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Review

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New pharmacological strategies to fight enveloped viruses

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Enveloped viruses pose an important health threat because most of the persistent and many emerging viruses are enveloped. In particular, newly emerging viruses create a need to develop broad-spectrum antivirals, which usually are obtained by targeting host cell factors. Persistent viruses have developed efficient strategies to escape host immune control, and treatment options are limited. Targeting host cell factors essential for virus persistence, or immune-based therapies provide alternative approaches. In this review, we therefore focus on recent developments to generate antivirals targeting host cell factors or immune-based therapeutic approaches to fight infections with enveloped viruses.

Enveloped viruses

Viruses can be divided into nonenveloped and enveloped viruses. The latter have a host-derived membrane, called an envelope, covering the capsid, which protects the viral genome. A membranous envelope is associated with a relatively low resistance to desiccation, heat, alcohols, or detergents, limiting survival of virus particles outside their host. However, carrying an envelope offers distinct advantages for a virus: (i) envelopment allows the virus to exit from its host cell using the cellular machinery for exocytosis, thus avoiding cell damage and preventing immune responses; (ii) the envelope increases the packaging capacity of a virus particle and allows for carrying additional viral proteins; (iii) the envelope hides structurally restricted capsid antigens from circulating antibodies; and (iv) envelope proteins on the surface of a virus have a higher structural flexibility than capsid proteins because they are not part of a rigid capsid structure; thus, enveloped viruses can escape neutralizing immune response better than their nonenveloped counterparts. Most zoonotic viruses are enveloped; apparently, variations of envelope proteins facilitate adaptation to different hosts, although of course additional adaptations are required.

Besides host cell phospholipids and proteins from cellular membranes, the viral envelope contains viral glycoproteins that play the major role in binding to specific receptors on host cells. Fusion of viral envelopes and cellular membranes upon virus uptake leads to release of the capsid into the cytoplasm, whereby the viral genome is finally released to initiate virus replication and progeny production (Figure 1).

In this review we focus on enveloped viruses and especially on those for which novel treatment approaches are being or have recently been developed. The most relevant enveloped viruses causing human diseases are listed in Table 1. Different strategies can be developed to fight enveloped viruses. On the one hand, viral infection may be prevented by prophylactic vaccinations. Indeed, successful vaccines against several enveloped viruses have been established (Table 1). On the other hand, newly emerging viruses cannot be targeted by vaccines, and there are no vaccines approved yet to prevent infections with several important human pathogens such as hepatitis C virus (HCV), HIV, cytomegalovirus (CMV), or Epstein–Barr virus (EBV). Furthermore, despite an effective vaccine against hepatitis B virus (HBV), >240 million people are still chronically infected and need curative therapeutic options. This illustrates the need to develop new antivirals against enveloped viruses.

The establishment of novel antivirals during the past 2 decades has proven that viral infections can be limited or even eliminated by the use of inhibitory molecules. Historically, antiviral drug discovery has mainly focused on viral targets, because fewer side effects are expected with this highly specific approach. Table 2 includes typical viral targets of clinically evaluated or approved antivirals. The disadvantages of using distinct viral targets, however, are the risk of developing resistance and the narrow spectrum of application to often only one particular virus.

An alternative therapeutic strategy is the targeting of host cell factors essential to support the viral life cycle. Using cellular targets helps to avoid rapid adaptation of viruses and development of drug resistance, and often allows targeting of emerging as well as known pathogens at the same time. Therefore, targeting host cell factors is becoming more popular in antiviral drug development. Another alternative is using immunomodulatory therapies that have proven to be very efficient in the control of viral infections. In this review, we therefore focus on new developments in antiviral strategies primarily targeting host factors (Figure 1) and on immunotherapies targeting the surface proteins of enveloped viruses (Figures 2 and 3).

Antiviral strategies targeting host factors

Inhibition of virus attachment to the host cell

Plasma membranes consist of glycoproteins, glycolipids, and proteoglycans that have been selected as attachment

