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Polyoma Virus-Human Cell Interactions: Persistence of T-Antigen in Two Cell Lines with and Without Transformation

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The interaction of polyoma virus and human cells was investigated. Abortive infection as evidenced by the synthesis of T-antigen was observed in normal fibroblast and abnormal (transformed) cells but not in normal epithelial cells. A high percentage of simian virus 40-transformed WI-18 Va2 and spontaneously transformed BE skin cells produced T-antigen after high-multiplicity infection, but most of the cells rapidly lost antigen-producing capacity upon cell passage, and the cultures became negative by passage 3. All fibroblast cells displayed varying degrees of susceptibility to infection, but most of the cell lines became negative for T-antigen except for two. In one, T-antigen persisted in a small percentage of the cells throughout the lifetime of the culture, without cellular transformation occurring. In the other, the entire culture became morphologically transformed and eventually consisted of 100% T-antigen-positive cells. This is the first time that normal diploid human fibroblast cells have been transformed by polyoma virus.

Polyoma (Py) virus, a mouse papovavirus, was the first DNA-containing virus reported to transform cells in culture (10). Unlike the simian papovavirus simian virus 40 (SV40), which transforms cells from a wide variety of species, Py virus has a restricted host range for transformation. Rodent cells, such as hamster and rat cells, are readily transformed by Py virus (6, 7), but cells from other species are relatively resistant to lytic or transforming infection by Py virus. The only nonrodent cells reported to have been transformed by Py are bovine cells (3, 9).

Information concerning the interaction of Py and human cells is meager. Earlier reports (1, 2) were concerned primarily with adsorption of Py to human cells; these studies showed that Py could adsorb to human cells. Very little is known about events subsequent to adsorption. Gruen et al. (4) failed to detect any Py virus replication in a human cell line. These workers infected human cells with intact virus or viral DNA by the microinjection technique and followed viral activity by examining cells for tumor (T)- or viral antigen synthesis by fluorescent-antibody (FA) methods. Viral antigen was not detected, but cells infected with either virus or viral DNA produced T-antigen for a limited period; cellular transformation was not observed. These workers reported that infection of human cells by infectious virus by using the usual adsorption procedures, or by viral DNA with DEAE-dextran, did not result in the synthesis of viral or T-antigen by the cells. From these experiments, they concluded that resistance of human cells by Py infection was due to a block in adsorption or penetration.

Since only a few cell lines were studied in the reports cited above, general conclusions cannot be drawn concerning the effects of Py virus infection of human cells. We have examined a number of human cell lines for their response to Py infection. Fibroblastic as well as epithelial-like cells were tested, and normal as well as transformed cells were included in the study. Our results differ from those previously reported. With the exception of normal epithelial cells, infection of all cell lines with an input multiplicity of approximately 100 PFU per cell resulted in abortive infection with T-antigen production. In one fibroblast cell line, T-antigen persisted in a small percentage of the cells throughout the life span of the cultures but cellular changes (transformation) were not observed. However, in another fibroblast line, the entire culture became morphologically transformed, and 100% of the cells produced the virus-specific T-antigen. To our knowledge, this is the first report of transformation of normal human cells by Py virus. These results form the basis of this report.

MATERIALS AND METHODS

Virus. The large-plaque strain of Py virus (obtained from Tom Benjamin) was used throughout these experiments. A stock was prepared in primary mouse kidney cells and had a titer of 6×10^8 PFU/ml.

Antiserum. Py T-antiserum was obtained from serum of hamsters bearing transplantable Py virus-induced tumors. It had a titer of 1:320 as measured by FA titration by the indirect procedure. It was carefully tested against normal cells as well as against other virus-induced tumors (SV40, BK, and JC tumors) to rule out nonspecific reactions or antinuclear antibody that has been detected recently in sera of some tumor-bearing hamsters (8). The pool of Py T-antiserum reacted only with Py-transformed cells.

Antiviral antiserum was made in hamsters by subcutaneous inoculation of purified Py virus. This serum had a hemagglutination inhibition titer of 1:2,560 and an FA titer of 1:640.

Cells and medium. The cell lines used in this study and their sources are as follows. Normal fibroblast lines included: Mc, skin of Wiskott-Aldrich patient (National Institutes of Health); Fl-5000, whole human embryo (Flow Laboratories, Rockville, Md.); and SK-36, SK-38, B102, B105, and B114, all skin (Biotech Laboratories, Rockville, Md.). Normal epithelial cells included: human embryonic kidney (3 different lots from Microbiological Associates, Inc., Bethesda, Md.); and FHS-74, human embryonic intestine, provided by J. Rhim, who obtained early-passage cells from the Naval Biological Laboratory, Oakland, Calif. Abnormal (transformed) cells included WI-18 Va2, human embryonic lung cells transformed by SV40 (Wistar Institute, Philadelphia, Pa.), and B.E., a spontaneously transformed skin cell line provided by M. Eisinger, Sloan Kettering Cancer Center, New York, N.Y.

