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Distribution of HSV-1 and VZV in Ganglia of the Human Head and Neck

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Abstract

The distribution of the neurotropic alphaherpesviruses (HSV-1, HSV-2, and VZV) was determined in autonomic and sensory ganglia of the head and neck from formalin-fixed human cadavers. HSV-1 and VZV DNA were found in 18/58 and 16/58 trigeminal, 23/58 and 11/58 pterygopalatine, 25/60 and 14/60 ciliary, 25/48 and 11/48 geniculate, 15/50 and 8/50 otic, 14/47 and 4/47 submandibular, 18/58 and 10/58 superior cervical, and 12/36 and 1/36 nodose ganglia, respectively. HSV-2 was not detected in any site. Viral DNA positivity and location were independently distributed among autonomic and sensory ganglia of the human head and neck.

Keywords

Herpes simplex virus (HSV); varicella-zoster virus (VZV); human ganglia; formalin-fixed tissue, DNA

INTRODUCTION

The neurotropic human herpesviruses, HSV-1, HSV-2 and VZV infect a large percentage of the world population and remain latent for the lifetime of the host. Virus can reactivate to cause symptoms ranging from vesicular lesions to encephalitis[1]. In the United States, serologic testing has determined the relative rates of prior infection at 57.7% (HSV-1), 17.0% (HSV-2), and 95.8% (VZV)[2,3]. However, the presence of antibodies does not provide information about where virus is latent.

The trigeminal ganglion is the traditional site of latency of HSV-1 and VZV in the head and neck; however, these viruses have been detected in other sensory and autonomic ganglia including the dorsal root, geniculate, vestibular, spiral, and nodose (inferior vagal), superior cervical, and ciliary ganglia[4]. Although HSV-2 has been postulated to have a preference for infection/latency in the sacral ganglia, HSV-2 has also been implicated in oral-labial infections [5].

Previous studies of the alphaherpesviruses in ganglia have usually reported results from a single donor or from a limited region of the body. Few studies have examined the interaction of

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different alphaherpesviruses with each other (i.e., if one virus is present, are others more likely to also be present in the same site?). The goal of the studies described herein was to determine the distribution of HSV-1, HSV-2, and VZV DNA in autonomic and sensory ganglia of the head and neck and to determine whether the presence of virus DNA in one site was predictive of the presence of virus DNA at another site in the same individual or of another virus in the same site.

MATERIALS AND METHODS

Human specimens

The ganglia used in these studies were collected from whole embalmed bodies that had been donated to the Medical College of Georgia for use in anatomical education and not from pathology specimens or from specimens collected for diagnostic purposes. The collection and study of donated human material was conducted under MCG HAC protocol #02-06-341. The average age of cadavers used in this study was 80.8 years (standard deviation ±10.3 years, range 48–96). The age, sex and presumed cause of death of the donors used in this study are listed in Table 1. Serological information was not available; no donor had evidence of an active herpesvirus infection. Donors were refrigerated within 4 hours of death and were embalmed within 20 hours of death.

To collect ganglia, the calvaria was removed circumferentially, and brain was removed by severing the cranial nerves and spinal cord as close to the brain as possible. The trigeminal ganglion (TG), superior cervical ganglion (SCG), nodose ganglion (NG), pterygopalatine ganglion (PTG), ciliary ganglion (CG), otic ganglion (OG), submandibular ganglion (SMG), and geniculate ganglion (GG) were removed separately using a new set of sterile instruments and gloves. Sections of selected samples were stained using hematoxylin and eosin to ensure that neuronal nuclei were present. Fifteen mg of each tissue sample (≈16mm^3) was briefly washed in PBS, flash-frozen in liquid nitrogen, and powdered using a stainless steel tissue pulverizer.

