Cysts in Human Thymus: Maturational Forms of Hassal's Corpuscles?

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Summary: Presence of extracellular cystic cavities in the thymus of many vertebrates has long been known. Various forms of such structures in human thymus were observed and examined thoroughly on immunostained and serial semithin sections. We grouped these structures into five categories according to their most characteristic features. The lympho-epithelial content of the cysts clearly reflected the structural features and antigenic profile of thymic cortical parenchyma in both elongated and ovo-spherical cysts. Our findings suggest that the various types of cystic structures observed in human thymus may represent maturational stages of classical Hassal's corpuscles. Presence of cortical lympho-epithelial content and its gradual replacement with debris material also suggests a unique mechanism of thymocyte disposal.

Presence of extracellular cystic cavities in the thymus of many vertebrates has been previously reported (1). Though such structures are usually termed as cysts, they were also described as ducts, alveoli, tubules, channels or vacuoles as well by several investigators (2, 3). With the exception of mucous acini which are believed to be derivatives of pharyngeal pouch epithelium, their function remains to be clarified. It is generally suggested that thymic cysts represent structural counterparts of thymic cell disposal. This suggestion usually arises from the structural features of thymic cysts which are reported to be containing varying amounts of cellular debris in addition to macrophages and some granulocytes. A detailed light and electron microscopic description of these structures was assessed by the examination of the serial sections of murine thymi by Khosla and Ovalle (4). These investigators reported that thymic cysts are spherical or cylindrical structures composed of three structurally distinct regions termed as cranial, central and caudal segments. The lining of the cranial part is reported to be resembling respiratory epithelium, while the remainder of the cyst is mainly lined by squamous epithelial cells. In the cavity of these cysts, presence of varying amount of cellular debris

and amorphous extracellular material in addition to intact cells which are predominantly lymphocytes together with varying amounts of macrophages especially in central and caudal zones of these cystic structures is also described. Though they are observed throughout the organ, they are reported to be more numerous in the rostral half of the gland concentrated at the corticomedullary region. Caudal end of cylindrical cysts is described to be confluent with thymic parenchyma in some sections. We observed similar structures during our screening examinations in human thymus using various monoclonal antibodies and we examined the serial sections of the plastic embedded samples of the same specimens. We present our findings reflecting the antigenic profile of both the surrounding epithelial cells and the lymphoid content of thymic cysts and also their light microscopic structures.

Material and Methods

Tissues

Normal infantile thymic tissues (of five patients whose ages were ranging between 4–13 years and having congenital cardio-vascular anomalies) were

obtained as specimens of partial thymectomy which was performed as a part of cardio-vascular surgical procedure. All tissue specimens were immediately frozen in liquid nitrogen, and stored at -30°C .

Cryostat sections (6–8 μm thick) were taken on gelatin-coated slides and kept in humidity-free containers at room temperature until the staining was performed within a week.

Antibodies and staining procedure

The monoclonal primary antibodies used in this study were listed Table 1. They were supplied from the Vth Leukocyte Typing Workshops-Boston-1993 (5) and VIth Leukocyte Typing Workshop-Kobe-1996 (6).

The immunostaining procedure (indirect immunoperoxidase) used in the study has been described in detail elsewhere (7).

Light microscopy

Serial semithin sections of plastic embedded material were obtained following routine electron microscopy fixation and embedding technique. Slides were then examined and photographed using an Olympus-BH2 photomicroscope.

Results

Light microscopical findings

In the examination of serial semi-thin sections

Table 1. Monoclonal antibodies used in the study

Specificity	Clone name	Source*
CD1	Leu-6	Wamer
CD1b	SN13	Seon
CD2	Leu-5b	Wamer
CD3	OKT3	Connelly
CD4	MEM-16	Horejsi
CD5	Leu-1	Wamer
CD6	SPV.L. 14	van Agthogen
CD7	LT7	Filatov
CD8	OKT8	Connelly
CD9	15C3	Marcil
CD63	115C6	Hidreth
CD36	CLB-IV C7	CLB-Amsterdam
CD31	TP1/15	Sachez-Madrid
CD151 (PETA-3)	11B1.G4	Ashman
CD105	1G2	Buhring
CD106	E1/6	Bevilacqua
CD29	5D9	Bankert
CD49a (VLA- α 1)	TS2/7	Qin
CD49b (VLA- α 2)	Gil4	Santosa
CD49c (VLA- α 3)	J143	Albino
CD49f (VLA- α 6)	BQ16	Liebert
CD98	J1 G3B	Skubitz
CD44	TA-8B1	Kikuchi
CD146	541/10B2	Knapp

of human thymus several types of cystic structures, some resembling Hassal's corpuscles, were determined. We grouped these structures according to their most characteristic features into five categories:

