ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1992, p. 1589–1595
0066-4804/92/081589-07$02.00/0

Vol. 36, No. 8

MINIREVIEW

Resistance of Herpesviruses to Antiviral Drugs

PAMELA A. CHATIS AND CLYDE S. CRUMPACKER*

Division of Infectious Diseases, Department of Medicine, Beth Israel Hospital, and Harvard Medical School, Boston, Massachusetts 02215

INTRODUCTION

The study of resistance of herpesviruses to antiviral drugs provides an important tool for understanding mechanisms of drug action and understanding clinical failures with antiviral therapy. The increasing numbers of patients with AIDS and recipients of organ transplants who are receiving antiviral drugs are bringing the study of drug resistance into the mainstream of clinical investigation. The antiviral drugs against the herpesviruses provide the best examples of effective antiviral therapy and represent the best models that can be used to design better therapies against other human viral diseases (18). Understanding of the mechanisms of resistance to a drug can provide a rational basis for using other drugs which work by different mechanisms and for combining drugs which act synergistically on different steps in viral replication. Most importantly, the location of a mutation in the gene of a viral enzyme which enables the virus to replicate in the presence of an active form of the antiviral drug provides solid evidence that the mechanism of action for that drug involves the specific viral gene product.

The study of drug resistance in herpesviruses is having an increasing impact on direct patient care. Since the first report of resistance to acyclovir in an immunocompromised, herpes simplex virus (HSV)-infected patient in 1982 (14), numerous other reports of resistance have been published (1, 6, 9, 11, 12, 22–24, 42, 53, 56, 58, 60, 69, 77). In the setting of patients with AIDS, resistance has clearly become clinically significant in HSV type 1 (HSV-1) and HSV-2 (2, 7, 8, 27, 33, 50, 55, 61, 63, 65), cytomegalovirus (CMV) (4, 19, 25), and varicella-zoster virus (VZV) (35, 40, 46, 57).

Prior to the advent of AIDS, acyclovir resistance occurred infrequently and only in immunocompromised hosts. The emergence of resistance as a clinically relevant problem is reflected by the severe immunocompromised state of patients with AIDS, which leads to an increase in viral burden and, therefore, a greater likelihood of the emergence of resistance. The clinical settings in which resistance is significant usually include extensive mucocutaneous HSV lesions which fail to heal with high-dose acyclovir (8, 27, 33, 55, 61, 63, 65), CMV retinitis which continues to progress with aggressive ganciclovir therapy (19, 25), and persistent disseminated hyperkeratotic VZV papules which fail to heal with high-dose acyclovir therapy (35, 40, 46, 57).

MECHANISMS OF RESISTANCE OF HSV-1 AND HSV-2

The deoxyguanosine analog acyclovir is the prototype of a group of anti-herpesvirus drugs which use a virus-specific enzyme to form an active inhibitor of viral DNA replication (17, 21, 31). Its effectiveness in treating many herpesvirus infections and its remarkable lack of toxicity indicate it is an antiviral drug which will be in use for a long time (17). Acyclovir is a preferred substrate for herpesvirus thymidine kinase (TK), which phosphorylates acyclovir to acyclovir monophosphate (54). Cellular deoxyguanosine-phosphorylating enzymes then convert acyclovir to its triphosphate form (31, 54). Acyclovir triphosphate is an effective competitive inhibitor of dGMP incorporation into DNA and is a chain terminator for elongating herpesvirus DNA (21, 31).

The mechanism of inhibition of HSV-1 DNA polymerase by acyclovir triphosphate had been described earlier as a suicide substrate for HSV-1 DNA polymerase, leading to inactivation of the enzyme (32). This mechanism of inhibition of HSV-1 DNA polymerase has been reexamined and is more correctly described as an induced substrate form of inhibition. The acyclovir monophosphate residue is incorporated into the template primer, and the addition of the next nucleotide (dCTP) results in the formation of a reversible "dead-end complex" which includes the DNA polymerase (59). The activated acyclovir triphosphate is a potent and specific inhibitor of herpesvirus DNA polymerase, exhibiting 30 times more preferential inhibition of the viral polymerase than of the cellular polymerase (21, 31).

Three mechanisms have been associated with the resistance of HSV to acyclovir (12, 17). Two mechanisms involve the viral TK enzyme, with the primary one being selection of TK-deficient (TK^-) mutants of HSV which fail to phosphorylate the drug (7, 66). The other mechanism involving the viral TK is the selection of viruses which induce an altered TK that phosphorylates thymidine but that fails to phosphorylate acyclovir (16, 23, 51).

Using a collection of TK mutants with distinct substrate specificities, Darby et al. (15) proposed an active center model for the HSV-1 TK polypeptide which consists of three highly conserved regions: the nucleotide-binding pocket (amino acid residues 49 to 66), the thymidine-binding site (amino acid residues 168 to 178), and an ATP- or nucleoside-binding site (amino acid residue 336). Darby et al. (15) showed that drug-resistant isolates which had alterations in nucleoside substrate affinities had amino acid changes in the thymidine-binding site region (amino acid residues 168 to 178). Specifically, mutant B3, the bromovinyldeoxyuridine-resistant mutant, had a substitution of threonine for alanine at amino acid residue 176. A third mutant, S1, had a dramatic effect on nucleoside and ATP binding. This mutant had a tyrosine-for-cysteine substitution at amino acid residue 336, the putative ATP- or nucleoside-binding site (15). Subsequently, mutations in other regions of the gene have been shown to be associated with a decreased phosphorylating ability of the viral TK (7).

An altered viral DNA polymerase enzyme may also be

* Corresponding author.

selected by acyclovir. This enzyme is capable of elongating viral DNA in the presence of high concentrations of acyclovir triphosphate (43). The altered enzyme contains point mutations in specific conserved regions of the viral polymerase (45).

The HSV DNA polymerase enzyme is a member of a large family of alpha DNA polymerases which show remarkably consistent structural features (80). The herpesvirus polymerase is 1,235 amino acids long and possesses three functional domains consisting of a 5′-3′ exonuclease (RNase H function), a 3′-5′ exonuclease (editing function), and a large catalytic domain involving amino acids 606 to 1135 in a single peptide (36). All of the drug resistance mutations have been located in the catalytic domain in the carboxyl-terminal portion of the enzyme and have helped to define important functions in this region of the enzyme (11–13, 18, 36, 45).

