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Regression of Transplantable Immunoglobulin-secreting Rat Tumors by Irradiation and Chemotherapy and Induction of Transplantation Resistance[1]

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SUMMARY

Immunoglobulin-secreting immunocytomas arose spontaneously in the LOU/c inbred strain of rats. The immunoglobulin G-producing immunocytoma $ISIS_{130}$ was sensitive to Cytoxan; 80% of rats with advanced tumors could be permanently cured. Sixty-seven % of cured rats developed specific transplantation resistance to $ISIS_{130}$.

INTRODUCTION

Only a minority of well-established transplantable tumors regress under the influence of a single radiation or chemotherapy treatment, or of a combination of both (1, 13, 15-18). There is evidence that host factors may be involved in these situations (7).

A spontaneous tumor that arose in our rat strain LOU/c[4] was serially transplanted. The transplantable tumors derived therefrom were sensitive to local irradiation and to chemotherapy. Well-established tumors also responded to these treatments, and macroscopic regression of transplanted tumors was observed. Thereafter the animals were resistant to a 2nd graft of the same tumor. The transplantation resistance remained for very long time periods.

MATERIALS AND METHODS

Rat Strain and Tumors. The adult rats used were from a strain that was random bred at the Cancer Institute from 1945 until 1956 and that was inbred thereafter. All tumors used in this work arose in the inbred strain.

The incidence of the immunoglobulin-secreting immunocytoma was 2.8% (9, 10). This type of tumor was first observed in our rat strain in 1955 and was described previously as a leukosarcoma (11). The animals developed these spontaneous tumors of the ileocecal lymph node between 7 and 20 months of age (age of animals at appearance of tumors). At present, the incidence of immunoglobulin-secreting immunocytomas has increased to 10% (2). The primary tumor grows rapidly in the peritoneal cavity, invades the mesenterial lymph nodes and the peritoneum, and provokes ascitic fluid containing isolated cancer cells. Metastatic involvement of mediastinal, axillary, and cervical lymph nodes and pleural effusion, as well as anemia and peripheral blood disorders, are seen in the terminal stage of the disease (2, 11).

Microscopically, the immunoglobulin-secreting immunocytoma structure is that of an undifferentiated reticulosarcoma (2, 11). (Dr. Dunn and Dr. Snell examined our tumor and described it as an undifferentiated lymphoreticular cell tumor.) The primary tumors secrete complete or incomplete immunoglobulins which can be detected in the serum, in the ascitic fluid, and in the pleural effusion by electrophoresis or immunoelectrophoresis (2-4).

Different strains of transplantable tumors were established from these immunoglobulin-secreting immunocytomas. From the 1st passage on, the paraprotein secretion that was observed in the host serum remained in some cases after more than 100 passages. In exceptional cases paraproteinemia diminished to very low levels during subsequent grafts (2).

In this work, we used the IgG-secreting immunocytoma $ISIS_{130}$ which originated from the inbred strain and which is easily transplanted (2).

The primary tumor was an ileocecal lymph node isolated from a male rat in 1965. The tumor is transplanted by s.c. administration of 10^6 viable cells or of small tumor fragments. These tumor fragments are given by s.c. injection in the flank (0.25 ml of a suspension of 1 g minced tumor tissue per 1 ml 0.9% NaCl solution).

Under these conditions, nearly 100% of the untreated animals develop a palpable tumor within 1 week which kills the animal in 2 to 4 weeks. All challenge injections consisted of 10^6 isolated viable cells.

Local Irradiation of the Tumor. The graft was irradiated by a narrow beam (about 2 x 2 cm) covering only the tumor. The animals were restrained on a special table during irradiation, which was given by a Maxitron unit (250 kV; 80-cm focus-to-target distance; filter, Cu 0.25/Al 1 mm).

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[4] Originally of Wistar origin, inbred at the Cancer Institute; more than 40 brother-sister matings in this line. Skin grafts are accepted between rats of this strain. Only inbred animals were used in this work. The name LOU/c is proposed in the new edition of the *International Index of Laboratory Animals*.

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Chemotherapy. In these experiments, Cy[5] was administered s.c. in a single dose on the 7th day after tumor transplantation.

Natulan [also designated as a methylhydrazine derivative, or Procarbazine, or RO 4-6467/1 (Roche Laboratories, Brussels, Belgium)] was administered i.p., also in a single dose. The drug, obtained as a dry powder, was solubilized in 0.9% NaCl solution immediately before use and was injected i.p. as a 5% solution. Dosages of the drugs are indicated on Charts 2 and 3.

