

Additive Effects of Acyclovir and Immune Transfer in Neonatal Herpes Simplex Virus Infection in Mice

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Acyclovir had a dose-dependent, mild, but significant, inhibitory effect on interferon-stimulated human antiviral natural killer cytotoxicity in vitro. In a murine model of neonatal herpes simplex virus infection, acyclovir significantly ($P < 0.05$) increased survival afforded by the injection of human interferon and human mononuclear leukocytes from 67.8 to 88.6%.

Natural killer cytotoxicity (NKC) is the ability of a subset of lymphocytes to destroy tumor cells or virus-infected target cells in the absence of antibody (2). Interferon is a potent stimulator of NKC (1, 2, 4). We have previously demonstrated that neonatal C57BL/6 mice have very low levels of NKC to herpes simplex virus (HSV)-infected target cells. The ontogeny of this activity correlated with murine resistance to HSV infection (5). More recently, we have demonstrated that neonatal mice can be partially protected from HSV infection by a combination of human natural interferon and human lymphocytes (6). This observation most likely represented protection by stimulated human NKC or cross-species active antiviral protein production. The present series of experiments were performed to determine whether acyclovir, a potent anti-HSV antiviral agent, affected anti-HSV NKC in vitro and could add to the improved survival from HSV infection achieved with immune transfer in vivo.

The virus used throughout was an acyclovir-sensitive HSV type 1 (HE strain; acyclovir 50% infective dose, 1.22 $\mu\text{g/ml}$, as determined by a dye-uptake assay, kindly performed in the laboratories of Colin McLaren, Burroughs Wellcome Co., Research Triangle Park, N.C.).

The 18-h cytotoxicity assay, in which human Ficoll-Hypaque (Pharmacia Fine Chemicals Inc., Piscataway, N.J.), purified mononuclear cells, and HSV type 1-infected, ^{51}Cr -labeled Chang liver target cells were used, was performed as previously described (4, 5). Effector cells were preincubated in 1,000 U of human alpha-interferon (produced by recombinant DNA technology, designated IFLrA, and kindly provided by Patrick Trown, Hoffmann-La Roche Inc., Nutley, N.J.) and acyclovir (kindly provided by Gertrude Elion, Burroughs Wellcome Co.) for 2 h before the assay. The interfer-

on and acyclovir were present throughout the entire assay period. Acyclovir had a slight, but significant, dose-dependent inhibitory effect on human interferon-stimulated NKC when present throughout the assay (Table 1). It can be seen that this effect was minimal at expected clinically achievable levels of less than 50 $\mu\text{g/ml}$.

To ascertain the in vivo effect of acyclovir in the infant mouse model (6), 1-week-old C57BL/6 mice received intraperitoneally (i.p.) various combinations of human Ficoll-Hypaque-purified mononuclear cells (5×10^6 cells) and 1,000 U of recombinant DNA-produced human alpha-interferon (IFLrA) in a volume of 0.1 ml 1 day before i.p. challenge with HSV type 1 (10^5 PFU; 100 50% lethal doses per mouse).

Animals receiving acyclovir alone or with the above agents received subcutaneously 0.30 mg (50 mg/kg of body weight per dose) beginning 1 day before and resuming 1 day after viral challenge for 7 days (Fig. 1).

Our previous studies have revealed that animals receiving human mononuclear cells alone or interferon alone uniformly succumbed to this HSV challenge (6). In the present study, no animals survived receiving acyclovir or interferon alone (Fig. 1). One animal receiving acyclovir plus mononuclear cells survived (not significant). In contrast, the expected protection was demonstrated in animals receiving human mononuclear cells plus interferon (67.8% survival; $P < 0.001$ compared with survival of animals receiving acyclovir, interferon, or acyclovir plus mononuclear cells by chi-square analysis). Animals receiving human mononuclear cells and interferon before HSV challenge and acyclovir beginning 1 day before and resuming 1 day after HSV challenge also had a significantly increased survival rate (88.6% survival; $P < 0.001$ compared with mice receiving acyclovir, interferon, or mononuclear cells plus acyclovir). In addi-

