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Induction of rat sarcomas in rats treated with antithymocyte sera after transplantation of human cancer cells

(antithymocyte sera immunosuppression/induced rat cancer cells)

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ABSTRACT **Human cancer cells that had had high (>160) tissue culture passages, when transplanted into antithymocyte-treated F344 newborn rats, caused induction of rat sarcomas in the rats within 2 or 3 subcultures, whereas human cancer cells with low (5–33) passages *in vitro* did not cause overt induction of rat sarcomas until after 5–10 subtransplantations. Because oncornavirus activity was not detected in either rat or human tumors, it is suggested that transforming sequences located on the human tumor cells may have been transferred to supporting rat reticulum cells in close contact with the human cancer cells.**

During serial subcutaneous transplantation of several types of human cancer cells into newborn F344 rats immunosuppressed by antithymocyte sera (ATS), we observed the local development of malignant rat-specific sarcomas which replicated readily when transplanted into newborn syngeneic rats. The number of subtransplants required for development of cancers in the rats varied according to the number of *in vitro* subcultures the human cells had previously experienced. Thus, ATS-immunosuppressed rats carrying a human rhabdomyosarcoma that had had 158–160 subcultures developed sarcomas in subtransplant 3, whereas three other human tumor cell lines, including a melanoma, a lung carcinoma, and sarcoma, with many fewer *in vitro* subcultures required 5–10 subtransplantations before the rat-specific tumors could be detected. The original human tumors and the subsequent rat tumors were characterized as human or rat by karyological, serological, and transplantation assays. All attempts to demonstrate oncornavirus activity in the rat or human tumors were negative.

Four human cancer cell lines were subcultured serially in ATS-treated F344 rats. All eventually developed lethal rat cancers (sarcomas) which were readily transplanted into newborn F344 rats after termination of ATS treatment and regression of the initial human cancers. The number of subtransplantations required for development of each of the four rat cancers appeared to be directly related to the number of prior *in vitro* tissue culture passages the human cancer cells had previously experienced.

MATERIALS AND METHODS

The human cancer cell lines grown in tissue culture and used for injection of ATS-treated newborn rats were as follows: RD rhabdomyosarcoma (1) at tissue culture passage 158; melanoma Hs294T (established in the laboratory of W. Nelson-Rees and provided by J. Fogh) (2) at tissue culture passage 33; a lung carcinoma at tissue culture passage 7; and TE 85 (clone F-5) cells, transformed by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), (3, 4) at passage 18.

The ATS inoculations were given before and after inoculation of the respective human cancer cells. The newborn F344 rats were given antiserum against rat thymocytes (lot 15192 from Microbiological Associates, Bethesda, MD) as follows: 0.1 ml intraperitoneally on the day of birth (day 0) and then on days 2, 4, and 10. This series was followed by 0.2 ml every 4 days as necessary, a regimen that reduced the peripheral whole white blood cells to 600–1200 cells/mm^3, as compared with the normal range of approximately 5000 cells/mm^3. In the initial transplant the rats were injected subcutaneously on the day of birth with approximately 10^7 freshly cultured human cancer cells. Subsequent transplantations of the tumor to additional ATS-treated newborn rats were accomplished by trocar injections of approximately 2×10^8 cells.

In each of four different experiments, the developing human tumors were first noted on day 10 (rhabdomyosarcoma), day 22 (lung), day 22 (melanoma), and day 8 (MNNG). During each experiment in the tumor-bearing rats, cell biopsies of the tumors were taken at regular intervals for the following purposes: (*i*) to determine whether the newly developed tumors were rat or human, by transplanting them into newborn rats not given ATS, a procedure that provided clear-cut specifications for rat cancer; (*ii*) to evaluate anti-human and anti-rat specific antibody reactions to the rat or human tumors or both with the use of complement fixation assays; (*iii*) to obtain karyological information specifying human or rat tumors (by W. Nelson-Rees, University of California School of Public Health, Oakland, CA); (*iv*) to demonstrate the presence or absence of rat sarcoma sequences in the cells of the respective tumors by molecular probes used by Howard Young (Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, MD); and (*v*) to histopathologically evaluate the rat cancers (by Melvin D. Reuber, Litton Bionetics, Frederick, MD).