The catalytic domain of all of the alpha DNA polymerases contains six highly conserved regions of nucleotide sequences which are ordered in the same relationship to each other. This highly conserved arrangement of nucleotide sequences in a large number of DNA polymerases of cellular (human alpha and yeast alpha), viral (herpesviruses, vaccinia virus, and adenovirus), and phage (T4, 029, PRD1) origin suggests that these structural similarities provide strong functional consequences (80). The similarities in the viral polymerases have also been extended to human immunodeficiency virus (HIV) reverse transcriptase. Region I in herpesvirus polymerases contains the highly conserved sequence Y-G-D-T-D, and in HIV type 1 (HIV-1) reverse transcriptase, the most highly conserved region with an active center consists of the sequence Y-M-D-D. In addition, the homology between DNA polymerase and HIV-1 reverse transcriptase has been extended to show that the RNase H domain of HIV-1 is spatially oriented in a manner very similar to that of the *Escherichia coli* polymerase I helices (72). In the HSV DNA polymerase, all of the drug-resistant mutations are found in the highly conserved regions, most notably in regions II and III. Resistance to acyclovir, adenine arabinosine, phosphonoacetic acid, and phosphonoformic acid appears to be conferred by mutations in the highly conserved regions (12, 17, 28, 45). A single-base change is usually associated with resistance. The most compelling evidence indicating that a single-base change leading to an amino acid change is all that is needed for resistance to occur results from site-specific mutagenesis studies on the cloned HSV-1 polymerase expressed in yeast (37, 52). As a result of the clinical use of acyclovir, selection of two distinct polymerase mutants has occurred, and the DNA polymerase gene of both mutants has been sequenced (11, 39, 58, 60). One DNA *pol* mutant contained a single-base change of glycine to serine at position 341 in region III on the polymerase (11, 58), and the other *pol* mutant contained a change of tyrosine to histidine at position 941 in a region designated VII (39). With the increasing recognition of polymerase mutants associated with antiviral drug use, it is likely that more mutations will be identified, and they may be identified in regions on the *pol* gene which have not yet been associated with resistance.

ANTIVIRAL DRUG RESISTANCE OF HSV-1 AND HSV-2 WITH CLINICAL USE OF ACYCLOVIR

Recently, there have been several reports describing chronic HSV infections in patients with AIDS which fail to heal with high-dose acyclovir therapy (8, 27, 33, 55, 61, 63, 65). Two reports have described the initial isolation (8, 27)

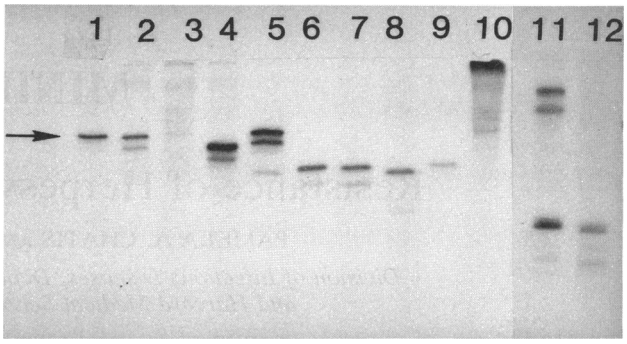

FIG. 1. Immunoprecipitation of TK polypeptides from ACV^r clinical isolates was carried out with a monoclonal antibody (K. Powell) to the HSV-2 TK polypeptide and Sepharose 4B-protein A agarose beads (Genzyme). The heterogeneity in the TK peptide induced by the TK-deficient mutants of HSV-2 is shown. HSV-infected Vero cell monolayers were labeled with [^{35}S]methionine (25 μCi/ml) for 20 min at 4.5 h postinfection. Immunoprecipitates were subjected to sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis. The gel was dried and exposed to Kodak XAR film. An autoradiograph of wild-type HSV-2 333 (lane 1), ACV^r 86012 (lane 2), ACV^r 87011 (lane 3), ACV^r 87040 (lane 4), ACV^r 87050 (lane 5), ACV^r 87034 (lane 6), ACV^r 85012 (lane 7), ACV^r 87020 (lane 8), ACV^r A590 (lane 9), uninfected cells (lane 10), ACV^r 88080 (lane 11), and ACV^r 88071 (lane 12) is shown. The position of the HSV-2 TK polypeptide is indicated by an arrow. Reprinted from *Virology* (7), with permission.

and characterization (7) of 13 acyclovir-resistant (ACV^r) HSV-2 viruses isolated from patients with AIDS. All of these isolates were shown to be TK$^-$ by a biochemical assay for TK which measures [^{3}H]thymidine phosphorylation with an extract of infected cells (27). The median inhibitory dose (50% effective concentration [EC_{50}]) of acyclovir for these ACV^r isolates ranged from 7.1 to 91.4 μg/ml, whereas it was 0.27 μg/ml for a wild-type reference strain (27). There has not been uniform agreement on what level of in vitro susceptibility indicates a resistant virus. In another report, an EC_{50} of 1 μg/ml was taken to describe acyclovir-susceptible HSV isolates, and a value of greater than 2 μg/ml defined resistance (24). This definition of resistance to acyclovir is unusually high, because most laboratories would find an EC_{50} of greater than 1 μg/ml as clearly indicating resistance. All but one of the clinical isolates synthesized a TK protein with altered electrophoretic mobility compared with that of the wild-type strain, as detected by immunoprecipitation (8) (Fig. 1). The mutant (ACV^r 86012) which induced a full-length TK protein (Fig. 1, lane 2) was neuropathogenic for mice and contained a single amino acid change from glutamine to proline at position 105 on the TK polypeptide, a region outside of the putative nucleotide-binding region (8). The multiple forms of the TK proteins which were selected with acyclovir use illustrate the remarkable heterogeneity of viruses and their proteins which may occur in patients with AIDS. From this analysis and results from other investigators (1a), it is possible to conclude that most TK$^-$ mutants selected with acyclovir therapy produce aberrant TK proteins which either are truncated and have altered mobility on polyacrylamide gel electrophoresis analysis or are completely undetectable. The best data on the prevalence of ACV^r HSV isolates are found in a report by Englund et al. (24) from a tertiary-care hospital. They found ACV^r HSV in 7 of 148 (4.7%) immunocompromised patients

and in none of the isolates from 59 immunocompetent patients treated in a tertiary-care hospital. They assayed all of the HSV isolates obtained in the virology laboratory over 1 year by a DNA hybridization assay. Resistance to ACV was defined as an EC_{50} of greater than 2 μg/ml. That study (24) may have seriously underestimated the incidence of resistance to acyclovir, because the cutoff for resistance of an EC_{50} of 2 μg/ml is much greater than that which would be selected in other studies, and therefore, some of the conclusions must be interpreted with caution. Clinical disease occurred in all seven patients, and the occurrence of disease in association with the presence of drug-resistant virus was more severe in pediatric patients. All the resistant isolates had absent or altered TK activities. Other reports have documented the clinical occurrence of resistance caused by viruses that possess altered TK activities (23, 51).

