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Gumming Up the Works: DNA Polymers as HCV Entry Inhibitors

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Amphipathic DNA Polymers Inhibit Hepatitis C Virus Infection by Blocking Viral Entry

Infection with hepatitis C virus (HCV) is a global health concern due to the lack of effective treatments. Currently, there are no prophylactic or therapeutic vaccines, and antiviral therapies (interferon and ribavirin combinations) are associated with suboptimal response rates. Clearly, there is a need for novel HCV-specific antivirals to treat the 3–4 million new infections each year. Although many elements of HCV replication have not been fully studied, the recent ability to culture the virus in vitro has led to a better understanding of HCV replication, and has uncovered some promising new strategies for treating infection. In this issue of *GASTROENTEROLOGY*, Matsumura et al¹ discuss amphipathic oligodeoxynucleotides as putative antivirals to inhibit entry of HCV into host cells.

HCV Attachment and Entry

HCV is an enveloped, positive-strand RNA virus within the family *Flaviviridae*. Our understanding of the early stages of HCV replication has remained limited until only recently, because of the inability to efficiently propagate the virus in culture. Nevertheless, since the first receptor for HCV was discovered in 1998,² HCV entry has been the subject of intense research. Common tools used to study HCV entry include recombinant, soluble forms of the E2 glycoprotein, retroviral particles pseudotyped with HCV glycoproteins (HCVpp), cell culture-derived HCV (HCVcc), and HCV isolated from the serum of infected primates. To date, there have been 4 co-receptors and several attachment factors identified, but there are still many aspects of HCV entry that are unknown.

Initial association of HCV with host cells may involve nonspecific attachment factors that function to concentrate virions to subdomains of the cell surface. Heparin sulfate, a glycosaminoglycan, is 1 such attachment factor; it was identified as a docking site for HCV and the related flaviviruses.^{3,4} It is unclear whether heparin sulfate associates with HCV particles, or the lipoproteins (low-density lipoproteins [LDL] and very low-density lipoproteins [VLDL]) commonly found in association with the virus. The LDL receptor is also implicated as an attachment factor, and adsorption of HCV particles can be inhibited by antibodies specific for either purified VLDL or its receptor.^{5–7} C-type lectins, including DC-SIGN and L-SIGN, are also implicated in HCV attachment.^{8–12} The role for each of these attachment factors in HCV entry is unclear; they have not been shown to be required for productive infection. The effects of inhibiting heparin sulfate and LDL-R have not been substantial, and studies have not

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Conflicts of interest

The authors disclose no conflicts.

yet been reported using HCVcc. The role for DC/SIGN and L/SIGN in productive entry is also questionable, because these receptors are not expressed on hepatocytes. However, even when present on nonhepatic cells, these receptors may play a role in cell-to-cell spread of HCV.^{8,11}

After initial attachment, HCV may then bind to specific receptors that can actively initiate virus internalization via endocytosis. The first and, therefore, most well-studied receptor for HCV is the tetraspanin CD81,^{2,13–15} but it was soon realized that other factors were required. Indeed, scavenger receptor B1 (SR-B1), together with claudin-1 and occludin-1 (both tight junction proteins) were later identified as additional factors required for HCV entry.^{13,16–20} SR-B1, occludin-1, and claudin-1 expression are more restricted than the almost ubiquitous CD81, thus may better account for the tissue tropism of HCV. Interestingly, SR-B1 is also a receptor for LDL; hence, its involvement in HCV attachment may be mediated via interactions with virus-associated lipoproteins. Despite the identification of multiple components involved in HCV entry, the kinetics of virus attachment and internalization remain unknown (Figure 1). It is difficult to determine the sequence of binding events, because our current knowledge is based on indirect studies using a wide range of in vitro models. Additionally, it is difficult to draw conclusions from studies with HCVcc or HCVpp, because circulating HCV is associated with serum lipoproteins, so has a different composition than in vitro particles (Figure 1). Several outstanding questions remain for HCV entry, such as why the virus requires multiple receptors for entry, and what are the roles of tight junctions in infection. Despite recent advances in identifying HCV entry factors, our understanding of HCV entry is far from complete, and there are probably additional unidentified host factors awaiting discovery.

