# 13 General Mechanisms of Antiviral Resistance

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## 13.1    Introduction

Mammalian viruses represent a diverse group of infectious agents. The viruses that cause the common diseases of man and domestic animals comprise approximately 25 known families, which fall into groups according to their genome and replication strategies. Some important examples of these viruses are summarized in Table 13.1.

Over recent years, knowledge of the complete nucleotide sequences has enhanced our understanding of the interrelationships between virus nucleic acids and relevant host genes. Such molecular studies (see Chapter 3) indicate that there are homologies between viral and host proteins; of particular interest to the antiviral field are those involved with genome replication and other virus enzymes. Although no host enzymes exist in eukaryotes to replicate RNA (a prerequisite for all RNA viruses) and reverse transcriptase (RT) has no corresponding host function, many viral and host genes appear to have common origins. These findings support the view that, during the coevolution of virus and host cell, there have been exchanges of functional modules, mediated by several forms of genetic recombination. Further evolution of modern viruses is continuing with mutations (substitutions, additions, deletions), recombinations, or reassortments (Holland and Domingo, 1998).

Modern viruses have developed, through their evolutionary history, an extraordinary diversity of strategies for their efficient replication and survival, counteracting both innate and adaptive immune responses. For example, herpes simplex virus (HSV) and varicella-zoster virus (VZV) have a latent state enabling the virus to survive lifelong in the host. Reactivation at intervals allows the herpesvirus to spread to new individuals, enabling transmission through space and time. In contrast, influenza virus is usually cleared from the host within days or weeks, but has the ability to spread rapidly from person to person, potentially worldwide. Only in the relatively recent past, since Jenner's first use of cowpox as a vaccine for human smallpox, have viruses faced a new threat to their replication—human intervention. Faced with vaccines and specific antiviral compounds, some viruses appear to be

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**Table 13.1** Examples of Human Viruses and their Genome Structures

| DNA | Double strand | **Adenoviruses** |
|---|---|---|
| | | **Herpesviruses** |
| | | HSV |
| | | Cytomegalovirus |
| | | VZV |
| | | **Papillomaviruses** |
| | | **Poxviruses** |
| | Single strand | **Parvoviruses** |
| | Partial double strand, replicating via RNA | **Hepadnavirus** |
| | | HBV |
| RNA | Double strand, segmented | **Reoviruses** |
| | | Rotavirus |
| | Single strand, positive strand | **Flaviviruses** |
| | | HCV |
| | | West Nile virus |
| | | Yellow fever virus |
| | | Dengue virus |
| | | **Picornaviruses** |
| | | hepatitis A or HAV |
| | | Rhinovirus |
| | | **Togaviruses** |
| | | Rubella virus |
| | Single strand, negative strand, segmented | **Orthomyxoviruses** |
| | | Influenza virus |
| | Single strand, negative strand, nonsegmented | **Rabies virus** |
| | | **Paramyxoviruses** |
| | | Mumps, measles, RSV |
| | Replicating via DNA | **Retroviruses** |
| | | HIV |

poorly adapted to survive. A well-known example is smallpox, which was eliminated from the human population by vaccination. Similarly, poliovirus has been eradicated from most countries of the world. The use of vaccines has greatly reduced the burden of human disease caused by several other human viruses (e.g., rubella, mumps, measles, hepatitis A [HAV], and hepatitis B [HBV]). Nevertheless, there seems to be no prospect of eradicating these viruses in the near future and patients with chronic HBV cannot be cured by vaccination. In complete contrast, several human viruses have, through their evolutionary history, developed survival strategies which happen to enable them to resist vaccines (e.g., human immunodeficiency virus [HIV] and hepatitis C [HCV]). Specific antiviral compounds have been developed for several of those viral infections that have not been adequately controlled by vaccines. Examples of widely licensed compounds are given in Table 13.2.

This review aims to explore general mechanisms of virus resistance. We start by summarizing those evolutionary outcomes that have enabled human viruses to resist mankind's best efforts at control. The review then focuses on how viruses acquire resistance to compounds that specifically target a virus protein (e.g., polymerase,

**Table 13.2** Illustrative Examples of Commonly Used Antiviral Compounds

*A: Primarily active against Herpes viruses*

| Generic Name (Abbreviation) Trade Name (Company) | Structure[a] | Mechanism Viral Target | Target Viruses |
|---|---|---|---|
| Valaciclovir (VACV) Valtrex® (GSK) | Prodrug of acyclovir (ACV) Zovirax® | Activated by viral TK, inhibits viral polymerase | HSV-1 and 2 VZV |
| Famciclovir (FCV) Famvir® (Novartis) | Prodrug of penciclovir (PCV) Denavir®/Vectavir® | Activated by viral TK, inhibits viral polymerase | HSV-1 and 2 VZV |
| Foscarnet (PFA) Foscavir® (Astra Zeneca) | Pyrophosphate analog | Polymerase inhibitor | HSV-1 and 2 VZV |
| Valganciclovir (VGCV) Valcyte® (Roche) | Prodrug of ganciclovir (GCV), Cymmevene® | Activated by kinase encoded by UL 97, polymerase inhibitor | CMV |

a For prodrugs, parent antiviral drug name, abbreviated name and trade name are given.

*B: Primarily active against RNA viruses*

| Generic Name Trade Name (Company) | Structure | Mechanism Viral Target | Target Viruses |
|---|---|---|---|
| Zanamivir Relenza® (GSK) | Sialic acid analog | Neuraminidase inhibitor | Influenza A and B |
| Oseltamivir Tamiflu® (Roche) | Sialic acid analog | Neuraminidase inhibitor | Influenza A and B |
| Ribavirin Virazole® (Schering-Plough) | Nucleoside analog | Possibly no direct viral target | HCV RSV |

**C: Primarily active against Hepadnaviruses (HBV)**

| Generic Name (Abbreviation) Trade Name | Structure | Mechanism Viral Target | Company |
|---|---|---|---|
| Lamivudine (LMV or 3TC) Zeffix®, Heptovir® | Nucleoside analog | Polymerase | GSK |
| Adefovir dipivoxil (ADV) Hepsera® | Prodrug of adefovir nucleotide analog | Polymerase | Gilead |
| Entecavir Baraclude® | Nucleoside analog | Polymerase | BMS |

**D: Primarily active against Retroviruses (HIV)**

| Generic Name (Abbreviation) Trade Name | Structure | Mechanism Viral Target | Company |
|---|---|---|---|
| Zidovudine/Lamivudine (AZT/3TC) Combivir® | Two NRTIs | Polymerase | GSK |
| AZT/3TC/abacavir Trizivir® | Three NRTIs | Polymerase | GSK |
| Emtricitabine/tenofovir/ efavirenz Atripla® | Two NRTIs and one NNRTI | Polymerase | Gilead and BMS (Jointly) |
| Nevirapine Viramune® | NNRTI | Polymerase | Boehringer |
| Fosamprenavir Lexiva® | gag cleavage site mimic | Protease | GSK |
| Saquinavir mesylate Invirase® Fortovase® | gag cleavage site mimic | Protease | Roche |
| Lopinavir/ritonavir Kaletra® | gag cleavage site mimic/PK enhancer | Protease | Abbott Lab |
| Indinavir Crixivan® | gag cleavage site mimic | Protease | Merck |
| Darunavir Prezista® | gag cleavage site mimic | Protease | Tibotec |
| Raltegravir Isentress® | Dihydropyrimidine derivative | Integration of HIV DNA into chromosome | Merck |

**D: Primarily active against Retroviruses (HIV)** **(Continued)**

| Generic Name (Abbreviation) Trade Name | Structure | Mechanism Viral Target | Company |
|---|---|---|---|
| Efuviritide (T-20) Fuzeon® | Polypeptide | Envelope protein gp41 | Roche |
| Maraviroc Selzentry® | CCR5 ligand mimic | Blocks receptor on host cell membrane | Pfizer |

*Source*: Table adapted from Field and Vere Hodge (2008).

protease, integrase, sialidase). For the therapy of chronic viral infections, such as HIV, the concept of the genetic barrier has emerged as key factor for delaying antiviral resistance. In some cases, the price to the virus of gaining resistance may be reduced "fitness." There may, however, be other less obvious effects.