To reverse formaldehyde cross-linking of nucleic acids, the powdered tissue was washed 3X in 10mM Tris, 10mM glycine, and 1mM EDTA solution, pH 8.0 at 55°C; 0.2M NaCl was added to the final wash solution to facilitate separation. The tissue was pelleted between washes by centrifugation for 10 seconds at 2200 × g [6]. The tissue was digested with 30mAU/ml Proteinase K overnight at 55°C, and DNA was extracted (Qiagen, Valencia, CA).

Verification of amplifiable DNA

DNA obtained was quantified spectrophotometrically and verified using primers specific for a 149bp single-copy, non-coding region of human chromosome 18 (D18S1259) (see Table 2) [7]. The identity of the PCR products was confirmed by separation in agarose gels and by the presence of a band at the expected molecular weight.

Detection of viral DNA

Nested PCR was performed for each virus on 200ng of sample DNA using primers and conditions listed in Table 2. HSV-1 and VZV primers were verified previously in studies of DNA from fixed human trigeminal ganglia[8]. HSV-2 external primers were obtained from published sequences, and nested primers were designed to be within this original 285bp sequence using Primer3 software[9]. PCR reactions for each virus were performed in 96 well plates using the FailSafe PCR system reagents (Epicentre, Madison, WI). For the nested reaction, 2μl of the first reaction was transferred to a second reaction plate containing the appropriate buffer, Taq polymerase, and the second set of primers. The identity of the final product was confirmed by electrophoretic separation and UV visualization.

The sensitivity of nested PCR amplification was determined for each virus using a ten-fold dilution series of viral DNA (10^6–10^0 genomes) mixed with 200ng human DNA (Promega, Madison, WI). Quantified viral DNA for PCR standards was obtained from Randall Cohrs, Ph.D. (University of Colorado Health Sciences Center, Denver, CO). The sensitivity of the HSV-1, HSV-2, and VZV nested primer sets was 39, 1000, and 100 genome copies, respectively (not shown). All primer sets were specific for only one viral type.

Statistical assessment

The data set included 415 ganglia categorized with respect to location, anatomical side, and positivity for HSV-1 and VZV. HSV-2 virus was excluded from any comparison since no sample was positive for HSV-2. Statistical Analyzing Software (SAS Institute, Cary, NC) was used to develop a log-linear model of the data. Variables analyzed for this study included virus type (V), positivity (P), side (S) and location (G). All variables were nominal-level variables and were dichotomous except for G which had eight categories. All cadavers (including ones that were negative for virus in all locations) were included in the statistical calculations.

A hierarchical log-linear model was fit to the data using backward elimination to assess the associations of the variables and to determine the dependence structure of the occurrence of the two viruses. Once this model was complete, a detailed analysis of residuals was conducted to assess the differences in the observed counts and the expected counts for the above model and to graphically assess model fit and model assumptions.

RESULTS

Herpesvirus prevalence

The results of testing all samples for HSV-1 and VZV DNA are shown in Table 1. No sample (0/415) was positive for HSV-2 DNA. Overall, 36% (150/415) of the samples were positive for HSV-1 DNA and 18% (75/415) of the samples were positive for VZV DNA. All ganglionic locations had HSV-1 DNA and VZV DNA in at least one sample. Eighty-nine percent (32/36) of the cadavers had HSV-1 DNA and 61% (22/36) had VZV DNA in at least one ganglion.

Distribution of virus DNA among ganglia and between types

Results were analyzed for dependence between sites, sides, and virus type using a step-down log-linear model. The final log-linear model of the complete data set (8 ganglion sites × 2 sides × 2 virus types) included the following significant terms: Constant + G + P + V + G × P + V × P. After step-down elimination of all non-essential terms, only G, P, V, G × P (dependence of ganglion and positivity) and V × P (dependence of virus type and positivity) were needed to accurately model the data. All other higher-order terms were not significant and were considered non-essential to the model suggesting independence from all other factors. The likelihood-ratio chi-square of the final model was 26.04 (46 degrees of freedom; P=0.992) indicating excellent fit of the model. Three-way and higher-order effects were not significant (likelihood-ratio chi-square=21.92, 29 degrees of freedom, P=0.823)(Supplemental table). The maximum absolute adjusted residual was 1.6. Residual plots for the model included adjusted residuals vs. expected normal values and adjusted residuals vs. deviations from normality; no anomalies were evident in either plot (not shown).

