Review

How does Cellular Heparan Sulfate Function in Viral Pathogenicity?*

ZHU WuYang, LI JiangJiao, and LIANG GuoDong#

Department of Viral Encephalitis, Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention and State Key Laboratory for Infectious Disease Prevention and Control (SKLID), Beijing 100052, China

Abstract

Heparan sulfate (HS) is ubiquitously expressed on the surfaces and in the extracellular matrix of virtually all cell types, making it an ideal receptor for viral infection. Compared with wild-type viruses, cell culture-adapted laboratory strains exhibit more efficient binding to cellular HS receptors. HS-binding viruses are typically cleared faster from the circulation and cause lower viremia than their non-HS-binding counterparts, suggesting that the HS-binding phenotype is a tissue culture adaptation that lowers virus fitness *in vivo*. However, when inoculated intracranially, efficient cell attachment through HS binding can contribute to viral neurovirulence. The primary aim of this review is to discuss the roles of HS binding in viral pathogenicity, including peripheral virulence and neurovirulence. Understanding how heparan sulfate functions during virus infection *in vivo* may prove critical for elucidating the molecular mechanism of viral pathogenesis, and may contribute to the development of therapeutics targeting HS.

Key words: Heparan sulfate; Viral pathogenicity; Receptor

Biomed Environ Sci, 2011; 24(1): 81-87 doi:10.3967/0895-3988.2011.01.011 ISSN:0895-3988

www.besjournal.com (full text) CN 11-2816/Q

INTRODUCTION

Heparan sulfate (HS) is a negatively charged linear carbohydrate polymer composed of repeating disaccharides of glucosamine and hexuronic acids that are sulfated at various positions[1]. Apart from the essential functions in animal development and homeostasis, demonstrated by targeted disruption of the enzymes involved in the biosynthesis of HS[2-3], HS is drawing attention as a potential target for the prevention of viral infection and pathogenicity. As shown in Table 1[4-28], cell surface HS, found in both vertebrate and invertebrate species, has been shown to serve as a receptor for a growing number of viruses from many different families, including some important pathogens causing infectious epidemics, such as herpes simplex virus (HSV)[4], human papillomavirus (HPV)[10], hepatitis B virus (HBV)[11], respiratory syncytial virus (RSV)[14], foot-and-mouth disease virus (FMDV)[16] and human immunodeficiency virus type 1 (HIV-1)[26]. In addition, several alphaviruses were also found to use cellular HS as a receptor, such as Sindbis virus (SINV)[22], Ross River virus (RRV)[23], Venezuelan equine encephalitis virus (VEEV)[24] and Semliki Forest virus (SFV)[25].

It is well established that HS is also involved in pathological processes by mediating infection of diverse microbial entities including viruses. The most direct evidence for this was obtained through studies of infection by HSV that required a specific fine structure of HS to interact[4]. Generally, a viral HS-binding phenotype was obtained by multiple passages in tissue culture, or by constructing mutant viruses harboring a mutation conferring a positive charge. HS-binding viruses are typically cleared faster from the circulation and cause lower viremia than their non-HS-binding counterparts, suggesting

*This work was supported by grants from the National Natural Science Foundation of China (No. 30970160), the Major Science and Technology Project for Infectious Disease (No. 2008ZX10004-001; 2009ZX10004-705) and the Development Grant of the State Key Laboratory for Infectious Disease Prevention and Control (2008SKLID105).

#Correspondence should be addressed to: LIANG GuoDong, MD, Tel: 86-10-63510124; Fax: 86-10-63532053; E-mail: gdliang@hotmail.com

Biographical note of the first author: ZHU WuYang, PhD, associate professor, specialized in molecular virology.

Received: July 15, 2010; Accepted: January 7, 2011

that HS-binding is a tissue culture adaptation that lowers virus fitness *in vivo*. However, when viruses are inoculated intracranially, efficient HS-binding can contribute to viral neurovirulence in an animal model[24]. The effect of HS affinity on viral peripheral virulence and viral neurovirulence indicates that viral pathogenicity may be correlated to the manner of inoculation, and that HS binding may attenuate viral infection that is dependent on high-titer viremia. However, efficient interaction with HS can increase virulence, possibly through enhancing viral replication within specific host tissues such as the brain. Elucidation of the relevance of HS binding on viral pathogenicity may therefore lead to insights into the molecular mechanisms of HS-related infectious diseases.

