infection, as well as CMV infection, in a patient deficient in NK cells (Biron et al., 1989). In vivo depletion of IFN, using polyclonal antisera to IFN α/β, decreased natural resistance to both MCMV and HSV-2 (Chong et al., 1983; Kunder et al., 1992). It is not clear if the involvement of IFN is due to a direct antiviral role or IFN-mediated activation of cellular effectors such as NK cells or Mϕ (Morahan et al., 1985; Bukowski et al., 1987; Pyo et al., 1991). The antiviral BRM-mediated mechanisms described against herpesviruses and other viruses include activation of Mϕ and NK cells, and induction of IFN. However, it is not clear if there is a single antiviral mechanism common to all BRMs. There has been limited investigation of BRM therapy against MCMV and no systematic studies. Protective effects have been demonstrated with prophylactic treatment with pIC (Kern et al., 1977), OK-432 (Ebihara and Minamishima, 1984), and IFN-τ (Fennie et al.,1988), indicating that this herpes virus infection is sensitive to immunomodulation. There has been considerable investigation of BRM prophylactic treatment for HSV infections in mice, which appear to be quite sensitive to treatment with a variety of immunomodulators, including microbially derived, biological and chemical

immunomodulators (Okada and Minamishima, 1987; Gangemi et al., 1989; Harada et al., 1989; Pinto et al., 1988).

The present study was undertaken to determine whether NK cells are required as a common mediator of BRM antiviral resistance against MCMV and HSV-2 infections. Several BRMs with diverse chemical structure were selected for study including the synthetic polyanionic divinyl ether-maleic anhydride (MVE-2) (Carrano et al., 1984); polyriboinosinic polyribocytidylic acid (pIC) (Kern et al., 1977; Pyo et al., 1991) and its nuclease resistant form complexed with carboxymethyl cellulose and poly l-lysine (pICLC) (Kern et al., 1977); the small molecular weight compound CL246,738 (Sarzotti et al., 1989; Wang et al., 1986); and the recombinant products rmIFN-τ (Fennie et al., 1988) and rHuIFNα B/D (Gangemi et al., 1989), composed of segments from HuIFN-αB and HuIFN-αD, lacking the species specificity of many IFNs. Our results demonstrate marked antiviral activity in immunocompetent mice and a minimal role for NK cells in immunomodulator-mediated antiviral protection against both MCMV and HSV-2 herpesvirus infections.

Materials and Methods

Mice

Barrier raised and specific pathogen-free 6–7 week-old CD-1 (Charles River, Montreal, CN or Portage, MI) female mice were used in these studies. Mice were maintained in microisolator cages in a barrier facility (ALAAC accredited) as previously described (Kunder et al., 1992) 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).

Production of monoclonal antibody α-NK1.1

The mab anti-NK1.1 was produced in female Swiss athymic nude mice, 6–8 weeks old (National Cancer Institute, Bethesda, MD). The mice were injected i.p. with 0.5 ml of pristane (Sigma, St. Louis, MO) 10 days prior to i.p. injection with 1×10^7 PK136 hybridoma cells (Koo and Peppard, 1984). 10 days later the ascites fluid was harvested and centrifuged, the supernatant collected, filtered, aliquotted and stored at $-70°C$. HL1610 hybridoma cells (Koo and Peppard, 1984) were used to produce isotype control antibody.

Immunomodulators

All immunomodulators were injected i.p. except for CL246,738 which was administered orally (p.o.). MVE-2 (lot #XA471-19-4) was obtained from Hercules, Wilmington, DE, stored at room temperature, and prepared as required in saline at pH 7 to provide an injection concentration of 50 mg/kg. The rmIFN-τ compound (lot # 2271-68) was obtained courtesy of Genentech, San Francisco, CA and stock solutions were stored at 4°C. It was prepared at a

concentration of 13000 U per 0.2 ml dose, in physiologic saline containing 0.2% bovine serum albumin (BSA). The rHuIFN-α B/D compound (lot 14/170/1), (gift of Dr. J.D. Gangemi, University of South Carolina, Columbia, SC) was prepared at a concentration of 24000 U per 0.2 ml dose, in saline containing 0.2% BSA. Stock solutions and aliquots were stored at −70°C. pIC (lot #11H40411), was purchased from Sigma, St. Louis, MO and was prepared in saline to provide an injection concentration of 5 mg/kg. Stock solutions and aliquots were stored at −70°C. The stock solution of pICLC (lot # UI 84-101, gift of Dr. H. Levy, NCI, Frederick, MD) was stored at 4°C and prepared in saline to provide an injection concentration of 1 mg/kg. The streptococcal preparation OK-432 (kind gift of M. Saito, Chugai Pharmaceutical, Tokyo, Japan) was prepared in saline, at a stock concentration of 1 mg/ml and stored at 4°C. CL246,738 (lot # PC 0594) was obtained courtesy of Dr. Fred Durr, Lederle Laboratories, Pearl River, NY and stored as a powder at room temperature and prepared in distilled water as needed to provide an injection concentration of 100 mg/kg.

