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Cytomegalovirus infection in immunocompromised guinea pigs: a model for testing antiviral agents in vivo

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Summary

An experimental model for testing antiviral agents against severe cytomegalovirus (CMV) infection in immunocompromised hosts was developed. The model consisted of cyclophosphamide (Cy) treatment of CMV-infected guinea pigs to simulate CMV infection in immunodeficient individuals. Of the 3 Cy regimens tested, a single 300 mg/kg dose administered one day after virus inoculation resulted in the most severe CMV infection considering mortality rates, mean day of death and loss of body weight. Evaluation of responses to both T and B cell mitogens suggested that the severe and lethal CMV infection resulted from the combined immunosuppressive effect of Cy and CMV. The nucleoside analog [9-(1-3-dihydroxy-2-propoxymethyl)guanine (DHPG) was used to assess the usefulness of the CMV-infected immunocompromised host model. DHPG (100 mg/kg/day for 8 days) prevented death but did not reduce virus infectivity titers in blood of Cy-treated, CMV-infected guinea pigs. This model of CMV infection in immunocompromised guinea pig is a relevant and convenient experimental tool for the assessment of candidate anti-CMV agents under well-defined experimental conditions, such as appropriate CMV inoculum and Cy regimen.

Cytomegalovirus infection; Immunocompromised guinea pig; 9-(1-3-Dihydroxy-2-propoxymethyl)guanine; AIDS; Bone marrow transplant; Antiviral testing

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Introduction

Cytomegalovirus (CMV) is capable of producing a broad spectrum of clinical diseases in humans. While CMV infections are frequently asymptomatic, their occurrence in immunocompromised hosts such as transplant and AIDS patients can be clinically significant (Armstrong et al., 1985; Drew, 1988; Dworsky et al., 1984; Lerner and Tapper, 1984; Macher et al., 1983; Masur et al., 1986; Palestine et al., 1984). Because of the association of CMV with morbidity and mortality in the immunocompromised host, there is an urgent need for the development of both preventive and therapeutic interventions against this viral infection.

Evaluation of the efficacy of candidate antiviral compounds requires not only testing in vitro, but also in vivo studies in a well-defined experimental model. Because of the strict species specificity demonstrated by human CMV (HCMV), testing of potential antiviral agents for activity against HCMV in animals is not possible. The guinea pig model of CMV infection is well suited for testing antiviral agents because the pathogenesis of the viral infection in this animal model closely approximates that in the human host. Most importantly, transplacental transmission of CMV, which does not occur in mice, has been demonstrated in guinea pigs (Choi and Hsiung, 1978; Griffith et al., 1986; Johnson and Connor, 1979; Kumar and Nankervis, 1978). An experimental syndrome similar to human CMV-associated mononucleosis has been described in immunocompetent guinea pigs with acute CMV infection (Griffith et al., 1981). This model has been used to test antiviral agents (Chen et al., 1988; Fong et al., 1987; Lucia et al., 1984). However, candidate antiviral compounds have not been evaluated systematically in immunocompromised guinea pigs infected with CMV. To date, a convenient model has not been defined for clinically relevant testing of antiviral drugs although severe generalized CMV infections have been shown to occur in guinea pigs treated with cyclosporine (Bia et al., 1985; MacGregor et al., 1986; Markham et al., 1987).

This study was designed to define an experimental model of CMV infection in immunocompromised guinea pigs suitable for antiviral agent testing. The severity of CMV infection was evaluated in guinea pigs given various regimens of cyclophosphamide (Cy) and virus inocula. In addition, non-specific immune cell responses were evaluated in the various groups of Cy- and CMV-treated and untreated animals. Finally, the acyclic nucleoside analog 9-(1-3-dihydroxy-2-propoxymethyl)guanine (DHPG) was assessed as to its ability to alter the course and severity of CMV infection in immunocompromised guinea pigs.

Materials and Methods

Animal inoculation

Two- to three-week-old female Hartley guinea pigs (Camm Research Institute, Wayne, NJ), weighing 220–250 g were inoculated subcutaneously in the left axilla with 1 ml of salivary gland-passaged (passage level 31) guinea pig CMV (Prototype

strain No. 22122, American Type Culture Collection, Rockville, MD). Animals received either undiluted, 1:10, 1:100 or 1:1000 dilution of the virus stock containing 10^7 PFU/ml on the day of inoculation designated as day 0. Control animals received no virus.

