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Association of mast cells with calcification in the human pineal gland

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Abstract

Increased pineal calcifications and decreased pineal melatonin biosynthesis, both age related, support the notion of a pineal bio-organic timing mechanism. The role of calcification in the pathogenesis of pineal gland dysfunction remains unknown but the available data document that calcification is an organized, regulated process, rather than a passive aging phenomenon.

The cellular biology and micro-environmental conditions required for calcification remain poorly understood but most studies have demonstrated evidence that mast cells are strongly implicated in this process. The aim of the present study was to examine the phenotype of mast cells associated with early stages and with the progressive development of calcification in the human pineal gland. The study was performed on pineal samples of 170 fetuses and children whose brains were autopsied and diagnosed during 1998-2002. The representative cerebral and pineal specimens were stained with haematoxylin and eosin or the von Kossa staining technique and for the distribution of mast cell tryptase, mast cell chymase, histamine H4 receptor and vascular network using biotinylated Ulex europaeus agglutinin.

Tryptase mast cells were found in all stages of pineal gland development independently of the presence of local tissue lesions. All of them were always localized in the close vicinity of the blood vessels and expressed immunoreactivity to histamine H4 receptor antibody. Immunolocalization of mast cells by chymase antibody (and following dual immunostaining with both chymase and tryptase antibodies) demonstrated that these cells were few in number and were located in the subcapsular region of the gland. In our study, all functional mast cells that underwent activation and were co-localized with deposits of calcium did not contain chymase. All of them were stained with tryptase and represent the MC-T phenotype. Tryptase mast cells and extracellular tryptase were often associated with areas of early and more advanced stages of calcification. Our results lead to the conclusion that the tryptase mast cells play a major role in the pineal calcification process as sites where this process starts and as a source of production of numerous biologically active substances including tryptase that participate in calcification.

Key words: *mast cells, tryptase, calcifications, pineal gland.*

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Introduction

The pineal gland is considered as a neurohumoral transducer, accepting photic information from the retinae and converting this neural message into chemical mediators, the best documented of which is melatonin. The calcium ions are necessary for the multiplicity of intracellular and extracellular events that interact to produce the melatonin. Based on the well-known age-related decrease in melatonin, it has been hypothesized that aging is a pineal failure [15] and that melatonin is an anti-aging hormone [13]. Increased pineal calcifications and decreased pineal melatonin biosynthesis, both age related, support the notion of a pineal bio-organic timing mechanism. Intracranial calcifications are well known calcium and magnesium deposits that in the pineal gland are called 'brain sand' or acervuli (corpora arenacea) and are numerous in adult and aging patients. The role of calcification in the pathogenesis of pineal gland dysfunction remains unknown but the available data document that calcification is an organized, regulated process, rather than a passive aging phenomenon [3-5,10]. The cellular biology and micro-environmental conditions required for calcification remain poorly understood but most studies have demonstrated evidence that mast cells (MC) are strongly implicated in this process. In contrast to various mammalian species, in the human brain mast cells are not numerous.

Most of them are located in the perivascular area of arterioles and venules of the leptomeninges, choroid plexus and pineal gland. This location allows mast cell products easy access to regulation of the microcirculation by releasing rapidly diffusible mediators such as histamine, nitric oxide, and arachidonic acid metabolites, which profoundly affect vascular permeability. Thus, mast cells residing throughout the brain are considered to be involved in regulation of the blood-brain barrier. However, mast cell products not only enhance vascular permeability but also promote adhesion of circulating cells to microvasculature endothelium, and induce transmigration of inflammatory cells into tissue sites. These pro-inflammatory effects may assist the host in defence; in contrast, host reactions may not require mast cells' participation and their presence may even be deleterious. The number of mast cells within a given tissue under normal conditions is tightly regulated and relatively constant; thus the finding of localized mast cell hyperplasia is often viewed as evidence of a contribution to disease pathogenesis.