PATHOGENICITIES OF ACV^r HSV STRAINS

Previous reports have suggested that TK-deficient HSV isolates have diminished neuropathogenicities and diminished abilities to establish infections in the central nervous system (20, 28, 29, 56, 57, 69, 73–75). In neuropathogenicity studies with a murine model, Schinazi (64) determined that there are differences in the degree of virulence displayed by HSV-1 isolates in comparison with those displayed by HSV-2 isolates. Schinazi (64) determined that TK-deficient HSV-1 isolates are, in general, severely impaired in their ability to induce neuropathogenesis in mice in comparison with TK-deficient HSV-2 isolates. Coen et al. (10) addressed the questions of latency and reactivation by constructing TK mutants with defined deletions in the TK gene which were able to establish latent infections in mice but could not be reactivated. In mice ganglia infected with this mutant, only 20% of the expression of the latency-associated transcript was detected compared with that in a wild-type infection, and this decreased infection may account for the inability of the HSV mutant to be reactivated. In a recent study, Wilcox et al. (79) used TK deletion mutants of HSV-1 which were carefully constructed so that the TK function was eliminated without affecting other known transcripts encoded in that region of the genome. That study showed that the TK deletion mutants were able to establish latency and could be fully reactivated in sensory neurons in culture, suggesting that HSV-1 TK activity facilitates viral replication, but that TK activity is not essential for either replication or reactivation from latent infections in neurons in vitro. In the study of 13 patients with AIDS and ACV^r HSV-2 infection mentioned above, Erlich et al. (27) demonstrated that at least one isolate was fully neurovirulent in a mouse model of encephalitis. In addition, 6 of 10 ACV^r isolates established latent infections in murine trigeminal ganglia. Thus, the ACV^r virus that develops in the mucocutaneous lesions of patients with AIDS may be capable of infecting the sensory ganglia, establishing neural latency and affecting disease sequelae in the population with AIDS.

Gately et al. (33) described a patient with AIDS who developed meningoencephalitis while undergoing acyclovir therapy for a persistent perirectal herpesvirus lesion. Initially, an HSV-2 TK$^+$ viral isolate was isolated from a brain biopsy specimen, and the patient responded with combined acyclovir and vidarabine therapy. However, a fatal relapse of the meningoencephalitis occurred. The meningoencephalitis failed to respond to intravenous acyclovir therapy. An HSV-2 ACV^r TK$^-$ virus was isolated from the patient's cerebrospinal fluid. Pathogenicity studies were conducted in a mouse model. The studies showed that the TK$^-$ isolate had greater pathogenic potential than previously studied isolates. As a result, the authors thought that the isolate was sufficiently neurovirulent to be the cause of the death of the patient (33). Another report described three bone marrow transplant patients who developed pneumonia because of ACV^r HSV-1 (47). Two additional reports described the characterization of ACV^r (TK$^-$) isolates from two patients with chronic lymphocytic leukemia and chronic myelogenous leukemia (56, 60). All of the reports underscore an underlying immunoincompetency in all of the patients from whom ACV^r HSV was isolated (9, 22, 42, 47, 53, 56, 76, 77). No reproducible well-documented cases of ACV^r HSV have occurred in patients with normal immune system defenses.

In the report by Englund et al. (24) of ACV^r HSV in a tertiary-care hospital, three of the patients with resistant HSV, including two infants, had progressive and life-threatening disease associated with the TK$^-$ virus strain. The conclusion from the several separate reports indicates that ACV^r TK-deficient mutants of HSV have the capacity to produce progressive neurovirulent disease in patients, and this is an important area for further study.

ALTERNATIVE THERAPY FOR ACV^r HSV

Alternative therapies for HSV mucocutaneous infections that fail to resolve with acyclovir therapy are being studied. Vidarabine (adenine arabinoside) is phosphorylated directly to arabinosyl-ATP (ara-ATP) by cellular enzymes (mainly adenosine kinase). Ara-ATP is a potent competitive inhibitor of HSV DNA polymerase and is incorporated into DNA by internucleotide linkage. It does not require specific activation by viral enzymes. Ara-AMP becomes incorporated into the primer terminus and produces a significant decrease in the rate of primer elongation. The 3′-terminal ara-AMP residues are removed by action of the HSV-1 DNA polymerase-associated exonuclease activity. The HSV exonuclease, however, does not remove terminally incorporated acyclovir monophosphate and does not appear to possess an editing function for this nucleoside analog (12, 53).

Foscarnet (trisodium phosphonoformate) is an inhibitor of PP_i exchange and can act directly as a noncompetitive inhibitor of viral DNA polymerase. This compound is not incorporated into viral DNA. Foscarnet is a noncompetitive inhibitor of viral DNA polymerase when deoxynucleotide triphosphates are used as variable substrates but is uncompetitive with respect to the template as the variable substrate (26). All of the triphosphates of nucleoside analogs inhibit viral DNA synthesis directly as competitive inhibitors of viral DNA polymerase, by chain termination of viral DNA elongation, or by incorporation into viral DNA; foscarnet is an exception to this mechanism (8, 12, 17, 53, 78). Foscarnet is difficult to administer because of many toxic side effects. These include renal failure, seizures, hypocalcemia, hypomagnesemia, and severe nausea.

In the study of 13 patients with AIDS and ACV^r (TK$^-$) mucocutaneous HSV mentioned above (27), all isolates were susceptible to foscarnet (EC_{50}s, 1.9 to 23.5 μg/ml) in vitro. Foscarnet can successfully be used to treat infections caused by ACV^r HSV infections (8, 76). In a well-documented study of three successive outbreaks of recurrent genital herpesvirus infections in one patient with AIDS, the first outbreak was associated with an acyclovir-susceptible virus with a full-length viral TK enzyme, and the lesion healed with acyclovir therapy (8). A second outbreak with an ACV^r

mutant containing a truncated viral TK occurred, and this cleared with foscarnet therapy but was followed by a third outbreak in an anatomically distinct site by an acyclovir-susceptible virus with a full-length TK enzyme. All three isolates were of the same virus strain, as determined by the identical restriction endonuclease patterns of DNA fragments from all three isolates (8). This indicates that an episode of HSV infection caused by an ACV^r mutant that possesses an altered viral TK may be followed by a subsequent episode with an acyclovir-susceptible virus strain, probably following reactivation of the initially infecting virus from the sacral ganglion.