Statistical analyses revealed that (1) virus occurrence was independent of side and there were no significant interactions of side with any other factors (S not in final model), (2) virus occurrence differed by type of ganglion (significant G × P term in model), (3) virus occurrence differed by type of virus (significant V × P term in model), and (4) there was no significant association of virus and ganglia with respect to virus occurrence (i.e., virus type and type of

ganglion operate independently on occurrence and not jointly). There were no other higher-order terms in the final model; in particular there was no G × V × P term.

DISCUSSION

The results of these studies demonstrate that HSV-1 and VZV DNA are independently distributed among ganglia of the human head and neck. The presence of HSV-1 and VZV genomes in both sensory and autonomic ganglia suggests that latency (and perhaps, reactivation) of these viruses extends beyond the trigeminal ganglion. Reactivation of HSV-1 or VZV from a non-trigeminal site may help to explain unusual types of suspected herpesvirus infection in the head and neck such as Meniere's disease, Bell's palsy, and acute retinal necrosis (ARN)[1]. Independent distribution of alpahaherpesvirus DNA in ganglia of the head and neck and between sides may explain the clinical observation that herpetic keratitis usually occurs unilaterally[10]. Most cases of ARN are also unilateral and are not associated with concomitant herpesvirus infection at a non-ocular site, suggesting that virus enters the eye after reactivation and spread from a non-trigeminal site[11].

The prevalence of 89% (32/36 cadavers), 0% (0/36), and 61% (22/36) for HSV-1, HSV-2, and VZV DNA, respectively, in this study differs from previously reported averages of seropositivity of 57.7%, 17.0%, and 95.8%[2,3]. One factor that might account for the difference of prevalence of virus in our study is the relative difference in the age of subjects. The age range of our subjects (48–96 years) only minimally overlapped with that of the subjects involved in the larger NHANES assessment of HSV-1 and HSV-2 (14–49 years[2]. As would be expected with longer time of exposure to viruses in the environment, the prevalence of HSV-1-is significantly higher in advanced age (i.e., >60 years)[12]. In these studies, HSV-2 DNA was not detected in any of the head and neck ganglia, which is similar to previous studies in the head and neck showing that only 1/15 TG samples and 0/14 muscle biopsy specimens were positive for HSV-2[13].

The prevalence of VZV genomes was much lower than that of HSV-1 (18% versus 36%). One reason for this discrepancy may be that the number of virus episomes decreases as the time after establishment of latency increases, eventually falling below our limit of detection (100 copies). The lower prevalence of VZV DNA in ganglia of the head and neck in our studies may also provide information about the incidence of VZV reactivation in the face. Although reactivation of VZV can occur in any of the dermatomes supplied by the trigeminal nerve, shingles in dermatomes supplied by the trigeminal nerve occurs less frequently (14–20%) compared to truncal reactivation[1]. Comparison studies of VZV in ganglia of the head and neck and of the dorsal root ganglia of the trunk are needed to determine whether there are regional-specific patterns of VZV neuronal latency.

Although only formalin fixed material was used in this study, long term losses of DNA from formalin fixation have been shown to be minimal14]. Formalin fixation results in crosslinking of proteins to DNA which impairs the ability of PCR primers to bind to and amplify the sample [15]. However, since all cadavers were subjected to the same fixation process, the effects of the fixation on the results of these studies would be expected to be similar for all samples.

