Histidine Decarboxylase Activity of the Rat Spleen Following Skin Allografting

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AN increase in the urinary excretion of histamine has been observed by Moore and Chang [14] to occur following skin allografting in the rat. The 24-hour excretion of histamine in the rat was found by Kahlson [7] to be a useful means of monitoring the rate of intracellular histamine formation from histidine decarboxylase enzyme activity. Direct isotopic measurement of the histidine decarboxylase activity of allografted rat skin by the authors [16] has shown a 33-fold increase above normal levels during rejection. The peak elevation was found 10 days after allografting. No such change was observed in autografted rat skin.

The use of established inhibitors of histidine decarboxylase has been found by Moore [10, 11, 13] to prolong markedly the survival of skin allografted between inbred rat strains. The survival of second-set allografts carried out between inbred rat strains was prolonged when the histidine decarboxylase inhibitors were given during both first and second-set allografting but not when given only at the time of the second set grafting.[12] Histidine decarboxylase inhibitors also were found by Smellie and Moore [24] to be effective in prolonging the survival of canine renal allografts.

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The inhibitors of histidine decarboxylase found to be most effective in prolonging the survival of allografts of rat skin have been found by Moore and Lawrence [15] to suppress markedly the formation of antibody to strong antigens in rats and mice. Salmonella flagellar antigen was employed in rats and sheep red blood cells were used in mice for the agar plaque assays of Jerne [6] and of Dresser and Wortis.[2] The suppression of antibody formation in mice was associated with lymphoid depletion as judged by spleen weights on sacrifice at various intervals following sheep red blood cell immunization. In addition to lymphoid depletion, there was a reduction in the capacity of remaining cells to produce antibody. The suppression involved both 19S and 7S antibody formation.

The above observations suggest that the involvement of intracellular histamine metabolism in transplant rejection may be complex. Alterations in histidine decarboxylase activity may occur at the site of the allograft and, in addition, may involve tissues remote from the area of rejection. These remote tissues may include such immunologically involved lymphoid tissues as spleen, lymph nodes, Peyer's patches and thymus.

This paper presents observations relative to histidine decarboxylase activity of rat spleen and Peyer's patches following skin allografting and of rat spleen following skin autografting.

Experimental Method

Female rats of the inbred Lewis and PA strains were used. The rats weighed approximately 200 grams. Full-thickness grafts of relatively thin abdominal skin were cleared of subcutaneous tissue by scraping and were secured to the backs of recipient rats by means of small Michel clips as described by Millington and Moore.[9] Grafts were of a standard size, cut from a circular pattern measuring 3.5 cm. in diameter. Skin allografts were from Lewis to PA rats.

Because the allografts were of thin abdominal skin to the backs of the recipients, it was not possible to carry out comparable control studies with autografts. Earlier studies of graft histidine decarboxylase activity had shown little change in activity of autografts as compared with marked elevation in activity of allografts. The autografts performed were of thin abdominal skin transplanted to the backs of PA recipients. This required an additional abdominal skin resection and wound closure, not required in allografts, and prevented these animals from being true autograft controls as far as the systemic response to grafting was concerned. The abdominal wounds were closed with a series of small Michel clips.

Grafts were left open and animals were kept in individual cages. A standard diet of Purina Laboratory Chow for mice, rats and hamsters was provided for all animals. Animals were sacrificed daily from 1 through 10 days after allografting and spleens and small intestinal Peyer's patches were removed and quick frozen for subsequent assay of histidine decarboxylase activity. Approximately 18 Peyer's patches were removed from each rat small intestine. All Peyer's patches from each animal were pooled for assay. Autografted animal spleens were removed 1 through 7 days.

Histidine decarboxylase activity was determined by the following method. Spleen and Peyer's patches samples were minced, frozen and thawed three times. A small amount of phosphate buffer, pH 7.4, was added and the freezing and thawing was repeated once more. Additional buffer then was added to give four times the weight of the tissue in each sample. This process extracts histidine decarboxylase which is a soluble enzyme. After centrifuging, aliquots of the supernate were incubated with microgram quantities of ^{14}C L-histidine. To determine the ^{14}C histamine formed, a large excess of nonisotopic histamine carrier was added, extracted, converted into benzenesulfonyl histamine (BSH), weighed, counted in a Packard Tri-Carb Scintillation Spectrometer and corrected for background and weight of sample. Enzyme activity was expressed as c.p.m. for each gram of spleen studied and as total c.p.m. for each spleen studied. In each group of spleens studied, normal splenic tissue was carried through the assay as a control. Full details of the method have been published.[21]

Results

The histidine decarboxylase activity of normal, unstimulated reticulo-endothelial tissue has been found to be low. The activity of Peyer's patches lymphoid tissue remained low in this study after skin allografting. It was detectably greater than blanks only at one, 3 and 7 days after grafting. On these days it was 630, 310 and 850 c.p.m. per gram of tissue. Even those detectable levels were within the normal range for lymphoid-reticulo-endothelial tissue and were not considered to be elevated.