Virus preparation, titration and DNA slot blot hybridization

The Smith strain of MCMV (ATCC VR-194) was passaged by i.p. infection of 4–6 week old female CD-1 mice and harvesting salivary glands 17–19 days after infection as previously described (Selgrade and Osborn, 1974; Kunder et al., 1992). 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.

For some studies, livers and lungs were harvested and pooled on day 3 after viral infection. Total DNA extraction and subsequent slot blot hybridization were performed as described (Sambrook et al., 1989). The MCMV DNA probe containing an insert of *Hin*dIII fragment κ (immediate early gene region) was a kind gift of Drs. Ann Campbell and Richard Steinberg, Eastern Virginia Medical School, Norfolk, VA. Plasmids were used as nick translation probes, prepared using a kit purchased from Bethesda Research Laboratories (Grand Island, NY).

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 routinely performed during preparation of dilutions for both viruses.

NK cytotoxicity assay

Splenic NK cell cytotoxicity was measured in a standard 4 h ^{51}Cr-release assay using ^{51}Cr-labeled YAC-1 target cells (10^4 cells/ml) at effector/target cell ratios from 200:1 to 50:1 on a 96-well microtiter plate at 37°C and 5% CO_2. Target cells were labeled with 100 μCi $Na_2{}^{51}CrO_4$ (ICN Radiochemicals,

Irvine, CA) for 1 h followed by a 1.5 h incubation to minimize spontaneous ^{51}Cr release, which did not exceed 20%. Cytotoxicity was expressed as: % ^{51}Cr-release = (observed cpm − spontaneous cpm)/(total cpm − spontaneous cpm) × 100.

Antiviral protection assays

Mice were treated with either mab NK1.1 or control antibody 48 h prior to infection with either MCMV or HSV-2. All immunomodulators were administered 24 h prior to infection excepting IFN-α B/D and IFN-τ, which were injected 24 h and 4 h prior to infection. Survival was monitored for 21 days following infection.

Statistical methods

Statistically significant differences ($P < 0.05$) in mortality were determined by chi-square test, using the ABStat program (Anderson-Bell, 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) as previously described (Kunder et al., 1992). The Student's *t*-test was used to determine significance between groups in cytotoxicity and viral plaque assays.

TABLE 1

Establishment of schedule and dilution for NK cell depletion of splenic NK cell cytotoxicity with mab NK1.1 treatment of CD-1 mice

			% Cytotoxicity ± S.E.[c]		
Group[a]	pIC[b]	*n*	200:1	100:1	50:1
Experiment 1					
Saline	−	4	7.0 ± 1.7	5.8 ± 1.8	4.8 ± 1.7
Saline	+	4	18.5 ± 2.1	14.5 ± 3.1	8.6 ± 1.8
Ab control	+	4	16.3 ± 2.2	12.5 ± 1.9	9.2 ± 2.4
NK1.1, 2×	−	4	1.6 ± 1.6	0	1.1 ± 1.1
NK1.1, 2×	+	5	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.2
NK1.1, 1×	−	5	0	0	0
NK1.1, 1×	+	4	3.2 ± 1.9	0.2 ± 0.7	1.3 ± 0.8
Experiment 2					
Saline	−	5	7.1 ± 1.5	5.8 ± 1.1	3.1 ± 1.0
Saline	+	5	29.7 ± 2.8	22.0 ± 2.8	10.3 ± 1.8
NK1.1 (1:50), 2×	+	5	0.9 ± 0.0	0	0
NK1.1 (1:50), 1×	+	5	2.2 ± 1.3	3.6 ± 1.6	0.8 ± 0.6
NK1.1 (1:25), 1×	+	4	0.1 ± 0.1	0	0
NK1.1 (1:5), 1×	+	5	0	0	0

[a]CD-1 female mice were injected i.p. 4 days prior to assay with mab NK1.1, saline, or an antibody control (Koo et al., 1986). Mice receiving 2 injections of mab NK1.1 received a second injection on day −3 relative to assay. For injection, mab NK1.1 or the control monoclonal antibody was diluted 1:5 or as noted from unpurified ascites fluid.