I. Huge ovo-spherical cysts surrounded by usually a single layer of flattened cells, occupied almost entirely with a lympho-epithelial content. The lympho-epithelial content reflected the structural features of thymic cortical parenchyma being composed of numerous small lymphocytes, thymic epithelial cells, macrophages, few capillaries and mitotic figures (Fig. 1). All of these cysts were entirely surrounded by medullary tissue.

II. Large cysts containing predominantly debris material and varying amounts of lympho-epithelial elements. Cysts in this group were entirely located in the thymic medulla and separated from the medullary tissue by a flattened epithelial capsule. The intact lymphoid content reflected the structural features of thymic cortical parenchyma as in the first group (Fig. 2).

III. Cysts almost entirely occupied with debris material, which were usually smaller in diameter when compared to the first two groups. Epithelial lining of these cysts was composed of larger, rounded cells rather than flattened cells, which resemble the peripheral cells of Hassal's corpuscles (Fig. 3). Few degenerating lymphoid figures were also observed in the cavity of these cysts.

IV. Smaller cysts which gradually assumed the structure of Hassal's corpuscles as they diminish in diameter (Figs. 4, 5). Some of these cysts still contained degenerating lymphoid figures (Fig. 4).

V. Cysts with a lympho-epithelial content which was confluent with cortical parenchyma. Very few samples of such cysts were observed. The epithelial capsule was surrounding the cyst is separating it entirely from the medullary tissue but it was discontinuous at the corticomedullary junction (Fig. 6). Smaller such openings were also observed in some cysts representing the probable entry site for the lympho-epithelial content.

Immunohistochemical analysis

In addition to certain T cell markers, we used some monoclonal antibodies of known specificity which clearly distinguish different compartments of the thymus, in addition to CD designated mAbs reactive with thymic epithelial cells like CD1, CD1b, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD63, CD36, CD31, CD151, CD105, CD106, β_1 integrins (CD49a, CD49b, CD49c, CD49f) CD98, CD44 and CD146. Immunoreactivity of thymocytes, thymic epithelial cell types, epithelial capsule of thymic cysts and lympho-epithelial con-

tent of the cysts is briefly described below. Our observations clearly revealed the identical immunostaining pattern of lympho-epithelial content with the cortical parenchyma (Fig. 8). Encapsulating epithelial cells exhibited mostly the immunophenotype of medullary epithelial cells. They also shared determinants with type -1 (perilobular) epithelial cells as observed with certain antibodies.

T-cell markers

CD1 and CD1b: With anti-CD1b mAb (Leu-6) all cortical thymocytes except very few cells in the subcapsular zone were strongly reactive. Lympho-epithelial content of the cysts exhibited the same staining pattern. There were few reactive thymocytes in the medulla, adjacent to cortico-medullary junction. Anti-CD1 mAb (Leu-6) exhibited a similar staining pattern, somehow differing in the stronger reactivity of Hassal's corpuscle epithelium. Fewer cortical thymocytes were reactive when compared to the CD1b reactivity.

CD2: Almost all cortical and majority of medullary thymocytes were strongly reactive.

CD3: Cortical thymocytes in the deeper half of cortex and almost all thymocytes in the medulla were reactive. Approximately 50% of thymocytes in the superficial cortex were also reactive.

CD4: Majority of cortical thymocytes and approximately one third of medullary thymocytes were reactive.

CD5: Scattered cortical thymocytes and all medullary thymocytes were reactive. Diffuse staining in the medulla suggested an epithelial staining as well.

CD6: Majority of the cells in the medulla and a few cells in the cortex were reactive.

CD7: Majority of the cortical thymocytes were weakly reactive with scattered strongly reactive ones. Almost all medullary thymocytes were also reactive.