Drug treatment

Cyclophosphamide (Cy) was dissolved in phosphate-buffered saline (PBS) at a concentration of 30 mg/ml and administered intraperitoneally according to the following regimens: (a) a single dose of 300 mg/kg, two days prior to CMV inoculation (group Cy 300, day −2), (b) a single dose of 300 mg/kg, one day after virus inoculation (group Cy 300, day 1), and (c) regimens of daily single doses of 30 mg/kg/day starting one day before or after CMV inoculation and lasting for one, four or five days.

The compound 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) was kindly provided by Dr. Thomas Matthews (Syntex Research, Mt. View, CA). It was solubilized in warm PBS at a concentration of 4 mg/ml and given at a dose of 100 mg/kg/day for eight days starting one day after virus inoculation. All DHPG treatments were administered subcutaneously at 4 different sites (i.e., both axillae and groin areas) for even distribution. The sham-treated group was treated likewise with PBS. A localized superficial induration was observed at the site of multiple injections of DHPG.

Animal evaluation

Body weights were recorded and animals were observed for death daily. In some experiments, spleen and lung weights were also recorded.

Virus infectivity titers in blood, spleen, lung and brain were determined on day 7–10 post CMV inoculation. Blood samples were collected via cardiac puncture on the 7th day post CMV inoculation (Griffith et al., 1981). Blood, spleen, lung and brain cells were collected, 10% (weight/volume) tissue suspensions were made and serial 10-fold dilutions were prepared. Each dilution (0.1 ml) was inoculated onto guinea pig embryo cell monolayers on 24-well plates (Costar, Cambridge, MA) (Hsiung et al., 1976). Virus adsorption was allowed to proceed for 90 min after which 1 ml/well of minimal essential medium containing Earle's balanced salt solution and 5% calf serum was added. Four consecutive weekly examinations of cells for evidence of virus-induced cytopathic effect were done. Selected viral isolates were blind-passaged and identified by neutralization with specific antisera (Bia et al., 1979). In some experiments, leukocyte counts were determined microscopically using a hemocytometer after lysis of erythrocytes with acetic acid (Unopette Microcollection System, Becton-Dickinson Company, Rutherford, NJ).

Assay of proliferative responses to mitogens

Spleen cells were subjected to mitogen stimulation using concanavalin A (Con A), which stimulates predominantly T cells, and lipopolysaccharide (LPS), which stimulates predominantly B cells in the guinea pig (Elfenbein et al., 1973; Gregerson et al., 1975). It has been shown that splenic lymphocytes obtained from animals aged 1 day to 12 months respond well to phytohemagglutinin (PHA) and Con A (Merikanto et al., 1979). Spleens were aseptically removed and placed in RPMI containing 10% inactivated calf serum, antibiotics and glutamine, perfused with 30 ml of the same media, then gently passed through a sterile 230 μm mesh sieve (Cellector, Bellco, Vineland, NJ). The spleen cells were serially washed and centrifuged as described before (Griffith et al., 1984).

Spleen cell suspensions adjusted to 10^6 cells/0.1 ml/well were dispensed in flat-bottomed 96-well plates (Costar). Stock solutions of Con A and LPS were prepared as previously described (Griffith et al., 1984). Cultures were prepared in triplicate and received no mitogen or final concentrations of either Con A at 20 or 40 μg/ml or LPS at 50 μg/ml.

Cells were harvested after 2 days of culture with LPS and 4 days of culture with Con A. A solution (0.05 ml) containing 16 μCi/ml of methyl-[^{3}H]thymidine (New England Corp., Boston, MA) was added to each well 4 h prior to harvesting onto glass-fiber filter strips. [^{3}H] TdR uptake was determined using a Beckman Scintillation spectrometer.

Statistical analysis

The unpaired *t*-test was used to test for statistical significance unless otherwise indicated.