[b]Mice were injected i.p. with polyinosinic-polycytidylic acid (pIC) at 5 mg/kg on the day prior to assay day −1).

[c]Spleen cells were assayed for NK cell cytotoxicity against YAC-1 cells using effector/target cell ratios of 200:1, 100:1, and 50:1.

Results

Marked depletion of spontaneous and BRM enhanced splenic NK cell cytotoxicity in CD-1 mice by treatment with mab αNK1.1

Outbred CD-1 mice were injected with mab NK1.1 control antibody or saline to determine whether NK cell activity could be suppressed for a period over which NK activity might be induced following BRM treatment or MCMV infection. Treatment with mab NK1.1 significantly depleted both spontaneous and pIC-induced NK cell cytotoxicity levels using either one or two injections of mab (Table 1). One dose at a 1:25 dilution of ascites fluid was chosen for subsequent experiments.

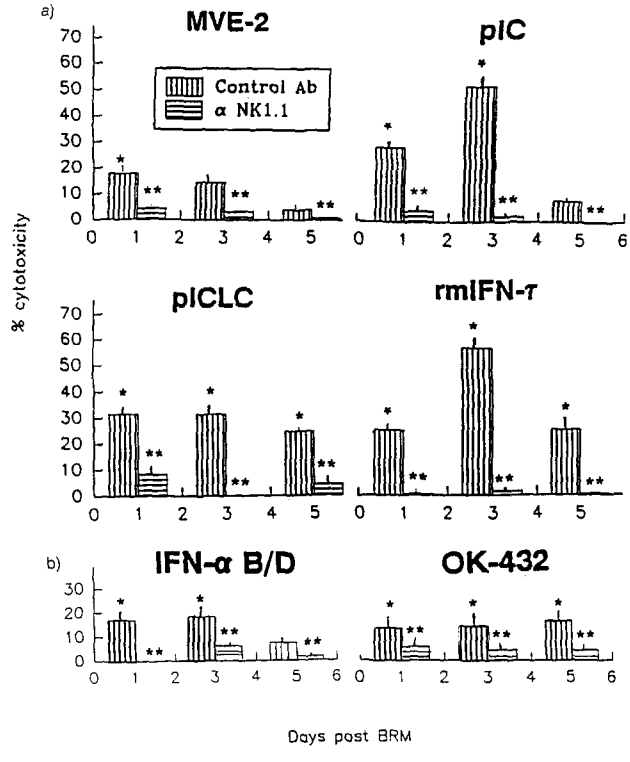


Fig. 1. CD-1 female mice (5–7 weeks old) were injected i.p. with either mab NK1.1 or an isotypic control on day −1. Mice (3–4/group) were injected i.p. with BRM on day 0, except rmIFN-τ and, rHuIFN-α B/D, which were injected twice, 20 h apart on day 0. A standard 4 h ^{51}Cr release assay with YAC-1 target cells was performed on separate sets of mice on days +1, +3, +5 following BRM treatment. The % cytotoxicity ± S.E. is given for an effector/target cell ratio of 100:1. Control cytotoxicity for panel a: Saline + control Ab = 13.8 ± 1.3%, saline + NK1.1 = 4.0 ± 2.3%. Control cytotoxicity for panel b: Saline + control Ab = 6.2 ± 0.1%, saline + NK1.1 = 0%.

*Significant increase ($P < 0.05$) in mice treated with control Ab, as compared with the spontaneous NK cell cytotoxicity in the saline control.

**Significant decrease ($P < 0.05$) in mice treated with mab NK1.1 as compared with cytotoxicity in corresponding mice treated with control Ab.

The treatment with mab NK1.1 was effective in depleting enhanced NK cell activity following treatment with all the BRMs used in this study (Fig. 1). Whether the kinetics of NK cell activation showed a peak at 1 or 3 days, or was shortlived or prolonged for 5 days, mab NK1.1 significantly decreased BRM-induced NK cell cytotoxicity.

Depression of natural resistance to MCMV following splenic NK cell depletion by mab NK1.1