Recording of the Growth of the Tumor after Treatment. The growth of the tumor was followed by measurement daily of the 2 largest dimensions of the tumor. These figures are given on the charts. Surface values correlate the weights of the tumors. For other measurements, the tumor-bearing animals were killed on the 7th or 8th day after treatment and records were made of the weight of the excised tumor in comparison with untreated controls. These values are included in the charts.

The paraprotein level was determined in some experiments and also correlated well with tumor volume (C. Deckers, in preparation).

RESULTS

Inhibition of IgG-secreting Immunocytomas by Radiotherapy and Chemotherapy Treatment

Growth Curve of $ISIS_{130}$

In the 1st experiment, the growth rate of $ISIS_{130}$ was determined in untreated hosts. Tumor grafts, established as described, could already be detected after 4 to 5 days of growth and were followed by daily measurement as recorded in Chart 1.

The growth of this tumor was rapid and, after 2 to 3 weeks, most of the animals died. With 10^7 cells, tumor development was more rapid whereas, with fewer tumor cells, the latent period was slightly increased.

Sensitivity of $ISIS_{130}$ to Radiotherapy

Growth Curve after a Single Dose of Radiotherapy. As seen on the growth curve obtained by daily measurement of the tumor, a dose of 100 R (Chart 1) administered on the 8th day of tumor growth did not essentially modify the growth curve; a dose of 500 R produced a "plateau" effect. The tumor diameter remained constant for 2 days and thereafter the growth rate was similar to that of the control tumor. At 1000 R, a significant reduction was obtained for several days; afterward, the tumor regrew and the animals died a few days later than the untreated controls (Chart 1). With larger doses, skin necrosis occurred in the irradiated area.

Comparison of Tumor Weights on Day 7 after Radiotherapy. The graft was excised from animals that were sacrificed on the 7th day after radiotherapy. The tumor weight was recorded and compared with that of untreated controls. Thus, a dose of 1000 R gave a 38.5% reduction in tumor weight.

Sensitivity of $ISIS_{130}$ to Cy and Natulan Treatment

The same method was used to test the sensitivity of $ISIS_{130}$ to Cy treatment.

The growth curve of the tumor of animals treated with Cy (1 dose of either 0.12 or 1.5 mg/100 g body weight on the 8th day after the tumor graft) is given in Chart 2.

Chart 3 gives similar data for $ISIS_{130}$ tumors treated with Natulan, also given as a single dose on the 8th day of tumor growth.

By recording the tumor weights at Day 8 after Cy treatment, the reduction of tumor volume by different levels of the drug could be determined. A dose of 0.12 mg Cy per g body weight reduced the graft weight to 30% of the control. A dose of 1.5 mg Cy per 100 g body weight provoked a nearly complete tumor regression at Day 8. The regression of the tumor was not yet complete by Day 8 but was complete only 10 to 15 days after Cy treatment. Some animals were observed for 12 months without recurrence of the tumor.

In conclusion, a well established graft of our $ISIS_{130}$ can be easily destroyed by chemotherapy alone. The tumor is more sensitive to 1 dose of Cy or Natulan than to 1 dose of radiotherapy.

Specific Transplantation Resistance after Tumor Treatment

In the following experiment, 1.5 mg Cy per 100 g rat weight was administered to produce a permanent resorption of the graft in most of the animals (Table 1).

Transplantation resistance after tumor destruction by Cy treatment was investigated. Fifteen days after Cy treatment, *i.e.*, 23 days after tumor grafting, or later, the animals were challenged with a 2nd graft of 10^6 $ISIS_{130}$ viable cells. This number of $ISIS_{130}$ cells induced a transient growth in most of the animals of this group. In some animals, nodes of 2 cm in diameter appeared and regressed spontaneously after 10 to 15 days. Untreated control animals that received the same graft had 100% positive grafts and died. The number of animals rejecting the graft is given in Table 1.

To test the specificity of this rejection, a syngeneic tumor was injected at a different site in the $ISIS_{130}$-rejecting animals, together with a challenge $ISIS_{130}$ graft. These experiments showed that a transplantable hepatoma which secretes α-fetoprotein grows in $ISIS_{130}$-rejecting as well as untreated animals. The growth of the hepatoma was followed by histological analysis and by the appearance of α-fetoprotein in the host serum. No IgG paraprotein was found in these animals, indicating the absence of $ISIS_{130}$.

