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# Amino acid differences in glycoproteins B (gB), C (gC), H (gH) and L(gL) are associated with enhanced herpes simplex virus type-1 (McKrae) entry via the paired immunoglobulin-like type-2 receptor $\alpha$

Sona Chowdhury, Misagh Naderi, Vladimir N Chouljenko\*, Jason D Walker and Konstantin G Kousoulas\*

## Abstract

**Background:** Herpes simplex virus type-1 (HSV-1) enters into cells via membrane fusion of the viral envelope with plasma or endosomal membranes mediated by viral glycoproteins. HSV-1 virions attach to cell surfaces by binding of viral glycoproteins gC, gD and gB to specific cellular receptors. Here we show that the human ocular and highly neurovirulent HSV-1 strain McKrae enters substantially more efficiently into cells via the gB-specific human paired immunoglobulin-like type-2 receptor- $\alpha$  (hPILR- $\alpha$ ). Comparison of the predicted amino acid sequences between HSV-1(F) and McKrae strains indicates that amino acid changes within gB, gC, gH and gL may cause increased entry via the hPILR- $\alpha$  receptor.

**Results:** HSV-1 (McKrae) entered substantially more efficiently than viral strain F in Chinese hamster ovary (CHO) cells expressing hPILR- $\alpha$  but not within CHO-human nectin-1, -(CHO-hNectin-1), CHO-human HVEM (CHO-hHVEM) or Vero cells. The McKrae genes encoding viral glycoproteins gB, gC, gD, gH, gL, gK and the membrane protein UL20 were sequenced and their predicted amino acid (aa) sequences were compared with virulent strains F, H129, and the attenuated laboratory strain KOS. Most aa differences between McKrae and F were located at their gB amino termini known to bind with the PILR $\alpha$  receptor. These aa changes included a C10R change, also seen in the neurovirulent strain ANG, as well as redistribution and increase of proline residues. Comparison of gC aa sequences revealed multiple aa changes including an L132P change within the 129-247 aa region known to bind to heparan sulfate (HS) receptors. Two aa changes were located within the H1 domain of gH that binds gL. Multiple aa changes were located within the McKrae gL sequence, which were preserved in the H129 isolate, but differed for the F strain. Viral glycoproteins gD and gK and the membrane protein UL20 were conserved between McKrae and F strains.

**Conclusions:** The results indicate that the observed entry phenotype of the McKrae strain is most likely due to a combination of increased binding to heparan sulfate receptors and enhanced virus entry via gB-mediated fusion of the viral envelope with plasma membranes.

## Introduction

Herpes simplex type 1 (HSV-1), Herpes simplex type 2 (HSV-2) and Varicella-zoster virus (VZV) are human neurotropic viruses that belong to the *Alphaherpesvirinae* subfamily and are a major cause of worldwide morbidity [1-4]. Neurovirulence, establishment of latency in sensory neurons and intermittent reactivation are some

of the unique properties of these viruses [5,6]. Reactivation of latent virus from trigeminal ganglia can lead to recurrent ocular infections and is a leading cause of blindness in developed countries [7,8]. In very rare cases HSV-1 can spread spontaneously to the brain, causing life threatening herpes encephalitis [3].

Herpes virus initiates infection by binding to heparan sulfate (HS) moieties on cell surfaces using viral glycoproteins gC and gB [9]. Moreover, viral glycoprotein D (gD) binds to different cellular receptors including the herpesvirus entry mediator (HVEM, or HveA), nectin-1

\* Correspondence: vchoul1@lsu.edu; vtgusk@lsu.edu  
Division of Biotechnology and Molecular Medicine and Department of Pathobiological Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803, USA

(HveC), or 3-O-sulfated HS [10-12]. Apparently, gB can also bind to additional receptors including the paired immunoglobulin-like type 2 receptor alpha (PILR $\alpha$ ), non-muscle myosin heavy chain IIA (NMHC-IIA), and myelin-associated glycoprotein (MAG) that function in virion attachment and virus entry [13-15]. HSV-1 enters into epithelial and neuronal cells via a pH-independent fusion of the viral envelope with plasma membranes, while it can enter into a wide range of non-neuronal cells via either pH-independent or pH-dependent endocytosis. Binding of gD and gB to their cognate receptors is thought to trigger sequential conformational changes in gH/gL and gB causing gB-mediated fusion of the viral envelope with cellular membranes during virus entry, as well as fusion among cellular membranes [16-18].

