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Incidence of herpes simplex virus types 1 and 2 isolated in patients with herpes genitalis in Sheffield

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SUMMARY Thirty-one strains of herpesvirus (HSV), isolated from patients presenting with the clinical features of herpes genitalis, were typed by polypeptide analysis of virus proteins in sodium dodecyl sulphate polyacrylamide gels. Nineteen (61·3%) of the isolates were shown to be HSV type 1 and 12 (38·7%) HSV type 2. There was no obvious difference in the incidence of HSV-1 in primary or recurrent infections and no apparent correlation between the genital site of isolation and virus type. The high incidence of genital HSV-1 infection in this group of patients is probably due to the increased practice of oro-genital contact and has possible implications for the future development of drugs and vaccines in the control of genital herpes.

Introduction

Herpesvirus hominis (HSV) is becoming an increasingly common cause of genital infections. Despite the development of circulating antibodies to the virus many people develop recurrent infections, usually at or close to the site of primary infection but sometimes remote from it.[1]

Herpesvirus hominis may be divided into HSV type 1 (HSV-1) and HSV type 2 (HSV-2) by serological criteria, and it is generally accepted that there is a difference in the site of involvement and modes of transmission for the two serotypes.[2 3] Thus, HSV-1 is usually transmitted non-venereally and affects non-genital sites, including the mouth, lips, skin above the waist, eyes, and brain; HSV-2 is most often transmitted venereally causing infection of the genitalia and skin below the waist. Nahmias and Starr[1] found that 89% of female and 97% of male urogenital infections were due to type-2 virus. Other reports, however, indicate that HSV-1 may play a greater part in genital infections than was previously thought. In a survey in Edinburgh, Smith, Peutherer, and Robertson[4] found 14 of 64 (22%) female and 11 of 124 (9%) male genital isolates to be HSV-1; in the United States Kaufman[5] isolated HSV-1 from nine of 67 (13·4%) genital infections; and in a Japanese study 23 of 53 (43%) genital isolates from female patients were found to be HSV-1.[6]

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Previous studies of the incidence of HSV-1 in genital infections have relied on the use of kinetic or quantal neutralisation tests or immunofluorescence to type the virus[7 8]; these tests are often difficult to perform, and the results are not always clear-cut. In this study viruses were typed by analysis of the polypeptides present in infected cells using sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE), since there are many reports of distinct intertypic differences between HSV-1 and HSV-2 structural and infected cell polypeptides.[9 10] This technique allows unequivocal identification of HSV-1 and HSV-2 strains. In addition, 25 of the isolates described in this report have also been typed by digestion of virus DNA with restriction enzymes and separation of the resultant fragments on agarose gels. Since HSV-1 and HSV-2 virus DNA have been shown to have no coincident sites for any of the restriction enzymes which have been tested, they give easily identifiable patterns on electrophoresis, and this typing method again gives unequivocal results.[11-13] Using these techniques the incidence of HSV-1 and HSV-2 in genital lesions was studied in a series of patients attending the sexually transmitted diseases clinic in Sheffield.

Materials and methods

TISSUE CULTURE

BHK-21 (C13) cells (obtained from Flow Laboratories, Irvine, Scotland) were grown as monolayers in Glasgow modified Eagle's medium containing 10% (w/v) tryptose phosphate broth and 10% (v/v) newborn-calf serum. Cells were subcultured at regular intervals on a split ratio basis.

VIRUSES

HSV-1 (71-15) and HSV-2 (333) (obtained from F Rapp, Milton S Hershey Centre, Pennsylvania, USA) were propagated at low multiplicity in BHK-21 cells.

ISOLATION FROM SWABS

Swabs were taken of vesicular fluid or secretions from ulcerated genital lesions and immediately placed in a transport medium. On receipt this was divided into aliquots and stored at −80°C until used for inoculation of cells.