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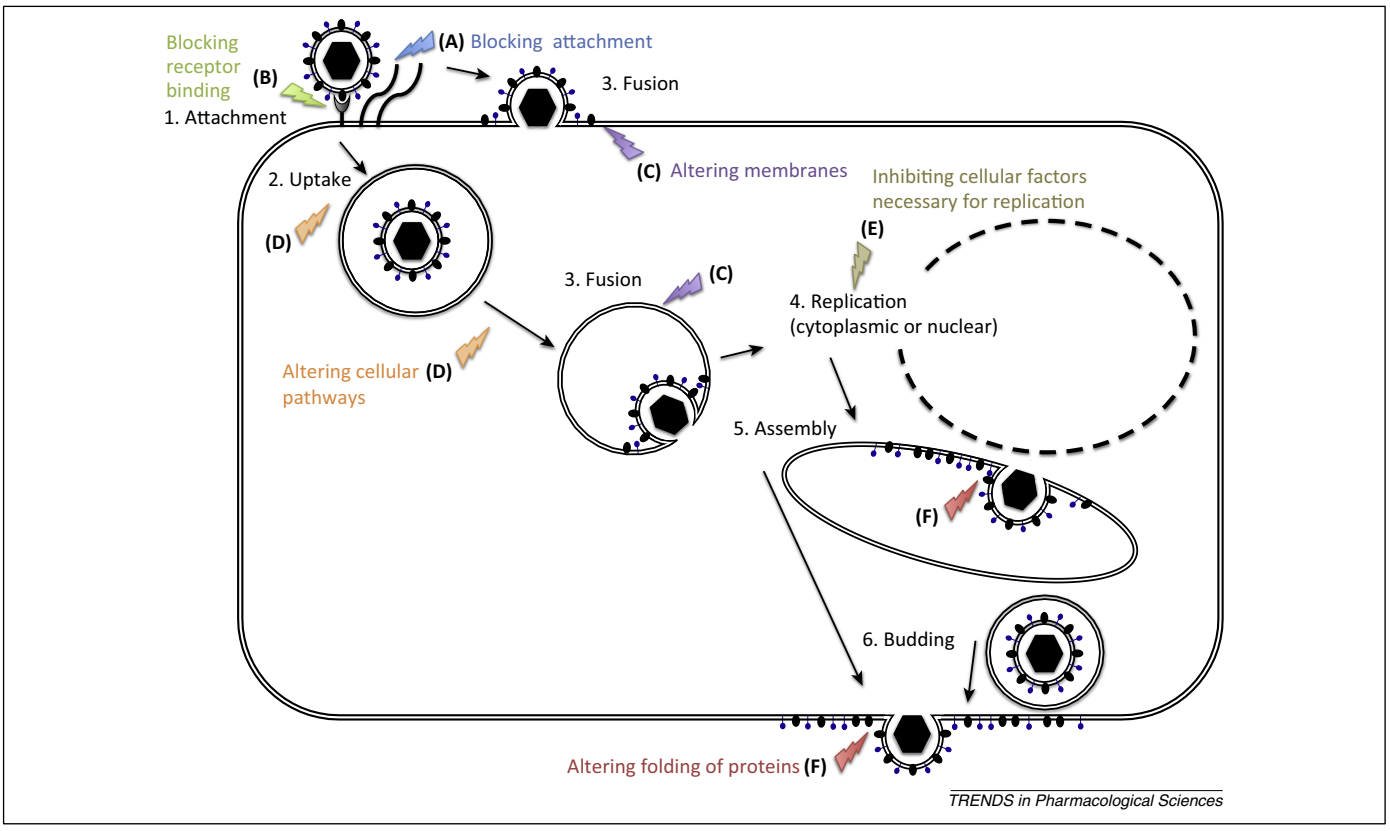

Figure 1. Antiviral strategies targeting host factors. Attachment of a virus to the host cell can be blocked by molecules binding to membrane components, such as SALPs binding to heparin sulfates **(A)**. Virus entry requires high-affinity receptor binding and membrane fusion, which can be inhibited by blocking the specific receptor **(B)** or by disrupting the viral membrane integrity, such as LJ001 or Arbidol **(C)**, respectively. Molecules can also modify intracellular transport pathways hijacked by the virus, such as ezetimibe altering the lipid metabolism **(D)**. Inhibition of cellular factors involved in viral replication, for example, cyclophilin A or miR122 **(E)**, or in viral morphogenesis, such as glucosidases **(F)**, can decrease production and release of infectious viral particles. Abbreviation: SALPs, synthetic anti-lipopolysaccharide (LPS) peptides.

sites by different enveloped viruses. Molecules binding to these membrane components compete with the virus for its attachment to the host cell and can thus inhibit infection at the earliest step. For example, a new class of synthetic anti-lipopolysaccharide (LPS) peptides, named SALPs, significantly interfere with *de novo* infection of various enveloped viruses *in vitro*, such as HIV, herpes simplex virus (HSV)-1, HSV-2, HCV, and HBV, by binding to heparan sulfate proteoglycan (HSPG) moieties covering the cell surface [1]. Further molecules that bind or mimic HSPG have been used to inhibit infection with distinct enveloped viruses: peptide 3-O-HS for HSV-2 [2], peptide SB105-A10 for respiratory syncytial virus (RSV), lactoferrin for severe acute respiratory syndrome coronavirus (SARS-CoV) [2,3], chondroitin sulfate for Dengue virus [4], and chemically sulfated derivatives of the *Escherichia coli* K5 capsular polysaccharide for CMV [5].

Entry inhibition

Productive virus entry requires high-affinity receptor binding and membrane fusion. If the receptor and/or co-receptors of the virus are known, they can be blocked by specific inhibitors. HIV, for example, uses cluster of differentiation (CD)4 and the chemokine receptors CCR5 and CXCR4 as entry receptors. HIV fusion inhibitors (e.g., T-20, enfuvirtide) and CCR5 inhibitors (e.g., maraviroc) are already in clinical use (Table 2). Synthetic compounds that simultaneously inhibit attachment and entry of HIV are interesting candidate antivirals: a molecule consisting of a CD4-mimetic peptide linked to a dodecasaccharide HSPG has been designed to simultaneously target two critical and highly conserved regions of gp120 from HIV-1. Linkage of two functional moieties provides strong cooperative effects, resulting in low-nanomolar antiviral activity towards both CCR5- and CXCR4-tropic HIV-1 strains [6].

For other viruses, such as HCV and HBV, (co-)receptors have only been identified more recently. Epidermal growth factor receptor and ephrin receptor A2 serve as critical host determinants of HCV entry by regulating CD81–claudin-1 co-receptor associations and membrane fusion. Blocking their tyrosine kinase activity with clinically approved inhibitors broadly impairs infection by all major HCV genotypes and known viral escape variants [7]. Recently, a myristoylated peptide derived from the N-terminal part of the large surface protein of HBV, named Myrcludex-B, has been shown to bind to the sodium-taurocholate transporter peptide NTCP serving as HBV and hepatitis D virus (HDV) receptor. Myrcludex B efficiently prevents HBV and HDV infection [9], and is currently being evaluated in clinical trials. An alternative option that does not require any knowledge about virus receptors is the use of peptides derived from the envelope protein of a virus for competitive inhibition of receptor binding. Such peptides have proven antiviral effects against dengue virus, HSV, and HIV-1 [8].