HSV-1 clinical isolates, such as the McKrae and H129 strains, are known to be highly virulent in rodents and rabbits in comparison to other laboratory strains such as KOS [19-21]. Several viral proteins and glycoproteins contribute to neurovirulence and latency in vivo, however their mode of action is not well elucidated [22-30]. HSV-1 gK is known to be involved in neurovirulence [30-34], and is a structural component of the virion particle functioning in virus entry into epithelial cells [35,36], cytoplasmic virion envelopment, virion egress and virus-induced cell fusion [37-39]. Recently, we showed that that HSV-1 gK and UL20 physically bind to gB and gH and modulate gB-mediated membrane fusion [40,41]. Also, we reported that gK is essential for virus spread in the cornea of mice, neuroinvasiveness and establishment of latency into ganglionic neurons [34].

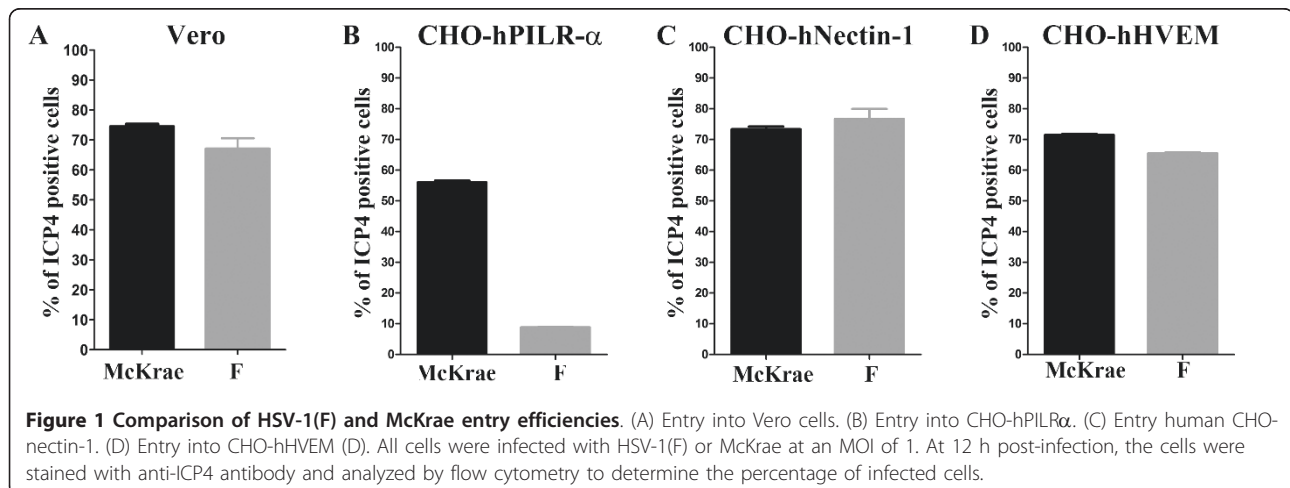
In this manuscript, we compared the relative efficiency of virus entry between HSV-1 McKrae and F strains and found that McKrae entered substantially more efficiently into Chinese hamster ovary (CHO) cells expressing the hPILR $\alpha$ . We sequenced all viral genes encoding viral glycoproteins involved in entry and cell-to-cell fusion

and identified aa differences between McKrae and F strains that may cause the observed enhanced entry of McKrae over other viral strains.

## Results

Both McKrae and F viruses appeared to enter with similar efficiency into Vero and CHO cells expressing either nectin-1 or HVEM (Figure 1: A, C, D, respectively). In contrast, F entered into CHO cells expressing PILR $\alpha$  substantially less efficiently than McKrae (Figure 1: B). Comparison of McKrae, F, KOS and H129 gB aa sequences (Figure 2) revealed that most of the aa differences between the four strains were located within the N-terminal 80 aa of gB. Amino acid comparison of four strains revealed that KOS had a number of aa that differed from the other HSV-1 strains. McKrae gB had a unique aa (A28V) within its predicted signal sequence (Figure 2). Comparison of F and McKrae gB sequences showed the following aa changes: C10R, A28V, P61A, A62P, T67P, N77P, and P79K (Table 1).

Comparison of the aa sequence of gC of HSV-1 McKrae, F, KOS and H129 revealed that aa variations between the four strains were distributed throughout the molecule (Figure 3). Although McKrae and KOS were more similar to each other, McKrae differed from all three strains at aa positions 289 (A289D) and 299 (F299L). Specifically, there were 20 nucleotide differences between gC of F and McKrae strains. Out of 20 nucleotide differences only 8 resulted in the aa changes V16L, Q75K, D116G, L132P, A289D, F299L, H306R, R421H (Table 2). Nucleotide comparison between gD of HSV-1 F and McKrae strain revealed no aa differences between the McKrae and F strains (data not shown). Comparison of the McKrae, F and H129 gH aa sequences revealed that these proteins were highly conserved (Figure 4). There were only three aa differences in the H1 domain of gH and two aa differences within



