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The *in vivo* and *in vitro* effects of interleukin-1 and tumor necrosis factor on murine cytomegalovirus infection

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Abstract

The effects of tumor necrosis factor (TNF) and interleukin-1 (IL-1) on infection with murine cytomegalovirus (MCMV) were investigated *in vitro* and *in vivo*. The addition of each of these cytokines (at 1 ng/ml) to tissue culture monolayers 24 hr prior to MCMV challenge produced a reproducible decrease in vital titer (from 1×10^8 pfu to approximately 4×10^6 pfu for both cytokines). There was no further increase in this effect when a 10 or 100 ng/ml of each of these cytokines was employed. Despite these *in vitro* effects, the pretreatment of suckling, weanling, or adult mice with 80 or 400 ng of TNF or IL-1 alone, or 80 ng of each cytokine together, had no effect on the survival of mice following MCMV. Similarly, neither of these cytokines adversely influenced the protective effects of hyperimmune anti-MCMV antiserum; that is, they did not attenuate the protection conferred by the antiserum nor affect the protective effects of subtherapeutic doses of the antiserum. We conclude that despite promising antiviral effects against MCMV *in vitro*, these agents do not result in a useful therapeutic effect *in vivo*. Moreover, despite the ability of IL-1 to induce ACTH and corticosterone in mice, IL-1 treatment did not increase the mortality to CMV.

Interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF) are cytokines produced by mononuclear phagocytes in response to inflammatory stimuli which mediate the acute phase response [1, 2]. A deleterious role for these cytokines has been postulated to occur in the pathogenesis of the protean clinical manifestations of gram negative sepsis. However, we have previously demonstrated that the administration of a low dose of human recombinant IL-1 is capable of protecting neutropenic mice with a lethal *Pseudomonas aeruginosa* or *Candida albicans* infection [3, 4]. In addition, increased nonspecific resistance to *Klebsiella pneumoniae* infection has been induced with recombinant IL-1 [5, 6] and recombinant TNF [6, 7].

These studies raise the possibility that cytokines might have potential utility in the treatment of life-threatening infection, particularly infection occurring in immunocompromised individuals. A particularly challenging form of infection in this clinical context is that caused by cytomegalovirus (CMV). Recently, several groups [8–14] have reported that TNF has significant antiviral effects, both by itself and in synergistic combination with gamma interferon, when assayed *in vitro*. In these studies, one of the viruses most intensively studied was herpes simplex virus, a herpes group virus closely related to CMV. In addition, IL-1 and TNF have been shown to induce beta interferon in fibroblasts in vitro [15]. Because of these observations, and

because current therapy of life-threatening CMV syndromes (even with the antiviral drug gancyclovir) remains difficult [16], we embarked on a series of studies to evaluate the effects of IL-1 and TNF on the course of CMV infection. Utilizing the murine CMV system we were able to demonstrate a significant anti viral effect *in vitro*, but not *in vivo*. The course of murine CMV (MCMV) in mice of varying ages appears to be unaffected by the exogenous administration of recombinant IL-1 or TNF.

Materials and methods

Animals

Female BALB/c mice obtained from Cumberland View Farms (Clinton, TN) were used in all *in vivo* experiments. One week old mice were used in the experiments in which suckling animals were employed, three week old mice in the experiments in which weanling mice were employed, and six week old mice in the experiments in which adult mice were employed. Infected mice were kept in bonneted cages to prevent cross infection, with no more than six animals per cage. They were maintained on Purina Laboratory Chow and water *ad lib.*

Virus

The Smith strain of MCMV, originally obtained as a 10% (wt/vol) homogenate of infected salivary gland tissue from Dr. John D. Shanley (Veterans Administration Medical Center, University of Connecticut, Newington, CT), was used in these experiments. The virus is maintained in this laboratory by serial passage in CD-1 Swiss Webster mice (Charles River Breeding Laboratories, Inc., Wilmington, MA). Three week old CD-1 mice were inoculated intraperitoneally with 10^4 plaque forming units (pfu) of MCMV. Twenty-one days after infection, the salivary glands were harvested and homogenized in Dulbecco's Medium containing 10% fetal calf serum and 10% dimethyl sulfoxide. The virus stock was a 10% (wt/vol) homogenate of infected salivary gland with a titer of 2×10^8 pfu/ml. Uninfected salivary gland homogenates (NSG) were prepared in a similar fashion from weanling mice inoculated with saline. Both stocks were stored at -70°C until used [17].