To examine whether NK cell depletion with mab NK1.1 enhanced MCMV

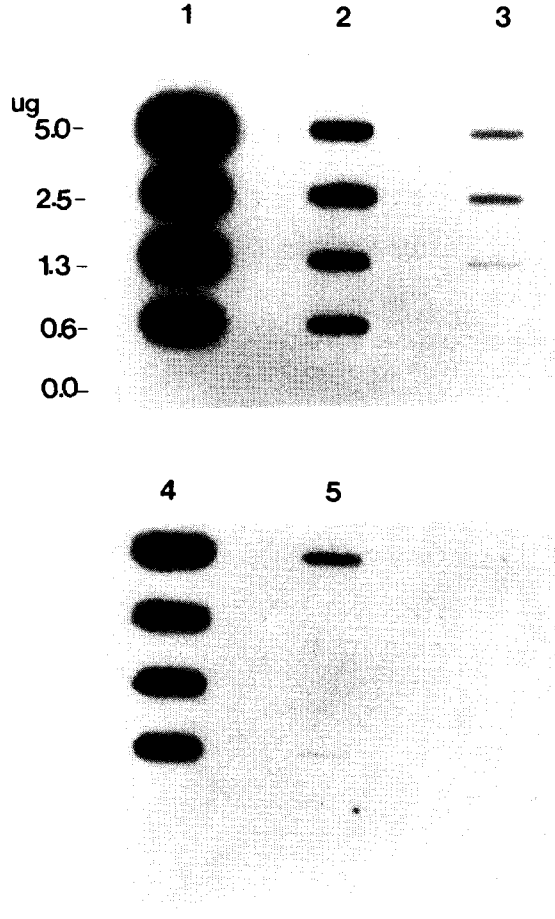


Fig. 2. Depletion of NK cells in CD-1 mice increased MCMV DNA replication. Livers and lungs were harvested and total cellular DNA extracted. DNA slot blot hybridization was performed to detect viral DNA with probes containing an insert of *Hin*dIII fragment K (immediate early gene region). Lane 1 = *Hin*dIII fragment plasmid DNA control; Lane 2 = liver, no mab NK1.1 treatment; lane 3 = lung, no mab NK1.1 treatment; lane 4 = liver, with mab NK1.1 treatment; lane 5 = lung, with mab NK1.1 treatment; because insufficient DNA was obtained from this sample, 5.0 μg DNA was spotted on top well only and no DNA was spotted in remaining wells.

