Immunocytochemical Localization of Mast Cells in Lymphocytic Hypophysitis

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Abstract

We studied 15 transphenoidally resected pituitary tissues diagnosed by histologic examination as chronic lymphocytic hypophysitis. Six autopsy-obtained pituitaries of patients who died of nonendocrine diseases also were studied. Tryptase immunohistochemical analysis, which specifically identifies mast cells, demonstrated numerous, randomly distributed multifunctional cells throughout the inflammatory reaction. Several mast cells were located in the vicinity of capillaries; several others were distributed far from the blood vessels. Occasional mast cells also were noted in the nonpathologic anterior and posterior pituitary lobes. Morphometric analysis confirmed that in lymphocytic hypophysitis, the number of mast cells per volume of tissue was significantly increased compared with that of nonpathologic anterior and posterior pituitary lobes. To elucidate the possible role of mast cells in chronic lymphocytic hypophysitis, microvessel densities were assessed quantitatively using immunohistochemical analysis for CD34, a sensitive marker of endothelial cells. The strong positive correlation between numeric density of mast cells and microvessel density per volume of pituitary tissue suggests that mast cell–derived products may influence capillary permeability and angiogenesis, thereby facilitating the access of inflammatory cells to adenohypophysial cells.

Lymphocytic hypophysitis is a rare disorder, the pathogenesis of which is not fully resolved. Given its occurrence mainly in young women, typically late in pregnancy or during the postpartum period, it is assumed to have an autoimmune basis.1,2 Light microscopy reveals extensive lymphocytic infiltration of the adenohypophysis by lymphocytes and, to a lesser extent, plasma cells and macrophages. Some cases feature lymphoid follicle formation.3 The presence of other inflammatory cells, particularly mast cells, has not been explored.

In the overall spectrum of inflammatory cells, mast cells are among the most functionally diverse, having a pivotal role in the following: (1) the immune response; (2) host defense against parasites, neoplasms, and nonspecific inflammatory disorders; (3) angiogenesis; and (4) tissue remodeling. Mast cells are distributed widely in different organs and tissues. As a rule, they are concentrated around small blood vessels, a finding consistent with the view that mast cells are involved in regulation of vascular permeability, blood flow, and angiogenesis. Histamine, serotonin, heparin, a variety of cytokines, growth factors, proteolytic enzymes, and numerous angiogenic factors have been identified in mast cells.4,5 Despite their widespread distribution in various organs, previous studies in rodents have not identified mast cells in the pituitary.6

The goals of our study were as follows: (1) determine whether mast cells are present in the normal human pituitary, (2) investigate their distribution and speculate regarding their role in the progression of lymphocytic hypophysitis, and (3) assess microvascular density in lymphocytic hypophysitis.
Materials and Methods

Six normal pituitaries, all removed at autopsy from equal numbers of men and women ranging in age from 40 to 60 years, served as study controls. All 6 patients had died of nonendocrine causes. Fifteen pituitary biopsy specimens obtained by transphenoidal resection from 11 women and 4 men (age range, 24-75 years) all showed classic light microscopic features of lymphocytic hypophysis. The 2 groups were the basis for the present study.

Both the autopsy-obtained and surgically obtained pituitaries were processed routinely, fixed in 10% buffered formalin, dehydrated in graded ethanol, and embedded in paraffin. Sections, 5-μm thick, were stained with H&E and the periodic acid–Schiff method. For immunocytochemical analysis, the streptavidin-biotin-peroxidase complex technique was used. Details of the immunocytochemical method; antibody types, sources, dilutions, and duration of exposure; and control procedures were described in previous publications.7,8

For the immunocytochemical demonstration of mast cells in the human pituitary, a monoclonal mouse antibody directed against human mast cell tryptase (Novocastra, Vector Laboratories, Newcastle upon Tyne, England) served as the primary antibody. Preliminary titration experiments determined the optimal working dilution of 1:100. To ensure specificity, control tests included replacement of tryptase antibody with phosphate buffered saline. The streptavidin-biotin-peroxidase complex technique was applied with slight modifications.9 After routine deparaffinization, rehydration, and blocking of endogenous peroxidase activity, microsections were pretreated for the purpose of antigen retrieval using pepsin (0.4% in 0.01N hydrochloric acid) at 37°C for 30 minutes as previously described.10 Subsequently, slides were incubated overnight with the tryptase antiserum at room temperature and then were exposed to the streptavidin-biotin-peroxidase complex. Diaminobenzidine served as the chromogen. Slides then were counterstained with hematoxylin, dehydrated, mounted, and examined with a light microscope.

