Virus induced chromosomal abnormalities in Chinese hamster lung cell line and human peripheral blood leukocyte culture

Smita P Paranjpe
National Institute of Virology, 20-A Dr. Ambedkar Road, Pune 411 001, India
and
U V Wagh
Interactive Research School For Health Affairs, [IRSHA], Bharati Vidhyapeeth Deemed University, Dhankawadi, Pune, Pune 411 043, India

Received 11 June 2002; revised 17 December 2002.

Chinese hamster lung (CHL) cells were susceptible to Herpes Simplex type-1 and Chandipura viruses; which induced chromosomal abnormalities in these cells. Chromosomal changes induced in these cells were specific. The cells were refractory to measles virus and chromosomal abnormalities were not detected after inoculation of the virus. On the other hand human peripheral blood (HPB) leukocytes were susceptible to all the 3 viruses studied and exhibited chromosomal abnormalities upon infection. The aberrations induced in HPBL cultures were random. The results suggest that a virus could induce chromosomal changes only in susceptible cells. This is the first report of comparative in vitro study on chromosomes.

For the past few years extensive use of permanent cell lines and primary cultures in various fields of biology, medicine and biotechnology has stimulated cytogenetical studies of cells in culture1. During the cultivation of cells in vitro high dose of antibiotics, presence of viral and bacterial contaminants, non-physiological conditions like alteration in ionic strength, pH, temperature or nutritional stress etc. may lead to genotypic changes, cellular transformation and cytotoxicity2. After the pioneer discovery of virus induced chromosomal aberrations by Hamper and Ellison3 virus induced chromosomal damage has been well established. It was shown that live replicating, live non-replicating and inactivated influenza viruses were equally capable of inducing chromosomal aberrations4. Chromosomal damages were induced after infection with polio virus5. Viruses causing infectious hepatitis, serum hepatitis, aseptic meningitis, chickenpox, mumps, and measles were reported to induce chromosomal aberrations in lymphocytes of patients. Viruses like Adeno types 2,4,7,12 and 18, SV-40, Herpes simplex and Vaccinia induce chromosomal aberrations in cultured mammalian cells6. It was also reported that inactivated human viral vaccines induced chromosomal aberrations7. The present study has been undertaken to investigate the effects of virus replication on chromosomes of Chinese hamster lung (CHL) cell line and human peripheral blood leukocyte (HPBL) cultures.

Materials and Methods

Cells—(1) CHL cell line from the National Institute of Virology (NIV) cell repository at passage levels ranging from 36 to 47 grown in Minimum Essential Medium Eagles {(MEM (E)} containing Earle's Salts supplemented with 5% goat serum (GS) was used. The confluent monolayers were maintained in the same medium without GS. Subcultures were prepared by splitting the trypsinized monolayers in 1:10 ratio.

(2) HPBL suspension cultures were set from vein-punctured heparinised blood of a healthy donor in medium 199 containing Hank's salts supplemented with 5% phytohaemagglutinin (PHA) solution.

Viruses—The following virus strains from our repository were used:

(1) Herpes Simplex Virus-1(HSV-1) at passage level 4, (2) Chandipura (CHP) virus at passage level 7, and (3) Measles virus (Edmonton strain) at passage level 3. The plaque purified virus stocks were prepared in Vero cell line. The infectivity titres of HSV-1, CHP and Measles virus strains were $10^{5.5}$, $10^{7.5}$ and $10^{3}$ PFU/ml respectively.

Infectivity assay—Belleo tube cultures of CHL cell line were prepared seeding 0.2 million cells/ml/tube. The cultures were inoculated with various dilutions of virus suspensions and incubated at 37°C. These cultures were then observed daily for cytopathic effect (CPE) for 10 days. Suspension cultures of heparinised
peripheral whole blood were set; inoculated with the above mentioned virus suspensions and incubated at 37°C. Then the supernates from the cultures were assayed in susceptible cell line (Vero) at different time intervals. The TCID<sub>50</sub>s were calculated by Karber's formula<sup>10</sup>.

Chromosomal studies — (I) Cover slip cultures in Leighton tubes were set with 0.2 million CHL cells/ml/tube and inoculated with 2 logs TCID<sub>50</sub> of virus suspension at different time intervals prior to chromosome preparations. Cultures (30 hr old) were used for this purpose. The method followed was as described by Hsu and Zenzes<sup>11</sup>. The preparations were stained with Giemsa stain and altogether 150 metaphases from each type of culture were screened under the microscope for chromosomal abnormalities.

(II) HPBL cultures were set and inoculated as mentioned above. The infected cultures were harvested at 72 hr for chromosomal studies. The preparations were made as described by Moorhead et al<sup>12</sup>. In brief the cultures were treated with colchicine solution (0.4 μg/ml) and incubated at 37°C for 2 hr. Then the colchicine solution was removed by low centrifugation and the cells were treated with hypotonic solution (0.5% KCl Solution) at 37°C for 20 min. After removing the hypotonic solution the cells were fixed in acetic alcohol (1.3 acetic acid and methanol). The slides were prepared by air-drying method, stained with Giemsa stain and observed as mentioned above.

