Acta virol. **28** : 97—106, 1984

EFFECTS OF HERPES SIMPLEX VIRUS STRAINS ON HUMAN FIBROBLAST AND LYMPHOCYTE CHROMOSOMES AND THE LOCALIZATION OF CHROMOSOMAL ABERRATIONS

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Received January 20, 1983; revised June 6, 1983

Summary. — The effects on human chromosomes of types 1, 2 and of intermediate serotype of herpes simplex virus (HSV) was compared in fibroblast and lymphocyte cultures. The karyological changes due to HSV were shown to depend on the serotype used as well as on the kind of cells examined (agent-specificity and cell-reaction specificity). Differences were noted among the strains in relation to the degree and character of the aberrations induced. Conventional Giemsa staining and the trypsin G-banding techniques were used to localize aberrations in the length of human fibroblast and lymphocyte chromosomes after HSV infection. A non-random damage of chromosomes 1 and 3 displaying the same pattern in either cell type was established. The distribution of chromosomal abnormalities was independent of the chromosome length. The topographic banding analysis of lesions induced by strains of HSV-1, HSV-2 and intermediate serotypes showed that the most frequent aberrations were localized in bands p32, p34, q21 and q32 of chromosome 1 and in the band q21 of chromosome 3. The localization of the most frequently occurring aberrations in the chromosomes belonging to other groups was also determined.

Key words: *herpes simplex virus*; *human chromosomes*; *band staining techniques*; *aberrations*; *human lung fibroblasts*; *lymphocytes*

Introduction

It is well known that HSV induces chromosomal aberrations in cultured cells of both animal and human origin (Hampar and Ellison, 1961; Stich *et al.*, 1964; Huang, 1967; Shubladze *et al.*, 1967; O'Neill and Milles, 1969; Huang *et al.*, 1972; Webber and Whalley, 1977). The available data on the action of HSV on chromosomes are mainly concerned with strains belonging to the 1st serotype. Comparative studies on the effect on human chromosomes of HSV-1 and HSV-2 strains are limited (O'Neill and Milles, 1969; Nachtigal and Li, 1980). They revealed some differences in the action of the

two serotypes as well as in the action of HSV strains of the same serotype (Duff and Rapp, 1971, 1973; Seth *et al.*, 1974; Dundarov *et al.*, 1980; Nachtigal and Li, 1980). Therefore, we decided to explore the pattern of cytopathic action on the chromosomes of infected cells of various HSV strains belonging to different serotypes. All strains examined were isolated in Bulgaria and some of them were used for preparation of the Bulgarian inactivated HSV vaccines. The homogeneous staining of chromosomes in cytogenetic studies as performed until 1971 allowed only the identification of chromosome groups B, C, D and E and the approximate localization of aberrations in chromosomes group A (Hampar and Ellison, 1963; Stich *et al.* 1964; Tanzer *et al.*, 1964; Zacepin *et al.*, 1970; Huang *et al.*, 1971). Introduction of various methods for band staining of chromosomes extended the range of cytogenetic analysis. This report presents data concerning the distribution of HSV-induced aberrations along the length of chromosomes from human fibroblasts and lymphocytes and their band localization.

Materials and Methods

Cell cultures. Human diploid embryonic lung fibroblasts (HL) were used in the 11th or 12th passages. The cells were obtained from the Laboratory of Cell Cultures, Research Institute of Infectious and Parasitic Diseases, Sofia. The cultures met the international standard requirements for diploid cell strain (Minutes, 1970). Cells were grown in Eagle's medium (Difco) supplemented with 15% bovine serum, 100 U/ml penicillin and 250 g/ml kanamycin. The lymphocytes obtained from peripheral blood of healthy female and male donors were cultured in medium RPMI-1640 (Flow) containing phytohaemagglutinin (PHA, Wellcome) at concentration 0.04—0.05 mitogenic units/ml. The fibroblasts and lymphocytes were infected at 22 and 65 hr after seeding, respectively.

