Effects of Adenine Arabinoside on Lymphocytes Infected with Epstein-Barr Virus

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Low concentrations of adenine arabinoside inhibited growth of two Epstein-Barr virus producer cell lines in culture, while not significantly affecting a nonproducer cell line and a B-cell-negative line. These observations were extended to include freshly infected cells. Mitogen-stimulated human umbilical cord blood lymphocytes were unaffected by the drug at concentration levels that inhibited [³H]thymidine incorporation into the DNA of Epstein-Barr virus-stimulated cells. DNA synthesis in Epstein-Barr virus-superinfected Raji cells was also adversely affected by adenine arabinoside. However, these same low concentrations of adenine arabinoside in the triphosphate form produced less effect on DNA synthesis in nuclear systems and DNA polymerase assays than on growth or DNA synthesis in whole cells. Therefore the effects reported here of low concentrations of the drug on whole cells may be only in part related to DNA polymerase inhibition. The work reported here suggests that adenine arabinoside has multiple sites of action in infected cells.

The synthesis of 9- β -D-arabinofuranosyladenine (adenine arabinoside, or ara-A) as a potential anticancer agent was first reported in 1960 (18). Since then ara-A has been reported to have antitumor and antiviral effects for tissue culture cells and laboratory animals and in clinical use in treatment of certain virally caused diseases. Reports between 1964 and 1968 have shown that ara-A has specific antiviral effects in tissue culture for herpes, pox, and Rous sarcoma viruses (11, 29, 32; R. W. Sidwell, G. Arnett, and G. J. Dixon, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 7th, Chicago, Ill., Abstr. no. 64, 1967). Furthermore, inhibition of mammalian ribonucleotide reductase and DNA polymerase by ara-A were demonstrated (12, 22). Certain studies have shown that mammalian α polymerases are sensitive to ara-A (23-25; C. M. Reinke, J. C. Drach, C. Shipman, Jr., and A. Weissbach, in G. de Thé, ed., *Oncogenesis and Herpesviruses III*, in press), although the reported effective drug levels vary widely. The herpes simplex-induced DNA polymerase has also been reported to be inhibited by ara-A (25; Reinke et al., in *Oncogenesis and Herpesviruses III*, in press).

In 1965, Brink and LePage (4) showed that TA3 ascites tumors in mice underwent remission after ara-A treatment, although the TA3 cells as

solid subcutaneous tumors responded less well. In 1968, Sidwell et al. (35) showed that ara-A treatment increased the survival number and mean survival time of vaccinia-infected mice. Schabel (32) reported that herpes simplex-induced keratitis in hamsters and encephalitis in mice responded favorably to ara-A treatment at dose levels where little toxicity was demonstrated. In fact, to our knowledge the only report that ara-A may be toxic to uninfected cells in vivo or in vitro was that of Nichols (26), who demonstrated the existence of chromosomal breaks in cultured human leukocytes after brief exposure to high doses of ara-A. Recent clinical trials have demonstrated that ara-A is effective against various manifestations of herpes simplex, herpes zoster, and cytomegalovirus in humans at dose levels that show little detectable toxicity (1, 6-8, 19, 40).

Although there are clearly documented anti-herpes effects of the drug, no reports of possible action against Epstein-Barr virus (EBV) have appeared until recently, despite the suggestion by Schabel (32) in 1968 that it might be effective in treating two EBV-associated diseases, Burkitt's lymphoma and infectious mononucleosis. Coker-Vann and Dolin (9) have now shown that exposure of the EBV-producing lymphoblastoid cell line P3HR-1 to ara-A causes inhibition of the cells' expression of viral capsid antigen.

In the present report we have examined a

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variety of EBV-infected cells and have found that ara-A has a selective toxicity at low concentrations for cell populations which are either actively producing or freshly infected with EBV. It is shown that ara-A is a partial inhibitor in vitro of an EBV-associated DNA polymerase at concentrations at which the drug is very active in culture. However, the data suggest that the marked anti-EBV action of ara-A is due only in part to DNA polymerase inhibition, and that other factors mentioned in the discussion must be considered.

