Proc. Nat. Acad. Sci. USA
Vol. 71, No. 1, pp. 148–152, January 1974

Tumor–Host Cell Hybrids in Radiochimeras

(*in vivo* hybrid/enzyme-deficient solid tumors/ascites tumors/macrophage)

FRANCIS WIENER, EVA MARIA FENYÖ, AND GEORGE KLEIN

Department of Tumor Biology, Karolinska Institutet, 104 01 Stockholm 60, Sweden

Contributed by George Klein, September 14, 1973

ABSTRACT **F_1 hybrid mice syngeneic or semiallogeneic with respect to the relevant tumor were lethally irradiated and then reconstituted with hemopoietic cells from strain CBAT6T6 mice. After chimerism had been established, the animals were inoculated with solid or ascites tumors. Tumor-host cell hybrids were selected from enzyme-deficient solid tumors by explanting the tumor cell suspension into hypoxanthine-amethopterin-thymidine containing medium. The selection of hybrid cells from ascites tumors was achieved by exploiting the difference between the ascites tumor cells and hybrid cells in their ability to adhere to the surface of culture vessels. T6T6 chromosomal and H-2 antigenic markers served to distinguish between the hemopoietic cells derived from the donor graft and the cells of the host. All solid tumors tested fused with cells of the irradiated host, whereas ascites tumors fused with repopulating cells of hemopoietic origin.**

In two previous publications (1, 2) we have described the successful selective isolation of tumor–host cell hybrids from solid and ascites tumors *in vivo*. The tumors studied included polyoma- and methylcholanthrene-induced sarcomas, an ascites carcinoma of mammary origin, and sarcoma lines derived from spontaneous transformation of normal fibroblasts. Unequivocal evidence was obtained for fusion between the tumor cells and host cells. Hybrid cells were isolated containing tumor-derived chromosomal and antigenic (H-2) markers together with host-derived H-2 or translocation (T6T6) markers. The chromosomal constitutions of the selected hybrid line were often only slightly reduced in comparison with what might be expected from the fusion of one tumor cell with one host cell.

The type of host cell involved in the fusion is unknown. In order to assess the biological significance of the phenomenon, it was obviously important to define this, at least in outline. Radiation chimeras in which the host and the repopulating cells carried different markers appeared to present a possible approach to this question.

MATERIALS AND METHODS

The experimental design was as follows. F_1 hybrid mice syngeneic or semiallogeneic with respect to the test tumor were lethally irradiated and then reconstituted with CBAT6T6 hemopoietic cells. After chimerism had been established, the animals were inoculated with solid or ascites tumors. Tumor–host cell hybrids were selected as previously described (1, 2). T6T6 chromosomal and H-2 antigenic markers served to distinguish between the hemopoietic cells derived from the embryonic liver graft and the cells of the host.

Production of Chimeras. C3H × DBA/2, C3H × C57Bl, and C3H × C57 leaden F_1 hybrid mice, 6–8 weeks old, were lethally irradiated with 800 R (3). About 5×10^5 hemopoietic cells obtained from the liver of newborn CBAT6T6 mice were suspended in 0.5 ml of sterile Eagle's minimal essential medium and inoculated into the tail vein of an irradiated recipient. The development of chimerism was confirmed by examining metaphase plates of spleen or lymph node cells. As a rule, lymphopoietic and hemopoietic organs were repopulated with T6T6 marker positive cells within 2–3 weeks.

Tumors. (*a*). *Solid tumors.* A9HT and B82HT are highly malignant variants selected from the L cell sublines, A9 and B82 (4). Like the original *in vitro* lines from which they were derived, A9HT and B82HT are enzyme-deficient; A9HT lacks hypoxanthine - guanine - phosphoribosyltransferase (HGPRT), while B82HT lacks thymidine kinase (TK). Due to these enzyme deficiencies both lines are unable to grow on hypoxanthine–amethopterin–thymidine (HAT) medium. With inocula of 4×10^4 to 4×10^4 cells, both lines grew progressively in newborn x-irradiated (4 J kg) syngeneic or semisyngeneic C3H mice and killed 85–95% of the recipients (4). Both lines carry the $H\text{-}2^k$ histocompatibility complex, characteristic of the C3H mouse strain from which the original L-cell line was derived. The karyotypes of A9HT and B82HT are similar to those of A9 and B82, but show slightly reduced chromosome numbers (5). The modal chromosome number of A9HT and B82HT are about 53, compared with 57 for A9 and B82. The 23–28 biarmed chromosomes present in both lines served as chromosomal markers.

