Proteolytic activity, its inhibitors, and the blastic reaction in graft rejection

The role of proteolytic enzymes in eliciting reactions to transplantation have been concerned with changes in serum enzymatic activity after making allografts (Patkowski, 1968; Patkowski, Halawa & Giełdanowski, 1969), or with prolongation of graft survival under the influence of the proteolysis inhibitor ϵ-aminocaproic acid (EACA) and its acetamide (Bertelli & Frontino, 1963; Gillette, Findley & Conway, 1963; Bertelli, Bisiani & others, 1964; Cramer, Hinshaw & Spar, 1964; Zukoski, Sachatello & Tinsley, 1965). Because of the discussion on the mechanisms of the action of EACA in suppressing the transplantation barrier, we have investigated the relation between enzyme activities in the serum and lymph nodes and morphologic changes in the regional lymph node with respect to the graft in animals treated chronically with the natural inhibitor trasylol (Bayer) (a trypsin and kallikrein inhibitor from animal glands) and synthetic ϵ-aminocaproic acid (Polfa). The same parameters were observed in animals not treated with the drugs.

Experiments were made with 52 young, randomly bred, rabbits weighing 2–3 kg each. The drugs were injected twice daily, intravenously, beginning one day before the transplantation and to the end of the period of graft survival. EACA was injected in doses of 0·2 and 0·5 g/kg body weight, and trasylol in doses of 5000 u/kg. Allogenic skin grafts were made in typical manner on the dorsal side of the auricula of the rabbits. Enzyme activities in the blood serum and lymph nodes were measured (Heuson, 1959) and blastic changes in the draining lymph nodes were also evaluated (Woolf, 1950).

Results. Skin allografts evoked a biphasic increase in serum proteolytic activity, about 3–4 and 10–14 days after the operation. Early changes in enzymatic activity after transplantation were observed also after autografts and seem to be immunologically non-specific. The rise of activity after 10–14 days, on the other hand, occurred only in allografts, indicating a specific immunologic reaction (Fig. 1).

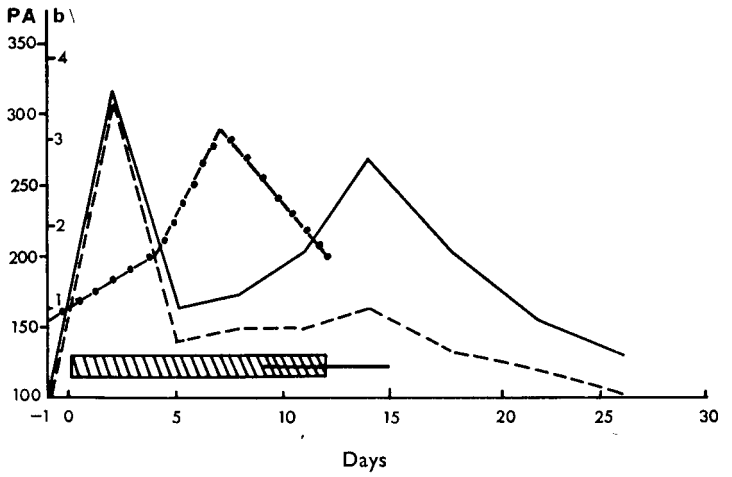

FIG. 1. Proteolytic activity and blastic activity in rabbits after transplantation. Ordinate axis: PA = proteolytic activity, as percentage. b = blastic cells, as percentage. Abscissa axis: time in days/transplantation on "O" day. ——— allograft. – – – – autograft. —●—●—●— blast cells. Hatched area = mean survival time of skin allografts with rule showing the extreme range of results.

Changes in proteolytic activity in the regional lymph node were erratic and permitted no conclusions to be reached.

The blastic reaction in the draining lymph nodes reached a peak after about 7 days. At that time the number of blasts was 3·18%; the number of plasma cells was not affected.

Administration of the proteolysis inhibitors trasylol and EACA in the lower dose gave similar effects. The survival of the skin allografts increased from 10·5 to 13–14 days, and the number of blasts in the lymph node stimulated by the graft decreased slightly, from 2·59 to 2·28%. Suppression of the blastic reaction in the strictest sense, was therefore unlikely; only the time after which the changes appeared was prolonged. Serum proteolytic activity behaved similarly. The rise in enzymatic activity after transplantation was less pronounced, but was not suppressed (Table 1).

Table 1. *Survival of skin allografts and recorded transformative changes in the regional lymph node*

Compound/dose		Survival time of skin allografts	Blastic reaction	
			Blast cells	Plasma cells
Trasylol	5·000 u/kg	13·2 ± 0·5	2·29 ± 0·53	0·48 ± 0·13
EACA	200 mg/kg	13·9 ± 2·1	—	—
EACA	500 mg/kg	13·5 ± 1·9	2·28 ± 0·30	0·48 ± 0·13
Control		10·5 ± 1·4	2·59 ± 0·45	0·58 ± 0·24

After the higher doses of EACA, survival of the allografts was not proportionally prolonged, and the blastic reaction was not significantly weakened. Proteolytic activity, however, was suppressed more strongly, persisting at the level of the initial changes (Table 2).

Table 2. *Serum proteolytic activity in animals treated after transplantation with trasylol or* EACA *(mg of digested casein/ml)*

Compound/dose		Initial	Days after allografting		
			5	10	15
Trasylol	5·000 u/kg	0·056 ± 0·013	0·077 ± 0·008	0·068 ± 0	0·053 ± 0·008
EACA	200 mg/kg	0·066 ± 0·007	0·076 ± 0·022	0·094 ± 0·015	0·076 ± 0·011
EACA	500 mg/kg	0·056 ± 0·010	0·055 ± 0·015	0·056 ± 0·019	0·062 ± 0·033
Control		0·081 ± 0·018	0·133 ± 0·046	0·157 ± 0·037	0·205 ± 0·028

In the control animals without grafts, both drugs caused 60–65% reduction of the physiologic enzymatic activity.

Cramer (1965) has suggested that EACA acts by competing for lysine, and Taylor & Fudenberg (1964) that it suppresses complement activity, which plays an important role in the transplantation reaction (Hager, Du Pay & Wallach, 1964; Cramer, 1965). On the basis of our observations on the enzymatic and blastic reactions, it must be concluded that the absence of any correlation of the degree of inhibition of proteolysis and the graft reaction arises from the manifold points of attack of EACA.

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