Except for the WI-38 cells, which were used from passage 24, all normal cell lines were used before passage 10. Transformed cells were used at high passages. Cell lines were grown in Eagle medium supplemented with 10% fetal bovine serum and antibiotics.

Infection of cells with Py. The experimental plan was as follows. Trypsinized cell suspensions were plated onto 60-mm dishes containing cover slips for FA studies; at the same time, 25-cm^2 flasks were also seeded. Subconfluent cultures were infected with 6×10^8 PFU of Py virus and, after 1 h of adsorption, fresh medium was added. Cover slips were removed at various times after infection, air dried, fixed in methanol-acetone (1:1), and stored at −20°C until tested. The flask cultures that had been infected at the same time as the cover slip cultures were generally kept for 10 to 14 days before the next cell passage was initiated. At that time, the cells were split at a 1:2 ratio, and cover slips were also seeded to monitor the cells for T-antigen production by the FA test. Cell passages were continued for each cell line until T-antigen was no longer detected.

RESULTS

T-antigen synthesis. Table 1 summarizes the results of infection of the various cell lines by Py virus as monitored by FA tests. The response to Py infection was highly variable among the different types of cells, and, even among fibroblast lines, varying degrees of sensitivity to infection were noted. The different cell lines can be grouped according to their susceptibility as high (transformed cells), intermediate (fibroblast cells), and resistant (epithelial cells). The SV40-transformed human cells, WI-18 Va2, and the spontaneously transformed human skin cells, BE, were quite sensitive to high-multiplicity Py infection. In several different experiments, the number of T-antigen-producing cells varied from 50 to 100% within 5 days after infection. However, the number of T-antigen-producing cells declined precipitously after a single passage (to levels of 1 to 5%), and, by passage 2, only a few cells were positive. By passage 3, the cultures were completely negative.

TABLE 1. *Response of various human cell lines to Py virus*

Cell line	T-antigen positive at 7 days (%)	Cell passages T-antigen positive
Fibroblast cells		
Mc	1–5	28
Fl-5000[a]	<0.01	2
WI-38	0.3	3
SK-36	<0.01	1
SK-38	<0.01	1
B102	0.5	Indefinite[b]
B105	0.05	1
B107	0.02	1
B114	0.04	1
Epithelial cells		
Embryonic kidney[c]	0	
FHS-74[d]	0	
Transformed cells		
BE[e]	50–75	3
WI-18 Va2[f]	50–95	3

[a] Whole human embryo.
[b] Cells transformed.
[c] Three different lots.
[d] Embryonic intestine.
[e] Established from adult skin, spontaneously transformed in vitro.
[f] Embryonic lung cells transformed by SV40.

Normal epithelial cells from human embryonic kidney (three different lots) and FHS-74, an embryonic intestine cell line, were completely resistant to Py infection. Cells were negative up to 15 days after infection and remained negative during subsequent cell passages.

Nine fibroblast cell lines were tested for susceptibility by Py infection. Most of the lines were derived from skin biopsies of normal individuals. One (Mc) was from the skin of Wiskott-

Aldrich patient who had defective cellular and humoral immunity. As judged by their capacity to synthesize T-antigen, the fibroblast cell lines exhibited varying degrees of susceptibility. These ranged from cells with intermediate susceptibility (0.1 to 5% positive cells) to low susceptibility (less than 0.01% positive cells). With two exceptions, Mc and B102, all cell lines became negative for T-antigen by passage 4. Viral antigen was not detected in any of the cell lines infected with Py virus when tested by the FA procedure.

Fate of T-antigen-producing cells in Mc cell line. The Mc culture, containing approximately 5% T-antigen-positive cells, was transferred at a 1:2 split ratio through 12 passages and at 1:3 in passages 12 through 27. The cells were examined for T-antigen at each passage, and the number of positive cells remained at a fairly constant level of 1 to 5% throughout the lifetime of the culture. There were no obvious differences from uninfected control cells, either in cellular morphology or in growth behavior. Growth rate declined after passage 25; by passage 28, the cells grew extremely slowly, and further transfers could not be made. Thus, although T-antigen-synthesizing cells were always present in low numbers throughout the life span of the culture, cellular transformation did not occur. Control uninfected Mc cells carried at the same time as the Py-infected cultures also grew slowly after passage 25, and could not be transferred after 27 passages.