Confluent cultures of cells in 2-oz or 4-oz glass medical flats were inoculated with 0·5-1·0 ml of transport medium. The inoculum was adsorbed for one hour at 37°C, the monolayer washed once with Eagle's minimal essential medium containing 2% (v/v) newborn-calf serum (EMM), and 10 ml EMM then added to each bottle. The cells were maintained at 37°C with changes of medium every 3-5 days and observed daily for cytopathic changes (CPE). The monolayers were observed for a minimum of 14 days or until cell degeneration occurred. Cultures which showed 75% CPE were frozen at −80°C until harvested.

PREPARATION OF VIRUS STOCKS

Positive cultures were thawed at 37°C, the cells scraped into the medium, and the cell suspension sonicated at full power for 30 seconds using an MSE ultrasonic disintegrator. After cell disruption to release intracellular virus, the suspension was centrifuged for 10 minutes at 2000 × *g* and the supernatant containing the released virus stored in 1-2 ml aliquots at −80°C. Viruses were passed 1-3 times in BHK-21 to increase the titre; the final virus pool was prepared by inoculating confluent monolayers of BHK-21 cells in 4-oz medical flats with 0·01-0·1 plaque-forming units (pfu) per cell, and the cells harvested after 48 hours' incubation.

VIRUS TITRATION

For the determination of an infectivity titre, confluent monolayers in 5-cm plastic Petri dishes (Sterilin, Middlesex) were inoculated with 0·1 ml of an appropriate virus dilution. The virus was allowed to adsorb for one hour at 37°C and then 5 ml of EMM containing 0·8% carboxymethylcellulose was added to each dish. The dishes were further incubated at 37°C in an atmosphere of 5% CO_2 in air for 3-4 days; after this time the cells were fixed with 10% formol saline, stained with Leishman's stain, and the plaques counted.

LABELLING OF INFECTED CELL POLYPEPTIDES

The labelling and preparation of infected cell polypeptides with ^{35}S-methionine in BHK-21 cells was performed as described by Lonsdale *et al.*[14]

GEL ELECTROPHORESIS

Samples containing equal numbers of trichloracetic acid precipitable counts were analysed on 18 × 14 cm slab gels of 8·5% acrylamide with a 5% stacking gel using the discontinuous buffer system of Laemelli.[15] A set of proteins of known molecular weight (obtained from Pharmacia, Hounslow, Middlesex) were included in each gel together with the ^{35}S-methionine-labelled infected cell polypeptides of the laboratory strains of HSV-1 (71-15) and HSV-2 (333). Electrophoresis of the gels and the procedures used in staining and destaining were the same as described by Lonsdale *et al.*[14] Gels were dried under vacuum using a Bio-Rad gel drier and exposed to *x*-ray film for varying lengths of time.

PATIENTS

The first 21 specimens were taken from consecutive patients with clinically obvious genital herpes seen between March and May 1980. This selection favoured the inclusion of patients with severe primary genital infections. The remaining 10 patients were seen between 29 October and 11 November 1980; during this period consecutive patients who presented with any form of genital ulceration were routinely tested for herpes virus. Except for patients 13 and 28 the cases were unrelated.

Results

VIRUS IDENTIFICATION

Virus samples labelled with ^{35}S-methionine were electrophoresed in 8·5% polyacrylamide gels as described above; a typical gel is shown in the figure. The principal feature used to identify the strain of virus was the molecular weight of the major capsid protein which is greater in HSV-2 than HSV-1.[9 10] All the strains of HSV-1 analysed also gave rise to a heavily labelled band of molecular weight 122K which was absent from all the strains of HSV-2 examined. Although several differences between both virion and infected cell polypeptides have been reported,[9 10] these were not always apparent in the gel system used. To confirm the accuracy of this method of typing the virus, 25 isolates were also typed by the restriction enzyme technique of Lonsdale.[16] In all the 25 isolates examined the polypeptide and restriction endonuclease analysis were in agreement.