Conceptually, one way to avoid virus resistance is to use a compound to target a host protein rather than a viral protein. Such an approach seems to risk causing unacceptable toxicity, although recently it has been shown that there can be specificity for the virus-infected cell. We end by asking the question, how will viruses respond to such an indirect challenge?

## 13.2 Evolutionary Outcomes that have Enabled Viruses to Resist Control

Viral resistance is usually discussed in the context of antiviral therapy. However, through the long process of evolution, viruses have acquired various attributes that happen to limit our ability to control the burden of disease and resist mankind's best efforts to control the viral infections. Examples of such attributes are the following.

### 13.2.1 The Virus Has Developed the Ability to Enter Latency

Herpesviruses establish a latent state that enables the virus to remain in the host for a lifetime despite normal adaptive immune responses. The latent virus can reactivate at intervals with or without clinical signs. Antivirals are effective at reducing virus replication during an acute episode but, currently, there are no therapies that remove latent herpesvirus infections. HSV, VZV, and human cytomegalovirus (HCMV) have been major and successful antiviral targets for three decades, acyclovir (ACV) being the first antiviral drug to be both potent and selective. ACV was followed by famciclovir (FCV), prodrug of penciclovir (PCV), and valaciclovir (VACV), prodrug of ACV, which are used to treat or suppress HSV-1, HSV-2, and VZV and ganciclovir (GCV) for HCMV. Although these have been used clinically

worldwide and have helped patients manage their herpes infections, the latent virus remains as a potential source of reactivation.

### 13.2.2    Integration

Another way in which a virus can establish a form of latency is by means of integration of a DNA copy of the genome. Soon after infecting a cell, HIV RNA is the template for the viral RT to synthesize HIV DNA, which is then integrated into cellular DNA. Those current therapies, which inhibit the viral RT (RTIs) or which target the viral protease (PIs), have no direct effect on integrated viral DNA. The integrase inhibitors prevent the integration process but have no effect on viral DNA already integrated into host DNA. Because some cells contain integrated HIV DNA which remains quiescent (latent), it has been impossible to "cure" HIV-infected patients by clearing the HIV completely, notwithstanding the fact that combination therapy has given good clinical control of the symptomatic disease.

### 13.2.3    The Virus Has Over 100 Serotypes/Genotypes

There are two well-studied examples: rhinovirus (common cold) and papillomavirus (wart virus).

Even at the research stage with rhinoviruses, no compound showed activity against all serotypes (e.g., pleconaril is active against about 70% of serotypes). Although this compound was selected for development, one of the potential problems was that it could not be clinically effective against all rhinovirus serotypes, let alone against all viruses causing similar symptoms. (The development of pleconaril was terminated due to toxicological considerations.)

For the second example, papillomavirus vaccines (designed to prevent carcinoma of the cervix) seem very effective against the targeted virus strains (papilloma types 16 and 18) but give little or no protection against those remaining strains which are associated with a minority of carcinomas. Types 16 and 18 are associated with 70−75% of cervical cancers, 70% of vaginal cancers, and 50% of vulvar cancers. To protect against essentially all these cancers, it would be necessary to have vaccines for about 13 types of papilloma, with types 45, 31, 33, 52, 58, and 35 being the most important after 16 and 18. The current vaccines, Cervarix (GSK) and Gardasil (MSD), contain antigenic proteins from types 16 and 18. In addition, Gardisil includes types 6 and 11, which cause 90% of genital warts not associated with cancers. Will other strains, not countered by the vaccines, now become more prominent?

### 13.2.4    Rapid Mutation Rates and Quasi-Species

The mutation rate of a virus has been described as the probability that during a single replication of the virus genome a particular nucleotide position is altered (Smith and Inglis, 1987). While mutation frequencies are directly measurable, in practice, it is extremely difficult to convert this to a "rate" (Drake and Holland,

1999). The "rate" may, however, be reduced if many potential mutations lead to nonviable virions. For example, HBV has overlapping reading frames for the surface antigen and the polymerase. As a consequence, some mutations in the surface antigen may cause the polymerase to be nonfunctional.

There is a consensus that RNA viruses have relatively high mutation rates compared with DNA viruses by two orders of magnitude or more. Average misincorporations per nucleotide base in RNA viruses have been reported to be of the order $10^{-4}$ to $10^{-5}$ (Holland and Domingo, 1998). This is thought, at least in part, to be a consequence of the lack of proofreading and mismatch repair.

Conventionally, DNA viruses are considered to have low mutation rates relative to RNA viruses; even so, this may be perhaps a 100-fold higher than that of host DNA. As a consequence, there will be low proportions of mutant viruses, sometimes referred to as polymorphisms, within an infected individual. It is becoming recognized that pre-existing polymorphisms may include resistant mutants that greatly increase the rate (in tissue culture) at which DNA viruses develop antiviral resistance compared with the appearance of resistance due to spontaneous mutations. However, with rapidly mutating RNA viruses (e.g., HIV or HCV), there may be no practical distinction between pre-existing and *de novo* resistance mutations.

Mutation rate alone does not determine how soon resistant virus will appear in clinical practice. There are other important factors including the number of virions formed per day in the patient and the proportion of progeny that are "fit." Furthermore, in some cases "fitness" may require compensating mutations (section 13.5). This combination of factors we shall call "resistance rate."

HIV and HCV are two viruses that produce huge numbers of virions each day (ca. $10^9$ and $10^{12}$ virions/day, respectively) in untreated patients (Field and Vere Hodge, 2008). The large population of new virions, coupled with high rates of mutation (ca. $10^{-4}$), can quickly lead to enormous genetic diversity within a single infected host. For example, HIV has a single-strand RNA genome of approximately 9,000 nucleotides. The replication rate in an infected individual has been estimated to be approximately $10^9$ daily, thus $10^{-4} \times 9,000 \times 10^9 = 9 \times 10^8$ mutants occur each day. This means that, in theory, every point mutation occurs $10^5$ times per day in an HIV-infected individual and every double mutant 10 times per day! As a result, HIV actually exists as a quasi-species or "swarm" around a particular consensus sequence. Similarly, HCV exists as quasi-species; it has the fastest known daily replication rate of $10^{12}$ virions daily.

Among different viruses, there is a huge range of "resistance rates" and this has clinical consequences (Table 13.3).

With the production rate of new virions being a key factor in resistance rate; this emphasizes the importance of reducing viral replication (e.g., HIV and HCV) as quickly as possible after commencing therapy, since a large (e.g., 8 $\log_{10}$) reduction in replication will give a corresponding reduction in the formation and selection of resistant mutants. It is crucially important to keep viral replication at the lowest possible level both throughout a single day and during a long course of therapy. Missed doses and "drug holidays" can give the virus a better chance to mutate and so become resistant.