MATERIALS AND METHODS

Cell cultures. Lymphoblastoid lines B95-8 and Raji were obtained from Pfizer, Inc. P3HR-1 was from J. Pagano and from M. Nonoyama, and Ramos negative was from G. Klein. B95-8 is an EBV-transformed marmoset B cell line that produces transforming virus (21). The Raji B cell line was derived from a Burkitt's tumor biopsy and carries EBV genomes without producing viral particles (30). P3HR-1 is also a B cell line derived from Burkitt's tumor tissue, but undergoes production of superinfecting virus in culture (15). Ramos is a B cell line devoid of detectable EBV antigens or genomes and is derived from an African lymphoma biopsy (16).

Cells were maintained in RPMI 1640 medium (Microbiological Associates) supplemented with 10% fetal calf serum and 300 μ g of glutamine per ml. No penicillin or streptomycin was used. Cells were fed every 3 to 4 days and maintained at 37°C in a humidified 5% CO₂ atmosphere. All cell lines were routinely checked for mycoplasma by plating on PPLO agar (Gibco) and allowing the plates to incubate under anaerobic and humidified aerobic conditions at 37°C for up to 2 weeks. No mycoplasma were detected in any cells used for these experiments.

Reagents. Ara-A and Pronase were from Calbiochem. Phosphonoacetic acid (PAA) was from Abbott Laboratories. Fluorescein-isothiocyanate-conjugated goat anti-human immunoglobulin G was from Hyland Laboratories or Gibco. The anti-EBV sera were gifts of G. Klein and W. Henle. [8-³H]dATP (15 Ci/mmol), [methyl-³H]TTP (55 Ci/mmol), and [methyl-³H]thymidine (18 Ci/mmol) were from Schwarz/Mann. Unlabeled deoxynucleoside triphosphates were from Sigma. Ficoll was from Pharmacia, and Hypaque (Isopaque) was from Sterling. Phytohemagglutinin was from Gibco or Burroughs Wellcome.

Testing for viral antigen production. Indirect immunofluorescence assays were performed both for EB viral early antigen and for viral capsid antigen according to the method of Henle and Henle (14).

Preparation of fresh lymphocytes from cord blood. The Ficoll-Hypaque method of Böyum (3) was employed to isolate leukocytes from freshly obtained human umbilical cord blood. The cells were then washed two to three times in RPMI 1640 medium with 10% fetal calf serum, finally resuspended to about 2×10^6 to 3×10^6 cells per ml in RPMI 1640 with 20% fetal calf serum, and placed in culture in wells of Linbro MicroTest II plates.

Preparation of EB viral stocks. Virus from B95-8 cells was prepared by leaving cultures for 10 to 12 days at 37°C after the last feeding and then sedimenting the cells at low speed; the supernatant was filtered through a 0.45- μ m filter and diluted 1:1.5 with RPMI 1640 containing 20% fetal calf serum. This filtered supernatant was then further diluted 1:1 with the cord blood lymphocytes for experiments in which viral infection was to be studied. Virus from P3HR-1 (Nonoyama) cells was produced by maintaining the cells at 34°C and diluting them with fresh medium 1:1 once a week. When a desired volume of culture was attained, the cells were sedimented at low speed, and the virus in the resulting supernatant was pelleted in the GS-3 rotor of a Sorvall RC-5 centrifuge by sedimentation for 2 h at 9,000 rpm at 5°C. The pellet was resuspended to 1% of the original volume in RPMI 1640 and used in superinfection experiments.

Superinfection of Raji cells with P3HR-1 virus. Superinfection was performed according to the method of Shaw et al. (34), with the following modifications: Pronase treatment was at 50°C, and the superinfection was at 34°C after the initial adsorption step.

Preparation of partially purified DNA polymerases. Host α and β polymerases and the EBV-associated polymerases were isolated from P3HR-1 cells or viral material. The purification methods included DEAE-cellulose and phosphocellulose column chromatography, as described by Weissbach et al. (39), and are discussed in detail elsewhere (S. R. Goodman, C. Prezyrna, and W. C. Benz, *J. Biol. Chem.*, in press).

Preparation of nuclei. Isolated nuclei were prepared from B95-8 and Raji cells and tested for ability to synthesize DNA as previously described (2).

Synthesis of ara-ATP. Ara-A triphosphate (ara-ATP) was synthesized from ara-A monophosphate (Sigma) according to the method of Symons (37), with the following modifications. The reaction mixture included the following volumes: 80 μ l of distilled water, 25 μ l of monophosphate kinase (Sigma), and 90 μ l of the diphosphate kinase (Sigma).