Disruption of viral membrane integrity

Viral membranes are susceptible to specific disruption because they lack metabolic turnover and cannot be

Table 1. Overview of enveloped viruses and available antiviral strategies

Virus family	Genome[b]	Relevant viruses	Targets of directly acting antivirals	Vaccine
Herpesviridae	dsDNA	Herpes simplex viruses 1/2 Varicella zoster virus Epstein–Barr virus Cytomegalovirus Human herpesviruses 6A/B and 7 (HHV 6A/B, HHV 7) Kaposi's sarcoma-associated herpesvirus (KSHV)	DNA-dependent DNA Polymerase (HSV 1/2, VZV, CMV, HHV 6, HHV 7) mRNA transcripts[a], terminase complex[a], kinase[a] (CMV)	VZV, EBV[a]
Poxviridae	dsDNA	Smallpox virus, vaccinia virus, molluscum contagiosum virus	Envelope protein (orthopoxviruses)	Smallpox virus
Hepadnaviridae	Circular partially dsDNA	Hepatitis B virus	Reverse transcriptase mRNA transcripts[a] capsid formation[a] subviral particle formation and release[a]	HBV
Retroviridae	ssRNA	HIV 1/2 Human T-lymphotropic viruses 1/2 (HTLV 1/2)	gp41 (HIV 1) reverse-transcriptase (HIV 1/2) integrase (HIV 1/2) protease (HIV 1/2)	
Virusoid	ssRNA	Hepatitis D virus (HDV)		(HBV, indirectly)
Flaviviridae	ssRNA	Dengue virus, hepatitis C virus, Japanese encephalitis virus (JEV), yellow fever virus (YFV), West Nile virus, tick born encephalitis virus (TBEV)	RNA-dependent RNA Polymerase (HCV) NS3/4 protease (HCV) NS5A[a] (HCV/Dengue)	JEV, YFV, TBEV
Paramyxoviridae	ssRNA	Measles virus, mumps virus, respiratory syncytial virus, Nipah virus, parainfluenza viruses 1-3, human metapneumovirus (HMPV)	RNA-dependent RNA polymerase (RSV) mRNA transcripts[a] (RSV)	Measles virus, mumps virus
Orthomyxoviridae	ssRNA	Influenza A/B viruses	M2 ion channel (Influenza A) neuraminidase (Influenza A/B)	Influenza A/B viruses
Filoviridae	ssRNA	Ebola virus, Marburg virus	mRNA transcripts[a] (Ebola)	
Coronaviridae	ssRNA	Corona viruses (including SARS-CoV and Middle East respiratory syndrome (MERS)-CoV)	RNA-dependent RNA polymerase[a]	
Arenaviridae	ssRNA	Lymphocytic choriomeningitis virus, Lassa virus		
Togaviridae	ssRNA	Rubella virus, Chikungunya virus, Sindbis virus etc.		Rubella virus
Bunyaviridae	ssRNA	California encephalitis virus, Hanta virus, Rift Valley fever virus, Toscana virus, Crimean–Congo hemorrhagic fever virus (CCHFV)		
Rhabdoviridae	ssRNA	Rabies virus		Rabies virus

[a]In clinical evaluation.

[b]Abbreviations: dsDNA, double-stranded DNA; ssRNA, single-stranded RNA.

repaired once the virus has budded from the host cell membrane. Although this has been exploited by disinfectants for a long time, molecules altering viral membranes are now under evaluation as new antivirals. Arbidol, a potent broad-spectrum antiviral, also inhibits membrane fusion of several enveloped viruses including influenza virus and HCV. It was recently demonstrated *in vitro* that Arbidol interacts with the polar head of phospholipid membranes and protein motifs enriched in aromatic residues thereby preventing fusion of HCV [10].

LJ001, a small molecule that intercalates into lipid membranes, was reported to inhibit *in vitro* virus–cell fusion and entry of many enveloped viruses including Nipah, Ebola, Marburg, Influenza, HIV, HCV, West Nile virus (WNV), and yellow fever and vaccinia viruses [11]. LJ001 binds to both viral and cellular membranes generating singlet oxygen in the membrane bilayer, which changes the biophysical properties of the viral membrane and thus prevents virus–cell fusion. These changes do not become apparent on cellular membranes due to their repair by cellular lipid biosynthesis [11].

A family of synthetic compounds named rigid amphipathic fusion inhibitors (RAFIs), which have the shape of phospholipids (with hydrophilic heads larger than their hydrophobic tails), were shown to inhibit in cell culture experiments the infectivity of several enveloped viruses including HCV, HSV-1, and HSV-2. RAFIs alter the negative curvature of the viral membrane required to initiate fusion [12]. Like LJ001, RAFIs act as membrane-binding photosensitizers because their antiviral effect requires activation to generate singlet oxygen. Photosensitization of viral membranes thus appears to be an interesting mechanism to develop broad-spectrum antivirals against enveloped viruses [13].