**Table 13.3** Clinical Consequences due to Varying "Resistance" Rates

| Virus (DNA/RNA) | "Resistance Rate"[a] | Clinical Outcome |
|---|---|---|
| Vaccinia (DNA) | Very slow | Vaccine has eliminated virus from human population. Selective antiviral agents (e.g., ST 246) being developed as anti-bioterrorism agent. Resistance can be obtained in the laboratory but no clinical data available. |
| Polio (RNA) | Very slow | Vaccine has eliminated virus in most countries. |
| Varicella zoster (DNA) | Moderately slow | Vaccine expected to be effective for decades; antiviral therapy has not led to an increase (<1%) of resistant isolates among the immunocompetent patients (no increase in three decades) but some increase in immunocompromised patients. |
| Herpes simplex types 1 and 2 (DNA) | Moderately slow | No efficacious vaccine yet available but resistance to antiviral therapy similar to that with VZV. |
| Rubella, mumps, measles, HAV (RNA viruses), and HBV[b] | Slow | Vaccines have remained clinically effective for years; antiviral resistance to therapy of HBV with single antiviral agents may occur (within one or a few years). |
| Influenza (RNA) | Fast | Vaccine needs to be updated at least annually. Resistance to antiviral compounds occurs in the population at various rates for different compounds (days to years). |
| HIV (RNA) | Very fast | No vaccine successful. Monotherapy leads quickly to resistance in individual patients. Combination therapy (3 or 4) gives low "resistance rate" (several years). |
| HCV (RNA) | Very fast | No vaccine successful. As for HIV, monotherapies lead to quick appearance of resistance. Antiviral combinations being evaluated. |

[a]See text for definition of "Resistance rate"
[b]Hepatitis B is a DNA virus but replicates via an RNA intermediate.

## 13.3 One Principle Mechanism for Development of Resistance

In spite of the many strategies for viral replication and transmission, as summarized briefly above, all viruses have one main mechanism for development of resistance to antiviral compounds and vaccines—the selection of random mutations. Darwin's theory of evolution—random changes followed by survival of the fittest—is well illustrated in the virus field. At least for some viruses, the evolution of resistance can be followed in days or weeks as the genome replication and mutation rates, leading to random changes, are so high. Sequence analysis of the DNA or RNA shows that a particular resistant variant may have one or more base changes that account for resistance (usually confirmed by the introduction of the same mutation(s) by means of site-directed mutagenesis into

a wild type (wt) background). Other mutations may be random changes with no particular consequences for the viability of the virus. For example, there may be base changes that neither alter the encoded amino acid nor cause significant change to the RNA secondary structure. In DNA viruses, such variants are commonly referred to as polymorphisms. In those RNA viruses which mutate rapidly, the huge number of variants are called quasi-species. In a natural infection, under pressure from antiviral therapy, the proportion of wt virus decreases markedly whereas the resistant variant, either as a pre-existing minor variant or a newly formed mutant, becomes dominant.

## 13.4 Viruses with Segmented Genomes: Additional Resistance Mechanism

Several families of RNA virus have segmented genomes (Table 13.1). The clinically most important is influenza, which has eight segments. These viruses have an additional mechanism of acquiring resistance. When two strains co-infect a cell, in theory, the gene segments may re-assort in every possible combination. This gives the possibility for a drug-resistant virus, which has a poor ability to transmit, to re-assort with a highly infectious but drug-sensitive virus, so that some of the progeny viruses will be highly infectious and drug-resistant.

In the 2009 pandemic $H_1N_1$ influenza, the eight RNA segments or genes were recently derived from avian (two segments), swine from two continents (five segments) and human (one segment) viruses, presumably in a series of re-assortments. In this case, the resulting virus was not a drug-resistant strain but one to which the general human population, at least those under about 60 years old, did not have effective immunity. It was fortunate that the initial widespread transmission of this virus did not cause devastating burden of illness and deaths. There were only a few reports of oseltamivir-resistant influenza (section 13.6.4) during the first year of the pandemic.

## 13.5 Evolution of Resistant Mutants

In some instances, a single mutation leads to high-level resistance to the antiviral compound and the virus appears to remain fully "fit." An example is the M2 channel-blocking inhibitors, amantadine and rimantadine, which had activity against influenza viruses. Resistant variants are selected so quickly that a treated person can pass on resistant virus to contacts. Being fully fit, the resistant virus is easily spread. During the 2005−2006 season in the United States, 109/120 (91%) of H3N2 clinical isolates were resistant to amantadine and rimantadine. This has severely limited the clinical usefulness of these drugs.

In contrast to amantadine and rimantadine, there is often a slower evolution of resistance to antiviral compounds that act as substrate mimics for a viral enzyme and so bind to the catalytic site. Most potential mutations will give a nonfunctional enzyme and a nonviable or "less fit" virus. There may be very few (even a single) specific mutation(s) that interfere with the binding of the antiviral compound to the

enzyme yet does not reduce by too much the catalytic activity. Such mutations, which usually appear first, are therefore termed "primary mutations." Initially, the degree of resistance may be modest and so there is pressure to create additional "secondary mutations," which enhance the level of resistance to the drug. These structural changes often result in reduced catalytic activity and probably will affect the fitness of the virus (the term "fitness" embraces not only the viability or replication rate of the virus but may also include effectiveness of immune evasion genes, transmission, etc.). So yet further mutations may appear which are apparently unrelated to the protein sites that interact with the drug. Such "tertiary mutations" may have no direct effect on biochemical drug−protein binding but may increase enzyme efficiency so as to compensate for the deleterious effects of the primary and secondary mutations. Many compensating mutations are suspected but often their precise role has yet to be elucidated. Of course, virus mutations do not always fit tidily into these human concepts. There may be a "step" mutation which then allows further mutations. In the case of HIV protease, both the protease and the corresponding cleavage sites can co-mutate to give cross-resistance to PIs (section 13.6.3).

## 13.6 Illustrative Examples of Resistance to Specific Antiviral Drugs

### 13.6.1 Poxvirus

Cidofovir (HPMPC) is used clinically to treat AIDS-associated cytomegalovirus retinitis but has also been shown in cell culture and animal tests to be an effective therapy against poxviruses. It has been suggested that cidofovir (or a less toxic prodrug with improved bioavailability) could be stockpiled for use in the event of malicious introduction of smallpox. A study by Andrei et al. (2006) investigated the mutations giving resistance to HPMPC and if the drug resistance was inextricably linked to reduced virulence. If this were the case, then there would be no reason for malicious introduction of mutations conferring resistance.

Drug-resistant vaccinia virus (VV) was obtained by serial passage of the virus in cell cultures with increasing concentrations of HPMPC. In parallel, wt virus was passaged in drug-free cultures. From the final passage, seven plaque-purified HPMPC-resistant (HPMPC$^R$) isolates and five plaque-purified wt isolates were obtained. As it was thought that resistance to HPMPC would be due to mutations in the viral DNA polymerase gene (E9L), this gene was sequenced for each of these isolates. The results are summarized in Table 13.4.