RESULTS

Effects of ara-A on viral antigen production. Immunofluorescence assays for EB viral capsid antigen and early antigen were performed on B95-8 cells exposed to various concentrations of ara-A. Table 1 shows that although ara-A concentrations as low as 1 μ g/ml significantly inhibited viral capsid antigen production in cultures of B95-8 cells, concentrations as high as 25 μ g/ml did not cause 100% inhibition. Early antigen inhibition was less marked, never reaching the 50% level. Since it had previously been demonstrated that the antiviral activity of PAA is manifested by an inhibition of viral capsid antigen production, PAA controls were performed using a drug concentration that gave substantial viral capsid antigen inhibition (100 μ g/ml) in accord with previous reports (28, 36). The data in Table 1 show that by the initial criterion of

viral antigen inhibition, ara-A is similar to PAA in its antiviral effects.

Effect of ara-A on growth of various B cell lines. While the above experiments were in progress, it was noted that the drug was very

TABLE 1. *Effect of ara-A on EB viral antigen production in B95-8 cells*

Ara-A concn ($\mu\text{g/ml}$)	Percent inhibition of immunofluorescence after 72 h in culture ^a	
	VCA	EA
1	64	12
2.5	79	25
10	75	46
25	87	38
PAA control (100 $\mu\text{g/ml}$)	69	35

^a Each number represents the average of at least four experiments. In every experiment, a total of 500 cells were examined for each concentration of the drug. VCA, Viral capsid antigen; EA, viral early antigen.

toxic to B95-8 cells in culture. As a consequence, at higher drug concentrations it was only with great difficulty that sufficient cells could be counted to obtain statistically accurate data for Table 1. Therefore, growth curves in the presence of varying drug concentrations were performed with several lymphoblastoid B cell lines to see whether the toxicity was a general feature of the interaction of the drug with B cells or was selective for B95-8. Figure 1 shows growth curves of four B cell lines in the presence of 0, 2.5, 5, 7.5, and 10 μg of the drug per ml. The growth of the two virus producer lines was strongly inhibited by as little as 2.5 μg of ara-A per ml (approximately 8.8 μM) in the culture. However, Raji, an EBV-positive nonproducer line, and Ramos, an EBV-negative line, showed far less sensitivity, and even exhibited significant growth in the presence of 10 μg of drug per ml (approximately 35 μM). It was also noted that B95-8 or P3HR-1 cells after exposure to ara-A in culture had greatly altered morphology. The cells were

FIG. 1. *Effect of ara-A on the growth rate of various cultured B cell lines. The four cell lines were grown at 37°C without drug or at the indicated concentrations of ara-A. Samples were counted using a hemacytometer in the presence of trypan blue at various intervals.*

smaller and had a slightly shriveled appearance despite the ability to exclude trypan blue. This was not true of Raji or Ramos cells in the presence of the drug. In these experiments the EBV-producer B cell lines tested were significantly more sensitive to ara-A in culture than B cell lines that were not producing virus.

Effect of ara-A on [^3H]thymidine incorporation by freshly infected cells. The above observations on producer cell lines were extended to include freshly infected lymphocytes. These experiments included use both of fresh cord blood cells infected with B95-8 virus and of Raji cells superinfected with P3HR-1 virus.

There are long-standing observations that human umbilical cord blood lymphocytes, when exposed in culture to either mitogens or certain transforming strains of EBV, undergo a burst of DNA synthesis that can be measured by adding [^3H]thymidine to the culture and determining its incorporation into acid-insoluble material (13, 31). Relative to our studies here, PAA has been reported to inhibit outgrowth and [^3H]thymidine incorporation of adult or cord blood lymphocytes infected with the B95-8 strain of EBV, while not affecting mitogen-stimulated control cells (38). Because of this effect of PAA, we decided to test the effect of ara-A on the incorporation of [^3H]thymidine in freshly infected cells. Fresh human lymphocytes separated from whole umbilical cord blood were placed in culture with either B95-8 virus or phytohemagglutinin, which has been shown to be a mitogen for B as well as T cells (38). Figure 2 shows that even 10 μg of ara-A per ml did not inhibit [^3H]thymidine incorporation by umbilical cord blood lymphocytes exposed to phytohemagglutinin. However, [^3H]thymidine incorporation observed in cells placed in culture with EBV was both delayed and significantly reduced by as little as 2.5 μg of ara-A per ml. In summary, these data indicate that the stimulation of DNA synthesis in fetal lymphocytes exposed to a mitogen is not affected by ara-A at drug concentrations which markedly inhibit the stimulation seen when EBV is added to the culture. However, unlike the action of PAA, the outgrowth of transformed cells in the virally infected culture was not inhibited indefinitely despite the periodic feeding of the cells with medium containing fresh ara-A. After 3 or 4 weeks in culture, cells started to grow out even in wells containing 10 μg of drug per ml of medium. This phenomenon may represent the appearance of a class of drug-resistant cells.

