Transplantation of Allogeneic Tumors in Rats and Mice Treated with Azathioprine, Prednisone and Antilymphocyte Serum[1] (36377)

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The survival rate of organ transplants through suppression of the immune response mechanism of the host is increased by utilizing 2 chemical immunosuppressive drugs, the purine analogue, azathioprine (Imuran), and the corticosteroid, prednisone, and antilymphocyte serum (ALS) (1, 2).

The purpose of this investigation was to study the effect of azathioprine, prednisone, and ALS singly, and in binary and ternary combinations on the acceptance rate of several allogeneic tumor transplants in mice and rats. This end point was used instead of skin transplantation technique [see ref. (3)] to evaluate the effect of immunosuppression in systems related to specific properties of tumors, rather than the antigenic characteristics of tissues generally. Thus, tumor transplant methods might be applied to questions of immune status in relation to the induction and development of primary induced neoplasms, and also the occurrence of spontaneous tumors under some conditions. Recently a number of laboratories have begun to utilize a similar approach (4–7), and others have noted an effect of immune status on metastasis (8, 9).

Materials and Methods. Three mouse and two rat tumors were used: (a) a transplantable line of the spontaneous mammary adenocarcinoma of the C_3H mouse (C_3H Breast); (b) hepatoma 129 (Hep 129) of the C_3H mouse; (c) adenocarcinoma 755 (Ca 755) of the BDF_1 mouse (C57BL/6 × DBA/2); (d) prostate carcinoma 11095 (Prost Ca 11095) of the Fischer rat; and (e) the transplantable mammary adenocarcinoma 1/C (Mam Ad. 1/C) from a 7,12-dimethylbenz[*a*]anthracene-induced cancer in the Fischer rat. All tumors were maintained in serial passage in their "compatible" syngeneic host strains. Random-bred Swiss mice (18–20 g) served as the "incompatible" host, for the mouse tumors, and Sprague-Dawley rats (90-100 g) for the rat tumors.

The antimouse lymphocyte serum, the antirat lymphocyte serum and the normal rabbit serum (NRS) were prepared by a modification of the procedure of Monaco *et al.* (10). New Zealand albino rabbits (2–3 kg) were injected via the foot pads with a suspension of 1×10^8 thymus cells suspended in Freund's complete adjuvant (Difco Laboratories, Detroit). Three weeks later, the rabbits were rechallenged intravenously with a suspension of 1×10^8 thymus cells in TC 1066 (Grand Island Biological, Grand Island, NY) once a day for 3 days. One week later, the rabbits were exsanguinated, the sera were pooled, heat-inactivated (56° for 30 min), and sterilized by Millipore filtration prior to storage at −20°. The control NRS was processed likewise.

Each tumor was transplanted subcutaneously by trocar into 10 "compatible" syngeneic host animals and into 12 groups of 10 "incompatible" host animals (Swiss mice or Sprague-Dawley rats) treated with the various combinations of azathioprine, prednisone, ALS, and NRS (Table I). ALS and NRS were given at 0.1 ml/mouse/dose and 0.2 ml/rat/dose, azathioprine and prednisone at 5.0 mg/kg/dose in mice and 20 mg/kg/dose in rats. All agents were injected ip on days −2, 0, 3, 7, 10, 14, 17, 21, and 24. Animals were observed daily and weighed

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TABLE I. Tumor Incidence at the Termination of Immune Suppression Therapy (day 25).[a]

Tumor	Compatible host	Incompatible host	Compatible host control	Incompatible host											
				Control	NRS[b]	ALS[c]	Aza.[d]	Pred.[e]	NRS[b] +Aza.[d]	ALS[c] +Aza.[d]	NRS[b] +Pred.[e]	ALS[c] +Pred.[e]	Aza.[d] +Pred.[e]	NRS[b] +Aza.[d] +Pred.[e]	ALS[c] +Aza.[d] +Pred.[e]
C_3H breast	C_3H mouse	Swiss mouse	9/10	1/10	0/9	4/9	0/10	0/10	0/8	5/9	0/10	2/10	0/10	0/10	6/8
Hepatoma 129	C_3H mouse	Swiss mouse	7/7	0/10	2/10	6/9	2/10	2/9	2/10	5/9	1/9	5/7	1/9	2/10	4/8
Ca 755	BDF$_1$ mouse	Swiss mouse	4/4	1/10	0/9	7/9	0/10	2/10	0/9	7/9	0/10	5/10	0/10	0/9	7/10
Prost Ca. 11095	Fischer rat	S-D rat	9/9	3/7	2/6	3/7	2/8	1/7	3/8	5/7	1/5	5/7	2/8	3/5	2/7
Mam Ad. 1/C	Fischer rat	S-D rat	8/8	2/10	1/10	5/10	2/10	4/10	2/7	7/8	1/7	7/9	1/10	2/10	6/10
Av take-rate (%):			97	15	11	57	12	20	17	69	9	56	8	16	58

[a] All agents were injected intraperitoneally 2 days prior to tumor implant, on the day of implant, and on days 3, 7, 10, 14, 17, 21, and 24. Initially each group consisted of 10 animals.

[b] Normal rabbit serum: 0.1 ml/mouse/dose; 0.2 ml/rat/dose.

[c] Antilymphocyte serum: 0.1 ml/mouse/dose; 0.2 ml/rat/dose.