Microvessel density in lymphocytic hypophysis samples was assessed after immunostaining for CD34 (monoclonal; DAKO, Glostrup, Denmark), a sensitive marker of endothelial cells. Before staining, sections underwent microwave antigen retrieval in a 0.1-mmol/L citrate buffer for 15 minutes. Immunostains were performed using a 1:25 dilution of antibody at room temperature overnight. The reaction product was visualized with diaminobenzidine. Slides were counterstained with methyl green and mounted.

Morphometric study was undertaken using a computer analysis system (Microimage, Media Cybernetics, Silver Spring, MD) following the principles established by Weibel.11 The number of tryptase-immunoreactive mast cells per unit volume of tissue (Nv) was determined using the Floderus equation as previously described.12,13 In each control pituitary, a total of 20 randomly chosen fields from both the adenohypophysis and neurohypophysis representing 6.7 mm² of both tissues were assessed. All immunoreactive cells with a recognizable nucleus were counted. The same approach was applied to estimate the number of mast cells per unit of pituitary volume in the lymphocytic hypophysis samples.

Microvascular density, expressed as percentage of tissue area consisting of vessels in lymphocytic hypophysis samples, excluding fibrotic areas, was determined by measuring their cumulative area per microscopic field. In each sample, a total of 20 randomly selected fields totaling 6.7 mm² of adenohypophysial tissue were assessed.

Data were tested for statistical significance using the SPPS statistical computer program (SPSS, Chicago, IL). Since assumptions for a parametric test were not valid (Shapiro-Wilk, P < .05), all data were evaluated by Kruskal-Wallis analysis of variance and by the Mann-Whitney U test as a multiple comparison method. The Spearman test was used to assess the statistical significance of the correlation between mast cell numbers per unit volume of adenohypophyseal tissue and vascularity in the lymphocytic hypophysis samples assessed. Only differences of P < .05 were considered statistically significant.

Results

Tryptase immunostaining was moderate to strong, affecting the entire cytoplasmic area of mast cells. Cell nuclei were nonreactive Image II.

Immunohistochemical analysis revealed the presence of only a few tryptase-immunoreactive cells in the anterior and posterior lobes of the normal control pituitaries. These cells lay scattered throughout the pituitary gland. In the adenohypophysis, they were mainly perivascular, surrounding the vessels between adenohypophysial acini. Few also were identified throughout the neurohypophysis, usually in the vicinity of blood vessels (Image 1). Although mast cells per unit volume of tissue were slightly more numerous in the neurohypophysis than in the adenohypophysis, the morphometric study demonstrated no statistically significant differences in their densities in the 2 lobes Figure II.

Immunostaining for tryptase showed that in cases of lymphocytic hypophysis, mast cell distribution differed considerably from that in control pituitaries. Thus, in lymphocytic hypophysis, tryptase mast cells were numerous and randomly distributed within the inflammatory infiltrate, both in the vicinity of and far from blood vessels.
Mast cells appeared interspersed with other inflammatory cells including lymphocytes, plasma cells, and macrophages. The morphometric analysis confirmed striking differences in the number of mast cells in normal control pituitary samples and lymphocytic hypophysitis samples. In lymphocytic hypophysitis, the Nv was significantly higher than in the adenohypophysis (P = .001 or less) and neurohypophysis (P = .05 or less) control pituitaries (Figure 1).

In lymphocytic hypophysitis, CD34 immunoreactivity was confined entirely to endothelial cells. Positivity was well defined and easy to quantify. Considering previous findings in normal pituitary and pituitary adenomas, the present morphometric study showed that the pituitary in lymphocytic hypophysitis is well vascularized. It is of note that a strong positive correlation (r = 0.857; P = .014) existed between microvessel density and the Nv of pituitary tissue.
Image 2 A, Lymphocytic hypophysitis showing immunostaining for tryptase. B, Immunopositive cells were numerous and distributed randomly throughout the inflammatory reaction. (x430)

Image 3 Immunostaining for CD34 in lymphocytic hypophysitis (x320).

