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SELECTIVE CHROMOSOMAL DAMAGE CAUSED BY HUMAN CYTOMEGALOVIRUS

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Summary. — The effect of human cytomegalovirus (CMV) on human foetal cell chromosomes was investigated. Cultures of foetal skin fibroblasts were infected with human CMV (AD-169 strain) and their chromosomes analysed at intervals. The distribution of chromosomal abnormalities was independent of chromosome length.

Key words: *cytomegalovirus*; *human chromosomes*; *Giemsa banding method*

Introduction

The effect of viruses on the chromosomes of higher organisms has been known for years. The first studies involved the demonstration of chromosomal changes in Chinese hamster cells by herpes simplex virus (Hampar and Ellison, 1961). In the early sixties the effects of measles, SV40 and adenovirus have been documented (Cooper and Black, 1963; Nichols *et al.*, 1964; Štich *et al.*, 1964*a*, *b*). Analysis of blood cell chromosomes in patients with viral infections (Makino and Aya, 1968) and cytogenetic evaluation of congenital abnormalities presumed to be caused by intrauterine viral infections has been performed (Nusbacher *et al.*, 1967). Development of Giemsa banding techniques for chromosome identification helped considerably to study the specificity to virus-chromosome interactions. Since the integration of viral nucleic acids into the host chromosomes seems to be the main step in viral carcinogenesis, interaction of virus and host chromosomes should have a great importance in the outcome of the infection of the cells by potentially oncogenic viruses. It is believed that the cytogenetic analysis might contribute to the solution of the virus-cancer problem (Joachim, 1977; Sakízlí and Günalp, 1977).

As there are some hints indicating a possible oncogenic role of human CMV, we became interested in the distribution of chromosomal damages in CMV-infected cultured human cells to show whether there is any selectivity of CMV for human chromosomes.

Table 1. Number of abnormal metaphases at intervals p. i.

Hr p.i.	Normal	Abnormal	Total	Per cent
24	20	9	29	31
48	23	19	42	45
72	18	9	27	33
97	18	48	66	72
120	16	10	26	38
Control	70	6	76	8

Materials and Methods

Cell cultures were prepared by the fragmentation technique from foetal skin tissues. The cells were used between the 5th to the 10th passage. Minimum essential medium (MEM) supplemented with 10% foetal bovine serum and antibiotics was used throughout.

Cytomegalovirus (AD-169 strain) was obtained through the W.H.O. and maintained in primary human fibroblast cultures in our laboratory. The titre of the stock virus was $10^{5.5}$ TCD_{50}/ /0.1 ml. Human foetal cell cultures were infected at a multiplicity of 1. Uninfected cell cultures of the same passage level were used as control. Chromosome studies were performed at 24, 48, 72, 96 and 120 hr after inoculation (p.i.).

Mitotic cells were separated from the culture flask surface by shaking firmly and incubated with a 1 μg/ml solution of colcemide (CIBA), at 37° C for 15 min. The method of Moorhead *et al.* (1960) was used to prepare the chromosomes for staining. The metaphase chromosomes were stained by the Giemsa banding method (Finaz and Grouchy, 1972; Seabright, 1972) with minor modifications. Metaphases were photographed and karyotypes were prepared according to the Giemsa banding scheme standardized at the International Conference in Paris in 1971 (Paris Conference, 1972).

Results

The number of the abnormal metaphases in infected cultures at different intervals and in corresponding control cultures is shown in Table 1. The numbers of the anomaly types (Fig. 1 — see Plate XXVI) encountered at

Table 2. Anomaly types induced by CMV infection in the chromosomes of foetal human cells

Anomaly type	No. of anomaly types at hr p.i.					
	24 hr	48 hr	72 hr	96 hr	120 hr	Total
Gap	5	10	5	36	6	62
Break	3	11	4	59	5	82
Deletion	2	6	2	20	7	36
Translocation	3	7	4	23	2	39
Fragmentation	—	1	1	5	1	8
Deformation	—	—	—	5	—	5
Ring	—	1	—	4	1	6
Aneuploidy	—	—	—	2	—	2
Polypoidy	—	—	1	5	—	6
Endoreduplication	—	—	1	—	—	1
Markers	—	2	—	3	—	5
Total	13	37	18	162	22	252

Table 3. Distribution of chromosome anomalies in CMV-infected foetal human cell cultures

