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ScienceDirect
Antiviral Research 75 (2007) 83–86
Antiviral Research
www.elsevier.com/locate/antiviral

Short communication

Herpes glycoprotein gL is distantly related to chemokine receptor ligands

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Received 11 June 2006; accepted 27 November 2006

Abstract

Glycoprotein L (gL) is one of the critical proteins involved in transmission of *Herpesviridae*. We applied the methodology of protein structure prediction to shed a light on the so far unknown molecular mechanism of its action. Here we show that gL forms a chemokine-like protein. *Alphaherpesvirinae* gL as well as CMV functional homolog (UL130) create a novel CX chemokine-like protein, while *Gammaherpesvirinae* gL (HHV8 and EBV) adopt a regular CC beta-chemokine fold.

We conclude that gL may interact with specific cellular chemokine receptors during the invasion of *Herpesviridae*. The proposed mechanism has a potential impact on future development of novel therapeutic and prophylactic strategies.

Keywords: Herpes; Cellular receptor; Cell invasion

Herpesviridae is a family of dsDNA vertebrate viruses involved in pathogenesis of various human infectious diseases including herpetic stomatitis (HSV1), genital herpes (HSV2), chickenpox (VZV), mononucleosis (EBV) and opportunistic infections (CMV, HHV8) (Whitley, 1996). Species of herpes are classified into three different evolutionary subfamilies—*Alphaherpesvirinae*: HSV1, HSV2, VZV; *Betaherpesvirinae*: CMV, HHV-6, HHV-7; *Gammaherpesvirinae*: EBV, HHV8.

Transmission of virions into cells is a limiting step of infection spread. The exploitation of specific entry receptors determines the tissue specificity of the infection (Spear, 2004). In HSV1 genome – the model organism of herpetic infection – 11 out of 80 genes encode glycoproteins localized on the outer membrane of the virion. Previous studies determined the minimal set of proteins required for fusion, comprising glycoprotein D (gD), glycoprotein B (gB) and the complex of glycoproteins L (gL) (Roop et al., 1993) and H (gH) (Geraghty et al., 1998).

Glycoprotein L is a small protein that was postulated to act as a chaperonin of gH and be involved in its secretion and proper folding (Roop et al., 1993). The gL/gH complex is located on the surface of virion, but there was no clear evidence whether this complex is involved in recognition of specific cells (tissues) or directly involved in membrane fusion. To identify the exact molecular function of the gL/gH complex in human pathogenic *Herpesviridae* we applied state-of-art methods of protein structure prediction.

Protein sequences of human pathogens from *Herpesviridae* were retrieved from genome entries of GenBank database. Open reading frames obtained from genome annotations were submitted to MetaBasic (Ginalski et al., 2004). Sequences of human chemokines were retrieved from GenBank. Protein families of glycoprotein L homologs were collected with Psi-Blast (Altschul et al., 1997) ($p < 0.001$, three iterations) and aligned with ClustalW (Thompson et al., 1994) independently for *Alpha-*, *Beta-* and *Gammaherpesvirine*. Minor manual corrections of alignments guided by structural predictions were performed with BioEdit. Signal peptides were trimmed prior to the subsequent analyses (Bendtsen et al., 2004). Templates for homology modeling were selected according to the results of 3D-Jury assessment method (Ginalski et al., 2003) with subsequent manual corrections as described previously (von Grotthuss et al., 2003). Homology models were obtained with Modeller (Version 6.2).

Various state-of-the-art profile-profile [MetaBasic (Ginalski et al., 2004), FFAS3 (Jaroszewski et al., 2005)] and threading [3D-PSSM (Kelley et al., 2000), mGenThreader (McGuffin and Jones, 2003), INUB] methods predicted that the glycoprotein L encodes a fold similar to interleukin 8 (Fig. 1A). The prediction was supported by relative high scores provided by each method.