Mast cells in different anatomical sites, and even in a single site, can have substantial differences in mediator content, sensitivity to agents that induce activation, mediator release, and responses to pharmacological agents. Such heterogeneity is regulated by many factors, including certain cytokines, which influence the cells' stage of maturation, differentiation, and proliferation. Experimental studies indicate that phenotypic characteristics of mast cell populations can change, sometimes reversibly, in response to alterations in the microenvironment. The phenotypic plasticity of mast cell populations may permit these cells to respond to changes in the microenvironment produced by diseases or immunological responses. In human tissues at least two phenotypes of mast cells have been detected: TC mast cells containing both tryptase and chymase (MC-TC), and T-type mast cells containing only tryptase (MC-T). Therefore, tryptase and chymase are the best markers of mast cells and well reflect the heterogeneity of these cells in humans. Both these enzymes are stored in an active form, held in check at the acid pH inside mast cell secretory granules. Thus, the presence and distribution of one or both phenotypes of mast cells in the tissue may play a role in some pathological events including calcification.

The aim of the present study was to examine the phenotype of mast cells associated with early stages and the progressive development of calcification in the human pineal gland.

Material and methods

Samples of pineal glands of fetuses and children were collected from the files of the Department of Developmental Neuropathology (Polish Academy of Sciences), the Department of Reproductive Pathology (Medical University of Warsaw), and the Department of Clinical Pathomorphology (Institute of Polish Mother Health Centre, Łódź). The study was done on 170 cases autopsied and diagnosed in the above centres during 1998-2002. Brains were fixed in 10% neutral buffered formalin and processed to paraffin blocks. The representative cerebral and pineal specimens were sectioned at 5 µm, placed on glass slides treated with poly-L-lysine (Sigma), dewaxed and rehydrated, and stained with haematoxylin and eosin or the von Kossa staining technique [1], followed by counterstaining with Safranin O. Specimens which showed signs of calcification were subsequently exa-

mined for the distributions of the following: mast cells using monoclonal antibodies to mast cell tryptase (Chemicon, USA, dilution 1 : 100); mast cell chymase (Chemicon, USA, dilution 1 : 100); vascular network using biotinylated *Ulex europaeus* agglutinin (Vector, USA, dilution 1 : 500); histamine H4 receptor using polyclonal antibody (Santa Cruz, USA, dilution 1 : 50).

All the secondary antibodies and the alkaline phosphatase and peroxidase-avidin-biotin conjugates were purchased from Sigma (USA). Dual localization of chymase and tryptase was also studied as previously [12].

For negative controls, primary antibodies were replaced with an appropriate isotypically normal goat or rabbit immunoglobulin fraction at matched protein concentration. These were included for the examination of each specimen and consistently produced negative results.

Comparative staining techniques for mast cells

The tryptase immunolocalization technique was compared to other conventional staining using formalin-fixed tissue specimens. Consecutive tissue sections from one specimen were each stained with the following procedures: acidified toluidine blue, Alcian blue/safranin and tryptase immunolocalization. Each staining procedure was evaluated on three different sections from each specimen.

Results

Previous neuropathological studies performed on pineal gland specimens revealed different types of tissue lesions including haemorrhagic, necrotic and cystic changes [11]. In the group of fetuses haemorrhagic and necrotic changes were found. Cystic changes predominated in older patients (newborns, infants and children up to 11 years of age), as in adults [16]. Some of the oldest children from the last group were affected by a systemic disease (leukaemia, parasitic infection or paraneoplastic cerebellar degeneration) as well.

Analysis of the different staining procedures used for detection of mast cells confirmed the observations [10] that tryptase immunolocalization was far superior to all other methods. Tryptase mast cells were found in all developmental stages of pineal gland independently of the presence of local tissue lesions. This means that some non-activated mast cells are permanently resident in the gland. Immunolocalization of mast cells by chymase antibody (and following dual immunostaining with both chymase and tryptase antibodies) demonstrated that these cells were very few in number and were located in the subcapsular region of the gland. Numerous were tryptase pineal mast cells in children with some systemic diseases and local tissue lesions such as leukaemia, parasitic infections (cysticercosis) or paraneoplastic cerebellar degeneration. Those cells infiltrated the central part of the pineal parenchyma. All of them were always localized in the close vicinity of the blood vessels (Fig. 1A) and expressed immunoreactivity to histamine H4 receptor antibody. Although mast cells were observed in all pineal specimens, there were marked variations in regional distribution for each specimen. In some specimens mast cells that contained intracellular tryptase were usually surrounded by well ordered stroma tissue (Fig. 1A-B). In contrast, other specimens showed numerous mast cells with extracellular grains or "halos" of tryptase, or diffuse staining of the stroma or blood vessels (Fig. 1C-H). Such observations, indicative of mast cell activation/degranulation, were commonly associated with histological evidence of stromal disruption. As to whether some observations of extracellular tryptase might have been artificially induced by the physical trauma of sampling the tissues, our concerns were alleviated to some extent by the reproducibility afforded by having several specimens from each paraffin block.

