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# Mast Cell Kinetics during Tumor Growth

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**Abstract.** The behavior of mast cells was studied during tumor growth. Sarcoma 13, and normal syngeneic kidney, as control, were implanted subcutaneously in BALBc mice. The number of mast cells in the hypodermis and peritumoral tissue increased 3-fold, 20 days after implantation. In the peritumoral tissue, mast cell degranulation increased together with tumor growth, while in the dermis and hypodermis, it first decreased and only became evident on day 20. Mitosis and mast cell degranulation, more conspicuous in tissues near the tumor, seem to indicate the existence of tumoral factors which spread slowly from the tumor to distant zones. The role of mast cells in peritumoral tissues will be evaluated in the near future.

## Introduction

Mast cells are widely distributed in connective tissues and the presence of these cells in a variety of tumors as well as in inflammatory processes is universally known. However, there is no unanimity of opinion concerning the functional significance of this relationship. Some authors assigned a protector role against tumor invasion to mast cells. This point of view is supported, among others, by Csaba et al. [1], who suggest that mast cells inhibit tumor growth by neutralizing the polysaccharides necessary for tumor development. Fisher and Fisher [2] suppose that mast cells exert an inhibitory influence on tumor growth by serotonin secretion. Farram and Nelson [3] describe a mast cell cytotoxic action on a mouse methylcholanthrene-induced fibrosarcoma (C4). In mice severely depleted of mast cells, the tumor incidence increased either, in mice bearing a 3-methylcholanthrene-induced fibrosarcoma [4], or in mice implanted with tumor cells [5]. Ueda et al. [6] correlated a high mast cell number with a favorable prognosis in patients with soft tissue sarcoma. In contrast with these reports, authors like Roche [7, 8], developing in vitro investigations, and Ionov [9] with in vivo experiments, demonstrate that mast cells are able to enhance tumor growth.

Having these two different viewpoints in mind when investigating the role of mast

cells during tumor invasion, we have focused our attention on analyzing: (1) whether our model is suitable for examining mast cell behavior during tumor growth; (2) whether the presence of mast cells in the peritumoral tissues is related to an inflammatory mechanism or with specific tumor characteristics; (3) whether during tumor growth there are any changes in the number of mast cells, and (4) whether tumor facilitates mast cell degranulation, as has been described by some authors.

## Materials and Methods

*Animals.* Female 9- to 12-week-old inbred BALB/c mice were obtained from our Animal Breeding Center.

*Tumors.* A vascularized syngeneic mammary adenocarcinoma S13, which appeared spontaneously in BALB/c mice, was used. It was maintained by serial subcutaneous transplantations. Approximately 30 days after tumor implantation, when the tumor reached 25–30% of the body weight the mice died.

*Mast Cells Proliferation Assay.* S13 tumor was subcutaneously implanted by trochar in the middle of the right lateral side of 10 animals. Ten mice implanted by trochar with normal syngeneic kidneys were used as controls. On days 5, 8, 13, 16 and 20 from implantation, 2 tumor-bearing mice and 2 mice from the control group were sacrificed. The tumor and the peritumoral tissues including the skin were excised and processed for histologic analysis.

*Histology.* Each biopsy was fixed in Mota's fixative for 48 h, paraffin-wax-embedded and sectioned perpendicularly to the skin surface. Three nonserial sections from each biopsy were stained with basic fuchsin (Cajal-Gallego method) and were employed for mast cell counting. Other sections were stained with alcian blue 8GX (pH 0.5) [10], to confirm the presence of heparin.

*Statistical Analysis.* In each of these three sections, mast cells (granulated and degranulated), in the dermis, hypodermis, and peritumoral tissue were counted in 10 randomly selected high-power fields (HPF). The data presented in figure 2 represent the mean of the number of mast cells present in 10 HPF in each layer of all the three sections of the 2 animals analyzed.