The Structural Characteristics of HS that Confer its Role as a Receptor

Proteoglycans carrying HS chains are ubiquitously expressed at cell surfaces and in extracellular matrices, and HS chains interact with numerous proteins, including growth factors, morphogens, extracellular matrix proteins and many pathogens, such as bacteria, protozoa, and viruses. Although the virus–receptor interaction is needed to initiate infection, pathogenesis is a multi-step process involving cellular functions, such as the capacity of the host to develop a proper immune response, the velocity of virus replication, cytopathogenicity and the spread of infection within and between organs, which again may or may not depend on the presence of specific cellular receptors. These interactions form the basis of HS-related biological phenomena, which regulate key events in embryonic development and homeostasis, and the ability of viruses to bind HS could affect viral pathogenicity, thus mediating disease progression.

It has recently been shown that cell surface HS is involved in viral infection and pathogenesis through being used as a receptor by a number of viruses (Table 1). Three major characteristics confer cellular

Table 1. Viruses Using HS as Receptor

Virus Genome	Virus Family	Virus Genus	Virus Specie	Receptor Type
DNA	Herpesviridae	Simpexvirus	Herpes simplex virus[4]	Specific receptor
		Varicellovirus	Varicella-zoster virus[5]	Unknown
		Cytomegalovirus	Human herpesvirus 5[6]	Unknown
			Pseudorabies virus[7]	Unknown
			Bovine herpesvirus 1[8]	Unknown
	Poxviridae	Orthopoxvirinae	Vaccinia virus[9]	Unknown
	Papovaviridae	Papillomavirus	Human papillomavirus[10]	Initial receptor
	Hepadnaviridae	Orthohepadnavirus	Hepatitis B virus[11]	Unknown
	Parvoviridae	Dependovirus	Adeno-associated virus type 2[12]	Initial receptor
RNA	Paramyxoviridae	Paramyxovirus	Human parainfluenza virus type 3[13]	Unknown
		Pneumovirus	Human respiratory syncytial virus[14]	Initial receptor
	Picornaviridae	Cardiovirus	Theiler's virus[15]	Unknown
		Aphthovirus	Foot-and-mouth disease virus[16]	Initial receptor
		Enterovirus	Swine vesicular disease virus[17]	Initial receptor
	Flaviviridae	Flavivirus	Dengue virus[18]	Initial receptor
			Tick borne encephalitis virus[19]	Initial receptor
		Hepatitis C virus	Hepatitis C virus[20]	Unknown
		Pestivirus	Swine fever virus[21]	Unknown
	Togoviridae	Alphavirus	Sindbis virus[22]	Initial receptor
			Ross River virus[23]	Unknown
			Venezuelan equine encephalitis virus[24]	Unknown
			Semiliki forest virus[25]	Unknown
	Retroviridae	Lentivirus	Human immunodeficiency virus type 1[26]	Initial receptor
		BLV-HTLV retroviruses	Human T-cell leukemia virus[27]	Unknown
	Coronaviridae	Coronavirus	A vian coronavirus infectious bronchitis virus[28]	Unknown

HS selectivity for protein binding. First, HS is a linear carbohydrate polymer with a negative charge, which is composed of repeating disaccharides of glucosamine and hexuronic acid[29]. Most protein–HS interactions are mediated by the electrostatic interaction between clusters of basic amino acids arranged in a three-dimensional array on the ligand and a concentrated negative charge on the sulfated polysaccharide chain. Thus, the phenotype of HS-dependent infection through tissue culture was sometimes conferred by the selective advantage of an adaptive mutation for positively charged amino acids (aa), such as Arg, Lys, and His. Second, clustering of these modifications along the HS chain yielded highly N-sulfated domains (NS domains) of approximately 12-20 residues that alternate with typically larger sized, relatively unmodified, N-acetyl-rich domains (NA domains). The NS domains can assume several different conformations, and thus influence the orientation of the sulfate residues in space. This domain organization places relatively flexible NA domains adjacent to relatively rigid NS domains, thus facilitating protein interactions with the sulfate residues. Finally, this micro-sequence diversity and macro-organization are cell type specific, and do not appear to be core protein specific, presumably the result of the cell type-specific repertoires of HS chain-modifying enzymes.

