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Effect on Tumor Homografts of Treating Hosts with Antiproteolytic Enzyme Compounds.* (28222)

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The exact role of proteolytic enzymes in delayed hypersensitivity reactions is not clearly defined and has stimulated controversy. Recently, however, studies utilizing antiproteolytic enzyme factors established that proteolytic enzymes are at least partially responsible for the development of some hypersensitivity responses. Substances such as epsilon amino-n-caproic acid (EACA)(1) and soy bean trypsin inhibitor (SBTI)(2) suppress proteolytc enzymes of the chymotrypsin type. Zweifach(3) demonstrated that EACA modifies both the reaction to solubilized antigen-antibody complex and the cutaneous reaction to mixtures of endotoxin and epinephrine. It also exerts a protective effect against acute endotoxemia and Noble Collop drum shock. Chryssanthou(4) showed that SBTI can abrogate the delayed Schwartzmann reaction. Recent experiments in our laboratory using these compounds indicated that enzymes of the chymotrypsin type also participate in the rejection of homografts of normal tissue(5). Specifically, administration of EACA to host mice attenuated the rejection reaction as evidenced by the extended survival time of skin homografts and the modified cytologic reactions observed in the host bed.

Tumor immunity differs in several respects from normal tissue immunity. For example, the enhancement phenomenon can be produced with certain types of tumor tissue indicating that different mechanisms are involved. Therefore, we decided that treating animals that have received tumor homografts with EACA and SBTI might further delineate the role of proteolytic enzymes in the mechanisms of rejection.

Materials and methods. A mammary adenocarcinoma, *H2712*,† which is strain spe-

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† Tumor obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

TABLE I. Effect on Tumor Homografts of Treating Hosts with Antiproteolytic Enzyme Compounds.

Compound	Dose/day, mg/g b.w.	Mouse strain	Evidence of tumor growth No. with tumors/No. inoculated		
			Treated	Untreated controls	Untreated C_3H controls (isografts)
EACA	3	$C_{57}Bl$	11/25	0/25	9/9
		St. A/Jax	6/11	0/11	3/3
SBTI	4	$C_{57}Bl$	3/6	0/6	3/3
Combination EACA & SBTI	3 & 4	St. A/Jax	15/18	0/6	6/6

cific and, therefore, only freely transplantable among C_3H/HeJ mice, was used. Homogenates of this tumor injected subcutaneously into the dorsum of C_3H/HeJ mice resulted in palpable tumors within 11 days and the demise of the host in 3 to 4 weeks. "Takes" occurred in 100% of the C_3H mice *isografted* with this tumor. Similar homogenates were inoculated into $C_{57}Bl$ mice and strain A mice. These tumor *homografts* were rejected uniformly in all cases, *i.e.*, no tumor growths were seen. Then, 3 experimental regimens were designed to test the effects of antiproteolytc enzyme factors on the rejection of homografts of this tumor. First, strain A and $C_{57}Bl$ mice received daily i.p. injections of EACA. The dose was 3 mg/g b.w. On the same day as the initial injection of EACA, the animals were homografted with 1 cc of tumor homogenate. The host animals were treated with EACA for the duration of the experiment. Second, the same procedure was followed in a group of $C_{57}Bl$ mice; but these animals were treated with SBTI in daily doses of 4 mg/g b.w. Third, the same procedure was followed in a group of strain A mice, but these animals were treated daily with combined doses of EACA, 3 mg/g b.w., and SBTI, 4 mg/g b.w. The doses used in these studies were determined by previous tests(5) to be the optimum levels. In each experiment both C_3H/HeJ mice and untreated control animals, either strain A or $C_{57}Bl$, were inoculated with tumor homogenate.