gB_McKrae	MRQGAPARGR	RWFVVWALLG	LTLGVLVSSA	APSSPGTPGV	AAATQAANGG	PATPAPPAPG	APPTGDPKPK	KNKKPKPKPK	80
gB_H129	.....	.....	.....A.....	.....	.....	.....	PA...T...	.....N.P.	80
gB_F	.....C.....	.....	.....A.....	.....	.....	.....	PA...T...	.....N.P.	80
gB_KOS	.H...SW.	.....	.....A.....	.....	.....	.....L.	.....A.....	.....N.T.	80
gB_McKrae	PRPAGDNATV	AAGHATLREH	LRDIKAENTD	ANFYVCPPT	GATVVQFEQP	RRCPTRPEGQ	NYTEGIAVVF	KENIAPYKFK	160
gB_H129	.....	.....	.....	.....	.....	.....	.....	.....	160
gB_F	.....	.....	.....	.....	.....	.....	.....	.....	160
gB_KOS	.....	.....	.....	.....	.....	.....	.....	.....	160
gB_McKrae	ATMYKDVTV	SQVWFGHRYS	QFMGIFEDRA	PVPFEEVIDK	INAKGVCRST	AKYVRNNLET	TAFHRDDHET	DMELKPANAA	240
gB_H129	.....	.....	.....	.....	.....	.....	.....	.....	240
gB_F	.....	.....	.....	.....	.....	.....	.....	.....	240
gB_KOS	.....	.....	.....	.....	.....	.....	.....	.....	240
gB_McKrae	TRTSRGWHTT	DLKYNPSRVE	AFHRYGTTVN	CIVEEVDARS	VYPYDEFVLA	TGDFVYMSPF	YGYREGSHTE	HTSYAADRFK	320
gB_H129	.....	.....	.....	.....	.....	.....	.....	.....	320
gB_F	.....	.....	.....	.....	.....	.....	.....	.....	320
gB_KOS	.....	.....	.....	.....	.....	.....	.....T.....	.....	320
gB_McKrae	QVDGFYARDL	TTKARATAPT	TRNLLTTPKF	TVAWDWVPKR	PSVCTMTKWQ	EVDEMLRSEY	GGSRFRSSDA	ISTFTTNLT	400
gB_H129	.....	.....	.....	.....	.....	.....	.....	.....	400
gB_F	.....	.....	.....	.....	.....	.....	.....	.....	400
gB_KOS	.....	.....	.....	.....	.....	.....	.....	.....	400
gB_McKrae	EYPLSRVDLG	DCIGKDARDA	MDRIFARRYN	ATHIKVGQPQ	YYLANGGFLI	AYQPLLSNTL	AELYVREHLR	EQSRKPPNPT	480
gB_H129	.....	.....	.....	.....	.....	.....	.....	.....	480
gB_F	.....	.....	.....	.....	.....	.....	.....	.....	480
gB_KOS	.....	.....	.....	.....	.....	.....	.....	.....	480
gB_McKrae	PPPPGASANA	SVERIKTSS	IEFARLQFTY	NHIQRHVNDM	LGRVAIAWCE	LQNHELTSWN	EARKLNPAI	ASATVGRRS	560
gB_H129	.....	.....	.....	.....	.....	.....	.....	.....	560
gB_F	.....	.....	.....	.....	.....	.....	.....	.....	560
gB_KOS	.....	.....	.....	.....H.....	.....	.....	.....	.....V.....	560
gB_McKrae	ARMLGDVMAV	STCVPVAADN	VIVQNSMRIS	SRPGACYSRP	LVSFRYEDQG	PLVEQGLGEN	NELRLTRDAI	EPCTVGHRRY	640
gB_H129	.....I.....	.....	.....	.....	.....	.....	.....	.....	640
gB_F	.....	.....	.....	.....	.....	.....	.....	.....	640
gB_KOS	.....	.....	.....	.....	.....	.....	.....	.....	640

**Figure 2 Alignment of the predicted glycoprotein B (gB) amino acid sequences specified by HSV-1 strains McKrae, H129, F and KOS.** Amino acids that match the McKrae sequence are represented by dots. Amino acid changes that are different with respect to the HSV-1 McKrae strain are shown. The region of gB (aa 1-640), which contains aa substitutions is shown.

the C terminal H3 domain of gH. There were two unique aa substitutions (S670N and C720R) in McKrae gH that were not present in either strain F or H129. Moreover, HSV-1(F) and McKrae strains differed by four aa in gH (Y147H, V150A, N670S, and R720C) (Table 3). Amino acid alignment of gL encoded by McKrae, F, H129 and KOS revealed a number of aa differences that were spread across the entire molecule (Figure 5). The aa sequence of strain McKrae was similar to strain H129 except at aa residues 171,181 and 212. Comparison between nucleotide sequence of gL of HSV-1 F and McKrae strain revealed 19 nucleotide differences, ten of which coded for different aa (S22P, K90R, V100G, N115D, P168L, G171R, P181S, P196S, L202S, A212T) (Table 4). Seven of these aa changes were conserved in the H129 strain (P22, R90, G100, D115, L168, S196, S202) (Table 4). F and McKrae gK sequences were absolutely conserved despite four nucleotide changes within their genes. Similarly, there were four nucleotide changes between the UL20 gene of HSV-1 F and McKrae strains without causing any aa changes.