Amphipathic DNA Polymers as Entry Inhibitors

As we continue to learn more about HCV receptors, viral entry is becoming an increasingly attractive target for HCV antiviral drug development owing to the conserved entry mechanism across different genotypes. Despite being an active area of research, there are currently no entry inhibitors available for HCV on the market, but amphipathic DNA polymers (AP) have recently emerged as an attractive option for further investigation. Phosphorothioate oligonucleotides (PS-ON) can block binding and entry of a range of pathogens including HIV-1, human cytomegalovirus, varicella zoster virus, arenaviruses, and scrapie.^{21–25} These compounds are oligonucleotides that have been chemically modified by phosphorothioation to produce a structure that is more stable and hydrophobic than naturally occurring DNA. Although the exact mechanism of action for these compounds is not fully elucidated, it is thought that they prevent viral attachment via interaction with the α helices of viral glycoproteins such as HIV-1 gp41.^{21,22,25}

HCV Entry Inhibitors

In this issue, Matsumura et al¹ demonstrate that APs have antiviral activity against HCV by blocking viral entry. These findings are significant because they represent the first example of small molecules that specifically target HCV entry. A recent study has validated HCV entry as an antiviral target by showing that prophylactic anti-CD81 antibodies protect against HCV challenge in vivo.²⁶ In the current study, the authors demonstrate that nanomolar amounts of PS-ONs result in decreased accumulation of core protein, and both intracellular and extracellular RNA in cell culture. The inhibitory action of PS-ONs was dependent on the length of the oligonucleotide, but not the sequence, and these findings are consistent with previous studies with other viruses.^{21–25} The antiviral activity of these compounds cannot be attributed solely to the increased stability they confer on oligonucleotides; control compounds that lack phosphorothioation but remain stable in vivo have minimal effects on HCV entry. To investigate the actions of PS-ONs in vivo, uPA/SCID mice engrafted with human hepatocytes were inoculated with infectious HCV. The majority of mice treated with PS-ONs were resistant to

de novo HCV infection, thus supporting in vitro results. PS-ONs are proposed to act at a post-binding but pre-replication step of the HCV lifecycle because viral attachment and RNA replication were unaltered in PO-ON-treated cells, but virus internalization was reduced. The authors suggest that the compounds act at a similar step to concanamycin, a drug known to prevent acidification of endosomes.

This study demonstrates that HCV entry is a viable target for antivirals, and that PO-ONs warrant further investigation as potential drug candidates. Importantly, Matsumura et al¹ show that the effects of PO-ONs are not limited to certain genotypes, a problem that plagues other classes of HCV antivirals. The findings in this paper are promising, but further investigations of PS-ONs are required for 2 reasons. The first is to better understand their mode of action. PS-ONs were demonstrated to inhibit HIV-1 entry by interfering with gp41-mediated fusion of the viral and host membranes.²⁵ The PS-ONs interact with α -helical domains in the HIV fusion protein, and are proposed to act similarly in other type I membrane fusion proteins. However, because HCV fusion is most likely via a type II fusion process, the mechanism of action may not be conserved. The second, and perhaps equally important, reason for studying the action of PS-ONs on HCV entry is to better dissect the HCV entry process. As mentioned, entry of HCV is a complex and multistep process that is not well understood. Although several important players required for viral attachment and internalization have been identified, there are still unknown factors implicated in the process. By specifically identifying the molecule(s) targeted by PS-ONs, we may be able to identify intermediate steps in viral entry, especially those involved in the internalization and fusion process. It is also essential to determine if APs can inhibit cell-to-cell spread of HCV, which may play an important role in natural infections.

Although the results presented by Matsumura et al¹ are promising, there may be limitations associated with oligonucleotides as antivirals. Firstly, there is concern that APs may induce an endogenous interferon response in vivo when administered, although the authors state that hepatocytes do not express large quantities of the receptor required to sense nucleic acids (Toll-like receptor 9). In their study, the authors suggest that they did not detect any interferon induction in cells exposed to either PS-ON or control compounds. For HIV-1, the antiviral effects of PS-ONs have been attributed to their ability to inhibit fusion of viral proteins with cellular membranes. Thus, these compounds may cause adverse effects by nonspecifically interacting with other hydrophobic molecules. Consequently, these compounds must undergo rigorous testing before deemed suitable for human use.

HCV entry into host cells is a complex process and we have only recently begun to understand all the components involved. The inhibitory action of PS-ONs on HCV infection has further validated viral entry as a viable target for antiviral development. Although the mechanism of action for these compounds remains to be determined, further investigations may lead to the development of a greatly needed treatment option, and a tool to better understand the HCV entry process.

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References

1. Matsumura T, Hu Z, Kato T, et al. Amphipathic DNA polymers inhibit hepatitis C virus infection by blocking viral entry. *Gastroenterology* 2009;137:673–681. [PubMed: 19394333]

