

Letter to the Editor

Human mast cells are capable of serotonin synthesis and release

To the Editor:

Increased levels of mast cell-associated mediators, such as tryptase and histamine, have been reported in patients with mastocytosis. Recently, we observed abnormal levels of peripheral blood serotonin in a subset of patients with mastocytosis. Total blood levels of serotonin and peripheral blood platelet numbers were determined in patients admitted to the Intramural National Institutes of Health protocol 02-I-0277 after providing informed consent. Serotonin levels were correlated with platelet counts ($\times 10^3$ per microliter) and compared with a theoretic curve generated from published normal values.¹ The correlation coefficient was calculated by using Pearson correlation and a linear regression curve with Prism software (Graph-Pad Software, San Diego, Calif). We found a bimodal distribution of serotonin in patients with mastocytosis (Fig 1, A). Ten patients had levels less than the detection limit (50 ng/mL). Fourteen patients had serotonin levels of greater than 140 ng/mL, and 9 of these had levels of greater than 210 ng/mL. Three patients had serotonin levels in the midrange (70-139 ng/mL) of values. Reported levels in a normal population followed a normal distribution, with a mean level 130 ng/mL. No correlation was found with platelet numbers.

Serotonin is known to be produced and stored by murine mast cells. Rare studies have suggested the presence of serotonin in human mast cells.² To determine whether human mast cells produce serotonin, we examined serotonin levels using ELISAs in mast cells derived from CD34⁺ peripheral blood progenitors and cultured in serotonin-free media (Table I). Mouse mast cells grown in media depleted of serotonin were used for comparison.

TABLE I. Serotonin measurement in human and murine mast cells by means of ELISA*

Sample	Human mast cells	Serotonin (ng/10 ⁶ cells; mean $\pm$ SD)	Mouse mast cells	Serotonin (ng/10 ⁶ cells; mean $\pm$ SD)
Cell associated	Donor 1	12.4 $\pm$ 0.7	Culture 1	81.5 $\pm$ 3.0
	Donor 2	8.6 $\pm$ 0.5	Culture 2	75.6 $\pm$ 3.5
	Donor 3	14.4 $\pm$ 0.5	Culture 3	174.8 $\pm$ 4.9
Supernatant	Donor 1	2.8 $\pm$ 0.0	Culture 1	9.3 $\pm$ 5.5
	Donor 2	2.7 $\pm$ 0.3	Culture 2	16.9 $\pm$ 7.0
	Donor 3	1.8 $\pm$ 0.1	Culture 3	12.2 $\pm$ 0.8
Culture media		0.9 $\pm$ 0.4		6.3 $\pm$ 1.3

Human and murine cells were cultured at 1×10^6 per milliliter, and thus values in nanograms per 10^6 cells of serotonin in supernatant are from 10^6 cells minus the serotonin measured in 1 mL of culture media.

*Culture media serotonin content was measured in nanograms per milliliter.

The cell-associated serotonin content of human mast cells was 8.6 to 14.4 ng/ 10^6 cells; supernatant serotonin content was 1.8 to 2.8 ng/ 10^6 cells. Media used for human mast cell cultures contained less than 1 ng/mL serotonin, and thus we concluded that the remaining 1.8 to 2.8 ng/ 10^6 cells of serotonin found in the supernatants was produced by mast cells. Mouse mast cell serotonin averaged 75.6 to 174.8 ng/ 10^6 cells; supernatant content was 9.3 to 16.9 ng/ 10^6 cells. We also found that tryptophan hydroxylase, a specific serotonin synthesis enzyme, was present in human mast cells by using RT-PCR (SuperScript III One-Step RT-PCR system; Invitrogen) with specific primers (Bio-Synthesis, Inc, Lewisville, Tex) and by using Western immunoblotting with mouse anti-tryptophan hydroxylase (Sigma-Aldrich, Saint Louis, Mo) with

