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Mast Cells and Their Degranulation in the Tsk Mouse Model of Scleroderma (42183)

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Abstract. The Tsk mouse is a genetically transmitted example of cutaneous fibrosis which has been compared with human scleroderma. During a systematic histopathological study of the Tsk mouse, both an increased number and an increased proportion of degranulated mast cells were observed. The consistent association of mast cells and fibrosis in scleroderma, graft-vs-host reactions (GVHR), and now the Tsk mouse raises the question of a pathogenetic role for mast cells in fibrotic disorders in general.

Mast cells, abundant in the interstitial connective tissue of skin, are usually situated near small blood vessels, hair follicles, and fat cells. They are also present in the submucosa of the small intestine, in sheathes of peripheral nerves and meninges, and in the reticuloendothelial system. From both contiguous and remote anatomic sites, mast cells may differ widely in both morphologic and functional characteristics (1).

These cells are functionally associated with anaphylactic and allergic phenomena in which bridging of pairs of membrane IgE molecules by antigen perturbs the cell membrane, and is associated with energy-dependent entry of calcium, phospholipid metabolism alterations, and an increase in cAMP. These processes are followed by fusion of intracellular granular membranes with cell membranes and subsequent release of a cascade of biologically active factors, among which are vasoactive/smooth muscle cell reactive mediators such as histamine, slow-reacting substance A (leukotrienes LTC_4, LTD_4, LTE_4, and possibly LTF_4), serotonin, platelet-activating factor and prostaglandins; chemotactic mediators such as eosinophil chemotactic factors, neutrophil chemotactic factor, histamine, and several lipid chemotactic factors; glycosaminoglycans such as heparin, chondroitin sulfates, and dermatan sulfate; and enzymes such as chymase, arylsulphatase, glucosaminidase, and β-glucuronidase. These substances can be demonstrated *in vitro* to have roles in vasopermeability, smooth muscle contraction, and chemotaxis as well as in tissue damage and repair (2). Serotonin has been associated with platelet aggregation in patients with scleroderma (systemic sclerosis) (3).

In 1976, Green *et al.* described a new mutant mouse called tight skin (Tsk). The mutant gene (located on chromosome 2), when homozygous, causes death *in utero* and, when heterozygous, causes excessive deposition of connective tissue (4). Menton *et al.* demonstrated irregular dermal collagen fibers in the Tsk/+ mouse and compared the cutaneous abnormalities of Tsk/+ mice with those of scleroderma in man (5). Ross *et al.* also found changes in the hexosamine, uronic acid, and glycosaminoglycan content of Tsk skin similar to known changes in scleroderma skin and suggested that the Tsk mouse may be a model for human scleroderma (6).

We have observed an increased number and an enhanced degree of degranulation of mast cells in the skin of Tsk mice, raising the possibility that the fibrotic disease of Tsk mice may be associated with this effector cell. We have also quantified the increase in width of a subcutaneous fibrous layer in Tsk mice with increasing age. The widths of the fibrous layer and a comparison of mast cell number and degree of degranulation between Tsk and +/+ littermate mice are reported herein.

Materials and Methods. Tsk and syngeneic +/+ mice (C57BL/6 Tsk/+ and C57BL/6 +/+) were obtained from Jackson Laboratories (Dr. Sid Lane, Bar Harbor, Maine) between the ages of 4 weeks and 12 months,

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housed in the MUSC animal facilities and sacrificed at the ages indicated for each experiment. The number of mice used for each experiment is shown in Figs. 1 and 3. The number of Tsk mice used always equaled the number of +/+ mice used in each experiment. At the desired age, mice were anesthesized with 0.2 ml Avertin followed by cervical dislocation. A sample of shaved skin was taken from the dorsal surface between the shoulder blades with a 6-mm biopsy punch. Samples were fixed and processed for light microscopy. Five micrometer sections were cut; hematoxylin and eosin and Giemsa stains were performed. Sections were projected onto graph paper, outlines drawn, and the paper cut out and weighed to two decimal places. A transparent measure was projected similarly to obtain a curve of standard areas from which the total area in square millimeters of each section was determined. Mast cells in the entire section were counted and the following equation was used to determine the number of mast cells per mm square millimeter:

$$\text{mast cells/mm}^2 = \frac{\text{total mast cell number}}{\text{total section area}}.$$

Mast cells were also classified as to the degree of degranulation from 0 to 4+ as follows:

0 = no extracellular granules visible
1+ = cell and nucleus clearly distinguishable with 2–10 extracellular granules visible
2+ = cell and nucleus clearly distinguishable with 11–25 extracellular granules visible
3+ = cell and nucleus barely visible through extracellular granules numbering 26–50
4+ = cell and/or nucleus difficult to find; massive number of granules (>50) clustered in an area two to five times the size of the cell, and many times accompanied by a diffuse interstitial distribution of granules.