Transplantation resistance to $ISIS_{130}$ occurs very soon after Cy treatment and remains for a very long time. In some animals, transplantation resistance was still found 12 months after Cy administration. In the interim, we never observed recurrence of the $ISIS_{130}$ graft.

[5] The abbreviation used is: Cy, cyclophosphamide (Cytoxan or Endoxan).

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Table 1

Incidence of $ISIS_{130}$

The animals were grafted on Day 0 with 10^6 $ISIS_{130}$ cells.

	Tumor incidence on day of Cy[a] treatment		Tumor regression after Cy treatment			Transplantation resistance after $ISIS_{130}$ challenge[b]		
Experiment	Day after transplantation	No. of tumors/ no. of animals	Day after transplantation	No. of tumors/ no. of animals	% regressed graft	Day after transplantation	No. of tumors/ no. of animals	% rejected graft
7057	8	40/40	31	16/40	60	105	5/6[c]	16
7045	8	40/40	30	12/40	70	100	0/5	100
						147	3/5	40
						168	0/4	100
7134	10	36/36	35	9/36	75	55	3/27	89
7036	8	60/60	30	12/57[d]	79	111	9/9	0
						167	1/4	75
						197	0/3	100
7032	7	36/36	21	6/36	84	44	8/8	0
						73	6/6	0
						94	2/3	33
7130	8	32/32	28	4/32	87	52	6/28	78
7210	9	42/42	36	4/42	88	56	10/38	73
7039	8	11/11	30	0/11	100	77	1/7	86
7241	8	20/20	21	0/20	100	44	0/10	100
Total		317/317		63/314[d]	80		54/163[c]	67

[a] Dosage: 1.5 mg/100 g bodyweight.
[b] Cells (10^6) were given 20 days before the tumor incidence was recorded.
[c] Some animals were not challenged; eventually, late recurrences were observed.
[d] Three animals died of infection.

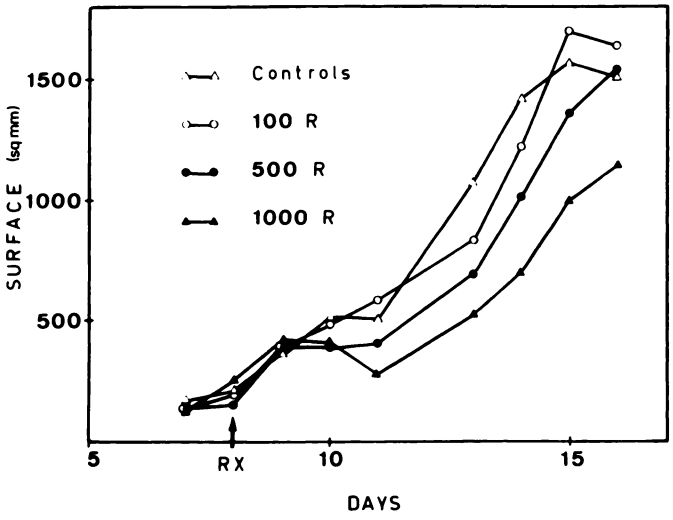

Chart 1. Effect of various single doses of irradiation on the growth of $ISIS_{130}$. *Arrow*, radiotherapy (*RX*).

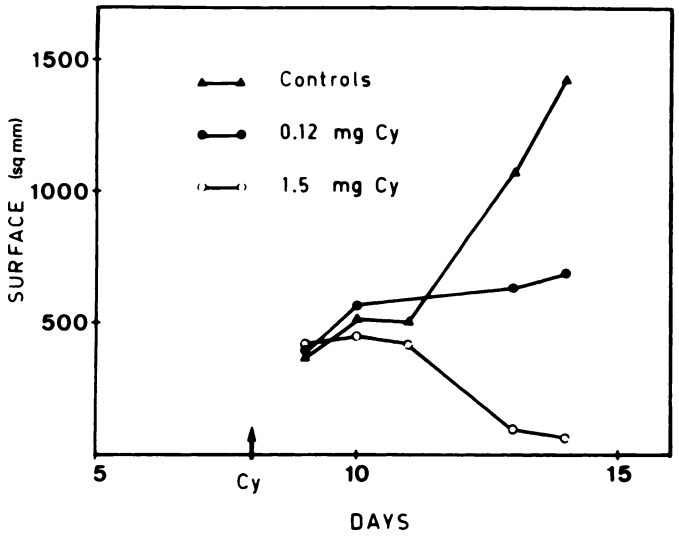

Chart 2. Effect of various single doses of Cy on the growth of $ISIS_{130}$.