The second method used in obtaining cells freshly infected with EBV was that of superinfecting the nonproducer line Raji with P3HR-1

virus. Large amounts of viral DNA as well as viral early antigen are synthesized in Raji cells that have been exposed to P3HR-1 virus (27), and it was of interest to determine whether ara-A interfered with this DNA synthesis. As previously shown in Fig. 1, growth of Raji cells in culture was only slightly affected by ara-A at 5 $\mu\text{g}/\text{ml}$. [^3H]thymidine incorporation by Raji cells in culture was also only slightly affected by a 24-h period of exposure to ara-A at 5 $\mu\text{g}/\text{ml}$; log-phase cells incorporated approximately 80% as much ^3H into acid-insoluble material in the presence of the drug as in its absence. However, when Raji cells superinfected with P3HR-1 virus were exposed to ara-A at either 2.5 or 5 $\mu\text{g}/\text{ml}$ for a period of 24 h after superinfection, the total ^3H incorporation was only 10% of that in superinfected cells not exposed to ara-A. Separation of viral and host cell DNA in CsCl density gradients (Fig. 3) indicated that synthesis of both types of DNA normally seen in superinfected Raji cells (Fig. 3A) was greatly decreased in the presence of 5 μg of the drug per ml (Fig. 3B). Similar results were obtained when DNA from Raji cells superinfected in the presence of 2.5 μg of ara-A per ml was sedimented in CsCl gradients (data not shown).

Effect of ara-A on DNA-synthesizing activity of subcellular preparations. Since ara-A has been reported to be an inhibitor of various eucaryotic and viral DNA polymerases (12, 24, 25; Reinke et al., in *Oncogenesis and Herpesviruses III*, in press), we have investigated the effects of ara-A on DNA synthesis at the subcellular level using the triphosphate form of the drug, ara-ATP. In the work reported here, ara-ATP was used in two different subcellular systems. First, lymphocyte nuclei from producer (B95-8) and nonproducer (Raji) cells were tested for DNA-synthesizing activity in the presence of various concentrations of ara-ATP. No significant inhibition or stimulation of nuclear DNA-synthesizing activity was observed at drug concentrations of over 5 $\mu\text{g}/\text{ml}$ (10 μM) for either Raji or B95-8 nuclei (data not shown). This data for B95-8 is in marked contrast to the data for the whole cells in culture as shown in Fig. 1. In those experiments B95-8 was strongly sensitive to concentrations of the drug significantly less than 10 μM .

Second, certain partially purified DNA polymerases were tested in a DNA polymerase assay system containing various concentrations of ara-ATP. As described in great detail elsewhere (Goodman et al., *J. Biol. Chem.*, in press), EBV has been shown to induce a new 8.3S DNA polymerase activity in producer cell lines P3HR-1 and B95-8, whereas such an enzymatic activity

FIG. 2. Effect of ara-A on DNA synthesis in cultured cord blood lymphocytes. Cord blood lymphocytes were placed in culture at 37°C either with phytohemagglutinin (0.25 μ g/ml) (A) or EBV (B). At intervals, incorporation of [3 H]thymidine into the DNA of the cells was determined as previously described (38). Each point represents the average of incorporation of [3 H]thymidine of three wells in the Linbro plate. In (B), * represents cord blood cells in culture without virus, phytohemagglutinin, or ara-A.

is lacking in virus nonproducer or negative cell lines such as Raji or Ramos. Also, a 9.5S DNA polymerase activity has been shown to copurify with detergent-treated EB viral nucleocapsids. Both of these enzymes have been partially purified through DEAE-cellulose and phosphocellulose column chromatography and have been shown to be different in many respects from comparably purified host α and β polymerases. All four of these DNA polymerase activities were tested in DNA polymerase assays with various concentrations of ara-ATP. Both stan-

dard assays testing [3 H]TTP incorporation (Fig. 4B) and similar assays testing [3 H]dATP incorporation (Fig. 4A) by the various polymerases indicated that ara-ATP does not markedly inhibit DNA polymerase activity. Furthermore, the utilization of dATP as labeled substrate was not strongly interfered with even when ara-ATP was present in six- to sevenfold higher concentrations than the dATP. It is interesting that the EB virion-associated polymerase was 60 to 70% inhibited in respect to TTP incorporation at 10 μ M ara-ATP, but that inhibition largely disap-

