HISTOGENESIS OF MOUSE SARCOMAS INDUCED BY IMPLANTATION OF POLYVINYL CHLORIDE FILM IN RADIATION CHIMERAS

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Sarcomas were induced in CBA/CBA-T6T6 mouse radiation chimeras by implantation of polyvinyl chloride film subcutaneously 13 months after irradiation and injection of donor's bone marrow. Of the 12 tumors studied 11 had the recipient's karyotype and one the donor's. The formation of connective-tissue cells from bone-marrow precursors thus, evidently, does not play an essential role in the histogenesis of sarcomas induced by plastics.

KEY WORDS: sarcoma; precursor cells; radiation chimeras in mice; karyotyping.

Sarcomas developing in mice at the site of subcutaneous implantation of pieces of plastic film arise, to judge from their morphology [7], from undifferentiated precursors of connective-tissue cells, particularly fibroblasts; the histogenesis of these sarcomas is thus linked with the problem of the origin of fibroblasts. There are two possible ways in which precursors of fibroblasts can arise: 1) from bone marrow stem cells identical with the hematopoietic stem cells [4]; 2) from an independent line of stem cells for mechanocytes [3].

In previous experiments [5, 7], sarcomas induced in mice by implantation of plastic film into radiation chimeras arose from the recipient's cells and not from transplanted bone marrow cells. These observations support the second of these two possibilities, but they do not completely prove the independence of sarcoma precursor cells from the stem cells of bone marrow. In these experiments the plastic was implanted into the chimeras a comparatively short time (30-40 days) after injection of the donor's bone marrow. The possibility cannot therefore be ruled out that the sarcoma precursor cells, although originating from bone marrow, unlike blood cells and macrophages were not a rapidly renewed population, i.e., in a short time interval the precursor cells from the newly injected bone marrow could not have replaced a large proportion of the sarcoma precursor cells formed previously from the recipient's cells. If, therefore, sarcomas nevertheless do arise from cells whose precursors are contained in bone marrow, this can only be detected by implanting pieces of plastic film into chimeras as late as possible after injection of the donor's bone marrow.

The object of this investigation was to discover whether a prolonged stay of transplanted bone marrow in the irradiated animal leads to the formation of sarcomas from the donor's cells.

EXPERIMENTAL METHOD

To obtain radiation chimeras, female CBA mice aged 3 months were irradiated with γ rays in a dose of 850-950 R. Immediately after irradiation the animals were given an intraperitoneal injection of 10^7 bone marrow cells from syngeneic CBA-T6T6 mice, differing by having two small marker chromosomes in their karyotype. Thirteen months after irradiation, one piece of polyvinyl chloride film measuring 1.5 × 2.2 cm was implanted subcutaneously into each radiation chimera in the region of the flank.

When the animals' condition worsened they were killed and the pieces of plastic film were removed together with the capsules which had formed around them and were transplanted at the rate of one piece each into female CBA or (CBA × CBA-T6T6)F_1 hybrid mice, as secondary carriers. The time during which the

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TABLE 1. Cytogenetic Analysis of Tumors in Radiation Chimera Mice

No. of chimera	% of cells with T6 markers in bone marrow of chimera	No. of tumor	Karyotype of tumor		Strain of secondary tumor carrier	Time from implanta. of plastic to transplanta. into second. carrier	Time from transplanta. of plastic with capsule into second. carrier to appearance of tumor	Time from subcutaneous implantation of plastic to appearance of tumor
			presence of T6 marker	number of chromosomes		months		
1	96	84	None	40—42	CBA	4,5	10,5	15
2	—	80	»	44:85	CBA	2	10,5	12,5
		85	»	39—42	CBA	2	13	15
	100	81	»	40—45	CBA	3	12	15
3		87	»	44—50	CBA	3	13	16
		92*	»	43—47	F_1†	2,5	11	13,5
6	—	83	»	40—44	CBA	3	12	15
	92	90	»	49—53	CBA	3	11	14
7		94	»	47—54	CBA	3	18	21
	90	78	»	70—72	F_1	5,5	3,5	9
8		96	»	82—98	F_1	5,5	15,5	21
9	98	86	T6	40—45	CBA	4,5	11	15,5
10	94	79	4T6	85—94	F_1	5,5	6,5	12

*Tumor arose from plastic reinjected into chimera (see text).

†F_1(CBA × CBA-T6T6) — one T6 marker present in karyotype.

pieces of plastic film remained in the chimeras varied from 2 to 5.5 months. The bone marrow of the killed chimeras was examined cytogenetically. In one case (chimera No. 3), after removal of the subcutaneous plastic from a chimera which was then 16 months old, a fresh piece of plastic was implanted, and this in turn was transplanted into a second carrier 2.5 months layer (Table 1, tumor No. 92).

