*PROPERTIES OF HYBRIDS BETWEEN POLYOMA-TRANSFORMED AND NORMAL MOUSE CELLS**

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Hybridization of somatic cells provides a useful tool for the study of interactions between the genomes of different cells and of the regulation of their phenotypic expression. Tumor cells differ from their normal counterparts in many properties, some of which may be determined by structural alterations of the genetic material, while others could result from regulation of gene expression. Hybrids between neoplastic and normal cells may yield some information as to the nature of changes involved in carcinogenesis.

In a previous communication it was shown that hybrids between cells (from a noninbred Swiss mouse) transformed by polyoma virus and mouse cells of the low cancer line NCTC 2555 (of C3H origin) maintained the polyoma-induced transplantation (PV-ITA) and complement-fixing (PV-ICFA) antigens characteristic of the former.[1] Inoculations of the hybrid cells by various routes into C3H and A mice did not result in the production of tumors, but it was impossible to decide whether this negative result was due to the intrinsic inability of the hybrids to produce tumors or to their antigenic constitution which was different from that of the hosts.

Experiments of a similar design but involving cells from two inbred strains of mice were performed by Gershon and Sachs.[2] Hybrids were obtained between polyoma-transformed (SWR) mouse cells and L cells (C3H). These hybrids were shown to be neoplastic in appropriate (F_1C3H $\times$ SWR) mice and, very probably, to have the PV-ITA.

The interpretation of the quoted results in terms of genetic changes underlying neoplasticity is rendered particularly difficult by the fact that *both* parents of the hybrids were cells with more or less pronounced neoplastic properties. The aim of the present investigation was therefore to determine the tumorigenicity and antigenic make-up of hybrid lines obtained by "crossing" polyoma-transformed mouse cells with *normal* diploid mouse cells.

Experimental.—The two polyoma-transformed "parental" lines used in this work are clones 27–6 and 27–6–8. The former was derived by us from clone Py 27, kindly given by Dr. R. A. Weisberg (California Institute of Technology) who isolated it from a clone of (A/Sn) mouse embryo cells transformed by a large plaque variant of polyoma virus.[3] Cl. 27-6-8 is a subclone of Cl. 27-6 also isolated for the experiments to be described. The relationship between the three clones is shown in Figure 1.

The normal "parental" cells were derived from primary cultures of skin and lung of newborn CBA mice carrying the T6 translocation.[4] Hybridization of these cells with cells of the high cancer line NCTC 2472 has been described by Scaletta and Ephrussi.[5] The hybrid cells used in the present work were obtained essentially by the same technique (growth of mixed cultures at 29°C). Their exact derivations are shown in Figure 1.

