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Flow Cytometry Analysis of Early DNA Content Changes in Human and Monkey Cells Following Infection With Simian Virus 40

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Simian virus 40 (SV_{40}) is capable of inducing cellular DNA synthesis in permissive and nonpermissive cells. Utilizing flow cytometry, we analyzed the DNA content changes in two diploid human cell strains and two monkey cell lines. The osteogenesis imperfecta (OI) human skin fibroblasts were induced into DNA synthesis, and within one to two cell generations, a polyploid cell population was produced. With WI-38 phase II cells, a similar pattern of increased cycling of cells into DNA synthesis was observed; however, the majority ($\sim$60%) of the cells were blocked in the G_2 + M phase of the cell cycle. At later time intervals, an increase in the G_1 population was demonstrated. The two monkey cell lines responded to SV_{40} virus with an accumulation of cells in the G_2 + M phase of the cell cycle. Thus, two diploid human cell strains exhibited different cell cycle kinetics early after infection with SV_{40} virus. The one strain (WI-38) behaved similarly to the two monkey cell lines studied. The other strain (OI) responded similarly to nonpermissive (transforming) cells infected with SV_{40} virus.

Key words: simian virus 40, flow cytometry, DNA synthesis induction, transformation, human diploid cells

SV_{40} virus is capable of transforming numerous strains of diploid rodent and human cells. Previous studies with diploid mouse and Chinese hamster embryo cells demonstrated the production of a tetraploid-polyploid population within 24–48 h after infection that resulted from two or more consecutive S periods without a mitosis [1, 2]. Therefore, the early changes observed in cellular DNA synthesis regulation may be an important step in the mechanism of neoplastic transformation.

Human diploid fibroblasts infected with SV_{40} virus may replicate the virus, which

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leads to lysis, or some cells may be transformed. Changes in human cells were first detected 4–6 weeks after infection, which is prior to morphological transformation. These changes included an increase in the tetraploid population and an increase in chromosome breaks. There was no evidence of chromosome changes early on (1–2 days) [3, 4]; however, cellular DNA synthesis as measured by ^{3}H-thymidine incorporation was observed within the first week after infection [5]. Monkey cells permit the complete replication of SV_{40} virus and are induced into the S phase of the cell cycle [6, 7].

This paper reports the early DNA content distribution of monkey cells and human cells following infection with SV_{40} virus. These analyses were performed with flow cytometry which allowed a quantitation of DNA content per cell and was capable of measuring a large population of cells within the first few cell generations following infection.

MATERIALS AND METHODS

The strains of human cells used in this study were obtained from patients with osteogeneis imperfecta (OI) and from WI-38 at passage 20–30 in vitro. Three strains of OI cells at the 20–25th passage were used for these studies (C, H, J) and all strains responded similarly. The infection and DNA content studies were performed at two passages for each cell strain. The monkey cell lines (BSC-1 and CV-1) at passage 30–40 were obtained from American Type Tissue Culture Collection. All cells were maintained in minimal essential medium (Gibco) supplemented with 5% fetal calf serum (Microbiological Associates). Confluent cultures were subcultivated with 0.25% trypsin–0.1% versene in phosphate-buffered saline (minus Ca^{++} and Mg^{++}). The RH-911 strain of SV_{40} virus was used for infection and was grown in CV-1 cells [1]. Cells and virus pools were checked for the presence of mycoplasma [8]. Assays for the SV_{40}-specific intranuclear T antigen and viral antigen were performed as previously reported [1].

The cells were subcultured into T-75 Costar flasks, infected 24 h later, and harvested at the various time intervals for DNA content measurement. DNA content measurements were performed with the flow cytometry system developed at the Los Alamos Scientific Laboratory. The cells were harvested as previously described by Tobey et al [9] and stained with acriflavin-Feulgen or mithramycin [9, 10]. Stained cells were introduced into the laminar flow stream of the flow cytometer, where they crossed an argon-ion laser beam at the rate of 5×10^4 cells per minute. The fluorescent intensity of the acriflavin or mithramycin dye was measured for each cell with a photomultiplier tube and recorded in a multichannel pulse-height analyzer. Details of this instrumentation have been previously described [11].