(*b*). *Ascites tumors.* The ascitic form of the SEWA sarcoma was used. SEWA was originally induced by polyoma virus in an A.SW mouse (6) and carries the $H\text{-}2^s$ histocompatibility complex. It also carries the polyoma-specific transplantation antigen. It has a near-diploid chromosome constitution with a mode of 43 and a narrow range of 42–44 subtelocentric or telocentric chromosomes. The majority of the metaphase plates contain a very long telocentric marker, a marker with a secondary constriction, and several minute chromosomes or fragments (7).

SEYF is a polyoma-induced fibrosarcoma of an A.BY mouse, converted to the ascites form. It contains the $H\text{-}2^b$ complex and the polyoma-specific transplantation antigen (8). It has an aneuploid chromosome constitution ranging between 57 and 69 chromosomes, with a mode of 67. Between 6 and 10 biarmed chromosomes are present (mode: 7); these serve as chromosomal markers.

The TA3Ha tumor is the ascitic form of a spontaneous mammary adenocarcinoma of the A/Sn strain (9). It carries the $H\text{-}2^a$ isoantigen complex. It has a near diploid chromosome number with a mode of 41. An 8-azaguanine-resistant variant designated $TA3Bimp^-$ was produced in Oxford. This variant line is deficient in hypoxanthine-guanine-phosphoribosyltransferase and does not grow on HAT medium.

Abbreviation: HAT, hypoxanthine–amethopterin–thymidine.

Chromosome Preparations. Metaphase spreads of cultured cells were prepared by the air-drying technique (10). Colcemid (GIBCO) was added to the culture in a final concentration of 0.04 μg/ml 4–5 hr before the cells were harvested. After hypotonic treatment (0.075 M KCl solution for 5 min), the pellet was fixed in acetic acid–methanol (3:1), spread on chilled wet slides, air dried, and stained with alkaline Giemsa solution. The total number of chromosomes and the proportion of biarmed chromosomes were estimated by direct counting under the microscope. Randomly selected metaphase plates were photographed, and karyograms prepared for detailed analysis.

Isoantisera and Adsorption Tests. Groups of 10–12 adult mice of the appropriate genetic constitution were immunized with pooled cell suspensions prepared from spleen, kidney, and liver of mice carrying the requisite H-2 complex. The animals were injected subcutaneously once every 2 weeks for 12–14 weeks. The activity and specificity of all isoantisera were checked by cytotoxicity tests against lymph node cells of the appropriate genotype as described (11).

To measure the concentration on H-2 antigens on the surface of the cell lines, quantitative adsorption tests were done as described previously (11). The ratio of bound to free antibody, (100-P)/P, was calculated by the method of Reif (12).

RESULTS

Chromosome Constitution and Antigenic (H-2) Characteristics of the Hybrid Cells. Ten tumor-host cell hybrids were isolated from tumors growing intraperitonally and subcutaneously. The isolation procedures described in detail in our previous publications (1, 2) can be summarized briefly as follows.

The selection of hybrid cells from enzyme-deficient tumors was achieved by explanting the tumor cell suspension into HAT medium. Neither hypoxanthine-quanine-phosphoribosyltransferase-deficient cells (A9HT and TA3Bimp$^-$) nor thymidine kinase-deficient cells (B82HT) can survive in HAT medium. The explanted tumor cells therefore died, but after 8–14 days some HAT-resistant colonies appeared. The cells in these colonies proved to be hybrids.

In the case of the ascites tumors SEWA and SEYF, which lack biochemical markers, the selection of hybrid cells was achieved by exploiting differences between the ascites tumor cells and hybrid cells in their ability to adhere to the surface of culture vessel. The ascitic fluid was sucked out of the peritoneum, suspended in minimal essential medium, and then seeded into Roux bottles. The explanted ascites cells attach loosely to the surface of the culture vessels and can be removed completely either by shaking or treating with trypsin. Within 24 hr of explantation, however, a few firmly attached cells were visible and some of these formed small colonies between 8 and 20 days after explantation.

Tables 1 and 2 summarize the chromosome constitutions and the antigenic characteristics of the original tumor lines and the tumor–host cell hybrids derived from them. Fig. 1 illustrates individual immune absorption results obtained in tests for tumor- and host-derived H-2 antigen typing. Fig. 2 shows the adsorptive capacities of tumor and hybrid cells from the various antisera.