CLINICAL ISOLATES

A total of 31 isolates from both male and female patients was typed; the source and the identification of virus type is shown in table 1. Nineteen (61%) of the strains were of HSV-1, as shown by virus polypeptide and restriction enzyme analysis. Analysis of the relationship between the virus type and the nature

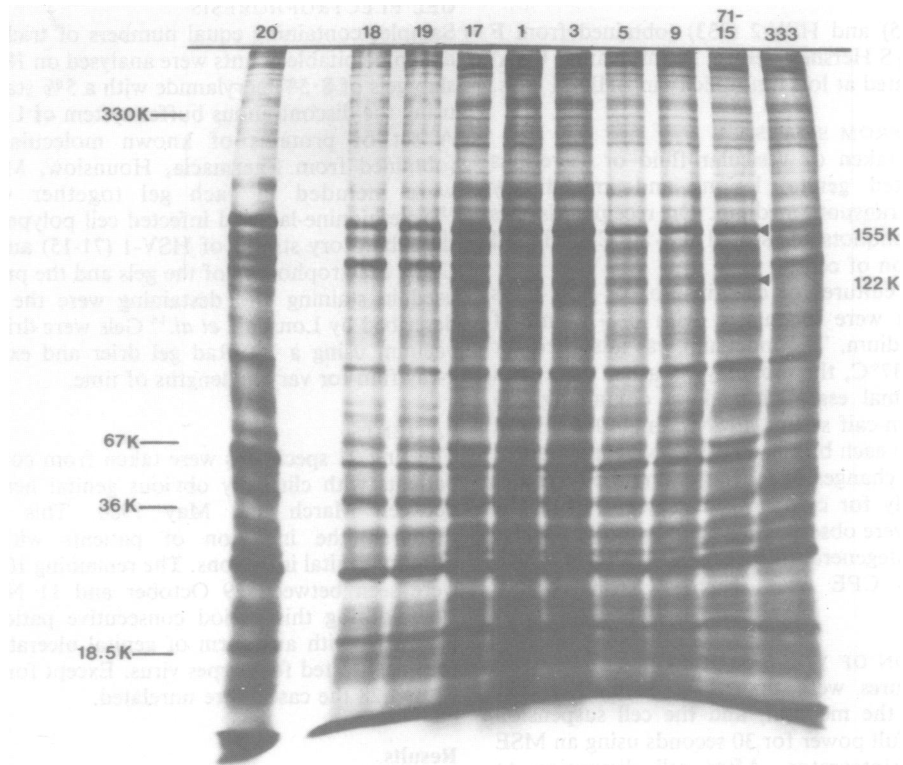

FIGURE *Sodium dodecyl sulphate polyacrylamide gel electrophoresis showing two strains of HSV-2 (18 and 19), six strains of HSV-1 (20, 17, 1, 3, 5, and 9), and the laboratory strains of HSV-1 (71-15) and HSV-2 (333). The position of the standard proteins of known molecular weight (thyroglobulin 330 K, bovine serum albumin 67 K, lactate dehydrogenase 36 K, and ferritin 18·5 K) are shown together with the major capsid polypeptide of HSV-1 (molecular weight 155 K) and the highly labelled band of molecular weight 122 K used in typing the isolates.*

of the genital lesion showed no apparent correlation; however, most of the isolates from men with primary infections were type 1. The distribution of types among primary and recurrent infections is shown in table II. There was no marked difference between the incidence of HSV-1 in primary (14/22) and recurrent (5/9) infections.

Discussion

Herpes genitalis is a disease which has increased in incidence in recent years; the infection is traditionally held to be associated with HSV-2, and this virus serotype is also considered an aetiological agent of carcinoma of the cervix.[17] The results of the present study do not confirm the marked association of genital herpes with the HSV-2 serotype reported by other workers. Thus, HSV-1 was recovered from genital sites with a much greater frequency than has previously been supposed and was the agent of both primary and recurrent genital infections in both sexes.

