

## Mononuclear Cell Clusters Observed in Pars Intermedius of Human Hypophysis

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**ABSTRACT.** We observed cell clusters morphologically resembling lymphocytes in the pars intermedius of human hypophyses, and investigated their immunohistochemical properties. These morphologically lymphocyte-like cells were not immunostained by any of the antibodies to hormones known to be present in the adenohypophysis or antidody to S-100 protein. However, immunostaining using antibodies to T cell membrane and B cell membrane showed that the cells were mainly stained by anti-B cell membrane antibodies. To investigate the stage of maturity of these B cell membrane-positive cells, we performed immunostaining using antibodies to IgG, IgM and IgA, and obtained negative results for all three. However, the present study did not answer the questions of why these cells are found only in the pars intermedius, why they are mainly B cells, and what functions they possess. Although there is so far no evidence suggesting a relationship between this cell cluster and functions of the pars intermedius in the hypophysis, the pars intermedius in human is considered more degenerate compared to amphibians, birds and rodents, and is likely to possess some yet undiscovered functions.

**Key words :** Pars intermedius — lymphocytes — immunohistochemistry — B cells

The adenohypophysis is well known to be the site of production of several hormones which regulate growth, development, and function of the thyroid gland, adrenal cortex, gonads, and breasts. In comparison, the pars intermedius is smaller in size than the adenohypophysis and is embryologically a residual structure of the Rathke pouch with almost unknown function.<sup>1)</sup> Recently, Gurevich *et al*<sup>2)</sup> studied the function of the pars intermedius, and reported that the epithelium of the pars intermedius possesses a secretory component (SC) that secretes Ig throughout the embryonic stage. However, the function of the pars intermedius remains largely unknown. In the present study, we observed cell clusters

morphologically resembling lymphocytes in the pars intermedius of human hypophysis, and investigated their immunohistochemical properties. Our findings suggest that the pars intermedius of hypophysis, which is conventionally considered to have practically no function, may actually possess some yet undiscovered functions.

## MATERIALS AND METHODS

### Materials

The hypophyses obtained from autopsies of three fetuses (33 to 38 gestational weeks) and 150 subjects (75 males and 75 females, aged from 0 to 98 years) were studied. The hypophyses were fixed in phosphate buffered formalin and embedded in paraffin. Four- $\mu$ m serial sections were cut from the anterior 1/3, middle 1/3 and posterior 1/3 of each hypophysis.

### General staining for morphological study

After deparaffinization with xylene, the sections were passed through a graded series ethanol and finally to distilled water. The first section was stained with hematoxylin and eosin (HE). The structures of the peripheral tissues and clusters of mononuclear cells were observed.

### Identification of cells by immunohistochemistry

From the second section onward, immunostaining was performed using antibodies against six types of hormones known to be present in the adenohypophysis, anti-S-100 protein antibody, and antibodies to T cell, B cell and red cell surface antigens. The avidin biotin complex (ABC) method was used, and color was developed with 3,3'-diaminobenzidine

TABLE 1. Types of antibodies used in the immunohistochemical study

Antibody	Dilution	Manufacturer	City	country
Rabbit anti-human growth hormone (GH)	1 : 8,000	NIDDK	MD	USA
Rabbit anti-human adrenocorticotrophic hormone (ACTH)	1 : 8,000	Anatomy, Jikei-University	Tokyo	JPN
Rabbit anti-human follicle-stimulating hormone (FSH)	1 : 7,000	Scantibodies Laboratory	CA	USA
Rabbit anti-human prolactin hormone (PRL)	1 : 8,000	Biogenesis	New Hampshire	UK
Rabbit anti-human thyroid-stimulating hormone (TSH)	1 : 5,000	NIDDK	MD	USA
Rabbit anti-bovine S-100 ( $\alpha$ , $\beta$ ) protein	1 : 5,000	Anatomy, Jikei-University	Tokyo	JPN
Rabbit anti-human CD-45	1 : 5,000	DAKO	Tokyo	JPN
Rabbit anti-human CD-147	1 : 5,000	DAKO	Tokyo	JPN
Rabbit anti human CD-20	1 : 5,000	DAKO	Tokyo	JPN
Rabbit anti-leukocyte B-cells (MB-1)	1 : 10	SIGMA	Saint Louis	USA
Rabbit anti-Red cell Wrb Antigen	1 : 10	DAKO	Tokyo	JPN
Rabbit anti-human IgA	1 : 300	DAKO	Tokyo	JPN
Rabbit anti-human IgM	1 : 300	DAKO	Tokyo	JPN
Rabbit anti-human IgG	1 : 500	DAKO	Tokyo	JPN