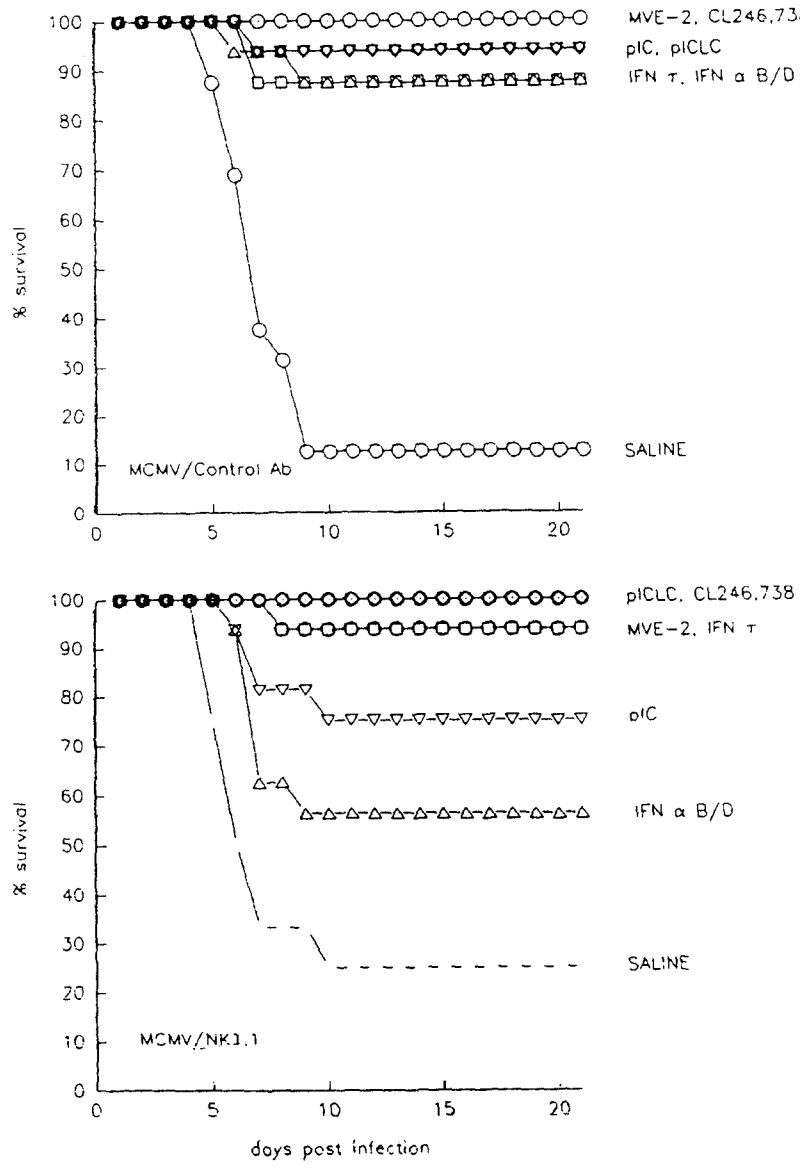


Fig. 3. CD-1 female mice (5–7 weeks old) were treated with mab NK1.1 on day −2 and infected i.p. with 8 × 10^5 PFU MCMV on day 0. BRMs were injected at −24 h i.p. except CL246,738, which was administered p.os., and rmIFN-τ and rHuIFN-α B/D, which were injected i.p. at −24 h and −4 h. Mice not receiving mab NK1.1 received an isotype antibody. Survival was monitored for 21 days. All BRMs provided significant ($P < 0.05$) protection compared to the corresponding saline-treated control. Survival of mice receiving rHuIFN α B/D and treated with mab NK1.1 was significantly ($P < 0.05$) less than the corresponding group receiving an isotype control Ab.

replication, mice were killed 3 days after infection when MCMV replication is easily detectable in the liver, spleen and lungs. NK cell depletion resulted in increased MCMV titers in the spleen (6.3×10^5 PFU/g) as compared with a titer of 3.2×10^4 PFU/g in mice receiving control antibody. The MCMV titer in NK cell depleted mice was also increased in the liver, to 1.6×10^6 PFU/g from 6.0×10^2 PFU/g MCMV in control antibody treated mice. These results were confirmed by direct detection of MCMV DNA from liver and lungs of infected mice (Fig. 2). Viral replication was clearly increased in both organs in mice depleted of NK cells. Thus there was definite enhancement of early replication and spread of MCMV in NK cell depleted mice, although there was no significant effect on the ultimate outcome of the infection (Fig. 3). There was 10% survival with 5 days median survival time (MST) in mice treated with control antibody and 22% survival with 5 days MST in mice treated with mab NK1.1.

Lack of requirement for NK cells in BRM-mediated protection against MCMV and HSV-2 infection

In intact mice, prophylactic treatment with all seven BRMs tested provided

TABLE 2

OK-432 dose-related requirement for NK cells in protection against MCMV infection

Exp #	Group[a]	dose (mg/kg)	NK1.1	Mortality Median Survival		
				Dead/Total	%	Time (days)
1	Saline		–	7/8	87.5	5
			+	7/8	87.5	5
	OK-432	10	–	0/8	0	>21
			+	0/8	0[b]	>21[b]
		5	–	0/8	0[b]	>21[b]
			+	0/8	0[b]	>21[b]
		1	–	0/8	0[b]	>21[b]
			+	0/8	0[b]	>21[b]
		0.5	–	0/8	0[b]	>21[b]
			+	2/8	25.0	>21[b]
1	Saline		–	7/8	87.5	5
			+	7/8	87.5	5
	OK-432	0.5	–	2/8	25.0	>21[b]
			+	5/8	62.5[c]	5[c]
		0.1	–	1/8	12.5[b]	>21[b]
			+	8/8	100.0[c]	5[c]
		0.05	–	7/8	87.5	5
			+	7/8	87.5	5

[a]CD-1 female mice (5–7 weeks old) were injected i.p. with either mab NK1.1 (+) or an isotypic control (–) on day –1. Mice (3–4/ group) were injected i.p. with OK-432 on day –1. Mice were infected on day 0 with MCMV, 8×10^5 PFU/mouse and monitored for survival for 21 days.

[b]Significant increase ($P < 0.05$) as compared with survival of the appropriate saline control group treated with either control Ab or mab NK1.1.

[c]Significant decrease ($P < 0.05$) in mice treated with mab NK1.1 as compared with survival in corresponding mice treated with control Ab.

significant protection as compared to control animals receiving saline. Thus, a wide variety of chemically diverse BRMs can provide protection against MCMV (Fig. 3, Table 2) and HSV-2 (Fig. 4) infections in mice, at doses comparable to those effective against other infections (Pinto et al., 1988; Pinto