HS as a Viral Receptor

HS on the cell surface and in the extracellular matrix normally binds to a wide variety of growth factors, chemokines, enzymes and matrix components[30-31], but is also important in the attachment of a number of bacteria, protozoa and viruses[32]. Recently, much attention has been focused on the interaction of viral surface proteins with HS, which are present almost ubiquitously on cell surfaces. The first viral strain reported to use HS as a receptor was HSV type 1, for which the interaction with HS carrying a specific sulfation pattern can functionally substitute for a protein receptor[4]. Although the overall picture is still far from complete, it has become clear during the past few years that cellular HS is used as a receptor by a growing number of viruses, including five DNA virus families and six RNA virus families (Table 1), some of which can cause severe disease epidemics in humans, such as HIV, HBV and HPV. Thus it has been proposed that the binding of the viral surface to HS may play an important role in viral pathogenicity[23,33-38].

Proteins typically bind electrostatically to HS via stretches of positively charged aa, such as Lys and Arg, and attachment of viruses to HS is presumably mediated in the same fashion. During the past few years research into a number of viruses, including alphaviruses[39-40], pestiviruses[20], picornaviruses[41-42] and retroviruses[43-44], has demonstrated that adaptation to certain cell lines results in the selection of mutants that bind HS with high affinity. This suggested that the ability of a virus to bind HS could be an adaptation that arose in laboratory strains during repeated passaging in tissue culture, and that non-tissue culture-adapted strains infect host cells by a HS-independent mechanism. Thus, the HS-dependent phenotype has a selective advantage through the adaptive mutations for positively charged aa acquired during tissue culture, and these adaptive mutations have been found to increase viral infectivity by enhancing the binding or attachment to HS on the cell surface.

It should be noted that HS is commonly exploited by multiple viruses for the initial attachment to host cells. In most cases, the binding of the virus to HS seems to be relatively low-affinity, and may serve the purpose of concentrating the virus on the cell surface to facilitate subsequent binding to one or more high-affinity receptors[45-49]. This model is supported by results obtained using the flaviviruses, such as dengue virus and tick-borne encephalitis virus. Dengue virus binds first to HS and then to a high-affinity receptor, which induces endocytosis and subsequent cell membrane fusion[17,47,50]. Thus, HS proteoglycans on the cell surface can be used as initial attachment receptors by several viruses. In contrast, HSV-1 is unique because it can use HS for both attachment and penetration, provided specific binding sites for the HSV-1 envelope glycoprotein, gD, are present[51]. Therefore, future studies to investigate the interaction between cellular HS and other co-receptors will be important in elucidating the additional roles of HS in viral pathogenicity.

HS Binding and Peripheral Virulence

It is easy to demonstrate that a viral HS-dependent phenotype, either in cell-tissue cultured strains or in recombinant viruses carrying mutations conferring a positive charge, can increase viral infectivity via efficient attachment to cultured cells. However, investigations into how HS binding influences viral infection *in vivo* remain more difficult. However, how HS-binding proteins behave

in vivo is well characterized and pharmacokinetic studies on HS-binding proteins, such as bactericidal/ permeability-increasing protein, extrace llular superoxide dismutase and hepatocyte growth factor/scatter factor, have demonstrated rapid biphasic clearance from the circulation after intravenous injection[52–54]. This biphasic decay can be modeled as the sum of two exponential equations[55]. The early, rapid phase of clearance is strongly influenced by binding to HS and clearance during this phase can be decreased by co-injecting heparin[56-57], digesting tissue HS with intravenous heparinase[58], or mutating basic residues so that the protein losses its capacity to bind HS[54,59]. Because the liver contains large amounts of highly sulfated HS [60], a large percentage of the protein removed from the circulation can be found in this organ[56,58,61-63], and it is thought that viruses able to bind HS are mediated in the same fashion.

Previous investigations on the clearance of alphaviruses from the circulation showed that HS-binding variants were typically cleared faster after intravenous injection than non-HS-binding variants[33,38,64-66]. One of these studies demonstrated the accumulation of VEEV in the liver, with virions deposited in the sinusoids and the spaces of Disse, as well as within vacuoles of Kupffer cells[66]. In contrast, recombinant SINVs with lower binding to heparin or cellular HS were cleared more slowly from the circulation and caused higher viremia than the parental virus[33]. In addition, several studies have demonstrated that non-HS-binding strains of SINV[22,40], VEEV[24], FMDV[36], tick-borne encephalitis virus[19] and classical swine fever virus[21] are more virulent in animal models than their HS-binding counterparts, suggesting that for these viruses, the HS-binding phenotype is a tissue culture adaptation that lowers virus fitness *in vivo*. The selective adaptation of HS-binding has also been shown to be a common and frequent phenomenon during the propagation of flaviviruses, which attenuated HS-binding variants, suggesting the major role of HS dependence in flavivirus attenuation. Given what is now known about the clearance of HS-binding proteins from the circulation, it seems likely that the differences in clearance rates in these studies were due to differences in viral binding to HS. These findings, together with what is known about the behavior of HS-binding proteins *in vivo*, provide strong evidence that the ability to bind HS has a negative impact on virus production *in vivo*.

