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## The road to new antiviral therapies

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### Abstract

Viral diseases continue to pose some of the greatest challenges to modern medicine. For many viral diseases, prophylactic vaccines are unlikely to be developed in the near future. Fortunately, effective antiviral therapies have been developed for many of these viruses. In this review, I will focus on antiviral therapy for herpes simplex virus, human immunodeficiency virus, hepatitis C virus, and human papillomavirus. The development of compounds targeting these viruses illustrates many of the principles driving current antiviral development. It is likely that our increasing understanding of viral replication and the virus-host interaction will lead to more rapid development of new antivirals in the future.

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*Keywords:* virus; antiviral; herpes simplex virus; human immunodeficiency virus; hepatitis C virus; human papillomavirus

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### 1. Introduction

Viruses have caused some of the most devastating diseases to afflict humanity. Smallpox is thought to have arisen more than 5000 years ago, and killed millions of people before it was eradicated. Similarly, poliomyelitis has been a cause of paralytic disease since ancient times [1]. Fortunately, effective vaccines have been developed for both these diseases. Vaccines led to the eradication of smallpox in 1979 [2], and seem likely to achieve the eradication of wild-type poliomyelitis in the not-too-distant future [3].

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*Abbreviations:* HIV, human immunodeficiency virus; HCV, hepatitis C virus; AIDS, acquired immunodeficiency syndrome; HPV, human papillomavirus; NRTI, nucleoside reverse transcriptase inhibitor; HAART, highly active antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; TLR, toll-like receptor.

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However, for some of the most pressing viral pathogens of today, no vaccine is available. Thus, despite the fact that 40 million people are currently living with human immunodeficiency virus (HIV) infection (UNAIDS/WHO 2003 estimate), no prophylactic vaccine is available to break the cycle of new infections. Similarly, no vaccine is available to prevent hepatitis C virus (HCV) infection, which is estimated to infect 170,000,000 people worldwide [4], leading to millions of deaths due to cirrhosis or hepatocellular carcinoma. No vaccine is available to prevent infections with herpes simplex virus type 2, which affect one in five Americans [5]. In fact, much effort has been expended in attempts to develop vaccines for these diseases, with a notable lack of success. It might be argued that the “easy” viral candidates for vaccine development have been exhausted. Viruses such as HCV and HIV pose a unique challenge due to their rapid antigenic variation, while other viruses such as the herpes simplex viruses have potent immune escape mechanisms, including the establishment of lifelong latency. Thus, the development of new vaccines for such viruses is likely to be a slow and laborious process. Together with the increased awareness of emerging viral agents such as West Nile Virus, the SARS coronavirus, and Ebola, the tortuous path to new vaccines is a sobering prospect. Fortunately, another path to the management of viral infections has opened with the development of specific antiviral compounds. The advent of these compounds has allowed effective management of some viral diseases, such as herpesvirus infections and HIV infection (AIDS), and has even made possible the cure of some viral infections such as HCV.

The purpose of this article is to review the development of some of the most important antiviral compounds, to illustrate the principles and processes guiding current development of antiviral therapy, and to highlight areas likely to yield significant progress in the future. I will focus mainly on four viruses: the alpha-herpesviruses, because these were among the first viruses to be successfully treated with targeted therapy; HIV, because there are more drugs available for HIV than any other viral infection; HCV, because of the magnitude of its clinical impact and the fact that HCV may be amenable to true cure; and human papilloma virus (HPV) infections, because these illustrate the concept of immune response modifiers in clinical treatment.

## 2. Historical aspects

The path to the development of effective antivirals was in many ways opened by the earlier development of antibacterial compounds. Although not the first antibacterial, the introduction of penicillin into clinical use was in many ways a watershed event, in that it demonstrated the power of therapy specifically directed at an infectious agent. The development of other effective antibacterials progressed rapidly, but little progress was made against viruses. In fact, a prevailing notion was that viruses, which commandeer the life processes of the cell itself, would not be susceptible to the kinds of attack that had proved so effective against bacteria. As late as 1978, Whitley and Alford [6] wrote:

*antiviral chemotherapy remains a major challenge to medical science, particularly when contrasted to the satisfactory approaches currently available for bacterial infection.*

Fortunately, these authors and others had the foresight to understand that the development of effective antivirals was going to require more than serendipity - what was needed was a deep understanding of the workings of the viral replication cycle and its relationship to the host cell. As they put it:

*To comprehend the difficulties of the current approach to antivirals, it is appropriate to review the rationale and complexities of the development of available compounds. Yearly, numerous compounds are synthesized on a random basis by structural alteration of known cellular inhibitors. These manipulations are undertaken with the hope of producing effective anticancer agents; yet the compounds are also screened for antiviral activity.*

*Specific antiviral chemotherapy is dependent upon the identification and inhibition of the viral-specific events of replication, events that only now are becoming understood.*

This approach has proven remarkably fruitful, as the following examples will attest. Furthermore, the approach is likely to continue to be effective in the future, as our understanding deepens of the fundamental biology of viral infections and the corresponding response of the host.

