ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1985, p. 600–604
0066-4804/85/040600-05$02.00/0

Vol. 27, No. 4

Inhibition of Human Cytomegalovirus by Combined Acyclovir and Vidarabine

STEPHEN A. SPECTOR* AND EILEEN KELLEY

Department of Pediatrics, University of California Medical Center, San Diego, California 92103

Received 13 July 1984/Accepted 29 January 1985

The inhibition of human cytomegalovirus (HCMV) isolates by acyclovir (ACV) and vidarabine (ara-A) was assessed by using an infectious-center plaque-reduction assay. When fixed concentrations of 4.5 μg of ACV and 250 ng of ara-A per ml were compared singly and in combination, the viral inhibition resulting from the ACV–ara-A combination was synergistic for three of four HCMV clinical isolates studied and additive for one HCMV isolate. An additional four HCMV strains obtained at postmortem examination from the lungs of bone marrow transplant patients were assessed for sensitivity to ACV–ara-A by using the dose required for 50% viral inhibition (ID_{50}) as the endpoint. The mean ID_{50} of ACV for the four HCMV isolates was 12.3 μg/ml, whereas the mean ID_{50} of ara-A was 3.4 μg/ml. When 1 μg of ara-A per ml (which yielded a mean plaque reduction of 23.6%) was combined with ACV, a mean of 5.2 μg of ACV per ml was required for 50% viral inhibition. The sum of the fractional inhibitory concentrations for each of the four HCMV isolates was <1, indicating synergy by the ACV–ara-A combination. Although DNA synthesis in growing human embryonic lung fibroblast (HEL) cells, as determined by [^{3}H]thymidine incorporation, was diminished to 61% of that in untreated control cells when 22.5 μg of ACV and 1 μg of ara-A per ml were used, there was no additive inhibition of DNA synthesis when the two-drug combination was used. HEL cell growth remained at 97% of control cell growth at 72 h when concentrations as high as 45 μg of ACV combined with 1 μg of ara-A per ml were used.

Human cytomegalovirus (HCMV) is a major cause of disease in newborns, infants, and immunocompromised individuals, including cancer patients, organ transplant recipients, and persons with acquired immunodeficiency syndrome (3, 9, 16–18, 22). Particularly devastating infections occur in bone marrow transplant patients; as many as 25% of recipients having survived the rigors of transplantation succumb to HCMV-induced interstitial pneumonia (11).

Three antiviral agents, leukocyte interferon (IFN-α), vidarabine (ara-A), and acyclovir (ACV), have been beneficial in the treatment of certain herpes simplex virus and varicella-zoster virus infections (1, 4, 7, 28, 29). None of these antiviral agents used singly has improved the outcome of HCMV disease. In vitro studies have indicated that combinations of ACV with IFN-α (8) or IFN-β (23) generally have additive inhibitory effects, and one study suggested possible synergism at high concentrations of ACV (20). When applied clinically, however, the combination of IFN-α with either ara-A (10) or ACV (27) has not altered the fatal outcome of HCMV pneumonia in bone marrow transplant recipients.

Previous in vitro studies in our laboratory indicated that combinations of ACV with either trifluorothymidine or phosphonoformic acid were synergistic against most clinical HCMV strains (23, 24). However, both trifluorothymidine and phosphonoformic acid may be too toxic to use in humans, and neither is approved for intravenous administration in humans. In a further attempt to develop an effective antiviral therapy for HCMV infections, we examined in vitro the capacities of combinations of ACV and ara-A to inhibit the multiplication of clinical HCMV isolates. Both drugs are currently approved for intravenous thereapy of certain systemic herpesvirus infections and have a low toxicity in humans.

* Corresponding author.

(This material was presented in part at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy [S. A. Spector and E. Kelley, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 920, 1983].)

