THE ACTION OF INFLUENZA VIRUS ON HUMAN CHROMOSOMES

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The action of the A2/Hong Kong/68 strain of influenza virus on the chromosomes of human cells was studied in peripheral blood cultures from patients with influenza and in cultures of human diploid cells (HDC) infected with a reference strain of influenza virus. During epidemics a significant increase in the level of aneuploidy and of chromosomal aberrations was observed in the acute stage of the disease in various groups of patients with influenza, returning to the normal level in the period of convalescence. A significant increase in the level of aneuploidy (13-21%) and of chromosomal aberrations was discovered in infected HDC cultures between 6 and 24 h after infection compared with the control. The principal type of aberration both in peripheral blood cultures of influenza patients and in HDC cultures was of the chromatid type: deletions, single fragments, chromatid translocations, gaps.

The genetic aspects of interaction between viruses and cells continues to attract the attention of geneticists, virologists, and epidemiologists because of the unsolved problems of human intrauterine pathology [1, 7, 8]. So far as influenza virus is concerned, its role in the development of embryopathies has not yet been finally established.

The pandemic character of influenzal outbreaks, affecting large groups of the population and, in particular, women in various stages of pregnancy, makes the problem of the mutagenic action of influenza virus one of the utmost importance in modern human genetics. An important role in the investigation of this problem from all its aspects is played by the analysis of the cytogenetic changes arising in cells infected with influenza virus in vivo and in vitro.

TABLE 1. Frequency of Chromosomal Aberrations and Aneuploidy in Pregnant Women with A2/Hong Kong Influenza

Patient	No. of cells analyzed	No. of aneuploid cells			No. of cells with aberrations			
					total			
		total	hyperploid	hypoploid	abs.	%	chromosomal	chromatid
1	60	18,2±5	—	18,2	10	16,6±4,8	1	9
2	40	25±6,7	—	25	3	7,5±4,2	1	2
3	50	16±5,4	—	16	5	10±4,2	3	2
4	50	18±5,6	—	18	3	6±3,2	1	2
5	50	16±5,4	—	16	3	6±3,2	1	2
6	50	16±5,4	—	16	4	8±3,9	1	3
7	50	16±5,4	—	16	10	20±5,8	3	7
8	50	16±5,4	—	16	6	12±4,4	5	1
9	50	18±5,6	2	16	4	8±3,9	—	4
Total	450	17,2±1,8	0,8	16,4	48	10,6±1,5	16	32

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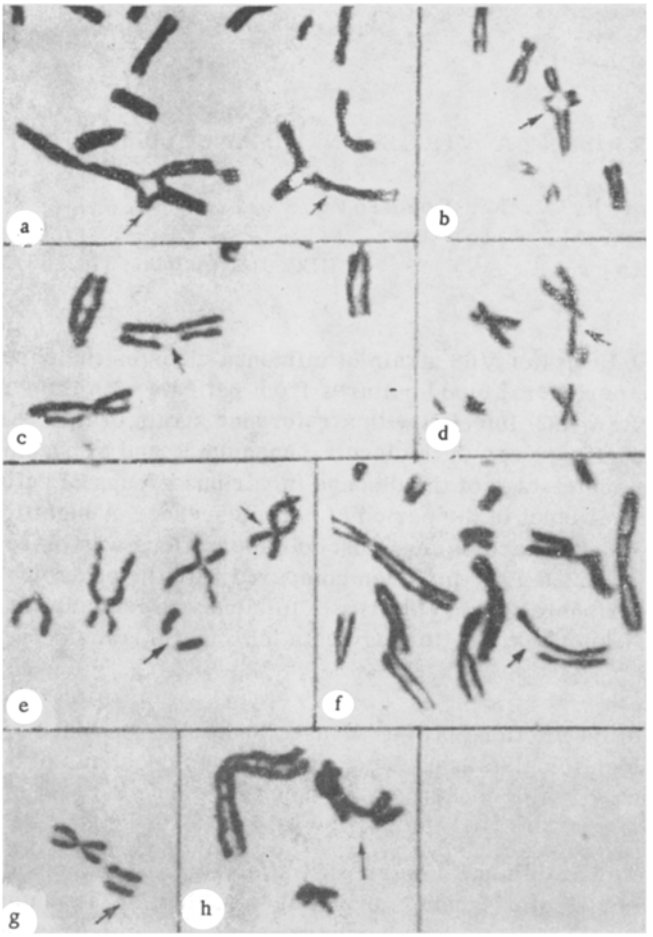

Fig. 1. Structural changes in chromosomes of human peripheral blood leukocytes and in a diploid culture of human embryonic lung: a,b) chromatid translocations (in culture of peripheral blood leukocytes); c) chromatid break (culture of peripheral blood leukocytes); d) chromatid gap (culture of peripheral blood leukocytes); e) dicentric and paired fragment (HDC culture); f) inverted chromatid deletion (HDC culture); g) chromatid break (HDC culture); h) chromatid break (HDC culture). Giemsa–Romanovsky, 10 × 90 immersion.

This paper gives some results of a comparative study of the action of A2/Hong Kong influenza virus on the genetic apparatus of human cells in vivo and in vitro.