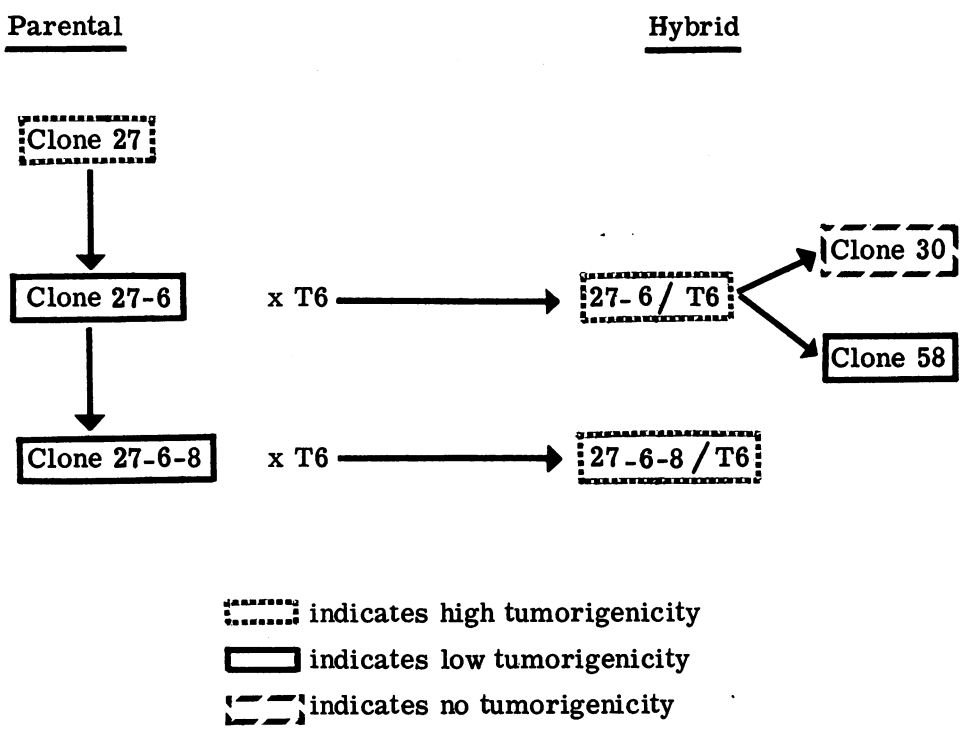

FIG. 1.—History and tumorigenicity of parental and hybrid lines.

The detailed description of the procedure and of the karyological identification of the hybrids has been given elsewhere.[6] With respect to the latter, it will suffice here to say that (*a*) the modal chromosome numbers of clones 27-6 and 27-6-8 were, respectively, 63 and 58, with 7 biarmed chromosomes used as markers of these cells;

TABLE 1

TUMORIGENICITY OF PARENTAL AND HYBRID CELL LINES

Cells	Dosage	A/Sn(Jax)	CBA/T6	CBA × A/Sn (KI)	CBA × A/Sn (Jax)
			Parental Lines		
T6	1×10^6	...	0/5*,†	...	...
T6‡	5×10^6	...	0/10	...	...
Cl 27	2×10^6	...	...	2/3§	...
	5×10^6	8/8§	...	...	8/8§
Cl 27-6	2×10^6	...	0/3**	0/3	...
	5×10^6	3/8§	...	...	0/8
Cl 27-6-8	5×10^6	0/4	...	2/7	1/4§
			Hybrid Lines		
27-6/T6	3.7×10^6	...	0/3**	3/3	...
	5×10^6	0/8	...	...	8/8§
27-6/T6 (clone 30)	2×10^6	...	0/3**	0/3	...
	5×10^6	0/8	...	...	0/8
27-6/T6 (clone 58)	5×10^6	...	...	2/5§	...
27-6-8/T6	5×10^6	0/4	...	7/7§	4/4

* Animals with tumors/animals inoculated.
† Inoculated into F_1(C3H × CBA) mice.[5]
‡ Whole embryo secondary cultures.
§ At least one tumor was analyzed for cytological identification of origin.
** Mice were treated with X ray (350 r) prior to cell inoculation.

(*b*) the two very characteristic T6 chromosomes served as the complementary markers of the normal parent; (*c*) the hybrids were identified by the simultaneous presence of the above parental markers; (*d*) although, by the time of inoculations, all hybrids had undergone the usual decrease in chromosome number,[7] their initial chromosome number and the presence in each cell of just two T6 chromosomes indicated that they were indeed products of fusion with normal diploid cells of the T6 culture (however, see *Discussion*).

TABLE 2

TEST FOR DEMONSTRATION OF PV-ITA IN HYBRID CELLS

Inoculated cells	Control		Immunized*	
	3×10^6	5×10^5	3×10^6	5×10^5
Parental Lines				
Cl 27	8/8†	7/9	2/7	0/9
Cl 27-6	3/8	0/9	0/9	0/9
Cl 27-6-8	5/9	1/10	0/10	0/11
Hybrid Lines				
27-6/T6	5/8	4/7‡	0/8	0/9
27-6/T6 (clone 30)	0/8	0/9	0/8	0/9
27-6-8/T6	6/9	6/8‡	0/7	0/12

* Panels of ♂CBA/J ♀ × A/Sn ♂ were immunized by intraperitoneal inoculation of P172 virus three times, 1 week apart. Cells were inoculated 1 week after the final immunization.
† Animals with tumors/animals inoculated.
‡ At least one tumor was analyzed for cytological identification of origin.