Results

Effect of various Cy regimens on the severity of acute guinea pig CMV infection

In the first group of experiments, guinea pigs were inoculated with 2.4×10^6 PFU of CMV and treated with various regimens of Cy to assess the effect of various Cy treatments on the severity of acute guinea pig CMV infection. Death rates are shown in Fig. 1. Mortality rates in uninfected guinea pigs treated with 300 mg/kg Cy and in sham-treated, infected animals were 5% and 0%, respectively. Mortality rates were higher in Cy-treated than in sham-treated, infected guinea pigs and were highest (100%) in the infected guinea pigs treated with 300 mg/kg Cy on either day −2 or day 1. Cy given at 30 mg/kg/day consecutively for 4 days starting a day prior to virus inoculation resulted in more deaths (83%) than when given singly on either day −1 or day 1 (17%). Earliest deaths were seen in Cy 300, day 1 group (mean day of death = 9.5). Mean days of death in the other groups ranged from day 10–13 with the exception of the group given 30 mg/kg Cy on day 1 where the

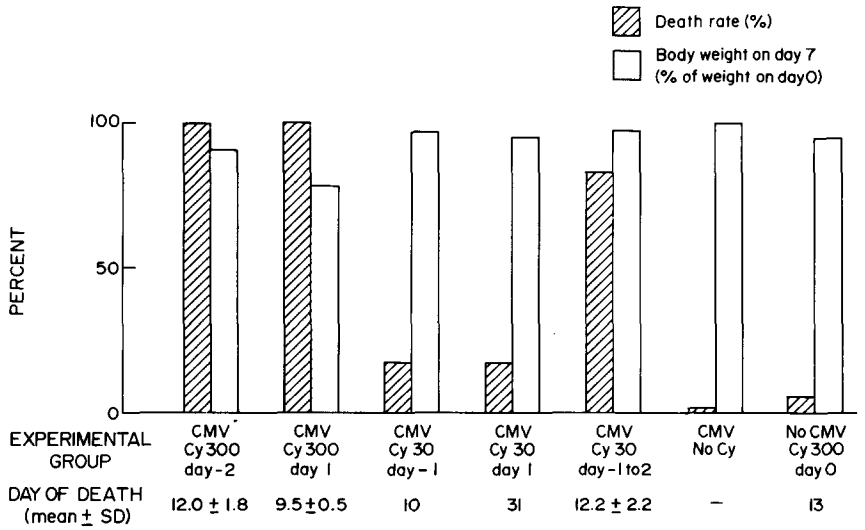

Fig. 1. Death rates and body weight changes in guinea pigs inoculated with 2.4×10^6 PFU of CMV and given various regimens of Cy. $N = 6$ in each group except Cy 300 group without CMV ($N = 13$). Body weight on day 7 is expressed as % of weight on day 0 (base 100).

mean day of death was 31. Body weight changes expressed as % weight loss on day 7 compared to day 0 are also shown in Fig. 1. Cy-treated infected guinea pigs lost weight as compared to sham-treated infected guinea pigs. Among the Cy-treated, CMV-infected guinea pigs, those treated with either 300 or 30 mg/kg Cy on day 1 lost weight significantly compared to their sham-treated counterparts ($P<0.0001$ and $P<0.05$, respectively). The greatest weight loss seen in the Cy 300, day 1 group differed significantly from that in the different Cy-treated groups ($P<0.01$) except the Cy 300, day −2 group. As determined in a different set of experiments (Table 1), virus infectivity titers in spleen and lung of Cy-treated, CMV-infected guinea pigs were significantly different from those of sham-treated, infected animals ($P<0.0001$). However, virus infectivity titers in the brain of Cy-treated, CMV-infected guinea pigs did not differ significantly from those of sham-treated, infected animals. As shown in Table 1, a significant decrease in body weights, total leukocyte counts and spleen to body weight ratios in Cy-treated, infected compared to sham-treated guinea pigs was also noted ($P<0.001$). Lung to body weight ratios in both groups were not significantly different from each other.

Effect of different virus inocula on the severity of acute CMV infection in immunocompromised guinea pigs

The effect of various inoculum doses (10^7, 10^6 and 10^5 PFU of CMV) on the severity of CMV infection in immunocompromised animals was determined in other groups of experiments. Guinea pigs given 3 different Cy regimens were used for study. Death rates are shown in Table 3. Guinea pigs treated with 300 mg/kg Cy

TABLE 1

Effect of cyclophosphamide (Cy) on the severity of guinea pig CMV infection on days 7–10 days post-CMV inoculation