It was known that the original stock of VV contained polymorphisms, in particular at amino acid residue 420. A second polymorphic locus was found at positions 936 to 938. Two clones suffered a small in-frame deletion. However, all wt clones were equally sensitive to HPMPC and so these polymorphisms were unlikely to be related to drug resistance. Therefore it seemed likely that only two point mutations, A314T and A684V, were potentially associated with resistance. These are within the 3′−5′exonuclease proofreading domain and the polymerase catalytic domain,

**Table 13.4** Mutations in the E9L Gene of HPMPC[R] Vaccinia Virus (strain Lederle)

| Virus | Amino Acid Present at Position(s) (Vaccinia Virus)[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 246 | 314 | 420 | 684 | 845 | 857 | 936-937-938 |
| HPMPC[R] 7 clones[b] | R | T | S | V | M | R | A-N-V |
| Lederle wt clones | | | | | | | |
| Clone 1 | R | A | S | A | M | R | N-Δ[c]-G |
| Clone 2 | R | A | L | A | M | R | N-Δ-G |
| Clone 7 | R | A | S | A | M | R | A-N-V |
| Clone 8 | Q | A | L | A | M | R | A-N-V |
| Clone 11 | R | A | S | A | M | R | A-N-V |
| VV strains | | | | | | | |
| Ankara | R | A | S | A | T | G | A-N-V |
| Copenhagen | R | A | L | A | T | G | A-N-V |
| WR | R | A | L | A | T | G | A-N-V |

[a]The amino acid numbering refers to the numbering system for vaccinia E9L gene. The residue numbering differs slightly for homologous residues in other orthopoxvirus genes.
[b]All seven clones were identical.
[c]Δ symbol for deletion.
*Source*: Table adapted from Andrei et al. (2006).

respectively. Marker rescue methods were used to investigate the role of each of these in drug resistance. The cloned DNA encoded A314T, A684V, or both A314T and A684V mutants. As a control, wt DNA was included to test for any of the polymorphisms having an effect on drug resistance. The results showed that A314T and A684V contributed to resistance but both together gave the greater resistance (about 10-fold) to HPMPC. During the serial passaging, it seems that the A314T mutation appeared first followed by the A684V mutation, an example of primary and secondary mutations.

All three HPMPC[R] recombinant viruses exhibited reduced virulence in mice (i.e., the mutants were "less fit"). With both mutations together, the reduction in virulence was about 100-fold. To test for efficacy of HPMPC against this resistant virus, mice were challenged with 4,000 pfu, which caused considerable loss in body weight but nearly all mice survived. Mice treated with HPMPC at 10 mg/kg daily had a small transient weight loss whereas those treated with 50 mg/kg daily were similar to uninfected controls. Although one must be cautious that this result has been shown only for one animal species, it is encouraging that HPMPC would be expected to give useful cover against malicious vaccinia release even if the resistant mutations were introduced.

## 13.6.2 Herpesvirus

The nucleoside analogs, VACV, FCV, and GCV, owe their high selectivity to the fact that their activity requires phosphorylation by a viral enzyme. For ACV and PCV, it is the viral thymidine kinase (TK) enzyme, for GCV the UL97 kinase. The corresponding monophosphate is then further phosphorylated to the triphosphate by

cellular enzymes. It is the triphosphate that interacts with the viral DNA polymerase and terminates viral DNA replication. The pyrophosphate analog, foscarnet, and the cyclic phosphonates (e.g., adefovir) do not require the initial phosphorylation step by a viral enzyme and so their selectivity depends solely on their preferential inhibition of the viral DNA polymerase. The recently described VZV inhibitor, FV100, requires phosphorylation by the VZV TK, but too little of the triphosphate is formed to account for its activity; its mechanism remains a puzzle.

Mutant viruses with acquired resistance to all these compounds can be selected in tissue culture. Resistance-conferring mutations can be detected in the target proteins involved in the mechanism of action of each of the drugs. Mutations in the TK gene may lead to an ablation of this enzyme, thus conferring cross-resistance between ACV, PCV, and other compounds that require this phosphorylation step. A wide variety of different mutations can give rise to a truncated or nonfunctional TK polypeptide and loss of enzyme activity. Clinical and laboratory isolates of HSV typically contain of $\geq 10^{-4}$ TK-defective variants. Since a single plaque produced in a tissue culture contains $\geq 10^5$ infectious virions, it may be seen that TK-mediated resistance develops rapidly in tissue culture. Early work on ACV demonstrated that clinical isolates also contain TK-defective variants at high frequency (Paris and Harrington, 1982).

Another mechanism for the development of resistance are mutations leading to single amino acid residue substitutions in TK or DNA polymerase, which reduces the affinity of the drug to the enzyme but maintains, at least in part, the enzymic activity. Such changes occur at about two orders of magnitude less frequently (ca. $10^{-6}$) in tissue culture-grown virus but have been shown to account for clinical drug resistance in HSV, VZV, and HCMV (Andrei et al., 2007).

The helicase-primase inhibitors (HPIs) represent a new generation of antiviral compounds active against HSV and VZV. It was shown that resistance mutations to BAY 57-1293 occur in the helicase gene, most being located to a group of residues just downstream from the fourth functional motif. For example, the substitution K356N accounts for >5,000-fold resistance. Such mutations are apparent at a frequency of $\leq 10^{-6}$ in tissue culture for many virus isolates. Surprisingly, it was observed that both laboratory isolates and some recent clinical isolates contain HPI resistance mutations at 100-fold higher frequency (Sukla et al., 2010). PCR amplification experiments and other evidence shows beyond doubt that such mutations exist at high frequency as polymorphisms in the virus population prior to drug exposure (Biswas et al., 2007).

While herpesvirus drug-resistance occurs at relatively high frequency in tissue culture, the widespread clinical use of herpesvirus antivirals is rarely limited by the emergence of resistance in immunocompetent patients. Indeed, large-scale screening of isolates of both HSV-1 and HSV-2 obtained from typical lesions of labial or genital herpes show no obvious trends to resistance (<1%) after extensive use over the period from the early 1980s to date (Bacon et al., 2003). However, resistance to nucleoside analogs does appear to be more common in patients with neonatal HSV and herpes keratitis. In the former, resistance may be observed in 5% of patients and for ocular isolates from herpes keratitis, Duan

et al. (2008) reported that 11/173 (6.4%) patients yielded resistant isolates and 10/11 of these isolates mapped to TK, with these authors arguing that the cornea represents an immunologically privileged site. In immunocompromised patients, resistance to ACV and similar drugs has commonly been reported in about 5% of patients and in some cases up to 20%. Often, such viruses comprise mixtures of wild-type together with one or more different resistant mutants. There may be two reasons underlying the apparent divergence between results in immunocompetent and immunocompromised patients. Most important, many resistance mutations clearly result in loss of virus "fitness." This is most easily demonstrated for TK-defective strains in a variety of animal models. Such strains are much less neuropathogenic (Field and Wildy, 1978) and, while they can establish a latent infection, these seem unable to reactivate efficiently to produce infectious virus (see 13.8.3). Also, other resistance mutations undoubtedly result in subtle defects that may diminish the ability of the virus to reactivate efficiently from latency and/or replicate successfully. Secondly, the establishment of neuronal latency with wt virions during primary infection means that subsequent reactivations originate from the pool of sensitive virus.

The discovery of high frequency of resistance mutations (or polymorphisms) in clinical isolates of HSV challenges the dogma that large DNA viruses display high genetic stability. While the genome is generally highly conserved, it is still not clear why frequency of particular resistance mutations leading to single amino acid substitution at defined loci may be as high as $10^{-4}$ in some strains. Perhaps herpesviruses are able to generate some constrained genetic flexibility during DNA replication to overcome host heterogeneity, provide tropism for biological sites, and/or enable immune avoidance? However, the mechanism for this intriguing ability has yet to be determined.

Finally, where herpesvirus resistance has become a recognized problem, such as in herpes keratitis, neonatal herpes, and herpes in the immunocompromised patient, the lessons learned from HIV and hepatitis viruses will be applied in the form of drug combinations. These will most likely involve nucleoside analogs in combination with the ether-lipid analog of cidofovir, CMX001, HPIs, and other novel compounds.