In conclusion, the results of these studies show that HSV-1 and VZV genomes are distributed independently among autonomic and sensory ganglia in the human head and neck and also that the presence of virus DNA in one location cannot predict/eliminate the presence of virus DNA in another location. As a consequence, each ganglion should be considered as a separate and perhaps, independent site of latency and virus reactivation in humans. The studies described herein provide additional information about non-trigeminal sites of herpesvirus latency which may, in turn, provide clues about how the neurotropic herpesviruses behave in their natural

host and perhaps also about from where these viruses reactivate to cause unusual herpesvirus infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Kleinschmidt-DeMasters BK, Gilden DH. The expanding spectrum of herpesvirus infections of the nervous system. Brain Pathol 2001;11:440–451. [PubMed: 11556690]
2. Xu F, Sternberg MR, Kottiri BJ, et al. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. JAMA 2006;296:964–973. [PubMed: 16926356]
3. Jerant AF, DeGaetano JS, Epperly TD, Hannapel AC, Miller DR, Lloyd AJ. Varicella susceptibility and vaccination strategies in young adults. J Am Board Fam Pract 1998;11:296–306. [PubMed: 9719352]
4. Mitchell BM, Bloom DC, Cohrs RJ, Gilden DH, Kennedy PG. Herpes simplex virus-1 and varicella-zoster virus latency in ganglia. J Neurovirol 2003;9:194–204. [PubMed: 12707850]
5. Lafferty WE, Coombs RW, Benedetti J, Critchlow C, Corey L. Recurrences after oral and genital herpes simplex virus infection. Influence of site of infection and viral type. N Engl J Med 1987;316:1444–1449. [PubMed: 3033506]
6. Cohrs RJ, Randall J, Smith J, et al. Analysis of individual human trigeminal ganglia for latent herpes simplex virus type 1 and varicella-zoster virus nucleic acids using real-time PCR. J Virol 2000;74:11464–11471. [PubMed: 11090142]
7. Bustos DE, Atherton SS. Detection of herpes simplex virus type 1 in human ciliary ganglia. Invest Ophthalmol Vis Sci 2002;43:2244–2249. [PubMed: 12091423]
8. Cohrs RJ, Laguardia JJ, Gilden D. Distribution of latent herpes simplex virus type-1 and varicella zoster virus DNA in human trigeminal Ganglia. Virus Genes 2005;31:223–227. [PubMed: 16025248]
9. Rozen, SSH. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, SMS., editor. Bioinformatics Methods and Protocols: Methods in Molecular Biology. Totowa, NJ: Humana Press; 2000. p. 365-386.
10. Liesegang TJ. Herpes simplex virus epidemiology and ocular importance. Cornea 2001;20:1–13. [PubMed: 11188989]
11. Atherton SS. Acute retinal necrosis: insights into pathogenesis from the mouse model. Herpes 2001;8:69–73. [PubMed: 11867023]
12. Motani H, Sakurada K, Ikegaya H, et al. Detection of herpes simplex virus type 1 DNA in bilateral human trigeminal ganglia and optic nerves by polymerase chain reaction. J Med Virol 2006;78:1584–1587. [PubMed: 17063515]
13. Pevenstein SR, Williams RK, McChesney D, Mont EK, Smialek JE, Straus SE. Quantitation of latent varicella-zoster virus and herpes simplex virus genomes in human trigeminal ganglia. J Virol 1999;73:10514–10518. [PubMed: 10559370]
14. Jerome KR, Huang ML, Wald A, Selke S, Corey L. Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR. J Clin Microbiol 2002;40:2609–2611. [PubMed: 12089286]
15. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol 2002;161:1961–1971. [PubMed: 12466110]

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TABLE 1

Cadaver information and positivity for HSV-1 and VZV DNA by PCR assessment.