	CMV + CY[a] (N = 8) mean ± SD	CMV (N = 13) mean ± SD
Clinical parameters		
Weight change[b]	86 ± 9*	108 ± 6
Total leukocyte count[c]	495 ± 469*	3032 ± 720
Spleen to body weight ratio $\times 10^{-2}$	3.4 ± 1.3*	6.9 ± 1.1
Lung to body weight ratio $\times 10^{-2}$	12.1 ± 1.7	11.6 ± 1.6
Virus infectivity titers[d]		
Spleen	3.8 ± 0.7*	1.8 ± 0.8
Lung	2.9 ± 0.4*	1.9 ± 0.5
Brain[e]	1.1 ± 0.7	0.1 ± 0.2

[a]Cy (300 mg/kg) was administered on day 1 after CMV inoculation.
[b]Weight on day 7 post-guinea pig CMV inoculation expressed as % of weight on day 0 (base 100).
[c]Expressed as number of leukocytes per mm^3.
[d]Mean Log_{10} $TCID_{50}$ per 0.01 g of tissue.
[e]Only 4 animals tested in CMV + Cy group and 5 animals tested in CMV group. 4/4 in CMV + Cy group and 2/5 in CMV group were virus-positive.
*Significantly different from sham-treated animals ($P<0.001$, unpaired t-test).

(day −2 or day 1) and given 10^7 PFU of virus had the highest mortality rates (71–100%). In contrast, guinea pigs treated with Cy 300 mg/kg (day −2 or day 1) and inoculated with 10^6 or 10^5 PFU had mortality rates of 44–67% and 50–75%,

TABLE 2

Virus infectivity titers in blood[a] of guinea pigs given various Cy[b] regimens and virus inocula

Virus inoculum (PFU/ml)	Cy treatment		% Positive (N)	Virus titers (mean log_{10} $TCID_{50}$/ml) ± SD
	Dose (mg/kg)	Timing		
10^7	300	day −2	100 (7)	4.1 ± 0.9
	300	day 1	100 (4)	3.5 ± 0.8
	30	days −1 to 3	100 (4)	3.8 ± 0.8
	0		75 (3)	1.3 ± 0.3
10^6	300	day −2	100 (7)	3.8 ± 1.3
	300	day 1	100 (4)	2.3 ± 0.2
	30	days −1 to 3	100 (4)	2.9 ± 0.4
	0		88 (4)	2.0 ± 1.0
10^5	300	day −2	100 (8)	4.1 ± 1.1
	300	day 1	100 (4)	3.4 ± 0.9
	300	days −1 to 3	100 (4)	3.8 ± 1.0
	0		100 (4)	2.3 ± 0.2

[a]Viremia was assessed on day 7 post-CMV inoculation.
[b]Cyclophosphamide.

TABLE 3

Mortality rates and body weights in guinea pigs given various Cy[a] regimens and virus inocula

Virus inoculum (PFU/ml)	Cy treatment		(% Mortality) No. deaths/No. tested	Mean body weights[b] ± SD		
	Dose (mg/kg)	Timing		D_0	D_7	% Change[c] (*N*)
10^7	300	day −2	5/7	284 ± 24	292 ± 12	103 ± 9 (8)
	300	day 1	4/4	296 ± 19	289 ± 22	98 ± 3 (4)
	30	day −1 to −3	4/4	214 ± 105	285 ± 15	101 ± 5 (4)
	0		0/4	291 ± 16	296 ± 23	102 ± 7 (4)
10^6	300	day −2	4/9	282 ± 20	279 ± 17	100 ± 5*(11)
	300	day 1	4/6	294 ± 16	270 ± 28	92 ± 8*(7)
	30	day −1 to −3	6/6	294 ± 19	282 ± 19	96 ± 4*(8)
	0		1/6	286 ± 22	299 ± 23	105 ± 3 (8)
10^5	300	day −2	4/8	294 ± 11	311 ± 15	106 ± 4*(8)
	300	day 1	3/4	301 ± 10	313 ± 22	104 ± 5*(4)
	30	day −1 to −3	1/3	299 ± 4	331 ± 14	111 ± 5*(4)
	0		0/4	311 ± 11	374 ± 12	121 ± 3 (4)

[a]Cyclophosphamide.
[b]Obtained on D_0 (day 0, day of CMV inoculation) and D_7 (day 7, post-CMV inoculation).
[c]Weight on day 7 post-guinea pig CMV inoculation expressed as % of weight on day 0 (base 100). (*N*) = no. of animals per group.
*Significantly different from sham-treated animals ($P<0.0001$ to $P<0.05$, unpaired *t*-test).