TABLE 1. *Chromosomes and H-2 antigens of hybrids between host cells and SEWA, SEYF, and TA3 ascites tumor cells*

Tumor*	Genotype of host	Total chromosome no.		No. biarmed chromosomes		T6T6 markers†		H-2 isoantigen complex	
		Range	Mode	Range	Mode			Present	Absent
SEWA	A.SW	42–44	43	—	—	−	−	H-2^a	
SEYF	A.BY	57–69	67	6–10	7	−	−	H-2^b	
TA3Bimp$^-$	A	40–42	41	—	—	−	−	H-2^a	
SEWA-H7A	C3H × C57Bl F_1	81–84	83	—	—	+	+	H-2^a H-2^k	H-2^b
SEWA-H7B	C3H × C57Bl F_1	63–86‡	—	—	—	+	+	H-2^a H-2^k	H-2^b
SEYF-YHA	C3H × ABY F_1	82–93	—	3–7	5	+	+	H-2^b H-2^k	
TA3Bimp$^-$-H7A	C3H × C57Bl F_1	41–119‡	—	—	—	+	+	H-2^a	H-2^b
TA3Bimp$^-$-H7B	C3H × C57Bl F_1	91–122	104	—	—	+	+	H-2^a	H-2^b
TA3Bimp$^-$-H7C	C3H × C57Bl F_1	80–124	116	—	—	+	+	H-2^a	H-2^b
TA3Bimp$^-$-HDB	C3H × DBA/2 F_1	108–121	119	—	—	+	+	H-2^a	
TA3Bimp$^-$-HDC	C3H × DBA/2 F_1	98–120	109; 115	—	—	+	+	H-2^a	

* The second part of the name designates the genotype of the host. H: C3H, F: C5FBl, D:DBA/2. The third letter is the serial designation of the individual selected line.

† +: Both T6T6 markers were present; − − both were absent.

‡ Markedly reduced chromosomal numbers relative to the expected. The T6T6 markers were only present in metaphase plates showing a nearly complete bi-parental chromosome constitution.

TABLE 2. *Chromosomes and H-2 antigens of hybrids between host cells and A9HT or B82HT solid tumors*

Tumor	Genotype of host	Total chromosome no.		No. biarmed chrs.		T6T6 markers		H-2 isoantigen complex
		Range	Mode	Range	Mode			
A9HT	C3H	50–55	53	23–27	24	−	−	H-2^k
B82HT	C3H	51–56	54	22–29	25	−	−	H-2^k
A9HT-HDE	C3H × DBA/2 F_1	87–94	93	21–25	23	−	−	H-2^k H-2^d
B82HT-HDA	C3H × DBA/2 F_1	82–91	86	22–30	24	−	−	H-2^k H-2^d
A9HT-HLA	C3H × C57 leaden F_1	63–95	—	15–25	—	−	−	H-2^k H-2^b

Conventions as in Table 1. L: C57 leaden in tumor designation.

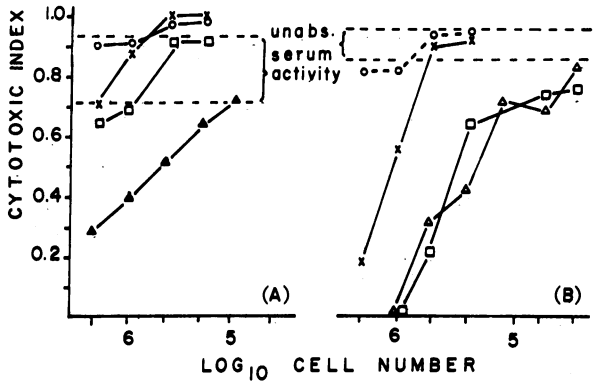

FIG. 1. Adsorption of antisera by tumor cells. (*A*) Antisera detecting the H-2^b antigen complex, made in strain A mice immunized with cells from strain C57 leaden. (*B*) Antisera detecting H-2^d or H-2^s.D antigen complexes. The sera were made by immunizing C3H × C57 Bl F_1 mice. (——), or ASW × C57Bl F_1 mice (- - -) with DBA/2 cells. Hybrid tumor cells were: O, SEWA-H7A; ×, TA3Bimp$^-$-H7A; △, A9HT-HDE; ▲, A9HT-HLA; □, B82HT-HDA.