The cylindrical skin biopsy was cut in half along its long axis perpendicular to the epidermis. The tissue was placed in the embedding block with the flat surface down, and cut parallel to the flat surface with epidermis up. The measurement of the width of the subcutaneous fibrous layer was performed on a Videoplan image analysis system coupled to a photomicroscope (Zeiss, West Germany). The actual measurements were performed interactively on a digitizing pad using the Videoplan's y-x software.

Except for the recognized difference in hair color (Tsk mice have black coats; +/+, beige) and skin thickness, no differences were observed in growth, behavior, appetite, or age-corrected weights between Tsk and +/+ mice.

Results. In both Tsk and +/+ mice, as has been shown in other species, the total number of mast cells decreases with age. In the Tsk mouse, the number of mast cells and/or the degree of degranulation differ from +/+ mice at comparable ages. Figure 1 shows the num-

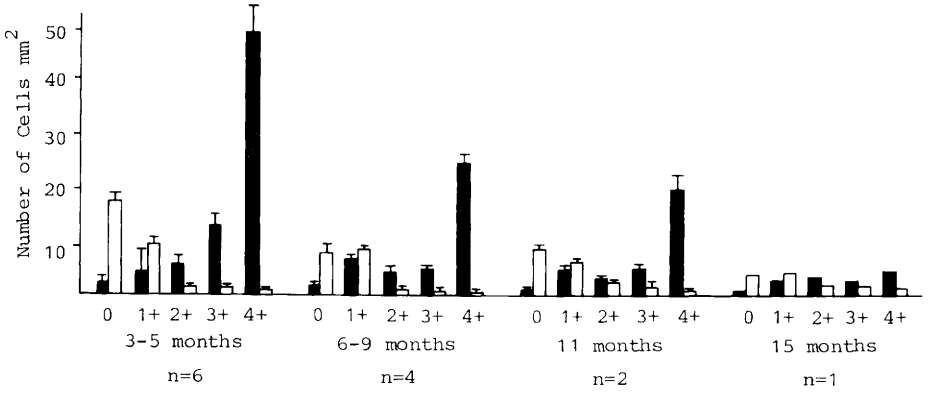

FIG. 1. Number of mast cells/mm² skin in each degranulation state 0 to 4+ for Tsk (closed bars) and +/+ (open bars) mice. *n* = number of sections quantified and number of mice studied. Brackets represent one standard deviation.