RESULTS

The OI (C, J, H) and WI-38 cells were infected with a multiplicity of 200 plaque-forming units (PFU) per cell and the monkey cells were infected with a multiplicity of 10 PFU per cell. Within 48–72 h, 40–50% of the human cells (OI and WI-38) were expressing the SV_{40}-specific intranuclear T antigen and 10–20% of the cells were producing virion capsid antigen. At 24 h after infection 80–85% of the cells of both monkey cell lines were expressing T antigen and 20–30% were expressing V antigen. Within this time period, a large percentage of the OI cells and WI-38 cells were infected with

SV_{40} virus, thus allowing an analysis of the changes in the cell cycle resulting from SV_{40} infection.

Figure 1 shows the DNA content distribution of control and infected cells (OIC) at 48 and 96 hours after infection. A semilog plot was employed to emphasize the cells in the polyploid range. The G_1 (2C) peaks of both control and infected cultures were normalized and plotted in semilog to emphasize the cells in S, G_2 + M, and tetraploid G_2. It was evident at the earliest time point, 48 h, that there were more cells in the S phase (2C to 4C) of the cell cycle in the infected population. The G_2 + M population (4C) was also considerably greater in the infected population and there was a twofold to fourfold

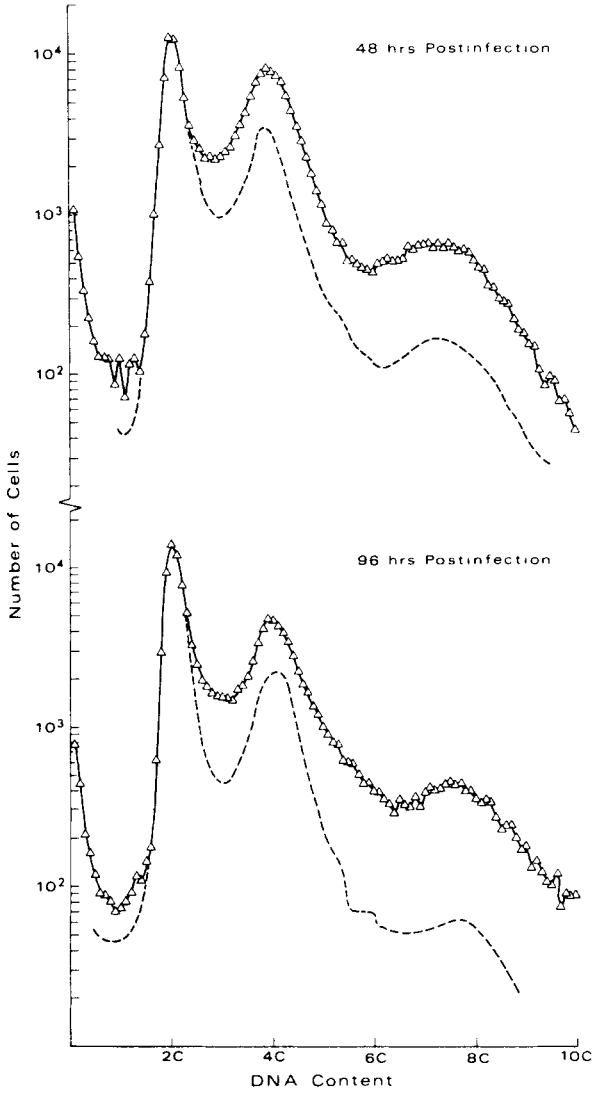

Fig. 1. DNA content distribution of control (- - -) and infected (△-△-△) OI-infected cells at 48 and 96 h after infection. The cells were dispersed, fixed, stained with mithramycin, and measured with the flow cytometer. The data are presented in semilog to emphasize the S and G_2 + M populations.

increase in the number of cells with DNA content between 4C and 8C. The tetraploid G_2 + M cells (8C) in the infected population were increased (threefold to fourfold) over the control (Table I). The other cell strains of OI (H and J) were analyzed for this DNA content shift and showed a similar pattern to the OIC. Thus, SV_{40} virus induced these human cells into DNA synthesis and increased the number of polyploid cells within 3–4 days following infection.