(DAB). The antibodies used are shown in Table 1.

### Control experiments

After centrifugation of human blood samples from healthy volunteers, the buffy coat was collected and used as positive controls for various lymphocyte surface antigens. Red blood cells were also used as positive controls for red cell Wrb antigen. Human adenohypophysis was used as positive controls for various hormones. Cerebral tissue was used as positive control for S-100 protein.

## RESULTS

### Morphology of mononuclear cells

On the morphological examination of 153 hypophysis samples, 8 samples showed clusters of mononuclear cells in the pars intermedius. Table 2 lists the age, gender, cause of death and results of immunostaining with various antibodies for the eight cases. As shown in the table, lymphocytes were identified in the pars intermedius of subjects aged 15 years or above, comprising 7 males and 1 female. The proportion of elderly subjects aged 65 years or above was apparently high, although statistical analysis was not done. There was no common cause of death or underlying disease among the cases showing mononuclear cells in the pars intermedius. The mononuclear cells observed in the present study showed no reddish coloration suggesting the presence of heme protein in unstained sections. In HE-stained sections, the mononuclear cells had a large N/C ratio, and contain a relatively large proportion of chromatin in the nucleus, which is consistent with the morphology of lymphocytes (Fig 1a, b). In some cases, the mononuclear cell clusters were circumscribed by fibers and were separated from the adenohypophysial cells and neurohypophysial tissue. Whereas in other cases, the mononuclear cell clusters were not separated by fibrous tissue from the adenohypophysis. However, we found absolutely no destruction of the basement membrane in the adenohypophysial parenchyma, and no irregular infiltration of mononuclear cells into the adenohypophysial tissue at all. In areas where these cell clusters were observed, blood vessels were found in some cases. In these cases, the vascular wall was further encircled by a single layer of lymphocyte-like mononuclear cells (Fig 2).

TABLE 2. Results of immunohistochemical study of mononuclear cells

Case	Cause of death	sex	age	CD45	CD147	CD20	MB1	E6	BIII-136	IgG	IgM	IgA
1	Acute heart failure	M	15	+	+	++	+	—	—	—	—	—
2	Brain crush wound	M	49	+	+	+++	+++	—	—	—	—	—
3	Coronary arteriosclerosis	M	59	—	—	+++	++	—	—	—	—	—
4	Myocardial infarction	M	66	++	+	++	++	—	—	—	—	—
5	Drowning	M	68	—	—	++	++	—	—	—	—	—
6	Drowning	F	71	+	+	+++	++	—	—	—	—	—
7	Burn	M	73	+	+	++	++	—	—	—	—	—
8	Burn	M	74	—	—	++	++	—	—	—	—	—