Virologic techniques

Quantitative assays for infectious virus were performed on tissue homogenates (10% wt/vol) prepared in Dulbecco's medium containing 10% fetal calf serum. These were then assayed in secondary mouse embryo fibroblasts prepared from 12–15 day CD-1 Swiss Webster embryos under an overlay with 1% methylcellulose according to previously described methods [10].

Experimental design

In the *in vivo* experiments, adult or weanling mice were administered one of the following regimens intraperitoneally (ip): 400 or 80 ng of recombinant IL-1 beta (obtained from Cistron Biotechnology, Inc., Pinebrook, NY or Glaxo Institute of Molecular Biology, Geneva, Switerland), 80 ng of human recombinant TNF alpha (Biogen, Inc., Cambridge, MA), or a combination of 80 ng of both cytokines. In one series of experiments, the cytokine administration was followed two hours later by the ip administration of 0.2 ml of a variety of dilutions of a hyperimmune anti-MCMV antiserum prepared as previously described [12]. Twenty-four hours later, mice were challenged ip with a variety of doses of MCMV or with an equal volume of NSG. Mice were then monitored on a daily basis for mortality. In experiments in which suckling mice were employed, the same experimental design was employed, except that lower doses of the cytokines were employed: 40 ng of either IL-1, TNF, or both.

In the *in vitro* experiments, secondary mouse embryo fibroblasts (MEF) were prepared from embryos of 12–15 day CD-1 Swiss Webster mice as described above. Cells were maintained

in Dulbecco's minimum essential medium containing 10% fetal calf serum, 200 U of penicillin per ml, and 200 mcg of streptomycin per ml. Secondary MEF monolayers were plated in six or twelve well tissue culture plates (Falcon Plastics, Oxnard, CA) with Dulbecco's medium. Cell layers were treated with purified recombinant human gamma interferon (Biogen, Inc., Cambridge, MA), IL-1 beta, TNF alpha, or combinations of these, at concentrations of 1, 10, or 100 ng/ml in Dulbecco's medium. After 24 hr incubation at 37°C and 5% CO_2, supernatants were removed and the cell layer washed twice with fresh medium. MCMV was then added with an MOI = 1. Control wells did not receive any virus or received only virus. Cells were incubated for 90 min at 37°C in 5% CO_2 with rocking of the plate every 15 minutes to ensure the spread of virus and maximize adsorption. Two ml of media were then added to each well, and plates were incubated for 5 days under the same conditions. On day 5, supernatants were collected and frozen at −70°C. These were subsequently thawed and quantitatively assayed for the amount of virus present as described above.

Results

The addition of TNF, IL-1, and gamma interferon to tissue culture monolayers 24 hr prior to challenge with MCMV produced a reproducible decrease in viral titer (from 9.1×10^7 pfu/ml to approximately 2.5×10^6 pfu/ml for all three cytokines). There was no significant difference in the magnitude of the effect, whether 1, 10, or 100 ng/ml of each of these cytokines was employed (Table 1).

The pretreatment of suckling, weanling, or adult mice with IL-1 or TNF either alone or together had no effect on the survival of mice following challenge with MCMV. Similarly, there were no discernible adverse effects of these cytokines when they were administered to animals challenged with noninfected salivary gland homogenate (NSG). In addition to the lack of effect of these cytokines on animal survival (Table 2), there was no effect on the time of death in animals (data not shown).

Table 1. Effects of interleukin-1 (IL-1) tumor necrosis factor (TNF), and interferon (IFN) on murine cytomegalovirus *in vitro*.*

Treatment	Dose	Viral titer (pfu)
None	—	9.1×10^7
TNF	1 ng	4.2×10^6
TNF	10 ng	2.1×10^6
TNF	100 ng	2.2×10^6
IL-1	1 ng	2.5×10^6
IL-1	10 ng	2.9×10^6
IL-1	100 ng	2.6×10^6
IFN	1 ng	2.8×10^6
IFN	10 ng	1.7×10^6
IFN	100 ng	1.1×10^6

* Results are the mean values obtained in three separate experiments in which each determination was carried out in duplicate.

Pretreatment of animals with hyperimmune anti-MCMV antiserum provided full protection against lethal challenge when a 1 : 4 dilution of the antiserum was employed, but no protection when a 1 : 16 dilution was administered. The addition of 80 ng of IL-1 to these therapies had no discernible effect in that it did not detract from the efficacy of the 1 : 4 dilution of antiserum and did not improve the results obtained with the 1 : 16 dilution (Table 3).