### 3. Herpes simplex virus

The development of effective antiherpetic compounds was perhaps the event that set the stage for all later efforts at antiviral therapy, and even today this stands as one of the great success stories in medical virology. As noted above, in the 1960s and 1970s a large number of nucleoside analogs were developed and screened for antineoplastic and antiviral activity. Some of these, such as 5'-iodo-2'-deoxyuridine (IDU) and trifluorothymidine (TFT), came into use for topical treatment of herpes simplex virus (HSV) keratitis. Unfortunately, these compounds were unacceptably toxic for systemic use [7]. Another of these so-called "first generation" antiherpetics, vidarabine (Ara-A), was sufficiently well-tolerated for systemic therapy, and found use in herpes encephalitis, neonatal herpes, and varicella zoster infections [8–10]. Vidarabine is an analog of adenine that is activated by cellular enzymes to form a triphosphate form, which then inhibits the HSV DNA polymerase.

Despite the utility of vidarabine, toxicity remained a concern, as the triphosphate form would be generated in all cells, infected or not. Thus, the search continued for compounds with true selectivity for virus-infected cells. The culmination of these efforts resulted in the synthesis of 9-(2-hydroxyethoxymethyl)guanine. In this guanine derivative, the 9 position is replaced by a acyclic side chain. Originally termed acycloguanoside, the compound which would come to be known as acyclovir has become the standard of treatment for alpha-herpesvirus infections. Acyclovir is converted to a monophosphate by a virus-encoded thymidine kinase, and subsequently converted to di- and tri-phosphates by cellular kinases [11]. In contrast, in the uninfected cell, the lack of the viral thymidine kinase prevents generation of the triphosphate form. In the infected cell, the triphosphate form is incorporated into the viral growing viral DNA chain, leading to chain termination. Acyclovir also directly inhibits

the viral DNA polymerase, 10–30 times more effectively than it does the cellular polymerase [11]. Thus, acyclovir has a high degree of viral selectivity, and is a remarkably well-tolerated drug.

The adoption of acyclovir into clinical practice was rapid. After demonstration of activity in an animal model [12], the drug was used in immunosuppressed patients with cutaneous or systemic herpes simplex infections, with remarkable efficacy and lack of toxicity [13]. It has subsequently become a mainstay of therapy of genital herpes simplex virus infections, in which it shortens the time to lesion healing and shortens the period of viral shedding [14]. Acyclovir remains an important compound in the treatment of alpha-herpesvirus infections; in 2003 over 9000 scientific and medical articles were published regarding acyclovir and its use. Acyclovir has also given rise to related compounds such as valaciclovir, penciclovir, famciclovir, which feature slightly different spectra of antiviral activity and/or improved pharmacodynamics. Unfortunately, these compounds do not eradicate latent virus, and in immunocompetent people the treatment of recurrent lesions provides only a modest clinical benefit. These compounds can be used as suppressive therapy over the long term to prevent viral reactivation [15,16], but once the drug is stopped, reactivation of the virus resumes. Clearly, treatment options that reduce the frequency of clinical recurrences and viral shedding are badly needed.

#### **4. Human immunodeficiency virus**

The clinical syndrome of AIDS was first described in 1981, and the human immunodeficiency virus (HIV) was identified shortly thereafter. Because it emerged relatively recently, a deeper understanding of the fundamental processes of viral replication was available at the start of the initial pharmacologic attacks on HIV. HIV is a retrovirus, and therefore its lifecycle requires the unique reverse transcription of viral RNA to DNA, through the action of a viral reverse transcriptase (RT). Because mammalian cells do not encode a reverse transcriptase, this offered an attractive target for therapeutics that might be expected to be relatively non-toxic. The first successful attempts at targeting the HIV RT were compounds analogous to acyclovir, in that they were nucleoside analogs. The first of this class of drugs, known as nucleoside reverse transcriptase inhibitors (NRTIs) was AZT [17,18], which had remarkable efficacy in patients with advanced AIDS [19]. AZT has a 100-fold higher affinity for the viral RT than it does for the cellular polymerases, and thus selectively inhibits RT function [18]. The NRTIs have been a highly successful class of anti-HIV compounds; as of 2004, 9 had been approved by the FDA, and several more are in late-stage development.