EXPERIMENTAL METHOD

To study the action of influenza virus on the chromosomes of human blood cells a cytogenetic investigation was made of 29 influenza patients and 18 healthy subjects during the period of the seasonal increase in incidence of the disease (February-March, 1968) and during two epidemics of influenza January-February, 1969 and January-March, 1972) in Moscow. The diagnosis of influenza was made on the basis of clinical, epidemiological, and laboratory data. The cytogenetic investigation was repeated at intervals during the disease: in the acute period (1st-3rd day) and during convalescence (15th-20th day of the disease). Blood cultures were prepared by Moorhead's method [14] and by Hungerford's micromethod [10]. No fewer than 50 metaphase plates were analyzed at each time of the investigation. In the experiments in vitro strains of human diploid cells (HDC-9 and HDC-11) were used. The culture was free from mycoplasmas. Strain A2/Hong Kong/68 of influenza virus was used for infection. The cells were infected 24 h after feeding. The multiplicity of infection was 10 EID_{50}/cell in the different series of experiments. The virological and

cytogenetic investigations of the cultures were carried out 3, 6, 24, and 48 h after infection. The frequency of aneuploidy and of structural disturbances of the chromosomes was studied. The statistical analysis of the results was carried out with the aid of Student's criterion.

EXPERIMENTAL RESULTS

To estimate the natural variation in the karyotype of the blood cells 1,400 metaphase plates were studied from healthy adults who had not undergone x-ray examination during the previous 6 months. The level of aneuploidy in the control group was 1.7% and the frequency of the chromosomal disturbances (mainly aberrations of chromatid type) averaged 2.1%. This is in agreement with results obtained by various workers who have studied the spontaneous level of chromosomal aberrations and of aneuploidy in cultures of human peripheral blood leukocytes [2-5].

During the seasonal increase in the incidence of influenza the frequency of aneuploidy in the patients studied averaged 11%. The chromosomal disturbances consisted of gaps, deletions, chromatid and isochromatid breaks, and chromatid translocations (Fig. 1) and their frequency was 5.0-14.8% (mean 9.1%). The difference between the control and experimental series was significant ($P < 0.01$).

During the first epidemic of A2/Hong Kong influenza the level of aneuploidy in 14 influenza patients investigated was 4-14% (mean 8%) and the frequency of structural changes in the chromosomes was 4-27.7% (mean 9.2%). In the same group of patients during convalescence the mean level of aneuploidy was 10.3% and the mean number of cells with structural disturbances of the chromosomes was 5%. The types of these disturbances corresponded to those in Fig. 1. The most marked changes in structure and number of the chromosomes were found in pregnant women with influenza during the 1972 epidemic (Table 1). The level of aneuploidy in this group was 16-25% (mean 17.2%) in the acute stage of the disease. The structural disturbances of the chromosomes included gaps, deletions and, less frequently, chromatid translocations, the frequency of which was 6-20% (mean 10.6%). Tests carried out in the stage of late convalescence revealed a decrease in the frequency of aneuploidy and structural disturbances of the chromosomes in most of the persons studied, down to the levels found in the control group. Statistical analysis of the results obtained from tests on various groups of influenza patients showed that the differences between values obtained in the acute period and the control were significant ($P < 0.01$).

The study of the action of A2/Hong Kong/68 influenza virus on HDC cultures, the biological properties of which are similar to those of the body cells in situ [1], revealed the following consecutive infectious titers: 0 h 10^2, 3 h 10^4, 24-48 h 10^6. Under these conditions a significant increase in the aneuploidy level from 6% 3 h after inoculation with the virus to 13-21% 24-48 h after infection of the cells compared with 2-6% in the control at all periods of observation was observed. Structural changes in the chromosomes were found 3-6 h after inoculation with the virus with a frequency of 14-16% and were recorded at the same frequency throughout the experiment. In the control series the level of aberration did not exceed 4-6%. True breaks were found in all groups of chromosomes in the infected cultures but more frequently in the centromere regions of chromosomes of the A and B groups. Unlike in experiments by other workers [6], no fragmentation or pulverization of the chromosomes could be detected at any time of the investigation.

In most influenza patients a statistically significant increase in the frequency of aneuploidy and of structural disturbances of the chromosomes was thus found in the acute stage of the disease, with a return of these parameters to the normal value during convalescence. In HDC cultures infected with influenza virus a significant increase in the level of aneuploidy and of chromosomal aberrations was observed after 3-24 h compared with the control cultures.

The wide range of variations in the indices found in different patients was evidently due both to the time when the blood was taken, for the viruses acted only briefly on the cell chromosomes, and to the physiological features of the individual patients [17]. Morphologically the chromosomal aberrations in the leukocyte cultures and the HDC cultures were identical: in all series of tests most disturbances were of the chromatid type: there is experimental evidence that this indicates damage to the chromosomes at the stage of DNA synthesis [15-17]. The absence of specificity in the character and distribution of the chromosomal aberrations by groups is in agreement with observations of other workers who discovered that the character of the abnormalities was the same after the action of different mutagens: x-rays, alkylating compounds, and bromodeoxyuridine [11, 12]. According to the scheme of the hypothetical mechanisms of intrauterine infection suggested by Catalano and Sever [8], inherited changes in a cell population, especially single fragments of chromosomes, can lead to the inhibition of mitosis, hypoplasia of organs, mongolism,

mosaicism, and so on. Hakosalo and Saxen [13], who studied an influenza epidemic in Finland and developmental defects in children whose mothers had developed influenza during the first 3 months of pregnancy, found correlation between the epidemic and congenital defects of central nervous system. A similar correlation had previously been observed by Coffey and Jessop [9].

In connection with the facts described above it would be interesting to study the late sequelae of the disturbances in the number and structure of chromosomes produced by the A2/Hong Kong/68 strain of influenza virus revealed by this investigation.

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