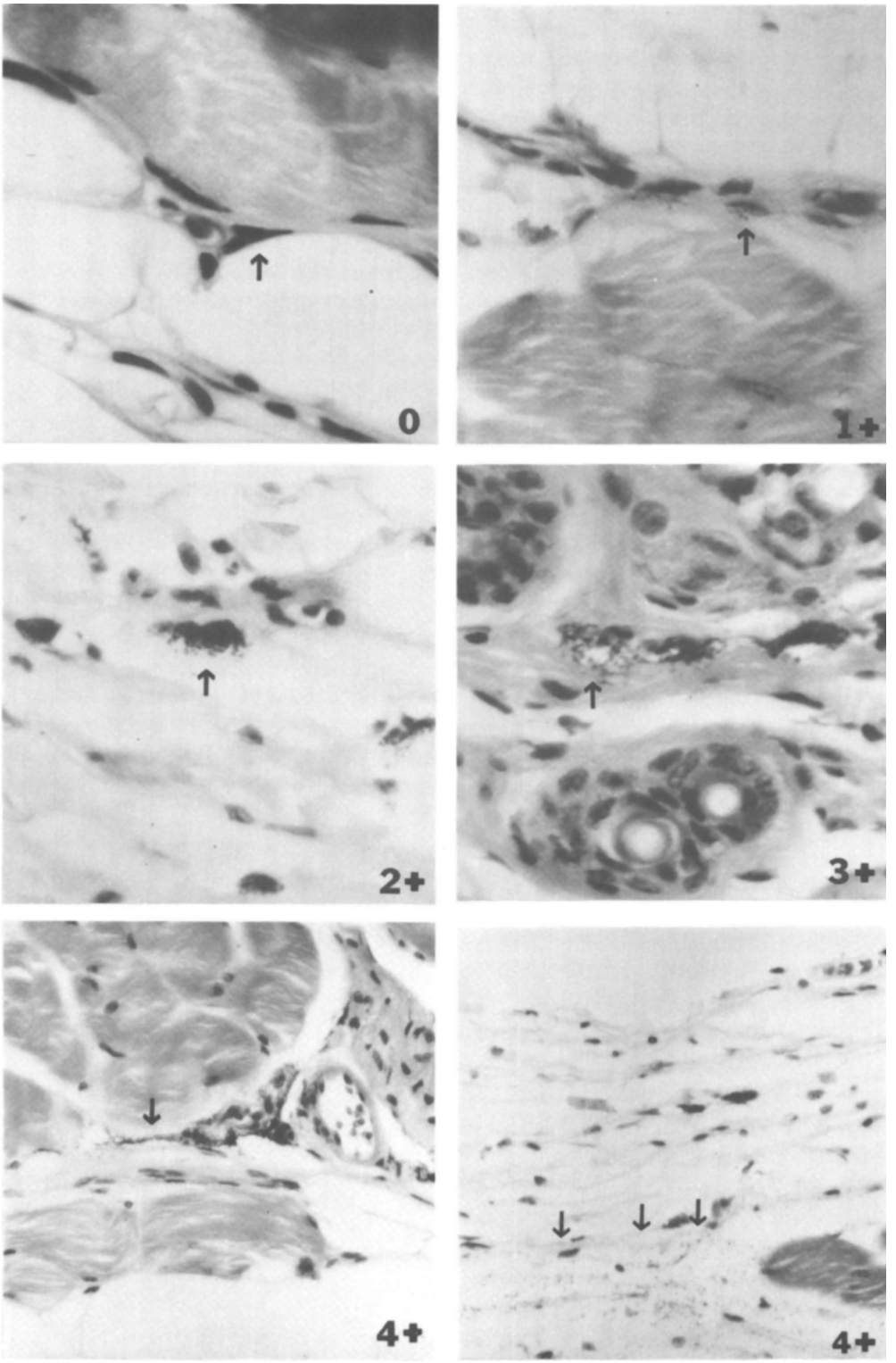

FIG. 2. Photomicrographs of mast cells in each of the degranulation states (0 to 4+) (400×). The 0 state shows no granules outside the cell. The 1+ cell has 2 extracellular granules. There are approximately 20 granules outside the 2+ cell. The 3+ cell has approximately 40 extracellular granules and the cell looks broken up compared to the 0 state cell. The 4+ cell (left picture) shows many granules in a large area and the cell is difficult to see. The 4+ area (right picture) is a large accumulation of granules. The cell(s) is not visible as a distinct entity.

ber of cells per square millimeter in Tsk and +/+ for each degranulation category (0–4+) at ages between 3 and 15 months. At 3, 5 and 6 months, mast cell numbers in Tsk skin are more than twofold greater than in +/+ skin. At 11 months, the proportion is 1.8, and at 15 months, the number of skin mast cells is similar in Tsk and +/+ and significantly reduced from the number in younger mice. That younger Tsk mice have more mast cells is consistent with a pathogenetic role for these cells in the fibrotic process.

Not only do the number of cells in the affected and normal mice differ, but also the degree of degranulation differs. At 5 months, the Tsk mouse has few cells exhibiting no visible degranulation with the majority exhibiting 4+ degranulation. At 5 months, most mast cells in the +/+ mouse are normal (0) and very few are degranulated (4+ state). At 15 months, even though the total mast cell number per square millimeter is nearly equivalent in Tsk and +/+, the proportion of highly degranulated cells in Tsk remains increased. Figure 2 shows representative examples of degranulation classified from 0 to 4+.