Tumorigenicity.—The tumorigenicity of the different cell lines was tested by inoculation of different dosages of cell suspensions, obtained by trypsinization of monolayers, into the subcutaneous tissue of the hind leg of two- to three-month-old mice. In earlier experiments,[5] inoculations into mice of appropriate genotype of 10^6 T6 cells prepared in a manner similar to that of the T6 cells used in the hybridization experiments did not result in the development of tumors (Table 1). Similarly, inoculations of cells of secondary whole embryo CBA T6 cultures did not produce tumors in syngeneic mice at an inoculum dosage as high as 5×10^6. As can be seen in Tables 1 and 2 ("Control"), and Figure 1, the original parental polyoma-transformed line (Py 27) produced tumors in the syngeneic (A/Sn) strain and in F_1 hybrid (CBA × A/Sn) mice, but the two subclones (27-6 and 27-6-8) were clearly less tumorigenic, producing fewer tumors (and after a longer latent period) than the line of origin. In view of this finding, the three clones Py 27, 27-6, and 27-6-8 were subjected to serological tests[8] which proved them all to possess the antigens characteristic of the H-2a allele carried by A/Sn mice. The hybrids of the latter two clones with T6 cells were clearly more "efficient" in producing tumors in F_1 CBA × A/Sn mice than their neoplastic parents. Of the two clones isolated from the 27-6/T6 mass hybrid culture, one (Cl. 30) did not produce tumors at the highest inoculation dose (5×10^6 cells), while the other (Cl. 58) was only occasionally tumorigenic (Tables 1 and 2). Therefore, it appears that both the parental polyoma-transformed lines and the population of their hybrids with normal cells are heterogenous with respect to the tumorigenicity of their component cells. Some tumors in each group were analyzed karyologically and found to be composed of donor-type cells, the tumors produced by both the parental neoplastic and the hybrid lines having the expected karyotypes.

Two of the three tumorigenic hybrid clones were implanted also into the parental

TABLE 3

TEST FOR DEMONSTRATION OF PV-ITA IN PARENTAL AND HYBRID CELLS*

Immunizing cells	Challenge Dose (C57/B1 tumor cells) 2×10^5	5×10^4
None	10/10†	9/10
Polyoma virus	1/5	1/9
Py 1‡	1/10	0/9
Cl 27	10/10	8/10
Cl 27-6	9/10	5/10
Cl 27-6-8	8/9	5/10
27-6/T6	9/9	7/8
27-6/T6 (clone 30)	8/9	5/8
27-6/T6 (clone 58)	9/10	5/7
27-6-8/T6	9/10	5/8

* Panels of C57/B1 mice were inoculated three times 1 week apart with 1.5×10^7 cells each time. In the case of polyoma virus, 0.2 ml in a single injection was given 1 month prior to challenge. Ten days after the last injection the animals were inoculated subcutaneously with the C57/B1 mouse polyoma tumor at two different doses.

† Animals with tumors/animals inoculated.

‡ Hamster cell line transformed *in vitro* by polyoma virus (from Dr. L. Diamond).

mouse strains A/Sn or CBA (Table 1), but none gave rise to tumors. Thus, clearly, the hybrid clones have histocompatibility antigens of both parental lines.