We found, however, that most specimens demonstrated both intact and degranulated mast cells within the same tissue section, thereby providing some reassurance that mast cell integrity was retained during the sampling processes.

Mast cell heterogeneity, indicated by the differential content of mast cell tryptase and chymase, is an established feature of mast cell biology, and specific tissues usually show that one of two mast cell phenotypes predominates. In our study, all functional mast cells that undergo activation and are co-localized with deposits of calcium did not contain chymase; all stained for tryptase and represent the MC-T phenotype. By contrast, some MCs localized at the periphery of the pineal gland (capsule) were positive for chymase, indicating the MC-TC phenotype. Such immunolocalisation of MC chymase revealed positive staining for all pineal specimens examined. However, the number of chymase-containing MCs was far low-

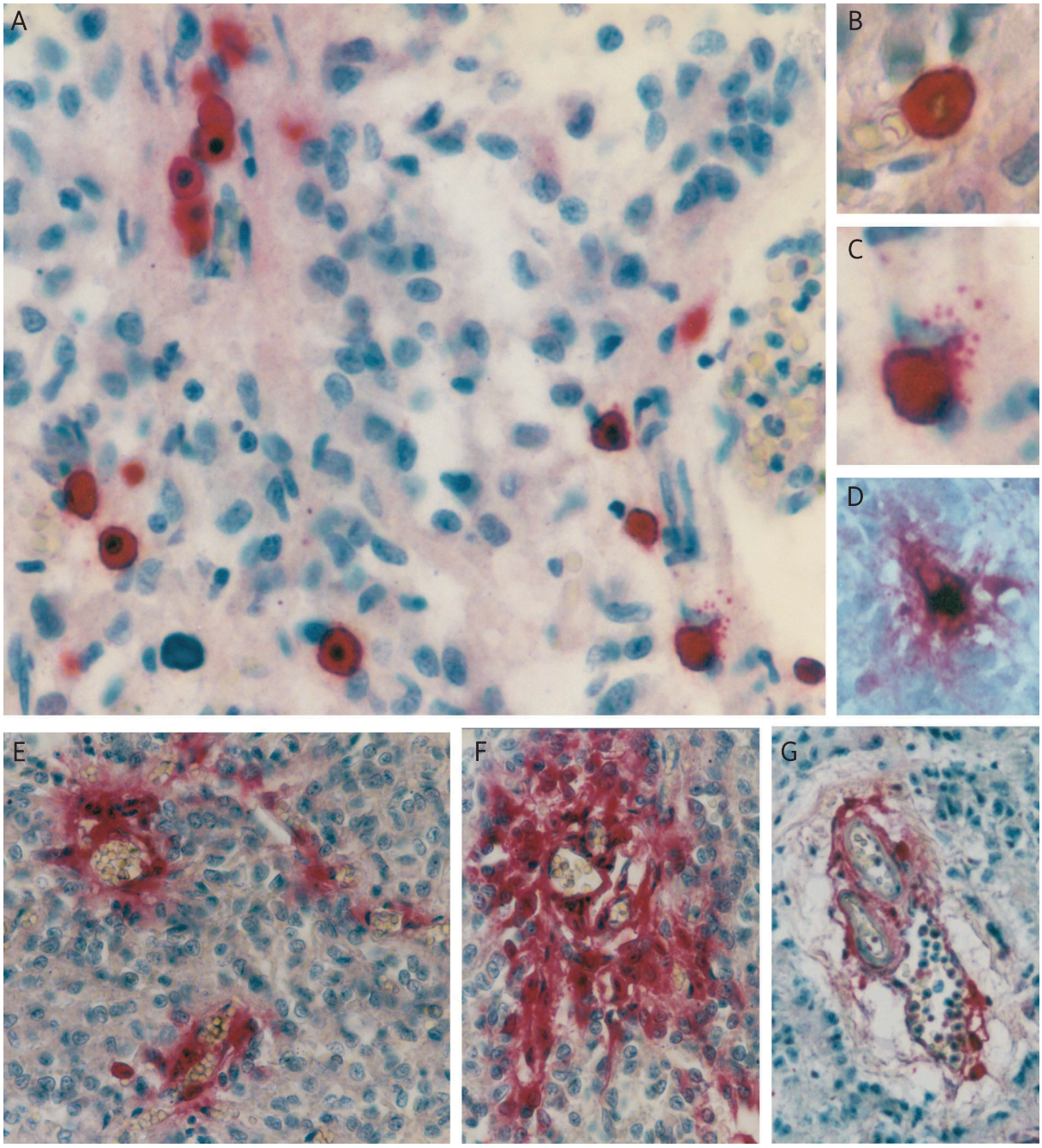

Fig. 1. A) Numerous tryptase mast cells in the pineal gland of newborn. **B)** Intracellular localization of tryptase. **C)** Mast cell with tryptase extracellular grains. **D)** Extracellular "halo" of tryptase. **E-F)** Diffuse staining of the pineal stroma near the vessels in older children. **G**) Diffuse staining of the vessel wall in child with leukaemia. Magn. A × 200, B-D × 400, E-G × 100.

er than that of tryptase MCs, especially in pineal glands with calcium deposits. These studies demonstrated and confirmed the existence of two distinct MC phenotypes in human pineal tissue.

Haematoxylin and von Kossa staining used to assess the extent and nature of calcium deposits showed different stages of calcification. The earliest and simplest calcium deposits were detected in newborns

as small "stipplings" scattered in the tissue (Fig. 2A). At high magnification these stipplings demonstrated internal laminations (Fig. 2B) or grains of calcium salts (Fig. 2C). Tryptase mast cells and extracellular tryptase were often associated with areas of the early calcium deposits. The more advanced stages of calcification as morula-type and large, solid calcified deposits were always infiltrated and/or surrounded by extracellular tryptase (Fig. 2D-F) some of such deposits contained fragments of tryptase immunopositive cells (Fig. 2E).