respectively. In animals given 30 mg/kg Cy from day −1 to 3, mortality rates were the same in guinea pigs given either 10^7 or 10^6 PFU (100%), while animals that received 10^5 PFU of CMV had much lower mortality rate (33%). Only 1 (which received 10^6 PFU of virus) of 13 CMV-infected animals that were not given Cy died. In each of the Cy-treated groups, there were no significant differences in mortality rates among animals given 10^7, 10^6 and 10^5 PFU/ml of CMV. Body weights of Cy-treated and sham-treated, CMV-infected guinea pigs were obtained 7 days post-CMV inoculation and compared with weights taken on day 0 (Table 3). Weight differences between Cy-treated and sham-treated guinea pigs given either 10^6 or 10^5 PFU of CMV were statistically significant ($P<0.0001$ to $P<0.05$). Regardless of CMV inoculum dose, the groups treated with 300 mg/kg Cy on day 1 had the greatest decrease or the least increase in weight.

As shown in Table 2, Cy-treated guinea pigs infected with 10^7 and 10^5 PFU of CMV had mean virus infectivity titers in blood that were significantly higher than those of sham-treated, infected animals ($P<0.001$, unpaired *t*-test to $P<0.05$, one tail test). However, significant differences in virus titers were less readily demonstrable in guinea pigs inoculated with 10^6 PFU of CMV because of large animal to animal variation in this Cy-treated group. Only the Cy 300, day −2 group, given 10^6 PFU of CMV had significantly higher mean virus titer than its sham-treated counterpart ($P<0.05$).

Proliferative responses to mitogens in immunocompromised guinea pigs with acute CMV infection

A group of experiments was performed to obtain baseline data on the time course of changes following one single Cy administration (300 mg/kg) in uninfected animals. Changes in blood leukocyte counts and spleen cell responses to mitogens in uninfected guinea pigs given 300 mg/kg Cy were determined on days 7–10 and 20 post Cy administration (Fig. 2). There was a significant decrease in blood leukocyte counts 7 days after Cy treatment with a return to normal levels on the 10th and 20th days after treatment. On days 7 and 10 post-Cy inoculation, spleen cell responses to LPS and Con A were reduced as compared to control values. Although spleen cell responses to both mitogens were low the response to LPS was relatively lower than that of Con A on days 7 and 10. By day 20, responses to Con A were restored, but responses to LPS remained lower than control values.

To determine the combined effect of Cy plus CMV on non-specific immune responses, experiments were also performed to assess the capacity of spleen cells from Cy- or sham-treated, CMV-infected or uninfected guinea pigs to respond to mitogens. Animals were tested 7 days post-virus inoculation. Results are expressed as mean ratios of counts in stimulated cultures over counts in unstimulated cultures (stimulation index, SI). CMV-infected guinea pigs treated with Cy were unable to respond to either Con A or LPS regardless of the Cy regimen (SI values ranged from 0.3 ± 0.1 to 1.2 ± 0.2; mean background CPMs ranged from 268 ± 12 to 6744 ± 3167). In contrast, uninfected guinea pigs treated with Cy showed inhibition of responses to LPS primarily (SI values ranged from 0.2 ± 0.0 to 1.1 ± 0.2; mean background CPMs ranged from 573 ± 356 to 12182 ± 3552). Responses to Con A (SI) in the same animals ranged from 2.9 ± 0.8 to 16.4 ± 15.6;

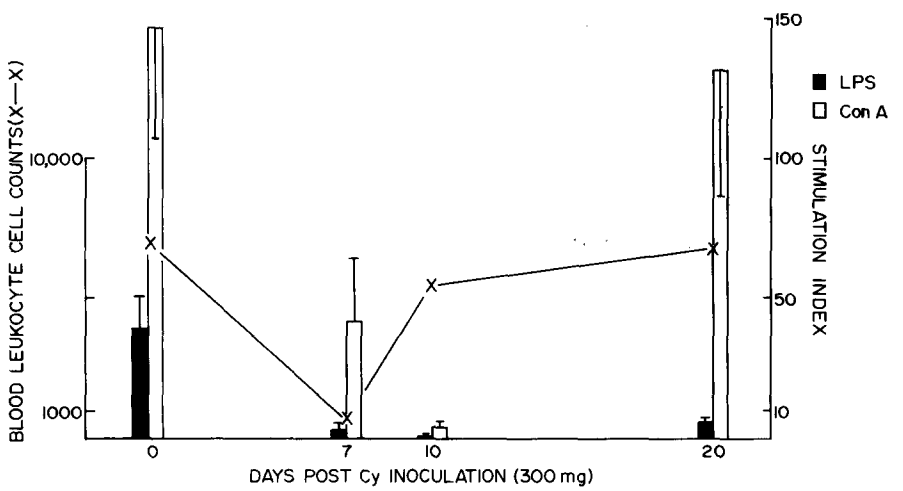

Fig. 2. Time course of changes in blood leukocyte counts (x–x) and spleen responses to mitogens in uninfected guinea pigs given Cy.