Viruses. The following HSV strains were used: DP, TM — type 1; DD_2, BJ — type 2 and MB_I — intermediate serotype (Dundarov *et al.*, 1980). The strains were isolated in Research Institute of Infectious and Parasitic Diseases, Sofia during the period 1963—1973. The multiplicity of infection (m.o.i.) varied from 1.5 to 1.9. The effect of infection on the cell karyotype was tested at 2, 4, 6 and 8 hr post-infection (p.i.).

Cytogenetic analysis. Cells were treated with 0.3 μg/ml colcemid (Ciba) for 2 hr followed with 0.075 mol/l potassium chloride for 15 min and fixed in 3 changes of a mixture of methanol-acetic acid (3 : 1). Finally the chromosome preparations were air dried. Uninfected cell cultures at each interval served as controls. Hundred to 300 metaphases were studied in each case. Polyploidy was assessed on 500 cells. The results were statistically treated by the chi square method of Pierson and the method of Fischer. After viewing the densely stained chromosomes, the same slides were destained in 80% ethyl-alcohol for 3—4 min until complete removal of the dye, then preheated at 90°C according to Manolov (Institute of Oncology, Sofia) and treated with trypsin G according to Wand and Fedoroff (1972). Cells with clearly expressed banding were used to determine the location of chromosomal damage. The latter was photographed at magnification 1000×. The aberrations were localized according to the diagrams of Manolov (in press) in agreement with the nomenclature of the Paris Bands System (Paris Conference, 1971). The analysis of the aberrations of both cultured fibroblasts and lymphocytes did not show any differences and thus the distribution of the lesions was limited only to the lymphocyte cultures.

Results

Chromosomal aberrations in HL cells and human lymphocytes

Various structural chromosomal aberrations were observed in HL cells and human lymphocytes after infection with the given five HSV strains.

Table 1. Chromosomal aberrations in HSV-infected HL cells

Aberration	HSV strain					
	DP	TM	DD_2	BJ	MB_1	Control
Chromatid breaks	4*	4	3	2	4	—
Chromosome breaks	2	2	1	—	2	—
Gaps	30	50	30	18	20	3
Endoreduplication	3	2	—	—	1	—
Pulverization	—	1	—	—	1	—
Despiralization in the centromere region	5	3	1	—	—	—
Metaphases with severe chrom. damage***	10	13	10	4	4	—
Acentric fragment	—	3	—	—	—	—
Cells with more than one aberration	9	7	4	—	4	—
Total	54 (25.2%)	85 (30.4%)	45 (15.7%)	24 (12.8%)	32 (26.6%)	3 (2.0%)
Number of cells examined	214	279	285	186	120	150
P(t)	$p < 0.001$					
Chromatid breaks	4**	2	3	—	4	—
Chromosome breaks	—	—	—	—	—	—
Gaps	3	6	2	5	8	5
Metaphase with severe chrom. damage	15	13	19	8	12	—
Cells with more than one aberration	5	4	—	2	4	—
Total	22 (33.8%)	21 (40.3%)	24 (30.7%)	13 (30.9%)	24 (53.3%)	5 (2.5%)
Number of cells examined	65	52	78	42	45	200
P(t))	$p < 0.001$					

* Number of aberrations at 6 hr p.i. (out of total).
** Number of aberrations at 8 hr p.i. (out of total).
*** Multiple chromatid and chromosome breaks and gaps, including exchanges.

Metaphases containing chromatid and isochromatid breaks and gaps, pulverizations, endoreduplications, exchanges and acentric fragments were seen in the infected cultures. The prevailing type of lesions were gaps in all samples of HSV-infected cells. All categories of aberrations were found except of metaphases with despiralized chromosome areas especially in pericentromeric regions of few or many chromosomes and of severe damage to chromosomes; the latter were evident only in fibroblast cultures.