In an uncontrolled study, 26 patients with mucocutaneous ACV^r herpesvirus lesions were treated with foscarnet (61). Clinical responses were noted in 81% of the patients; complete reepithelialization of the lesions occurred in 73% of the patients.

An AIDS Clinical Trial Group randomized study comparing foscarnet and vidarabine for the treatment of ACV^r mucocutaneous HSV infection in patients with AIDS demonstrates that all the viral isolates from the patients were TK deficient, and the study showed that virus clearing and complete healing occurred with foscarnet treatment but not with vidarabine treatment (63). The study demonstrated that even though ACV^r HSV may be susceptible to vidarabine in vitro, this was not associated with a clinical benefit, and in that setting, vidarabine had in vitro effects that were not borne out when it was used clinically. The study concluded that for the treatment of mucocutaneous ACV^r HSV infections in patients with AIDS, foscarnet has superior efficacy and less frequent serious toxicity than does vidarabine (63). Vidarabine, however, is still useful in the treatment of HSV encephalitis and neonatal HSV infections.

VZV ANTIVIRAL DRUG RESISTANCE

The treatment of choice for VZV infections is acyclovir (17, 48). As in HSV infections, acyclovir is activated to the monophosphate form by a viral-encoded TK protein. Until the AIDS epidemic, documented ACV^r in the setting of disseminated VZV infection had never been reported (35, 46), although ACV^r VZV can be selected for in the laboratory (68). Pahwa et al. (57) described continuous VZV infections associated with ACV^r in a child with AIDS. In addition, in a report by Jacobson et al. (40) four profoundly immunocompromised patients with advanced HIV disease developed cutaneous persistent disseminated VZV infections that were highly resistant to acyclovir in vitro but that were susceptible to vidarabine and foscarnet. All four of these patients initially received and responded to intravenous and oral acyclovir therapy, but the VZV infections subsequently recurred with persistent disseminated zoster lesions after the patients received 1 to 5 months of chronic suppressive oral therapy of 0.4 to 4 g of acyclovir per day. Talarico et al. (71) extended the data from these four patients by sequencing polymerase chain reaction-amplified DNA encoding the viral TK gene of plaque-purified virus from these clinical isolates. Two of the isolates had deletions in their TK genes. The two remaining isolates had nucleotide substitutions in the proposed nucleoside-binding sites which would be expected to affect the substrate specificity of the protein (15, 71).

ALTERNATIVE THERAPY FOR ACV^r VZV INFECTIONS

On the basis of in vitro susceptibility testing of clinical isolates from patients with persistent hyperkeratotic VZV papules, either foscarnet or vidarabine may be a useful therapy for ACV^r VZV infections (44, 67). Susceptibility testing might suggest that vidarabine and foscarnet would be effective in treating persistent ACV^r VZV. One of the four patients with AIDS and ACV^r VZV mentioned above (40) was treated with vidarabine, with no clinical improvement. On the basis of experience with ACV^r HSV infections, however, it is likely that foscarnet might be beneficial in patients with ACV^r VZV infections. This was addressed in a small uncontrolled study conducted by Safrin et al. (62). In four of five patients with ACV^r VZV infections and a dermatomal zoster eruption, foscarnet was an effective antiviral therapy, as determined by healing of the mucocutaneous VZV infection. On the basis of the failure of vidarabine therapy in a controlled trial in patients with ACV^r HSV infections and the neurotoxicity of vidarabine, vidarabine should not be routinely considered as alternative therapy for ACV^r HSV or VZV infections.

RESISTANCE OF CMV TO GANCICLOVIR

The guanosine analog ganciclovir is the first licensed drug for the treatment of severe CMV infections. It is effective treatment for CMV retinitis in patients with AIDS. It can also provide benefit for the treatment of colitis in patients with AIDS, but this has not been rigorously established. Ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl) guanine] is phosphorylated to ganciclovir triphosphate in CMV-infected cells, and high levels of ganciclovir triphosphate are present in CMV-infected cells (5). Ganciclovir triphosphate is an effective inhibitor of CMV DNA polymerase at concentrations much lower than those that affect the human cellular alpha polymerase. In contrast, acyclovir has little activity against CMV infection, and very little acyclovir triphosphate is found in CMV-infected cells (5). The complete nucleotide sequence of the CMV genome has been determined, and there are convincing data which indicate that the CMV genome does not encode a TK enzyme or any likely candidate for a nucleotide kinase. The mechanism by which CMV-infected cells produce high levels of ganciclovir triphosphate is obscure, and it is not clear whether a virus-encoded enzyme or a cellular enzyme which is induced by a viral protein is responsible for phosphorylation of ganciclovir. Recent work suggests that the product of the CMV UL97 gene may be able to phosphorylate ganciclovir (2a).

The study of a ganciclovir-resistant mutant of CMV selected by growth of CMV strain AD169 in the presence of ganciclovir indicates that the CMV-resistant mutant BW759-D100 fails to induce ganciclovir triphosphate (3). This is the main explanation for the failure of ganciclovir to inhibit infection with this CMV mutant, even though this mutant has recently been shown to possess a second mutation in a highly conserved region of the CMV polymerase which leads to an amino acid change in the viral DNA polymerase (70). This appears to be a double mutant of CMV, but the mutant remains susceptible to foscarnet. To date, all of the mutants of CMV which exhibit resistance to ganciclovir, either from mutants derived in vitro or CMV mutants which have arisen from clinical use of ganciclovir, have a common mechanism in that they fail to induce ganciclovir triphosphate following

infection (4, 25). All of the clinical isolates are susceptible to foscarnet, and there have been well-documented examples of effective clinical treatment with foscarnet (41). A previous study on the DNA polymerase of HSV-1 showed that mutants that were resistant to phosphonoacetic acid because of a mutation in the DNA polymerase gene were still susceptible to ganciclovir. This finding suggests that ganciclovir triphosphate and foscarnet may interact with different regions of the HSV-1 DNA polymerase enzyme (13). Synergy between ganciclovir and foscarnet has also been demonstrated for CMV infection in tissue culture (49). The combination of ganciclovir and foscarnet has also been studied in cell-free systems (30).