Cadaver ID#	Age & Gender	Presumed Cause of Death*	TG		PTG		CG		OG		SMG		GG		SCG		NG	
			L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
5249	75 M	N/A†	**hv**	n/a	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4915	81 F	cardiac arrest	ns	**h**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4899	86 M	heart failure	ns	ns	ns	**h**	n/a	**h**	ns	ns	ns	ns	ns	ns	**h**	ns	ns	ns
4903	79 F	N/A	-	-	-	ns	-	ns	ns	ns	ns	ns	ns	ns	-	-	ns	ns
5008	67 M	cardiopulmonary arrest	ns	ns	ns	ns	**h**	ns	ns	ns	ns	ns	ns	ns	**h**	-	ns	ns
4948	77 F	acute hemorrhagic stroke	-	ns	ns	-	-	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4909	80 M	lung cancer	**hv**	**h**	**hv**	ns	**h**	**hv**	ns	ns	ns	ns	ns	ns	**h**	**hv**	ns	ns
4922	75 F	N/A	-	-	ns	ns	-	-	ns	ns	ns	ns	ns	ns	-	-	ns	ns
4902	77 M	N/A	-	-	n/a	n/a	n/a	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
5015	79 M	N/A	ns	-	-	-	-	**v**	ns	ns	ns	ns	ns	ns	-	-	ns	ns
5062	85 M	COPD	ns	-	**h**	**h**	**h**	**h**	**h**	**h**	ns	**h**	**h**	**h**	**v**	**v**	ns	ns
5070	95 F	COPD	-	**hv**	**h**	**hv**	**hv**	**h**	**h**	**hv**	ns	n/a	**hv**	**h**	**h**	-	ns	ns
5063	91 F	myocardial infarction	-	**h**	**h**	**h**	**h**	**hv**	**h**	**h**	**h**	**h**	**h**	**hv**	**h**	**h**	ns	ns
5118	96 F	N/A	-	-	**h**	**hv**	-	-	-	-	-	-	**hv**	-	-	-	ns	ns
5057	72 F	N/A	**hv**	**h**	**h**	**h**	**hv**	**hv**	**h**	**hv**	**h**	**h**	**h**	**hv**	**hv**	**h**	**h**	**h**
5064	86 M	stroke, metastatic cancer	-	ns	-	**h**	-	**hv**	-	-	-	**v**	**h**	-	ns	ns	-	-
5051	82 M	cardiac arrest, prostate cancer	**h**	**h**	-	-	-	-	-	-	-	-	**h**	**h**	**h**	**h**	**h**	**h**
5117	80 M	pleural effusion	-	ns	-	-	-	**v**	-	-	-	-	-	-	-	-	**h**	-
5038	85 M	N/A	-	-	-	-	**h**	**h**	-	**h**	**h**	-	-	-	ns	-	**h**	**h**
5127	76 F	colon cancer	ns	ns	-	-	-	-	n/a	-	**h**	**v**	**h**	**hv**	**h**	ns	ns	ns
5134	94 M	congestive heart failure	ns	-	**h**	**h**	-	ns	-	-	-	-	**hv**	**hv**	-	-	ns	**h**
5024	59 F	N/A	-	-	-	-	ns	ns	-	**h**	**h**	**h**	**h**	-	**h**	-	-	-
5103	78 M	N/A	**hv**	**v**	**h**	**hv**	**h**	**h**	**v**	**v**	-	-	**hv**	**v**	-	**v**	**h**	ns
5081	72 M	prostate cancer	**v**	**v**	-	-	-	**h**	**h**	**h**	**h**	-	ns	**h**	-	-	-	-
5120	87 F	congestive heart failure	-	-	-	-	-	**h**	-	-	-	-	**h**	-	-	-	-	ns
5124	90 M	COPD	**h**	-	**h**	-	-	**h**	**h**	-	-	-	-	**h**	ns	-	**h**	-
5055	95 M	cardiac failure	-	-	-	**h**	-	**h**	ns	-	-	-	-	**h**	-	-	-	-
5130	81 M	mantle cell lymphoma	-	**v**	-	**h**	-	**h**	-	-	-	-	**v**	-	-	-	-	-
5092	83 F	N/A	-	**h**	-	**h**	-	-	-	-	-	-	-	-	-	**h**	-	ns
5125	79 F	chronic airway obstruction	-	**h**	-	-	-	**v**	**v**	**h**	-	**hv**	-	**h**	-	**v**	-	ns
5121	95 M	cardiopulmonary arrest	**h**	-	-	-	**v**	-	-	-	-	**h**	-	-	-	-	-	-
5053	82 M	lung cancer	**v**	**v**	**v**	**v**	**v**	**v**	**h**	-	**hv**	ns	-	-	**hv**	**hv**	-	ns
5302	48 M	amyotrophic lateral sclerosis	-	**v**	-	**v**	**h**	**h**	-	**v**	**h**	-	ns	ns	-	-	**v**	-
9991	N/A	N/A	**v**	**v**	-	-	-	-	-	**v**	-	-	-	**v**	**v**	**v**	-	-
9992	N/A	N/A	**hv**	**h**	**v**	**hv**	**h**	**v**	**v**	-	-	-	**h**	**h**	**h**	-	**h**	-
9994	N/A	N/A	-	**hv**	**v**	**hv**	ns	**v**	-	-	-	ns	ns	-	-	**h**	-	**h**
HSV-1 positive / total ganglia by site			**18/58**		**23/58**		**25/60**		**15/50**		**14/47**		**25/48**		**18/58**		**12/36**	
VZV positive / total ganglia by site			**16/58**		**11/58**		**14/60**		**8/50**		**4/47**		**11/48**		**10/58**		**1/36**	