Results. The data are summarized in Table I. All of the C_3H mice developed tumors. None of the untreated strain A or $C_{57}Bl$ mice showed any evidence of tumor growth. Nearly half of all the strain A and $C_{57}Bl$ mice treated with either EACA or SBTI developed sizable tumors within 12 days. The tumors ranged from 2 to 3 cm in diameter. The series of strain A mice that were treated with combined doses of EACA and SBTI showed the most dramatic results. Of these 18 animals, 15 developed tumors. In every series, however, the homografted tumors reached a maximum size in 18 to 20 days (Fig. 1), then began to regress. Despite continued injections of EACA, or SBTI, or both together, the tumors usually were only barely palpable 30 days after inoculation. Histologic sections revealed apparently viable tumor cells persisting 30 to 40 days after homotransplanta-

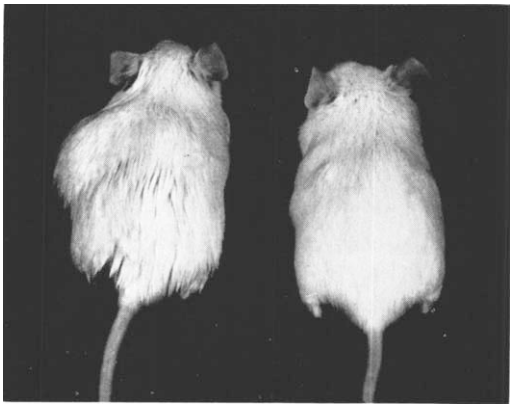

FIG. 1. Two strain A mice photographed 10 days after inoculation with 1 cc of tumor homogenate. Animal on left received a combined dose of EACA and SBTI daily. Animal on right was an untreated control. Note that although the entire homogenate was not resorbed by the control animals within 2 wk, no tumor growth was seen and histologic examination showed degenerating tumor cells infiltrated with reticulo-endothelial cells of the host.

tion to the treated hosts. The same tumor homogenate, when isografted to C_3H mice, continued to grow until the hosts died.

Discussion. This experiment revealed that anti-proteolytic enzyme factors interfere with the rejection of tumor homografts in about the same manner that they interfere with the rejection of normal tissue. Treatment with these agents permits the tumor to grow initially, but it inevitably regresses slowly. Similarly, homografts of skin survive for longer periods. It can be postulated that suppressing the activity of proteolytic enzymes interferes with only part of the rejection mechanism. Therefore, two possible features of the rejection phenomenon are indicated. First, proteolytic enzymes are important in rejection of normal and tumor tissue as they are in certain delayed hypersensitivity reactions. Possibly they act in the initial steps which bring the antigenic stimulus into contact with potentially reactive cells. The rapid initial growth of tumor homografts in the treated animals lends credence to this interpretation. Second, it appears that homograft rejection may be composed of a spectrum of independent mechanisms any one of which is capable of destroying the graft if given enough time. The eventual regression of tumor homografts, the protracted rejection of normal skin homografts, and the different cytologic picture observed in the treated hosts support this view. Additional evidence for such a theory can be found in a recent study by McKhann(6). This investigator found that the rejection of grafts between mice which differed only in the H-3 locus was much slower as compared to rejection of grafts between mice which differed at both the H-2 and H-3 loci. The changes observed in the lymph nodes of both groups were similar in type and magnitude, differing only in length of time required to achieve their maximum. Thus, independent pathways eventually can lead to rejection, but their combined effect results in rapid rejection while inhibition, or lack, of one of them results in an attenuated response.

Summary. Two compounds, EACA and SBTI, known suppressors of certain proteolytic enzymes, were administered daily to mice that had been inoculated with a tumor that they normally reject. Nearly half of all the Strain A and $C_{57}Bl$ mice treated with either agent developed large tumors within 12 days. No tumor growth was seen in any of the untreated control animals. Of the 18 mice given combined doses of both compounds, 15 developed sizable tumors. The tumors always reached a maximum size and then regressed. Therefore, it seems that proteolytic enzymes participate in the rejection of homotransplanted tumors, but that suppressing their effect merely attenuates the immune response, and other mechanisms eventually cause the demise of the tissue.

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