With the clinical use of ganciclovir, resistant CMV isolates have been observed in a patient with leukemia and two patients with AIDS (25). All three patients had progressive CMV disease which did not respond to treatment. By restriction endonuclease analysis, it was clear that one of the isolates was initially susceptible to ganciclovir, and a virus from the same patient with an identical pattern of restriction endonuclease fragments was isolated; that isolate exhibited resistance to ganciclovir (25). All three ganciclovir-resistant CMV mutants described above failed to phosphorylate ganciclovir in infected cells (4). To determine the prevalence of resistant CMV isolates in patients receiving ganciclovir, Drew et al. (19) prospectively monitored 72 patients with AIDS and CMV disease for the development of drug-resistant virus. No resistant strains were found in patients before therapy or in patients who received ganciclovir for less than 3 months. At the end of 3 months, 80% of the patients were not excreting virus. In the 20% of patients who were excreting virus after 3 months of ganciclovir treatment, however, 38% of the patients excreted resistant virus for which the EC_{50} was >12 μM. For all susceptible isolates, the EC_{50} was less than 6 μM. The overall prevalence of ganciclovir-resistant CMV was 7.6% after 3 months of therapy (19). All of the ganciclovir-resistant strains of CMV were clinically significant, as indicated by the progression of retinitis. The investigators (19) concluded that if the patients excreted CMV after 3 months of ganciclovir therapy, there was a 40% chance that they would excrete virus which was resistant to ganciclovir and that they would be candidates for alternative therapy. All of the ganciclovir-resistant strains of CMV remained susceptible to foscarnet (19).

ALTERNATIVE THERAPY FOR GANCICLOVIR-RESISTANT CMV

For well-documented ganciclovir-resistant CMV, foscarnet is the therapy of choice and has been licensed by the U.S. Food and Drug Administration for ganciclovir-resistant CMV (41, 78). To date, resistant strains of CMV fail to phosphorylate ganciclovir but remain susceptible to foscarnet. With the expanded use of foscarnet, it is likely that foscarnet resistance will also be encountered in CMV-infected patients, and a foscarnet-resistant HSV strain has been reported (2). The dose of foscarnet to be used requires an induction intravenous dose of 60 mg/kg of body weight three times a day. This is followed by a maintenance dose of either 90 or 120 mg/kg/day (41). It also appears that the toxicity on the 120-mg/kg maintenance regimen is not greater than the toxicity on the 90-mg/kg/day treatment regimen and is well tolerated. In other studies on foscarnet maintenance therapy, the median time to retinitis progression has been reported to be from 49 to more than 123 days with dosages of 60 to 120 mg/kg/day or 420 to 840 mg/kg/week (41, 78). The time to retinitis progression in patients on foscarnet therapy appears to be similar to the time observed for progression to retinitis in patients on ganciclovir maintenance regimens.

Another treatment strategy for patients with CMV retinitis who fail ganciclovir therapy because of severe neutropenia and an inability to take the drug is a regimen of ganciclovir and the granulocyte macrophage colony-stimulating factor (38). That regimen was tested in a randomized trial which showed that patients had fewer episodes of neutropenia which required the cessation of ganciclovir therapy. The patients were able to stay on the ganciclovir therapy for a longer period of time, and there was a prolonged time to the progression of CMV retinitis. The addition of granulocyte macrophage colony-stimulating factor to ganciclovir for the treatment of CMV retinitis enables successful treatment for patients who would otherwise not be able to receive ganciclovir. The first demonstration that the combination of ganciclovir and granulocyte macrophage colony-stimulating factor would produce an enhanced effect in humans with CMV infection was reported by Grossberg et al. in 1989 (34).

The review of the properties of resistant herpesviruses, their clinical significance, and alternative therapies presented here indicates the progress that has been made with herpesvirus therapy. This review also points out the remarkable heterogeneity that may occur in virus populations, particularly in severely immunocompromised patients. Clinical isolates of herpesviruses consist of multiple virion subpopulations with differential TK activities even in the absence of acyclovir therapy. Thus, it appears that clinical isolates that are able to grow to a high titer and that are resistant to acyclovir and other drugs most likely occur because of the selection of a virion subpopulation rather than the occurrence of a new mutation. This points the way to future studies on virus heterogeneity in human diseases and new antiviral agents directed at herpesviruses.

REFERENCES

1. **Balfour, H. H., Jr.** 1983. Resistance of herpes simplex to acyclovir. Ann. Intern. Med. **98:**404–406. (Editorial.)

1a. **Birch, C.** Personal communication.

2. **Birch, C. J., G. Tachedjian, R. R. Doherty, K. Hayes, and I. D. Gust.** 1990. Altered sensitivity to antiviral drugs of herpes simplex virus isolates from a patient with the acquired immunodeficiency syndrome. J. Infect. Dis. **162:**731–734.

2a. **Biron, K. K.** Personal communication.

3. **Biron, K. K., J. A. Fyfe, S. C. Stanat, L. K. Leslie, J. B. Sorrell, C. U. Lambe, and D. M. Coen.** 1986. A human cytomegalovirus mutant resistant to the nucleoside analog 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine (BW B759U) induces reduced levels of BW B759U triphosphate. Proc. Natl. Acad. Sci. USA **83:**8769–8773.

4. **Biron, K. K., S. C. Stanat, J. Reardon, A. Erice, H. Balfour, and M. C. Jordan.** 1989. Ganciclovir-resistant clinical isolates of CMV: antiviral susceptibility profiles and mode of resistance studies, abstr. 65. Abstr. Second Int. Cytomegalovirus Workshop. University of California, San Diego.

5. **Biron, K. K., S. C. Stanat, J. B. Sorrell, J. A. Fyfe, P. M. Keller, C. U. Lambe, and D. J. Nelson.** 1985. Metabolic activation of the nucleoside analog 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine in human diploid fibroblasts infected with human cytomegalovirus. Proc. Natl. Acad. Sci. USA **82:**2473–2477.

6. **Burns, W. H., R. Sanat, and G. W. Santos.** 1982. Isolation and characterization of resistant herpes simplex virus after acyclovir therapy. Lancet **i:**421–424.

7. **Chatis, P. A., and C. S. Crumpacker.** 1991. Analysis of the thymidine kinase gene from clinically isolated acyclovir-resistant herpes simplex viruses. Virology **180:**793–797.