Each row represents a different cadaver.

Age, sex and cause of death of last 3 donors could not be determined at time of retrieval (i.e., identification tag had been removed from the specimen or the head was separated from the rest of the body).

*as recorded on death certificate;

†no information available; COPD = chronic obstructive pulmonary disease

ns – no sample
n/a – not amplifiable for chromosome 18

(h) – sample positive for HSV-1
(v) – sample positive for VZV
(-) – sample negative for HSV-1 and VZV

TABLE 2

Primers and conditions used for PCR reactions.

Name	Location*	Size (bp)	Primers Sequence (5'-3')	PCR conditions
Chromosome 18	1259[7]	149	CTTAATGAAAACAATGCCAGAGC TGCAAAATGTGGAATAATCTGG	35 cycles of 94°C (30s), 51°C (30s), 72°C (1m)
HSV-1	U_L30[6]	345	CCAACACAGACAGGGAAAAG GGAACATGCTGTTCGACCAG	35 cycles of 94°C (30s), 52°C (30s), 72°C (1m)
		196	CAGACAGCAAAAATCCCCTGAG ACGAGGGAAAACAATAAGG	25 cycles of 94°C (30s), 48°C (30s), 72°C (1m)
HSV-2	U_S6[9]	285	AGGCCTACCAACAGGGCGTG CCTGGATCGACGGGATGTGC	35 cycles of 94°C (30s), 58°C (30s), 72°C (1m)
		210	CACCGTCGCCCTATACAGCTT ATCGACGGGATGTGCCAGTTT	25 cycles of 94°C (30s), 56°C (30s), 72°C (1m)
VZV	ORF29[6]	271	ACGGGTCTTGCCGGAGCTGGTAT AATGCCGTGACCACCAAGTATATT	35 cycles of 94°C (30s), 55°C (30s), 72°C (1m)
		207	TTCTGGCTCTAATCCAAGGCG ACTCACTACCAGTCATTTCT	25 cycles of 94°C (30s), 49°C (30s), 72°C (1m)

*Reference numbers for primers are included in brackets.

HSV-1 and VZV PCR amplifications were performed in triplicate and all HSV-2 reactions were done in duplicate. Primers specifically amplified sequences within the DNA polymerase gene in the unique long (U_L) segment of HSV-1, the open reading frame (ORF) 29 in the VZV genome, and the glycoprotein D gene in the unique short (U_S) segment of HSV-2.