CD8: Almost all cortical thymocytes and scattered medullary thymocytes were distinctly reactive (Fig. 8). Epithelial cells surrounding cysts and the periphery of Hassal's corpuscles were weakly reactive to some of these T cell markers, especially CD1b, CD2, CD5.

Platelet antigens

CD9: Subgroup of cortical epithelial cells and cortical thymocytes were distinctly reactive. Epithelial cells bordering perivascular compartment, medullary epithelial reticulum, Hassal's corpuscles and epithelium surrounding cysts were strongly reactive. Vascular endothelium and smooth muscle were also reactive (Fig. 2).

CD63: This mAb was strongly reactive with the

stroma of perivascular compartment. In addition to subgroup of cortical and medullary epithelium. The epithelial cells surrounding the cysts and Hassal's corpuscles were also reactive. *CD31*, *CD36* and *CD62P* were mainly reactive with microvascular endothelium but none of the lymphoid or epithelial elements.

CD151 (PETA-3) reactivity was determined in epithelial cells surrounding the cysts and forming the Hassal's corpuscles. Epithelial lining of the perivascular compartment subgroup of thymic epithelial cells and microvascular endothelium was also reactive.

Endothelial cell markers

CD105: Anti-endoglin mAbs reactive with vascular endothelium of all types. Hassal's corpuscles and epithelial cells surrounding cysts were also moderately reactive.

CD106: VCAM-1 reactivity was determined in subgroup of cortical epithelial cells which resemble in situ counterparts of thymic nurse cells and follicular dendritic cells of the thymic medullary follicles.

Beta-1 integrins: α -1 (CD49a), α -2 (CD49b), α -6 (CD49f) and β -1 chain CD29 were expressed by both type 1 (perilobular) epithelial cells and epithelial cells surrounding cysts and Hassal's corpuscles.

Various mAbs

CD98: Reactivity was present in cortical epithelial reticulum and thymocytes in addition to vascular endothelial cells. The thymic medulla exhibited a diffuse staining pattern. Hassal's corpuscles and epithelial capsule of cysts was reactive with this mAb (Figs. 6, 7).

CD44: Reactivity was observed in almost all medullary thymocytes few scattered cortical thymocytes and weakly on microvascular endothelium.

CD146 (MUC18) is reactive with vascular endothelium, strongly with vascular smooth muscle, perilobular epithelial cells and subgroup of medullary epithelial cells.

Discussion

Presence of uncommon structures in thymus is reported previously by several investigators (1, 2, 3, 4, 8, 9, 10). Mucous acini represent a well characterized sample of such structures but occurrence of cysts, cyst-like structures could not be described clearly especially regarding their functional significance though they are mostly believed to be related to cell disposal. We could reach to a single report

describing the detailed structure of cystic structures in murine thymus presented by Khosla and Ovalle. In the literature examining the several section of the gland at light and electron microscopic levels, these investigators configured out their structural features and their localization. Presence of such structures are not mentioned in full reviews on thymus covering the structure or function of the organ (11, 12). We observed various forms of cystic structures during our screening examinations on human thymus which led us to study the details (structural and immunophenotypic) of them to outline their occurrence and structure in human thymus.

We observed cysts of varying diameters and structure in the thymic medulla. They were usually outlined as cross-sections of ovo-spherical structures in serial sections. Infrequently, some of them were found to be elongated structures extending to the corticomedullary junction with a confluent cortical parenchymal content. Large cysts are always encapsulated by a flattened epithelial lining which completely separates them from the surrounding medullary tissue. They were observed to be discontinuous only in a few samples with a lympho-epithelial content that is continuous with the cortical parenchyma at cortico-medullary poles of these elongated cysts as described above. Khosla and Ovalle described a similar pattern in the murine thymus but we should point out that we observed such openings not in all cysts but only in a few samples. Remainder of the cysts appear to be ovo-spherical structures which may represent a maturational form of the infrequently seen elongated-open ended cysts. We never observed a respiratory epithelium in these cysts.

