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Growth of Human Cancer Cells as Lung Metastases in Immunologically Tolerant Rats (38759)

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Previous reports from this laboratory (1–3) described the induction of specific immunologic tolerance to subcutaneous heterotransplants of human cancer cells in baby rats which had been inoculated intravenously during the first day of life with living human cancer cell lines. The immune tolerance so produced was specific for human cells but was not restricted to the particular cell line used to induce the tolerance. The cells used to induce tolerance established widely disseminated implants of the tumor cells which grew progressively and killed the recipients by about 5–6 wk of age.

Subsequent studies, as yet unpublished, have shown that similar specific immunologic tolerance to human cancer cell lines can be induced in rats by neonatal intravenous inoculation of human cancer cell lines which have been irradiated by X-rays or gamma rays to a dose of 2000 or 10,000 rad, which prevents propagation of the intravenously inoculated cells. These animals remain healthy and grow as rapidly as their nontolerant litter mates. Persistence of tolerance (that is, receptivity to the subcutaneous tumor transplants) decreases after 3–4 wk and usually is gone by 6–8 wk of age. Tolerance has been induced using the nonmalignant human cell line "Amnion B," instead of malignant cell lines, although our limited attempts to induce tolerance by use of human leukocytes or cultured normal human fibroblasts were unsuccessful.

In the studies reported here, we demonstrate that baby rats made immunologically tolerant by use of the Amnion B cell line will subsequently accept *intravenous* heterografts of the human malignant cell line J-111 with production of multiple tumors in lungs which usually grow progressively and kill the recipient.

Materials and Methods. The Amnion B cell line (4) was initiated and maintained for the past 12 yr in this laboratory. It propagates rapidly as a monolayer culture on glass although it is of normal origin and does not produce tumors in the immunologically tolerant rat system, even when the same cell line is used to induce tolerance. The J-111 line (5) originated from blood buffycoat of a patient with monocytic leukemia but it grows as a monolayer of epitheloid cells on glass and produces subcutaneous nodules with the histologic characteristics of a malignant neoplasm on transplantation to immunologically tolerant rats (1). Both cell lines were cultured in Eagle's minimal essential medium (MEM) supplemented with glutamine and containing penicillin 200 units per ml and streptomycin 0.2 mg/ml. To harvest the cells they were exposed to 0.25% trypsin solution in isotonic saline for about 10 min, collected into centrifuge tubes, washed twice with solution A (6), counted in a hemocytometer and resuspended in solution A at the concentrations desired for transplantation.

The rats were bred in this laboratory of mixed Long-Evans and Wistar parentage. (Extensive studies with Fisher, Long–Evans and noninbred Wistar rats show that success in induction of tolerance is not related to the genetic constitution of the rats.) Pregnant rats due to deliver were checked twice daily and the babies were inoculated IV between 4 and 22 hr after birth with a "tolerogenic" inoculum of 2 million amnion B cells suspended in 0.25 ml solution A. Injection was via a 30 gauge needle and 0.25 ml syringe. An equal number of animals in each litter was reserved for use as nontolerant controls and marked by clipping off the tips of the tails. Each "challenge" inoculum contained 2 million cells of the J-111 cell line and was administered between 4 and 19 days of age. Each rat received both a subcutaneous challenge with 2 million cells in 0.1 ml in the left flank, and an intravenous challenge with 2 million cells in 0.25 ml. Intravenous injec-

TABLE I. GROWTH OF J-111 CELLS INOCULATED INTRAVENOUSLY AT VARIOUS AGES INTO IMMUNOLOGICALLY TOLERANT BABY RATS AND NORMAL LITTER MATE CONTROLS.

	Autopsy		Tolerant rats					Non Tolerant Controls				
Age when challenged (days)	Day of age	Days after challenge	No. of rats	SC Tumors mm diam	Lung tumor grades[a]	Body wt (g)	Lung wt % of body	No. of rats	SC tumors mm diam	Lung tumor grade[a]	Body wt (g)	Lung wt % of body
4	30	26	3	14, 8, 6	4, 4, 4	48, 36, 25		4	6, 6, 4 0	4, 1, 0, 0	36, 82, 78, 78	
7	28	21	5	20, 12, 10, 10, 0 0	4, 2, 4, 4, 0	55, 60, 35, 38 64	6.6, 1.7, 10., 10., 1.0	3	0, 0, 0	0, 0, 0	58, 62, 62	1.5, 1.0 1.3
12	41	29	3	20, 16, 14	1, 0, 0	142, 142, 115	0.8, 0.7, 0.7	3	0, 0, 0	0, 0, 0	120, 150, 160	0.9, 0.9, 0.8
14	41	27	4	20, 18, 15, 12	1, 1, 1, 1	94, 110 100, 116	1.0, 0.9, 0.9, 1.1	3	0, 0, 0	0, 0, 0	116, 126 130	0.9, 0.8, 0.9
19	33	14	2	8, 6	0, 0	95, 85		3	0, 0, 0	0, 0, 0	85, 90, 92	