SEWA–Host Cell Hybrids. The two SEWA–host cell hybrid lines were isolated by the selective adherence technique from tumors growing intraperitoneally in C3H × C57Bl F_1 chimeras repopulated with CBAT6T6 hematopoietic cells. The modal chromosome numbers were slightly lower than that expected from the sum of the two parental modes. The presence of both SEWA and T6T6 chromosomal markers in the majority of the metaphase plates analyzed clearly showed that both hybrid lines were derived from fusion between the SEWA tumor cells and the CBAT6T6 donor cells that had repopulated the irradiated host. The adsorption tests showed the presence of the H-2^s isoantigenic complex characteristic of the SEWA tumor. The absence of the H-2^b complex indicated that the C3H × C57Bl F_1 host cells had not participated in the fusion.

SEYF–Host Cell Hybrid. This hybrid was isolated by the selective adherence technique from a SEYF tumor growing intraperitoneally in a C3H × ABY F_1 mouse. The chromosome constitution was consistent with its arising from a fusion between a SEYF tumor cell and one of the CBAT6T6 cells used to repopulate the irradiated animal. The hybrid cells contained the SEYF biarmed marker chromosomes and the T6T6 markers (Fig. 3). The simultaneous presence of the H-2^k and H-2^b complexes confirmed the hybrid nature of the line. In this case, the adsorption tests cannot distinguish between host cells and repopulating cells, since the H-2^k complex is carried by both. However, the presence of the T6T6 chromosomes is decisive evidence for the participation of the repopulating donor cells in the fusion event that gave rise to this line.

TA3–Host Cell Hybrids. Five tumor–host cell hybrids were isolated on HAT medium from TA3Bimp$^-$ tumors growing intraperitoneally in C3H × C57Bl F_1 and C3H × DBA/2 F_1 mice. The expected modal chromosome number for a hybrid cell derived from the fusion of a TA3 cell with a host cell or a CBAT6T6 donor cell would be 81. However, the modes of the hybrid lines ranged between 115 and 118 chromosomes. Two T6T6 marker chromosomes were regularly present (Fig. 4). The H-2^d antigen complex characteristic of three of the five irradiated hosts was absent. These observations suggest that the hybrids originally contained two TA3 chromosome sets and one chromosome set from the repopulating CBAT6T6 donor cell population. It is noteworthy that all five independently isolated hybrids showed roughly the same total chromosome number, the same antigenic constitution, and the presence of the T6T6 markers, and that another TA3 line that had been used for previous cell fusion experiments *in vitro* (13) also showed a marked tendency to produce 2:1 hybrids.