### 13.6.3   HIV: Protease Inhibitors

Resistance to HIV protease inhibitors provides good examples of stepwise mutations (Molla et al., 1996): primary, secondary, and tertiary as defined above (section 13.3). At the time of a review by Schafer (2002), there were six protease inhibitors (PIs) approved in the United States—amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir. For all these, the primary mutation occurs in the substrate cleft of the protease, thereby reducing the affinity of the inhibitor. These primary mutations generally reduce the activity of the compounds by only 2- to 5-fold, not enough to be clinically resistant but sufficient to confer a selective advantage to the virus. These variants will overtake the wt HIV and allow other mutations to develop. For indinavir and ritonavir, the first mutation is V82A/T/F/S,

for nelfinavir, D30N. For other PIs, especially saquinavir, the first mutation is often L90M (Clavel and Hance, 2004). The second mutation for indinavir, ritonavir, saquinavir, and amprenavir is I84V and for nelfinavir N88D/S. Further mutations can occur in the protease flap, generally I54V but also I54T/L/M. By the time there are about six mutations, the new strain is likely to be highly resistant and have cross-resistance against several PIs.

There are many other reported mutations which may be considered to be tertiary mutations helping to restore viral "fitness" but their role has not been defined. Remarkably, viral "fitness" can also be increased by mutations in HIV gag, the main viral substrate of the protease. Such gag mutations, A431V and L449F, can improve the ability of the protease of resistant strains to interact with the substrate.

### 13.6.4 Influenza Virus

Rational design programs led to development of the neuraminidase (NA) (or sialidase) inhibitors, zanamivir, and oseltamivir. Both compounds block the action of the essential virus function, NA, which is required by influenza for efficient release of infectious progeny. These compounds are generally held to be efficacious (Dutowski, 2010).

Oseltamivir has been prescribed far more often in Japan than elsewhere, thus oseltamivir-resistance has been investigated in this population. There have been several reports of NA-inhibitor resistance among clinical isolates. For example, a study in Japan found that 9 of 50 children with influenza A (H3N2) virus infection who had been treated with oseltamivir had a virus with drug resistance, although it was suggested that these mutations were less fit than the wt viruses from which they were derived (Kiso et al., 2004). One study followed resistance from 1996 through 2007 (Tashiro et al., 2009). During the period 1996−2002, influenza A N2 viruses were circulating but no resistant viruses were detected (0/175). During the season 2003−2004, 0.3% (3/1180) of N2 samples were resistant. During the following three seasons, no N2 resistant viruses were detected but N1 virus started circulating. In 2004−2005 and 2006−2007, no resistant viruses were detected but in 2005−2006, 3% (4/132) were found. This survey confirms that resistance to oseltamivir occurs in the normal population far less readily than does resistance to amantadine. Unfortunately, the situation changed dramatically and globally within 3 years (Okomo-Adhiambo et al., 2010). Among seasonal $H_1N_1$ influenza, the proportions of oseltamivir-resistant viruses were low (ca. 1%) in 2006−2007 but then resistance emerged rapidly worldwide; in the United States, high-level resistance (100- to 3,000-fold) was found in about 20% of samples tested in 2007−2008 and about 90% in 2008−2009. Sequencing confirmed the H275Y mutation in resistant strains. It appears that a natural, spontaneously arising variant had spread globally, without drug selection pressure, during 2007−2008. Early work in cell culture and animals suggested that the H275Y mutant virus was somewhat disabled, but there seems to have been co-selection of other compensating mutations, perhaps to the hemagglutin gene (HA), to give a "fit" virus enabling this variant to spread globally.

Among the circulating human influenza viruses, there are three subtypes of neuraminidase (NA), influenza A types N1 and N2, and influenza B NA. These NAs differ in the structure of a pocket adjacent to the active site of the enzyme. Oseltamivir makes use of this pocket in binding to the NA and so resistance can occur with mutations. With N1, just a single mutation, H275Y, gives high-level resistance but the corresponding mutation in N2, H274Y, does not give resistance. Instead two mutations, E119V and R292K, give high-level resistance with N2. With Influenza B NA, R152K and D198N give resistance. Generally, these mutations affecting the pocket do not give cross-resistance to zanamivir but there are some NA mutations which do so, for example, R371K. With influenza B, R152K and D198N give cross-resistance. However, it seems that such strains may be disabled as zanamivir resistance has been isolated only rarely in the clinic.

In clinical studies on oseltamivir resistance, it has been noted that resistance occurs at a higher rate in influenza with N1 than N2, presumably because it takes just a single mutation, H275Y, to give high-level resistance with N1. For example, in a study of oseltamivir-treated children during 2005−2007 (Stephenson et al., 2009) resistance was detected in 3/11 (27%) with influenza A $H_1N_1$, 1/34 (3%) H3N2, and 0/19 with influenza B.

During the $H_1N_1$ pandemic of 2009−2010, resistance to oseltamivir has been reported in case studies of seriously ill patients. Fortunately, human-to-human spread has occurred sporadically in geographically dispersed regions. Virtually all the resistant viruses have had the H275Y mutation. Other than these few cases, oseltamivir has been used widely during the pandemic and seems to have not been associated with resistance in the general population. This situation could easily change. With such high proportion of resistance among the seasonal N1 influenza, and with influenza viruses having the ability to re-assort, it seems likely that the threat of resistant pandemic $H_1N_1$ is ever-present.

## 13.7    Optimizing Drug Combinations to Avoid Resistance

### 13.7.1    Genetic Barrier

When a virus is being inhibited by an antiviral compound, resistance mutations are selected, but the ease with which this is done depends upon how many potential mutations can give resistance without compromising virus fitness. This has become known as the genetic barrier.

The anti-influenza M2 channel blockers, amantadine and rimantadine, and anti-picornarvirus capsid-binding compounds, such as pleconaril, are examples of agents which present too low a genetic barrier to become useful clinical monotherapies. Such compounds may give added benefit if always used in drug combinations without ever being used alone.

The importance of the genetic barrier concept has been emphasized by experience from testing combinations of drugs active against HIV. There are three major classes of anti-HIV compounds: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and PIs.

More recently, a fusion inhibitor, HIV integrase inhibitors, and a receptor-binding blocker have also become available. With monotherapies, resistance appears quickly with NNRTIs but more slowly with NRTIs, which also target RT but at its catalytic site. It appears that mutations at an allosteric site are more readily accommodated than mutations at the catalytic site.

Similarly, for PIs, which target the protease catalytic site, the rate of appearance of resistance is about comparable to that for NRTIs. It was thought that combining one NRTI and one PI would delay the appearance of resistance greatly but clinical practice showed that the delay was modest. The gain from such combinations of drugs is probably due to the faster reduction in the rate of virus replication (virions/day), thus reducing the opportunities for creating resistant mutants. However, aside from this factor, it is as easy to form resistant mutants, one in the RT and one in the protease, as for the monotherapies. It is more effective to combine two or three compounds that target the same HIV enzyme. An ideal situation is when the resistance mutations to one drug confer enhanced sensitivity to the other. Even with drugs which have differing mutation patterns, the aim is to have no possible mutations without causing a large reduction in enzyme efficiency. In summary, one high genetic barrier is more effective in delaying resistance than two low genetic barriers.

An example of a commonly used combination therapy for HIV is Atripla® (Gilead and BMS jointly). This single pill contains three compounds: emtricitabine, tenofovir, efavirenz, or two NRTIs and a NNRTI, respectively. Another example is Trizivir® (GSK), combining the three NRTIs, zidovudine, lamivudine, and abacavir. These combination pills, targeting the HIV RT, are often used with an HIV protease inhibitor. When used correctly, these multi-drug therapies provide good control of HIV replication and symptoms and have prevented resistance development for at least several years.