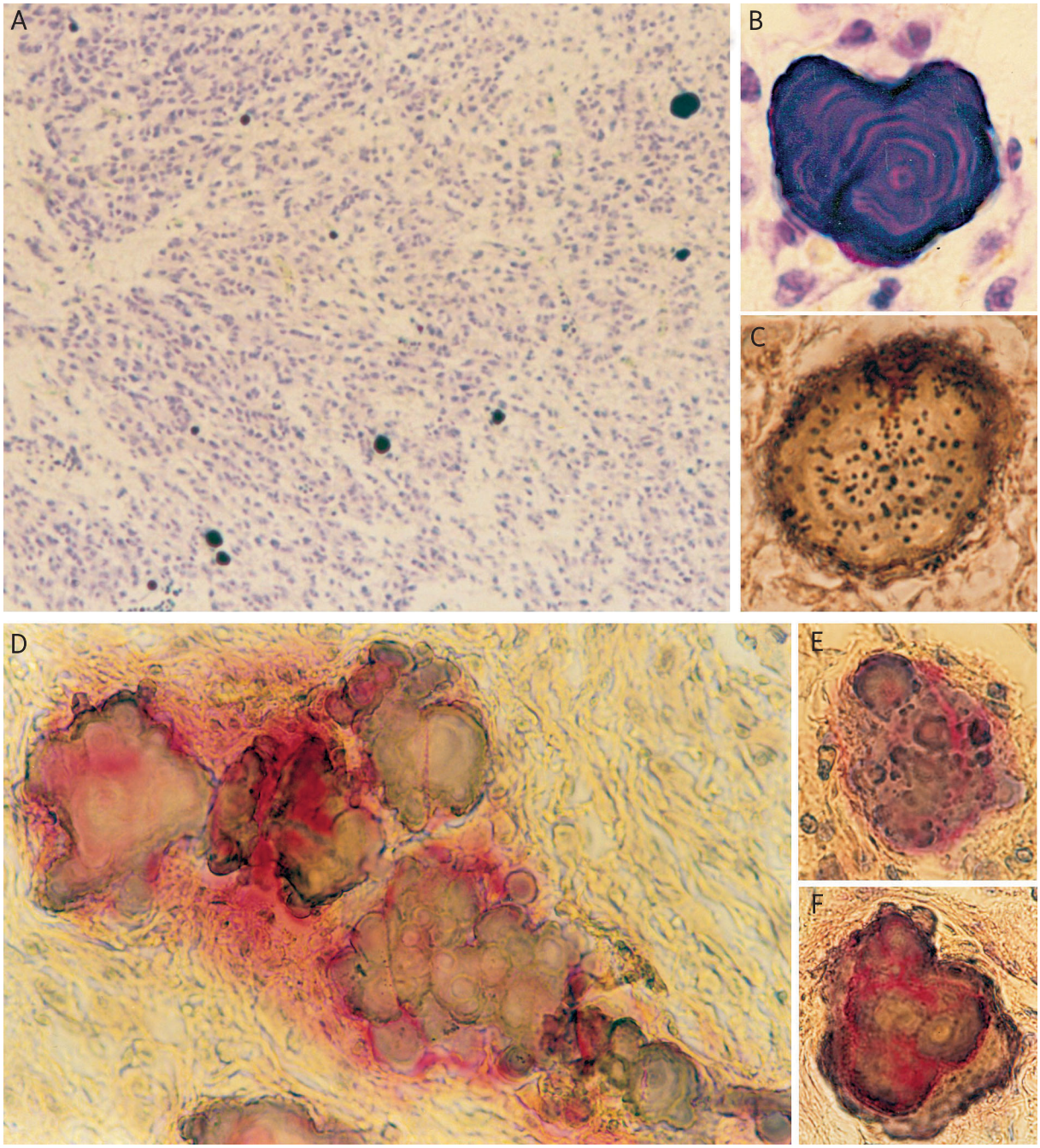

Fig. 2. A) Small calcium deposits ("stipplings") scattered in the pineal gland tissue of newborn. **B)** Lamination of the calcium deposits. **C)** Grains of calcium salts, van Kossa staining. **D)** Conglomerate of solid-type calcifications infiltrated and surrounded by extracellular tryptase. **E)** Large calcified deposit containing fragments of tryptase immunopositive cells. **F)** Morula-like calcium deposit. Magn. A) × 40, B-C) × 400, D-F × 100.

Discussion

Intracranial calcification occurs in physiological and pathological conditions. In abnormal states, calcium deposition is often categorized into dystrophic and metastatic types. By definition, dystrophic calcification develops in damaged CNS tissue that is bathed by extracellular fluid containing normal levels of calcium and phosphate. This can be seen in ischaemic infarction and degenerative disorders where the plasma membrane of cells has been rendered more permeable to calcium. In contrast, the metastatic changes are accompanied by hypercalcaemia, which predisposes the normal brain parenchyma to deposition of calcium salts. The calcium equilibrium across the membrane is presumably altered so that more calcium enters the cell. In both processes, the final result is the formation of an insoluble calcium phosphate mineral in the form of hydroxyapatite. When these calcium deposits reach a certain size, they can be imaged by neuroradiological methods. Calcification of the pineal gland has hitherto been considered to be a physiological phenomenon which occurs after a certain age. The results of our study demonstrate that already in newborns and young children the earliest calcification begins but only in those regions of the pineal gland where various types of cell degeneration and tissue lesions are present. Moreover, we observed that this phenomenon starts within the cytoplasm of mast cells which infiltrate the pineal area with pathological changes or are a part of a mast cell