FIG. 3. *CsCl* density gradients of DNA from Raji cells superinfected with P3HR-1 virus. Raji cells were superinfected with P3HR-1 virus as previously described (ref. 34 and the text). Virus was allowed to adsorb for 1 h, and infection proceeded for 4 h more, after which time [^3H]thymidine (10 $\mu\text{Ci}/\text{ml}$) was added. Infection was terminated at 24 h. Pronase treatment of Sarkosyl-lysed cells was followed by *CsCl* density centrifugation in a Sorvall 65 Ti vertical rotor at 33,000 rpm for 16 h. All fractions were precipitated with trichloroacetic acid before counting. (A) No drug added. (B) Cells were maintained in 5 μg of ara-A per ml throughout the 24-h infection period. $\times$, Grams per cubic centimeter; $\bullet$, ^3H counts per minute.

peared at higher drug concentrations. The range of ara-ATP concentrations giving significant inhibition of the virion-associated polymerase was not far above those concentrations of ara-A showing significant effects on whole virally infected cells (Fig. 1 to 3; Table 1). The other three enzymes tested did not show substantial inhibition in the 10 μM drug concentration range. However, it is important that, in general, a drug may be expected to be effective *in vitro* at the same or lower concentrations than it is *in vivo*. This is the case for PAA, which inhibits DNA synthesis in nuclei at 5- to 10-fold lower concentrations than it does in whole cells (W. G. Clough, unpublished data).

In summary, ara-ATP was not active against DNA synthesis in nuclei and was moderately active against DNA polymerase activities *in vi-*

FIG. 4. Effect of ara-A on various DNA polymerase activities *in vitro*. α , β , and the two EBV-associated DNA polymerases were tested in DNA polymerase assays with the indicated concentrations of ara-A. Each point represents an average of three assays. (A) [^3H]dATP incorporation measured. (B) [^3H]TTP incorporation measured. The DNA polymerase assay mixture contained the following: 80 mM Tris (pH 8.0), 6 mM magnesium acetate, 0.5% Triton X-100, 150 μg of activated calf thymus DNA per ml, and 10 μM each of three unlabeled deoxynucleoside triphosphates. The radioactively labeled triphosphate (either [^3H]dATP or [^3H]TTP) was present at 5 μM with a specific activity of 2,000 cpm/pmol. Incubations were for 60 min at 37°C, and polymerase activity of all four enzymes was linear over the time span and protein concentrations chosen. 100% values were as follows: (A) α , 7,020 cpm; β , 11,440 cpm; EBV-induced, 12,630 cpm; virion-associated, 1,420. (B) α , 8,747 cpm; β , 11,460 cpm; EBV-induced, 12,860 cpm; virion-associated, 1,844 cpm.

tro at concentrations at which it both strongly inhibited cell growth of P3HR-1 and B95-8 in culture and affected the infection process in cells freshly exposed to P3HR-1 or B95-8 virus.

DISCUSSION

The data reported here show that the virus-producer B cell lines B95-8 and P3HR-1 are markedly more susceptible to ara-A than are either the nonproducer Raji line or the virus-negative B cell Ramos line. Furthermore, DNA synthesis in freshly infected cells, particularly

Raji cells superinfected with P3HR-1 virus, was also far more sensitive to the drug than that in uninfected control cells. High levels of inhibition of cell growth or DNA synthesis were seen when the more sensitive cells are exposed to less than 10 μ M ara-A.