2. Pileri P, Uematsu Y, Campagnoli S, et al. Binding of hepatitis C virus to CD81. *Science* 1998;282:938–941. [PubMed: 9794763]
3. Barth H, Schafer C, Adah MI, et al. Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate. *J Biol Chem* 2003;278:41003–41012. [PubMed: 12867431]
4. Koutsoudakis G, Kaul A, Steinmann E, et al. Characterization of the early steps of hepatitis C virus infection by using luciferase reporter viruses. *J Virol* 2006;80:5308–5320. [PubMed: 16699011]
5. Agnello V, Abel G, Elfahal M, et al. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 1999;96:12766–12771. [PubMed: 10535997]
6. Germe R, Crance JM, Garin D, et al. Cellular glycosaminoglycans and low density lipoprotein receptor are involved in hepatitis C virus adsorption. *J Med Virol* 2002;68:206–215. [PubMed: 12210409]
7. Meyer K, Basu A, Ray R. Functional features of hepatitis C virus glycoproteins for pseudotype virus entry into mammalian cells. *Virology* 2000;276:214–226. [PubMed: 11022009]
8. Cormier EG, Durso RJ, Tsamis F, et al. L-SIGN (CD209L) and DC-SIGN (CD209) mediate transinfection of liver cells by hepatitis C virus. *Proc Natl Acad Sci U S A* 2004;101:14067–14072. [PubMed: 15371595]
9. Pohlmann S, Zhang J, Baribaud F, et al. Hepatitis C virus glycol-proteins interact with DC-SIGN and DC-SIGNR. *J Virol* 2003;77:4070–4080. [PubMed: 12634366]
10. Gardner JP, Durso RJ, Arrigale RR, et al. L-SIGN (CD 209L) is a liver-specific capture receptor for hepatitis C virus. *Proc Natl Acad Sci U S A* 2003;100:4498–4503. [PubMed: 12676990]
11. Lozach PY, Amara A, Bartosch B, et al. C-type lectins L-SIGN and DC-SIGN capture and transmit infectious hepatitis C virus pseudotype particles. *J Biol Chem* 2004;279:32035–32045. [PubMed: 15166245]
12. Lozach PY, Lortat-Jacob H, de Lacroix de Lavalette A, et al. DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. *J Biol Chem* 2003;278:20358–20366. [PubMed: 12609975]
13. Bartosch B, Vitelli A, Granier C, et al. Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. *J Biol Chem* 2003;278:41624–41630. [PubMed: 12913001]
14. Cormier EG, Tsamis F, Kajumo F, et al. CD81 is an entry coreceptor for hepatitis C virus. *Proc Natl Acad Sci U S A* 2004;101:7270–7274. [PubMed: 15123813]
15. Zhang J, Randall G, Higginbottom A, et al. CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. *J Virol* 2004;78:1448–1455. [PubMed: 14722300]
16. Scarselli E, Ansuini H, Cerino R, et al. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 2002;21:5017–5025. [PubMed: 12356718]
17. Zeisel MB, Koutsoudakis G, Schnober EK, et al. Scavenger receptor class B type I is a key host factor for hepatitis C virus infection required for an entry step closely linked to CD81. *Hepatology* 2007;46:1722–1731. [PubMed: 18000990]
18. Evans MJ, von Hahn T, Tschernie DM, et al. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007;446:801–805. [PubMed: 17325668]
19. Ploss A, Evans MJ, Gaysinskaya VA, et al. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009;457:882–886. [PubMed: 19182773]
20. Catanese MT, Graziani R, von Hahn T, et al. High-avidity monoclonal antibodies against the human scavenger class B type I receptor efficiently block hepatitis C virus infection in the presence of high-density lipoprotein. *J Virol* 2007;81:8063–8071. [PubMed: 17507483]
21. Bernstein DI, Goyette N, Cardin R, et al. Amphipathic DNA polymers exhibit antiherpetic activity *in vitro* and *in vivo*. *Antimicrobial Agents and Chemotherapy* 2008;52:2727–2733. [PubMed: 18505857]
22. Guzman E, Cheshenko N, Shende V, et al. Amphipathic DNA polymers are candidate vaginal microbicides and block herpes simplex virus binding, entry and viral gene expression. *Antiviral Therapy* 2007;12:1147–1156. [PubMed: 18240855]
23. Kocisko DA, Vaillant A, Lee KS, et al. Potent antiscrapie activities of degenerate phosphorothioate oligonucleotides. *Antimicrob Agents Chemother* 2006;50:1034–1044. [PubMed: 16495266]

24. Lee AM, Rojek JM, Gundersen A, et al. Inhibition of cellular entry of lymphocytic choriomeningitis virus by amphipathic DNA polymers. *Virology* 2008;372:107–117. [PubMed: 18022208]
25. Vaillant A, Juteau J-M, Lu H, et al. Phosphorothioate oligonucleotides inhibit human immunodeficiency virus type 1 fusion by blocking gp41 core formation. *Antimicrobial Agents and Chemotherapy* 2006;50:1393–1401. [PubMed: 16569857]
26. Meuleman P, Hesselgesser J, Paulson M, et al. Anti-CD81 antibodies can prevent a hepatitis C virus infection in vivo. *Hepatology* 2008;48:1761–1768. [PubMed: 19030166]

Figure 1.
Attachment and entry of HCV.