population which is activated following some systemic diseases. Such mast cells exhibit histamine H4 receptors that in activated cells mobilize calcium from intracellular calcium stores [8]. The laminations found in such calcified cells may be an effect of periodic activation of these receptors by histamine, which can be released by the cells participating in the inflammatory response to the pineal tissue injury [6,7,9,11]. Although as yet, the sequence of cellular events responsible for the calcification process remains uncertain, we suppose that a group of calcified mast cells forms morula-like structures. Large calcified deposits observed in the pineal gland could be, in addition, an effect of the progressive reorganization of the cells and matrix around groups of mast cells undergoing calcification. The observation of diffuse extracellular tryptase associated with the microzone of calcification suggests that mast cell activation could potentially contribute to the calcification process. Tryptase is an enzyme with numerous properties that may participate in tissue remodelling. It can degrade matrix components such as fibronectin and type VI collagen, can activate precursors of matrix metalloproteinases, is mitogenic for fibroblast and epithelial cells, stimulates collagen synthesis, and acts directly as a chemoattractant for neutrophils and eosinophils [2,14]. Although mast cells in our study were always localized in the close vicinity of the blood vessels, we did not observe (probably because of the young age of our patients) that the pineal calcification may be due in part to vascular lesions or reflect common alteration in calcium concentration.

All our results lead to the conclusion that the tryptase mast cells are the main players in pineal calcification as the sites where this process starts and as a place of production of the biologically active substances including tryptase that participate in calcification.

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