The number and the type of the chromosomal aberrations in infected cultures at 6 and 8 hr p.i. are shown in Tables 1 and 2. In contrast to the uninfected control cultures which showed no increase in chromosomal lesions, both infected cultures showed a significant increase in the number of cells with aberrations. The difference in the incidence of damage metaphases in HL cells by 6 hr p.i. was significant for the strains of both serotypes ($p < 0.05$). In HL cells, the strains MB_1 and TM caused the highest number of

Table 2. Chromosomal aberrations in HSV-infected lymphocytes

Aberration	HSV strain					
	DP	TM	DD_2	BJ	MB_1	Control
Chromatid breaks	5*	4	6	5	14	—
Chromosome breaks	3	1	3	1	8	—
Gaps	17	21	30	22	30	2
Endoreduplication	—	—	—	—	2	—
Acentric fragment	1	—	—	—	—	—
Exchanges	—	—	3	—	3	—
Cells with more than one aberration	6	3	8	3	12	—
Total	26 (22.8%)	26 (13.8%)	42 (25.9%)	29 (22.1%)	57 (23.5%)	2 (1.0%)
Number of cells examined	114	188	162	131	242	200
P(t)	$p < 0.001$					
Chromatid breaks	5**	3	5	3	10	—
Chromosome breaks	7	4	8	5	6	—
Gaps	12	10	17	14	21	4
Endoreduplication	—	2	—	—	—	—
Acentric fragment	4	3	3	2	—	—
Exchanges	2	—	1	—	4	—
Cells with more than one aberration	8	4	7	4	10	—
Total	30 (20.0%)	22 (12.5%)	34 (22.3%)	24 (17.4%)	41 (22.7%)	4 (2.1%)
Number of cells examined	150	175	152	140	180	190
P(t)	$p < 0.001$					

* Number of aberrations at 6 hr p.i. (out of total)
** Number of aberrations at 8 hr p.i. (out of total)

aberrations as compared to those produced by other HSV strains at 6—8 hr p.i. In some cases, the number of the cells examined at 8 hr was smaller than 50 due to the extreme cytopathic effect. Metaphases with severe chromosomal damage were more frequent at 8 hr p.i. In cultured lymphocytes the highest incidence of metaphases with aberrations was visible with strains MB_1 and DD_2 at 6—8 hr p.i. A significant difference ($p < 0.01$) in the frequency of aberrations induced was noted by all strains at 8 hr p.i.

Table 3. Assignment of breaks and gaps to particular chromosomes and chromosome groups in HSV-infected human lymphocytes

Strain		A-1		A-2		A-3		B		C		D		E	
	n_1/n_2	n	%	n	%	n	%	n	%	n	%	n	%	n	%
DP	93/430	24	25.80	20	21.50	12	12.90	16	17.20	9	9.67	2	2.15	10	10.75
DD_2	80/323	19	23.75	10	12.50	13	16.25	13	16.25	20	25.00	3	3.75	2	2.50
MB_1	85/441	18	21.10	7	8.23	18	21.10	14	16.40	16	18.80	7	8.23	5	5.80

n_1 — Aberrations (total)
n_2 — Cells examined (total)

Table 4. Distribution of breaks and gaps along the length of chromosomes of cultured human lymphocytes after infection with HSV strain DD_2

Chromosome No.	Number of aberrations		Difference	P (χ^2)
	observed	expected		
1	19	7.83	+11.17	<0.001
2	10	3.33	— 2.67	>0.05
3	13	6.15	+ 6.85	<0.001
4	13	10.95	+ 2.05	>0.05
5				
6—12, XX	20	34.84	—14.84	<0.01
13—15	4	9.08	— 5.08	>0.05
16—18	2	7.78	— 5.78	<0.01
19—20	0	4.09	— 4.09	
21—22	0	2.92	— 2.92	
Total	91	91.00		

The distribution of the aberrations along the length of the densely stained chromosomes group A and of the other chromosome groups following infection of lymphocytes with HSV strains DP, DD_2 and MB_1 is given in Table 3.

