On the mode of action of some immunosuppressant drugs

SIR,—I have investigated the influence of a number of immunosuppressant drugs of the alkylating group, such as nitrogen mustard, triazequone and the anti-metabolites methotrexate, azathioprine and 5-fluorouracil, on transplantation immunity in rabbits induced by allogenic skin grafts. Cytologic changes in the regional lymph node and a possible action on the activity of serum proteolytic enzymes were measured. The cytotoxic effect of the drugs was assessed by their influence on peripheral white blood cell counts.

The experiments were made with 177 young, randomly bred, male rabbits weighing about 2·5 kg each. The drugs were administered chronically in two doses related to the acute LD50 with the exception of methotrexate (Table 1).

In the experiments on enzymatic activity, the doses of the preparations were selected for their power to inhibit transplantation immunity.

Allogenic skin grafts were made on the dorsal surface of the ear of rabbits.

Seven days after making the allogenic skin graft, that is at the peak of blastic transformation and proliferative changes in the regional lymph node (André, Schwartz & Dameshek, 1962), the rabbits were killed by air embolism. A method elaborated by Zaleski, Rymaszewska & others (1964) was used to evaluate the morphologic changes in the regional lymph node, localized in rabbits at the base of the ear. The method consists in determining the percentage of blast cells in smears from a cellular suspension of the lymph node. The percentage of blast cells was calculated in the smears by the method of balanced fields described by Woolf (1950).

Serum proteolytic activity was assayed by the use of casein labelled with ^{131}I by a modification of the method of Henson (1959), giving a labelled substrate of pH 7·7 and content of free iodine less than 0·1%. Blood was drawn from fasting animals from the marginal ear vein, in amounts of 5 ml, into a test tube containing 100 units of heparin. The serum obtained by centrifugation was kept in a refrigerator at −2° for 24 hr. Radioactivity of the serum was measured with a well scintillation counter. Results were expressed in terms of mg of digested casein/ml of serum.

The mean survival time of allogenic skin grafts in the control animals was 10·5 ± 1·7 days. The experiments on the influence of immunosuppressant drugs on transplantation immunity in rabbits demonstrated that azathioprine and nitrogen mustard gave the strongest suppression of the immunologic response to transplantation, markedly prolonging the survival time of the skin

TABLE 1. SCHEME OF DOSAGE OF THE PREPARATIONS AND THEIR INFLUENCE ON THE SURVIVAL OF ALLOGENIC SKIN GRAFTS AND THE BLASTIC REACTION IN THE REGIONAL LYMPH NODE IN RABBITS

Preparation (route of administration)	Doses		Method of administration in relation to the day of transplantation (= day 0)	Prolongation of survival time of skin allografts (days)	Decrease in the number of blast cells (%)
	Part of LD50	mg/kg			
Nitrogen mustard (i.v.)	1/10	0·16	−3, −1, 1	7·0	63
	1/4	0·40		7·0	68
Triaziquone (i.v.)	1/10	0·0055	−4, −3, −1, 1, 3	1·0	72
	1/4	0·014		4·0	89
Methotrexate	—	1·0	−4, −3, −2, −1, 1, 2, 3	1·5	78
		2·5		2·0	88
Azathioprine (oral)	1/20	25·0	−3, −2, −1, 0, 3, 4, 5, 6	13·0	69
	1/10	50·0		14·0	83
5-Fluorouracil (i.v.)	1/10	2·0	−2, −1, 0, 2, 3, 5, 6, 7	1·0	65
	1/4	5·0		2·0	80

grafts in doses which did not elicit any severe toxic symptoms. The effect of triaziquone was much weaker, and only after larger doses. Methotrexate and 5-fluorouracil were practically ineffective (Table 1).

In contrast to these results, the lymphnodal blastic reaction test showed that all the preparations exerted a strong antitransformative effect. However, distinct correlation between the survival time of the allografts and degree of inhibition of the blastic reaction was not observed. Contrary to expectation, the weakly active preparations triaziquone and methotrexate (a preparation devoid of an effect on transplantation immunity) inhibited the blast reaction more strongly than compounds which effectively suppressed the transplantation barrier like azathioprine or nitrogen mustard (Table 1).