The general behavior of data was first investigated computing variances with respect to a linear regression. Then, variances between tumor-implanted and control mice were evaluated day by day, by means of the one-way variance analysis. The statistical significance of the percentage of degranulated mast cells was evaluated by the $\chi^2$ test.

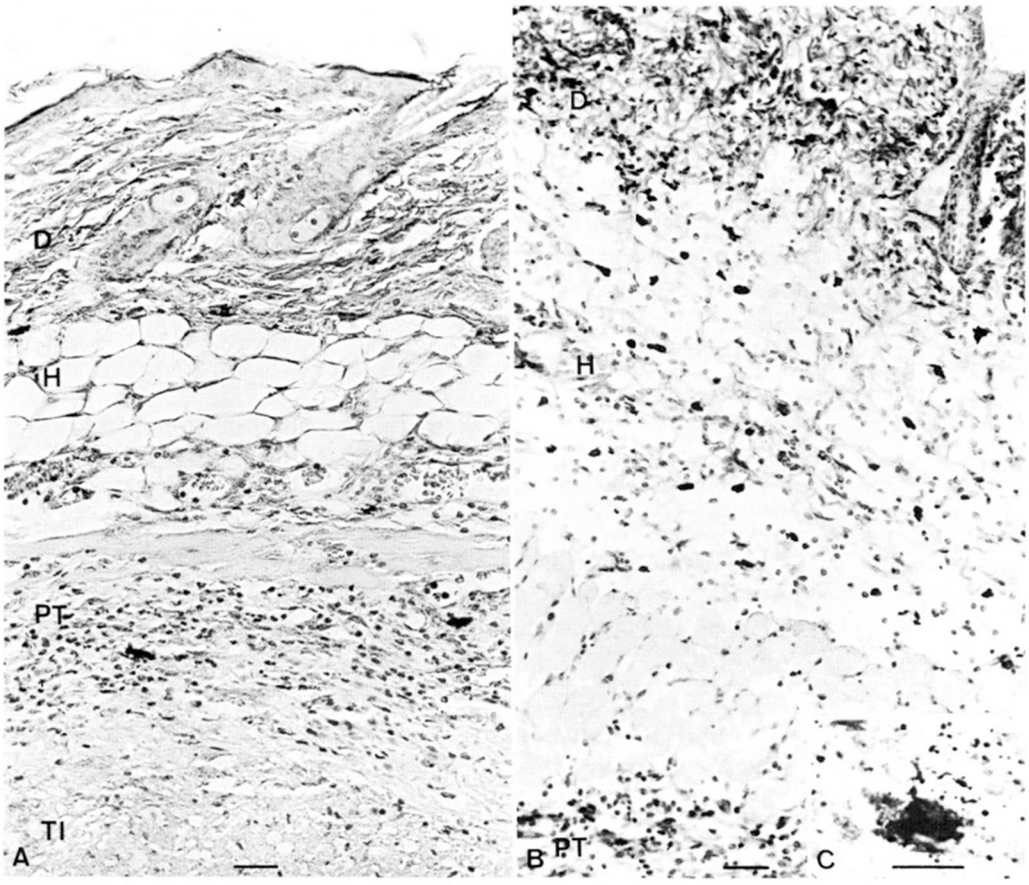
## Results

Mast cell number in tumor-implanted mice increased with time (fig. 1,2). This assessment was checked, fitting linear regression curves to the data and comparing curve slopes (table 1).

Data presented in figure 2 indicate an increase of mast cell number in peritumoral tissue from the 5th day ($F = 41.63$; d.f. = 1,10; $p < 0.001$) as well as in the hypodermic zone from the 8th day ($F = 30.25$; d.f. = 1,10; $p < 0.001$) after tumor implant, when compared with the controls. Mast cell number in the dermic zone shows no statistically significant differences with respect to control mice, except on day 13 where a smaller mast cell number is significant ($F = 16.43$; d.f. = 1,10; $p < 0.001$) and on day 20 where an increased number of mast cells is significant also ($F = 20.64$; d.f. = 1,10; $p < 0.001$).