8. **Chatis, P. A., C. H. Miller, L. E. Schrager, and C. S. Crump-**

acker. 1989. Successful treatment with foscarnet of an acyclovir-resistant mucocutaneous infection with herpes simplex virus in a patient with acquired immunodeficiency syndrome. N. Engl. J. Med. **320:**297–300.

9. **Christophers, J., and R. N. P. Sutton.** 1987. Characterisation of acyclovir-resistant and -sensitive clinical herpes simplex virus isolates from an immunocompromised patient. J. Antimicrob. Chemother. **20:**389–398.
10. **Coen, D. M., M. Kosz-Vnenchak, J. G. Jacobson, D. A. Lieb, C. L. Bogard, P. A. Schaeffer, K. L. Tyler, and D. M. Knipe.** 1989. Thymidine kinase negative herpes simplex virus mutants establish latency in mouse trigeminal ganglia but do not reactivate. Proc. Natl. Acad. Sci. USA **86:**4736–4740.
11. **Collins, P., B. A. Larder, N. M. Oliver, S. Kemp, I. W. Smith, and G. Darby.** 1989. Characterization of a DNA polymerase mutant of herpes simplex virus from a severely immunocompromised patient receiving acyclovir. J. Gen. Virol. **70:**375–382.
12. **Crumpacker, C. S.** 1988. Resistance of herpes viruses to nucleoside analogs: mechanisms and clinical importance. *In* E. De Clercq (ed.), Clinical use of antiviral drugs. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.
13. **Crumpacker, C. S., P. N. Kowalsky, S. A. Oliver, L. E. Schnipper, and A. K. Field.** 1984. Resistance of herpes simplex virus to 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine: physical mapping of drug synergism within the viral DNA polymerase locus. Proc. Natl. Acad. Sci. USA **310:**1545–1560.
14. **Crumpacker, C. S., L. E. Schnipper, S. I. Marlowe, P. N. Kowalsky, B. J. Hershey, and M. J. Levin.** 1982. Resistance to antiviral drugs of herpes simplex virus isolated from a patient treated with acyclovir. N. Engl. J. Med. **306:**343–346.
15. **Darby, G., B. A. Larder, and M. M. Ingliss.** 1986. Evidence that the "active centre" of the herpes simplex virus thymidine kinase involves interaction between three distinct regions of the polypeptide. J. Gen. Virol. **67:**753–758.
16. **Darby, G., H. Salisbury, and H. J. Field.** 1981. Altered substrate specificity of herpes simplex virus thymidine kinase confers acyclovir-resistance. Nature (London) **289:**81–83.
17. **Dorsky, D. I., and C. S. Crumpacker.** 1987. Drugs five years later: acyclovir. Ann. Intern. Med. **107:**859–874.
18. **Dorsky, D. I., and C. S. Crumpacker.** 1990. Site-specific mutagenesis of a highly conserved region of the herpes simplex type 1 DNA polymerase gene. J. Virol. **64:**1394–1397.
19. **Drew, W. L., R. C. Minor, D. F. Busch, S. E. Follansbee, J. Gullett, S. G. Mehalko, S. M. Gordon, W. F. Owen, T. R. Matthews, W. C. Buhles, and B. De Armond.** 1991. Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. J. Infect. Dis. **163:**716–719.
20. **Efstathiou, S., S. Kemp, G. Darby, and A. C. Minson.** 1989. The role of herpes simplex virus type 1 thymidine kinase in pathogenesis. J. Gen. Virol. **70:**869–879.
21. **Elion, G. B., P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp, and J. H. Schaeffer.** 1977. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)-guanine. Proc. Natl. Acad. Sci. USA **74:**5716–5720.
22. **Ellis, M. N.** 1987. Progressive mucocutaneous herpes simplex infection due to acyclovir-resistant virus in an immunocompromised patient: correlation of viral susceptibilities and plasma levels with response to therapy. Diagn. Microbiol. Infect. Dis. **7:**199–204.
23. **Ellis, M. N., P. M. Keller, J. A. Fyfe, J. L. Martin, J. F. Rooney, S. E. Straus, S. Nusinoff Lehrman, and D. W. Barry.** 1987. Clinical isolate of herpes simplex type 2 that induces a thymidine kinase with altered substrate specificity. Antimicrob. Agents Chemother. **31:**1117–1125.
24. **Englund, J. A., M. E. Zimmerman, E. M. Swierkosz, and H. H. Balfour.** 1990. Herpes simplex virus resistant to acyclovir. A study in a tertiary care center. Ann. Intern. Med. **112:**416–422.
25. **Erice, A., S. Chou, K. K. Biron, S. C. Stanat, H. H. Balfour, Jr., and M. C. Jordan.** 1989. Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. N. Engl. J. Med. **320:**289–293.