As successful as the NRTIs were, however, problems soon became apparent. The dramatic initial clinical benefits of AZT often proved transitory, as viral resistance soon began to appear in patients [20]. Drug resistance is a serious problem in HIV infections, due to the high mutation frequency of the HIV polymerase, coupled with the high rate of HIV replication. To make matters worse, many of the resistant viruses in fact showed significant cross-resistance to other NRTIs. By analogy with the multidrug regimens used to treat bacterial infections and cancers, combinations of NRTIs were tried [21], but cross-resistance continued to be a significant problem.

Clearly what was needed in the fight against HIV were drugs that acted against aspects of the viral life cycle different from those targeted by the nucleoside analogs. Again, what made the difference was the enormous body of basic knowledge regarding the replication of HIV. Several steps in the viral replication cycle made attractive targets, in that they were processes quite distinct from the processes necessary for the life of the normal uninfected human cell. Another target to be attacked was the viral protease, via the so-called protease inhibitors. In the replication cycle of HIV, translation of the coding RNA strand leads to production of a single polypeptide. This polypeptide must be cleaved by the HIV protease to generate the functional proteins required for viral replication. Without this cleavage, viral replication ceases. The first protease inhibitor drug approved for HIV, saquinavir, was introduced in 1995. The impact of this class of compounds was immediate and dramatic [22]. Since the protease inhibitors target a completely different aspect of the viral replication cycle than do the NRTIs, cross-resistance is a relatively infrequent occurrence. Using combinations of NRTIs and protease inhibitors, many patients near death experienced dramatic improvements, with viral loads often falling below the limit of detection [23]. Such combination therapy regimens have come to be known as highly active antiretroviral therapy (HAART), and the introduction of HAART regimens have made HIV infection a chronic condition that can be managed successfully for years in many patients. Currently, there are 9 protease inhibitors available for HIV infection, and others are in development.

With the successful introduction of the protease inhibitors, workers sought other mechanisms by which to selectively attack HIV replication. The success of the NRTIs demonstrated that the viral reverse transcriptase was an excellent target for pharmacologic attack. Therefore other, non-nucleoside drugs were sought that could inhibit RT function. The first of these non-nucleoside reverse transcriptase inhibitors (NNRTIs) to be clinically approved was nevirapine. The NNRTIs function by binding the hydrophobic pocket of the p66 subunit of the HIV RT [24]. The NNRTIs have become an extremely important part of the anti-HIV armamentarium for several reasons. Like the NRTIs, the NNRTIs are extremely effective drugs, due to the central role played by the viral RT during HIV replication. However, since they attack the HIV RT by a completely different mechanism, cross-resistance between the NNRTIs and the NRTIs is rare. In fact, a number of effective HAART regimens have been described that combine NRTIs and NNRTIs without the need for protease inhibitors [25]. Another attractive feature of the NNRTIs is that their toxicity profiles are quite different from the protease inhibitors. Despite the dramatic efficacy of protease inhibitor-containing HAART regimens, the long-term use of protease inhibitors has been associated with abnormalities of lipid and glucose metabolism, and changes in body shape [26]. For patients experiencing such side effects, the ability to halt protease inhibitors while maintaining viral control is critical. In addition, having multiple classes of drugs from which to choose is advantageous in patients treated for extended periods who eventually develop resistance to previously effective drugs.

Another aspect of the HIV replication cycle long eyed by investigators is the initial entry of the virus, during which the viral envelope fuses with the cell membrane. The first of this class of compounds, the fusion inhibitors, was recently approved by the FDA for clinical use. This compound, originally known as T-20 and now named enfuvirtide, consists of a 36 amino acid polypeptide that is identical to a portion of the HIV surface protein gp41. The

gp41 protein is critical for the fusion process, during which it is released from a high-energy configuration after binding of gp120 to its receptors [27]. Enfuvirtide interferes with this process, thus preventing the infection of new cells by HIV. Since it uses a completely different mechanism of action than the other classes of anti-HIV drugs, enfuvirtide is especially useful in treatment-experienced patients with virus resistant to other classes of drugs [28]. An important limitation of enfuvirtide in clinical practice, however, is the fact that because of its composition as a peptide, the drug is degraded in the gastrointestinal tract, and therefore must be administered by injection. The concept of fusion inhibitors as a class of compounds remains attractive, however, and will be even more so if orally available drugs are developed.