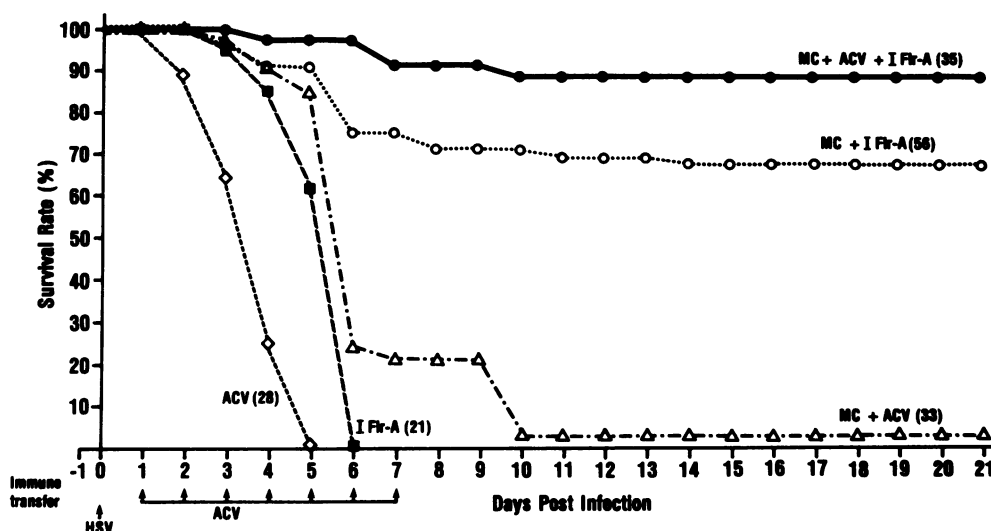


FIG. 1. Protection of mice from HSV infection. One-week-old mice received 0.30 mg of acyclovir (ACV) subcutaneously for 7 days, beginning 1 day before and resuming 1 day after HSV challenge (10^5 PFU i.p.) (\diamond); human DNA recombinant alpha-interferon (IFLrA), 1,000 U i.p. 1 day before HSV challenge (\blacksquare); human mononuclear cells (MC; 5×10^6 per mouse) i.p. 1 day before challenge and acyclovir before and after challenge (\triangle); human mononuclear cells plus interferon (1,000 U) i.p. 1 day before challenge (\circ); or human mononuclear cells and interferon 1 day before challenge and acyclovir beginning 1 day before and resuming 1 day after challenge (\bullet). The numbers in parentheses represent the number of mice in each group.

tion, these animals had a significantly ($P < 0.05$) higher survival rate than those receiving only mononuclear cells plus interferon. In experiments not shown, but performed simultaneously, none of 13 animals receiving acyclovir and interferon survived. Similarly, of over 100 infant mice, none receiving virus alone survived.

Thus, acyclovir had mildly inhibitory effects on human NKC in vitro (Table 1). This may

have been owing to the production of the active antiviral metabolites by the HSV thymidine kinase from the target cell virus, which then were inhibitory to the leukocyte effector cells (3). Experiments with acyclovir in nonviral NKC systems will enable the ascertainment of whether acyclovir or its antiviral-active metabolic products are responsible for the inhibition of NKC.

Despite the mild inhibition of NKC by acyclovir, in our infant mouse model of HSV infection, acyclovir improved the survival rate of mice reconstituted with human interferon and lymphocytes (Fig. 1). The lack of enhanced survival by acyclovir alone was probably due to the use in this model of a high (100 50% lethal doses), systemic (i.p.) viral challenge. In neonatal humans with disseminated HSV infection, as in this mouse model, antiviral therapy with vidarabine, the only therapy currently published, has had minimal beneficial effects (7). These data demonstrating an enhanced survival in animals receiving both antiviral and immunomodulating therapy would argue for the trial of a similar approach in human neonates, in whom the outcome of antiviral therapy alone is less than optimal (7).

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TABLE 1. Effect of acyclovir on interferon-stimulated NKC

Acyclovir concn ($\mu\text{g/ml}$) ^a	% NKC ^b	$P <^c$
0	37.4 \pm 11.2	
10	30.5 \pm 9.9	0.05
50	25.6 \pm 12.7	0.025
100	26.9 \pm 9.5	0.025
500	21.9 \pm 10.6	0.005

^a Concentration of acyclovir in natural killer cell assay.

^b Percentage of NKC in an 18-h assay in which 30 mononuclear leukocytes per target cell were used in the presence of 1,000 U of recombinant DNA interferon (IFLrA; Hoffmann-LaRoche Inc.) and the indicated concentration of acyclovir. Data are the mean \pm standard deviation of four separate experiments.

^c P is the significance of the difference between NKC at the indicated concentration of acyclovir compared with that obtained by assay with no acyclovir (0), by Student's paired t test.

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