Results and Discussion
From the susceptibility studies it was observed that the CHL cell line was susceptible to HSV-1 and CHP viruses (Figs 1-3). The infectivity titres of HSV-1 and CHP viruses were 5.5 and 7.5 dex/ml respectively. The chromosomal study revealed that the chromosomal aberrations were induced in CHL cells inoculated with HSV-1 and CHP viruses but not in measles virus inoculated CHL cultures. These cells were refractory to measles virus. The HPBL cultures were susceptible to all the three viruses used and the infectivity titres were 5.5, 7.5, and 5 dex/ml respectively (Figs 4 and 5). The cultures inoculated with all the three viruses exhibited chromosomal aberrations (Figs 6-10). From the above observation it appears that the cytogenetical changes occur only in susceptible cells. The present data support the earlier report that multiplication of virus was necessary to induce chromosomal abnormalities<sup>13</sup>.

Chromosomal aberrations were observed as early as 2 hr post infection. (HPI). This may be due to some early phase effects viz. action of the enzymes induced by the virus in the host-virus interaction. The aberrations were observed earlier in CHL culture, compared to that in HPBL cultures; because of shorter cell cycle of CHL cells (22 hr). Replication cycles of CHP, HSV-1 and measles viruses are 6–8 hr<sup>14</sup>, 23 hr<sup>15</sup>, and 41-46 hr<sup>16</sup> respectively. However the aberrations were observed before completion of one replication cycle. Hence it appears that the completion of replication was not necessary to induce chromosomal aberrations. The present data are in agreement with Zur Hausen<sup>17</sup> and Kato and Sandberg<sup>18</sup>. They reported that the viral DNA synthesis was not necessary to induce chromosomal aberrations. The types of aberrations were related to hpi elapsed between infection and the observation. The chromatid and chromosomal breaks were observed at an early stage of infection followed by fragmentation and pulverization of chromosome at the late phase of infection. The aberrations were frequent with the advancement of infection (Table 1). It may be due to continuous and repeated effect of the virus on host cells during the various phases of cell cycle.

The virus induced chromosomal aberrations may be either random or specific<sup>18,20</sup>. In the present study it was observed that HSV-1 exhibited specific breaks in chromosome No.1 and chromosomes from group 2 of CHL cells. Chandipura virus induced non-random breaks near the centromeric region in chromosome No.1 of CHL cell. Chromosomal aberrations induced in HPB leucocytes by all the three viruses were random with respect to specific chromosome involved and the region affected.

The chromosomal aberrations are visible portions of the damage, which may lead to repair and/or cause cell mutation that is not amendable for microscopic examination. Gene mutation is detected by screening many parameters. Chromosomal study is also a possible parameter to study cell mutation. Different scientists have used various methods to study genotoxicity. Cytogenetic effects of two inactivated viral vaccines (polio and antirabies) were studied in adult mice by micronucleus test<sup>19</sup>. Polio vaccine did not induce micronuclei formation. Whereas micronuclei were induced in bone marrow erythrocytes of mice vaccinated with antirabies vaccine. Classical karyotypic analysis is carried out by chromosome banding using dyes that differentially stain the chromosomes<sup>21</sup>. However this traditional technique cannot characterize many complex chromosome aberrations. Recently, new karyotyping method based on chromosome painting technique namely spectral karyotyping (SKY)<sup>22</sup>.
Figs 1-5 — (1) Normal CHL cell culture at 48 hpi (×50). (2) CHL cell culture inoculated with HSV-1 virus showing CPE at 48 hpi (×50). (3) CHL cell culture inoculated with CHP virus showing CPE at 18 hpi (×50). (4) Normal Vero cell culture at 96 hpi (×50) and (5) Vero cell culture inoculated with Measles virus showing CPE at 96 hpi (×50).
Figs 6-10—(6) Chromosome spread of CHL cell line at 30 hpi (x1000 oil immersion), (7) Chromosome spread of HPB leukocyte at 72 hpi (x1000 oil immersion), (8) HPB leukocyte showing chromosome and chromatid breaks at 8 hpi (x1000 oil immersion), (9) Fragmentation of chromosome in HPB leukocyte at 18 hpi (x1000 oil immersion) and (10) Pulverization of chromosome in HPB leukocyte at 24 hpi (x1000 oil immersion).
and multicolour in situ hybridization (M-FISH) have been developed. These techniques are proving to be highly successful in identification of new chromosomal alterations, which were previously unresolved by traditional approaches. In situ hybridization is most direct method of determining linear order of genes on chromosomes. By using chromosome and gene specific probes numerical and structural aberrations can also be analyzed within individual cells.

The micronuclei formation and comet assay techniques are conventional indirect techniques to study genotoxicity. Recent molecular techniques are more sensitive and have greater resolving power. However they need sophisticated and costly equipments and reagents. The technique used in the present study can be used in general hospitals of small towns of remote areas. The technique is handy, cheap and less time consuming.

Thus from the present study it can be concluded that chromosomal aberrations were exhibited only in the susceptible cells. The aberrations were induced before the completion of one replication cycle of virus. Chromatid and chromosome breaks were observed in the early phase and fragmentation and pulverization of chromosome were observed in the late phase of infection. Chromosomal aberrations were far from random in CHL cell line and random in HPBL cultures.

Acknowledgement

Thanks are due to the Director, National Institute of Virology, Pune, for the encouragement and Miss M.B. Thakker for editorial assistance.

References