Test for PV-ITA.—Panels of male F_1 CBA × A/Sn mice were immunized with two intraperitoneal injections of P 172 polyoma virus containing 320 HA units per ml. The virus used for immunization was derived from a large plaque polyoma variant (PV 242) originally obtained from Dr. R. Dulbecco. One week after the last injection, the immunized animals and an equivalent number of control mice of the same genotype and age were challenged with two different doses of parental and hybrid cells. As shown in Table 2, mice immunized with polyoma virus were almost completely resistant to implantation of the parental lines and completely resistant to the two hybrid lines 27-6/T6 and 27-6-8/T6. The results with hybrid line 27-6/T6 (Cl. 30) could not be evaluated because of its failure to induce tumors in nonimmunized mice. These results clearly indicate the presence of polyoma-induced transplantation antigen in the parental lines Py 27, 27-6, and 27-6-8 and in the hybrid clones derived therefrom by "crosses" with T6 cells.

In order to verify whether the nontumorigenic hybrid 27-6/T6 (Cl. 30) contained PV-ITA, a different testing method was used—one that had been previously successful in the characterization of antigenic properties of hybrid cells.[1] C57BL mice were immunized with suspensions of parental and hybrid cells. Each mouse received three intraperitoneal immunizing injections and was challenged, ten days after the last injection, with implantation of a polyoma-induced C57BL tumor. For control purposes, mice were also immunized with a polyoma-transformed hamster cell line (Py 1) and with polyoma virus (Table 3).

The results of the experiment shown in Table 3 are much less satisfactory than those of the experiment summarized in Table 2. Mice immunized with either polyoma virus of Py 1 tumor cells were highly resistant to challenge implantation of isogeneic tumor cells. However, the same degree of resistance was not observed in mice immunized with cells of the parental and hybrid lines grown *in vitro*. Thus, only partial resistance to the lesser of the two challenge tumor doses was observed. Within this context, the "immunogenic" properties of the nontumorigenic hybrid cells 27-6/T6 (Cl. 30) were the same as those of the other lines, and it is therefore quite probable that this hybrid clone also has the PV-ITA. From the comparison of the results shown in Tables 2 and 3, it is quite evident that challenging polyoma-

TABLE 4

TEST FOR DEMONSTRATION OF PV-ICFA IN PARENTAL AND HYBRID CELLS

Cells	Immunofluorescence method	Complement-fixation microtiter method
C57/B1 Tumor	+	1:8
L cells	−	0
Cl 27	+	1:8
Cl 27-6	+	N.D.
Cl 27-6-8	(+)	0
T6 cells	−	0
27-6/T6	(+)	1:16
27-6/T6 (clone 30)	+	1:8
27-6/T6 (clone 58)	N.D.	1:8
27-6-8/T6	−	1:8

N.D. = not done.

immunized mice with tumor cells is a more sensitive method of testing for PV-ITA than immunization with the cells themselves.

Test for PV-ICFA.—The different parental and hybrid cells were assayed for the presence of PV-ICFA by the microtiter method previously described,[9] the only modification being that 50 per cent cell suspensions were used. The cell extracts were tested for the presence of PV-ICFA several times over a period of one and a half years. The results given in Table 4 show the presence of ICFA in all polyoma-transformed parental cell lines and in all hybrid lines except 27-6-8. As expected, ICFA was not present in the parental normal T6 fibroblasts, nor in the control L cells.

Recently, hamster serum has become available which reacts specifically in the immunofluorescence test with polyoma-transformed cells.[10, 11] A number of cell lines, listed in Table 4, were therefore stained with polyoma anti-PV-ICFA serum and fluorescein-conjugated antihamster gamma globulin. By analogy with the SV40 system, the antigens demonstrated by immunofluorescence and by complement-fixation are thought to be identical.[12, 13] Nuclear immunofluorescence was observed in most of the parental and hybrid clones, all cells of one line showing similar intensity and distribution of immunofluorescence. But the intensity and distribution of the antigen varied between different cell lines. Thus, while in the cells of some lines the antigen had a fine, discrete distribution, in others it had a more diffuse and reticular appearance; in all cases the nucleoli were negative. The reason for the variations in the appearance of the antigen is not known, but differences in immunofluorescence staining have been observed among hamster, rat, and other mouse lines transformed by polyoma virus.[10]

As shown in Table 4, there is general agreement between the results obtained by the complement-fixing and immunofluorescence methods, except for hybrid line 27-6-8/T6 which showed complement-fixing activity by the microtiter test but was negative for immunofluorescence. Different sensitivities of the two methods could account for this discrepancy.