Inhibition of intracellular transport pathways

Most viruses hijack physiological transport pathways to enter their host cell or to deliver their genome. Compounds

Table 2. Targets of direct and indirect antivirals in clinical use (licensed or in clinical trial)

Step		Target		Mechanism	Example[a]
Entry	Attachment	gp120 (HIV) CD4 NTCP Scavenger receptor 1B Sialic receptor	Virus Host Host Host Host	Binding inhibition Blockage Binding inhibition Antagonism Cleavage	BMS-488043[b] (HIV 1) Ibalizumab[b] (HIV 1) Myrcludex B[b] (HBV) ITX 5061[b] (HCV) Fludase[b] (Influenza A/B)
	Co-receptor	CCR5	Host	Allosteric modulation	Maraviroc (HIV 1/2)
	Fusion	gp41 (HIV)	Virus	Conformational change inhibition	Enfuvirtide (HIV 1)
	Uncoating	M2 ion channel	Virus	Inhibition	Amantadin (Influenza A)
Replication		DNA-dependent DNA polymerase	Virus	Competitive inhibition	Aciclovir (HSV, VZV) Ganciclovir (CMV)
		Reverse transcriptase	Virus	Competitive inhibition	Tenofovir, Lamivudine[a] (HIV 1/2, HBV)
			Virus	Allosteric inhibition	Efavirenz[a] (HIV 1)
Replication/transcription		RNA-dependent RNA polymerase	Virus	Competitive inhibition	Sofosbuvir (HCV)
			Virus	Allosteric inhibition	BMS-791325[b] (HCV)
Genome processing		Terminase complex	Virus	Inhibition	Letermovir[b] (CMV)
Integration		Integrase	Virus	Inhibition	Raltegravir (HIV 1/2)
Translation		mRNA	Virus	Translation blockage	Fomivirsen (CMV)
			Virus	Degradation by RISC[c]	ALN-RSV01[b] (RSV) ARC-520[b] (HBV) TKM-100201[b] (Ebola)
Protein processing		Protease (HIV) NS3/4 protease (HCV)	Virus	Inhibition	Lopinavir[a] (HIV 1/2) Simeprevir (HCV)
		Cyclophilin A	Host	Inhibition	Alisporivir[b] (HCV)
		α-Glucosidase I	Host	Inhibition	Celgosivir[b] (HCV, Dengue)
Virus–host interaction		NS5A (HCV)	Virus	Inhibition	Daclatasvir[b] (HCV)
		miR122	Host	Antagonism	Miravirsen[b] (HCV)
Assembly		Capsid	Virus	Inhibition	BAY 41-4109[b] (HBV)
		Subviral particle	Virus/ host	Inhibition	Rep 9AC[b] (HBV)
Envelopment and egress		Neuraminidase envelope protein	Virus	Inhibition	Oseltamivir (Influenza A/B) Tecovirimat[b] (smallpox virus)
Innate immunity		Toll-like receptor 7	Host	Agonization	GS9620[b] (HBV)
		Interferon-stimulated genes	Host	Activation	Interferon-α 2a/b (HBV, HCV) interferon-λ[b] (HBV, HCV)
Adaptive immunity	Immunoglobulins	Viral surface antigens	Virus	Neutralization/activation of complement and effector cells	Available for CMV, VZV, HBV, RSV (palivizumab), measles virus, rabies virus
		Cellular marker	Host	Depletion of host cells/infected cells	Rituximab (EBV) Bavituximab[b] (HCV)
	Cellular response	Virus specific T cells	Host	Enrichment	CMV-specific $CD8^+$ T cells
		IL-7[c] receptor	Host	Agonization	IL-7/CYT107[b] (HBV, HCV, HIV 1)
	Combined	Virus specific B and T cells	Host	Therapeutic vaccination	DV-601 (HBV)

[a]Only selected examples according to the WHO guidelines of first-line treatment for adults and children are given.

[b]In clinical evaluation.

[c]Abbreviations: IL, interleukin; NTCP, sodium taurocholate cotransporting polypeptide (bile acid cotransporter); RISC, RNA-induced silencing complex.

inhibiting these cellular transport pathways without toxicity may thus be used as antivirals. HCV exploits and modifies the lipid metabolism of hepatocytes and enters hepatocytes by taking advantage of the cholesterol uptake pathway. This has been proven both *in vitro* and *in vivo* using ezetimibe, an FDA-approved selective inhibitor of intestinal cholesterol absorption [14]. Circulating HCV virions are embedded in triglyceride-rich lipoproteins, including very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), and are therefore referred to as lipoviro-particles. Inhibitors of lipoprotein lipase (LPL), the enzyme catalyzing conversion of VLDL to LDL, prevent HCV infection *in vitro* as well as in primary human hepatocytes transplanted into uPA-SCID mice by retaining the viral particles at the cell surface [15,16]. On the same line, we recently found that ezetimibe efficiently blocks a very early step in the HBV life cycle [17], indicating that HBV infection also involves the lipid transport pathway.

Inhibition of viral genome replication

Viruses are parasites that exploit the cellular machinery for their replication. Thus, targeting cellular components essential for virus replication is another promising option for the development of new antivirals.

A large-scale *in vitro* screen identified the small-molecular-weight compound A3, which blocks *de novo* pyrimidine synthesis and acts as a broad-spectrum antiviral by depleting cellular pyrimidine pools. Thereby, A3 inhibits a

number of DNA and RNA viruses *in vitro* including influenza A and B viruses, HCV, WNV, dengue virus, human adenoviruses, poxviruses, and HIV [18]. However, *in vivo* efficacy and toxicity of this interesting antiviral candidate still need to be determined.