The lympho-epithelial content of the cysts clearly reflected the structural features and antigenic profile of thymic cortical parenchyma in both elongated and ovo-spherical cysts. A group of cysts contained varying amount of debris material together with this lympho-epithelial content. Degenerating lymphoid figures within the debris material usually in the vicinity of intact lympho-epithelial content of these cysts were also frequently observed. The ratio of debris material to lympho-epithelial content was variable but appearingly increase when the diameter of the cyst decrease. In some sections cysts are observed to be consisting of only debris material. These may represent cysts on sections passing through only the debris content of the same cysts. But structural changes in the encapsulating epithelium, assuming the Hassal's corpuscle epithelial cells becoming rounded rather than flattened, suggested that the latter group of cysts represent another maturational form of the

cystic-structures. As their diameter diminish the structures with debris material as a luminal content gradually assume the Hassal's corpuscle's structure. Presence of degenerating lymphoid figures in them suggest that these cystic-structures may represent the early maturational forms of Hassal's corpuscles. Epithelial encapsulating cells share the antigenic profile of medullary and Hassal's corpuscle epithelium and a limited extent type-1 epithelium. These findings suggest a medullary thymic epithelial origin to the encapsulating epithelial lining of these cystic structures though they extend to the cortico-medullary junction.

Keeping all our observations in mind we suggest that the various types of cystic structures observed in human thymus may represent maturational stages of classical Hassal's corpuscles. Presence of cortical lympho-epithelial content and its gradual replacement with debris material also suggests a unique mechanism of thymocyte disposal at a large scale morphologically. Dynamic studies are certainly needed to assess such a mechanism. The preliminary report of this work was presented in the VIth International Workshop and Conference on Human Leukocyte Differentiation Antigens in Kobe 1996 (13).

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Explanation of Figures

Plate I

Fig. 1. A higher magnification of the cyst, reflecting the thymic cortical parenchyma pattern of lymphoid content is seen. Encapsulating epithelial cells (arrow), mitotic figures (arrow heads), cystic cavity (C), a capillary (double arrow) are distinguished methylene blue-Azure II counter-stained, original magnification $\times 40$). *Inset*: A large thymic cysts with a lymphoid content is seen in thymic medulla, ($\times 10$).

Fig. 2. Frozen section of human thymus stained with anti-CD9 mAb (15C3). Thymic cortex (C), medulla (M), perivascular compartment (P) all exhibited a unique staining pattern with this mAb. The epithelial cells lining the cyst and medullary epithelial network are CD9 (+). The cyst is largely occupied by debris material and a remnant of cortical parenchymal tissue is seen at the center (indirect immunoperoxidase/ hematoxylin counter-stained, original magnification $\times 10$).

Plate II

Fig. 3. Semi-thin section of a thymic cyst containing debris material and degenerating lympho-epithelial figures. Encapsulating epithelial cells are larger and rounded rather than flattened (methylene blue-Azure II counter-stained, original magnification $\times 40$).

Fig. 4. Semi-thin section of thymic medulla containing a small cyst which assume a newly forming Hassall's corpuscle structure. Lymphoid cells are still distinguished in its cavity (methylene blue-Azure II counter-stained, original magnification $\times 40$).

Plate III

Fig. 5. Another section from the same specimen. The Hassall's corpuscles can be compared with their internal structural features. The one on the left appears to be the mature form of the larger one on the right (methylene blue-Azure II counter-stained, original magnification $\times 40$).

Fig. 6. Frozen section of human thymus stained with J1G3B mAb. This mAb also distinguishes cortical (C), medullary (M) and perivascular compartments (P). A huge cyst with lympho-epithelial content and debris material is seen. The cortical parenchyma is confluent at the corticomedullary pole of this cyst (arrow) (indirect immunoperoxidase/hematoxylin counter-stained, original magnification $\times 10$).

Plate IV

Fig. 7. The frozen section of thymic medulla containing a large cyst with a lympho-epithelial content and debris material stained with J1G3B mAb. Note the diffuse staining pattern in the medulla (M). The lympho-epithelial content reflects the staining pattern of cortical parenchyma. The epithelial capsule of the cyst is also reactive (indirect immunoperoxidase/hematoxylin counter-stained, original magnification $\times 40$).

Fig. 8. Frozen section of human thymus stained with anti-CD8 mAb (OKT8). A giant cyst with a lympho-epithelial content exhibiting an identical CD8 reactivity with the cortical parenchyma. There are scattered CD8 (+) thymocytes in the medulla (indirect immunoperoxidase/hematoxylin counter-stained, original magnification $\times 10$).