Fate of T-antigen-producing cells in B102 culture. The percentage of T-antigen-positive cells in the B102 cell line remained at a low level of 0.1 to 1% through the first eight passages. At passage 9, a sudden increase in the number of positive cells was observed, and approximately 10% of the cells were positive. At each succeeding passage, the number of positive cells increased, and, by passage 13, virtually 100% of the cells were positive (Fig. 1). Other changes were also noted at this time. Many of the cells showed enlarged nuclei with prominent nucleoli, and the cells no longer grew in well-ordered, parallel alignment like the uninfected control cells (Fig. 2A). They grew, instead, in a highly random fashion with a criss-cross pattern (Fig. 2B). At passage 19, further morphological changes occurred, and most of the cells within the culture were now more epithelioid than fibroblastic (Fig. 2C). Thus, the cells were morphologically transformed with altered growth behavior, and they also synthesized a viral gene product, T-antigen. Viral antigen was not detected. This Py-transformed cell line, hereafter referred to as Py-B102, is now in its 23rd passage. The Py-B102 cells did not react with SV40, BK, or JC T-antibody.

Chromosomal analysis of Py-B102 cells. Although a Py-transformed hamster cell line was cultured in this laboratory at the time that these experiments were being conducted, cell contamination with this line was unlikely, since it was maintained in a separate facility by a different person. Furthermore, morphologically, the two kinds of Py-transformed cells are very different. Nevertheless, two different experiments were performed; they clearly showed that the Py-B102 cells were not hamster cells. In the first experiment, the Py-B102 cells were infected with type 1 poliovirus (5×10^6 PFU). Cytopathic changes occurred within 24 h, and the culture

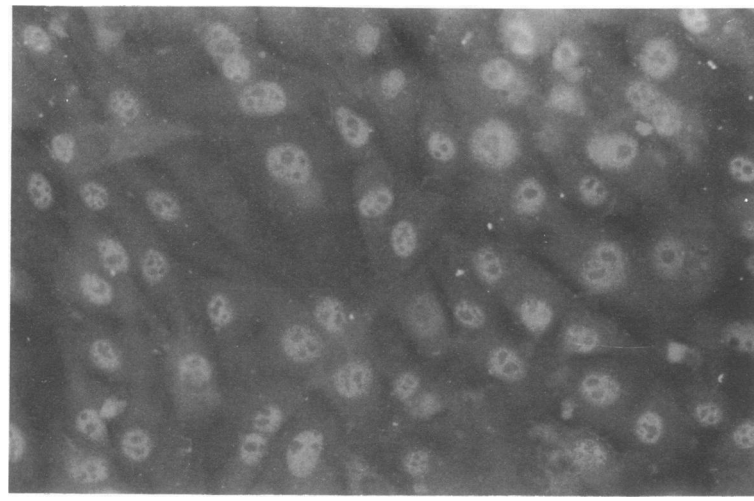

FIG. 1. *Immunofluorescent demonstration of Py T-antigen in Py-B102 cells, passage 19. Magnification.* ×280.