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doi:10.1016/j.antiviral.2006.11.015

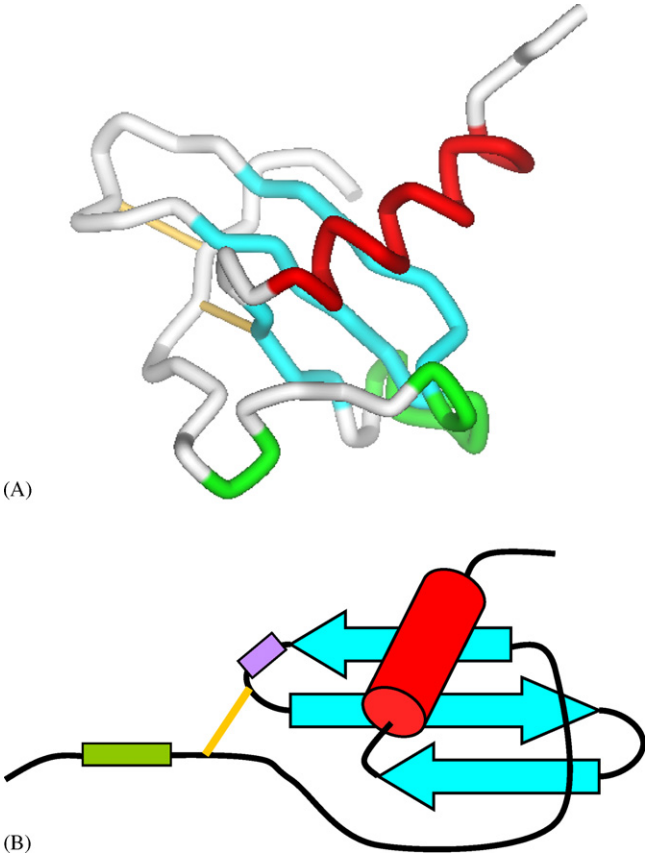

Fig. 1. Herpes glycoprotein L chemokine fold: (A) model of HHV8 glycoprotein L (CC beta-chemokine fold) created on a template of Eotaxin2 (PDB: 1EIG_A); (B) schema of CX fold: cystein bridge shown in orange, strands and helix shown in blue and red, respectively, site II (DxLK motif) shown in green, site I shown in violet (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.).

As it was shown previously (Rychlewski et al., 2003), the consistency of predictions between various methods strongly support the fold assignment. The 3D-Jury – prediction quality assessment system – confirmed the prediction (score 37.88 for HHV8 gL). Although the score falls below the cutoff (previous tests exhibited less than 5% of incorrect assignments for 3D-Jury cutoff above 50 for average protein length). As the standard confidence threshold exhibits linear correlation with number of correctly assigned amino acids it is expected to fall below the cutoff in the case of small folds (e.g. chemokine fold) (Ginalski et al., 2003).

Proteins from species of the Gamma subdivision of herpes are homologous to the CC type of chemokines with typical conserved cysteine bridges (Table 1C). Proteins from *Alphaherpesvirinae* contain only one disulphide bond (Table 1A), creating a novel CX type of the beta-chemokine fold (Fig. 1B). The importance of cysteines for gL function was previously demonstrated (Cairns et al., 2005). Glycoprotein L retrieved from *Betaherpesviridae* were not homologous to either CC or CXC chemokines at both sequence and structural level. These proteins were not assigned to cytokine-like fold in MetaBasic and other applied methods and additionally it did not contain a conserved pattern of cysteine residues.

To find homologous proteins in *Betaherpesvirinae* we scanned CMV, HHV-6 and HHV-7 genomes with MetaBasic. Proteins exhibiting similarity to chemokines were selected for further analyses. We identified UL130 (GenBank id: NP_040067) open reading frame in CMV genome as a putative functional homologue of chemokine-like molecules based on the consistent protein fold assignments and sequence similarity to *Alphaherpesvirinae* gL. Similarly to HSV1 gL protein-UL130 contains a signal peptide followed by CX type beta-chemokine (Table 1B) and non-globular C-terminal tail. The structural assignment of beta-chemokine fold was supported by 3D-Jury