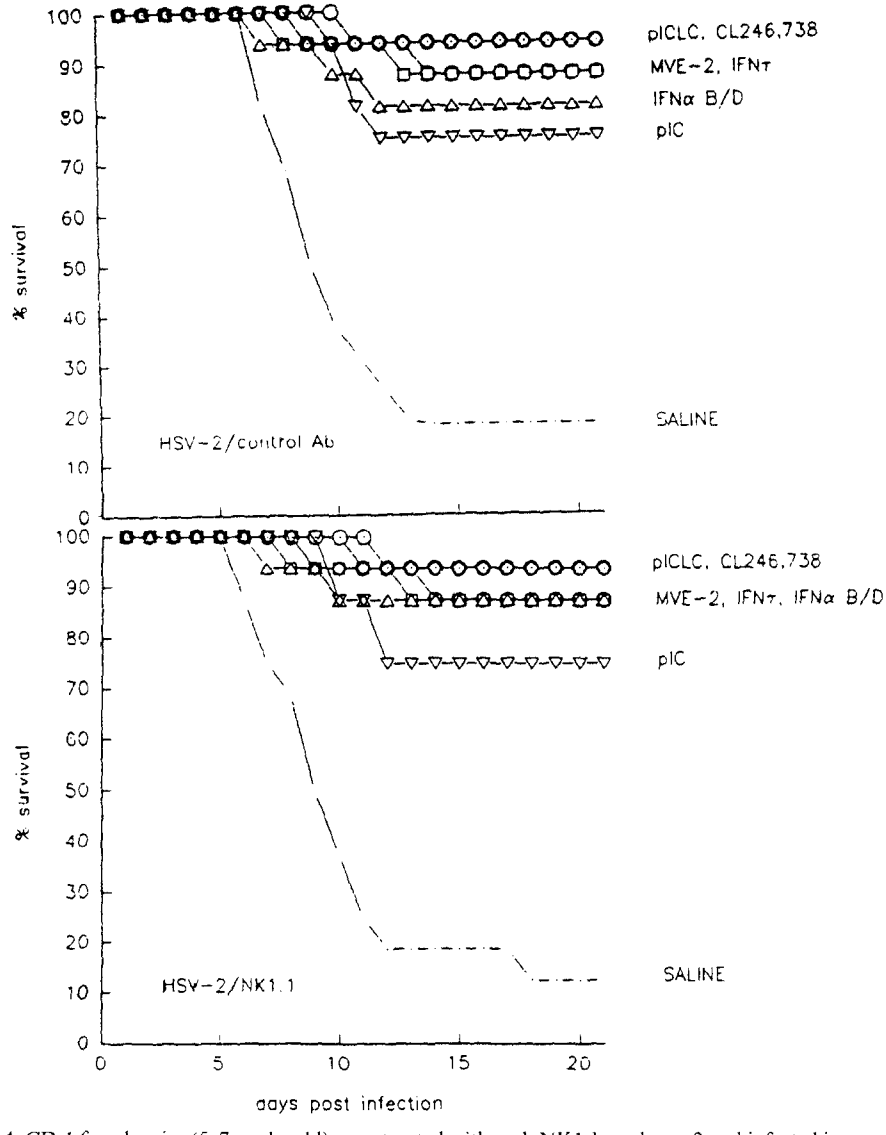


Fig. 4. CD-1 female mice (5–7 weeks old) were treated with mab NK1.1 on day −2 and infected i.p. on day 0 with 1.3×10^4 PFU HSV-2. BRMs were injected at −24 h i.p. except CL246,738, which was administered p.os., and rHuIFN-α B/D and rmIFN-τ, which were injected at −24 h and −4 h. Mice not receiving mab NK1.1 received an isotype antibody. Survival was monitored for 21 days. All BRMs provided significant ($P < 0.05$) protection compared to the corresponding saline-treated control.

et al., 1990; Morahan et al., 1987).

Depletion of NK cells with NK1.1 generally did not diminish protection provided by these doses of BRMs against MCMV or HSV-2 infection. With all BRMs, survival against either virus was still significantly enhanced in NK cell depleted mice. However, treatment with IFN-α B/D (25 000 U) against MCMV infection in NK cell depleted mice was clearly less effective than in intact mice (Fig. 3). In intact mice rHuIFN-α B/D treatment resulted in 87.5% survival, while in NK cell depleted mice it was reduced to 56% survival ($P < 0.05$).