mean background CPMs ranged from 576 ± 262 to 742 ± 281. CMV-infected animals not treated with Cy showed only reduction of responses to Con A (SI = 3.2 ± 2.2; mean background CPM = 664 ± 418) was compared to control untreated animals (SI = 64.8 ± 84.1; mean background CPM = 291 ± 143).

Effect of DHPG on the severity of acute CMV infection in Cy-treated, CMV-infected guinea pigs

In order to assess the usefulness of this model, the ability of the antiviral agent DHPG to alter the course and severity of CMV infection in the immunocompromised guinea pigs was evaluated. Based on earlier results of this study, guinea pigs treated with 300 mg/kg Cy on day −2 and 300 mg/kg Cy on day 1 were chosen for study as they exhibited the most severe disease and immunodepression. Fig. 3 shows the mortality rates in Cy-treated, CMV-infected guinea pigs in the presence or absence of DHPG treatment. Five to 7 animals were examined in each group except those inoculated with 10^7 PFU of CMV where only 2 animals were examined. Mortality rates were lower in all DHPG-treated as compared to sham-treated guinea pigs except in animals inoculated with 10^4 PFU of virus and treated with 300 mg/kg Cy on day 1 and in animals given 10^7 PFU of virus. Differences between DHPG- and sham-treated guinea pigs reached statistical significance in the group infected with 10^6 PFU/ml of CMV and treated with 300 mg/kg Cy on day −2, 86% among sham-treated animals as compared to 14% in DHPG-treated animals ($P<0.05$, Fishers' exact test). Two of the ten guinea pigs given DHPG alone died. Guinea pigs given DHPG alone had 107% mean body weight change on day 7 as compared to day 0 (data not shown). Virus infectivity titers in blood obtained 7 days post-CMV inoculation of Cy-treated, CMV-infected guinea pigs treated with DHPG were not significantly different from titers in sham-treated animals (data not shown).

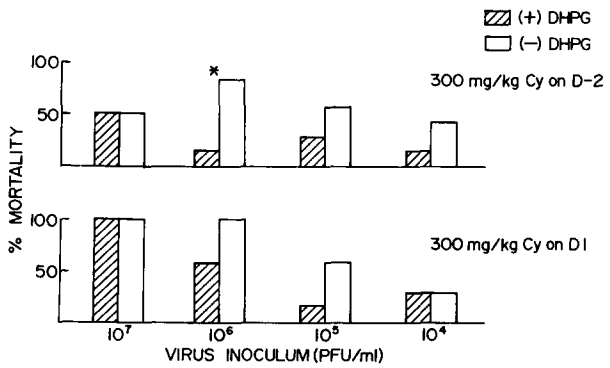

Fig. 3. Mortality rates in Cy-treated, CMV-infected guinea pigs in the presence or absence of DHPG treatment. *$P<0.05$ for sham-treated vs. DHPG-treated, CMV-infected guinea pigs given Cy.

Discussion

This study has characterized a model of CMV infection in immunocompromised guinea pigs, which parallels that of human CMV infection in immunodeficient individuals. The model was found to be an appropriate and convenient experimental tool for the assessment of candidate anti-CMV agents as to their effect on CMV infection in immunocompromised hosts. However, well-defined experimental conditions, such as appropriate CMV inoculum and Cy regimen, must be used.

Acute CMV infection of immunocompetent guinea pigs is generally sub-clinical and self-limited (Griffith et al., 1981). In the present study, administration of Cy before or after CMV inoculation resulted in a severe and generalized CMV infection in guinea pigs. Of the 3 Cy regimens used in this study, 300 mg/kg single dose administered a day after virus inoculation resulted in the most severe disease considering mortality rates, mean day of death and body weight loss. This finding supports earlier studies indicating that Cy as an immunosuppressive agent is active if given before or after an antigenic stimulus, but its effect is greater when given after (Makinodan et al., 1970).

