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Models of antiviral resistance

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1. Herpes simplex virus (HSV)

The use of animal model systems in evaluating HSV disease has provided fundamental information regarding the development of antiviral resistance, as well as the biologic characteristics of resistant viruses. Such models have proven valuable in evaluating cross-resistance of new antiviral agents to acyclovir (ACV)-resistant strains. In addition, they have been used to study both virulence and pathogenesis of these ACV-resistant isolates. An understanding of latency and reactivation has been enhanced by the evaluation of genetically engineered viruses in animal models. Furthermore, animal systems have been used to evaluate other types of therapies, including drug combinations. Notably, the development of new antiviral agents with activity against HSV must include both in vitro and in vivo evaluation of their efficacy against drug-resistant isolates.

The nude mouse was the first animal model of cutaneous HSV infection to prove useful in the evaluation of antiviral agents against resistant HSV isolates (Ellis et al., 1989a,b). However, the utility of this model system is limited by the expense and difficulty related to special housing requirements. As a result, additional animal models have been perfected.

The Balb/C mouse model has been used in studies of HSV encephalitis and vaginal disease. Intranasal inoculation of 3-week-old Balb/C mice results in local viral replication followed by infection of the central nervous system (CNS) and the establishment of latency in the trigeminal ganglia. Introduction of wild-type (thymidine

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kinase-positive, or TK^+) and TK-altered (TKa) virus into this model system can result in mortality and the establishment of latency. Low TK producers (TK-partial, or TKp), such as the AG3 strain of HSV, have also been shown to produce mortality and latency in inoculated animals. The pathogenesis of ACV-resistant mutants is similar to TK^+ virus, with a similar pattern of replication in brain and visceral tissues. Importantly, unlike certain resistant bacteria, drug-resistant viruses are not "supervirulent" in animal systems.

Intravaginal inoculation of female 6-week-old Balb/C mice with TK^+ virus results in viral replication in the vaginal tract, the development of genital lesions, and the establishment of latency in the spinal ganglia of surviving animals. All TK-negative (TK) and TKa viruses evaluated to date in this model replicate to levels about two logs lower than TK^+ HSV. All TKa isolates and the one TKp mutant evaluated to date have produced mortality and established latency. While most TK mutants are unable to establish latency, some can become latent but fail to reactivate. Polymerase mutants resistant to foscarnet (PFA) also have been found to replicate well in vaginal tract. All isolates with in vitro resistance to ACV or PFA have shown a lack of response to therapy in vivo, further validating the use of this animal model in the evaluation of antiviral resistance.

The guinea pig provides another model in which resistant isolates of HSV can be analyzed. As with the Balb/C mouse model, intravaginal inoculation of female adolescent guinea pigs with TK^+ HSV results in viral replication in the vaginal tract, development of genital and perianal lesions, and establishment of latency in the spinal ganglia of surviving animals. Acyclovir- and PFA-resistant viruses replicate to levels about two logs lower than TK^+ isolates in this model. All TKa isolates and the one TKp mutant evaluated to date produce disease and establish latency. While some TK isolates have been reported to cause genital lesions in guinea pigs, it is likely that these will prove to be TKp mutants with such low levels of TK present as to be below the sensitivity of some of the existing assay systems. In addition to evaluating the biochemical characteristics of such mutants in a TK-deficient cell line, direct genomic analysis by means of sequencing of the TK gene may give additional vital information.

Table 1 summarizes the biologic characteristics of ACV-resistant mutants which have been evaluated in animal model systems. General agreement exists that animal models can provide valuable information in the evaluation of new antiviral therapies for the treatment of drug-resistant viruses, especially TKa viruses. In addition, such models can be used to investigate the virulence, pathogenicity, and latency of resistant isolates (Hill et al., 1991; Field and Darby, 1980; Ellis et al., 1989a).

2. Influenza

Data obtained from the evaluation of influenza A virus in animal models suggest

Table 1
Biologic characteristics of ACV-resistant mutants in animal model systems

Biologic characteristic	Phenotypic characterization			
	TK^+	TKa	TKp	TK^-
In vitro susceptibility	Sensitive	Resistant	Resistant	Resistant
In vivo response	Yes	Variable	No	No
Vaginal replication	Yes	Yes	Yes	Yes
Ganglionic replication	Yes	Yes	Yes	No
Lesion development	Severe	Variable	Yes	No
Latency and reactivation	Yes	Yes	Yes	No
Pathogenicity *	Yes	Yes	Yes	No
Mortality	Yes	No	Yes	No

* Replicates in murine tissues.