[d] Azathioprine: 5 mg/kg/dose in mice; 20 mg/kg/dose in rats.

[e] Prednisone: 5 mg/kg/dose in mice; 20 mg/kg/dose in rats.

weekly. Tumors were measured by calipers twice weekly and their weight was estimated. Results were considered negative when no tumor growth could be detected by palpation at the end of 25 days. In such cases, the implant failed to develop or early growth was followed by regression.

Results. Injection of the tumor grafts into compatible hosts led to progressive growths in virtually all cases with the three mouse and two rat neoplasms. On the other hand, insertion into nonsyngeneic systems was almost always followed by initial slow growth, then resorption. As might be expected in random-bred animals, some tumors were not rejected in a few of the animals, but even in these instances the tumor mass was much smaller than in a compatible host. Furthermore, in the syngeneic system with certain of the tumors, the animals succumbed to the rapidly developing cancer; whereas survival was usually better, through the experimental period, with the other groups. Again, treatment with immunosuppressive agents led to increased early mortality owing to tumor growth, in some cases acting together with drug toxicity.

The data summarized in Table I were accumulated on day 25, one day after cessation of immunosuppressive treatment. Compatible host controls had an average tumor acceptance rate of 97% (range 90–100%) compared to an average 15% (range 0–43%) in the incompatible host animals. Treatment of the nonsyngeneic hosts with ALS, or combinations of ALS–azathioprine, ALS–prednisone, or ALS–azathioprine–prednisone produced higher average tumor acceptances and ranges of 57% (43–78%), 69% (56–88%), 56% (20–78%), and 58% (29–75%), respectively. Poor growth enhancement by the ALS treatment was noted with the prostatic carcinoma 11095 and by ALS-prednisone with the C_3H breast tumor. No response was seen to the three agent combination with the prostatic carcinoma 11095.

Average tumor acceptance of those animals treated with NRS, azathioprine, prednisone, or combinations of these agents was not markedly greater than the incompatible host untreated controls. One individual exception

was the 60% acceptance rate of the prostatic carcinoma 11095 given the NRS–azathioprine–prednisone combination.

In addition to enhanced tumor acceptance rates, tumor weights were usually indicators of the effectiveness of immunosuppression. For example, following ALS treatment, the median weight of Mam Ad. 1/C was 3.7 g (50% takes) compared to an average control value of 0.6 g in the untreated group (20% takes), and 1.5 g in the single take (10%) in the NRS control. However, the series involving ALS treatment of Swiss mice bearing C_3H breast tumors resulted in a median tumor weight of 0.3 g (44% takes) compared to 0.7 g in the single take (10%) of the untreated control. No take, however, was seen in the NRS control, and the average tumor weight in the 9 takes (90%) of the compatible strain transplants was 0.2 g. Also with few such exceptions, the weights of tumors growing in the incompatible hosts treated with immunosuppressants were lower than those in the compatible hosts.

Discussion. ALS, azathioprine, and prednisone have all been reported to be potent immunosuppressive agents in a variety of animals and man (2, 11). The present results indicate that ALS and the combinations of ALS–azathioprine, ALS–prednisone, and ALS–azathioprine–prednisone increased average allogeneic tumor acceptance rate in Swiss mice and Sprague-Dawley rats with several tumor systems. A combination of ALS–azathioprine produced the largest and most consistent increase which corresponds favorably with the results of Woodruff (2) and MacDonald *et al.* (12).

Although azathioprine and prednisone have both been shown to be effective immunosuppressive drugs either alone (12, 13) or in combination (11), they were generally unable to enhance the tumor takes in this study. The drug dosages utilized were selected on the basis of tolerated levels established in a concurrent long-term toxicity study (14) and also on dosages used in clinical practice. Perhaps higher doses, possible in a short experimental period according to our data, or more frequent administration, might develop a positive effect with azathioprine and/or prednisone. Two abstracts, one by Nemoto *et al.* (15), reported enhanced tumor acceptance and development in mice with a different azathioprine regimen, but the other by Dargent, Bourgoin, and Noel (16) indicated that azathioprine delayed the induction of mammary tumors in rats.

Allogeneic tumor acceptance, such as we demonstrate, may have a counterpart in human transplant recipients treated with immunosuppressants. A number of investigators [see (17, 18)] have observed the development of neoplasms in renal transplants, inadvertently containing tumor foci, while the patients were maintained on immunosuppressive therapy. Regression of tumors occurred upon cessation of treatment.

The increased acceptance rate of allogeneic tumor implants in animals treated with ALS provides another method of evaluating the activity of potential immunosuppressive agents with particular reference to problems of tumor induction and development. In addition to providing a more precise end point, the acceptance and growth or rejection of a tumor, this procedure is technically much less complicated and demanding than other techniques such as skin-graft methods.

Summary. In a study of the effects of antilymphocytic serum (ALS), azathioprine, and prednisone on the acceptance-rates of three mouse and two rat tumors in allogeneic hosts, marked increases in average tumor acceptance rates, and usually but not always in terminal tumor weights, were observed following treatment with ALS alone, or in combination with either or both or the other two agents. The technique of allogeneic tumor grafts offers a simple semiquantitative procedure to evaluate the effect of drugs, sera, and related agents on the immunocompetence of animal systems, especially in relation to control mechanisms of tumor induction and development.

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