## Discussion

HSV-1 utilizes multiple receptors to attach and enter into a variety of cells including neurons. Recently, it was shown that gB binds to cellular receptors that are required for gB-mediated membrane fusion during virus

entry and virus-induced cell fusion. We show here that the HSV-1 McKrae strain utilizes the gB receptor PIRL $\alpha$  more efficiently than HSV-1(F). DNA sequencing of all viral glycoprotein genes involved in membrane fusion indicates that there are a number of aa differences between HSV-1(F) and McKrae in gB, gH and gL that may affect PIRL $\alpha$ - mediated virus entry.

Initial entry experiments revealed that PIRL- $\alpha$  did not function as efficiently in facilitating virion entry in comparison to the gD receptors nectin-1 and HVEM when strains HSV-1(F) and KOS were used (not shown). Recently, we cloned the HSV-1(McKrae) strain as a bacterial artificial chromosome (bac) that has enabled us to rapidly produce mutant viruses, as we have previously done with HSV-1(F) bac [42,43]. We have found that this viral strain efficiently enters into a variety of cells including CHO cells constitutively expressing PIRL $\alpha$ . PIRL $\alpha$  binds gB and membrane fusion can be affected by interactions of gB with viral glycoproteins gD, gH/gL and gK/UL20 [18,40,41]. Therefore, we compared the predicted aa sequences between F and McKrae strains and found a number of aa that could contribute to the observed increased efficiency of entry of McKrae over F and other strains. Of particular importance were aa changes between F and McKrae located within the amino terminus of gB, known to bind to PIRL $\alpha$  and gK [41,44]. Specifically, gB binds to PIRL $\alpha$  via O-linked glycans located at aa positions 53 and 480 [44]. However,

**Table 1 HSV-1 (F) and McKrae gB Nucleotide and Amino acid Differences**

Base#	F	McKrae	Amino acid #	Amino acid change
28	T	C	10	Cys → Arg
83	C	T	28	Ala → Val
150	A	C		
181	C	G	61	Pro → Ala
184	G	C	62	Ala → Pro
199	A	C	67	Thr → pro
229	A	C	77	Asn → Pro
230	A	C	77	Asn → Pro
235	C	A	79	Pro → Lys
236	C	A	79	Pro → Lys
966	T	C		
1,513	C	A		
1,899	C	T		
1,932	C	T		
2,073	C	A		
2,196	C	T		
2,373	T	C		

**Table 2 HSV-1 (F) and McKrae gC Nucleotide and Amino acid Differences**

Base#	F	McKrae	Amino acid #	Amino acid change
46	G	T	16	Val → Leu
177	G	A		
223	C	A	75	Gln → Lys
279	A	G		
347	A	G	116	Asp → Gly
393	T	C		
394	T	C	132	Leu → Pro
395	T	C		
594	A	G		
619	G	A		
636	C	T		
866	C	A	289	Ala → Asp
897	T	G	299	Phe → Leu
917	A	G	306	His → Arg
981	A	C		
1,071	G	A		
1,254	G	A		
1,262	G	A	421	Arg → His
1,365	G	A		
1,485	A	G		

gB binding to PIRL $\alpha$  is also dependent on the conformation of the amino terminus of gB, since aa insertions in gB could reduce this binding [45]. Insertional mutagenesis has revealed that the structure and function of gB is not particularly flexible in tolerating aa insertions [46]. The McKrae gB contains additional proline residues at aa positions 67 and 77, while other proline residues have been re-arranged. Specifically, the F gB has a

proline at aa 61, which is an alanine for the McKrae strain, but the McKrae gB contains a proline at aa 62 instead of alanine. The structure of the amino terminus of gB is not known, although the x-ray structure of gB (aa 111-726) was obtained [17]. The additional proline