+++ strong positivity; ++ moderate; + weak positivity; — negative

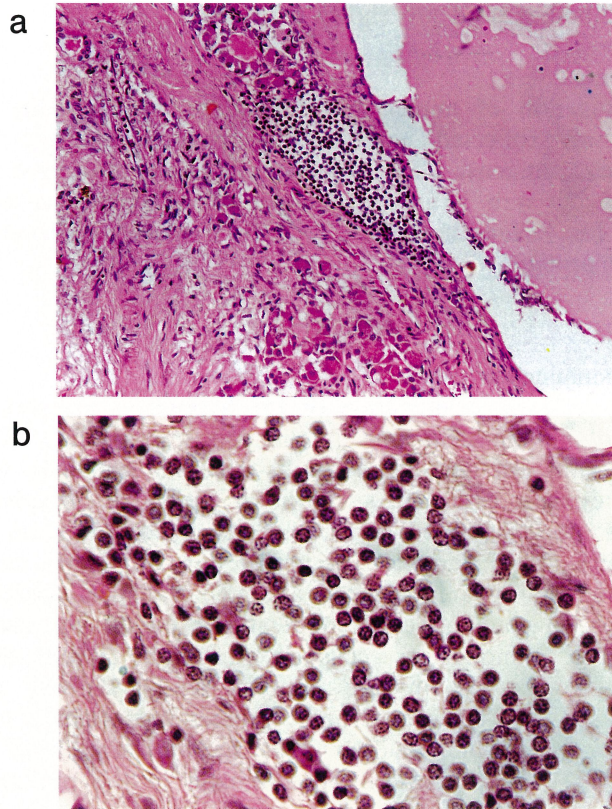


Fig 1. Mononuclear cells found in the pars intermedia of hypophysis. The mononuclear cells have large N/C ratio and the chromatin occupies a relatively high proportion of the nucleus (H.E. a:  $\times 100$ , b:  $\times 400$ ) (Case 2).

#### Results of identification of cells using immunohistochemical staining

The morphologically lymphocyte-like mononuclear cells demonstrated in the pars intermedia were not immunostained by any of the antibodies to hormones in the adenohypophysis or anti-S-100 protein antibody. However, they were immunoreactive with the antibody to T cell membrane (CD-45) and also the antibody to B cell membrane LB-26 (CD-20). In immunoreactivity to CD-45 and CD-20 encircled the cell membrane, which was consistent with the mode of staining for membrane proteins. However, CD-20 stained the whole lymphocyte cluster (Fig 3a, b), while CD-45 only stained a few cells within the cluster (Fig 4a, b). In addition, the result of immunostaining of adjacent sections showed that the two antibodies did not stain the same cell. Since the cells were immunostained by antibody to B cell membrane, we further performed immunostaining with antibodies to IgG, IgM and IgA to confirm the stage of maturity of the cells, but obtained negative results for all three. To exclude the possibility of non-specific reactions to the two antibodies (CD-20 and CD-45), we used another T cell marker (CD-147) and B cell marker (MB-1) to repeat the immunostaining, and obtained the same modes of staining as for CD-45 and CD-20 (Table 2).



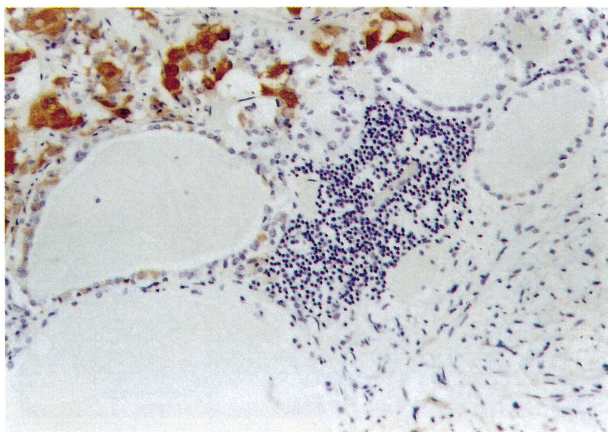


Fig 2. Mononuclear cell in the pars intermedius of hypophysis (ACTH immunostaining). In some cases, blood vessel is found in the center of the cell cluster, and a single layer of mononuclear cells encircle the blood vessel ( $\times 100$ ) (Case 3).