The general conclusion that strong binding to a ubiquitous carbohydrate such as HS causes attenuation *in vivo* may apply only to viruses that cause plasma viremia, and to instances in which viral spread through the circulation contributes to dissemination within the infected host. High viremia is also an important factor in the transmission from host to host for insect-borne arboviruses, such as SINV and dengue virus. In contrast, for viruses such as HSV type 1, infection is spread primarily from cell to cell and strong binding to HS is not necessarily deleterious. Besides accelerated clearance, another mechanism that might prevent HS-binding viruses from achieving high viremia is interaction with HS in the extracellular matrix near the site of viral production. The amount of virus in the blood required for equilibrium is a function of both the rate of release of new virus into the circulation and the rate of clearance, both of which may be decreased if the virus can bind HS. Sa-Carvalho *et al*. have shown that variants of FMDV that bind well to HS are attenuated in cattle, showing a decreased ability to spread from the site of inoculation[42]. HS-binding variants attach better to cultured cells, but are attenuated in mice and cattle, apparently because of a reduced ability to spread from the site of inoculation. After injection of cattle with high doses of an attenuated HS-binding variant, disease and systemic dissemination of the virus were observed, but were due to the development of non-HS-binding revertants[42]. It is proposed that binding to HS controls both the plaque size and the circulating half-life of the virus and that variants are cleared more quickly from the circulation, because they bind more effectively to HS. Therefore, the viral HS-dependent phenotype, resulting from either cell tissue culture or from recombinant viruses carrying mutations conferring a positive charge, generally result in increased specific infectivity, small plaque formation and significant attenuation of peripheral virulence.

HS Binding and Viral Neurovirulence

The mechanism behind the effect of HS binding on viral neurovirulence involves the entry of viruses into the central nervous systems (CNS) in the case of viruses that bind to cellular HS after peripheral inoculation, and involves the replication capacity of viruses after intracranial inoculation. In the case of HIV, there is *in vitro* evidence that HS improves the efficiency of binding to brain microvascular endothelial cells, and this is postulated to facilitate

the entry of this virus into the CNS[67-68]. However, higher levels of virus in the blood were not sufficient to confer increased virulence, and the differences observed in viral replication in the CNS were not predicted by binding to HS. Entry of alphaviruses into the CNS has been assumed to occur via the infection of endothelial cells[69-71], but there is also evidence for viral entry through axonal transport by nerves innervating either a peripheral site of replication or the olfactory mucosa[72]. Both the endothelial cell and olfactory routes of entry require spread to those sites through the blood, and it is reasonable to assume that the amount of virus in the blood and the length of time it circulates will influence the likelihood of infecting these sites and gaining entry into the CNS. Thus, viral replication and clearance in the periphery is correlated with the HS-binding phenotype, but does not totally account for differences in viral neurovirulence and other properties of the virus involved with neurovirulence; for example, the genetic background of the host could affect the outcome of viral infection.

Several descendant viruses from prototype SINV AR339 with HS-binding mutations showed low virulence after subcutaneous inoculation, but high virulence when inoculated directly into the brains of mice[38,64-66]. In contrast, the highly neurovirulent Theiler's murine encephalomyelitis virus (TMEV) strain GDVII uses HS as a co-receptor to enter target cells. GDVII virus with a non-HS-binding phenotype was obtained by adaptive growth in HS-deficient cells, which exhibited two aa substitutions (R3126L and N1051S) in the capsid[73]. When intracerebrally inoculated, the neurovirulence of the adapted virus in mice was substantially attenuated. Moreover, severe poliomyelitis, but not acute encephalitis, was observed in infected mice. The reason for this was that the adapted virus showed altered cell tropism in the CNS of mice, shifting from cerebral and brainstem neurons to spinal cord anterior horn cells, suggesting that the use of HS as a receptor by GDVII virus facilitates cell entry and plays an important role in cell tropism and neurovirulence *in vivo*[73]. These results indicated that viral variants with efficient cell attachment through HS binding exhibited increased viral neurovirulence, presumably through altered cell tropism or enhanced replication within specific host tissues, such as the brain.