Histidine decarboxylase activity of splenic tissue, on the other hand, was elevated after skin allografting. In the first group of spleens assayed, the peak elevation to 4,150 c.p.m. per gram of splenic tissue occurred in the initial five days after skin allografting (Fig. 1). This was 8.9 times greater than the control value for normal

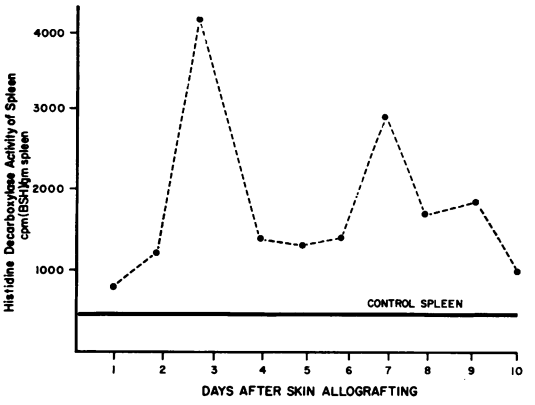

FIG. 1. Graph showing elevation of splenic histidine decarboxylase enzyme activity above that of normal splenic tissue at various intervals after skin allografting between inbred rat strains in the first group of spleens studied.

splenic tissue assayed at the same time as this study. The average elevation in splenic histidine decarboxylase activity per gram of spleen in the initial five days after allografting in this first group was to 1,720 c.p.m. This was 3.7 times the control value for normal spleen. When the activity was calculated as c.p.m. per total spleen, the activity in the initial five days after allografting in this group was 3.9 times greater than that for normal spleen.

Similar findings were encountered in the second group of spleens assayed. The peak and greatest elevation in histidine decarboxylase activity clearly occurred in the initial five days after allografting (Fig. 2). The peak elevation to 3,890 c.p.m. per gram of spleen was 4.3 times greater than the control value for normal splenic tissue assayed at the same time as this second group of spleens. The average elevation in the initial five days after skin allografting was 2,874 c.p.m. per gram of spleen. This was 3.2 times greater than the value for normal splenic tissue. When the activity as c.p.m. was calculated for the total spleen, the activity in the initial 5 days after grafting in this group of spleens was 4.0 times greater than that for normal spleen.

The splenic histidine decarboxylase activity of spleens following skin autografting also is of some interest although these cannot be regarded as true autograft controls since each animal had an additional abdominal skin resection and wound closure with Michel clips not carried out in allografted animals. This additional operative procedure might be expected to increase rather than decrease any nonspecific inflammatory systemic response such as an elevation in splenic histidine decarboxylase activity.

In the initial 7 days after autografting the splenic histidine decarboxylase activity as c.p.m. per gram of tissue ranged from 520 to 1,390. The study was carried out at the time of the second allograft spleen study. When the splenic histidine decarboxylase activity for the initial five days after autografting, $1,122 \pm 156$ c.p.m. per gram of spleen, is compared with splenic activity in the initial five days after allografting, $2,874 \pm 314$ c.p.m. per gram of spleen, an elevation of 2.6 times is found for allograft spleens as compared with autograft spleens. This is a significant difference ($p < 0.01$).

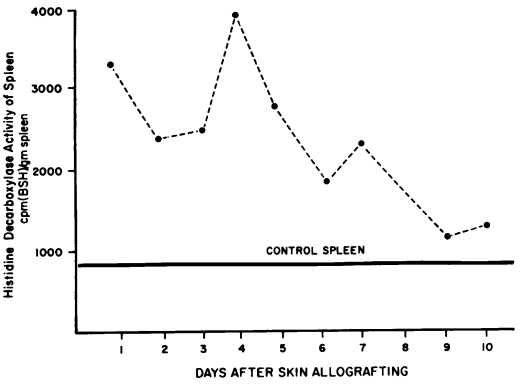

FIG. 2. Graph illustrating elevation in histidine decarboxylase activity of the rat spleen after skin allografting in comparison with normal splenic tissue in the second group of spleens studied.

Discussion

The development of a ^{14}C radioisotopic method for the measurement of tissue histidine decarboxylase activity in the 1950's provided a new and useful means for the study of intracellular histamine metabolism and its role in physiological and pathological processes.[20, 21] Virtually all tissue histamine is formed intracellularly through the activity of the specific histidine decarboxylase enzyme which requires pyridoxal phosphate as its coenzyme.[23] It may be purified from rat hepatoma or from homogenates of whole fetal rats (19 to 20 days gestation) as described by Hakanson.[5]

Kahlson,[7] employing the ^{14}C isotopic method and the level of urinary excretion of histamine in rats, found a marked increase in the histidine decarboxylase activity of rapidly growing fetal and tumor tissue and in tissue undergoing reparative and regenerative growth.

Schayer and Ganley,[23] in 1959, reported that treatment with pertussis vaccine produced an elevation in the histidine decarboxylase activity of mouse lung. They also found a twofold increase at 24 hours in the histidine decarboxylase activity at the site of the tuberculin reaction in the guinea pig. Graham and Schild,[3] in 1967, reported a peak tenfold increase in histidine decarboxylase activity at the site of a skin tuberculin reaction in the rat at 12 hours, with a threefold increase at 24 hours.