Chr. No.	24 hr		48 hr		72 hr		96 hr		120 hr		Total		
	I	II	I	II	I	II	I	II	I	II	I	II	III
1	1	0.94	5	2.66	3	1.01	12	11.75	3	1.64	24	18.02	12.70
2	2	0.89	0	2.53	2	0.96	18	11.16	5	1.56	27	17.12	15.04
3	1	0.76	5	2.15	1	0.82	17	9.51	1	1.33	25	14.58	16.35
4	0	0.70	5	1.98	1	0.76	13	8.77	2	1.22	21	13.45	14.89
5	2	0.67	5	1.91	0	0.73	11	8.46	1	1.18	19	12.98	13.96
6	1	0.56	1	1.86	1	0.71	10	8.21	1	1.15	14	12.59	10.60
7	0	0.59	1	1.69	0	0.64	9	7.46	1	1.04	11	11.44	9.17
X	0	0.57	0	1.61	0	0.61	4	7.13	1	0.99	5	10.93	4.36
8	0	0.54	0	1.55	1	0.59	10	6.86	1	0.96	12	10.52	10.87
9	0	0.53	1	1.51	0	0.57	5	6.68	1	0.93	7	10.25	6.51
10	1	0.51	5	1.44	1	0.55	6	6.39	1	0.89	14	9.80	13.63
11	0	0.51	0	1.45	1	0.55	2	6.42	0	0.89	3	9.84	2.90
12	0	0.51	0	1.47	0	0.56	3	6.49	1	0.90	4	9.95	3.83
13	0	0.41	2	1.81	1	0.45	4	5.20	0	0.72	7	7.98	8.36
14	0	0.39	0	1.21	1	0.42	7	4.95	0	0.69	8	7.60	10.04
15	0	0.38	1	1.09	0	0.21	2	4.81	0	0.67	3	7.38	3.87
16	1	0.37	1	1.06	0	0.40	3	4.67	0	0.65	5	7.17	6.65
17	1	0.36	1	1.02	0	0.39	3	4.52	0	0.63	5	6.94	6.87
18	0	0.32	1	0.92	0	0.35	0	4.08	0	0.57	1	6.25	1.52
19	1	9.29	0	0.84	0	0.32	1	3.71	0	0.52	2	5.70	3.34
20	0	0.28	0	0.80	0	0.30	0	3.56	0	0.49	0	5.46	0.00
21	0	0.21	0	0.59	0	0.22	6	2.64	1	0.37	7	4.05	16.46
22	1	0.22	0	0.64	0	0.24	4	2.84	1	0.39	6	4.35	13.14
Y	0	0.23	0	0.67	0	0.25	0	0.25	0	0.45	0	4.59	0.00
Total	12		34		13		150		21		230		

I — Anomaly values observed.

II — Expected anomaly values calculated by the following formula (Makino and Aya, 1968; Paris Conference, 1972):

$$\frac{\text{Standard relative chromosome length} \times \text{observed total anomaly number}}{\text{Total chromosome length (107.3)}}$$

III — Length-corrected anomaly values:

$$\frac{\text{observed anomaly value} \times \text{average chromosome length}}{\text{standard relative chromosome length}}$$

different intervals are given in Table 2. Non-identifiable abnormal chromosomes probably formed by translocation, deletion or deformations were classified as "marker chromosomes".

Distribution of anomaly types among different chromosomes was also studied. The number of observed and expected anomalies for each chromosome at different intervals and in total was compared to see whether there was any preference of CMV for different chromosomes (Table 3). Statistical analysis showed a significant difference between the expected and observed anomaly frequencies of the chromosomes. This difference seemed to be independent of the chromosome length.

Discussion

Several herpesviruses possess oncogenic properties. CMV was reported to transform cells (Rapp, 1974; Rapp and Li, 1974) and to have the characteristics of a DNA tumour virus. Since virus-host interactions occur at the chromosomal level during viral oncogenesis it was of interest to know whether any specific chromosomal aberration, as reported earlier for adenoviruses (zur Hausen, 1967), occurs on CMV infection of human foetal cell cultures.

Our results clearly indicate that despite of the absence of specific chromosomal aberrations, the distribution of chromosomal anomalies induced by CMV shows striking differences among the chromosomes. Chromosome Nos. 2, 3, 4, and 21 showed more anomalies as compared to other chromosomes. The number of anomalies was not proportional to the length of chromosomes. This finding may suggest some specific relation between these chromosomes and CMV. But it is not possible to conclude that the selective chromosomal damage induced by CMV has some direct relation to its oncogenicity.

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