[a] Grading of lung tumors: 4, complete replacement of lungs by tumor; 3, over 50% replaced; 2, less than 50% replaced; 1, a few scattered nodules; 0, no tumor found. Entries for individual rats are in same sequence in each of the 10 experimental groups.

tions, which are much more difficult at this age than at birth, were given through veins of the tail or the dorsal or plantar surface of the feet. Animals in which IV inoculation was attempted unsuccessfully were discarded. Thereafter, each rat was checked twice each week for 4–6 wk for general appearance, body weight, and development

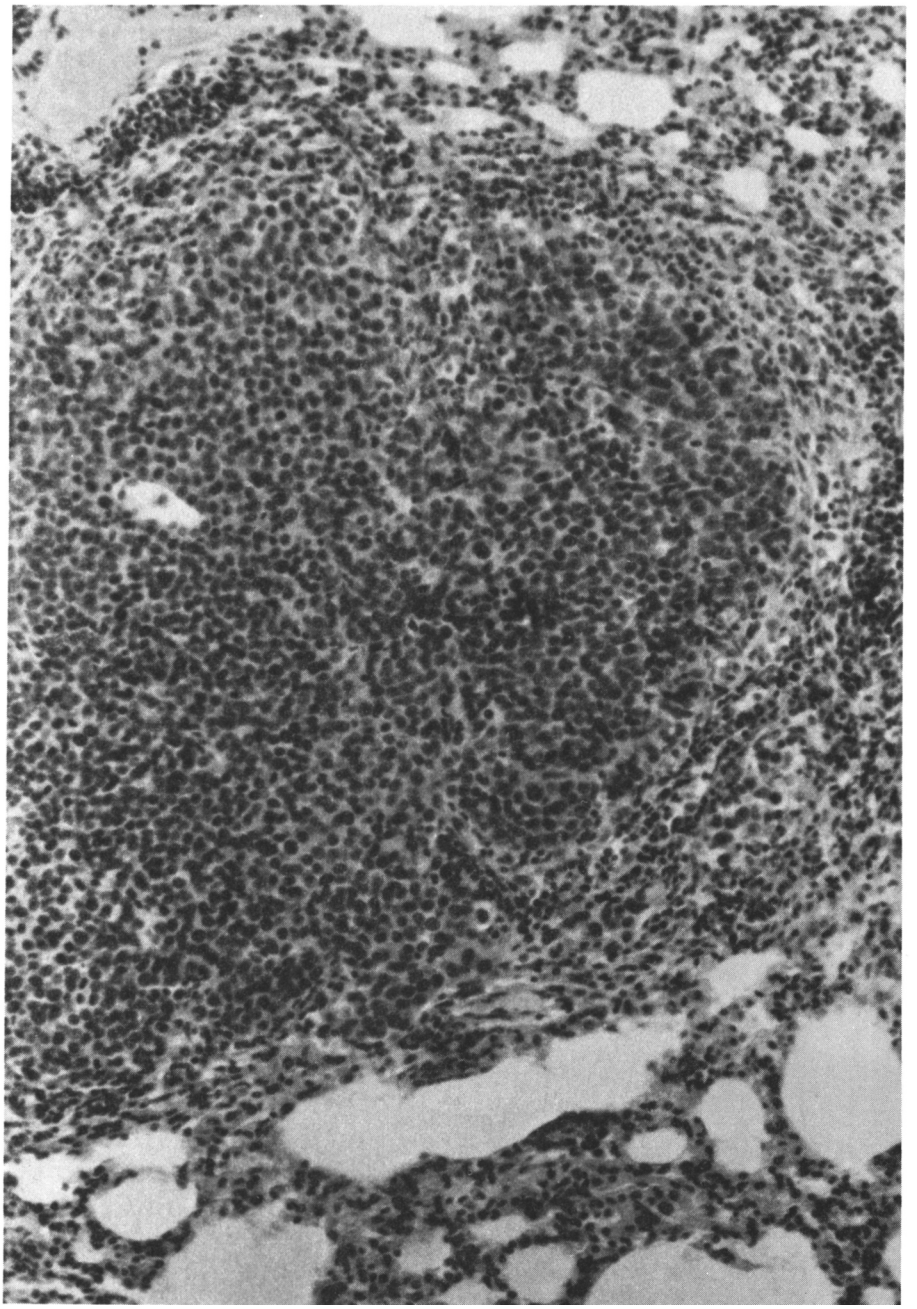

FIG. 1. a. Tumor nodule of J-111 cells in lung of a 41 day old tolerant rat injected IV with the malignant human cell line at age 14 days. The macroscopic tumor grade was 1+ (as defined