The experiments on the influence of the immunosuppressant drugs on the activity of proteolytic enzymes in the serum revealed a distinct correlation of the degree of inhibition of the immunologic transplantation response and the reduction of proteolytic activity. Azathioprine and nitrogen mustard, both highly active in suppressing transplantation immunity in rabbits, specifically or nonspecifically inhibited activity of the serum proteolytic enzymes. On the other hand, triaziquone, methotrexate, and 5-fluorouracil, with weak or no effect on the transplantation reaction, exhibited a weak inhibitory component in relation to the serum proteolytic enzymes (Table 2).

TABLE 2. SERUM PROTEOLITIC ACTIVITY AFTER ALLOGENIC SKIN TRANSPLANTATION AND ADMINISTRATION OF IMMUNOSUPPRESSANT DRUGS

Preparation, mg/kg	Days before/after transplantation (= day 0)	Mean serum proteolitic activity (mg digested casein per ml serum)
Nitrogen mustard, 0·16	−4	0·039 ± 0·022
	9	0·055 ± 0·013
	16	0·034 ± 0·017
Triaziquone, 0·014	−5	0·038 ± 0·013
	8	0·094 ± 0·013
	15	0·119 ± 0·015
Methotrexate, 2·5	−5	0·051 ± 0·018
	7	0·079 ± 0·014
	15	0·101 ± 0·023
Azathioprine, 25·0	−4	0·050 ± 0·018
	8	0·046 ± 0·016
	18	0·083 ± 0·009
	23	0·039 ± 0·025
5-Fluorouracil, 5·0	−3	0·060 ± 0·016
	7	0·044 ± 0·007
	15	0·104 ± 0·011
Control	−1	0·037 ± 0·019
	8	0·083 ± 0·018
	15	0·115 ± 0·022
	25	0·046 ± 0·016

In addition, it was observed that azathioprine and nitrogen mustard also inhibit serum proteolytic activity in non-grafted animals.

Besides suppression of the blastic reaction, inhibition of proteolytic activity is an additional favourable factor in the complex mode of action of immunosuppressant drugs, contributing to pharmacologic transplantation tolerance.

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The continuous recording of arterial blood pressure in the conscious unrestrained rat

SIR,—Although indirect cuff methods are available to monitor blood pressures of large groups of animals, they suffer from the disadvantages that they give an interrupted record and the values obtained are influenced by the fact that the animals are restrained during the measurement. Insertion of cannulae into the aorta or carotid artery is time-consuming and difficult. Fujita & Tedeschi (1968) have recently reported blood pressure measurements using cannulation of the caudal artery with a fine polythene cannula, in rats of more than 300 g. We wish to report a similar method in smaller rats and without surgery.

Male or female Sprague-Dawley rats (Carworth Farm E strain), 80–200 g, were lightly anaesthetized with ether and a 20 s.w.g. needle connected by fine polythene tubing (Portex PP 60) to a Devices CEI transducer was inserted into the ventral caudal artery 2–2·5 cm from the base of the tail and retained in position with a strip of adhesive tape. A lateral tail vein was also cannulated with a 26 s.w.g. needle, inserted into polythene tubing (Portex PP 10), approximately 4–6 cm from the tip of the tail. A rigid plastic tube (internal diameter, 10–12 mm, length 3–4 cm longer than the rat tail) was slid over the two cannulae and anchored by a thread from the adhesive tape holding the arterial cannula (Fig. 1).

The rats may be trained to accept the tube over the tail, the length of the tube preventing the rat from chewing the cannulae, which may then be carried through the lid of a deep cage. Over a period of 5 hr no necrosis of the tail artery occurred and the patency of the cannulae is ensured by using heparinized saline (1000 u/ml) as the hydrostatic link to the transducer.

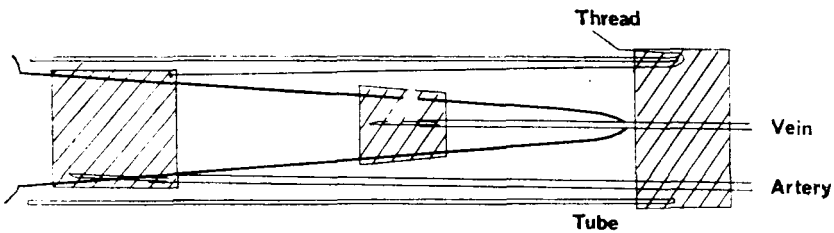

FIG. 1. Diagram showing the location of the cannulae and the protective tube for cannulation of a caudal artery and vein.

FIG. 2. Caudal and carotid arterial wave forms.