The experience with HIV should guide rational choice of compounds for combination therapies for influenza. Of the two neuraminidase inhibitors (NIs), oseltamivir has been much the more widely prescribed than zanamivir (inhaled) but, fortunately, zanamivir retains activity against the H274Y (H275Y in N1 numbering) mutant resistant to oseltamivir. Zanamivir is now being developed as an IV drug for use in seriously ill patients. Peramivir, another NI, is also being developed as an IV drug. There is cross-resistance between oseltamivir and peramivir, so this combination would not increase the genetic barrier. However, zanamivir and peramivir have differing resistance mutations although Q136K alone gives reduced sensitivity to both zanamivir (36-fold) and to peramivir (80-fold). As these two compounds are being developed as IV therapies, it may be beneficial to combine these into one IV product.

Favipiravir (T-705) is the first influenza RNA polymerase inhibitor to reach phase III clinical trials. In combination with oseltamivir and zanamivir, T-705 has given additive to synergistic activity in a mouse model. The combination of oseltamivir and T-705 has been evaluated in a Phase I study. Clearly, it is hoped that this combination would give highly effective control of influenza in seriously ill patients. But experience from HIV indicates that this would not be an optimum combination to delay resistance, especially in immunocompromised patients. The

greater reduction in virus replication with the combination therapy will help to delay resistance but the genetic barrier is the same as for the two individual therapies. For an optimum combination, we need a second viral RNA polymerase inhibitor, with resistance mutations differing from those for T-705. However, in otherwise healthy individuals, one may hope that the immune system would clear a small population of resistant influenza virus. Combination therapy is a strategy that was proposed by Hayden (1986). However, while there is a paucity of candidate compounds, obtaining combinations with an appropriate virological and pharmacological match remains a major challenge to this day.

Generally, vaccines could be considered as "drug combinations" as they may induce many antibodies, each specific for a different part (epitope) of the viral protein surface. To become resistant, the virus would have to change many parts of the protein surface (i.e., it presents a high genetic barrier). In contrast, therapy with a monoclonal antibody (e.g., palivizumab for respiratory syncytial virus [RSV]) which targets a single epitope, would be more susceptible to virus resistance. In a study in immunosuppressed cotton rats (Zhao and Sullender, 2005), palivizumab resistance was detected in 3/5 animals. Within the F gene, one mutation, A816T was sufficient to give resistance. This is similar to the situation following drug monotherapy.

## 13.8 Unexpected Consequences of Resistance Mutations

### 13.8.1 Multiple Changes Arising from One Mutation

Some viruses make very efficient use of their small genome size by using not just one of the three possible reading frames but two or all three reading frames. An example is HBV, in which the polymerase and capsid protein reading frames overlap. For treating HBV infections, the commonly used antiviral compounds are lamivudine (LMV or 3TC), adefovir dipivoxil (ADV), and telbuvidine. All these three inhibit the viral polymerase, and so resistant mutations arise in the gene coding for the polymerase. The same mutation can, however, also change the viral surface protein due to the overlap of the reading frames. Conversely, the immune system would exert pressure on the virus to generate mutations in the surface protein but such mutations may give rise to nonfunctional viral polymerase.

### 13.8.2 Carbohydrate-Binding Agent Leads to Greater Immunogenicity

Although many viral infections are short-lived and the host is able to clear the virus, HIV infections continue despite a vigorous antibody response. Could the antibody response be made more effective in clearing the virus and perhaps limit the progression of HIV infection? A novel approach to therapy uses compounds that bind to carbohydrate moiety of the HIV gp120. The concept is that this would lead to a high genetic barrier to resistance whilst making resistant mutants more susceptible to neutralizing antibody (Balzarini, 2005).

HIV gp120 is highly glycosylated ($\sim 50\%$), many of the carbohydrate chains being high-mannose type, which are rare on human cells. The virus envelope glycosylation is required for the proper folding of the gp120, for efficient entry of the virus into target cells, and for hiding the potentially highly immunogenic protein surface of gp 120. Dendritic cells have a receptor, DC-SIGN, which captures HIV via the (high-mannose) glycans and then directs transmission of HIV to T-lymphocytes. The expression of gp120 in the cell membrane of virus-infected cells allows fusion with uninfected cells, resulting in giant multinucleated cells. Carbohydrate-binding agent (CBA) have the potential to inhibit all these steps. As proof of concept, several plant lectins, with binding preference to mannose-containing glycans, have been shown to inhibit all the above steps. More encouraging for drug potential, Pradimicin A (PRMA) is a non-peptidic, small molecular weight CBA. Although less active, on a $\mu$M basis, than the plant lectins, it has similar broad-spectrum activity against a variety of HIV-1, HIV-2, and SIV strains.

Selection of CBA-resistant HIV can be achieved in cell culture but only after many passages. The mutations are predominantly in gp120, notably not in gp41, and result in loss of glycosylation sites, mainly the high-mannose glycan sites. There is a high genetic barrier due to the possibility that many PRMA molecules can bind to each single gp120 molecule and there has to be multiple glycan deletions for significant phenotypic resistance. When in the presence of an immune system, it is hoped that these CBA-resistant strains of HIV will be rendered susceptible to neutralizing antibody due to the exposure of the gp120 protein surface which is normally hidden under a protective glycan cover. There is now some evidence for this. Hu et al. (2007) used cyanovirin-N (CV-N), a CBA, to generate strains of HIV resistant to CV-N and other CBAs so that they could investigate the impact of the immune system on these CBA-resistant strains. One of the isolated resistant clones, GCV4, had five mutations resulting in the loss of glycosylation at amino acid residues 289, 332, 339, 392, and 448, all these being in the constant regions C2, C3, and C4 of gp 120. When used to infect cells with control serum, wt and GCV4 infectivities were not changed by the concentration of the serum. In contrast, when serum from HIV$^{+ve}$ patient was used, the serum had a greater potency against GCV4 than wt HIV. Furthermore, GCV4 was more sensitive to monoclonal antibodies (MAbs) directed to the V3 loop of gp120, a major determinant of viral entry. There was about an 8-fold higher sensitivity to MAb 1101 and over 200-fold for MAb 447-52D. As controls, there were no changes in sensitivities to MAbs directed at other parts of gp120. Furthermore, when wt and mutant SIV (lacking several gp120 glycans) were compared in monkeys; the wt gave long-lasting viremia (about 7 $\log_{10}$) whereas the mutant virus gave a short period of high virus levels which dropped as antibody levels rose.

CBA may be effective therapy, not just for HIV but also several other families of enveloped viruses. For example, CBA have shown marked activity against HCV, influenza and coronaviruses (but not HSV, VZV, RSV, or parainfluenza). The lack of activity against the latter viruses may be due to less mannose-rich glycans being present. The challenge now is to discover a low-molecular-weight CBA with the right properties for a good and specific antiviral agent.

### 13.8.3 Herpesvirus Latency Potential

As mentioned in section 13.6.2, a characteristic of herpesviruses is that they establish a latent form which remains viable for the rest of the host's life. The commonly used antiherpesvirus compounds, ACV, VACV, and FCV, are activated only in herpesvirus-infected cells; the crucial first step in that activation requires the viral TK. The TK function is not required for efficient HSV replication in cell culture; wt and TK$^{-ve}$ strains replicate with similar rates to similar titers (Field and Wildy, 1978). This allows the virus in vitro an easy option to become resistant to all the TK-mediated compounds. Furthermore, clinical resistant strains in immunocompromised patients and herpes keratitis patients are most commonly TK$^{-ve}$ strains.

However, TK$^{-ve}$ strains exhibit a marked reduction in viral "fitness." In mice, this is manifested by a large ($>$1,000-fold) reduction in lethality and altered latency. It appears that TK$^{-ve}$ strains are able to establish latent infection but their ability to reactivate greatly impaired. It seems that in humans reactivation is similarly impaired. TK$^{-ve}$ viruses do not readily spread among the population. In contrast, wt virus often transmits while the subject is unaware of a sub-clinical reactivation. For an individual immunocompetent subject, if treatment of an episode leads to resistant TK$^{-ve}$ herpesvirus appearing, then the next episode of recurrent herpes will not be from the new TK$^{-ve}$ strain but from the original wt virus. This seems to account for the continuing low rate ($<$1%) of resistant herpesvirus in the general population even after several decades of antiviral therapy. As may be expected, resistant herpesviruses are a concern in immunocompromised patients.