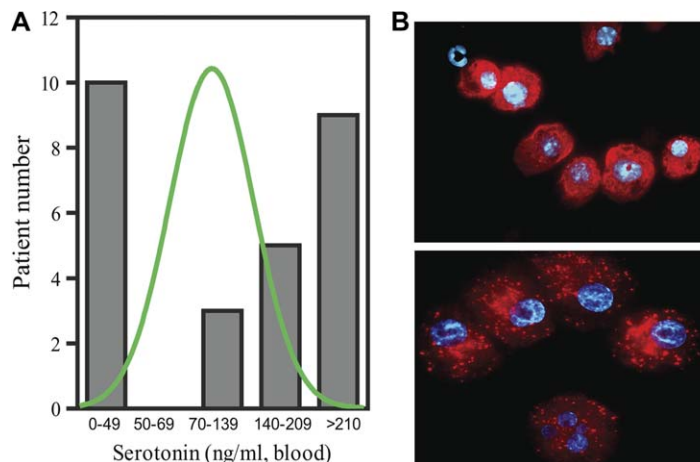


FIG 1. Total serum serotonin levels were determined in 27 patients with mastocytosis and compared with a theoretic curve (Gaussian distribution) based on published values¹ obtained from normal volunteers (n = 27, mean = 130, SD = 42). A, Bars represent patients with mastocytosis. B, Serotonin in cultured murine (upper panel) and human (lower panel) mast cells was demonstrated by means of immunofluorescent staining (red, anti-serotonin staining; blue, nuclear 4'-6-Diamidino-2-phenylindole [DAPI] staining).

secondary antibody horseradish peroxidase-conjugated anti-mouse IgG (Cell Signaling, Danvers, Mass). These studies (not shown) revealed the presence of mRNA and protein for tryptophan hydroxylase in human mast cells, which is consistent with the conclusion that human mast cells could synthesize serotonin.

Similarly, serotonin was found in human mast cells by means of immunofluorescence. Mouse bone marrow-derived mast cells were used as a positive control. Cytospin preparations of mast cells were fixed and permeabilized, blocked with BSA (to prevent nonspecific binding), and stained with monoclonal mouse anti-serotonin antibody (1:50; DakoCytomation, Glostrup, Denmark) or isotype control (mouse IgG1, Sigma-Aldrich). After washing, cells were stained with secondary goat anti-mouse phycoerythrin antibody and mounted with SlowFade Light Antifade reagent with 4'-6-Diamidino-2-phenylindole (DAPI) nuclear stain. Murine mast cells were strongly positive for serotonin, as anticipated.³ Human mast cells were also positive for serotonin: fluorescence was faint in the cytoplasm, and scattered bright areas of fluorescence could be observed (Fig 1, B). Fluorescence was absent when an isotype control antibody was used as a primary antibody (not shown).

When human mast cells were cultured for 32 hours with tritiated serotonin ($2 \mu\text{Ci}/10^6$ cells; Amersham, Piscataway, NJ), sensitized for 16 hours with 100 ng/mL biotinylated IgE, washed, and activated by the addition of streptavidin (Sigma-Aldrich), mast cells released this tritiated serotonin in concert with β -hexosaminidase. A 100 ng/mL dilution of streptavidin released 13% β -hexosaminidase and 17% tritiated serotonin in 30 minutes (data not shown; background subtracted).

Serotonin is a bioactive mediator implicated in immunomodulation,⁴ cell growth and development, mast cell adhesion and chemotaxis,⁵ tumorigenesis,⁶ and tissue regeneration.⁷ We did find that human mast cells contain and release serotonin, which has been demonstrated to contribute to allergic inflammation in mice.⁸ It is of interest that cyproheptadine, which has antiserotonergic properties, is of preferential use in the medical management of individuals with some forms of urticaria.⁹ Furthermore, the increased levels of serotonin found in some patients with mastocytosis could be associated with the increased mast cell burden and could help explain a report of a response to cyproheptadine in a pediatric patient with

bullous lesions.¹⁰ Serotonin thus joins a list of mast cell mediators with a possible role in mast cell-dependent processes.

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