**Table 1.** Slopes of linear regression curves computed for the quantity of mast cells in dermis, hypodermis and peritumoral tissues of kidney- and tumor-implanted mice

| | Dermis | Hypodermis | Peritumoral |
|---|---|---|---|
| Tumor mice | 0.27 | 1.81 | 0.89 |
| Control mice | −0.07 | −0.09 | −0.01 |

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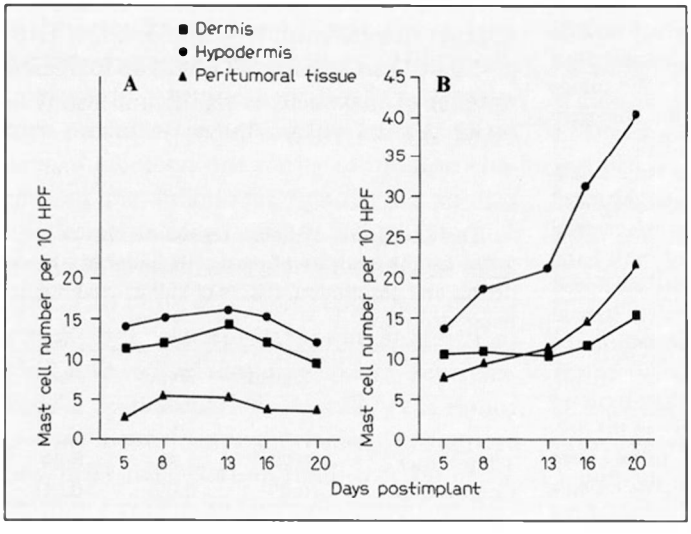


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**Fig. 1.** Mast cell in connective tissue near tumor implant. Basic fuchsin-stained mast cells after 5 (**A**) and after 20 days (**B**) of tumor implant. Bar = 50 µm. **C** Degranulated mast cell from hypodermis. Bar = 10 µm. D = Dermis; H = hypodermis; PT = peritumoral tissue; TI = tumor implant.

**Fig. 2.** Quantity of mast cells in kidney-implanted mice (**A**) used as control and in tumor-implanted mice (**B**). 10 HPF = 0.25 mm$^2$