26. **Erikson, B., A. Larsson, E. Helgotrand, N. G. Johansson, and B. Oberg.** 1980. Pyrophosphate analogues as inhibitors of herpes simplex virus type I DNA polymerase. Biochim. Biophis. Acta **607:**53–54.
27. **Erlich, K. S., J. Mills, P. Chatis, G. Mertz, D. F. Busch, S. E. Follansbee, R. M. Grant, and C. S. Crumpacker.** 1989. Acyclovir-resistant herpes simplex virus infections in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. **320:**293–296.
28. **Field, H. J., and D. M. Coen.** 1986. Pathogenicity of herpes simplex virus mutants containing drug resistance mutations in the viral DNA polymerase gene. J. Virol. **60:**286–289.
29. **Field, H. J., and P. Wildy.** 1978. The pathogenesis of thymidine-kinase deficient mutants of herpes simplex virus in mice. J. Hyg. **81:**267–277.
30. **Frank, K. B., and Y. C. Cheng.** 1985. Mutually exclusive inhibition of herpes virus DNA polymerase by aphidicolin, phosphonoformate, and acyclic nucleoside triphosphates. Antimicrob. Agents Chemother. **27:**445–448.
31. **Furman, P. A., M. H. St. Clair, J. A. Fyfe, J. L. Rideout, P. M. Keller, and G. B. Elion.** 1979. Inhibition of herpes simplex virus-induced DNA polymerase activity and viral DNA replication by 9-(2-hydroxyethoxymethyl)guanine and its triphosphate. J. Virol. **32:**72–77.
32. **Furman, P. A., M. H. St. Clair, and T. Spector.** 1984. Acyclovir triphosphate: inactivator of the herpes simplex virus DNA polymerase. J. Biol. Chem. **259:**9575–9579.
33. **Gately, A., R. M. Gander, P. C. Johnson, S. Kit, H. Otsuka, and S. Kohl.** 1990. Herpes simplex virus type 2 meningoencephalitis resistant to acyclovir in a patient with AIDS. J. Infect. Dis. **161:**711–715.
34. **Grossberg, H. S., E. M. Bonnem, and W. C. Buhles.** 1989. GM-CSF with ganciclovir for the treatment of CMV retinitis in AIDS. N. Engl. J. Med. **320:**1560. (Correspondence.)
35. **Gulick, R. M., M. Heath-Chiozzi, and C. S. Crumpacker.** 1990. Varicella zoster virus disease in patients with human immunodeficiency virus infection. Arch. Dermatol. **126:**1086–1088.
36. **Haffey, M. L., J. Novotny, R. E. Bruccoleri, R. D. Carol, J. T. Stevens, and J. T. Matthews.** 1990. Structure-function studies of the herpes simplex virus type 1 DNA polymerase. J. Virol. **64:**5008–5018.
37. **Haffey, M. L., J. S. Stevens, B. J. Terry, D. I. Dorsky, C. S. Crumpacker, S. M. Weinstock, W. T. Ruyechan, and A. K. Field.** 1988. Expression of the herpes simplex type 1 DNA polymerase in *Saccharomyces cerevisiae* and detection of virus-specific enzyme activity in cell-free lysates. J. Virol. **62:**4493–4498.
38. **Hardy, W. D.** 1991. Combined ganciclovir and recombinant human granulocyte-macrophage colony-stimulating factor in the treatment of cytomegalovirus retinitis in AIDS patients. J. Acquired Immune Defic. **4**(Suppl. 1)**:**S22–S28.
39. **Hwang, C. B., K. C. Ruffner, and D. M. Coen.** 1992. A point mutation within a distinct conserved region of the herpes simplex virus DNA polymerase gene confers drug resistance. J. Virol. **66:**1774–1776.
40. **Jacobson, M. A., T. G. Berger, S. Fikrig, P. Becherer, J. W. Moohr, S. C. Stanat, and K. K. Biron.** 1990. Acyclovir-resistant varicella zoster virus infection after chronic oral acyclovir therapy in patients with the acquired immunodeficiency syndrome. Ann. Intern. Med. **112:**187–191.
41. **Jacobson, M. A., and J. J. O'Donnell.** 1991. Approaches to the treatment of cytomegalovirus retinitis: ganciclovir and foscarnet. J. Acquired Immune Defic. **4**(Suppl. 1)**:**S11–S15.
42. **Kit, S., M. Sheppard, H. Ichimura, S. Nusinoff Lehrmann, M. N. Ellis, J. A. Fyfe, and H. Otsuka.** 1987. Nucleotide sequence changes in thymidine kinase gene of herpes simplex virus type 2 clones from an isolate of a patient treated with acyclovir. Antimicrob. Agents Chemother. **31:**1483–1490.
43. **Knopf, K. W., E. R. Kaufman, and C. S. Crumpacker.** 1981. Physical mapping of drug resistance mutation defines an active center of the herpes simplex DNA polymerase enzyme. J. Virol. **39:**746–757.
44. **Kunitomi, T., A. Akazai, M. Ikeda, M. Oda, and N. Kodani.** 1989. Comparison of acyclovir and vidarabine in immunocompromised children with varicella zoster virus infection. Acta