DISCUSSION

Spontaneous tumors similar in their macroscopic behavior to the ileocecal plasmocytomas observed in C3H mice (5, 6, 8, 13, 14) were observed in our rats. Initially, the incidence was 2.8%, but recently it has been increased to 10% (2, 9, 10, 11).

These tumors are easily transplantable at the present time. The histological type of these tumors was described as leucosarcoma (11) and, more recently, as immunocytoma (2). In this paper the name immunocytoma was used instead of leucosarcoma. The tumor cells are rather immature and in any case are not differentiated toward mature plasmocytes; they were classified by cytological criteria as reticulum cell neoplasms (5, 6). These cells probably can be considered as immature precursors of plasmocytes, as described by Rask-Nielsen *et al.* (14), in their grade IV group.

These tumors secrete a paraprotein in the serum of the original host, usually of the IgG type, rarely of the IgA type. More details about these immunoglobulins are published or in progress (2–4, 12).The paraprotein secretion

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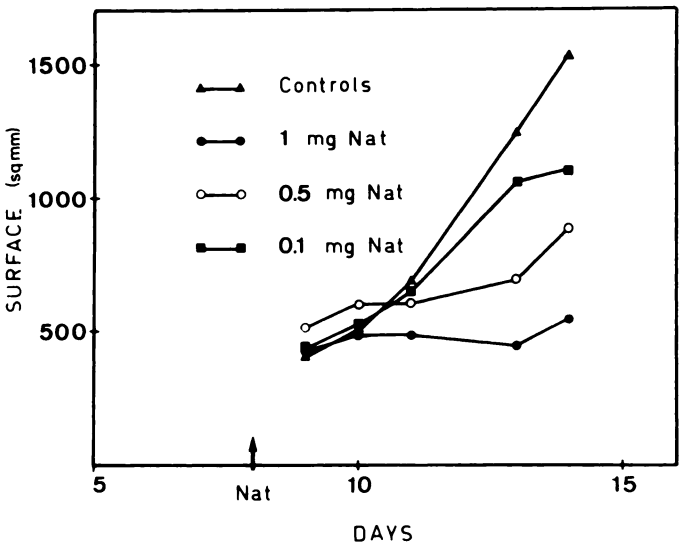

Chart 3. Effect of various single doses of Natulan (*Nat*) on the growth of tumor $ISIS_{130}$.

remains after serial transplantation and is an excellent marker for the tumor. The $ISIS_{130}$ used in this work is an IgG-secreting tumor.

One dose of local irradiation administered alone to the 8th-day tumor, a well-established nodule, temporarily controls growth of $ISIS_{130}$. However, a definitive "cure" of the nodule by a single dose of radiation was not obtained without injury to normal tissue (skin).

Conversely, Cy treatment alone led to tumor regression. A single dose of 0.12 mg Cy reduced the tumor volume to 30% of that in untreated controls. Complete regression of the immunocytoma was obtained by a single dose of 1.5 mg/100 g body weight. Thus, $ISIS_{130}$ is one of the rare well-established tumors with which regression can be obtained by chemotherapy. A similar situation was described in mice plasmocytomas, as recently reviewed by Potter (13). Complete regression was observed after a single dose of Cy, which result is similar to those reported in plasma cell neoplasms of the CAF_1 mouse (18), in the BALB/c mouse (1, 16, 17), or in immunosuppressed xenogeneic species with Walker 256 tumors (15).

The regression of $ISIS_{130}$ was followed by resistance to a 2nd graft of the same tumor; 3 weeks after the initial tumor graft (or 15 days after Cy treatment), 10^6 tumor cells induced a transient tumor growth followed by complete regression. The same number of cells killed untreated control animals.

Tumor immunity is usually induced by temporary growth of a tumor in an animal. Therefore, Cy-induced regression is a very easy way to obtain temporary growth without the need for surgery or other procedure to remove the tumor.

Additional evidence of the immunological nature of the transplantation resistance came from the demonstration of cellular immunity against $ISIS_{130}$ in $ISIS_{130}$-rejecting animals (C. Deckers and F. de Halleux, in preparation). This transplantation resistance could be passively transferred from resistant animals to untreated controls. Viable $ISIS_{130}$ cells inhibited the migration of spleen cells or peritoneal exudate cells of a resistant animal, indicating the presence of specific cellular immunity against $ISIS_{130}$ in the resistant animals.

The conclusion is that the transplantation resistance obtained after Cy treatment of the established tumor and regression of the nodule is due to specific cellular immunity against IgG-secreting immunocytomas.

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