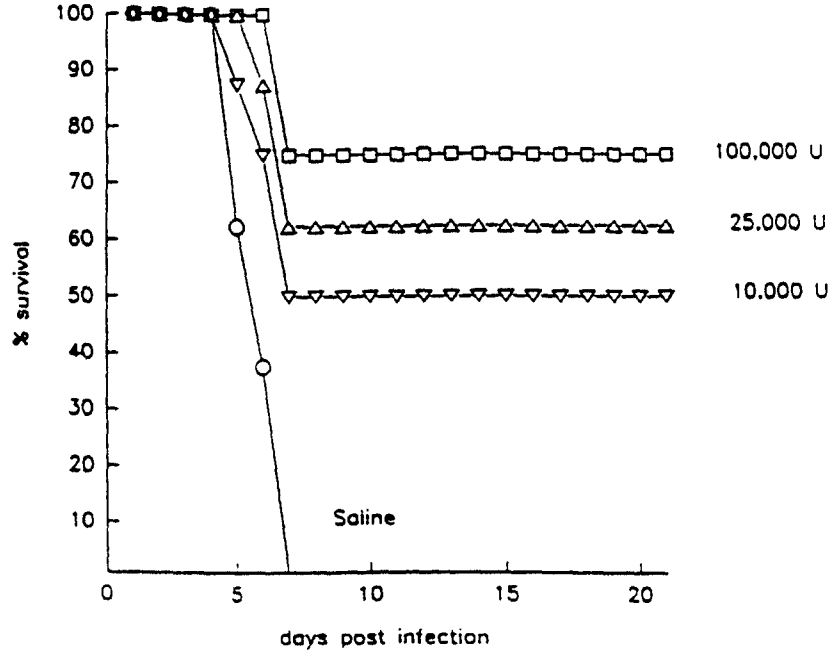
Dose-related requirement for NK cells in protection against MCMV by OK-432 and rHuIFN-α B/D

Because of the reduced effectiveness of rHuIFNα-B/D against MCMV in NK cell depleted mice, further studies were performed to define the role of NK cells in rHuIFN-α B/D antiviral effects (Fig. 5). There was no difference in protection at the 100 000 U dose between control and NK cell depleted groups. NK cell depletion, however, caused a significant loss of protection at the lower doses. At the 25 000 U dose there was a consistent decrease in activity in three separate experiments, as evidenced by either a decrease in protection from mortality and/or in median survival times (Figs. 3 and 5). The partial protection (50%) provided by the 10 000 U in intact mice dose was almost completely eliminated in the NK cell depleted mice (Fig. 5).

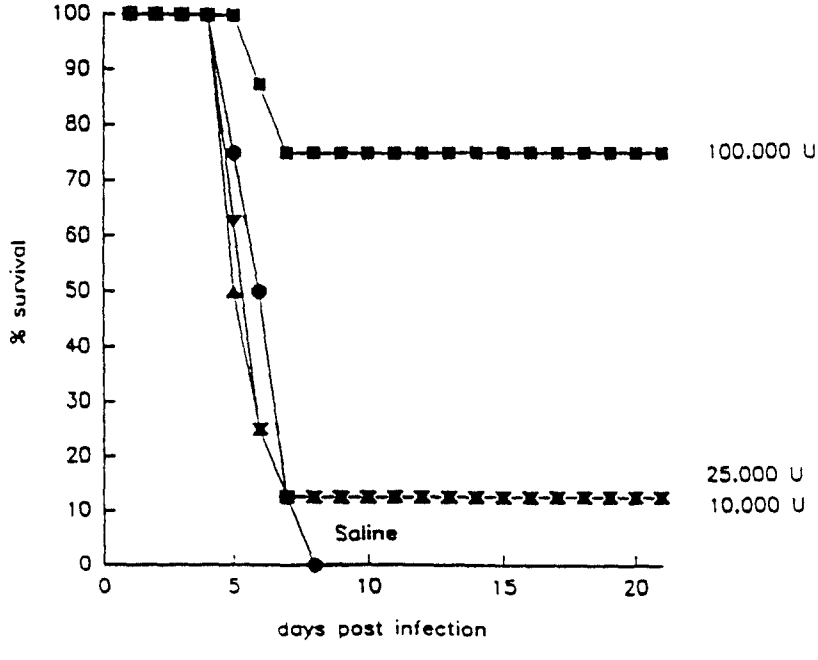
A previous report concluded that NK cells were required for the antiviral action of OK-432, demonstrated using the less specific anti-asialo GM1 antiserum NK cell depletion method (Ebihara and Minishima, 1984). Therefore, a range of doses was tested using the more selective mab NK1.1 (Table 2). When mice were treated with 10, 5 and 1 mg/kg doses of OK-432, survival against MCMV was unaffected by NK cell depletion. With treatment of 0.5 mg/kg, there was a variable decrease in protection in NK cell depleted mice. With the 0.1 mg/kg dose, there was almost complete protection in intact mice, while protection following NK cell depletion was completely lost. There was no protection against MCMV at the lowest dose, 0.05 mg/kg, in either intact or NK cell depleted mice.