gC_McKrae	MAPGRVGLAV	VLWSLLWLGA	GVSQSGSETAS	TGPTITAGAV	TNASEAPTSG	SPGSAASPEV	TPTSTPNPNN	VTQNKTTPT	80
gC_H129	.....	.....V.....	.....	.....	M.....	.....	.....	.....Q.....	80
gC_F	.....	.....V.....	.....	.....	.....	.....	.....	.....Q.....	80
gC_KOS	.....	.....G.....	.....A.....	.....	.....	.....	.....	.....	80
gC_McKrae	PASPPTPKP	TSTPKSPPTS	TPDPKPKNNT	TPAKSGRPTK	PPGPVWCDRR	DPLARYGSRV	QIRCFRFRNST	RMEFRLQIWR	160
gC_H129	.....	.....	.....	.....	.....	.....	.....	.....	160
gC_F	.....	.....	.....	.....D.....	.....	.....L.....	.....	.....	160
gC_KOS	.....	.....	.....	.....	.....	.....	.....	.....	160
gC_McKrae	YSMGPSPPIA	PAPDLEEVLT	NITAPPGLL	VYDSAPNLTD	PHVLWAEAG	PGADPPLYSV	TGPLPTQRLI	IGEVTPATQG	240
gC_H129	.....	.....	.....	.....	.....	.....	.....	.....	240
gC_F	.....	.....	.....	.....	.....	.....	.....	.....	240
gC_KOS	.....	.....	.....	.....	.....	.....	.....	.....	240
gC_McKrae	MYYLAWGRMD	SPHEYGTWVR	VRMFRPPLST	LQPHAVMEGQ	PFKATCTADA	YYPRNPVELV	WFEDDRQVFN	PGQIDTQTHE	320
gC_H129	.....	.....	.....	.....	.....A.....	.....F.....	.....H.....	.....V.....	320
gC_F	.....	.....	.....	.....	.....A.....	.....F.....	.....H.....	.....	320
gC_KOS	.....	.....	.....	.....	.....A.....	.....FD.....	.....	.....	320
gC_McKrae	HPDGFTTVST	VTSEAVGGQV	PPRTFTCQMT	WHRDSVTFSR	RNATGLALVL	PRPTITMEFG	VRHVVCTAGC	VPEGVTFAWF	400
gC_H129	.....	.....	.....	.....	.....	.....	.....	.....	400
gC_F	.....	.....	.....	.....	.....	.....	.....	.....	400
gC_KOS	.....	.....	.....	.....	.....	.....	.....	.....	400
gC_McKrae	LGDDPSPAAK	SAVTAQESCD	HPGLATVRST	LPISYDYSEY	ICRLTGYPAG	IPVLEHHGSH	QPPPRDPPTER	QVIEAIEWVG	480
gC_H129	.....	.....	.....	.....	.....	.....	.....	.....	480
gC_F	.....	.....	.....R.....	.....	.....	.....	.....	.....	480
gC_KOS	.....	.....	.....	.....	.....	.....	.....	.....	480
gC_McKrae	IGIGVLAAGV	LVVTAIVYVV	RTSQSRQRHR	R	511				
gC_H129	.....	.....	.....	.....	511				
gC_F	.....	.....	.....	.....	511				
gC_KOS	.....	.....	.....	.....	511				

**Figure 3 Alignment of the predicted glycoprotein C (gC) amino acid sequences specified by HSV-1 strains McKrae, H129, F and KOS.** Amino acids that match the McKrae sequence are represented by dots. Amino acid changes that are different with respect to the HSV-1 McKrae strain are shown.