MATERIALS AND METHODS

Propagation of virus and antiviral agents. HCMV was grown in human embryonic lung fibroblast (HEL) cells as previously described (23, 24). All clinical isolates were passaged in cell cultures ca. 10 times before they were used in these studies. HCMV isolates 1, 2, and 4 were cultured from urine specimens from premature infants who had acquired HCMV infections while hospitalized in an intensive-care nursery. HCMV isolate 4 was grown from a urine sample from a child with histiocytic medullary reticulosis. HCMV isolates 5 through 8 were isolated directly from lung specimens obtained at postmortem examination from bone marrow transplant patients who had died of HCMV-induced interstitial pneumonia.

ACV was obtained courtesy of Burroughs Wellcome Co. (Research Triangle Park, N.C.), and ara-A was obtained courtesy of Parke, Davis & Co. (Ann Arbor, Mich.).

Plaque-reduction assays. The procedures followed for the infectious-center plaque-reduction assays have been described previously (23, 24). Briefly, each well of a 24-well tissue culture plate was seeded with HEL cells. When growth was confluent, equal numbers of infectious centers of a specific HCMV isolate were added to each culture well and incubated at 37°C for 30 min before the antiviral agent(s) was added. Four to six duplicate wells were evaluated at each concentration of antiviral agent and for each set of controls. After incubation at 37°C for 4 to 5 days, discrete viral foci were counted with an inverted microscope. The 50% inhibitory dose (ID_{50}) of ACV and ara-A was calculated by using the linear regression equation of the linear portion of each

TABLE 1. Mean number of plaques in untreated virus control wells (VC), ACV (4.5 μg/ml)-treated wells, ara-A (250 ng/ml)-treated wells, and ACV (4.5 μg/ml)–ara-A (250 ng/ml)-treated wells

Isolate	Mean no. of plaques ± SD[a] in wells treated with:			
	VC	ACV	ara-A	ACV–ara-A
1	61.7 ± 4.5	37.3 ± 3.6	30.7 ± 4.0	10 ± 2.4
2	85.5 ± 6.2	43.3 ± 5.5	64.2 ± 8.0	19 ± 4.1
3	65.6 ± 5.0	50.5 ± 6.0	59.5 ± 3.8	45.2 ± 4.1
4	62.7 ± 6.0	39.7 ± 6.5	45.0 ± 4.9	19.5 ± 3.4

[a] Each value represents the analysis of six duplicate wells.

dose-response curve and determining the concentration of antiviral agent required for 50% plaque inhibition.

Analysis of inhibition by combined ACV and ara-A. Two methods were used to assess the inhibition of HCMV isolates by ACV and ara-A in combination. For experiments with HCMV isolates 1 through 4, the combination index (CI) was calculated as described in detail elsewhere (23). The absolute additivity is defined as the CI equalling 0 when CI = $\ln(\bar{A}_1) + \ln(\bar{A}_2) - \ln(\bar{A}_{1+2}) - \ln(\overline{VC})$, where $\bar{A}_1$, $\bar{A}_2$, and $\bar{A}_{1+2}$ are the mean numbers of plaques in wells treated with antiviral agents $\bar{A}_1$, $\bar{A}_2$, and the combination of $\bar{A}_1$ and $\bar{A}_2$, respectively, and $\overline{VC}$ is the mean number of plaques in virus control wells. Combinations are defined as additive, synergistic, or antagonistic based on the following definitions (SE is the standard error): additivity, $(0 + 2SE) \geq CI \geq (0 - 2SE)$; synergy, $CI > (0 + 2SE)$; and antagonism, $CI < (0 - 2SE)$.

For experiments with HCMV isolates 5 through 8, combined antiviral inhibition was assessed by using the fractional inhibitory concentration (FIC) method of Berenbaum (2). For this method of analysis, the concentrations of each of the agents producing a specific inhibition are determined. The degree of dilution of antiviral agents required is equal to the sum of the FICs. Thus, the sum of the FICs equals (Ac/Ae) + (Bc/Be), where Ae and Be are the concentrations of antiviral agents A and B, respectively, necessary for a specific inhibition when used alone and Ac and Bc are the concentrations necessary when the antiviral agents are combined. Combinations are defined as additive, synergistic, or antagonistic based on the following definitions: additivity, (Ac/Ae) + (Bc/Be) = 1; synergy, (Ac/Ae) + (Bc/Be) < 1; and antagonism, (Ac/Ae) + (Bc/Be) > 1.