TABLE I. Percentage of OI Cells in Various Phases of the Cell Cycle Following Infection With Simian Virus 40

Cells	Hours after infection	Between 4C and 8C	8C
OI control	48	3.9	1.3
OI infected	48	8	3.1
OI control	72	2.2	0.68
OI infected	72	9.1	2.8
OI control	96	2.1	0.83
OI infected	96	8.7	3.7

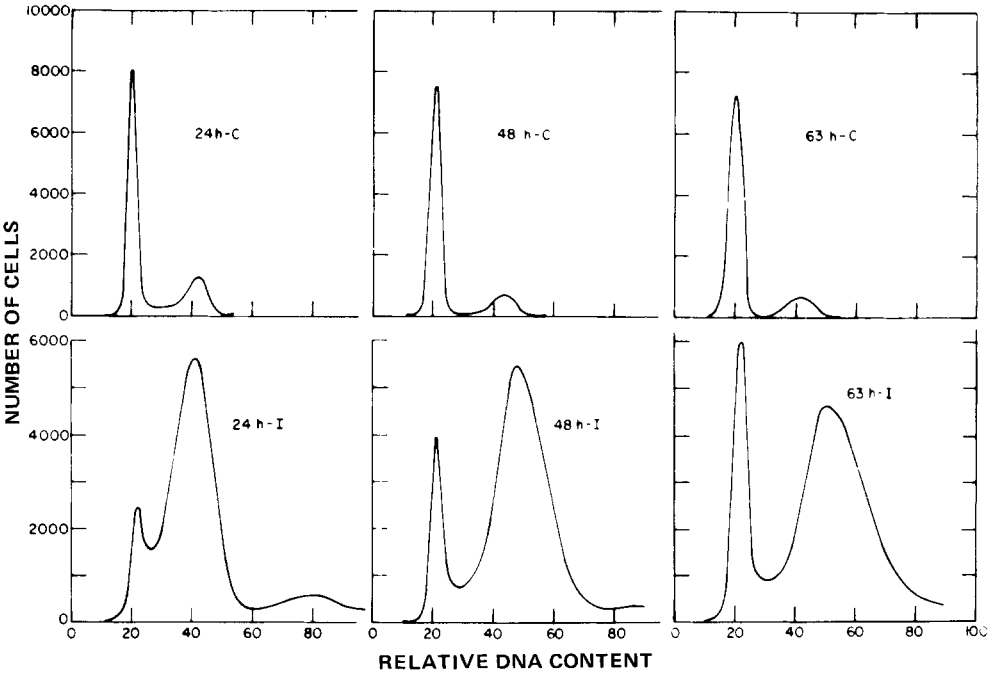

Fig. 2. DNA content distribution of control (upper) and infected (lower) WI-38 cells 24, 48, and 63 h after infection. The cells were dispersed, fixed, stained with mithramycin, and measured with the flow cytometer.

TABLE II. Percentage of WI-38 and BSC-1 Cells in Various Phases of the Cell Cycle Following Infection With Simian Virus 40

Cells	Hours after infection	G_1	S	$G_2 + M$
WI-38 control	24	66.7	10.0	23.3
WI-38 infected	24	14.1	3.7	82.2
WI-38 control	48	78.1	5.6	16.3
WI-38 infected	48	15.2	4.7	80.1
WI-38 control	63	82.4	2.7	14.9
WI-38 infected	63	21.8	5.3	72.9
BSC-1 control	48	64.9	9.8	25.4
BSC-1 infected	48	16.4	5.1	78.5

When the DNA content distributions of WI-38 control and infected cells were measured at 24, 48, and 63 h after infection, a pattern different from the OI cells was detected (Fig. 2). The mock-infected cells at all times had a normal DNA content distribution for G_1, S, and G_2 + M cells (Table II). The infected population at 24 h had a DNA content distribution markedly different from the control cells. There was a large increase in the number of cells in S and G_2 + M phases of the cell cycle (85%), and a decrease in the G_1 population (Fig. 2, Table II). At 48 and 63 h, there was an increase in the G_1 population; however, a considerable percentage of cells were in the G_2 + M phase. The WI-38 cells responded with a stimulation of DNA synthesis following SV_{40} virus infection; however, the majority of the cells were apparently blocked in the G_2 + M phase. The G_2 + M peak at 48 and 63 h was shifted to the right when compared to the 24-hour time point (40 vs 50), indicating an increase in DNA content in the G_2 + M phase. When later times – 2 weeks and 4 weeks – were assayed for DNA content distribution, the infected cultures were similar to the controls.

To determine whether the WI-38 cells in the G_2 + M phase were blocked in mitosis, we assayed for the number and type of mitotic figures at 48 and 96 h after infection. Similar multiplicities of virus were used, and a comparable number of cells producing T and V antigen were observed as described above. At 48 h the control culture had 0.5% mitotic cells and the infected culture contained 1.1%. The control and infected cultures at 96 h had 0.05% and 1.3% mitotic figures, respectively. A higher mitotic index was observed in the infected cultures; however, all stages of the mitotic cycle were observed, which suggests that the mitotic cells were cycling and not blocked in a particular stage of mitosis. These results suggest that the cells are probably blocked in the G_2 phase of the cell cycle.