Cyclophilins are a family of highly conserved cellular peptidyl–prolyl *cis–trans* isomerases, which are involved in many cellular processes such as protein folding and trafficking. Cyclophilins were proven to be essential for HCV replication. Inhibition of cyclophilin efficiently inhibits HCV replication, most likely by preventing cyclophilin A interaction with HCV NS5A. They also act on multiple other viruses such as HIV, HBV, influenza viruses, SARS-CoV, HSV, CMV, and vaccinia virus. Nonimmunosuppressive inhibitors of cyclophilins such as NIM811 and alisporivir are currently in clinical development for HCV therapy (reviewed in [19]).

miR122, which is the most abundant liver miRNA, has been shown to bind to HCV 5′ untranslated regions (UTRs) and to be essential for stability and replication of HCV RNA. Intravenous administration of a locked nucleic acid complementary to miR122 suppresses the propagation of HCV in chimpanzees chronically infected with HCV [20]. Recently, a stabilized DNA antisense oligonucleotide sequestering miR122 (Miravirsen) proved safe in a clinical phase II trial in patients with chronic HCV genotype 1 infection and showed prolonged dose-dependent reductions in HCV RNA levels without evidence of viral resistance [21].

Inhibition of viral morphogenesis and release

Preventing the release of mature and fully infectious enveloped viruses is an antiviral strategy exerted by neuraminidase inhibitors against influenza viruses. Using a similar strategy, valproic acid functions as a potent inhibitor of several enveloped viruses in cultured cells, including WNV, vaccinia viruses, and lymphocytic choriomeningitis virus (LCMV). It probably acts by causing alterations in the cellular membrane composition, which results in impaired infectious particle production [22,23].

Glucosidase inhibitors have proven their antiviral efficacy against HCV, HBV, and dengue virus by altering their morphogenesis (reviewed in [24]). Glucosidases are cellular enzymes involved in the biosynthesis of glycoproteins that, when expressed on the viral surface, are essential for virus–host interactions. One of these inhibitors, celgosivir, has reached clinical phase II for the treatment of chronic HCV infection as well as phase 1b for the treatment of dengue virus infection.

Amphipathic DNA polymers have shown antiviral activity against enveloped viruses including herpes viruses and HCV mainly by preventing entry [25]. Among them, REP 9AC, which is in phase I/II clinical trials for the treatment of chronic HBV infection, is thought to interfere with biochemical processes involved in the formation and release of HBV subviral particles. The exploratory use of REP 9AC in hepatitis B patients – without a controlled clinical trial setting – resulted in elimination of surface antigen from the blood associated with restoration of the capability of the immune system to fight the infection [26].

The development of new antiviral strategies to block virus release can also profit from naturally occurring antiviral defense mechanisms. For instance, tetherin, an interferon-inducible host factor, is known to limit enveloped virus spread and infection *in vitro* and *in vivo* [27]. The discovery of compounds boosting surface expression of tetherin and blocking viral antagonism would be a prime example of exploiting the natural, cellular antiviral defense.

Immunotherapies

In addition to the inhibitory molecules discussed above, passive and active immune modulatory therapies to treat viral infections are currently being explored. Passive immunotherapy comprises the infusion of antibodies. Alternatives comprise immune modulating molecules such as cytokines or immunostimulatory oligonucleotides, which activate e.g., toll-like receptors (TLR) resulting in cytokine release. Active immunotherapy is based on vaccination. A novel approach is the adoptive transfer of immune cells with defined antigen specificity. Here, we focus on targeted immunotherapies that directly target surface proteins of enveloped viruses or that interfere with functionality of envelope proteins of the virus.

Prophylactic application of neutralizing antibodies

Treatment with neutralizing antibodies can prevent viruses from entering the host cell in several ways. Binding of antibodies to proteins of the viral envelope will block binding of the virus to receptors on the cell surface or inhibit fusion of viral and cellular membranes. In addition, virus–antibody complexes will be recognized and scavenged via Fc receptors on immune cells, or activate the complement system (Figure 2).

Passive immunization with serum-derived, polyclonal antibodies has been used successfully as post-exposure prophylaxis or as treatment before transplantation to prevent (re-)infection with HBV, RSV, varicella zoster virus (VZV), measles virus, or rabies virus, but also nonenveloped viruses such as hepatitis A virus (HAV), and entero-, parvo-, and rotaviruses (reviewed in [28]). However, the quality of serum-derived antibody products varies. As an alternative, cell lines that produce well-characterized monoclonal antibodies can be generated either by fusion of B cells with myeloma cells (hybridoma technology), or after introducing the coding sequence of an antibody by genetic modification. For example, palivizumab is produced by recombinant DNA technology and approved for the prevention of RSV infection in children.

Problems of the prophylactic use of monoclonal antibodies are high costs and the sometimes rapid selection of viral escape mutants. To reduce costs, gene therapy is used allowing for constant secretion of neutralizing antibodies. HIV-neutralizing antibodies are expressed via viral vectors in hematopoietic stem cells [29] or in muscle tissue [30] for immunoprophylaxis of HIV infection. To reduce the risk of viral escape, highly conserved regions of the envelope glycoproteins are targeted [31], or antibodies are combined to increase selection pressure [31,32]. Nevertheless, an active, prophylactic vaccination strategy would still be preferred.