Distribution of chromosomal breaks and gaps

After infection with the chosen strains, the distribution of HSV-induced breaks and gaps was compared with the relative length of each chromosome

Table 5. Distribution of breaks and gaps in bands of the chromosomes of cultured human lymphocytes after HSV infection

Chromosome No.	short arm (p)	long arm (q)
1	1p32; 1p34	1q21; 1q32
2	2p15; 2p21	2q31; 2q33
3	3p21	—
4	—	4q31
5	—	5q31
6	—	6q21
7	7p13; 7p15	7q22; 7q32
8	—	8q22
9	—	9q32
11	11p13	—
12	—	12q24
13	—	13q14
14	—	14q24
15	—	15q22
16	—	16q24
17	—	17q22
18	—	18q21

Table 6. Distribution of break and gap points in chromosomes 1 and 3 in cultured human lymphocytes after HSV infection

Strain	Number of aberrations	Chromosome 1 p (short arm) region	n	region	n	q (long arm) region	n	region	n
DP	16	1p32	5	1p34	3	1q21	2	1q32	6
DD_2	12	1p32	3	1p34	2	1q21	2	1q32	5
MB_1	14	1p32	4	1p34	2	1q21	1	1q32	7
Total	42		12 (28.5%)		7 (16.6%)		5 (12.0%)		18 (43.0%)
		Chromosome 3							
DP	8	3p14	2	3p21	6	—			
DD_2	7	3p14	—	3p21	7	—			
MB_1	10	3p14	3	3p21	7	—			
Total	25		5 (20%)		20 (80%)				

of the human karyotype (according to Levan and Nichols, 1964). Table 4 shows the distribution of aberrations induced by the strain DD_2 only, because no significant differences were found. The results obtained with all three strains mentioned above showed statistically significant difference between the observed and expected distributions. That was demonstrated by additional aberrations seen in chromosomes 1 and 3, and in chromosomes of group B as well as by the defficiency of aberrations in groups C, D, E and F. The statistical analysis of these results indicated that the distribution of aberrations was non-random and was not proportional to the chromosomal length.

The localization of the most frequently observed aberrations was practically the same in both cell types as presented in Table 5 and Fig. 1 (Plate XIII). The percentage of break and gap points in the most frequently damaged chromosomes 1 and 3 is given in Table 6.

Discussion

Our observations showed an obvious increase of chromosomal aberrations in both cell cultures even in the first hours after infection with different HSV strains. These results were in accord with the suggestion that lesions induced by HSV result from the action of an early enzyme specified by the virus (Waubke *et al.*, 1968). The numbers and the types of chromosomal aberrations depended on the duration of virus effect. A marked incidence of metaphases with severe chromosomal damage and increased number of

altered chromosomes per metaphase were observed at later intervals. The effect in question was similar to that observed by other authors in human embryonic lung cells infected with HSV (Stich *et al.*, 1964; Shubladze *et al.*, 1967; Waubke *et al.*, 1968). In addition to the structural chromosomal lesions, endoreduplications were also observed in both cultures under study. Although the values relevant to endoreduplication were low, it was claimed that polyploidy was a very rare finding in vitro regarded for the expression of a disturbance in cell division (Obe, 1965). Chromatid gaps were the predominant aberration observed in the HSV-infected fibroblasts and lymphocytes. Taking the importance of these aberrations in consideration, we favour the concept that such lesions are suggestive of either a state preceding mutation with capacity to regain original structure or indicate a marked change in a constant mutation (Brøgger, 1971). The conventional system was used for differentiation of chromatid breaks from gaps as recommended by Chatham Conference, 1971 (Moorhead *et al.*, 1974). Significant variations were found among the capacity of the five HSV strains tested to induce chromosomal aberrations. Strain TM of the 1st and strain MB_1 of the intermediate serotypes produced the largest percentage of lesions in HL cells, while the highest incidence of abnormal metaphases as observed in lymphocytes infected with strain DD_2 of the 2nd and strain MB_1 of the intermediate serotypes. These differences could be ascribed to the different conditions of virus replication as well as to the strain specificity.