All 14 patients with primary HSV-1 infections admitted orogenital contact compared with four of eight of those with HSV-2 infections. Oro-genital contact was admitted less frequently by those with recurrent genital herpes. Patients with HSV-1 isolates admitted to fewer partners in the month before their attendance than did those with HSV-2 isolates. Fourteen of the 19 (74%) patients from whom HSV-1 was isolated admitted a single partner only compared with five of the 12 (42%) with HSV-2 isolates. In one instance HSV-1 was isolated from the cervix of a woman who had presented as the contact of a man who had previously been seen with primary genital herpes; in this case transmission was probably from the female patient's oral herpes via oro-penile contact, with subsequent transmission of virus to the cervix from the primary penile infection. The high incidence of genital infections with HSV-1 in this

TABLE I *Incidence of HSV-1 and HSV-2 isolated from a series of 31 patients*

Patient No (sex)	*Age (years)*	*HSV type**	*Restriction endonuclease†*
1 (M)	32	1	EcoRI, Hind III
2 (M)	27	1	Hind III
3 (F)	27	1	Hind III
4 (M)	25	1	Hind III
5 (M)	19	1	Hind III
6 (M)	22	1	Hind III
7 (M)	33	1	EcoRI, Hind III
8 (F)	23	1	ND
9 (M)	35	1	EcoRI, Hind III
10 (F)	16	1	EcoRI, Hind III
11 (F)	36	1	SmaI, Hind III
12 (F)	20	1	ND
13 (M)	19	2	Hind III
14 (M)	35	1	EcoRI, Hind III
15 (M)	51	2	EcoRI, Hind III
16 (F)	36	2	EcoRI, Hind III
17 (F)	16	1	EcoRI, Hind III
18 (M)	22	2	Hind III
19 (F)	17	2	ND
20 (F)	17	1	Hind III
21 (F)	17	2	Hind III
22 (M)	18	1	Hind III
23 (F)	20	2	ND
24 (F)	17	1	ND
25 (F)	42	2	EcoRI, Hind III
26 (F)	20	2	EcoRI, Hind III
27 (F)	26	1	ND
28 (F)	24	2	Hind III
29 (F)	25	2	EcoRI, Hind III
30 (F)	22	2	Hind III
31 (F)	22	1	EcoRI, Hind III

*Virus type as determined by analysis of infected cell polypeptides
†Restriction endonuclease used to confirm virus type.
ND = not done

TABLE II *Incidence of primary and recurrent infections with HSV*

HSV	*Primary infections*		*Recurrent infections*		*Total*
	Men	*Women*	*Men*	*Women*	*(%)*
Type 1	6	8	3	2	19 (16·3)
Type 2	1	7	2	2	12 (38·7)

group of patients may be the result of increasing acceptability and practice of oro-genital contact. This may be one factor which could explain why genital herpes infections appear to be increasing at a much greater rate than other sexually transmitted diseases. Transmission of HSV-1 by oro-genital contact may also explain the occurrence of herpes genitalis within sexually stable relationships where there have been no other partners.

The high frequency of HSV-1 isolation found in this group of patients has important implications if the results are confirmed in other centres. Firstly, the susceptibility of the two serotypes to treatment with antiviral agents varies; in general HSV-1 is more susceptible to antiviral drugs than HSV-2.[18 19] Therefore, in the assessment of antiviral compounds, reference must be made to the virus serotype since this may influence the results. Secondly, the proposed development of an HSV-2 vaccine for the control of herpes genitalis may have to be reconsidered should HSV-1 be shown to be an important aetiological agent of genital infection. Thirdly, the possible importance of HSV-2 as an aetiological agent of carcinoma of the cervix has been extensively reviewed[13 17]; but the present findings suggest that a similar role for HSV-1 should be investigated.

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