The studies in subcellular preparations showed a different picture from the studies of whole cells. Nuclei of both producer B95-8 and nonproducer Raji cells behaved similarly in the presence of ara-ATP; in vitro DNA synthesis was not significantly affected at drug concentrations which markedly inhibited growth of various types of whole cells. Furthermore, the EB virion-associated DNA polymerases were only partially inhibited in polymerase assays using drug concentrations which strongly affected the whole cells. Ara-A has been reported to significantly inhibit a partially purified preparation of calf thymus DNA polymerase at drug concentrations of 2 μ M or less in in vitro assays (12). In contrast, Reinke et al. (*in Oncogenesis and Herpesviruses III*, in press) have shown that HeLa cell α polymerase is 50% inhibited at 24 μ M. We have demonstrated about 30% inhibition of lymphocyte α at comparable drug concentrations. Therefore it is not known at this time how great a role DNA polymerase inhibition plays in the effects of ara-A on EBV-infected cells. It is possible, for example, that some other enzyme inhibited by ara-A, such as ribonucleotide reductase (22), is in part responsible for the effects reported here. This would explain why neither in vitro system, nuclei or DNA polymerase assays, was strongly affected by the drug at concentrations giving marked effects on cells in culture. This hypothesis is difficult to explore at this time, since ribonucleotide reductase activities or other virally induced enzymes involved with DNA synthesis which might be inhibited by the drug have not yet been characterized in EBV-containing lymphoblastoid lines. Still another possibility, however, is that the α or virion-associated polymerases may not show their maximal interaction with the drug under the in vitro conditions chosen here.

One final point concerns a metabolite of ara-A, hypoxanthine arabinoside. This metabolite is formed by the action of adenosine deaminase on ara-A. While data from several studies have shown that a portion of the ara-A added to tissue culture systems or administered to laboratory animals is converted to hypoxanthine arabinoside (4, 5, 10, 17, 20, 32, 33), these same studies have shown that ara-A is significantly more antiviral than hypoxanthine arabinoside in several different systems at similar concentration levels. In fact, evidence cited above and in other studies

clearly indicates that ara-A, not hypoxanthine arabinoside, is the effective compound in tissue culture and in whole animals. Relative to our findings reported here, there is the possibility that B95-8 cells, P3HR-1 cells, and freshly infected cells produce far less adenosine deaminase than the control cells, and thus could be more susceptible to lower concentrations of ara-A. If adenosine deaminase is present in the same relative amount in the nuclear systems compared with the amount of enzyme in whole cells, B95-8 nuclei should be somewhat more sensitive to the drug than Raji nuclei. However, both types of nuclei are equally insensitive. This again leads us to the suggestion that some other enzyme (such as ribonucleotide reductase) not required for DNA synthesis in a subcellular system is in fact responsible for the effects of the drug on whole EBV-infected cells.

In summary, the importance of the work reported here lies in the probability that ara-A may have multiple sites of antiviral action in infected cells.

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LITERATURE CITED

1. Abel, R., Jr., H. E. Kaufman, and J. Sugar. 1975. Intravenous adenine arabinoside against herpes simplex keratouveitis in humans. *Am. J. Ophthalmol.* 79:659-664.
2. Benz, W. C., and J. L. Strominger. 1975. Viral and cellular DNA synthesis in nuclei from human lymphocytes transformed by Epstein-Barr virus. *Proc. Natl. Acad. Sci. U.S.A.* 72:2413-2417.
3. Böyum, A. 1968. Isolation of leucocytes from human blood—further observations. *Scand. J. Clin. Lab. Invest.* 21(Suppl. 97):31-50.
4. Brink, J. J., and G. A. LePage. 1965. 9- β -D-Arabinofuranosyladenine as an inhibitor of metabolism in normal and neoplastic cells. *Can. J. Biochem.* 43:1-15.
5. Bryson, Y., J. D. Connor, L. Sweetman, S. Carey, M. A. Stuckey, and R. Buchanan. 1974. Determination of plaque inhibitory activity of adenine arabinoside (9- β -D-arabinofuranosyladenine) for herpesviruses using an adenosine deaminase inhibitor. *Antimicrob. Agents Chemother.* 6:98-101.
6. Ch'ien, L. T., N. J. Cannon, L. J. Charamella, W. E. Dismukes, R. J. Whitley, R. A. Buchanan, and C. A. Alford, Jr. 1973. Effect of adenine arabinoside on severe *Herpesvirus hominis* infections in man. *J. Infect. Dis.* 128:658-663.
7. Ch'ien, L. T., N. J. Cannon, R. J. Whitley, A. G. Diethelm, W. E. Dismukes, C. W. Scott, R. A. Buchanan, and C. A. Alford, Jr. 1974. Effect of adenine arabinoside on cytomegalovirus infections. *J. Infect. Dis.* 130:32-39.