Discussion

This is the first comparative study of the role of NK cells in antiviral immunomodulation by drugs with diverse structures, and the first using the very specific mab NK1.1. We have demonstrated that NK cells were not required for antiviral efficacy against either MCMV or HSV-2 at the doses tested for five chemically diverse BRMs, including the polyanionic BRMS, pICLC, pIC and MVE-2, the recombinant derived polypeptide IFN-τ, and the small molecular weight compound CL246,738. The few previous studies used asialo-GM1 polyclonal antibody or ^{89}Sr for NK cell depletion (Smee et al., 1990; Bukowski et al., 1983; Morahan et al., 1986). Protection by pIC against



MCMV : IFN α B/D + Control Ab



MCMV : IFN α B/D + NK1.1

sublethal MCMV infection appeared to be independent of NK cells (Bukowski et al., 1987), as was efficacy of 7-thia-8-oxoguanosine against Semliki forest alphatogavirus infection (Smee et al., 1990). Protection by the streptococcal preparation OK-432 against MCMV was found to partially require NK cells (Eihara and Minamishima, 1984).

In the present studies, NK cells were not required for antiviral protection with rHu IFN-α B/D and for OK-432 at the usual doses employed (Pinto et al., 1988; Ebihara and Minamishima, 1984). However, NK cells were required at lower doses. This requirement for NK cells for antiviral activity may reflect enhancement of NK cell activity by IFN-α B/D and OK-432 at low doses, without stimulation of other cells or mediators. At higher doses, at which NK cells are not required, there apparently is activation of other antiviral mechanisms, in addition to NK cells, that can compensate in the face of depletion of NK cells with mab NK1.1. For example, treatment of mice with the selective Mϕ activating agent, colony stimulating factor 1 (CSF-1) can provide antiviral activity against MCMV infection (Chong and Piazza, 1989), suggesting that Mϕ activation alone can be sufficient for BRM-mediated antiviral efficacy. With rHuIFN-α B/D, direct antiviral effects mediated through the IFN-induced oligo 2,5A synthetase system (Kirchner, 1986) may be sufficient at the higher doses, while indirect effects amplified through effector cells such as NK cells may be required at the lower doses of IFN. The present data suggest that there may be a hierarchy of activation within the host resistance system underlying the dose-related changes in requirement for NK cells but do not exclude other cytotoxic cells from the mediation of anti-viral protection against MCMV infection. These results emphasize the need for determining the mechanism of action of BRMs over a range of doses in order to develop optimal antiviral immunotherapy.

The difference in results with rHuIFN-α B/D against MCMV and HSV-2 infections is intriguing. In HSV-2 infection, the IFN treatment at 25 000 U was more effective and was completely independent of NK cells, while in MCMV infection there was partial requirement for NK cells. It is unlikely that differences in sensitivity to the direct antiviral effects of IFN alpha are involved, since depletion of alpha/beta IFN markedly decreases natural resistance to both of these herpesvirus infections (Kunder et al., 1992). Different mechanisms of pathogenesis may be involved, while the major target organ of HSV-2 infection is the CNS, the liver, spleen and lungs are the major targets in MCMV infection (Ho, 1991). The fact that depletion of NK cells did not affect rHuIFN-α B/D protection against HSV-2 infection may also reflect the minimal role of NK cells in natural resistance to HSV-2 as compared with a greater role in MCMV infections (Fig. 3 and Bukowski and Welsh, 1986).

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Fig. 5. CD-1 female mice (5–7 weeks old) were injected i.p. with either mab NK1.1 or an isotypic control on day −2. Mice (8/group) were injected i.p. with rHuIFN-α B/D, twice, 20 h apart on day −1. Mice were infected on day 0 with MCMV, 8×10^5 PFU/mouse and monitored for survival for 21 days.

As others have reported in other mouse strains, we found that depletion of NK cells decreased resistance of CD-1 outbred mice to MCMV infection. Treatment of mice with mab NK1.1 prior to lethal infection with MCMV increased both MCMV DNA synthesis and titers of infectious virus in target organs, but not overall mortality. Therefore, in both sublethal (Welsh et al., 1990; Shanley, 1990) and lethal infections (present data), NK cells are involved in limiting the early spread of MCMV. However, while NK cells may control viral replication in acute sublethal infection, their presence when a lethal viral inoculum is used does not affect survival. This situation has been seen in other infection models (Lipscomb et al., 1987).

This study helps clarify the role of NK cells in the antiviral activity of a group of seven chemically diverse BRMs against systemic herpesvirus infections. The minimal role of NK cells in mediating BRM-induced antiviral protection indicates the need to investigate other antiviral mechanisms, such as IFN and cytokine induction and Mϕ activation, in order to elucidate which antiviral mechanisms may be common to diverse BRMs and responsible for their antiviral protection.

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