Paediatr. Jpn. (Overseas Ed.) 31(Suppl. b):702–705.
45. **Larder, B. A., S. P. Kemp, and G. Darby.** 1987. Related functional domains in virus DNA polymerase. EMBO J. **6**:169–175.
46. **Linneman, C. C., K. K. Biron, W. G. Hoppenjans, and A. M. Solinger.** 1990. Emergence of acyclovir-resistant varicella zoster virus in an AIDS patient on prolonged acyclovir therapy. AIDS **4**:577–579.
47. **Ljungmann, P., M. N. Ellis, R. C. Hackman, D. H. Shepp, and J. D. Meyers.** 1990. Acyclovir-resistant herpes simplex virus causing pneumonia after marrow transplantation. J. Infect. Dis. **162**:244–248.
48. **Ljungmann, P., B. Lonngvist, G. Gahrton, O. Ringden, V. A. Sundgvist, and B. Wahren.** 1986. Clinical and subclinical reactivations of varicella zoster virus in immunocompromised patients. J. Infect. Dis. **153**(Suppl. b):840–847.
49. **Manischewitz, J. F., G. V. Quinnan, H. C. Lane, and A. E. Wittek.** 1990. Synergistic effect of ganciclovir in combination with foscarnet against cytomegalovirus replication in vitro. Antimicrob. Agents Chemother. **34**:373–375.
50. **Marks, G. L., P. E. Nolan, K. S. Erlich, and M. N. Ellis.** 1989. Mucocutaneous dissemination of acyclovir-resistant herpes simplex virus in a patient with AIDS. Rev. Infect. Dis. **11**:474–476.
51. **Martin, J. L., M. N. Ellis, P. M. Keller, K. K. Biron, S. N. Lehrman, D. W. Barry, and P. A. Furman.** 1985. Plaque autoradiography assay for the detection and quantitation of thymidine kinase-deficient and thymidine kinase-altered mutants of herpes simplex virus in clinical isolates. Antimicrob. Agents Chemother. **28**:181–187.
52. **Matthews, J. T., R. D. Carroll, J. T. Stevens, and M. L. Haffey.** 1989. In vitro mutagenesis of herpes simplex virus type I DNA polymerase gene results in altered drug sensitivity of the enzyme. J. Virol. **63**:4913–4918.
53. **McLaren, C., M. S. Chen, I. Ghazzouli, R. Saral, and W. H. Burns.** 1985. Drug resistance patterns of herpes simplex virus isolates from patients treated with acyclovir. Antimicrob. Agents Chemother. **28**:740–744.
54. **Miller, W. H., and R. L. Miller.** 1980. Phosphorylation of acyclovir (acycloguanosine) monophosphate by GMP kinase. J. Biol. Chem. **255**:7204–7207.
55. **Norris, S. A., H. A. Kessler, and K. H. Fife.** 1988. Severe progressive herpetic whitlow caused by an acyclovir-resistant virus in a patient with AIDS. J. Infect. Dis. **157**:209–210. (Letter.)
56. **Oliver, N. M., P. Collins, J. Van der Meer, and J. W. Van 'T Wout.** 1989. Biological and biochemical characterization of clinical isolates of herpes simplex virus type 2 resistant to acyclovir. Antimicrob. Agents Chemother. **33**:635–650.
57. **Pahwa, S., K. Biron, W. Lim, P. Swenson, M. H. Kaplan, N. Saick, and R. Pahwa.** 1988. Continuous varicella zoster infection associated with acyclovir resistance in a child with AIDS. JAMA **260**:2879–2882.
58. **Parker, A. C., J. I. Craig, P. Collins, N. Oliver, and I. Smith.** 1987. Acyclovir-resistant herpes simplex virus infection due to altered DNA polymerase. Lancet **ii**:1461.
59. **Reardon, J. E., and T. Spector.** 1989. Herpes simplex virus type 1 DNA polymerase: mechanisms of inhibition by acyclovir triphosphate. J. Biol. Chem. **264**:7405–7411.
60. **Sacks, S. L., R. J. Wanklin, D. E. Reece, K. A. Hicks, K. L. Tyler, and D. M. Coen.** 1989. Progressive esophagitis from acyclovir-resistant herpes simplex. Clinical roles for DNA polymerase mutants and viral heterogeneity? Ann. Intern. Med. **111**:893–899.
61. **Safrin, S., T. Assaykeen, S. Follansbee, and J. Mills.** 1990. Foscarnet therapy for acyclovir-resistant mucocutaneous herpes simplex virus infection in 26 AIDS patients: preliminary data. J. Infect. Dis. **161**:1078–1084.
62. **Safrin, S., T. G. Berger, I. Gilson, P. R. Wolfe, C. B. Wofsy, J. Mills, and K. K. Biron.** 1991. Foscarnet therapy in five patients with AIDS and acyclovir-resistant varicella-zoster virus infection. Ann. Intern. Med. **195**:19–21.
63. **Safrin, S., C. S. Crumpacker, P. Chatis, R. Davis, R. Hafner, J. Rush, A. Kessler, B. Landry, and J. Mills.** 1991. A randomized comparison of foscarnet versus vidarabine for treatment of acyclovir-resistant mucocutaneous herpes simplex virus infection in patients with AIDS. N. Engl. J. Med. **325**:551–555.
64. **Schinazi, R. F.** 1987. Drug combinations for treatment of mice infected with acyclovir-resistant herpes simplex virus. Antimicrob. Agents Chemother. **31**:477–479.
65. **Schinazi, R. F., V. del Bene, R. T. Scott, and J. B. Dudley-Thorpe.** 1986. Characterization of acyclovir-resistant and acyclovir-sensitive herpes simplex viruses isolated from a patient with an acquired immune deficiency. J. Antimicrob. Chemother. **18**(Suppl. B):127–134.
66. **Schnipper, L. E., and C. S. Crumpacker.** 1980. Resistance of herpes simplex virus to acycloguanosine: role of viral thymidine kinase and DNA polymerase loci. Proc. Natl. Acad. Sci. USA **77**:2270–2273.
67. **Shepp, D. H., P. S. Dandliker, and J. D. Meyers.** 1988. Current therapy of varicella zoster virus infection in immunocompromised patients. A comparison of acyclovir and vidarabine. Am. J. Med. **84**(Suppl. 2A):96–98.
68. **Shigeta, S., S. Mori, T. Yokota, K. Konno, and E. DeClercq.** 1986. Characterization of a varicella-zoster virus variant with altered thymidine kinase activity. Antimicrob. Agents Chemother. **29**:1053–1058.
69. **Sibracks, C. D., L. T. Gutman, C. M. Wilfert, C. McLaren, M. H. St. Clair, P. M. Keller, and D. W. Barry.** 1982. Pathogenicity of acyclovir-resistant herpes simplex virus type 1 from an immunodeficient child. J. Infect. Dis. **146**:673–682.
70. **Sullivan, V., K. Biron, M. Davis, S. Stanat, C. Talarico, and D. Coen.** 1991. Resistance of HCMV to DHPG, HPMC and HPMPA mapped to the viral DNA polymerase gene. Antiviral Res. **1**:84. (Abstract.)
71. **Talarico, C. L., W. C. Phelps, and K. K. Biron.** 1991. Sequence analysis of thymidine kinase genes of acyclovir-resistant varicella zoster virus isolates. Antiviral Res. **1**:112. (Abstract.)
72. **Taylor, E. W., and J. Joakkola.** 1991. A transposition of the reverse transcriptase gene reveals unexpected structural homology to *E. coli* DNA polymerase I. Genetics **84**:77–86.
73. **Tenser, R. B.** 1983. Intracerebral inoculation of newborn and adult mice with TK deficient mutants of HSV-1. J. Infect. Dis. **147**:956.
74. **Tenser, R. B., J. C. Jones, and S. J. Ressel.** 1985. Acute and latent infection by thymidine kinase mutants of herpes simplex type 2. J. Infect. Dis. **151**:548–550.
75. **Tenser, R. B., R. L. Miller, and F. Rapp.** 1979. Trigeminal ganglion infection by thymidine kinase-negative mutants of herpes simplex virus. Science **205**:915–917.
76. **Vinckier, F., M. Boogaerts, D. De Clerck, and E. De Clercq.** 1987. Chronic herpetic infection in an immunocompromised patient: report of a case. J. Oral Maxillofac. Surg. **45**:723–728.
77. **Wade, J. C., C. McLaren, and J. D. Meyers.** 1983. Frequency and significance of acyclovir-resistant herpes simplex virus isolated from marrow transplant patients receiving multiple courses of treatment with acyclovir. J. Infect. Dis. **148**:1077–1082.
78. **Walmsley, S. L., E. Chen, and S. E. Read.** 1988. Treatment of cytomegalovirus retinitis with trisodium phosphonophormate hexahydrate (foscarnet). J. Infect. Dis. **157**:569–572.
79. **Wilcox, C. L., L. S. Crnic, and L. I. Pizer.** 1992. Replication, latent infection and reactivation in a neuronal culture with a herpes simplex virus thymidine kinase-negative mutant. Virology **187**:348–352.
80. **Wong, S. W., A. F. Wahl, P. M. Yuan, and T. M. Wang.** 1988. Human DNA polymerase α gene expression is cell proliferation dependent and its primary structure is similar in both prokaryotic and eukaryotic replication DNA polymerases. EMBO J. **7**:37–47.