Table 1
The structural alignment of herpes gL and human beta-chemokines

Alpha / CX	EEEEEEEEE EEEEEEEEE EEEEE HHHHHHHHH (1VL9)
9629381 HSV1	(17)ILKVPCVPLPS(8)TPSAINYALIDG(4)YHCPGLDTVLWDR(1)AQKAYWVNP(1)LFVAGFLEDLSYP(115)
9629270_HSV2	(15)ILRVPCMRTPA(8)APSVIDYARIDG(4)YHCPGLDTFLWDR(1)AQRAYLVNP(1)LFAAGFLEDLSHS(114)
66866093 VZV	(19)IITEPCVSSVY(7)APPVSNLSEALS(5)TKCPVPEVILWFK(1)KQMAYWTNP(1)VTLKGLTQSVGEE(45)
15012099 CXCL10	(3)SRTVRCTCISI(6)PRSLEKLEIIPA(1)QFCPRVEIIATMK(1)KGEKRCLNP(2)KAIKNLLKAVSKE(6)
30582973 IL8	(6)AKELRCQCIKT(6)PKFIKELRVIES(1)PHCANTEIIVKLS( )DGRELCLDP(2)NWVQRVVEKFLKR(4)
	* # * #
Beta / CX	EEEEEEEEE EEEEEEEEE EEEEE HHHHHHHHH (1VL9)
9625818 CMV	(26)AATFYCPFLYP(2)PRSPLQFSGFQR(4)PECRNETLYLLYN(1)EGQTLVERS(1)TWVKKVIWYLSGR(94)
30582973 IL8	(6)AKELRCQCIKT(6)PKFIKELRVIES(1)PHCANTEIIVKLS( )DGRELCLDP(2)NWVQRVVEKFLKR(4)
15012099 CXCL10	( )SRTVRCTCISI( )PRSLEKLEIIPA( )QFCPRVEIIATMK( )KGEKRCLNP( )KAIKNLLKAVSKE( )
	* # * #
Gamma / CC	EEEEE EEEEEE EEEEE HHHHHHHHH (1RTO)
2246513 HHV8	( )YVALPCCAIQA(6)PLFFAVHSIHFA(2)NHCNGVCIAKLRS(4)ITVETCVNG( )FNLRSFLVAVVRR(76)
1334881 EBV	( )NWAYPCCHVTQ(6)LALENISDIYLV(2)QTCDGFSLASLNS(7)LVISRCANG( )LNVVSFFISILKR(41)
8118703 CCL5	(5)DT-TPCCFAYI(4)PRAHIKEYFYTS( )GKCSNPAVVFVTR( )KNRQVCANP(2)KWVREYINSLEMS( )
30582405 CCL3	(5)DTPTACCFSYT(4)PQNFIADYFETS( )SQCSKPSVIFLTK( )RGRQVCADP(2)EWVQKYVSDLELPS(1)
	*# * #

Cysteines creating disulphide bonds marked below the alignment. The residues of conserved properties were shown in colors (green for hydrophilic, yellow for hydrophobic, gray for small). Secondary structure retrieved from corresponding PDB entries was shown above the alignment (E, extended/beta; H, helix/alfa). Numbers in brackets refer to residues removed from alignment. (A) Alignment of *Alphaherpesvirinae* gL (HSV1, VZV) and two CXC beta-chemokines (CXCL10, IL8). (B) Alignment of *Betaherpesvirinae* gL (CMV) and two CXC beta-chemokines (CCL10, IL8). (C) Alignment of *Gammaherpesvirinae* gL (HHV8, EBV) and two CC beta-chemokines (CCL5, CCL3).

Table 2
Similarity between viral gL proteins (HSV1, VZV) and its putative cellular analogs (IL8, CXCL7, respectively)—similar residues marked with asterisk

ligand	Sequence	Receptor		
		IL8A	IL8B	Other
gL HSV1	(11)RVA-REVGDILKVPCVPL(176)			
IL8	( )AVLPRSAKE-LRCQCIKT(59)	+	+	
	** * * * ** **			
gL VZV	(13)KPLSDVSLI-ITEPCVSS(106)			
CXCL7	(17)SLDSDLYAE-LRCMCIKT(29)		+	
	*** * * ** *			
CXCL5	(1)GPAAAVLRE-LRCVCLQT(58)		+	
CXCL6	( )GPVSAVLTE-LRCTCLRV(59)	+	+	
CXCL1	( )---ASVATE-LRCQCLQT(58)		+	
CXCL2	( )---APLATE-LRCQCLQT(58)		+	
CXCL3	( )---ASVVTE-LRCQCLQT(58)		+	
CXCL9	( )---TPVVRK-GRCSCIST(88)			CXCR3
CXCL10	( )---VPLSRT-VRCTCISI(62)			CXCR3
CXCL11	( )---FPMFKR-GRCLCIGP(58)			CXCR3
CXCL4	( )----EEDGD-LQCLCVKT(54)			unknown
CXCL12	( )---KPVSLS-YRCPCRFF(56)			CXCR4
CXCL13	( )--VLEVYYTSLRCRCVQE(70)			CXCR5
CXCL14	( )----------SKCKCSRK(67)			unknown
CXCL16	( )----NEGSVTGSCYCGKR(208)			CXCR6

Known receptors of cellular analogs are listed.