Discussion

Mast cells are derived from bone marrow progenitor cells that undergo differentiation in various tissues, particularly nearby epithelial surfaces. In these areas they are located near potential allergens and other stimulating factors. Numerous studies have demonstrated that mast cells within the bronchial wall respond to inhaled substances and have a crucial role in the asthmatic reaction. Application of monoclonal antibodies directed toward specific mast cell enzymes makes it possible to study the distribution of mast cells in tissues more easily and accurately. Thus, the enzyme tryptase, the principal protein of human mast cells, has been used increasingly as their immunohistochemical marker in normal and abnormal tissues. The present study indicates that few tryptase-immunopositive mast cells lie within the anterior and posterior lobes of the human pituitary. Our findings are in agreement with those of Cromlish et al who, using biochemical methods, reported the expression of multiple tryptase isoforms in the normal human pituitary and suggested their expression with the presence of mast cells in connective tissue. In contrast with findings in the human pituitary, others have been unable to demonstrate mast cells in the rodent pituitary. The reasons for this difference are unclear. Rodent and human mast cells have a different biochemical composition. For example, rodent mast cell subsets store different chymase isoforms, whereas only a single chymase occurs in human pituitaries.

Although previous reports emphasize the presence of mast cells in the human neurohypophysis, our study found no significant differences in the numeric density of mast cells in the 2 lobes. Based on these findings, it is not possible to draw conclusions about the role of mast cells in pituitary function.

Their specific localization in perivascular regions suggests that mast cells have an important role in the regulation of blood flow and capillary permeability. It is known that stimulation of mast cells releases numerous mediators, including histamine, which causes vascular dilatation and increased vascular permeability. This is in keeping with the report of Gajkowska et al who stated that in the neurohypophysis,
mast cells avert postischemic damage by way of nitric oxide production with resultant vasodilation. Since blood flow, metabolism, and functional activity in endocrine organs are closely associated, one can assume that changes in pituitary blood flow affect the activity of adenohypophysial cells. In keeping with this notion are the results of previous studies demonstrating the active participation of intraglandular mast cells in the regulation of thyroidal blood flow and endocrine activity. It may also be that mast cells influence adenohypophysial cell activity through changes not only in blood flow and capillary permeability but also in a direct manner. For example, it is well known that mast cells are multifunctional and secrete a broad spectrum of biologically active cytokines and growth factors. It has become increasingly evident that various locally produced proteins, such as cytokines and growth factors, mediate adenohypophysial cell division and regulate pituitary hormone gene expression. Despite the fact that only few scattered mast cells can be found in the normal human pituitary, their possible participation in the direct control of adenohypophysial cell activity is intriguing. In support are various studies that have found the secretion products of mast cells to have an important role in the control of endocrine activity of the hypothalamic-pituitary-adrenal axis.

Since mast cell numbers are increased significantly in the pituitaries of lymphocytic hypophysitis, one may ask whether they have a role in the development and/or progression of this inflammatory process. Given the complex nature of the inflammatory reactions associated with autoimmunity, the exact relation between individual inflammatory cells and their mediator(s) and clinicopathologic manifestations remains unclear. Mast cells are thought to be essential to the development of the acute inflammatory reaction. Indeed, mast cell activation leads to release of inflammatory mediators, including histamine, heparin, prostaglandins, leukotrienes, proteases, and an array of cytokines as well as chemokines involved in recruitment and activation of leukocytes. Mast cells may be critical for the full expression of certain features of late phase autoimmune reaction and may contribute to clinically relevant aspects of the chronic inflammatory process. Mast cell enzymes might activate prometalloproteinase, thereby destabilizing connective tissue and affecting the process of fibrosis. The positive correlation between numeric density of mast cells and microvessel density suggests that in lymphocytic hypophysitis, mast cells-derived products also promote angiogenesis. Such neovasculature facilitates access of the inflammatory cells to the adenohypophysis.

Elucidating the mechanism underlying the cause of the increase in mast cells in lymphocytic hypophysitis will require a more thorough understanding of molecular signaling mechanisms. More work clearly is needed before novel therapies for this autoimmune process can be devised.

References