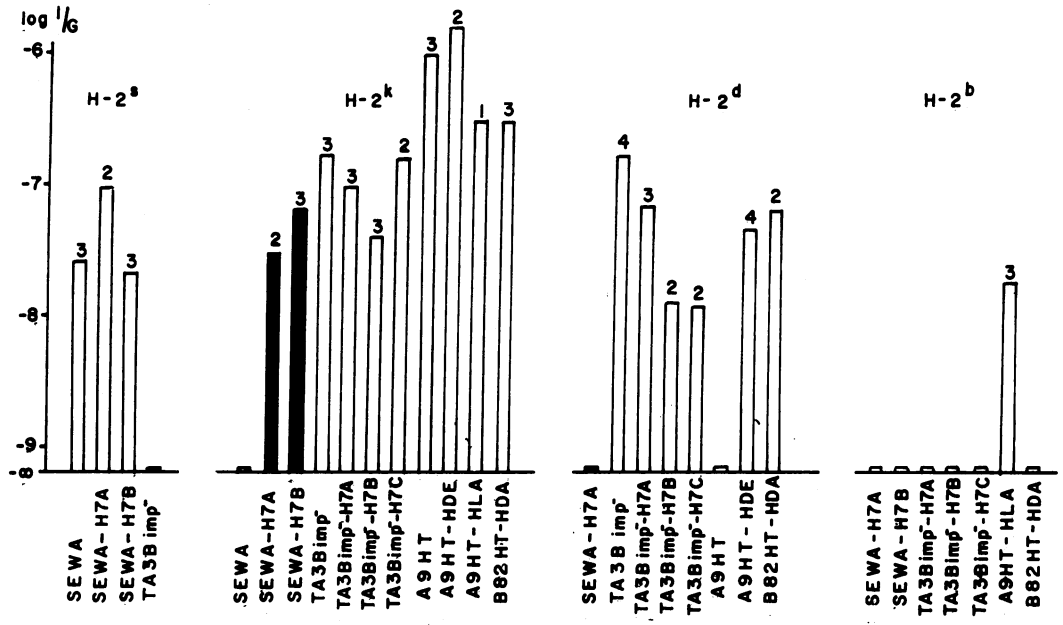

FIG. 2. Adsorptive capacities of cell lines for the following antisera: A × C57Bl anti-ASW (H-2^s); ASW anti-C3H ■ and DBA/2 × C57Bl anti-C3H □ (H-2^k); ASW × C57Bl anti-DBA/2 ■ and C3H × C57Bl anti-DBA/2 □ (H-2^d); and A anti-C57 leader (H-2^b). 1/G indicates the amount of antibody bound per cell when 50% of the cytotoxic activity of the antiserum had been bound. The numbers at the top of columns denote number of adsorption tests.

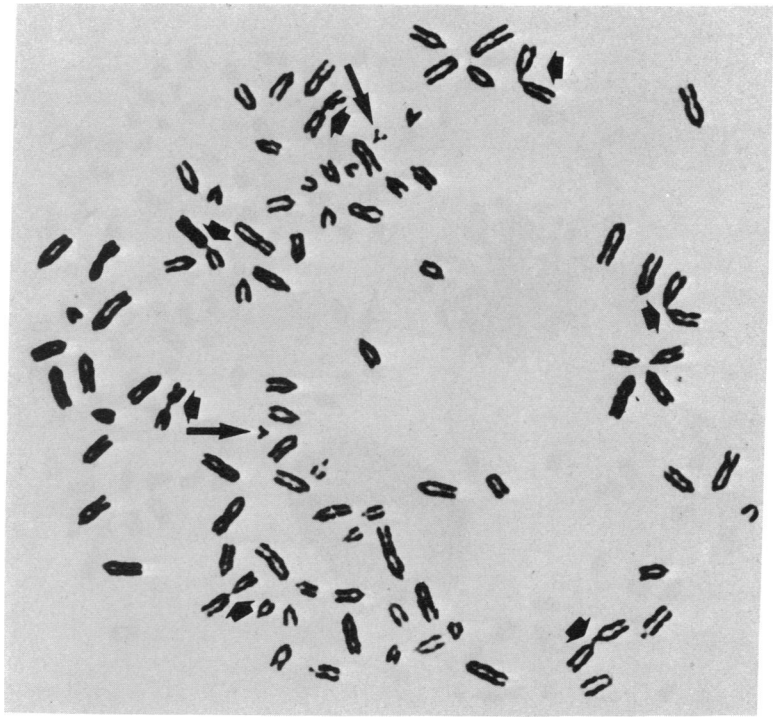

FIG. 3. Metaphase plate of SEYF–host cell hybrid isolated from a tumor produced in a C3H × ABY chimeric mouse. Note the presence of the SEYF-derived biarmed chromosomes (*thick arrows*) and of the two T6T6 markers (*thin arrows*) specific for the repopulating donor hemopoietic cells.

A9HT and B82HT–Host Cell Hybrids. Two hybrid lines were isolated on HAT medium from solid tumors growing in C3H × DBA/2 F_1 and C3H × C57 leaden F_1 mice. The hybrids contained the same number of biarmed chromosomes as A9HT and B82HT, and 30–35 additional telocentric chromosomes. Like other A9HT and B82HT tumor-host cell hybrids isolated previously (1), these new hybrids have the properties to be expected from the fusion of one A9HT or B82HT cell with one diploid host cell. The absence of the T6T6 marker chromosomes suggests, however, that the cells of these solid sarcomas fused with cells from the irradiated host and not with cells from the repopulating donor cell population. This view is confirmed by the regular presence of the host-derived H-2^d complex in the hybrid cells (Table 2). It thus appears that solid tumors fuse with cells of the irradiated host, whereas ascites tumors fuse with repopulating cells of hemopoietic origin.

DISCUSSION

These experiments raise two obvious questions. (*1*) What type of repopulating donor cells participate in the fusion? (*2*) Does the kind of tumor used, or its site of growth, determine if it fuses preferentially with hemopoietic or other host cells?