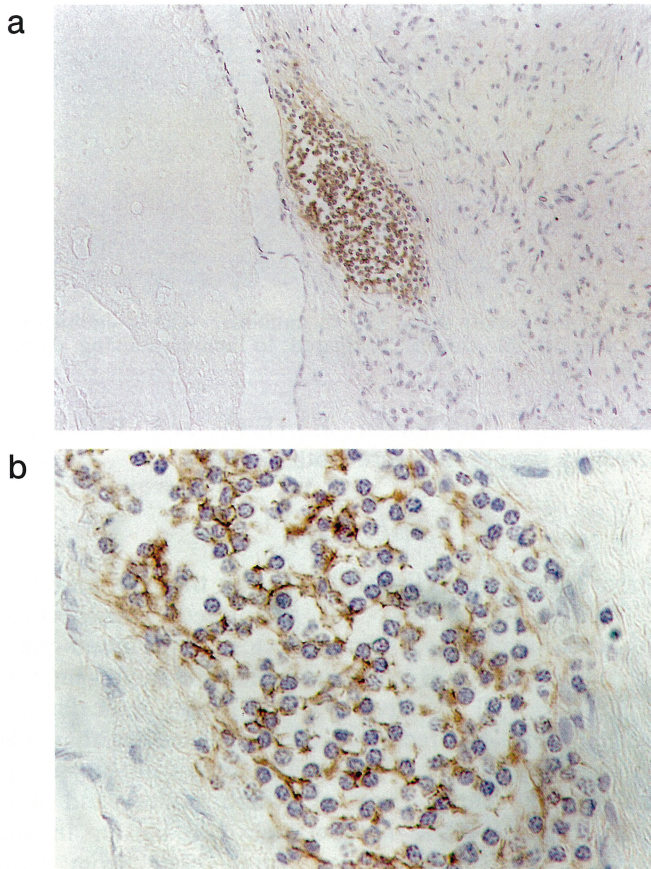


Fig 3. Immunohistochemical study using CD-20 antibody. Immunostaining by CD-20 encircles the cell membrane of the mononuclear cells forming a cluster in the pars intermedius (a:  $\times 100$ , b:  $\times 400$ ) (Case 2).

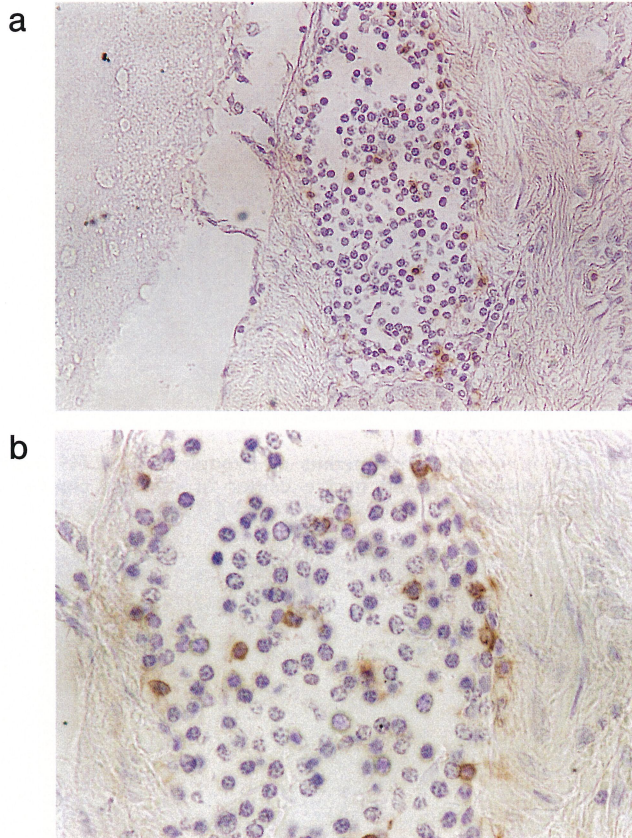


Fig 4. Immunohistochemical study using CD-45 antibody. CD-45 immunostained only a portion of the cells in the cluster, compared to immunostaining using CD-20 (a:  $\times 200$ , b:  $\times 400$ ) (Case 2).