PERSPECTIVE

Most protein-HS binding is mediated by electrostatic interactions between clusters of basic aa arranged in a three-dimensional array on the ligand, and a concentrated negative charge on the sulfated polysaccharide chain. Attachment of virus to cellular HS is presumably mediated via stretches of positively charged aa, such as Lys and Arg. Some viral envelope glycoproteins have been postulated to constitute heparin-binding domains, rich in positively charged aa. For example, E_2 glycoprotein from mature SINV particles have two heparin-binding domains located at aa 127-132 and 145-150, conforming to the XBBXBX and XBBBXXBBX (B, basic; X, any aa) heparin-interaction consensus motifs identified by Cardin and Weintraub[74]. In addition, adaptive mutations to positive charges scattered throughout the envelope glycoprotein sequence may play a role in the binding of virus to cell surface HS. Therefore, we speculate that the binding sites of virus to HS are composed of two parts: linear HS-binding domains similar to the XBBXBX or XBBBXXBBX consensus motifs and the scattered positively charged aa located in the envelope glycoprotein[22].

Taken together, viruses displaying a HS-binding phenotype, resulting from either tissue culture or recombination, exhibit attenuated peripheral virulence, but increased viral neurovirulence. Thus, HS binding may attenuate viral disease that is dependent on high-titer viremia, but efficient cell attachment through HS-binding can increase virulence, presumably through altering cell tropism or enhancing viral replication within specific host tissues, such as the brain. Understanding the roles of HS binding in viral pathogenicity may help us to obtain insight into the dynamics of viral behavior, and may also be important in the development of therapeutics targeting HS.

REFERENCES

1. Lindahl U, Li JP. Interactions between heparan sulfate and proteins design and functional implications. Int Rev Cell Mol Biol, 2009; 276, 105-59.
2. Lin X, Perrimon N. Role of heparan sulfate proteoglycans in cell-cell signaling in Drosophila. Matrix Biol, 2000; 19(4), 303-7.
3. Liu L, Xiang K, Zheng T, et al. The heparan sulfate proteoglycan gene polymorphism: association with type 2 diabetic nephropathy in Chinese. Mol Cell Biochem, 2003; 245(1-2), 121-6.
4. Wu Dunn D, Spear PG. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. J Virol, 1989; 63, 52-8.
5. Zhu Z, Gershon MD, Gabel C, et al. Entry and egress of varicella-zoster virus: role of mannose 6-phosphate, heparan sulfate proteoglycan, and signal sequences in targeting virions and viral glycoproteins. Neurology, 1995; 45, S15-7.
6. Akula SM, Wang FZ, Vieira J, et al. Human herpesvirus 8