The stem cell of the embryonic liver (after transfer to irradiated mice) can generate a variety of cell types, including erythrocytes, macrophages, granulocytes, and lymphocytes. Any cell type of the leucocytic series could be involved in the fusion, but a macrophage type cell seems a particularly likely candidate. Phagocytes derived from the granulocytic and monocytic series are probably essential for the survival of lethally irradiated repopulated animals (14–18). Gesner and Gowans (19) have shown that thoracic duct lymphocytes have little or no importance in this respect, since the injection of syngeneic lymphocytes from this source does not save the life of lethally irradiated animals. Furthermore, the overwhelming majority of the peritoneal cells of irradiated repopulated animals are macrophages, as judged by their size and phagocytic activity (20). Immunological and chromosomal studies have established unequivocally that peritoneal (free) macrophages originate from precursor cells in the bone marrow, probably of monocytic type (16, 18, 20–23).

The fact that the hybrids can be selected by their firm adherence to the surface of the culture vessel also suggests the possibility of a macrophage parent cell. Cells of the lymphoid series do not adhere readily to the surface of the vessels and are therefore unlikely to confer this property on the ascites tumor cells, which also do not adhere readily. Macrophages do confer this adhesiveness when they are fused with ascites tumor cells (24).

The participation of macrophages in the fusion would be proved by the demonstration of specific macrophage markers in the hybrid cells. Macrophage–melanocyte and macrophage–L tumor cell hybrids produced *in vitro* express some traits of both parent cells, but they do not express macrophage-specific markers (25–28). Our attempts to detect macrophage-specific markers (Fc receptors) in the present hybrid cell lines have also been unsuccessful.

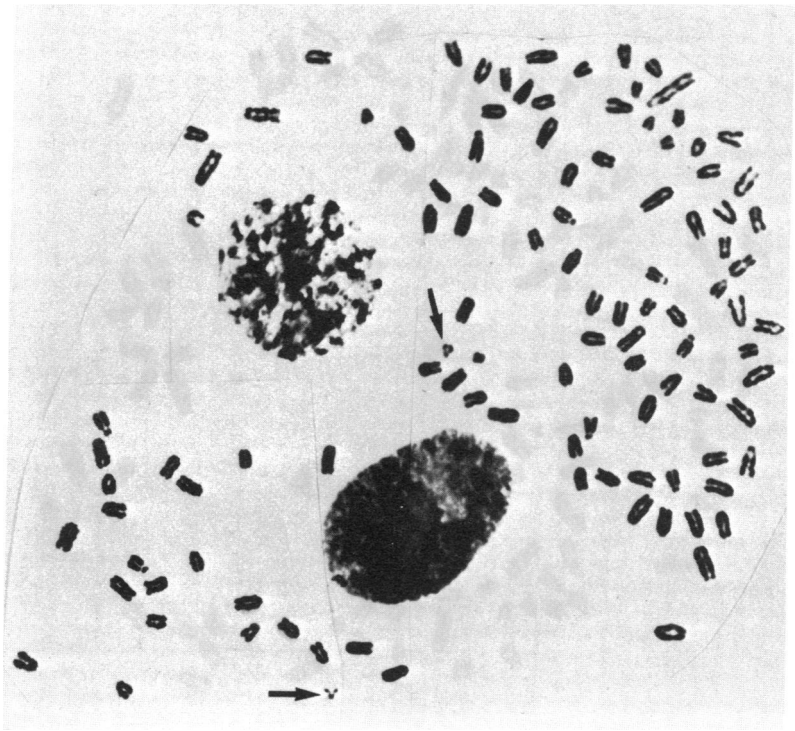

FIG. 4. Metaphase plate of TA3Bimp$^-$–host cell hybrid isolated from a tumor produced in C3H × C57Bl F_1 chimeric mouse. Note the presence of the two T6T6 markers (shown by *arrows*) specific for the repopulating donor hemopoietic cells.

There is a clear-cut difference in partner preference between the solid and the ascites tumors. It seems likely that there is no large number of macrophages in the solid tumors or in their subcutaneous environment, although the relative proportions of repopulating donor and host cells vary considerably in different tissues of radiation chimeras (20, 21, 30). Organs like the liver or the lungs are seeded secondarily with donor cells originating from the spleen and/or bone marrow of the reconstructed animals. Godleski and Brain (31) have demonstrated that bone marrow replacement precedes the replacement of the pulmonary macrophages with cells of donor origin. It is therefore possible that differences in the origin of free and fixed macrophages in the radiation chimeras might be responsible for the differences in fusion pattern between the solid and the ascites tumors.