Immunostaining with anti-human E (E-6) and BIII-136 antibodies specific to erythrocytic membrane were both negative.

#### DISCUSSION

To characterize the mononuclear cell clusters that we found in the pars intermedius of human hypophysis, we performed immunostaining using antibodies against cell membrane of lymphocytes. We found that while the cells were composed mainly of B cells, immunostaining for immunoglobulins (IgG, IgM and IgA) was all negative. These results indicated a possibility that the cells were B cells or precursors of B cells rather than plasmacytoid lymphocytes or plasmocytes. The lymphocyte clusters did not form a mantle zone or germinal center characteristic of lymph nodes, and blood vessels were found at the center of the mononuclear cell cluster in some cases. Therefore, they cannot be identified as lymph node from morphological ground. In addition, no vascular endothelial cells and erythrocytic cells were detected around the mononuclear cell clusters, and the cells were predominantly B cells, which exclude the possibility that we were looking at

the interior of the blood vessel.

Since lymphocytes are known to possess immune functions,<sup>3)</sup> the lymphocytes observed in the present study might migrate through the sinusoid as a result of some inflammatory stimuli. However, we found no gross findings of tumors or inflammation in other organs of 8 cases showing these lymphocytes. The causes of death in 8 cases were myocardial infarction, burn or drowning, and none of them accompanied by inflammatory disease, such as pneumonia, hepatitis and encephalitis. Furthermore, the lymphocytes did not always infiltrate into the whole hypophysis, and only clustered in a specific region facing the follicle in the pars intermedius. Hence, it is unlikely that the lymphocytes had migrated from the sinusoid as a result of inflammation.

Next, we examined the possibility of lymphocytic adenohypophysitis [LAH] which is a relatively common disease of the hypophysis. Since the first report by Goudie and Pinkerton,<sup>4)</sup> over 100 cases of LAH have been reported. LAH occurs mainly in females according to statistics. Various findings have been reported.<sup>5-7)</sup> A temporal relationship with pregnancy or parturition has been reported.<sup>8,9)</sup> Rare cases of LAH that originated from the sella turcica showed infiltration of inflammatory cells into the neurohypophysis or pituitary stalk.<sup>10,11)</sup> Immunoreactive findings has shown deficits of TSH, ACTH and PRL cells in the early stage.<sup>12-14)</sup> Horvath *et al*<sup>15)</sup> reported that in gestational lactotroph hyperplasia observed in postpartum patients, LAH developed in perivascular lumen and infiltration of inflammatory cells into the adenohypophysis together with an increase in PRL cells were observed. The mononuclear cell clusters we observed in the present study were found not only in female but also in male. There was absolutely no evidence of infiltration into the adenohypophysial tissue or the pituitary stalk. In addition, immunostaining using antibodies to adenohypophysial hormones showed no deficit or marked increase of adenohypophysial cells including PRL cells (data not shown). From these findings, LAH was also unlikely.

On the other hand, the secretory immune system (SIS) in the gastrointestinal, respiratory and urinary tracts is the greatest immune defense system in adults. The SIS, especially the immunoglobulin-secreting cells, is composed of multiple protein components. These components have been reported recently as Ig<sup>16)</sup> and secretory component (SC) in the adult thyroid.<sup>17)</sup> Although not all the SIS components are expressed equally in various endocrine glands, considering all the results together, it is possible that there might be a component in the hypophysis that functions as the SIS. Gurevich *et al*<sup>2)</sup> investigated the presence and location of the composing elements of the secretory immune system in endocrine gland and its precursor in the human fetus. They reported positive immunostaining of SIS in the precursor of endocrine gland in the 4 to 6 gestational-week samples, while the SIS immunostaining was found in the hypophysis, thyroid, pancreatic islet and adrenal gland in later samples. From these findings, it may be reasonable to hypothesize that the SIS was stimulated by some unknown causes resulting in activation of lymphocytes, mainly B cells, that pass through the sinusoid to appear in the pars intermedius, which accounts for the lymphocyte clusters that we observed.