interaction with target cells involves heparan sulfate. Virology, 2001; 282(2), 245-55.
7. Trybala E, Bergström T, Spillmann D, et al. Mode of interaction between pseudorabies virus and heparan sulfate/heparin. Virology, 1996; 218, 35-42.
8. Li Y, Littel-van den Hurk SD, Babiuk LA, et al. Characterization of cell-binding properties of bovine herpesvirus 1 glycoproteins B, C, and D: identification of a dual cell-binding function of gB. J Virol, 1995; 69, 4758-68.
9. Chung CS, Hsiao JC, Chang YS, et al. A27L protein mediates vaccinia virus interaction with cell surface heparan sulfate. J Virol, 1998; 72, 1577-85.
10. Giroglou T, Florin L, Schäfer F, et al. Human Papillomavirus infection requires cell surface heparan sulfate. J Virol, 2001; 75, 1565-70.
11. Cooper A, Tal G, Lider O, et al. Cytokine induction by the hepatitis B virus capsid in macrophages is facilitated by membrane heparan sulfate and involves TLR2. J Immunol, 2005; 175, 3165-76.
12. Summerford C, Samulski RJ. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. J Virol, 1998; 72, 1438-45.
13. Bose S, Banerjee AK. Role of heparan sulfate in human parainfluenza virus type 3 infection. Virology, 2002; 298: 73-83.
14. Hallak LK, Collins PL, Knudson W, et al. Iduronic acid-containing glycosaminoglycans on target cells are required for efficient respiratory syncytial virus infection. Virology, 2000; 271, 264-75.
15. Honey V R, Howard LL. Heparan sulfate mediates infection of high-neurovirulence Theiler's viruses. J Virol, 2002; 76, 8400-7.
16. Jackson T, Ellard F M, Ghazaleh R A, et al. Efficient infection of cells in culture by type O foot-and-mouth disease virus requires binding to cell surface heparan sulfate. J Virol, 1996; 70, 5282-7.
17. Escribano-Romero E, Jimenez-Clavero MA, Gomes P, et al. Heparan sulphate mediates swine vesicular disease virus attachment to the host cell. J Gen Virol, 2004; 85, 653-63.
18. Chen Y, Maguire T, Hileman RE, et al. Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. Nat Med, 1997; 3, 866-71.
19. Mandl CW, Kroschewski H, Allison SL, et al. Adaptation of tick-borne encephalitis virus to BHK-21 cells results in the formation of multiple heparan sulfate binding sites in the envelope protein and attenuation in vivo. J Virol, 2001; 75, 5627-37.
20. 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, 41003-12.
21. Hulst MM, van Gennip HGP, Moormann R J M. Passage of classical swine fever virus in cultured swine kidney cells selects virus variants that bind to heparan sulfate due to a single amino acid change in envelope protein E^{rns}. J Virol, 2000; 74, 9553-61.
22. Zhu W, Wang L, Yang Y, et al. Interaction of E2 glycoprotein with heparan sulfate is crucial for cellular infection of sindbis virus. PloS ONE, 2010; 5(3): e9656. doi:10.1371/ journal.pone. 0009656.
23. Heil ML, Albee A, Strauss JH, et al. An amino acid substitution in the coding region of the E2 glycoprotein adapts Ross River virus to utilize heparan sulfate as an attachment moiety. J Virol, 2001; 75, 6303-9.
24. Bernard KA, Klimstra WB, Johnston RE. Mutations in the E2 glycoprotein of Venezuelan equine encephalitis virus confer heparan sulfate interaction, low morbidity, and rapid clearance from blood of mice. Virology, 2000; 276, 93-103.