in Table I). Perinodular collections of lymphoid cells presage eventual rejection of the tumor cells. b. Higher magnification of another nodule of identical history but showing no lymphoid infiltrate.

of subcutaneous tumors. Each animal was then autopsied. Subcutaneous tumor nodules were removed and measured. Lungs, adrenals, and interscapular brown fat pads were examined for the presence of macroscopic tumors, since these organs were the most frequent site of implants of J-111 in previous studies in which J-111 cells were inoculated intravenously on the day of birth. Body weight and lung weights were recorded as a quantitative measure of the extent of tumor growth in the lungs.

Results. Data are presented in Table I. When challenged at age 4 days, all three

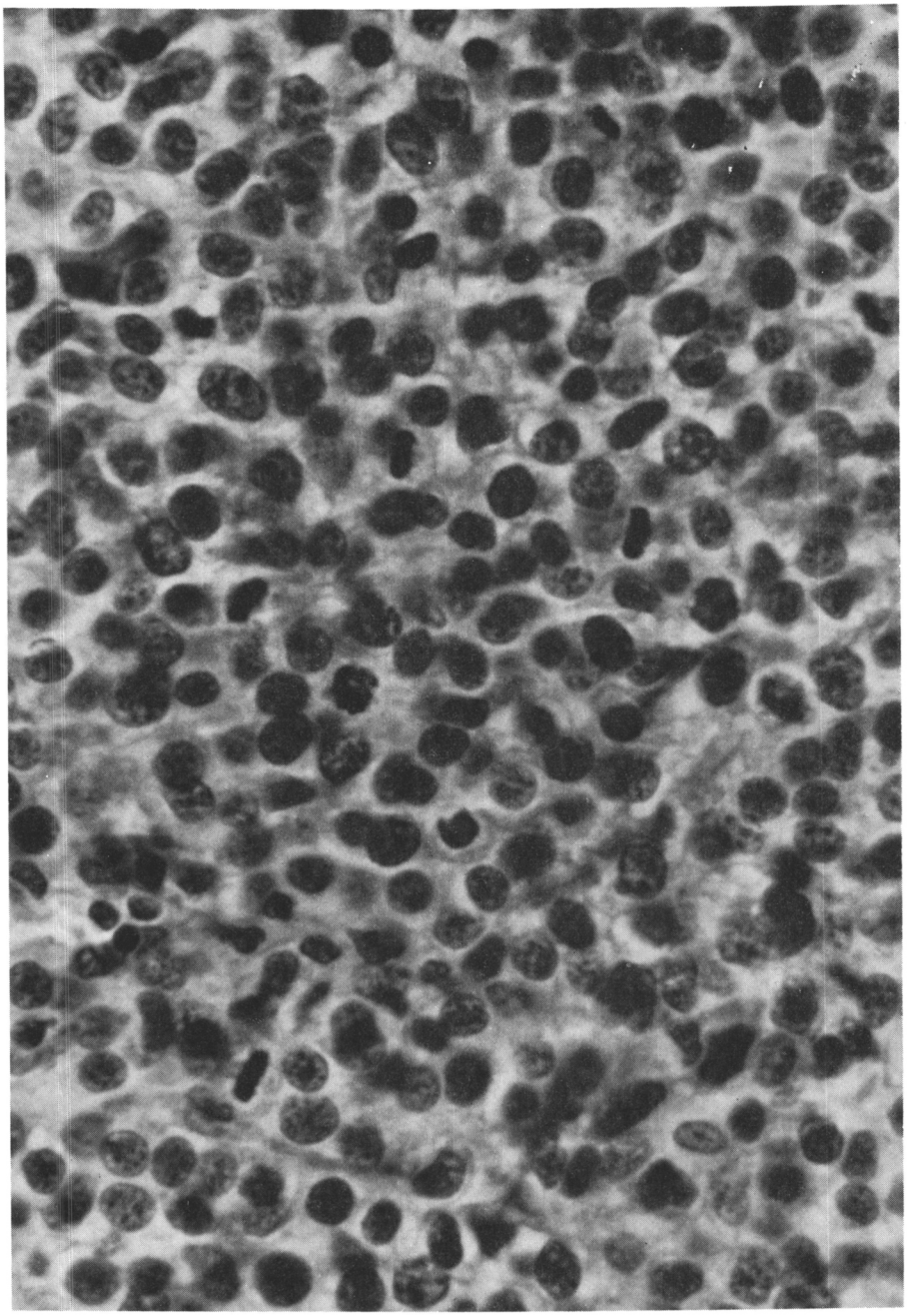

FIG. 1b.