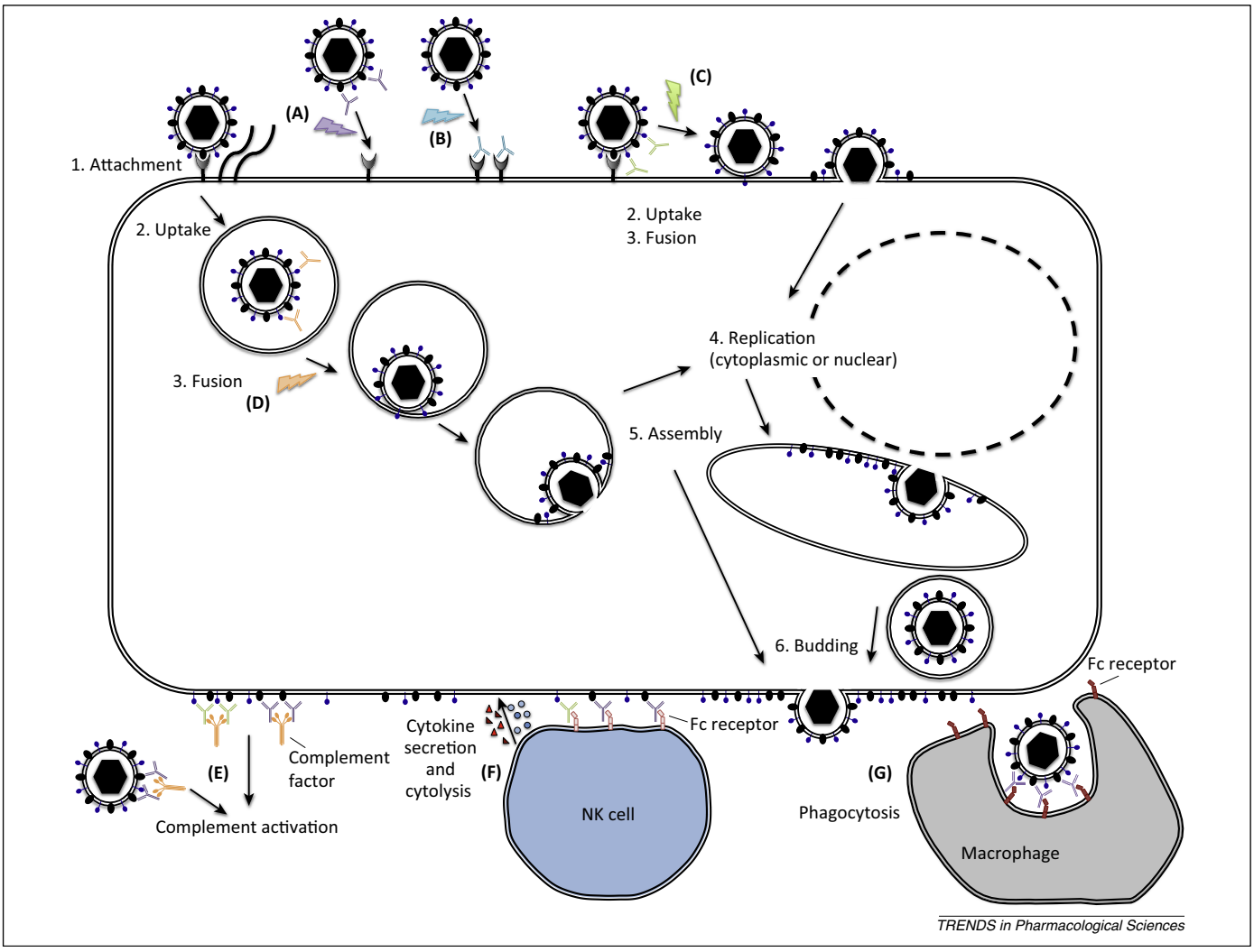

Figure 2. Antiviral mechanisms of monoclonal antibodies. Antibodies block attachment of the virus to the receptor on the host cell by either binding to viral envelope proteins **(A)**, or to the receptor **(B)**. Fusion of virus and cytoplasm **(C)**, or endosome membranes **(D)** is prevented by antibody blocking of the fusogenic peptide. Antibodies also mediate activation of the immune system via the Fc (fragment, crystallizable) region. Opsonization of infected cells or virus particles leads to activation of the complement cascade **(E)**. The Fc region is also recognized by Fc receptors on natural killer (NK) cells, which secrete cytokines and lyse infected cells **(F)**, or on macrophages, which phagocytize the virus–antibody complexes **(G)**.

Therapeutic application of monoclonal antibodies targeting viral envelope proteins

Monoclonal antibodies can also be used in a therapeutic setting. Recently, a humanized monoclonal antibody against glycoprotein B of HSV1 and HSV2, MAb hu2c, was described [33]. Treatment of HSV-infected mice with MAb hu2c led to a rapid decrease of virus titers and improved survival. MAb hu2c immobilizes glycoprotein B trimers, inhibits activation of the fusogenic signal, and hence abrogates viral entry as well as viral cell-to-cell spread. Similarly, monoclonal antibodies against hemagglutinin, the fusion peptide of the influenza virus, protect mice from lethal infection [34].

Treatment with the monoclonal antibody MBL-HCV1, which was generated in humanized HuMab mice and directed against the HCV envelope glycoprotein E2, was proven not only to prevent establishment of HCV infection in chimpanzees [35] and to delay HCV rebound in transplantation patients [36], but also to reduce viral RNA to undetectable levels for 2–3 weeks in chimpanzees with acute or chronic HCV infection [35].

Several monoclonal antibodies have been tested against HIV infection in clinical trials within the past three decades. In some studies, treatment with HIV-neutralizing antibodies during interruption of standard antiretroviral therapy could delay viral rebound by several weeks (reviewed in [37]). More recently, it was shown that administration of a cocktail of HIV-specific monoclonal antibodies led to a strong decrease of viral load and partial restoration of immune responses in primates chronically infected with SIV [38].