Two types of human cells used, i.e. fibroblasts and lymphocytes revealed some differences in the cell response to virus infection: a) metaphases with despiralized centromeric regions of few or many chromosomes; b) appearance of metaphases with severe chromosomal damage occurring only in cultured fibroblasts. Different chromosomal abnormalities were observed, when the effect of SV40 in cell cultures of different origin was investigated. This virus induces clear-cut endoreduplications in human lung fibroblasts (Mincheva, unpublished observations), although other authors (Lehman, 1974) failed to demonstrate them in Chinese hamster cell cultures and stressed their extremely rare occurrence.

An acentric fragment was demonstrated in both cells examined. We did not succeed in obtaining a well expressed banding of the acentric fragments in all slides which prevented its precise identification. Unidentified specific structure has been observed in group C of human lymphoid lines (Kohn *et al.*, 1967) carrying the genome of Epstein-Barr virus (Zur Hausen *et al.*, 1972). The acentric fragment observed as well as the despiralized pericentromeric regions of chromosome 1 and some other chromosomes require further attention.

The observation on densely stained chromosomes was a general indication of the degree to which HSV-induced chromosomal aberrations tended to accumulate in definite chromosomal groups. These results testify to specificity of the virus action. A common feature of the action of the strains belonging to three serotypes was the preferential damage of chromosomes 1 and 3 and the equal extent of damage of chromosome 2, followed by group B

chromosomes. The data obtained by different authors for densely stained chromosomes suggested certain non-randomness of the viral effect (Stich *et al.*, 1964; Markaryan *et al.*, 1969; Huang *et al.*, 1971; Webber and Whaley, 1977). It is known that the susceptibility of different hybrid cells to HSV infection is always connected with the human chromosome 3 (Carrit and Goldfarb, 1976).

The data indicate that certain regions of chromosomes 1 and 3 appear to be selectively affected by HSV infection. The specific regions in chromosome 1 appear to be p32 and p34 of the short arm and q21 and q32 of the long arm. As far as these are concerned it is noteworthy that some regions in chromosomes 1 and 17 in myeloid cells were preferentially damaged. The specific region of the long arm of chromosome 1 (q25—q32) was trysomic in each one of the 32 patients suffering from various diseases. Some clustering of aberrations at bands p32, p36, q32, q21 and q12 was also demonstrated (Rowley, 1977). The coincidence in the localization of HSV induced aberrations in chromosome 1 with some of the mentioned regions raises the question whether the locations of breaks and gaps are an expression of a definite aetiologic agent or whether they might be the indication of sites subjected to aberration.

Studies on the effect of adenovirus 12 on cultured human cells led to the recognition of four places of damage in chromosome 1 — bands p32, p36, q12 and q42 and two in chromosome 17 — q21 and q22, the latter being the locus for thymidine kinase (McDougal *et al.*, 1973). In the case of simian virus 40, chromosomes 7 and 17 were essential for the transformation of the cells (Croce and Koprowski, 1975; Croce, 1977). Supposing that chromosomal aberrations might represent gene locuses that are involved in cell regulation (Rowley, 1974), our data of non-random damage in definite regions of chromosomes 1 and 3 support the notion about a definite gene relation of these chromosomes to HSV infection. The presented findings on banding localization of the chromosomal aberrations could stimulate further investigation on HSV-induced chromosomal abnormalities and facilitate the mapping of enzymes in the host genome.

Acknowledgements. The authors are greatly indebted to Dr. George Manolov, Institute of Oncology, Sofia for his continuous interest and helpful suggestions.

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