Cellular toxicity assays. The toxicity of the combined antiviral agents for growing HEL cells was determined by using two tests of cellular inhibition. HEL cell growth was determined by seeding each 35-mm-diameter well of a six-well culture plate (Costar) containing minimal essential medium and 10% calf serum with the appropriate concentrations(s) of antiviral agent(s) as described previously (24). At 24-h intervals for 3 days, each set of controls and of cells treated with drug(s) was counted in triplicate with a hemacytometer. The mean of the three determinations was used to assess drug toxicity for growing HEL cells.

To estimate the effect of the combined antiviral agents on HEL cell DNA synthesis, we determined the incorporation of [^{3}H]thymidine (6). Equal numbers of HEL cells were added to each well of a 96-well flat-bottom microtiter plate (Falcon). After 24 h of incubation, when cell growth was subconfluent, the antiviral agent(s) was added to test wells. Three hours later, [^{3}H]thymidine (0.25 μCi per well; 2 Ci/mmol) was added. After 18 h, the monolayers were harvested by using an automated cell harvester. The counts per minute retained on the glass fiber filters were determined in a scintillation counter.

RESULTS

Inhibition of HCMV by fixed concentrations of ACV and ara-A. The combination of ACV (4.5 μg/ml) and ara-A (250 ng/ml) was synergistic for three of the four clinical isolates of HCMV (Tables 1 and 2). If the ACV–ara-A combination had been additive for these three isolates, the mean expected plaque reduction showing synergy would have been 62.1%; the mean observed plaque reduction for the three HCMV isolates was 76.8%. The mean number of SEs from 0 for the CIs when ACV and ara-A were combined for the three isolates was 4.31. The fixed combination of ACV and ara-A was additive for one HCMV strain, isolate 3. The mean expected plaque reduction if the ACV–ara-A combination had been additive for isolate 3 would have been 30.2%, which closely approximates the mean observed plaque reduction of 31.1%.

Inhibition of HCMV as determined by FICs. The activity of combinations of ACV and ara-A against four HCMV isolates obtained at postmortem examination from the lungs of bone marrow transplant recipients was assessed by the method of Berenbaum (2). The mean ID_{50} of ACV for the four HCMV isolates was 12.3 μg/ml, whereas the mean ID_{50} of ara-A was 3.4 μg/ml (Table 3). Because the dosage of ara-A is limited in adults to 10 to 15 mg/kg per day, achievable levels of ara-A, which is rapidly deaminated to arabinosyl hypoxanthine, are not in excess of 1 μg/ml. For this reason, we combined 1 μg of ara-A per ml with increasing concentrations of ACV. The mean ID_{50} of ACV for each of the four HCMV isolates studied was 5.2 μg/ml. The sum of the FICs for each of the four HCMV isolates was <1, indicating synergy. The inhibition of HCMV isolates by 1 μg of ara-A per ml and ACV at increasing concentrations indicated that the synergy was greatest at concentrations of ACV ranging from 5.6 to 22.5 μg/ml (25 to 100 μM) (Fig. 1). This concentration of ACV can be achieved in patients treated with 500 mg/m^2 every 8 h (S. A. Spector, M. Hintz, and J. D. Connor, unpublished data).

Assessment of the toxicity of combined ACV and ara-A for growing HEL cells. The combination of 45 μg of ACV and 1

TABLE 2. Analysis of inhibition of four clinical HCMV isolates by ACV (4.5 μg/ml) and ara-A (250 ng/ml) singly and in combination

Isolate	Plaque reduction (% of control) with:		% Plaque reduction with drug combination		CI ± SE	No. of SE of 0	Combined effect
	ACV	ara-A	Expected	Observed			
1	60.5	49.8	69.9	83.8	0.6184 ± 0.1206	5.13	Synergy
2	50.6	75.1	62	77.8	0.5372 ± 0.1215	4.42	Synergy
3	77	90.7	30.2	31.1	0.0133 ± 0.0725	0.36	Additivity
4	63.4	71.9	54.4	68.8	0.3808 ± 0.1130	3.37	Synergy