Treatment with neutralizing monoclonal antibodies has been proposed for many other viruses, such as VZV [39], CMV [40], dengue virus [41], WNV [42], Chikungunya virus [43] and many other athropod-borne viruses (reviewed in [44]). However, preclinical and clinical data proving a therapeutic effect or evaluating side effects are currently missing.

Development of the CMV-specific monoclonal antibody MSL-109, which had promising antiviral activity in preclinical models but then proved to be ineffective in patients, raised strong concerns: the antibody was taken up by

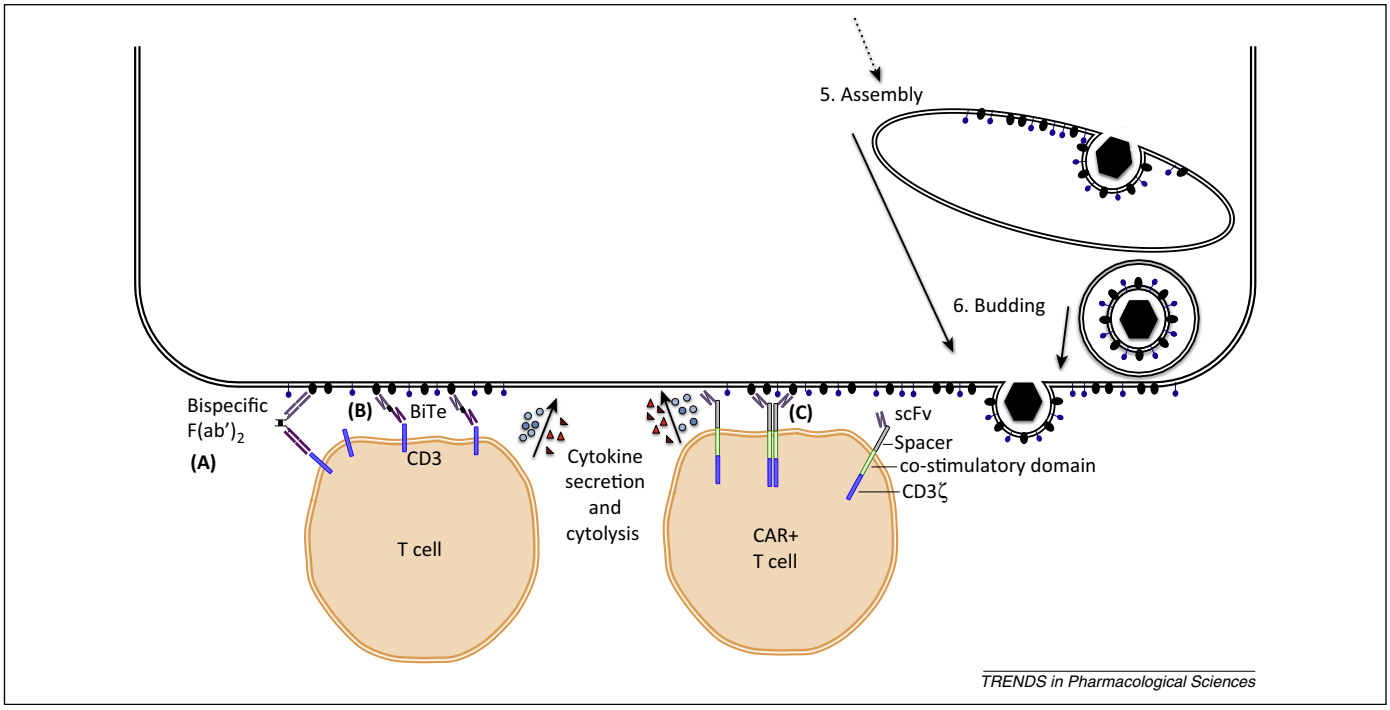

Figure 3. Redirection of T cells to virus-infected cells. There are several ways how T cells might be recruited to target cells, leading to the formation of an immunological synapse, secretion of cytokines and subsequent killing of virus-infected cells. Bispecific antibodies consist of two specificities: one targeting a viral envelope protein on the surface of the infected cell and the other one targeting a molecule on the immune cell, for example, CD3 on T cells. For example, bispecific antibodies are generated by chemically linking two F(ab) (fragment, antigen binding) regions **(A)**, or by genetically linking two scFvs (single chain fragment variable) with different specificities, so-called bispecific T cell engagers (BiTEs) **(B)**. Viral envelope proteins can also be targeted by T cells genetically modified to express a chimeric antigen receptor (CAR) **(C)**. This receptor consists of an scFv binding to the viral protein, a spacer domain, and intracellular CD28 and CD3ζ signaling domains. Upon antigen binding the CAR dimerizes and activation of the T cell is triggered.

infected cells and incorporated into assembling virions, which then infected even nonpermissive cells via the Fc domain of the virus–antibody complex [45].

Monoclonal antibodies targeting host factors

Similar to inhibitory molecules, antibodies that bind host factors interacting with viral envelope proteins have a minimal risk of developing resistance but bear the risk of toxicity. Host cell targets for antibodies can be lipid domains on the cell surface or distinct proteins that serve as virus receptors.

A potential target are phosphatidylserines, which become exposed on the cell membrane of cells infected with HCV, HIV-1, HSV-1, vaccinia virus, or influenza A virus [46]. In an initial clinical trial, the phosphatidylserine-specific antibody bavituximab induced a reduction of HCV RNA in HCV/HIV co-infected patients [47].

Remodeling of the host cell membrane to mask virus receptors or driving away co-receptors is an alternative option to inhibit entry. In this regard, a monoclonal anti-cholesterol IgG antibody recognizing clustered membrane cholesterol has been used to rearrange the lateral molecular organization of HIV-1 (co-)receptors, leading to a substantial inhibition of infection and HIV-1 production *in vitro* [48].