The two lines of monkey cells (CV-1 and BSC-1), which are permissive to SV_{40} replication exhibited an increase in the G_2 + M peak (78.5% vs 25.4% for the control) (Fig. 3, Table II). At later time intervals (>72 h), the majority of cells were dead. Both CV-1 and BSC-1 cells responded similarly. Stimulation of DNA synthesis was determined by autoradiography following 6-h pulses of ^{3}H-thymidine. Both the CV-1 and the BSC-1 used in these studies exhibited a stimulation of DNA synthesis with approximately 20–50% more cells in DNA synthesis. Grain counts were compared to minimize the background of viral DNA synthesis.

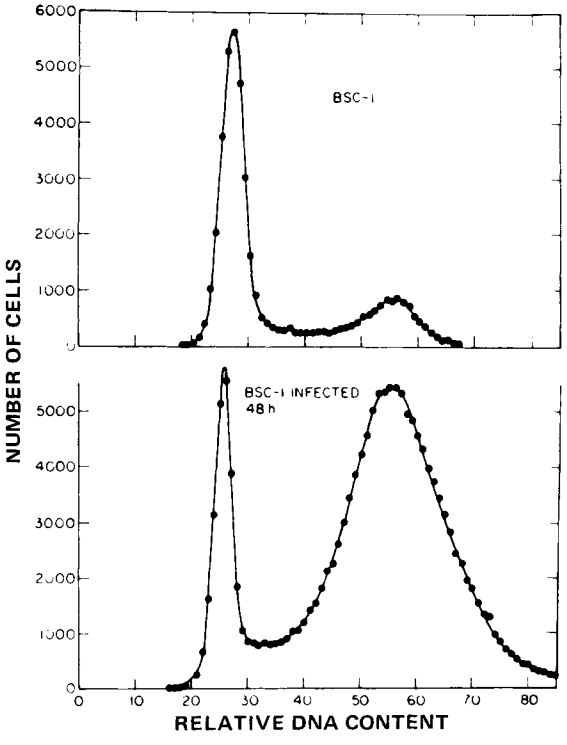

Fig. 3. DNA content distribution of control (upper) and infected (lower) BSC-1 cells at 48 h after infection.

DISCUSSION

Two strains of human diploid fibroblasts were induced into cellular DNA synthesis following infection with SV_{40} virus; however, these two human cell strains exhibited different DNA content distributions. The majority of the OI-infected cells were initially induced into DNA synthesis. A second round of DNA synthesis was induced in some cells, producing a tetraploid population similar to what has been observed with diploid Chinese hamster and mouse cells [1, 12, 13]. The WI-38 cells exhibited a DNA content distribution different from that observed for the three OI cell strains. There was a decrease in the G_1 population with a concomitant increase in the S and G_2 + M population. A portion of the G_2 + M cells were capable of mitosis, since at later time intervals an increase in the G_1 population was observed, suggesting that mitosis was possible in certain of the G_2 + M population. These different responses must be unique to the cell strains, since the same pool and multiplicity of virus were utilized. The monkey cells (CV-1 and BSC-1) exhibited an increase in the number of cells in the G_2 + M phase of the cell cycle similar to that of the WI-38 cells.

At present, there is no definitive explanation for this difference in distribution of cells about the cell cycle following SV_{40} infection. Both cell strains are fibroblastic but they are obtained from different sites. The WI-38 cells were cultured from the lung of a fetus [14] and the OI cell strains were initiated from skin biopsies of newborns. Whether age of the patient or site from which the cells were obtained is an important factor in

the response of these cells to induction of DNA synthesis with SV_{40} virus will be answered by an analysis of the responses of numerous strains of human cells to SV_{40} infection. Another explanation may involve the replication of the viral DNA (whole virion) and subsequent block of cells in the G_2 phase. The monkey cells which are permissive to SV_{40} replication accumulated cells in the G_2 phase; however, WI-38 cells assayed for virion antigen exhibited no increase above the OI cells. This can be explained if a significant amount of viral DNA was replicated without late protein synthesis. At present, this explanation cannot be validated unless the G_2 population was isolated and characterized for replicating viral DNA. The fact that these cells responded differently to virus-induced cellular DNA synthesis may be useful in defining steps in the neoplastic transformation of human cells with SV_{40} virus [15].

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