gH_McKrae	MGNGLWFVGV	I I L G V A W G Q V	H D W T E Q T D P W	F L D G L G M D R M	Y W R D T N T G R L	W L P N T P D P Q K	P P R G F L A P P D	E L N L T T A S L P	80
gH_H129	.....V.....	.....	.....	.....	.....	.....	.....	.....	80
gH_F	.....	.....	.....	.....	.....	.....	.....	.....	80
gH_McKrae	LLRWYEEFRC	FVLVTTAEFP	RDPGQLLYIP	KTYLLGRPPN	ASLPAPTTVE	PTAQPPPSVA	PLKGLLHNPA	ASVLLRSRAW	160
gH_H129	.....	.....	.....	.....	.....	.....	.....	.....	160
gH_F	.....	.....	.....	.....	.....	.....	.....Y..V	.....	160
gH_McKrae	VTFSAVPDPE	ALTFPRGDNV	ATASHPSGPR	DTPPPRPVVG	ARRHPTTELD	ITHLHNASTT	WLATRGLLRS	PGRYVYFSPS	240
gH_H129	.....	.....	.....	.....	.....	.....	.....	.....	240
gH_F	.....	.....	.....	.....	.....	.....	.....	.....	240
gH_McKrae	ASTWPGIWT	TGELVLGCDA	ALVRARYGRE	FMGLVISMHD	SPPVEVMVVP	AGQTLDRVGD	PADENPPGAL	PGPPGGPRYR	320
gH_H129	.....	.....	.....	.....	.....	.....	.....	.....	320
gH_F	.....	.....	.....	.....	.....	.....	.....	.....	320
gH_McKrae	VFVLGSLTRA	DNGSALDALR	RVGGYPPEEGT	NYAQFLSRAY	AEFFSGDAGA	EQGPRPPLFW	RLTGLLATSG	FAFVNAAHAN	400
gH_H129	.....	.....	.....	.....	.....	.....	.....	.....	400
gH_F	.....	.....	.....	.....	.....	.....	.....	.....	400
gH_McKrae	GAVCLSDLLG	FLAHSRALAG	LAARGAAGCA	ADSVFFNVSV	LDPTARLQLE	ARLQHLVAEI	LEREQSLALH	ALGYQLAFVL	480
gH_H129	.....	.....	.....	.....	.....	.....	.....	.....	480
gH_F	.....	.....	.....	.....	.....	.....	.....	.....	480
gH_McKrae	DSPSAYDAVA	PSAAHLIDAL	YAEFLGGRVL	TTPVVHRALF	YASAVLRQPF	LAGVPSAVQR	ERARRSLLIA	SALCTSDVAA	560
gH_H129	.....	.....	.....	.....	.....	.....	.....	.....	560
gH_F	.....	.....	.....	.....	.....	.....	.....	.....	560
gH_McKrae	ATNADLRTAL	ARADHQKTLF	WLPDHFSPCA	ASLRFDLDES	VFILDALQA	TRSETPVEVL	AQQTHGLAST	LTRWAHYNAL	640
gH_H129	.....	.....	.....	.....	.....	.....	.....	.....	640
gH_F	.....	.....	.....	.....	.....	.....	.....	.....	640
gH_McKrae	IRAFVPEASH	RCGGQSANVE	PRILVPIHNS	ASYVVTHSPL	PRGIGYKLTG	VDVRRPLFLT	YLTATCEGST	RDIESKRLVC	720
gH_H129	.....	.....	.....N	.....	.....	.....	.....	.....R	720
gH_F	.....	.....	.....N	.....	.....	.....	.....	.....R	720
gH_McKrae	TQNQRDLGLV	GAVFMRYP	TEPA	GEVMSVLLVD	TDNTQQQIAA	GPTEGAPSVF	SSDVPSTALL	LFPNGTVIHL	800
gH_H129	.....	.....	.....	.....	.....	.....	.....	.....	800
gH_F	.....	.....	.....	.....	.....	.....	.....	.....	800
gH_McKrae	IAPGFLAASA	LGVVMITAAL	AGILKVLRTS	VPFWRRE	838	.....	.....	.....	838
gH_H129	.....	.....	.....	.....	838	.....	.....	.....	838
gH_F	.....	.....	.....	.....	838	.....	.....	.....	838

**Figure 4** Alignment of the predicted glycoprotein H (gH) amino acid sequences specified by HSV-1 strains McKrae, H129 and F. Amino acids that match the McKrae sequence are represented by dots. Amino acid changes that are different with respect to the HSV-1 McKrae strain are shown.

residues suggest that the amino terminus of gB assumes a conformation that may affect binding to PIRL $\alpha$  [44,47-49]. In addition, this altered conformation of gB may affect interactions with gK, which binds to the amino terminus of gB and regulates gB-mediated membrane fusion [41]. Interestingly, six aa changes seen in McKrae versus F gB were conserved in the gB specified by the neurovirulent strain ANG [22] suggesting that these aa may contribute to neurovirulence.

Viral glycoproteins gD and gH have been shown to bind gB and modulate its ability to cause membrane fusion [18]. Therefore, mutations within the extracellular portions of gD and gH, as well as gL may affect the ability

of gB to utilize the PIRL- $\alpha$  receptor. In addition, since gH forms a functional heterodimer with gL [50], it is possible that the observed aa differences between McKrae and F within gH domain H1 may affect interaction with gL, known to bind exclusively to this domain [51]. The carboxyl terminus of gH has been shown to be important for virus-induced cell fusion [43]. Therefore, the observed aa changes N670S and R720C may alter virus entry kinetics. Multiple aa changes in gL between McKrae and F (S22P, K90R, V100G, N115D) are within the gL domain known to interact with gH and may affect gH/gL cell-surface expression, cell fusion and virus entry.

Positively charged aa residues are known to be critical for interaction with negatively charged sulfate/carboxylate groups of the HS chain [52]. Moreover, basic aa residues are known to be critical for HS-binding activity [53]. Therefore, the gC Q75K and H306R aa changes in McKrae compared to F may cause increased HS binding. Previous studies have delineated the HS-binding domain that interacts with gC amino terminal residues to be located between aa 33 and 123 [54], and between 129 and 247 [55]. Therefore amino acid changes in McKrae gC (aa 75, 116 and 132) may result in increased initial binding of McKrae gC.