The CBA female or (CBA × CBA-T6T6)F_1 hybrid mice developed tumors 3.5-20 months after transplantation of the pieces of plastic with capsules from the chimeras. The time between implantation of the plastic into the chimeras and the appearance of tumors around the pieces of film (in the secondary carrier) was 12-21 months. When the tumors had reached 1.5-2 cm in diameter the mice were killed and a cytogenetic analysis made of the tumors by the method described previously [1]. Depending on the presence or absence of T6 markers in the tumor cells the origin of the tumor from cells of the donor or recipient of the bone marrow was decided.

It was considered that it would be virtually impossible for a tumor to arise from the cells of the secondary carrier, for the present writers previously [2] and, independently, Brand and co-workers [6] showed that after transplantation of pieces of plastic film together with the intact capsule, tumors which subsequently grew in the recipient around the plastic arose from the "donor's" cells.

EXPERIMENTAL RESULTS

Cytogenetic analysis of 13 tumors showed that 11 of them had no T6 markers and, consequently, arose from cells of CBA mice (Table 1). In one tumor (No. 86), among the 40-45 chromosomes of the tumor cell karyotype, one chromosome similar to the T6 marker was seen. Tumor No. 86 may have arisen in two ways: from cells of bone marrow origin received by the chimera from the CBA-T6T6 mouse, followed by loss of one of the T6 markers during rearrangement of the karyotype in the course of malignant change, or from cells without markers (cells of the irradiated CBA mouse), with the appearance of a small chromosome resembling the T6 marker as a result of chromosomal structural changes. Since it was impossible to decide which of these two alternatives was correct, this tumor was not considered.

In tumor No. 79 four T6 markers were found in a near-tetraploid karyotype (89 chromosomes). This tumor arose in a secondary carrier which was a (CBA × CBA-T6T6)F_1 hybrid with one T6 marker. It was considered unlikely that the tumor could have arisen from the cells of the hybrid itself, with a subsequent doubling of the number of markers, considering the comparatively short period of stay of the plastic in the hybrid: From the time of transplantation of the plastic surrounded by its capsule from the chimera to the appearance of the tumor was 6.5 months. It is also difficult to imagine the appearance of four markers indistinguishable from T6 chromosomes as a result of chromosomal aberrations. Most probably tumor No. 79 developed from cells of the CBA-T6T6 mouse which was the donor of bone marrow for the chimera. Morphologically this tumor consisted of a typical spindle-cell sarcoma, indistinguishable from ordinary sarcomas induced by implantation of plastic film.

The results of this investigation thus show that sarcomas developing in radiation chimeras as a result of subcutaneous implantation of pieces of plastic film in the late periods after irradiation arise as a rule from cells of the irradiated animal and not from bone marrow cells transplanted into it. However, the presence of T6 markers in one of the tumors does not rule out the possibility that, in principle, sarcomas can develop through induction by plastic film from elements of bone-marrow origin.

If connective-tissue cells used up as material for the formation of these tumors in mice are in fact replaced by bone-marrow cells, the process takes place extremely slowly. Therefore, the formation of connective-tissue cells from bone-marrow precursors evidently does not play an essential role in the histogenesis of sarcomas induced by implantation of plastic film.

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ACTION OF SARCOLYSIN AND ASALINE ON INCORPORATION OF THYMIDINE-H^3 INTO DNA OF SARCOMA 45 AND TISSUES OF TUMOR-BEARING RATS*

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The action of sarcolysin and asaline on DNA synthesis in sarcoma 45 and in the spleen, thymus, bone marrow, and liver of tumor-bearing rats was studied in relation to the time of administration of the compounds. The selectivity of action of sarcolysin and asaline, which differ in the structure of the carrier, was found to be directly dependent on the ability of these compounds to depress DNA synthesis in the tumor and in normal tissues.

KEY WORDS: sarcolysin; asaline; DNA synthesis; sarcoma 45.

Larionov's view [5] that the effectiveness of antitumor alkylating compounds may depend substantially on the structure of the carrier has now obtained adequate experimental confirmation [3, 6]. Meanwhile the search for new chemotherapeutic agents with more selective action calls for the study of the molecular mechanisms of their cytostatic action [3]. One possible approach to the solution of this problem would be by studying DNA synthesis, which is easily disturbed by alkylating agents [7] and ionizing radiation [9].

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