(confident score of 52.00). With this approach we could not determine the corresponding open reading frames in HHV-6 and HHV-7 genomes.

To evaluate the results of predictions we compared herpes glycoproteins L with the complete repertoire of human CC and CXC chemokines. The assignment of the beta-chemokine fold was supported by conserved patterns of hydrophobic and polar residues within the aligned protein families and consistent predictions of secondary structure among herpes gL and human chemokines (Table 2). Residues were colored based on physicochemical characteristics of amino acids and shaded at 60% similarity. Conserved residues between viral proteins and its cellular analogues (IL8, CXCL7) were marked with asterisk.

Previous analyses of CXC chemokines (IL8, CXCL10) identified two regions (site I and II) responsible for binding and activation of their corresponding receptors (IL8R and CXCR3, respectively) (Wells et al., 1996). Site I represents a structural element without preserved sequence pattern and it is impossible to infer receptor specificity based on sequence conservation within this region. We observed sequence similarity between HSV1 gL and IL8 within site II, which is responsible for specific recognition of the IL8 receptor (Wells et al., 1996). To maintain its binding activity the ligands of IL8 receptor (CXCL1-3, CXCL5-7, IL8) contain the ELR motif (glutamic acid–leucine–arginine) within close proximity to cysteines (CXC) at the N-terminal end of protein. HSV1 and HSV2 gL have a similar site at the corresponding locus–HSV1: DXLK (aspartic acid–x–leucine–lysine), HSV2: DXLR (aspartic acid–x–leucine–arginine). The alignment of the region around site II was shown in Table 2.

VZV gL lacks a corresponding motif suggesting different receptor specificity. The amino acid composition of its site II resembled that of the other IL8RB ligand-CXCL7, but since the motif important for binding ([ED]-L-[RK]) was not observed, the interaction with IL8RB is not certain.

Glycoprotein L encodes a critical function for preservation of the ability of the herpesvirus to infect host cells. The utilization of described algorithms allowed the formulation of the highly reliable description of glycoprotein L structure. Based on it we conclude that herpes gL can resemble the host ligand and may interact with chemokine receptors during the invasion of *Herpesviridae*.

The previously identified set of glycoproteins critical for the transmission consists of specific ligands of cellular receptors: gD interacting with several cellular membrane proteins called herpesvirus entry mediators (HveA–HveD) (Spear et al., 2000), gB interacting with both heparan proteoglycans and an unknown specific cellular receptor (Bender et al., 2005) and complex of gL/gH. We postulate that gL acts as an interface between gH anchored within viral capsid and the chemokine receptors. The divergence within the family (beta-chemokine for *Alpha-* and *Betaherpesvirinae,* alfa-chemokine for *Gammaherpesvirinae*) and divergence within so called "site II" suggests that gL may be important factor of tissue selectivity.

We identified that CMV protein UL130 is homologous to HSV1 gL. UL130 was previously shown to be important for the infection of leukocytes (Hahn et al., 2004). Therefore we may assume that usage of either UL130 or CMV gL can result in a selective infection of given subset of host cells.

The proposed mechanism in transmission of *Herpesviridae* has a potential impact on the development of novel therapeutic and prophylactic strategies, where analogous peptides could be used as binding inhibitors or vaccine targets and will be subject of further studies. Although the structure-function assignment allowed for general description of the molecular mechanisms of gL activity, the bioinformatic methodology is unable to prove the interaction between given gL protein and selected beta-chemokine receptors. Since the structure of glycoprotein H cannot be currently solved by methods of the protein structure prediction (Wyrwicz L, unpublished data) we were also unable to give insight into the composition of gH/gL complex. Further functional studies are needed to answer these questions important for understanding the role of gL in the infection.

Acknowledgements

This work has been supported by MNiSW and the European Commission with grants for LR (LSHG-CT-2004-512035 and LSHG-CT-2003-503265) and MNiSW grant for LSW (2P05A00130, PBZ-MNiI-2/1/2005). LSW is supported by the Foundation for Polish Science within the Program for Young Researchers.

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