Discussion.—The described results indicate that, within the limits of the sensitivity and nature of the methods used, the properties of polyoma-transformed parental cells (tumorigenicity and production of polyoma-induced antigens) appear as dominant in the hybrid cells. Thus, *the interaction of normal and tumor cell genomes does not result in the repression of these tumor cell properties.*

The validity of this interpretation may be applied only to cell populations, since

the test for tumorigenicity selects for the more virulent cells, and depends on (1) the T6 cells being indeed normal at the time of fusion and (2) at the time of testing no significant losses of the normal alleles contributed by the T6 parent having occurred in the hybrid cells.

The first possibility cannot be excluded *a priori* because it is known that *in vitro* cultured diploid mouse cells eventually give rise to abnormal variants which are the origin of usually neoplastic permanent lines. The fact that inoculations of T6 cells, similar to those used for the "crosses," did not result in the development of tumors cannot be regarded as an entirely meaningful control, because the presence of few neoplastic cells could escape detection, and because the incidence of cell fusions in the crosses is also a relatively rare event. However, the T6 cells used for hybridization underwent, prior to mating, only very few generations *in vitro* and, as shown by Rothfels *et al.*,[14] young cell strains, even when containing nests of altered cells, are not neoplastic. Furthermore, the fact that cell hybrids contained the expected two T6 chromosomes gives strong support to the assumption that, by the time of mating, the parental T6 cells had not undergone major karyotypic alterations which appear to precede neoplastic conversion.[14] In view of the above considerations, it appears to us extremely unlikely that the T6 parents could have been neoplastic at the time of mating.

The second possibility is equally difficult to disprove but appears also to be unlikely because (*i*) all hybrid cells were tested very soon after isolation and before extensive chromosomal losses had occurred and (*ii*) all hybrid cultures exhibited, from the very outset, the characteristic pattern of growth of transformed cultures.

With these reservations in mind, it seems reasonable to conclude that the investigated properties of polyoma-transformed cells are expressed in their hybrids with *normal* cells.

The results are compatible with the general hypothesis that viral oncogenesis results from the persistence and expression in the cells of added genetic information.[15, 16] They appear, on the contrary, incompatible with hypotheses ascribing viral oncogenesis to the deletion of *structural* information or to virus-induced *recessive mutation(s) of structural genes* in the normal cell genome.

Explanation of the enhancement of the tumorigenic properties of clones 27-6 and 27-6-8 after hybridization with normal mouse cells may be sought along several lines. As indicated above, our results suggest that polyoma-transformed lines are heterogeneous populations comprising cells of different degrees of oncogenicity. Because of different properties of their cell surface, the more tumorigenic cells may fuse more readily with normal cells than the less tumorigenic ones. On the other hand, the frequency of fusion of all cells of the polyoma-transformed lines with normal cells may be the same, but the hybrids resulting from the fusion of the more neoplastic cells may have a selective advantage over the other components of the hybrid populations propagated *in vitro*. The hybrid cells could also be less antigenic from the point of view of histocompatibility antigens as a result of increased ploidy.[17] The data at hand do not permit the choice between the possible mechanisms.

Whatever the mechanism turns out to be, its result, the enhancement of the tumorigenicity of the parental cells following hybridization with normal cells, leads one to wonder whether fusion of tumor cells with adjacent normal cells might not be a possible pathway of tumor progression *in vivo*. The various reports of host-

induced adaptation in transplantable tumors, in which immunoselection of the original population could be critically excluded[18–20] and the peculiar karyotypic evolution of some Ehrlich ascites tumors[21] could be interpreted in the light of this possibility.

Summary.—Somatic hybrids resulting from the fusion of polyoma-transformed and normal mouse cells are neoplastic and have the polyoma-induced transplantation and complement-fixing antigens.

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