We thank Mrs. Mai-Lis Solberg, Mrs. Margareta Hagelin, Miss Mona Hansson, Miss Bodil Lidin, and Mrs. Gunilla Issakson for excellent technical assistance. This work was supported by the Public Health Service Research Grant no. 1 RO1 CA 14054-01 GEN., The Swedish Cancer Society, and the Jane Coffin Childs Memorial Fund for Medical Research.

1. Wiener, F., Fenyö, E. M., Klein, G. & Harris, H. (1972) *Nature* **232,** 155–159.
2. Fenyö, E. M., Wiener, F., Klein, G. & Harris, H. (1973) *J. Nat. Cancer Inst.*, in press.
3. Ford, C. E., Hamerton, J. L., Barnes, D. W. M. & Loutit, J. F. (1956) *Nature* **177,** 452–454.
4. Wiener, F., Klein, G. & Harris, H. (1973) *J. Cell Sci.* **12,** 253–261.
5. Allderdice, P. W., Miller, O. J., Miller, D. A., Warburton, D., Pearson, P. L., Klein, G. & Harris, H. (1973) *J. Cell Sci.* **12,** 263–274.
6. Sjögren, H. O., Hellström, I. & Klein, G. (1961) *J. Nat. Cancer Inst.* **26,** 707–717.
7. Klein, G., Bregula, U., Wiener, F. & Harris, H. (1971) *J. Cell Sci.* **8,** 659–669.
8. Sjögren, H. O. (1964) *J. Nat. Cancer Inst.* **32,** 375–393.
9. Klein, G. (1951) *Exp. Cell Res.* **11,** 518–573.
10. Rothfels, K. H. & Simonovitch, L. (1958) *Stain Technol.* **33,** 73–77.
11. Klein, G., Gars, U. & Harris, H. (1970) *Exp. Cell Res.* **62,** 148–160.
12. Reif, A. E. (1966) *Immunochemistry* **3,** 267–278.
13. Klein, G., Friberg, S., Wiener, F. & Harris, H. (1973) *J. Nat. Cancer Inst.* **50,** 1259–1268.
14. Nowell, P. C., Cole, L. J., Habermeyer, J. G. & Roan, P. L. (1956) *Cancer Res.* **16,** 258–261.
15. van Bekkum, D. W. & Vos, O. (1957) *J. Cell Comp. Physiol. (Supp. 1),* **50,** 139–156.
16. Volkman, A. & Gowans, J. L. (1965) *Brit. J. Exp. Pathol.* **46,** 50–61.
17. Goodman, J. W. (1963) *Transplantation* **1,** 334–346.
18. Goodman, J. W. (1964) *Blood* **23,** 18–26.
19. Gesner, B. M. & Gowans, J. L. (1962) *Brit. J. Exp. Pathol.* **43,** 431–440.
20. Balner, H. (1963) *Transplantation* **1,** 217–223.
21. Shand, F. L. & Bell, E. B. (1972) *Immunology* **22,** 549–556.
22. Virolainen, M. (1968) *J. Exp. Med.* **127,** 943–952.
23. van Furth, L. & Cohn, Z. A. (1968) *J. Exp. Med.* **128,** 415–435.
24. Watkins, J. F. & Grace, D. M. (1971) *J. Cell Sci.* **2,** 193–204.
25. Gordon, S. & Cohn, Z. (1970) *J. Exp. Med.* **131,** 981–1003.
26. Gordon, S. & Cohn, Z. (1971) *J. Exp. Med.* **133,** 321–338.
27. Gordon, S. & Cohn, Z. (1971) *J. Exp. Med.* **134,** 935–946.
28. Gordon, S., Ripps, C. S. & Cohn, Z. (1971) *J. Exp. Med.* **134,** 1187–1200.
29. Gordon, S. & Cohn, Z. (1971) *J. Exp. Med.* **134,** 947–962.
30. Pinkett, M. O., Cowdrey, C. R. & Nowell, P. C. (1966) *Amer. J. Pathol.* **48,** 859–867.
31. Godleski, J. J. & Brain, J. P. (1972) *J. Exp. Med.* **136,** 630–643.