The Cell Site of the Immunological Defect in Tumor-Bearing Mice[1] (35299)

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(Introduced by S. Malkiel)

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An interaction of at least two cell types—surface adherent cells with morphological and functional characteristics of macrophages and nonadherent cells with the characteristics of lymphocytes—appears to be required for the primary immune response *in vitro*. The processing of antigen by macrophages and the transmission of information from these cells to the antibody-producing lymphocytes may be essential for this reponse (1, 2). The availability of an *in vitro* system in which the two cell populations can be separated (3–6) has permitted us to study more precisely in a rodent tumor model the cell site of the immunological deficit associated with certain advanced malignant diseases—a defect well documented in patients with these neoplastic diseases (7–10).

Methods. For the *in vitro* study of the primary immune response, a modification (5) of the cultural conditions recommended by Mishell and Dutton (6) was adopted. Briefy, cell suspensions from spleens of 4-week-old AKR mice (2×10^7 cells/ml) were maintained in culture for 4 days at 37° in an atmosphere of 10% CO_2, 7% O_2, and 83% N_2 on a rocking platform in plastic petri dishes. For the cell separation studies, advantage was taken of the ability of macrophages to stick to plastic surfaces. The method described by Mosier and Coppleson (4) was adopted in which cells from 8–10 pooled spleens were cultured in petri dishes for 30 min at 37°. Those cells remaining free in the culture medium were designated the nonadherent population. Morphologically they were lymphocytes. These cells were aspirated and subjected to two further separation procedures. The cells remaining firmly adherent to the dish after three washings had the morphological attributes of macrophages. Functionally, these cells were seen to phagocytose sheep red blood cells and ingest lithium carmine or latex particles. After cell separation, the adherent cells obtained from tumor-bearing animals were recombined with nonadherent cells obtained either from the same group or from normal mice (and vice versa).

The recombined spleen cell suspensions were cultured with washed sheep RBC (2×10^7 spleen cells with 1×10^7 SRBC) for 4 days. The antibody-producing cells were assayed by the microchamber technique described by Cunningham and Szenberg (11). In all studies, the experiments were controlled by the simultaneous plaque assay of spleen cell suspensions from mice immunized *in vivo* 4 days previously by the intraperitoneal injection of 4×10^8 washed SRBC and/or of spleen cell suspensions from control animals cultured *in vitro* with SRBC for 4 days.

The Ridgeway osteogenic sarcoma (ROS) was implanted intramuscularly in 1-month-old AKR mice (2.5×10^7 cells in 0.1 ml of Locke's solution). The tumors grew in all of the animals, reaching a mean tumor weight of 3.3 g by the 22nd day after implantation. These mice were debilitated by the large tumor mass and would have been expected to die some 4–6 days later. In none of the animals studied was the tumor ulcerated. For

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TABLE I. ROS Tumor and Primary Immune Response *in Vivo*.

Day	No. of plaques: Per 10^6 spleen cells ($\times 10^3$)	No. of plaques: Per spleen ($\times 10^5$)	Cells/spleen ($\times 10^8$)	Spleen wt (g)	Tumor wt (g)	n
Control	2.91 ± 0.18[a]	5.8 ± 0.3	2.0 ± 0.11	0.28 ± 0.01		14
10	2.51 ± 0.15	5.0 ± 0.8	1.98 ± 0.21	0.26 ± 0.02	0.15 ± 0.04	5
12	2.53 ± 0.28	4.6 ± 0.6	1.83 ± 0.16	0.28 ± 0.03	0.38 ± 0.18	5
14	2.53 ± 0.21	5.5 ± 0.5	2.27 ± 0.39	0.23 ± 0.03	1.23 ± 0.14	5
18	1.63 ± 0.26	6.1 ± 0.5	4.14 ± 0.63	0.45 ± 0.05	2.15 ± 0.45	5
21	0.48 ± 0.11	2.1 ± 0.4	4.47 ± 0.26	0.50 ± 0.02	2.97 ± 0.39	10
22	0.08 ± 0.01	0.3 ± 0.06	4.37 ± 0.40	0.48 ± 0.03	3.34 ± 0.27	5

[a] Mean ± SE.

this study, control mice were implanted with the same number of irradiated (15,000 rad) tumor cells: the tumors did not grow in any of the animals. The *in vivo* primary immune response of tumor-bearing and control mice was assessed by plaque assays of spleen cell suspensions from mice immunized 4 days previously with 4×10^8 washed sheep red cells.

Results and Discussion: (a) *In vivo* studies of the primary immune response in animals bearing the ROS tumor demonstrated that when the tumor was far advanced, the capacity of the host spleen cells to respond to injected sheep red cells was impaired (Table I). Thus, by day 21, when the tumor weighed 3 g the number of plaques per spleen was 36% of the control value, while the number of plaques per spleen 1 day later was 5% of the control value. This "depletion" of cells competent to respond to injected sheep red cells occurred despite a more than 2-fold increase in the total number of splenic cells available.