that the virulence, pathogenesis, and transmissibility of amantadine- and rimantadine-resistant influenza A virus are unaltered compared with wild-type virus. In the avian model, intranasal inoculation of virulent influenza A/chicken/Pennsylvania/1370/83 (H5N2) virus results in death, with amantadine treatment significantly reducing occurrence of disease (Bean et al., 1989; Beard et al., 1987). During and after amantadine therapy, drug-resistant mutants can be detected in tracheal and fecal samples. Such mutants demonstrate cross-resistance with rimantadine. In addition, they are both virulent in and transmissible to contact birds. Intranasal inoculation of ferrets with rimantadine-resistant human influenza A (H3N2) viruses isolated from patients has demonstrated that the resistant viruses are unaltered in their growth characteristics and virulence (Sweet et al., 1991). Furthermore, no significant differences in the ability of resistant virus to produce disease in the weanling mouse model have been noted (R. Sidwell, manuscript in preparation).

Animal models are important in studying emergence of resistant influenza A viruses. The results of such studies have correlated well with clinical data. In the avian model, drug-resistant strains emerge rapidly during treatment with amantadine or rimantadine. Genetic analysis of the amantadine-resistant avian virus has demonstrated identical amino acid substitutions as in human virus, with substitutions at residues 27, 30, and 31 of the M2 protein (Bean et al., 1989). Resistant viruses are genetically stable, transmissible to untreated birds, and virulent (Bean et al., 1989). However, differences between avian and human disease exist with respect to pathogenesis. The avian virus replicates in the gastrointestinal tract with large quantities of virus excreted in the feces. In addition, the avian virus disseminates to other internal organs, whereas human virus generally does not. The avian model has also been employed to evaluate of efficacy of amantadine plus vaccine

for prevention of transmission of avian influenza virus in chickens. The combination of amantadine and vaccine given immediately before exposure to virus prevents disease and death in the infected birds and their contacts, whereas either used alone does not (Webster et al., 1985).

Amantadine-resistant isolates also emerge rapidly in the mouse model. In one experiment, influenza A2/Singapore/1/57 (H2N2) was passaged intranasally through mice treated with amantadine (Oxford et al., 1970; Oxford and Potter, 1973). A loading dose of drug was delivered intraperitoneally, followed by administration of amantadine in the drinking water. A 10-fold increase in resistance was noted after a single passage. After six passages, a stable amantadine-resistant isolate was identified. This mutant was 10–1000-fold less sensitive to amantadine than was wild-type virus, and was cross-resistant to rimantadine and to 2-adamantanamine sulphate. Stability of the isolate was confirmed by passage of this resistant virus through untreated eggs. Another experiment evaluated intranasal passage of influenza A/Port Chalmers/1/73 (an H3N2 virus) through amantadine-treated mice (R. Sidwell, manuscript in preparation). Drug in doses ranging up to toxic levels was administered in the drinking water beginning 2 h before exposure to the virus, and the animals were sacrificed on day 5. Treated mice were found to have lower viral titers, reduced lung scores, and reduced lung consolidation as determined by decreased lung weight. Despite the treatment pressure of near-toxic dosage levels, virus recovered from treated mice was at least 100-fold less susceptible to amantadine than wild-type virus. Cross-resistance was noted with rimantadine but not ribavirin. Individual plaque isolates of resistant viruses had susceptibilities which varied significantly, with some isolates remaining susceptible to the drug. Administration of resistant virus to weanling mice demonstrated that the mutant influenza virus maintained its virulence and pathogenicity. Similar results have been obtained utilizing rimantadine administered in the drinking water or intraperitoneally to mice infected with the same virus.

Thus, amantadine and rimantadine resistance occurs readily, despite alterations in drug dosage, route of administration, time of initiation of therapy, or use of loading doses. While it appears that resistance develops regardless of treatment strategy, these animal models will continue to prove useful in the quest for therapeutic options that will delay or prevent emergence of antiviral resistance in influenza infection.

3. Feline immunodeficiency virus (FIV)

Feline immunodeficiency virus (FIV) is a lentivirus that causes an acquired immunodeficiency syndrome (AIDS)-like syndrome in cats that is clinically similar to human immunodeficiency virus (HIV) infection in humans (Pedersen, 1993). The FIV reverse transcriptase (RT) and the HIV-1 RT share many of the same physical properties and catalytic activities, as well as susceptibility to zidovudine tri-

phosphate (AZTTP), ddATP, ddCTP, ddGTP, ddTTP, and 2′,3′-dideoxydidehydrothymidine triphosphate (d4TTP). However, the FIV RT is not affected by the non-nucleoside RT inhibitors (NNRTIs).