### 13.8.4 Reduced Replication Fitness

A common consequence of resistant mutations in various viruses, such as influenza virus, HBV, or HIV, is that the resistant virus has reduced ability to replicate in the patient. For example, when only lamivudine was available for the treatment of HBV infections, highly resistant virus was sometimes present within a year of starting therapy but it was better to continue therapy as the resistant virus was partially disabled. Now that other drugs are available, switching to another drug, such as adefovir, is usually the best option. Were it available, however, a combination would be preferable, since in principle, sequential switching from drug to drug is undesirable as it may more readily lead to multiple resistance.

## 13.9 A Role for Compounds Targeting Host Proteins for Antiviral Therapy

For some years there have been attempts to target a host function essential for virus replication. This seemed to provide an attractive way of circumventing virus resistance mutations, although this approach risks unacceptable toxicity. Recently this problem seems to have been addressed for influenza and other viruses by targeting only virus-infected cells.

For example, TSG101 is a host protein which is part of the system regulating transport within the cell. Importantly, TSG101 is normally found only in the

cytoplasm of uninfected cells, but an influenza viral protein binds to TSG101, and this results in TSG101 being localized to the cell membrane. Using TSG101-specific antibodies, it was shown that at time of infection with influenza virus, there was no TSG101 on the surface of the cell but by 24 hours after infection, TSG101 was on the surface. This was confirmed with different cells and various strains of influenza. Furthermore, TSG101 monoclonal antibodies reduced the release of influenza virus from infected cells, indicating that TSG101 plays a vital role in the replication cycle of influenza virus. In cell culture, it has been possible to add TSG101 antibody and natural killer cells to target specifically influenza infected cells.

This approach could provide a broad-spectrum therapy against many different strains of influenza. As TSG101 is normally resident within the cytoplasm of the cell, it is envisaged that TSG101 antibody would be safe to use. The same approach could be useful with other enveloped viruses (HIV, RSV, HSV-1, and 2, Ebola and parainfluenza) which, like influenza, "hijack" TSG101 to help transport the virus from the cell interior to the outer membrane. It seems remarkable that viruses from different families have evolved to use this single mechanism.

In a search for potential compounds, Kinch et al. (2009) used computer modeling to select a panel of low-molecular-weight compounds that may disrupt the binding of TSG101 to viral proteins. These compounds were screened for activity in a range of viruses. One compound, FGI-104, was active against all the tested viruses (including HBV, HCV, HIV, Ebola, and cowpox) in cell culture assays. FGI-104 was then evaluated in a mouse model of Ebola virus; dosing at 10 mg/kg daily gave 100% survival of the treated mice whereas there were 90% deaths in the control group. Although it remains to be demonstrated that FGI-104 is acting via TSG101, it seems that this is an encouraging result.

The budding of HIV has been shown to be dependent on the binding of HIV gag to TSG101. The binding site on TSG101 is highly conserved, Pro-Thr-Ala-Pro (Chen et al., 2010). Using similar strategy as for research leading to PIs, compounds which mimic the protein structure at the gag-TSG101 binding site are being evaluated for inhibition of HIV budding. A disadvantage of this approach is that the selectivity for the infected cell is lost when the anti-HIV compound binds to TSG101 inside the cell rather than on the cell surface.

How would viruses counter such an attack on their replication? To think that the blocking of TSG101 would permanently inhibit virus replication is both overly optimistic and unwise. The virus may mutate to increase its binding to the host protein so that it outcompetes the inhibitor. Alternatively, it is possible that there is a secondary mechanism for the release of virions from the cell. The efficiency of such a secondary mechanism could be enhanced by mutations, the new virus variant then becoming dominant.

## 13.10   Conclusion

The origins and evolution of viruses may be shrouded in mystery but one current aspect is certain. Only in the relatively recent past, since Jenner's first use of

cowpox as a vaccine for human smallpox, have viruses faced a new threat to their replication—mankind's intervention. Through the course of evolution, viruses have developed many hugely varying strategies for their highly successful survival. Now faced with this new threat posed by vaccines and specific antiviral compounds, some viruses are poorly adapted to survive. The human smallpox virus has been eliminated from the human population. The global polio eradication initiative has been highly successful. Its aim, to eradicate polio worldwide, seems achievable but remains elusive. The use of vaccines has been successful in preventing many viral infections, including rubella, mumps, measles, HAV and HBV. However, there seems to be no prospect of eliminating these viruses in the near future. More recently, specific antiviral compounds have been developed to control those human viruses for which, generally, no effective vaccines are available. ACV, active against herpesviruses (HSV-1 and -2, VZV), was the first truly active and selective antiviral agent. Some three decades later, it is still being used but has been joined by just two other drugs, the prodrug of ACV, VACV, and FCV. Although these drugs can limit the symptoms of acute infection, the incidence of latent infection has not been reduced. The mainstay for therapy and prevention of HCMV is just one drug, valganciclovir (VGCV). The spread of HIV has prompted a huge search for effective drugs and combinations of three or four drugs are providing at least several years of clinical control. From this research, drugs against HBV were developed. As for HIV, a similar combination approach is being developed for HCV. The very high mutation rates for HIV and HCV, combined with their high replication rates ($10^9$ and $10^{12}$ virions/day, respectively, in an infected patient without therapy), means that the threat of breakthrough remains ever-present especially if drug doses are missed. The concept of genetic barrier has been helpful in guiding combination therapies to give effective control of patient symptoms for at least several years. Although HIV has spread across the world and caused so much human disease in just a few decades, perhaps the virus with most potential to cause a rapid pandemic is influenza virus. The 2009−2010 pandemic, caused by $H_1N_1$ strain of influenza is the first influenza pandemic that may have been constrained by the use of antiviral drugs and the rapid development of a vaccine.

The last three decades have seen many advances but also highlighted the limitations of mankind's attempts to control viruses. Truly active and safe antiviral compounds seemed rather a remote possibility until ACV was discovered. Even then, when HIV was identified as the cause of AIDS, a vaccine approach was seen as the preferable way forward with an effective vaccine expected in 2 years. Instead, it has been remarkable how combination pills have given HIV patients an easy-to-use, once-daily dosing regimen, which is well tolerated. In too many publications, the *Introductions* state that virus resistance is limiting the use current antiviral compounds, and therefore new compounds with a different virus target and new mode of action are required. Even better to delay resistance, look for compounds which raise the genetic barrier. So far, the best combinations have been with compounds that target the virus polymerase. Similar combinations with protease inhibitors have not been so successful because the protease and the virus polypeptide cleavage sites can co-mutate, an option not available to the virus polymerase. Although the genetic barrier needs to be increased for long-term delay in resistance in

chronic infections, with any drug combination used in naturally self-limiting infections, the extra effect in reducing viral load quickly may well be a useful benefit.

Our current antiviral therapies have been successful in reducing the burden of human diseases but many viruses have evolved strategies for countering new threats to their replication. These strategies pose an ever-present threat to our modern human therapies. We need to use our antivirals wisely.