In addition, several monoclonal antibodies directed against the HIV (co-)receptors have been developed. Safety and antiviral activity of monoclonal antibodies against CD4 or CCR5 were proven in early clinical trials (reviewed in [37]). Alternatively, bispecific antibody derivatives consisting of two antibodies with different antigen recognition sites in CCR5 could be used to block two alternative docking sites of CCR5-tropic HIV strains and reduce the risk of resistant mutants occurring [49].

Although antibody-based therapies show great promise in cancer therapies, their role in antiviral therapies has not been established yet and it remains open whether clinical results will justify the high costs.

Antiviral T cell therapies

A lack of functional T cell response towards viral antigens is a hallmark of chronic infection with enveloped viruses such as HBV, HCV, and HIV, as well as reactivation of CMV or EBV under immunosuppression. Its restoration would help the host to control the infection (reviewed in [50]), therefore, the idea of redirecting T cells to the site of infection is intriguing.

Redirection of T cells to a defined target can be achieved using artificial chimeric molecules. For instance, bispecific antibodies consisting of two antibody fragments can bind a viral antigen as well as an immune cell antigen and thereby guide immune cells to infected cells. Bispecific antibodies have first been exploited to attract T cells to influenza-virus-infected cells. For HIV, a bispecific antibody with scFv directed against CD4 and the HIV envelope protein gp120 was generated [51]. This approach may be risky to use *in vivo* because the attracted CD4 T cells are infected by HIV, and hence other molecules on immune cells might be better suited for redirection to infected cells. To this end, an anti-gp41/anti-CD89 bispecific antibody to target neutrophils to virus particles and infected cells was developed and successfully tested in co-culture experiments [52].

Another interesting option to redirect T cells is to modify them genetically to express a chimeric antigen receptor (CAR). CARs are composed of a single chain antibody fragment binding to viral envelope proteins on the surface of infected cells, and intracellular signaling domains. Due to the necessity of viral envelope proteins being incorporated into cellular membranes, this therapeutic approach can exclusively be used to target enveloped viruses. CARs have been shown to activate specifically T cells against CMV *in vitro* [53] or influenza virus *in vivo* [54], and T cells expressing a CAR that binds the surface protein of HBV (HBs) efficiently kill infected primary hepatocytes and control viral replication in transgenic mice [55]. HBV, unlike other viruses, does not have a stage-dependent protein expression (a so-called early late shift), which would allow targeting only for a limited time span. HBV-infected cells constantly produce HBs even if virus replication is controlled, therefore, targeting HBs by immunotherapy represents a promising approach to cure hepatitis B.

Antiviral T cell responses might also be induced by therapeutic vaccination. A prominent example are recent experiments with CMV-based vaccine vectors that conferred sustained control and even clearance of SIV in macaques used to model HIV infection [56].

Immune modulatory therapies

Virus-infected cells can also be targeted indirectly by boosting the host's immune response. In the form of interferon α, this concept is part of the standard of care treatment for chronic viral hepatitis. Currently, several new immune modulatory substances are being tested. Interferon λ initially proved to be at least as effective for the treatment of chronic hepatitis C with fewer side effects (EMERGE study, Zeuzem *et al*. ILC 2012, oral presentation), but its further clinical development was halted because all-oral treatment regimens for hepatitis C are becoming available. Antibodies directed against programmed cell death protein 1 (PD1) or its ligand (PD-L1), so-called "immune checkpoint inhibitors", which revert an exhausted phenotype of virus-specific T cells [57], or TLR agonists that induce an antiviral innate immune response [58] are promising candidates for immunotherapy of viral infections (Table 2).

Combination of immune with antiviral therapies

Antibodies directed against viral envelope proteins or cellular entry receptors can be linked to immune modulators or antiviral molecules that were discussed in the first part of this review. For example, antibodies specific for glycoproteins of herpes viruses have been fused to toxins to induce lysis of infected cells [59]. These immunoconjugates could be used to target cells infected with enveloped viruses and allow for a more efficient delivery of an antiviral drug or an immunomodulator to the site of infection and thus reduce site effects.

Concluding remarks

This review focuses on novel therapeutic approaches against enveloped viruses because they include most of the important human pathogens, and major advances in related antiviral strategies have been and continue to be developed. The success of recently available HIV and HCV therapies demonstrate how efficient therapy development can be when suitable infection systems are available and research efforts are combined with drug-development efforts within the pharmaceutical industry.

This review emphasizes the need for increasing research and developmental activities to fight other important human pathogenic viruses by targeted antiviral strategies. Viral infections may be prevented by prophylactic vaccination. If no vaccine is available, however, or despite vaccination efforts a significant number of humans still suffer from infection (e.g., in the case of hepatitis B), antiviral treatments are needed.

The establishment of directly acting antivirals targeting viral enzymes or proteins has proven to be able to limit or – if the virus does not establish a persistence reservoir – even eliminate viral infection. The disadvantages of targeting distinct viral proteins or enzymes, however, are the high risk of resistance development and the narrow spectrum of application to often only one particular virus. Alternative therapeutic strategies therefore often aim at targeting host cell factors essential for supporting the viral life cycle. This can avoid rapid development of resistance, but also allows targeting of emerging pathogens that use similar infection strategies as the known viruses. Especially for chronic and persistent viral infections, immune therapies provide an attractive alternative that also has been proven to be very efficient in the control of viral infections but has not yet been broadly exploited.

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