8. Ch'ien, L. T., R. J. Whitley, A. J. Nahmias, E. B. Lewin, C. C. Linnemann, Jr., L. D. Frenkel, A. Bellanti, R. A. Buchanan, and C. A. Alford, Jr. 1975. Antiviral chemotherapy and neonatal herpes simplex virus infection: a pilot study—experience with adenine arabinoside (ARA-A). *Pediatrics* **55**:678–685.
9. Coker-Vann, M., and R. Dolin. 1977. Effect of adenine arabinoside on Epstein-Barr virus *in vitro*. *J. Infect. Dis.* **135**:447–453.
10. Connor, J. D., L. Sweetman, S. Carey, M. A. Stuckey, and R. Buchanan. 1975. Susceptibility *in vitro* of several large DNA viruses to the antiviral activity of adenine arabinoside and its metabolite, hypoxanthine arabinoside: relation to human pharmacology, p. 177–196. In D. Pavan-Langston, R. A. Buchanan, and C. A. Alford, Jr. (ed.), *Adenine arabinoside: an antiviral agent*. Raven Press, New York.
11. Freeman, G., A. Kuehn, and I. Sultanian. 1965. Tumorigenic virus and cell responses to biologically active compounds. II. Pharmacologic specificity in cell-virus relationships. *Ann. N.Y. Acad. Sci.* **130**:330–342.
12. Furth, J. J., and S. S. Cohen. 1968. Inhibition of mammalian DNA polymerase by the 5'-triphosphate of 1- β -D-arabinofuranosylcytosine and the 5'-triphosphate of 9- β -D-arabinofuranosyladenine. *Cancer Res.* **28**:2061–2067.
13. Gerber, P., and B. H. Hoyer. 1971. Induction of cellular DNA synthesis in human leukocytes by Epstein-Barr virus. *Nature (London)* **231**:46–47.
14. Henle, G., and W. Henle. 1966. Immunofluorescence in cells derived from Burkitt's lymphoma. *J. Bacteriol.* **91**:1248–1256.
15. Hinuma, Y., and J. T. Grace. 1967. Cloning of immunoglobulin producing human leukemic and lymphoma cells in long term cultures. *Proc. Soc. Exp. Biol. Med.* **124**:107–111.
16. Klein, G., T. Lindahl, M. Jondal, W. Leibold, J. Menezes, K. Nilsson, and C. Sundstrom. 1974. Continuous lymphoblastoid cell lines with characteristics of B cells (bone-marrow derived), lacking Epstein-Barr virus genome and derived from three human lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* **71**:3283–3286.
17. Koshiura, R., and G. A. LePage. 1968. Some inhibitors of deamination of 9- β -D-arabinofuranosyladenine and 9- β -D-xylofuranosyladenine by blood and neoplasms of experimental animals and humans. *Cancer Res.* **28**:1014–1020.
18. Lee, W. W., A. Benitez, L. Goodman, and B. R. Baker. 1960. Potential anti-cancer agents. XL. Synthesis of the β -anomer of 9-(D-arabinofuranosyl)-adenine. *J. Am. Chem. Soc.* **82**:2648–2649.
19. Luby, J. P., T. Johnson, R. A. Buchanan, L. T. Ch'ien, R. Whitley, and C. Alford. 1975. Adenine arabinoside therapy of varicella-zoster virus infections: summary of phase II studies, p. 237–245. In D. Pavan-Langston, R. A. Buchanan, and C. A. Alford, Jr. (ed.), *Adenine arabinoside: an antiviral agent*. Raven Press, New York.
20. Luby, J. P., S. R. Jones, M. T. Johnson, and D. Mikulec. 1975. Sensitivities of herpes simplex virus types 1 and 2 and varicella-zoster virus to adenine arabinoside and hypoxanthine arabinoside, p. 171–175. In D. Pavan-Langston, R. A. Buchanan, and C. A. Alford, Jr. (ed.), *Adenine arabinoside: an antiviral agent*. Raven Press, New York.
21. Miller, G., T. Shope, H. Lisco, D. Stitt, and M. Lipman. 1972. Epstein-Barr virus: transformation, cytopathic changes, and viral antigens in squirrel monkey and marmoset leukocytes. *Proc. Natl. Acad. Sci. U.S.A.* **69**:383–387.