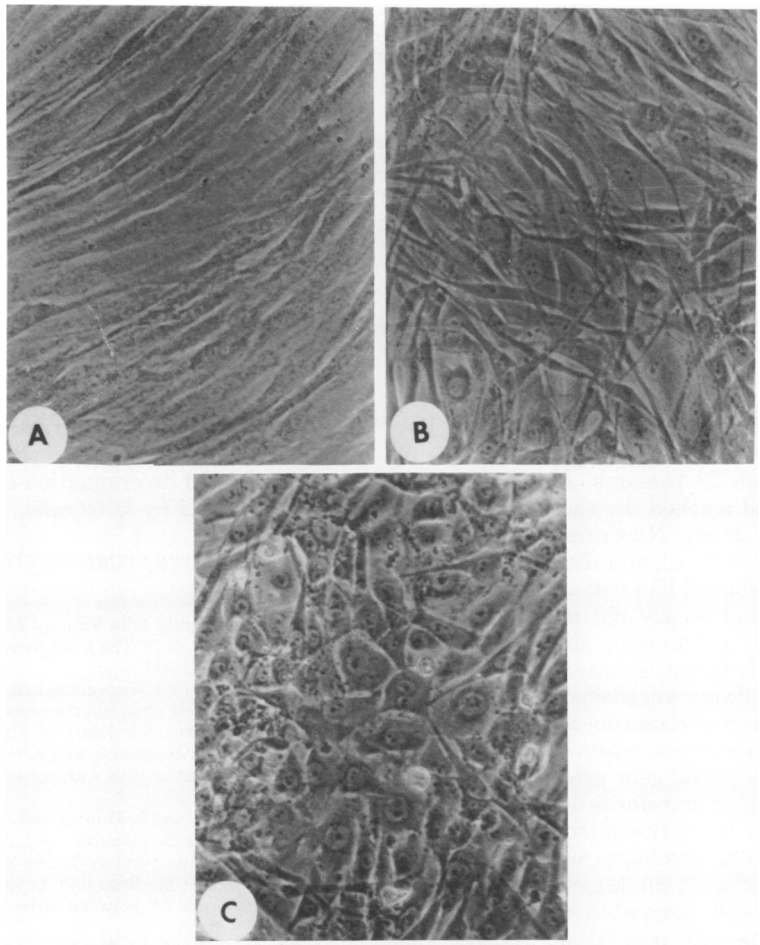

FIG. 2. *Morphology of (A) normal, untransformed B102 fibroblast cells and B102 cells transformed by Py virus at passages (B) 13 and (C) 20. Phase contrast, ×160 magnification.*

was destroyed by 48 h. Since poliovirus replicates only in primate cells, this test proved that the cells were not hamster cells, which are resistant to this virus.

In a second, definitive experiment, the Py-B102 cells were subjected to chromosomal analysis. The B102 cells were derived from a skin biopsy of an adult male. The Py-B102 cells at passage 15 had a diploid number of chromosomes (46) and still retained the male chromosome marker. Therefore, it was apparent that the transformed cells were of human origin and similar to the parental cells.

Properties of transformed Py-B102 cells. Early passage (passage 15 to 18) transformed Py-B102 cells were examined for some of the properties generally associated with transformation.

(i) Ability to form colonies from single cells on solid substrate. Trypsin-dispersed single cells were plated at varying cell numbers from 1×10^2 to 5×10^3 cells in 60-mm plastic dishes. Medium was changed every 5th day. After 21 days, dishes were examined for cell colonies, and none were observed.

(ii) Ability to form colonies in soft agar. The technique of MacPherson and Montagnier (5) was used to test the ability of Py-B102 cells to form colonies in soft agar. Visible colonies did not develop when cells varying in number from 5×10^2 to 1×10^5 were suspended in soft agar.

(iii) Tumor production in nude mice. The oncogenicity of Py-B102 cells was tested by inoculation of transformed cells into nude mice. Five mice were each inoculated subcutaneously with 2×10^7 cells (16th passage). Tumors have not developed during a 2-month period of observation.

DISCUSSION

The experiments reported here show that different human cell cultures exhibit markedly different response to high-multiplicity infection

with Py virus as monitored by T-antigen synthesis, ranging from complete resistance to high frequency of antigen synthesis. Among fibroblast cell lines, differences in sensitivity to Py were also noted. In all cell lines, infection was abortive, and only T-antigen was synthesized. With the exception of two cell lines, all fibroblast lines became T-antigen negative within a few passages.

The two cell lines that were continually positive were the Mc cell line, which was established from the skin of a patient with Wiskott-Aldrich syndrome, and the B102 cells, which were from the skin of an adult male. The consequences of abortive Py infection in these two cell lines were quite different. In the Mc cell line, a low but constant number of cells were positive for T-antigen through 28 passages. At this passage, the culture had reached the end of its life span and ceased to divide. No evidence of transformation was ever noted, and the T-antigen-producing cells behaved like normal cells in every respect. They obviously did not have an increased rate of cell division, since the number of positive cells never increased during successive passages. To our knowledge, this is the first example of cells that retain oncogenic DNA viral information during prolonged cell passages and that synthesize a viral gene product, T-antigen, without acquiring transformed characteristics.

On the other hand, the outcome of Py infection of the B102 fibroblasts was dramatically different from that in the Mc cells. The T-antigen-producing cells comprised only a small percentage of the cell population initially and through eight passages (ca. 0.5%). Thereafter, the number of positive cells increased markedly, until by passage 13 the culture was almost 100% T-antigen positive. Concomitantly, cellular alterations typical of transformed cells were observed. The cells retained their fibroblastic appearance, but their growth pattern became highly disoriented. Upon further passages, the cells continued to undergo changes, and by passage 19, the cells tended to be more epithelial-like.

Although the Py-B102 cells were morphologically altered and were 100% T-antigen positive, analysis of the cells for other transformed characteristics revealed that they have not acquired such properties as ability to form colonies either on plastic substrates or in soft agar. They also did not grow in medium containing low serum concentrations, and the cells did not form tumors in nude mice. Whether the cells will acquire these characteristics at later cell passages and whether stably transformed, permanent lines of Py-B102 cells can be derived remain to be determined. Studies are underway to characterize the Py-B102 cells further. These studies include ability to rescue Py virus from transformed cells and determination of the status of viral genes in the Py-B102 cells.

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