The PIRL alpha gene is expressed on cells of the immune system (monocytes, dendritic cells, NK cells, B cells, macrophages, neutrophils, eosinophils, mast cells), as well as neurons [13,56]. Moreover, HSV-1 enters into corneal epithelial cells (HCE) via the nectin1, HVEM

**Table 3** HSV-1 (F) and McKrae gH Nucleotide and Amino acid Differences

Base#	F	McKrae	Amino acid #	Amino acid change
439	T	C	147	Tyr $\rightarrow$ His
449	T	C	150	Val $\rightarrow$ Ala
558	A	G		
1,596	G	T		
1,650	A	C		
1,899	C	T		
2,009	A	G	670	Asn $\rightarrow$ Ser
2,067	C	G		
2,158	C	T	720	Arg $\rightarrow$ Cys
2,217	A	G		
2,301	G	A		

gL_McKrae	MGILGWVGLI	AVGVLCVRGG	LPSTEYVIRS	RVAREVGDIL	KVPCVPLPSD	DLDWRYETPS	AINYALIDGI	FLRYHCPGLD	80
gL_H129	.....	.....	.....	.....	.....	.....	.....	.....	80
gL_F	.....	.....	S.....	.....	.....	.....	.....	.....	80
gL_KOS	.....	.....	S.....	.....	.....	.....	.....	.....	80
gI_McKrae	TVLWDRHAQR	AYWVNPFLFG	AGFLEDLSHP	AFPADTQETE	TRLALYKEIR	QALDSRKQAA	SHTPVKAGCV	NFDYSRTRRC	160
gI_H129	.....	.....	.....	.....	.....	.....	.....	.....	160
gI_F	.....	.....	.....	N.....	.....	.....	.....	.....	160
gI_KOS	.....	K.....	V.....	N.....	.....	.....	.....	.....	160
gL_McKrae	VGRQDLGLTN	RTSGRTPVLP	SDDEAGLQPK	PLTTPSPIIA	TSDPTPRRDA	ATKSRRRRPH	SRRRI	224	
gL_H129	.....	G.....	P.....	.....	.....	A.....	.....	224	
gL_F	.....	P.....	G.....	.....	P.....	L.....	A.....	224	
gL_KOS	.....	P.....	G.....	.....	P.....	.....	L.....	224	

**Figure 5 Alignment of the predicted glycoprotein L (gL) amino acid sequences specified by HSV-1 strains McKrae, H129, F and KOS.** Amino acids that match the McKrae sequence are represented by dots. Amino acid changes that are different with respect to the HSV-1 McKrae strain are shown.

and PIRL $\alpha$  receptors [57]. Additional experiments are needed to determine the biological and pathogenetic implications of increased utilization of the PIRL $\alpha$  receptor by the HSV-1(McKrae). The availability of the McKrae strain as a bacterial artificial chromosome will enable the rapid construction of mutant viruses that could be used to elucidate the role of each viral glycoprotein in PIRL $\alpha$  mediated virion entry.

## Materials and methods

### Cells and Viruses

The clinical ocular isolate and neuroinvasive strain of HSV-1 (the parental wild-type), McKrae strain, was obtained from Dr. J. M. Hill (Louisiana State University Health Sciences Center, New Orleans, LA, USA). African green monkey kidney (Vero) cells were obtained from the American Type Culture Collection (Rockville,

MD, USA) and grown and propagated in Dulbecco's modified Eagle Medium (DMEM) supplemented with 7% fetal bovine serum (FBS) and antibiotics. The HSV-1 McKrae strain was maintained as a low passage stock on Vero cells. CHO-neo cells and CHO-nectin 1 were a kind gift from Dr. Yasushi Kawaguchi, (The University of Tokyo, Tokyo, Japan) and were propagated in Ham's F12 medium supplemented with 10% fetal calf serum (FCS) and 250  $\mu$ g G418/ml. The CHO-hPLIR $\alpha$  cells were obtained from Dr. Hishashi Arase, (Osaka University, Osaka Japan), were grown in Ham's F12 medium supplemented with 10% fetal calf serum (FCS) and puromycin, 1  $\mu$ g/ml. The human CHO-HVEM was created in our laboratory using PiggyBac Transposon system (System Biosciences). HVEM gene was cloned into PB514BL-1 PiggyBac Dual Promoter Vector and CHO cells expressing HVEM were selected using puromycin, 12  $\mu$ g/ml. All cells were cultured in non-selective medium prior to use in infectivity assays.