The final anti-HIV target to be discussed here has not yet come to clinical fruition, but deserves mention as a uniquely attractive target. Inherent to the HIV replication cycle is the integration of HIV DNA into the host chromosome, and this process is dependent upon a virally-encoded integrase. Several pharmaceutical companies have integrase inhibitors in various stages of development.

Despite the large numbers of compounds available for treating HIV infection, it must be stressed that these drugs are not a cure for HIV, and therapy must be continued for life. As noted before for HSV, there is a pressing need for new therapies that can eradicate the virus, or at least provide long-term control with a minimum of drug exposure and inconvenience.

## **5. Hepatitis C virus**

The worldwide health impact of hepatitis C virus (HCV) should not be underestimated. Although estimates vary, the global prevalence is thought to be about 170 million cases, making hepatitis C one of the most important chronic viral infections affecting humans. Approximately 4 million Americans have been infected with HCV, and of these, about 2.7 million have chronic infection [29–31]. According to the Centers for Disease Control and Prevention, there are approximately 25,000 to 30,000 new cases of HCV infection each year in the United States [32] and 8,000 to 10,000 deaths. Although the annual incidence of new infections in the United States appears to be declining, the death rate is expected to triple in the next decade, underscoring the importance of this epidemic.

No vaccine is presently available for the prevention of HCV infection. The first FDA-approved treatment for HCV infection was interferon- $\alpha$ , which likely acts through a combination of pro-inflammatory and direct anti-viral effects. At best, about 25% of patients treated with a 12-month or longer course of interferon- $\alpha$  achieved a sustained viral response; that is, they remained without detectable virus six months after completion of therapy [33]. Subsequently, interferon- $\alpha$  was combined with ribavirin, a synthetic nucleoside with antiviral activity of unknown mechanism. This regimen increased the rate of sustained viral responses to about 50% [34]. A recent advance has been the development of pegylated forms of interferon- $\alpha$ . In these forms, interferon- $\alpha$  is covalently conjugated to monomethoxy polyethylene glycol (PEG). This form has a greatly increased half-life compared to the standard preparations, allowing less-frequent injections and avoiding periods of subtherapeutic blood levels of interferon- $\alpha$ . Large studies have recently been completed evaluating 6 to 12 months of combination therapy with pegylated interferon- $\alpha$  and ribavirin; the sustained viral response rates in these studies approached 60% [35,36].

Other drugs are in development that target distinct mechanisms in the viral replication cycle. Farthest along of these are inhibitors of the NS3 protease of HCV. Translation of the coding RNA strand of HCV leads to production of a single polypeptide, which must be cleaved by the HCV NS3 protease to generate the functional proteins required for viral replication. In a small proof-of-concept study, a prototypical protease inhibitor, BILN2061 (Boehringer Ingelheim), was given twice daily for two days to HCV-positive volunteers [37]. Viral loads fell two to three logs or more for all patients within 24–48 hours of treatment, with the viral loads in some patients falling below the limit of detection. Despite the fact that all patients subsequently rebounded, such results are highly encouraging for potential future combination therapy.

Perhaps the most exciting aspect of developing new therapies for HCV infection is the prospect of true cure. This is in contrast to infections with viruses such as HIV or HSV, for which our current understanding of viral and host biology suggests that curative treatments are unlikely in the foreseeable future. As noted above for HCV, though, up to 60% of infected individuals can be cured even with currently available therapy (pegylated interferon plus ribavirin). It is reasonable to predict that as protease inhibitors and other targeted therapies enter clinical practice, true cures can be expected in an ever-higher percentage of patients.

## 6. Human papillomavirus

The human papillomaviruses (HPV) are a group of non-enveloped double-stranded DNA viruses that infect epithelial cells of the skin and mucosa. More than 100 types of HPV are known, and it is likely that many more remain to be discovered [38]. Individual types of HPV favor particular anatomic sites, and tend to cause characteristic manifestations. For example, a subset of HPV types preferentially infect the anogenital mucosa. Some of these types (especially type 16) are strongly associated with cervical dysplasia and carcinoma. A vaccine for HPV type 16 has recently shown good efficacy in preventing infection [39]. The use of such a vaccine may eventually decrease the incidence of cervical cancer. However, other HPV types, such as 6 and 11, are strongly associated with anogenital warts (condyloma) [40].