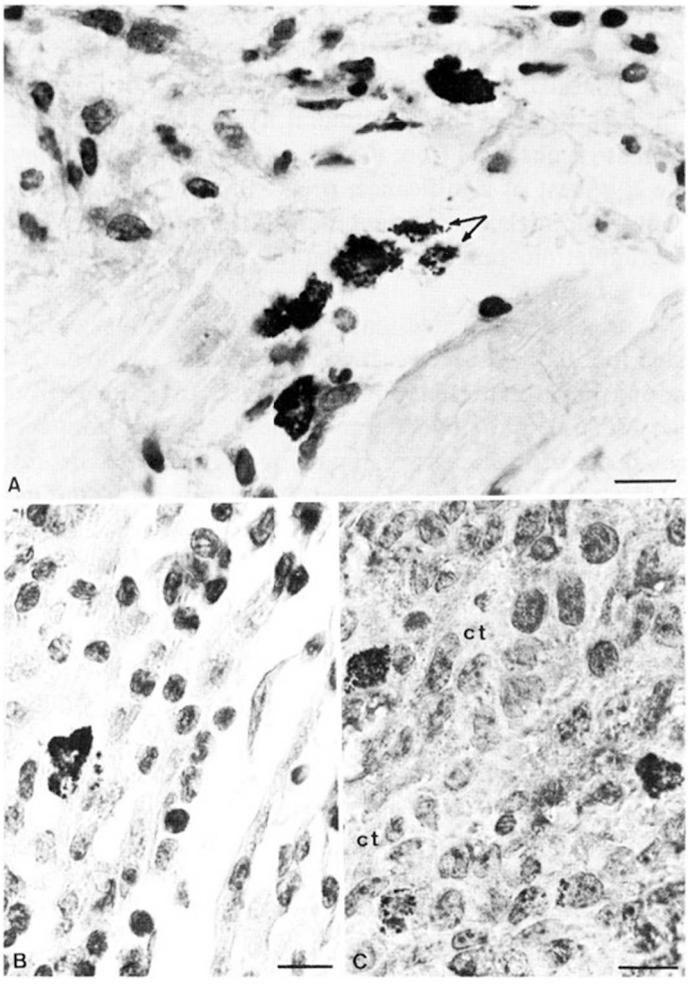


**Fig. 3.** In hypodermic zone (**A**), degranulated big mast cells as well as paired small ones (arrows), appear on day 16. In peritumoral tissue (**B**) two little mast cells closely packed and partially degranulated are shown. In tumor tissue (**C**) small mast cells are shown on day 20 near connective tissue (ct). All three sections stained following Cajal-Gallego method. Bar = 10 μm.

**Table 2.** Percentages of degranulated mast cells in dermis, hypodermis and peritumoral tissue of tumor-implanted mice, 5, 8, 13, 16 and 20 days after implant

| | Day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | | 8 | | 13 | | 16 | | 20 | |
| | $\bar{X}$ | SE | $\bar{X}$ | SE | $\bar{X}$ | SE | $\bar{X}$ | SE | $\bar{X}$ | SE |
| Dermis | 45.5 | 4.9 | 36.2 | 8.8 | 36.2 | 7.6 | 36.5 | 7.9 | 53.2 | 8.5 |
| Hypodermis | 43.6 | 3.7 | 42.6 | 12.1 | 43.1 | 8.1 | 37.8 | 12.2 | 71.5 | 6.9 |
| Peritumor | 43.3 | 15.4 | 52.6 | 8.4 | 53.6 | 15.0 | 67.0 | 14.1 | 72.1 | 9.5 |

In animals with tumor implants, degranulated mast cells in the dermic and hypodermic zones increase after the 16th day (fig. 3A), while in the peritumoral tissue an increase is observed from the 8th day on (table 2; $\chi^2$ test of significance; $p < 0.05$). In control animals, a constant percentage of degranulation ranging between 39.5 and 48.4% is found.

Around degranulated mast cells an alcian blue (pH 0.5) positive reaction appears, denoting heparin presence in the matrix. In peritumoral and hypodermic zones, we could detect mast cells of small size paired and packed (fig. 3A B). Concerning tumor tissue, a scarce number of mast cells was found only on the 20th day, usually placed in thin trabeculi next to blood vessels (fig. 3C).

## Discussion

The number of mast cells in hypodermic and peritumoral tissue of tumor-bearing mice increased 3-fold starting from the first day of implant, and during the tumor growth.

Control mice, with a normal kidney graft and scanty mast cell number, make it possible to disregard an inflammatory process as a mechanism for mast cell increase. The significant decrease in dermic layer mast cells in tumor-implanted mice, on day 13, could be due to mast cell migration towards the tumor, caused by a tumor chemoattractant factor [11]. The large number of mast cell cytoplasmic granules hinders the visualization of mitosis, but the presence of many pairs of closely coupled small mast cells, as well as the increase of mast cell numbers, permit the inference of an in situ mast cell division. This mitosis could be triggered either directly by a tumor growth factor [12], or indirectly by IL-4 [13].

The linear increase of degranulated mast cells in peritumoral tissue beginning on the 8th day, which is not observed in control mice, suggests that a tumor factor [7, 14] is responsible for this process, and the increase in number of degranulated mast cells in dermis and especially in the hypodermis on the 20th day suggest that this factor could spread slowly from the peritumoral tissue to more distant connective tissues.

The results of these experiments show that our model is appropriate for the study of mast cell kinetics and degranulation in order to provide information regarding the role of mast cells in tumor growth.

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