Figure 3 compares the widths of the fibrous layer at various ages. Even at young ages, there is more fibrous tissue in the Tsk than in the +/+ mouse; between 6 and 9 months a large increase in the width of the layer occurs in the Tsk mouse with little to no change in the +/+ skin.

Other histopathologic abnormalities of the Tsk mouse are being prepared for separate publication. Mast cell abnormalities have only been observed in the skin.

Discussion. We have demonstrated in Tsk mouse skin an absolute increase in the width of a subcutaneous fibrous layer, in the number of mast cells, and in the degree of mast cell degranulation, all compared to +/+ skin.

An increase in the density of dermal mast cells has been noted in various skin diseases, including urticaria pigmentosa, basal cell carcinoma, neurofibromatosis and lichenified atopic eczema; therefore, it seems reasonable that the mast cell density could be increased in other skin disorders as in the Tsk mouse and scleroderma in man.

Increased numbers of mast cells in scleroderma skin have been reported by Hawkins *et al.* (7). They noted that mast cell counts were greater in involved skin than uninvolved skin or in control skin; unusual degrees of mast cell degranulation were not seen. Skin lesions resembling those of scleroderma have been observed in chronic graft-versus-host disease (8–11); mast-cell dysfunction has been noted in a mouse model of chronic GVHD (12, 13).

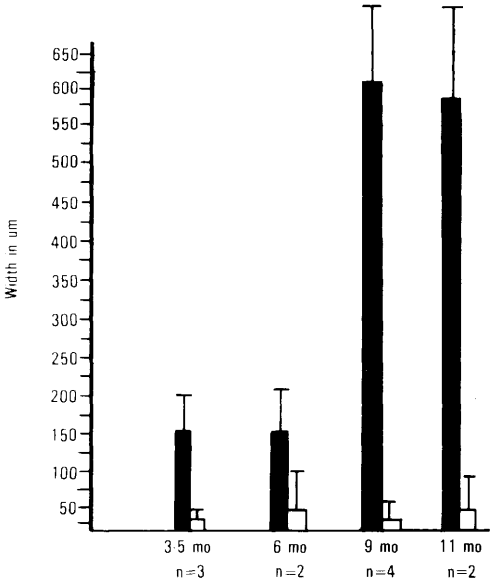

FIG. 3. Width of subcutaneous fibrous layer in Tsk (closed bars) and +/+ (open bars) skin. *n* = number of sections quantified. Brackets represent one standard deviation.

Greenberg and Burnstock have described a cell-to-cell interaction between mast cells and both fibroblasts and endothelial cells (14). Mast cells could affect the behavior of fibroblasts and be important in the pathogenesis of fibrosis in the Tsk mouse. The fact that these cells are present in abundance and exhibit massive degranulation at early age when the subcutaneous fibrous layer has not yet reached maximum width suggests an association between mast cells and fibrosis in the Tsk mouse. Experiments are underway to inhibit mast cell function followed by histological observation of the appearance of fibrosis.

There is evidence that mucosal mast cells may be under T-cell control, and that, in turn, they may modulate T cells and other immune cells (15–20). The mechanisms of regulation

of connective tissue mast cells are unknown. Mast cell differentiation appears to be regulated by factors from $Ly1^{+}2^{-}Ia^{-}$ T cells (20). Also, similarities have been found between mast cells and both natural killer and suppressor T cells (21–23). Furthermore, there is evidence that mast cell degranulation, triggered by compound 48/80 or Polymixin B, stimulates fibroblast proliferation and collagen deposition (24, 25). When immune responding animal strains are shown to have plentiful mast cells, it is difficult to determine whether mast cells lead to immunoresponsiveness or vice versa. In the Tsk mouse, fibrosis could be mediated directly by mast cell products or indirectly through IgE or cell-mediated immune responses which recruit mast cells. Further study is needed to dissect these interacting influences in the Tsk mouse.

In conclusion, we have observed an increased number of mast cells in the skin of Tsk mice with most of the cells exhibiting increased degranulation, accompanied by an increase in fibrosis with age, raising the possibility that mast cells may play a role in the initiation of fibrosis in the skin of Tsk mice. Thus, the Tsk mouse may be a suitable setting to explore the longstanding and well-studied interactions between mast cells, wound healing, and fibrosis.

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