Although there is so far no evidence suggesting a relationship between this cell cluster and functions of the pars intermedia in the hypophysis, the pars intermedia in human is considered more degenerate compared to amphibians, birds and rodents, and is likely to possess some yet undiscovered functions.

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#### REFERENCES

- 1) Ikeda H, Suzuki J, Sasano N, Niizuma H: The development and morphogenesis of the human pituitary gland. *Anat Embryol* **178**: 327-336, 1988
- 2) Gurevich P, Ben-Hur H, Moldavsky M, Szvalb S, Shperling I, Zusman I: An immunohistochemical study of the secretory immune system in human fetal endocrine glands and their precursors. *Early Pregnancy* **5**: 191-200, 2001
- 3) Alam R, Gorska M: 3.Lymphocytes. *J Allergy clin Immunol III (Suppl 2)*: 476-485, 2003
- 4) Goudie RB, Pinkerson PH: Anterior hypophysis and Hashimoto's disease in young woman. *J Pathol Bacteriol* **83**: 584-585, 1962
- 5) Kovacs K, Horvath E: The differential diagnosis of lesions involving the sella turcica. *Endocr Pathol* **12**: 289-395, 2001
- 6) Illueca C, Cerda-Nicolas M, Roldan P, Talamantes F, Ascaso J, Llombart-Bosch A: Idiopathic granulomatous hypophysis. Morphological and immunohistochemical study of a case. *Neurocirugia (Astur)* **13**: 137-141, 2002
- 7) Vidal S, Rotondo F, Horvath E, Kovacs K, Scheithauer BW: Immunocytochemical localization of mast cells in lymphocytic hypophysitis. *Am J Clin Pathol* **117**: 478-483, 2002
- 8) Jenkins PJ, Chew SL, Lowe DG, Afshar F, Charlesworth M, Besser GM, Wass JA: Lymphocytic hypophysis: unusual features of a rare disorder. *Clin Endocrinol* **42**: 529-534, 1995
- 9) Molitch ME: Pituitary diseases in pregnancy. *Semin Perinatol* **22**: 457-470, 1998
- 10) Cosman F, Post KD, Holub DA, Wardlaw SL: Lymphocytic hypophysis. Report of three new cases and a review of the literature. *Medicine* **68**: 240-256, 1989
- 11) Thodou E, Asa SL, Kontogeorgos G, Kovacs K, Horvath E, Ezzat S: Clinical case seminar: lymphocytic hypophysis: clinicopathological findings. *J Clin Endocrinol Metab* **80**: 2302-2311, 1995
- 12) Jensen MD, Handwerger BS, Scheithauer BW, Carpenter PC, Mirakian R, Banks PM: Lymphocytic hypophysis with isolated corticotropin deficiency. *Ann Intern Med* **105**: 200-203, 1986
- 13) Prager D, Braunstein GD: Pituitary disorders during pregnancy. *Endocr Metab Clin North Am* **24**: 1-14, 1995
- 14) Hashimoto K, Takao T, Makino S: Lymphocytic adenohypophysis and lymphocytic infundibuloneurohypophysitis. *Endocr J* **44**: 1-10, 1997
- 15) Horvath E, Vidal S, Syro LV, Kovacs K, Smyth HS, Uribe H: Severe lymphocytic adenohypophysis with selective disappearance of prolactin cells: a histologic, ultrastructural and immunoelectron microscopic study. *Acta Neuropathol* **101**: 631-637, 2001
- 16) Pritchard J, Horst N, Cruikshank W, Smith TJ: Igs from patients with Graves' disease induce the expression of T cell chemoattractants in their fibroblasts. *J Immunol* **168**: 942-50, 2002
- 17) Kondi-Paphitis A, Carvounis H, Kairi E, Frangou M, Papayanopoulou A, Deligeorgi H: Expression of a local immune defense system in the female genital tract. An immunohistochemical study. *Eur J Gynaecol Oncol* **20**: 141-143, 1999