Information obtained from in vitro studies of FIV resistance may improve the understanding of antiviral resistance in HIV infection (Barlough et al., 1993). In addition, it may assist in the development of therapeutic strategies to prevent the emergence of resistant isolates. The cell culture systems used with FIV permit selection of FIV mutants more easily than is possible for HIV mutants. A focal infectivity assay (FIA) permits the rapid and sensitive analysis of drug-resistant mutants selected by growth in the presence of drug. Feline immunodeficiency virus mutants resistant to zidovudine (AZT), 2′,3′-dideoxyinosine (ddI), 2′,3′-dideoxycytosine (ddC), 2′,3′-dideoxydidehydrothymidine (d4T), PFA, (−)-1-[(2*R*,5*S*)-2-hydroxymethyl-1,3-oxathiolan-5-yl]cytosine (3TC) and 3′-fluoro-3′-deoxythymidine (FLT) have been characterized (Remington et al., 1991, 1994; T. North, personal communication). However, passage of virulent isolates from cats in vitro selects for cell-adapted mutant viruses that are no longer pathogenic when reinoculated into cats. The need for a highly pathogenic molecular clone has limited the application of the observations in cell culture to the study of pathogenesis in the animal model.

In vitro studies of the emergence of drug-resistant mutants has shown that, as in HIV, the rapid emergence of genetic variants of FIV contributes to the development of drug resistance and to the ability of the virus to evade vaccines. In addition, some antiviral drugs can enhance the mutation rate of FIV. For instance, sub-inhibitory concentrations of AZT increase the mutation frequency of FIV. While the biological impact of this finding is unknown, it is possible that it could enhance the antiviral activity of AZT by inducing nonviable (lethal) mutants. Alternatively, it could contribute to viral pathogenicity or emergence of drug resistance. Notably, sub-inhibitory concentrations of ddI or ddC do not enhance the mutation frequency of FIV.

Feline immunodeficiency virus mutants resistant to the combination of AZT and ddI have been described. Other combinations of nucleoside analogs are being investigated with the goal of delaying emergence of resistance. In addition, alterations in the FIV RT are being pursued in order to make it susceptible to the NNRTI agents. And finally, in vitro evaluation of unique oligonucleotide-based inhibitors derived by the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) procedure are being carried out.

4. Human immunodeficiency virus-1 (HIV-1)

Though the simian immunodeficiency virus (SIV)/macaques model for human acquired immunodeficiency syndrome (AIDS) is promising for the study of primate lymphotropic lentiviruses (Wyand, 1992; Hirsch and Johnson, 1994), there

are only a few animal models that utilize the HIV virus, and all of these have significant weaknesses. While the SCID-hu mouse model has been utilized in the study of HIV-1 (McCune et al., 1990; Mosier et al., 1991), this model has not proven to be practical as a system in which HIV can be evaluated. This is due in part to the fact that the SCID-hu mouse lacks a fully functional systemic human immune system. The chimpanzee can be infected with HIV-1 (Alter et al., 1984; Fultz et al., 1986; Nara et al., 1987), but infected animals do not develop disease (Wyand, 1992). In addition, the chimpanzee model generally is not highly regarded for the study of antiviral agents. While rabbits can be infected with HIV-1 (Filice et al., 1988; Kulaga et al., 1989), they also do not develop disease. For these reasons, in vitro models, rather than in vivo ones, have been used for the selection of drug combinations for study in the management of HIV infections. The goals of such in vitro combination studies are to attain synergy and avoid antagonism. In addition, cross-resistance patterns can be evaluated.

Examples of in vitro experiments that may lead to development of novel clinical trials include those that have evaluated AZT/3TC co-resistance in HIV-1. Following three passages of AZT-resistant virus in the presence of 3TC, resistance develops to 3TC but the virus becomes more susceptible to AZT despite retaining AZT resistance mutations. When the AZT-resistant virus is passaged in escalating concentrations of 3TC plus a constant amount of AZT (to maintain AZT pressure on the virus), the virus remains susceptible to 3TC and resistant to AZT even after six passages. Following three passages of wild-type virus in the presence of 3TC and increasing concentrations of AZT, the virus attains resistance to 3TC but maintains its susceptibility to AZT. This suppression of AZT resistance by the M184 3TC resistance mutation provides a rationale for the clinical investigation of the combination of AZT and 3TC.

The ultimate goal of combination therapy is to suppress viral replication with the hope of delaying evolution of a resistant phenotype. Studies comparing the combination of AZT and either ddI or ddC with monotherapy (AZT or ddI or ddC) have found that combination therapy decreased viral load to a greater extent than monotherapy, but did not significantly delay the emergence of resistance to AZT.Studies comparing AZT and the combination of AZT and 3TC again found that combination therapy resulted in decreased viral load compared with monotherapy. Use of AZT and 3TC together does not prevent 3TC resistance, but virus remains susceptible to AZT.

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