RESULTS

Cancers with rat specifications were derived in each of the four immunosuppression experiments in which the different human cancers had been serially grown (Tables 1 and 2). The variable time factors required prior to development of the rat sarcomas appeared to be determined by the number of prior passages of the original tissue culture tumor cell lines and by the number of subsequent subtransplantations in newborn rats. Table 1 shows that the RD rhabdomyosarcoma, which had experienced 158 tissue culture passages prior to transplantation in the rat, resulted in the diagnosis of "rat" cancer in newborn transplant 3, which suggested that the rat tumors had developed late in the second transplant. The melanoma, lung, and MNNG cancer cells, which had many fewer tissue culture passages, required

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Abbreviations: ATS, antithymocyte sera; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Table 1. Influence of "high" and "low" subculture passages of four human cancer cell lines on transplant passage levels required for induction of rat cancers

Human tumor	Tissue culture passage	Transplant passage in which rat cancers were demonstrated
RD rhabdomyosarcoma	158	3
Melanoma	33	5
Lung	7	5
MNNG	5	10

5, 5, and 10 subtransplants, respectively, before cancers with specifications of "all rat" cancer were detected. Table 2 presents a typical protocol illustrating the onset of the rat cancer induced during or after (or both during and after) replication of the RD rhabdomyosarcoma cells.

DISCUSSION

The rationale for transplanting high and low passage human tumor cells in rats was based on earlier studies in which transformed rat cells grown exclusively *in vitro* lacked evidence for rat "*src*" transforming sequences when tested by Howard Young and Edward Scolnick (National Cancer Institute), using specific rat sarcoma probes. The tumor cells grown *in vitro* did not yield sarcoma viruses when attempts were made to rescue them with compatible oncornaviruses. However, after *in vivo* transplantation into syngeneic rats, the rat "*src*" sequences were regularly detected; when reestablished *in vitro*, the *src* sequences in the rat tumors were rescued by rat oncornavirus, thus yielding stable rat sarcoma viruses (5). These findings suggested that the human tumor cells, when subcultured in syngeneic rats, provided conditions for either activation of endogenous rat *src* sequences or transfer of human cell transforming sequences to the supporting rat cells. The findings suggested additional studies to find out if human cancer cells transplanted into newborn F344 rats (under ATS immunosuppression) would express human species-specific oncogenic sequences since it seemed logical to consider the possibility that the cancer cells identified as rat sarcomas after the removal of ATS may have been transformed by human transforming proteins (Tables 1 and 2). Because the probes for human sarcoma sequences have not been isolated, this question must be answered by future investigations since the tumor biopsies taken for cultivation and evaluation proved to be inadequate for this purpose. Reverse transcriptase (RNA-directed DNA nucleotidyltransferase) assays provided no evidence of oncornavirus activity in the human or rat tumors.

Table 2. Rat cancer induced during replication and rejection of RD rhabdomyosarcoma: Typical protocol

Transplant passage	Day tumor noted	Size, mm	Serological (complement fixation test) Anti-human antigen	Serological (complement fixation test) Anti-rat antigen
1	10	15	Not tested	Not tested
2	19	5	1:32 (human)	Negative (not rat)
3*	15	25	Negative (rat)	1:64 (rat)
5†	4	25	Negative (rat)	Not tested

ATS treatment: 0.1 ml intraperitoneally on days 0, 2, 4, and 10; 0.2 ml intraperitoneally on days 14, 18, 22, and 26. When ATS treatment was discontinued, the human and human–rat mixed tumors began to regress within several days, usually disappearing within 2 weeks. Many of the rats in which tumors regressed were held for several months, but no subsequent tumors appeared. Derivation of newly developed rat tumors was readily identified by successful subtransplantation in newborn syngeneic rats not treated with ATS.

* In the transplant bioassay test, the cell lines were passed in newborn rats without ATS, thus establishing the tumor as rat in subtransplant passages 3, 4, and 5. The serological data in transplants 3, 4, and 5 also confirmed the specifications for rat tumor cells.

† The tumors were rat sarcomas by histopathology and demonstration of rat chromosomes.

Addendum

Subsequent studies indicated that rat tumor cells, possibly in low numbers, were induced in the first transplant. This was confirmed by subculture of tumor cells isolated during the first transplantation and cultured 14 times *in vitro*. Karyology determinations were made by Walter Nelson-Rees.

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