22. Moore, E. C., and S. S. Cohen. 1967. Effects of arabinonucleotides on ribonucleotide reduction by an enzyme system from rat tumor. *J. Biol. Chem.* **242**:2116–2118.
23. Muller, W. E. G., A. Maidhof, R. K. Zahn, and W. M. Shannon. 1977. Effect of 9- β -D-arabinofuranosyladenine on DNA synthesis *in vivo*. *Cancer Res.* **37**:2282–2290.
24. Muller, W. E. G., H. J. Rohde, R. Beyer, A. Maidhof, M. Lachman, H. Taschner, and R. K. Zahn. 1975. Mode of action of 9- β -D-arabinofuranosyladenine on the synthesis of DNA, RNA, and protein *in vivo* and *in vitro*. *Cancer Res.* **35**:2160–2168.
25. Muller, W. E. G., R. K. Zahn, K. Bittlingmaier, and D. Falke. 1977. Inhibition of herpesvirus DNA synthesis by 9- β -D-arabinofuranosyladenine *in vitro* and *in vivo*. *Ann. N.Y. Acad. Sci.* **284**:34–48.
26. Nichols, W. W. 1964. *In vitro* chromosome breakage induced by arabinosyladenine in human leukocytes. *Cancer Res.* **24**:1502–1505.
27. Nonoyama, M., and J. S. Pagano. 1972. Replication of viral deoxyribonucleic acid and breakdown of cellular deoxyribonucleic acid in Epstein-Barr virus infection. *J. Virol.* **9**:714–716.
28. Nyormoi, O., D. A. Thorley-Lawson, J. Elkington, and J. L. Strominger. 1976. Differential effect of phosphonoacetic acid on the expression of Epstein-Barr viral antigens and virus production. *Proc. Natl. Acad. Sci. U.S.A.* **73**:1745–1748.
29. Privat de Garilhe, M., and J. Rudder. 1964. Effet de deux nucléosides de l'arabinose sur la multiplication des virus de l'herpès et de la vaccine en culture cellulaire. *C. R. Acad. Sci.* **259**:2725–2728.
30. Pulvertaft, R. J. V. 1964. Cytology of a Burkitt's tumor. *Lancet* **i**:238–240.
31. Robinson, J., and G. Miller. 1975. Assay for Epstein-Barr virus based on stimulation of DNA synthesis in mixed leukocytes from human umbilical cord blood. *J. Virol.* **15**:1065–1072.
32. Schabel, F. M. 1968. The antiviral activity of 9- β -D-arabinofuranosyladenine (ARA-A). *Chemotherapy* **13**:321–338.
33. Shannon, W. M. 1975. Adenine arabinoside: antiviral activity *in vitro*, p. 1–43. In D. Pavan-Langston, R. A. Buchanan, and C. A. Alford, Jr. (ed.), *Adenine arabinoside: an antiviral agent*. Raven Press, New York.
34. Shaw, J. E., T. Seebeck, H. L. Jui-Lien, and J. S. Pagano. 1977. Epstein-Barr virus DNA synthesized in superinfected Raji cells. *Virology* **77**:762–771.
35. Sidwell, R. W., G. J. Dixon, S. M. Sellers, and F. M. Schabel, Jr. 1968. *In vivo* antiviral properties of biologically active compounds. II. Studies with influenza and vaccinia viruses. *Appl. Microbiol.* **16**:370–392.
36. Summers, W. C., and G. Klein. 1976. Inhibition of Epstein-Barr virus DNA synthesis and late gene expression by phosphonoacetic acid. *J. Virol.* **18**:151–155.
37. Symons, R. H. 1974. Synthesis of α -³²P ribo- and deoxynucleoside 5'-triphosphates. *Methods Enzymol.* **29**:102–115.
38. Thorley-Lawson, D., and J. L. Strominger. 1976. Transformation of human lymphocytes by Epstein-Barr virus is inhibited by phosphonoacetic acid. *Nature (London)* **263**:332–334.
39. Weissbach, A., S. L. Hong, J. Aucker, and R. Muller. 1973. Characterization of herpes simplex virus-induced DNA polymerase. *J. Biol. Chem.* **248**:6270–6277.
40. Whitley, R. J., L. T. Ch'ien, R. Dolin, G. J. Galasso, and C. A. Alford, Jr. 1976. Adenine arabinoside therapy of herpes zoster in the immunosuppressed. *N. Engl. J. Med.* **294**:1193–1199.