TABLE 3. Inhibition by ACV and ara-A of HCMV isolates from bone marrow transplant recipients

Isolate	ID_{50} (μg/ml) of:			FIC
	ara-A	ACV	ACV–ara-A[a]	
5	3.5	15.6	6.5	0.702
6	4.6	10.4	5.0	0.692
7	3.7	12.9	7.5	0.85
8	1.8	10.3	1.7	0.734

[a] The concentration of ara-A was kept constant at 1 μg/ml, and the concentrations of ACV necessary to achieve 50% viral inhibition were as indicated (in micrograms per milliliter).

μg of ara-A per ml did not inhibit HEL cell growth over a 72-h incubation period (Table 4). With increasing concentrations of ACV, there was a progressive inhibition of DNA synthesis, as determined by the incorporation of [^{3}H]thymidine into the DNA of HEL cells (Table 5). However, when 1 μg of ara-A per ml was combined with increasing concentrations of ACV, no increased inhibition of HEL cell DNA synthesis was observed (Table 5). All HEL cells remained viable for the duration of the studies.

DISCUSSION

Single-drug antiviral therapy for HCMV infections has failed to alter the progression of HCMV disease in immunocompromised individuals. Combined antiviral therapy with two or more drugs could be beneficial in the treatment of HCMV disease. Our in vitro results indicate that combinations of ara-A and ACV are synergistic against most HCMV isolates. Synergy was observed at fixed concentrations of ara-A with either fixed or increasing concentrations of ACV. The synergy was greatest when 1 μg of ara-A per ml was combined with ca. 11.3 μg of ACV per ml (50 μM). Pharmacokinetic studies of ara-A suggest that levels of 1 μg/ml are transiently achieved when ara-A is administered at 10 mg/kg per day over a 12-h continuous infusion (5). However,

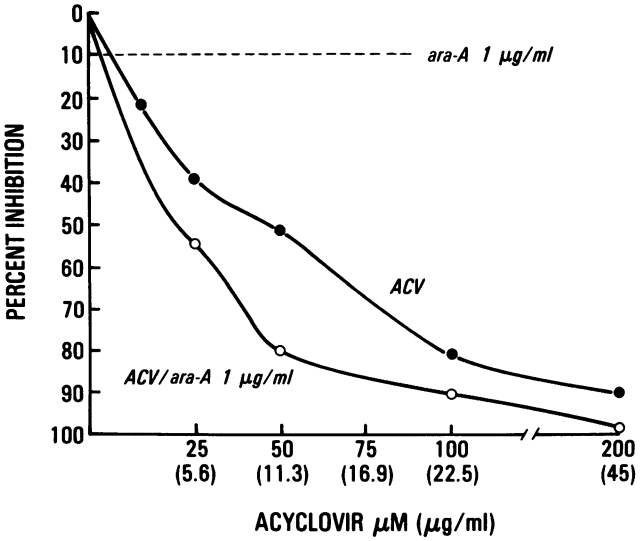

FIG. 1. Inhibition of HCMV isolate 6 (from a lung specimen from a bone marrow transplant recipient) by 1 μg of ara-A per ml (– – –), increasing concentrations of ACV (●), and 1 μg of ara-A per ml combined with increasing concentrations of ACV (○).

TABLE 4. Inhibition of HEL cell growth by 45 μg of ACV and 1 μg of ara-A per ml singly and in combination over 72 h

Drug	HEL cell growth (% of control) on indicated day after drug administration[a]		
	1	2	3
ACV	99	98	97
ara-A	96	97	98
ACV–ara-A	96	95	97

[a] On days, 1, 2, and 3, control cell counts were 1.79×10^5, 2.68×10^5, and 3.47×10^5, respectively.

in tissue cultures, ara-A is deaminated to arabinosyl hypoxanthine, mimicking the rapid deamination of ara-A in humans (25). Similarly, when ACV is administered to patients at 500 mg/m^2 every 8 h, ACV levels in plasma generally range from 11.3 to 22.5 μg/ml (50 to 100 μM). Therefore, the levels exhibiting the greatest synergy for the drug combination can be achieved readily in patients. In addition, we found no additive decrease in HEL cell growth or DNA synthesis with the ACV–ara-A combination.