**Table 4 HSV-1 (F) and McKrae gL Nucleotide and Amino acid Differences**

Base#	F	McKrae	Amino acid #	Amino acid change
21	C	T		
64	T	C	22	Ser $\rightarrow$ Pro
165	T	C		
261	T	C		
269	A	G	90	Lys $\rightarrow$ Arg
273	A	G		
294	A	G		
299	T	G	100	Val $\rightarrow$ Gly
327	C	T		
333	A	G		
343	A	G	115	Asn $\rightarrow$ Asp
489	A	C		
503	C	T	168	Pro $\rightarrow$ Leu
511	G	A	171	Gly $\rightarrow$ Arg
541	C	T	181	Pro $\rightarrow$ Ser
586	C	T	196	Pro $\rightarrow$ Ser
605	T	C	202	Leu $\rightarrow$ Ser
634	G	A	212	Ala $\rightarrow$ Thr
660	C	T		

### Viral DNA extraction and DNA sequencing

Viral DNA was isolated from infected Vero cells as described earlier for tissue samples [34]. Briefly, confluent monolayers of Vero cells were infected at a multiplicity of infection of 2 and harvested by scraping at 24 hour post-infection. Cell pellets were rinsed with PBS and viral DNA was extracted using the DNAeasy Blood and Tissue Kit according to manufacturer's instruction (Qiagen, Valencia, CA, USA).

Purified McKrae DNA and Fail Safe DNA polymerase (Epicentre Biotechnologies, Madison, WI) were used for PCR. Due to the high GC content of the genomic DNA, PCR was performed using a series of primers which generated overlapping products encompassing the entire gene to be sequenced. PCR products were column-purified (Zymo Research Corp., Orange, CA) and sequencing reactions were prepared using the Big Dye Terminator v3.1 Cycle sequencing Kit (Life Technologies). DNA sequencing was performed in both directions by a primer walking strategy for each gene using an automated DNA sequencer (3130 Genetic Analyzer, Applied Biosystems). The list of

all synthetic oligo-nucleotides used for amplification of the McKrae genes and sequencing is shown in the Additional file 1, Table 1.

### Sequence assembly and nucleotide and amino acid alignments

The Sequencher (4.10.1) software package was used to assemble the overlapping fragments using default parameters. Sequence assembly was performed using the HSV-1 (F) strain as the reference sequence (Gene Bank: GU734771.1). Nucleotide sequences of the following McKrae genes: gB, gC, gD, gH, gL, gK and UL20 were submitted to GenBank (accession numbers: JQ320080.1, JQ359758.1, JQ320083.1, JQ320081.1, JQ359759.1, JQ320082.1, JQ320079.1).

The CLC sequence Viewer version 6 software was used to align the aa sequences of H129 (GenBank: GU734772.1), F (GenBank: GU734771.1) and KOS strain (gB:AAG34116.1; gC: AAA45779.1; gL: AAA99790.1) against the derived McKrae sequences. Alignment was performed using the default parameters of the Clustal W alignment program (CLC sequence viewer software). To determine nucleotide and aa sequence variation between the McKrae and F strains, the sequences were aligned by the MegAlign program in the DNASTAR software package (Lasergene). The differences in nucleotides and aa in the coding region of each sequenced gene were tabulated in Tables 1, 2, 3 and 4.

### Virus entry assays

Confluent monolayers of CHO-neo, CHO-hHVEM, CHO-hPILR $\alpha$ , CHO-human nectin-1 and Vero cells were infected with HSV-1 strains (McKrae and F) at a multiplicity of infection of 1 for 1 hr at 34°C. The virus inoculum was subsequently removed, and the cultures were shifted to 37°C. Twelve hours post-infection (hpi), the cells were fixed and stained with anti-ICP4 antibody (Virusys, Inc., Taneytown, MD) and Alexa Fluor 647 goat anti-mouse IgG1 (Life Technologies, Grand Island, NY). The relative efficiency of virus entry was calculated by flow cytometry as the percentage of cells expressing ICP4 normalized to the CHO-neo entry values. Mean values and standard deviations of three independent experiments were calculated.

### Additional material

Additional file 1: Table S1. Sequencing and PCR primers.

### Abbreviations

HVEM, Herpes virus entry mediator; CHO, Chinese hamster ovary; hPILR $\alpha$ , Human paired immunoglobulin-like type 2 receptor  $\alpha$

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### Authors' contributions

SC was primarily responsible for virus entry experiments, sequence assembly of all genes and interpretation of amino acid differences. MN performed DNA sequencing reactions and assisted in virus entry experiments and gene sequence assembly. JW was responsible for designing the flow cytometry experiments. VC and KK were responsible for overall interpretation of the results and manuscript preparation. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

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