Treatment of all warts, and especially anogenital warts, has been problematic. Most standard therapies have been ablative, and rely upon destroying infected tissue by caustic agents, cryotherapy, laser treatment, etc., while hopefully minimizing damage to surrounding normal tissue. In general the response rate has been unsatisfactory, and what clinical success has been achieved has been complicated by the frequency of recurrences. On the other hand, spontaneous remission of warts is also common, and this has suggested that the immunologic response to HPV may be critical to its control. Thus, attention has focused upon the possibility of immune modulation as a treatment option for HPV. Of the various approaches attempted, two compounds, interferon- $\alpha$  and imiquimod, have achieved significant success.

Although interferon therapy for anogenital warts has been administered topically and systemically, the most effort has been focused on intralesional therapy. Topical treatment has had disappointing efficacy, while systemic therapy is associated with significant side effects [41]. Intralesional therapy is performed using either purified interferon- $\alpha$  or recombinant interferon- $\alpha$  2b. In controlled trials, intralesional therapy with interferon two or three



times per week for 3 to 8 weeks has been associated with complete wart remission rates ranging from 36% to 62% [42–44]. Recurrence rates average about 20–25% [42,44]. Even with intralesional therapy, systemic flulike symptoms are a common side effect [45]. In addition, the process of injecting individual lesions is both time-consuming and painful.

Despite its drawbacks, the efficacy of interferon therapy supports the concept of immune modulation as a treatment modality for anogenital warts. The most successful attempt in this regard is the immune response modifier imiquimod. Imiquimod is an imidazoquinoline derivative that stimulates multiple aspects of the innate and adaptive immune systems. Specifically, imiquimod induces multiple cytokines of the so-called T-helper type 1 (Th1) response, including interferon- $\alpha$ , interleukin-6, and tumor necrosis factor- $\alpha$  [46]. A similar Th1 response is seen in spontaneously regressing warts [47]. Although the molecular mechanism for these effects was initially unknown, it was later demonstrated that imiquimod stimulates the Th1-type response via Toll-like receptor 7 (TLR7). The TLRs are a family of receptors that recognize pathogen-associated molecular patterns, or PAMPs, and form the basis for detection of danger by the innate immune system [48]. TLRs are highly expressed in dendritic cells and other professional antigen presenting cells, and ligation of these receptors can strongly promote maturation and cytokine production by other cells of the immune system. Signaling via TLR7 after administration of imiquimod, specifically, results in generation of a vigorous Th1-type response.

Clinically, imiquimod was approved by the FDA in 1997 for the treatment of anogenital warts. It is administered as a 5% cream, which is applied topically approximately three times per week. The cream can be self-applied, and application is not painful, presumably increasing patient compliance [47]. The rate of complete elimination of warts after 8 to 16 weeks of therapy ranges from about 50% to 80%, depending on the exact population studied [49–52]. In general, women respond better to imiquimod than men, a difference that has been suggested to relate to the lower degree of keratinization in affected sites in females [47]. The time to complete remission of warts ranges from about 6 to 12 weeks, with women responding faster than men. Imiquimod also appears to be useful in reducing the rate of recurrence after surgical excision of anogenital warts [53,54].

The success of imiquimod is likely to presage development of other immune response modulators, with different spectra of action and different disease targets. One potentially important agent, resiquimod, is structurally related to imiquimod. Resiquimod also acts via an effect on TLRs, specifically TLR7 and TLR8 [55,56]. Resiquimod has shown some activity in suppressing recurrences of herpes simplex virus infection; in one study the mean time between recurrences of lesions was 169 days in resiquimod-treated patients, compared to 57 days in vehicle-treated patients [57]. The indications for immune response modulators may also extend beyond infectious diseases. Imiquimod has shown significant activity against basal cell carcinoma [58].

## 7. Concluding remarks

The development of new therapies for viral infections remains an active field, and is likely to remain so indefinitely. Certainly there is much still to be done before treatment for HSV,

HIV, HCV and HPV is considered adequate. Furthermore, the emergence of viruses such as West Nile virus, the SARS coronavirus, and Ebola suggests that new challenges will continue to arise. Fortunately, our ever-increasing understanding of viral replication, together with advances in cell biology and immunology, provide us a good basis for the development of new and more effective therapies to meet these threats.

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A regularly updated listing of FDA-approved anti-HIV drugs is available at: [http://www.hivandhepatitis.com/hiv\\_and\\_aids/hiv\\_treat.htm](http://www.hivandhepatitis.com/hiv_and_aids/hiv_treat.htm)

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