The synergy exhibited by the ACV–ara-A combination is consistent with previous findings of synergy with other combinations of antiviral agents that inhibit HCMV by blocking DNA synthesis (23, 24). It is at odds, however, with the results of in vitro studies which showed that combinations of ACV and ara-A effected no more than an additive inhibition of the growth of herpes simplex virus (19). However, Park et al. (14) recently showed that the combination of ACV and ara-A was significantly more effective than either agent alone in reducing the incidence of latent herpes simplex virus type 1 infections in the trigeminal ganglia of mice.

The testing of antimicrobial agents for synergy remains controversial, despite the fact that there is much literature on the subject. The two standard techniques used to test the interaction of combined antimicrobial agents, the checkerboard titration method and the time-kill curve method, correlate poorly when used to determine synergy (13). Several authors have stated that a more precise definition of antimicrobial synergy is needed (12, 13). The development of a precise definition is complicated, however, by the large

TABLE 5. Inhibition of HEL cell DNA synthesis, as determined by [^{3}H]thymidine incorporation (counts per minute) onto glass filters in the presence of 1 μg of ara-A per ml, ACV at increasing concentrations, and 1 μg of ara-A per ml combined with increasing concentrations of ACV

Drug (μg/ml)	[^{3}H]thymidine incorporation (cpm) ± SD[a]	Incorporation as a percentage of that in controls
None (control)	36,541 ± 8,066	
ara-A (1)	27,663 ± 3,508	76
ACV (45)	19,750 ± 5,131	54
ACV (22.5)	22,056 ± 7,972	60
ACV (11.25)	29,501 ± 861	81
ACV (5.6)	27,700 ± 5,945	76
ara-A (1)–ACV (45)	19,587 ± 5,766	54
ara-A (1)–ACV (22.5)	21,618 ± 1,799	59
ara-A (1)–ACV (11.25)	27,447 ± 1,440	75
ara-A (1)–ACV (5.6)	29,560 ± 5,693	81

[a] Each value is the mean of three determinations.

number of different mechanisms by which antimicrobial agents may interact. Some have suggested that it is unlikely that a single in vitro test will be adequate to detect all interactions (12). In this study, two methods were used to assess antiviral combinations. The first method, which used CIs, was developed in our laboratory to determine inhibition by antiviral combinations in an infectious-center plaque-reduction assay. This method extends the criteria of yield-reduction assays (19, 26) by placing strict definitions on antagonism, additivity, and synergy. The FIC method of analysis (2) uses different criteria to define synergy, additivity, and antagonism. As determined by both methods, the combination of ACV and ara-A is synergistic against HCMV isolates. Other methods of analysis, however, may not lead to the same conclusion and may indicate additivity and not synergy for the combined antiviral effects observed in this study. Some authors have avoided defining combinations of antimicrobial agents as being synergistic, additive, or antagonistic and have chosen to refer to the effects of combined agents as either enhancement or interference (8). According to these definitions, the combination of ACV and ara-A against HCMV isolates is enhancing.

In developing antiviral treatments for HCMV infections in immunocompromised patients, such as bone marrow transplant recipients or acquired immunodeficiency syndrome patients, the timing of antiviral therapy may be important. Patients with clinical evidence of diffuse HCMV-induced interstitial pneumonia are least likely to respond to antiviral therapy. Prophylactic antiviral therapy to prevent severe disease may prove effective but would require prolonged therapy for several months in patients receiving organ transplants. Studies in bone marrow transplant patients indicate that HCMV viremia occurs several weeks before HCMV pneumonia develops (15, 21). Antiviral therapy directed toward interrupting the progression of HCMV viremia to interstitial pneumonia and death may be successful in these patients. Nucleic acid hybridization techniques for the rapid identification of HCMV viremia in these patients may also prove useful (21).