Aures, Winquist and Hansson,[1] in 1965, reported a tenfold increase in the histidine decarboxylase activity of the bone marrow of guinea pigs treated with foreign protein. Kahlson, Rosengren and Thunberg,[8] in 1966, reported that a lethal type of intravenous antigenic challenge in sensitized rats and guinea pigs, protected from fatal anaphylaxis by antihistamines, produced an increase in the histidine decarboxylase activity in most tissues of the body. The increase in activity reached its peak in the initial 3 to 6-hour period in the liver, lung and aorta, but not until 24 hours in the spleen and small intestine.

As cited earlier, evidence of a marked increase in the histidine decarboxylase activity of allografted tissue during rejection was presented in the 1969 report of the authors.[16] This indicated that significant local changes in histidine decarboxylase activity were involve in the process of allograft rejection. The possibility that more than local transplant site changes in histidine decarboxylase activity also might be involved in the processes of rejection was suggested by the observation of Moore [12] that established inhibitors of histidine decarboxylase activity could prolong the survival of second-set skin allografts between inbred rat strains only when given during both first and second-set grafting. This possibility also was raised in the study of Moore and Lawrence [15] which demonstrated that the two means of drug induced histidine decarboxylase inhibition found to be most effective in prolonging the survival of first-set skin allografts also suppressed the formation of antibody to strong antigens in rats and mice and produced lymphoid depletion. Spleen weights of treated mice following sheep red blood cell immunization were approximately one half of those of control mice following the same immunization.

The present study was carried out to assess alterations in the histidine decarboxylase activity of lymphoid tissue anatomically remote from the site of the allograft. There was no change in the histidine decarboxylase activity of Peyer's patches lymphoid tissue of the small intestine. Splenic histidine decarboxylase activity, on the other hand, was elevated after skin allografting. The major elevation occurred in the initial 5 days after allografting. The peak elevations in the two studies were four and nine times control levels of nor-

mal spleen and occurred 3 and 4 days after allografting.

Elevations in splenic histidine decarboxylase activity after skin allografting in the initial 5 days after grafting in the two groups of spleens studied were remarkably similar in comparison with the activity of normal splenic tissue. The elevation as c.p.m. per gram of splenic tissue was 3.7 and 3.2 times that of normal splenic tissue and when calculated as c.p.m. per total spleen it was 3.9 and 4.0 times greater. The elevation in splenic histidine decarboxylase activity following skin allografting also was significantly greater than that encountered after skin autografting, despite the requirement of an additional abdominal wound and closure for autografts. It is of interest that the major changes in the histidine decarboxylase activity of the allografted skin undergoing rejection did not take place until 6 or more days following grafting, whereas the major elevation in splenic histidine decarboxylase activity after skin allografting appeared to occur in the initial 5 days.

The intracellular formation of histamine from histidine decarboxylase activity may exert some control over the immunologic function of lymphoid tissue. Gowans [4] has shown the importance of small lymphocyte recirculation and of the post-capillary venules of lymph nodes in this recirculation with respect to the immunologic activity of small lymphocytes. Schayer's [22] hypothesis is of interest in this regard. He has suggested a possible role for histidine decarboxylase in microcirculatory regulation and as an antagonist of glucocorticoids. The observed increase in the histidine decarboxylase activity of the spleen reported here to occur following skin allografting may be related to an increase in blood flow to and through the spleen, including possibly the immunologically important post-capillary venule areas.

Additional studies of changes in histidine decarboxylase activity of reticulo-endothelial tissues after allografting are in progress. Preliminary data suggests no change in histidine decarboxylase activity of thymic tissue after skin allografting in rats.[18] The effect of abdominal skin allografts in rats on histidine decarboxylase activity of regional and distant lymph nodes, in addition to spleen and thymus at various periods after grafting, currently is being studied and compared with changes produced by autografts. The effects of organ transplants such as renal and splenic on splenic and reticulo-endothelial tissue histidine decarboxylase activity also are under study. In addition, studies are in progress to evaluate the effect of antilymphocyte serum and of massive doses of corticosteroids on graft and reticulo-endothelial histidine decarboxylase activity following tissue and organ allografting and autografting. Preliminary observations suggest that when anti-thymocyte serum is effective in prolonging survival of skin allografts in rats, the histidine decarboxylase activity of the allograft may be substantially lower than otherwise might be expected.[17]

Summary

This study was undertaken to investigate changes produced by skin allografting and autografting in the histidine decarboxylase enzyme activity of reticulo-endothelial tissue anatomically remote from the site of the graft. Spleen and intestinal tract Peyer's patches were the reticulo-endothelial tissues selected for study.

Skin allografting produced no change in the normally low histidine decarboxylase activity of Peyer's patches tissue. It did result in an increase in the histidine decarboxylase activity of splenic tissue in comparison with normal splenic tissue and with splenic tissue obtained after skin

autografting. The peak elevation and period of greatest elevation in splenic histidine decarboxylase activity occurred in the initial 5 days after allografting.

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