Discussion

There are now several reports suggesting that tumor necrosis factor and other cytokines will selectively kill virus-infected cells in tissue culture systems [8–14]. Among the viruses shown to be susceptible to this effect are herpes simplex virus, vesicular stomatitis virus, adenovirus, and encephalomyocarditis virus. Because of the need for more effective treatment regimens in the management of cytomegalovirus infection, particularly in

Table 2. Effects of pretreatment with interleukin-1, tumor necrosis factor, on both the survival of mice challenged intraperitoneally with murine cytomegalovirus.*

Age of mouse**	Dose of virus (pfu)	Pretreatment	Survival # survival/ # challenged
A. Suckling	1×10^4	diluent	2/21
	1×10^4	40 ng IL-1	1/21
	1×10^4	40 ng TNF	2/21
	1×10^4	40 ng IL-1 & TNF	1/21
	NSG	40 ng IL-1 & TNF	21/21
B. Weanling	7.5×10^5	diluent	0/12
	7.5×10^5	400 ng IL-1	0/11
	7.5×10^5	80 ng IL-1	1/18
	7.5×10^4	inactivated IL-1	1/6
	7.5×10^4	400 ng IL-1	0/6
	7.5×10^4	80 ng IL-1	0/6
	7.5×10^5	80 ng TNF	0/6
	7.5×10^5	80 ng IL-1 & TNF	1/6
	NSG	400 ng IL-1	6/6
	NSG	80 ng TNF	6/6
	NSG	80 ng IL-1 & TNF	6/6
C. Adult	7.5×10^5	diluent	3/10
	7.5×10^5	80 ng IL-1	2/10
	7.5×10^5	80 ng TNF	1/11
	7.5×10^5	80 ng IL-1 & TNF	1/10
	NSG	80 ng IL-1 & TNF	10/10

* Mice were pre-treated with ip 24 hr prior to challenge with murine cytomegalovirus.
** Balb/c mice one (suckling), three (weanling) and six (adult) weeks old were employed in these experiments.

immunosuppressed hosts, we attempted to extend these *in vitro* observations to a murine CMV system and to compare the effects of cytokines *in vitro* and *in vivo*. The results

Table 3. Effects of pretreatment with interleukin-1 (IL-1) and anti-CMV antiserum on the survival of weanling mice challenged with murine cytomegalovirus.*

Dilution of antiserum used	Dose of IL-1	Survival # surviving/ # challenged
1 : 4	0	6/6
1 : 16	0	0/6
1 : 4	80 ng	6/6
1 : 16	80 ng	0/6

* Mice were pretreated 24 hr prior to lethal challenge with 7.5×10^5 pfu of murine CMV with either 80 mg of IL-1 or a comparable amount of heat inactivated IL-1, followed 2 hr later by 0.2 ml of a 1 : 4 or 1 : 16 dilution of anti-CMV hyperimmune antiserum.

reported in this study reveal, that *in vitro*, there are striking decreases in yield of virus when the cell monolayer is pretreated with IL-1, TNF, or gamma interferon. These effects are seen with doses of 1 ng/ml but did not increase at higher doses of 10 and 100 ng. The decrease in viral titer is approximately two logs of virus. This is less than that observed with the other viruses previously studied, including the closely related herpes simplex virus.

Regardless of the *in vitro* effects, however, there was no benefit of pretreatment with these cytokines individually or in combination when intact animals were challenged with lethal doses of MCMV. This remained true whatever age and degree of immunologic maturity of the animal studied. Similarly, the administration of these cytokines did not

produce adverse effects. This was despite the ability of IR-1 to increase ACTH and corticosterone in mice. In addition, the administration of IL-1 appeared to have no effect on the protection provided by hyperimmune anti-CMV antiserum. This series of experiments did not investigate the efficacy *in vivo* of alternate dosing regimens. It is possible that repetitive dosing schedules could have a protective effect *in vivo*.

The results reported in this study serve to emphasize an important point. The potential therapeutic benefits of cytokine administration or of an antiviral agent must be assessed in the intact animal, not only in cell or tissue culture systems. The complexity of the biologic effects of these agents, and the wide range of cell types that respond to them place a premium on their evaluation in intact animal systems that permit an assessment of the net biologic effect rather than just one aspect of their many actions.

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