tolerant rats developed both subcutaneous and lung tumors, but three of the four nontolerant controls also developed subcutaneous tumors and two of them had lung tumors, so growth of the J-111 cells cannot be attributed to tolerance induced by the injection of Amnion B cells, but rather to the immunologic immaturity of the animals at age 4 days. However, when challenged at age 7, 12, or 14 days, the nontolerant control rats had neither subcutaneous nor lung tumors, indicating that immune competence sufficient to reject the foreign cells had developed by 7 days of age; while most of the tolerant recipients had both subcutaneous and lung tumors, demonstrating that the neonatal inoculation of Amnion B cells had induced a state of immunologic tolerance. In the tolerant rats subcutaneous tumors grew to a similar size range regardless of age when challenged. In contrast, growth of the intravenously injected J-111 cells was strongly influenced by the time of injection. In rats which were challenged on day 7 growth of tumor in the lungs was massive and lethal. Those inoculated on day 12 or 14 had only scattered nodules in the lung, but histologic examination confirmed that these did consist of healthy appearing epithelioid cells with many mitoses (Fig. 1). When challenge was delayed until age 19 days, tumors grew subcutaneously but not in the lungs of the two tolerant rats, further confirming that the tolerant rats lose their receptivity for pulmonary implants of J-111 sooner than their receptivity for subcutaneous implants.

The tabulated data also show that body weight was inversely related to tumor growth in lungs, although not to subcutaneous tumor in the absence of lung tumors; and that the amount of tumor in lungs as graded macroscopically was paralleled by increased lung weight (expressed as proportion of body weight).

Discussion. In previous studies we observed that rats made tolerant to human cells accepted intramuscular or intraperitoneal transplants as well as subcutaneous transplants of J-111 cells (unpublished data), and we have now demonstrated that the tolerance extends to visceral implants established by intravenous injection. The visceral implants were all in the lungs except for two rats challenged on day 4 and one on day 7 which also had enlarged adrenals presumably, as judged by past experience, due to tumor implants. When J-111 cells were inoculated intravenously during the first day of life, in previous studies, many animals showed a more extensive distribution of the tumors, with gross involvement of adrenals, interscapular fat pads, heart, lymph nodes, brain and eyes, in addition to massive lung involvement. This difference suggests that the animals challenged intravenously at age 7 or more days are generally less susceptible or possibly show a different pattern of organ receptivity, than when inoculated on the first day of life. However, further studies with larger numbers of animals and with litter mate controls will be necessary to confirm this impression.

Comparison of growth from the subcutaneous and the intravenous injections in the present study shows that acceptance of subcutaneous implants persists longer than acceptance of lung implants. We do not know whether the lungs and other viscera are less receptive than subcutaneous tissue to the implantation and growth of the human tumor cells due to unknown physico-chemical conditions in the local milieu, or whether the difference is attributable to the high local concentration of cells deposited at the subcutaneous site in contrast to the wide scattering of intravenously injected cells.

It is hoped that this method of producing lung implants of human cancer in 1- to 2-wk old rats will be useful to study chemotherapy of "metastatic" human cancer in physiologically intact laboratory animals. We have demonstrated a chemotherapeutic effect from bleomycin in rats bearing disseminated J-111 tumors following inoculation on the day of birth (7), but the usefulness of that technique was limited by extreme toxicity of chemotherapeutic agents for newborn rats, whereas slightly older rats tolerate relatively larger doses of most drugs.

Summary. Newborn rats were made tolerant to human cell antigens by intravenous injection of Amnion B cells, a permanent human cell line of normal origin. At various

intervals thereafter each animal, and non-tolerant litter mate controls were challenged by SC and IV injections of the malignant human cell line J-111. Tumor nodules of J-111 cells grew SC and in the lungs of most of the tolerant rats challenged at ages from 7 to 14 days, but not in their controls. Challenge at age 19 days produced SC tumors but there was no growth from the IV inoculum.

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