# Abbreviations

Viruses/virus enzymes

**HAV** hepatitis A
**HBV** hepatitis B
**HCV** hepatitis C
**HSV** herpes simplex virus
**HCMV** human cytomegalovirus
**HIV** human immunodeficiency virus
**HK** herpes keratitis
**RSV** respiratory syncytial virus
**VV** vaccinia virus
**VZV** varicella-zoster virus
**TK** thymidine kinase
**RT** reverse transcriptase
**HA** hemagglutin
**NA** neuraminidase

Antiviral compounds/inhibitor type

**ACV** acyclovir
**VACV** valaciclovir
**ADV** adefovir dipivoxil
**HPMPC** cidofovir
**CV-N** cyanovirin-N
**FCV** famciclovir
**PCV** penciclovir
**T-705** favipiravir
**VGCV** valganciclovir
**GCV** ganciclovir
**HPIs** helicase-primase inhibitors
**LMV or 3TC** lamivudine
**PRMA** pradimicin A
**CBA** carbohydrate-binding agent
**NI** neuraminidase inhibitor
**NRTIs** nucleoside/nucleotide reverse transcriptase inhibitors
**NNRTIs** non-nucleoside reverse transcriptase inhibitors
**PIs** protease inhibitors

Others

**PK** pharmacokinetics
**wt** wild type
**iv** intravenous

# Cross References to Other Chapters

**(1) Molecular epidemiology and species definition of pathogens**
Michel Tibayrenc

**(2) Virus species**
Marc Van Regenmortel (University of Strasbourg, France)

**(3) Viral evolution**
Hiroshi Haeno and Yoh Iwasa (Department of Biology, Faculty of Sciences, Kyushu University, Fukuoka, Japan)

**(23) Genomics of infectious diseases and private industry**
Guy Vernet (Biomerieux company, Marcy-L'Etoile, France)

**(30) The origins of human immunodeficiency virus and implications for global epidemics**
Eric Delaporte, Martine Peeters (IRD, Montpellier, France)

**(31) Evolution of SARS coronavirus and the relevance of modern molecular epidemiology**
Zhengli Shi (Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China) and Lin-fa Wang (CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong, Victoria, Australia)

**(32) Ecology and evolution of avian influenza: the risk of a major pandemics**
Ron. A.M. Fouchier (Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands)

# References

Andrei, G., Gammon, D.B., Fiten, P., De Clercq, E., Opdenakker, G, Snoeck, R, et al., 2006. Cidofovir resistance in vaccinia virus is linked to diminished virulence in mice. J. Virol. 80, 9391−9401.

Andrei, G., Fiten, P, Froeyen, M., De Clercq, E., Opdenakker, G., Snoeck, R., 2007. DNA polymerase mutations in drug-resistant herpes simplex virus mutants determine in vivo neurovirulence and drug-enzyme interactions. Antivir. Ther. 12, 719−732.

Bacon, T.H., Levin, M.J., Leary, J.J., Sarisky, R.T., Sutton, D., 2003. Herpes simplex virus resistance to acyclovir and penciclovir after two decades of antiviral therapy. Clin. Microbiol. Rev. 16, 114−128.

Balzarini, J., 2005. Targeting the glycans of gp120: a novel approach aimed at the Achille heel of HIV. Lancet Infect. Dis. 5, 726−731.

Biswas, S., Smith, C., Field, H.J., 2007. Detection of HSV-1 variants highly resistant to the helicase-primase inhibitor BAY 57-1293 at high frequency in two of ten recent clinical isolates of HSV-1. J. Antimicrob. Chemother. 60, 274−279.

Chen, H., Liu, X., Li, Z., Zhan, P., De Clercq, E., 2010. TSG101: a novel anti-HIV-1 drug target. Curr. Med. Chem. 17, 750−758.

Clavel, F., Hance, A.J., 2004. HIV drug resistance. N. Engl. J. Med. 350, 1023−1035.

Drake, J.W., Holland, J.J., 1999. Mutation rates among RNA viruses. Proc. Natl. Acad. Sci. U.S.A. 96, 13910−13913.

Duan, R., de Vries, R.D., Osterhaus, A.D.M.E., Remeijer, L., Verjans, G.M.G.M., 2008. Acyclovir-resistant corneal HSV-1 isolates from patients with herpetic keratitis. J. Infect. Dis. 198, 659−663.

Dutkowski, R., 2010. Oseltamivir in seasonal influenza: cumulative experience in low- and high-risk patients. J. Antimicrob. Chemother. 65 (Suppl. 2), ii11−ii24.

Field, H.J., Vere Hodge, R.A., 2008. Antiviral agents. In: Mahy, B.W., Van Regenmortel., M.H.V. (Eds.), Encylopedia of Virology, third ed. I. Elsevier, Oxford, pp. 142−154.

Field, H.J., Wildy, P., 1978. The pathogenicity of thymidine kinase-deficient mutants of herpes simplex virus in mice. J. Hyg. 81, 267−277.

Hayden, F.G., 1986. Combinations of antiviral agents for treatment of influenza virus infections. J. Antimicrob. Chemother. Suppl. B 18, 177−183.

Holland, J., Domingo, E., 1998. Origin and evolution of viruses. Virus Genes 16, 13−21.

Hu, Q., Mahmood, N., Shattock, R.J., 2007. High-mannose-specific deglycosylation of HIV-1 gp120 induced by resistance to cyanovirin-N and the impact on antibody neutralization. Virology 368, 145−154.

Kinch, M.S., Yunus, A.S., Lear, C., Mao, H., Chen, H., Fesseha, Z., et al., 2009. FGI-104: a broad-spectrum small molecule inhibitor of viral infection. Am. J. Transl. Res. 1, 87−98.

Kiso, M., Mitamura, K., Sakai-Tagawa, Y., Shiraishi, K., Kawakami, C., Kimura, K., et al., 2004. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. Lancet 364, 759−765.

Molla, A., Korneyeva, M., Gao, Q., Vasavanonda, S., Schipper, P.J., Mo, H-M., et al., 1996. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nat. Med. 2, 760−766.

Okomo-Adhiambo, M., Nguyen, H.T., Sleeman, K., Sheu, T.G., Deyde, V.M., Garten, R.J., et al., 2010. Host cell selection of influenza neuraminidase variants: implications for drug resistance monitoring in A(H1N1) viruses. Antiviral Res. 85, 381−388.

Paris, D.S., Harrington, J.E., 1982. Herpes simplex virus variants resistant to high concentrations of acyclovir exist in clinical isolates. Antimicrob. Agents Chemother. 22, 71−77.

Shafer, R.W., 2002. Genotypic testing for human immunodeficiency virus type 1 drug resistance. Clin. Microbiol. Rev. 15, 247−277.

Smith, D.B., Inglis, S.C., 1987. The mutation rate and variability of eukaryotic viruses: an analytical review. J. Gen. Virol. 68, 2729−2740.

Stephenson, I., Democratis, J., Lackenby, A., McNally, T., Smith, J., Pareek, M., et al., 2009. Neuraminidase inhibitor resistance after oseltamivir treatment of acute influenza A and B in children. Clin. Infect. Dis. 48, 389−396.

Sukla, S., Biswas, S., Birkmann, A., Lischka, P., Zimmermann, H., Field, H.J., 2010. Mismatch primer-based PCR reveals that helicase-primase inhibitor resistance mutations pre-exist in herpes simplex virus type 1 clinical isolates and are not induced during incubation with the inhibitor. J. Antimicrob. Chemother. 65, 1347−1352.

Tashiro., M., McKimm-Breschkin, JL, Saito, T., Klimov, A., Macken, C., Zambon, M., et al., 2009. Surveillance for neuraminidase-inhibitor-resistant influenza viruses in Japan, 1996−2007. Antivir. Ther. 14, 751−761.

Zhao, X., Sullender, W.M., 2005. In vivo selection of respiratory syncytial viruses resistant to palivizumab. J. Virol. 79, 3962−3968.