A study was undertaken to examine the histological changes in the spleen in this system. For this purpose, 32 AKR mice were randomized into 4 groups: control mice, mice injected 4 days prior to sacrifice with SRBC, mice bearing a 21-day ROS tumor implant, and 21-day tumor-bearing mice injected 4 days earlier with SRBC. Histologically, the spleen showed a 3-step graded reaction. The control spleens had small evenly distributed well-defined follicles. The SRBC-injected normal mice showed slight enlargement of the spleen, some mitotic figures, slight sinusoidal congestion and a possible increase in the number of megakaryocytes. The tumor-bearing mice had striking splenomegaly with large confluent lymphoid follicles not clearly demarcated from the red pulp. Mitotic figures and plasma cells were prominent and the number of erythropoietic cells and megakaryocytes appeared to be increased. The tumor-bearing mice injected with SRBC showed similar changes to a slightly greater degree.

Thus, the major process producing splenomegaly in the tumor-bearing mice was cellular hyperplasia (confirmed by direct cell counts, Table I), affecting predominantly the lymphoid cells and to a much lesser degree the plasma cells, reticuloendothelial cells, and hematopoietic cells. No metastatic tumor cells were detected histologically in the spleen or liver of 21-day tumor-bearing animals and implantation of spleen cell suspensions (4×10^7 cells im) into each of 10 mice produced no tumor growth (Table II).

TABLE II.

Implant	Tumor wt (g)
Tumor[a] alone	4.8 ± 0.2[b]
Tumor + normal spleen cells[c]	5.6 ± 0.4
Tumor + "tumor" spleen cells[d]	5.8 ± 0.6
Normal spleen cells	0
"Tumor" spleen cells	0

[a] 2.3×10^7 ROS cells in 0.1 ml of Locke's solution im.

[b] Mean ± SE; $n = 10$; 21 days after implant.

[c] 4.0×10^7 spleen cells from normal AKR mice.

[d] 4.0×10^7 spleen cells from AKR mice implanted 21 days earlier with 2.3×10^7 ROS cells.

TABLE III. ROS Tumor and Primary Immune Response *in Vitro* (Two-cell system).

		Plaques/10^6 spleen cells		
	Expt.:	1	2	3
Macrophages (Tu)[a]	+ Lymphocytes (Tu)[a]	138	—	53
	+ Lymphocytes (N)[b]	498	—	168
Macrophages (N)[b]	+ Lymphocytes (Tu)	276	192	26
	+ Lymphocytes (N)	426	294	236

[a] Macrophages and lymphocytes from spleens of AKR mice implanted 21 days earlier with 2.3×10^7 ROS cells.

[b] Macrophages and lymphocytes from normal mice.

The splenic lymphoid hyperplasia in response to an implanted tumor could well represent an immunological response to the tumor antigens and might well account for the incapacity of the tumor-bearing mice to respond to the additional injection of another antigen—SRBC. In an attempt to demonstrate the efficiency of this immunological process, we implanted AKR mice im with 2.3×10^7 ROS tumor cells alone and in the presence of 4×10^7 spleen cells from normal and from 21-day ROS tumor-bearing mice. However, no protection against tumor growth was afforded by this procedure (Table II).

(b) *In vitro* experiments utilizing the cell isolation and recombination procedures revealed that the adherent macrophage cells from tumor-bearing animals behaved essentially normally when recombined with control lymphocytes (Table III). On the other hand, the lmphocytes from tumor-bearing mice produced fewer plaques when incubated with macrophages from control animals than the lymphocytes from control animals incubated under identical conditions.

We have reported (5) that the target cell of the well-established (12–14) immunological defect in neonatally thymectomized mice is the lymphocyte—the efferent limb of the immune response. It appears from the present study that the deficit in tumor-bearing mice is analogous. This is interesting in view of current opinion that neonatal thymectomy facilitates experimental carcinogenesis. However, further studies are necessary to define more clearly the similarities and differences in these two immunoincompetent states.

Summary. *In vivo* studies have shown that in spite of the reactive splenomegaly with lymphoid cell hyperplasia, which is a constant finding in tumor-bearing mice, the capacity of the spleen cells to respond to injected SRBC by hemolysin production is significantly impaired.

In vitro experiments, utilizing techniques which separate adherent macrophage and nonadherent lymphocyte populations of cells from the spleens of normal and tumor-bearing mice, have permitted the definition of the cell site of the immunological defect. The defect in tumor-bearing mice is analogous to that in neonatally thymectomized animals, residing in the nonadherent lymphoid population of spleen cells.

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