25. Smit JM, Waarts B-L, Kimata K, et al. Adaptation of alphaviruses to heparan sulfate: interaction of Sindbis and Semliki Forest viruses with liposomes containing lipid-conjug ated heparin. J Virol, 2002; 76, 10128-37.
26. Roderiquez G, Oravecz T, Yanagishita M, et al. Mediation of human immunodeficiency virus type 1 binding by interaction of cell surface heparan sulfate proteoglycans with the V3 region of envelope gp120-gp41. J Virol, 1995; 69, 2233-9.
27. Jones KS, Petrow-Sadowski C, Bertolette DC, et al. Heparan sulfate proteoglycans mediate attachment and entry of human T-cell leukemia virus type 1 virions into CD^{4+} T cells. J Virol, 2005; 79, 12692-702.
28. Madu IG, Chu VC, Lee H, et al. Heparan sulfate is a selective attachment factor for the avian coronavirus infectious bronchitis virus Beaudette. Avian Dis, 2007; 51, 45-51.
29. Bishop JR, Schuksz M, Esko JD. Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature, 2007; 446,1030-7.
30. Gruenheid S, Gatzke L, Meadows H, et al. Herpes simplex virus infection and propagation in a mouse L cell mutant lacking heparan sulfate proteoglycans. J Virol, 1993; 67 (1), 93-100.
31. Puvirajesinghe TM, Guimond SE, Turnbull JE, et al. Chemometric analysis for comparison of heparan sulphate oligosaccharides. J R Soc Interface, 2009; 6, 997-1004.
32. Lee JB, Hayas MK, Hashimoto M, et al. Novel antiviral fucoidan from sporophyll of Undaria pinnatifida (Mekabu). Chem Pharm Bull, 2004; 52(9), 1091-4.
33. Byrnes AP, Griffin DE. Large-plaque mutants of Sindbis virus show reduced binding to heparan sulfate, heightened viremia, and slower clearance from the circulation. J Virol, 2000; 74, 644-51.
34. Harwood LJ, Gerber H, Sobrino F, et al. Dendritic cell internalization of foot-and-mouth disease virus: influence of heparan sulfate binding on virus uptake and induction of the immune response. J Virol, 2008; 82, 6379-94.
35. Hilgard P, Stockert R. Heparan sulfate proteoglycans initiate dengue virus infection of hepatocytes. Hepatology, 2000; 32, 1069-77.
36. Neff S, Sa-Carvalho D, Rieder E, et al. Foot-and-mouth disease virus virulent for cattle utilizes the integrin alpha(v)beta3 as its receptor. J Virol, 1998; 72, 3587-94.
37. Ceballos A, Lenicov FR, Sabatté J, et al. Spermatozoa capture HIV-1 through heparan sulfate and efficiently transmit the virus to dendritic cells. J Exp Med, 2009; 206, 2717-33.
38. Ryman KD, Garder CL, Burke CW, et al. Heparan sulfate binding can contribute to the neurovirulence of neuroadapted and nonneuroadapted Sindbis virus. J Virol, 2007; 81(7), 3563-73.
39. Klimstra WB, Ryman KD, Johnston RE. Adaptation of Sindbis virus to BHK cells selects for use of heparan sulfate as an attachment receptor. J Virol, 1998; 72, 7357-66.
40. Klimstra WB, Heidner HW, Johnston RE. The furin protease cleavage recognition sequence of Sindbis virus PE2 can mediate virion attachment to cell surface heparan sulfate. J Virol, 1999; 73, 6299-306.
41. Escarmis C, Carrillo EC, Ferrer M, et al. Rapid selection in modified BHK-21 cells of a foot-and-mouth disease virus variant showing alterations in cell tropism. J Virol, 1998; 72, 10171-9.
42. Sa-Carvalho D, Rieder E, Baxt B, et al. Tissue culture adaptation of foot-and-mouth disease virus selects viruses that bind to heparin and are attenuated in cattle. J Virol, 1997; 71, 5115-23.
43. Moulard M, Lortat-Jacob H, Mondor I, et al. Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120. J Virol, 2000; 74,