New antiviral agents, such as 9-[(2-hydroxy-1-(hydoxymethyl)ethoxy)methyl]guanine and 1-(2-fluoro-2-deoxy-D-arabinofuranosyl)-5-iodocytosine, appear to be active in vitro against HCMV. However, each of these drugs has exhibited sufficient toxicity in animal studies to be limited in clinical usefulness. Our data suggest that until new antiviral agents are available for the treatment of HCMV infections, combinations of ACV and ara-A may be useful in patients with life-threatening HCMV disease.

ACKNOWLEDGMENTS

We thank Evon Hart John for typing this manuscript.

This work was supported by Public Health Service grants CA35048, HL/AI32471, and AI20736 from the National Institutes of Health and by the AIDS Task Force, University of California.

LITERATURE CITED

1. **Arvin, A. M., J. H. Kushner, S. Feldman, R. L. Baehner, D. Hammond, and T. C. Merigan.** 1982. Human leukocyte interferon for the treatment of varicella in children with cancer. N. Engl. J. Med. **306**:761–765.
2. **Berenbaum, M. C.** 1978. A method for testing for synergy with any number of agents. J. Infect. Dis. **137**:122–130.
3. **Betts, R. F.** 1982. Cytomegalovirus infection in transplant patients. Prog. Med. Virol. **28**:44–64.
4. **Bryson, Y. J., M. Dillon, M. Lovett, G. Acuna, S. Taylor, J. D. Cherry, B. L. Johnson, E. Wiesmeier, W. Growdon, T. Creagh-Kirk, and R. Kenney.** 1983. Treatment of first episodes of genital herpes simplex virus infection with oral acyclovir. N. Engl. J. Med. **308**:916–920.
5. **Bryson, Y. J., L. Sweetman, and J. D. Connor.** 1978. Simple sensitive microbioassay for adenine arabinoside and hypoxanthine arabinoside in human plasma. Antimicrob. Agents Chemother. **14**:909–915.
6. **Crumpacker, C. S., L. E. Schnipper, J. A. Zaia, and M. J. Levin.** 1979. Growth inhibition by acycloguanosine of herpesviruses isolated from human infections. Antimicrob. Agents Chemother. **15**:642–645.
7. **Hirsch, M. S., and R. T. Schooley.** 1983. Treatment of herpesvirus infections. N. Engl. J. Med. **309**:963–970.
8. **Levin, M. J., and P. L. Leary.** 1981. Inhibition of human herpesviruses by combinations of acyclovir and human leukocyte interferon. Infect. Immun. **32**:995–999.
9. **MacDonald, H., and J. O. Tobin.** 1978. Congenital cytomegalovirus infection: a collaborative study on epidemiological, clinical and laboratory findings. Dev. Med. Child Neurol. **20**:471–482.
10. **Meyers, J. D., R. W. McGuffin, Y. J. Bryson, K. Cantell, and E. D. Thomas.** 1982. Treatment of cytomegalovirus pneumonia after marrow transplant with combined vidarabine and human leukocyte interferon. J. Infect. Dis. **146**:80–84.
11. **Meyers, J. D., H. C. Spencer, Jr., J. C. Watts, M. B. Gregg, J. A. Stewart, R. H. Troupin, and E. D. Thomas.** 1975. Cytomegalovirus pneumonia after human marrow transplantation. Ann. Intern. Med. **82**:181–188.
12. **Moellering, R. C.** 1979. Antimicrobial synergism—an elusive concept. J. Infect. Dis. **140**:639–641.
13. **Norden, C. W., H. Wentzel, and E. Keleti.** 1979. Comparison of techniques for measurement of in vitro antibiotic synergism. J. Infect. Dis. **140**:629–633.
14. **Park, N.-H., J. G. Callahan, and D. Pavan-Langston.** 1984. Effect of combined acyclovir and vidarabine on infection with herpes simplex virus in vitro and in vivo. J. Infect. Dis. **149**:757–762.
15. **Quinnan, G. V., Jr., N. Kirmani, A. H. Rook, J. F. Manischewitz, L. Jackson, G. Moreschi, G. W. Santos, R. Saral, and W. H. Burns.** 1982. Cytotoxic T cells in cytomegalovirus infection. N. Engl. J. Med. **307**:7–13.
16. **Reynolds, D. W., S. Stagno, K. G. Stubbs, A. J. Dahle, M. M. Livingston, S. S. Saxon, and C. A. Alford.** 1974. Inapparent congenital cytomegalovirus infection with elevated cord IgM levels. N. Engl. J. Med. **290**:291–296.
17. **Rogers, M. F., D. M. Morens, J. A. Stewart, R. M. Kaminski, T. J. Spira, P. M. Feorino, S. A. Larsen, D. P. Francis, M. Wilson, L. Kaufman, and the Task Force on Acquired Immune Deficiency Syndrome.** 1983. National case-control study of Kaposi's sarcoma and *Pneumocystis carinii* pneumonia in homosexual men: part 2, laboratory results. Ann. Intern. Med. **99**:151–158.
18. **Rubin, R. H., P. S. Russell, M. Levin, and C. Cohen.** 1979. Summary of a workshop on cytomegalovirus infections during organ transplantation. J. Infect. Dis. **139**:728–734.
19. **Schinazi, R. F., and A. J. Nahmias.** 1982. Different *in vitro* effects of dual combinations of anti-herpes simplex virus compounds. Am. J. Med. **73**:40–48.
20. **Smith, C. A., B. Wigdahl, and F. Rapp.** 1983. Synergistic antiviral activity of acyclovir and interferon on human cytomegalovirus. Antimicrob. Agents Chemother. **24**:325–332.
21. **Spector, S. A., J. A. Rua, D. H. Spector, and R. McMillan.** 1984. Detection of human cytomegalovirus in clinical specimens by DNA-DNA hybridization. J. Infect. Dis. **150**:121–126.
22. **Spector, S. A., K. Schmidt, W. Ticknor, and M. Grossman.** 1979. Cytomegaloviruria in older infants in intensive care nurseries. J. Pediatr. **95**:444–446.
23. **Spector, S. A., M. Tyndall, and E. Kelley.** 1982. Effects of acyclovir combined with other antiviral agents on human cytomegalovirus. Am. J. Med. **73**:36–39.
24. **Spector, S. A., M. Tyndall, and E. Kelley.** 1983. Inhibition of human cytomegalovirus by trifluorothymidine. Antimicrob. Agents Chemother. **23**:113–118.
25. **Sweetman, L., J. D. Connor, R. Seshamani, M. A. Stuckey, S. Carey, and R. Buchanan.** 1975. Deamination of adenine arabi-