CMV-infected guinea pigs treated with Cy failed to respond to either B or T cell mitogen probably because of the combined depressive effect of CMV and Cy on the host's immune response. CMV infection by itself causes immunosuppression both in humans and experimental animals (Ho, 1984). Normal individuals with early CMV infection have decreased production of interferon induced by CMV or mitogens and reduced lymphocyte proliferative responses to heterologous viral antigens and mitogens (Ho, 1984; Rinaldo et al., 1980). In the guinea pig, an asynchronous depression of responses to the T and B cell mitogens has been shown to occur during acute CMV infection (Griffith et al., 1984). Despite this decrease in immune response, CMV-infected guinea pigs that are not given exogenous immunodepressing compounds experience self-limited, sub-clinical CMV infection. In the present study, the immunodepression caused by CMV infection exacerbated by the immunocompromising effect induced by Cy treatment resulted in severe and lethal CMV infection; however, the exact mechanism for such a response was not explored. Sham-treated, CMV-infected guinea pigs tested 7 days post-CMV inoculation had reduced proliferative responses of spleen cells to the T cell mitogen Con A. This is in agreement with a previous study which demonstrated reductions of T and B cell functions occurring 6–8 days and 11–15 days, respectively, post-CMV inoculation (Griffith et al., 1984). The finding of the most severe inhibition of responses to the B cell mitogen in uninfected guinea pigs treated with Cy is consistent with previous reports (Fauci et al., 1974; Horwitz, 1974; Poulter and Turk, 1972; Stockman et al., 1973; Turk and Poulter, 1972). It has been suggested that this B cell selectivity reflects differences in recovery rates of T and B cells, with the former being reconstituted at a more rapid rate (Winkelstein, 1977).

DHPG was used to assess the usefulness of the immunocompromised host model because it is the only established anti-CMV agent (Collaborative DHPG Treatment Study Group, 1986; Holland et al., 1986; Masur et al., 1986; Plotkin et al., 1985; Rosencan et al., 1986). DHPG given at a dose of 100 mg/kg/day for 8 con-

secutive days prevented death in Cy-treated, CMV-infected guinea pigs indicating a reduction in the severity of CMV infection in these animals. The efficacy of DHPG was most readily demonstrable in animals given the intermediate viral dose (10^6 PFU). Differences between DHPG and sham-treated groups were not significant in guinea pigs given low (10^4 PFU) and high (10^7 PFU) virus inocula. Whether lower DHPG doses will also be efficacious in limiting generalized CMV infections has not been determined. However, a recent study by Fong and coworkers showed that 50 mg/kg/day of DHPG given for 7 days to immunocompetent guinea pigs infected with CMV did not reduce infectivity titers in blood, spleen and lungs, but consistently lowered virus titers in the salivary gland of infected guinea pigs as compared to their sham-treated counterparts (Fong et al., 1987). While DHPG decreased mortality, it did not prevent establishment of CMV infection and did not significantly reduce virus infectivity titers in blood of Cy-treated, CMV-infected guinea pigs. A study of the efficacy of DHPG in murine CMV (MCMV) infection has also shown that DHPG prevented death but not the establishment of latent infection in MCMV-infected normal and immunosuppressed BALB/c mice (Wilson et al., 1987). Further, it has been reported that immunodeficient patients treated with DHPG showed virologic and clinical improvement; however, viral and clinical relapses occurred frequently, and leukopenia which appeared to be dose-related was the most common adverse reaction (Collaborative DHPG Treatment Study Group, 1986; Drew, 1988; Masur et al., 1986).

Both murine and guinea pig models of CMV infection exist as tools for antiviral studies. However, guinea pig CMV and HCMV infections have more features in common, notably intrauterine infection which does not occur in mice (Bia et al., 1983; Griffith and Hsiung, 1980). The use of a single Cy dose (300 mg/kg given 2 days before or a day after virus inoculation) has effected adequate immunosuppression and reproducible severity of CMV infection in guinea pigs. The prevention of death with DHPG treatment utilizing this CMV-infected immunocompromised host model indicates the usefulness of the model. Mortality rates and mean days of death can be more easily and objectively assessed in experimental groups as compared to virus infectivity titers in blood or tissues. Such a model is practical and convenient indeed. However, further immunologic characterization is needed to further establish this model as an investigational tool for antiviral testing.

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