1948-60.
44. Patel M, Yanagishita M, Roderiquez G, et al. Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. AIDS Res Hum Retrovir, 1993; 9, 167-74.
45. Gopal S, Bober A, James R, et al. Heparan sulfate chain valency controls syndecan-4 function in cell adhesion. J Biol Chem, 2010; 285, 14247-58.
46. Fry, EE, Lea SM, Jackson T, et al. The structure and function of a foot-and-mouth disease virus-oligosaccharide receptor complex. EMBO J, 1999; 18, 543-4.
47. Añez G, Men R, Eckels KH, et al. Passage of Dengue Virus Type 4 Vaccine Candidates in Fetal Rhesus Lung Cells Selects Heparin-Sensitive Variants That Result in Loss of Infectivity and Immunogenicity in Rhesus Macaques. J Virol, 2009; 83, 10384-94.
48. Qiu J, Handa A, Kirby M, et al. The interaction of heparin sulfate and adeno-associated virus 2. Virology, 2000; 269, 137-47.
49. Schneider-Schaulies J. Cellular receptors for viruses: links to tropism and pathogenesis. J Gen Virol, 2000; 81, 1413–29.
50. Putnak JR, Niranjan K-T, Innis BL. A putative cellular receptor for dengue viruses. Nature Medicine, 1997; 3, 828-829.
51. Shukla D, Liu J, Blaiklock P, et al. A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. Cell, 1999; 99, 13-22.
52. Abrahamson SL, Wu H-M, Williams RE, et al. Biochemical characterization of recombinant fusions of lipopolysaccharide binding protein and bactericidal/ permeability-increasing protein. J Biol Chem, 1997; 272, 2149-55.
53. Vigny M, Ollier-Hartmann MP, Lavigne M, et al. Specific binding of basic fibroblast growth factor to basement membrane-like structures and to purified heparan sulfate proteoglycan of the EHS tumor. J Cell Physiol, 1988; 137(2), 321-8.
54. Karlsson K, Sandstr Ö m J, Edlund A, et al. Pharmacokinetics of extracellular-superoxide dismutase in the vascular system. Free Rad Biol Med, 1993; 14, 185-90.
55. Gibaldi M, Perrier D. Pharmacokinetics, New York, N.Y. Marcel Dekker, Inc, 1975. 45-96.
56. Karlsson K, Marklund SL. Plasma clearance of human extracellular-superoxide dismutase C in rabbits. J Clin Investig, 1988; 82, 762-6.
57. Ugolini S, Mondor I, Sattentau QJ. HIV-1 attachment: another look. Trends Microbiol, 1999; 7, 144-9.
58. Ji Z-S, Sanan DA, Mahley RW. Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulfate proteoglycans. J Lipid Res, 1995; 36, 583-92.
59. Hartmann G, Prospero T, Brinkmann, Ö Ozcelik V, et al. Engineered mutants of HGF/SF with reduced binding to heparan sulphate proteoglycans, decreased clearance and enhanced activity in vivo. Curr Biol, 1998; 8, 125-34.
60. Lyon M, Deakin JA, Gallagher JT. Liver heparan sulfate structure: a novel molecular design. J Biol Chem, 1994; 269, 11208-15.
61. Bauer RJ, Der K, Ottah-Ihejeto N, et al. The role of liver and kidney on the pharmacokinetics of a recombinant amino terminal fragment of bactericidal/permeability -increasing protein in rats. Pharm Res, 1977; 14, 224-9.
62. Wells MJ, Blajchman MA. *In vivo* clearance of ternary complexes of vitronectin-thrombin-antithrombin is mediated by hepatic heparan sulfate proteoglycans. J Biol Chem, 1998; 273, 23440-7.
63. Yuge T, Furukawa A, Nakamura K, et al. Metabolism of the intravenously administered recombinant human basic fibroblast growth factor, trafermin, in liver and kidney: degradation implicated in its selective localization to the fenestrated type microvasculatures. Biol Pharm Bull, 1997; 20, 786-93.
64. Yin J, Gardner CL, Burke CW, et al. Similarities and differences in antagonism of neuron Alpha/Beta interferon responses by venezuelan equine encephalitis and sindbis alphaviruses. J Virol, 2009; 83, 10036-47.
65. Bear JS, Byrnes AP, Griffin DE. Heparin-binding and patterns of virulence for two recombinant strains of Sindbis virus. Virology, 2006; 347, 183-90.
66. Jahrling PB, Gorelkin L. Selective clearance of a benign clone of Venezuelan equine encephalitis virus from hamster plasma by hepatic reticuloendothelial cells. J Infect Dis, 1975; 132, 667-76.
67. Banks WA, Robinson SM, Wolf KM, et al. Binding, internalization, and membrane incorporation of human immunodeficiency virus-1 at the blood-brain barrier is differentially regulated. Neuroscience, 2004; 128, 143-53.
68. Bobardt MD, Salmon P, Wang L, et al. Contribution of proteoglycans to human immunodeficiency virus type 1 brain invasion. J Virol, 2004; 78, 6567-84.
69. Fazakerley JK. Pathogenesis of Semliki Forest virus encephalitis. J NeuroVirol, 2002; 8 (Suppl. 2), 66-74.
70. Johnson RT. Virus invasion of the central nervous system: a study of Sindbis virus infection in the mouse using fluorescent antibody. Am J Pathol, 1965; 46, 929-43.
71. Johnson RT. The incubation period of viral encephalitis. Slow latent and temperate virus infections. NINDB Monogr, 1966; 2, 119-24.
72. Cook SH, Griffin DE. Luciferase imaging of a neurotropic viral infection in intact animals. J Virol, 2003; 77, 5333-8.
73. Reddi HV, Manoj Kumar AS, Kung AY, et al. Heparan sulfate-independent infection attenuates high-neurovirulence GDVII virus-induced encephalitis. J Virol, 2004; 78, 8909-16.
74. Cardin AD, Weintraub HJ. Molecular modeling of protein-glycosaminoglycan interactions. Arteriosclerosis, 1989; 9, 21-32.

Correction

The third author of article "*Reported Willingness and Associated Factors Related to Utilization of Voluntary Counseling and Testing Services by Female Sex Workers in Shandong Province,China*" on page 466,Vol.23, No.6 (2010)in BFS should be XIAO-FANG WANG.

Authors' announcement

The authors' affiliation is Beijing Ditan Hospital, Capital Medical University for the article "*A(H1N1)Influenza Pneumonia with Acute Dissminated Encephalomyelitis: A Case Report*" published in BES 23(4), 323-326 (2010)by JUN YANG, YU-GUANG,YUN-LIANG XU,XIAN-LING REN, YU MAO AND XING-WANG LI. We apologize for this inconvenience that might have brought to the BES and its readers.