noside in cell cultures used for *in vitro* viral inhibition studies, p. 135–144. *In* D. Pavan-Langston, R. A. Buchanan, and C. Alford, Jr. (ed.), Adenine arabinoside: an antiviral agent. Raven Press, Publishers, New York.

26. **Valeriote, F., and H.-S. Lin.** 1975. Synergistic interaction of anticancer agents: a cellular perspective. Cancer Chemother. Rep. **59**:895–900.
27. **Wade, J. C., R. W. McGuffin, S. C. Springmeyer, B. Newton, J. W. Singer, and J. D. Meyers.** 1983. Treatment of cytomegaloviral pneumonia with high-dose acyclovir and human leukocyte interferon. J. Infect. Dis. **148**:557–562.
28. **Wade, J. C., B. Newton, C. McLaren, N. Flournoy, R. E. Keeney, and J. D. Meyers.** 1982. Intravenous acyclovir to treat mucocutaneous herpes simplex virus after marrow transplantation. Ann. Intern. Med. **96**:265–277.
29. **Whitley, R. J., S.-J. Soong, M. S. Hirsch, A. W. Karchmer, R. Dolin, G. Galasso, J. K. Dunnick, C. A. Alford, and the NIAID Collaborative Antiviral Study Group.** 1981. Herpes simplex encephalitis. Vidarabine therapy and diagnostic problems. N. Engl. J. Med. **304**:313–318.