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The ultrastructure and immunohistochemistry of the septum pellucidum in a case of thalamic low grade astrocytoma with review of literature

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Abstract

This article presents the results of light and electron microscopic (EM) findings of the septum pellucidum (Sp) in a case of thalamic low grade astrocytoma. Furthermore, it presents immunohistochemical staining of the Sp which has not been previously reported in the literature.
The tissue specimen was obtained from a 34 year-old male patient, who had undergone subtotal tumor excision operation by the neurosurgery department. The intact part of the Sp was taken for routine light, transmission (TEM), scanning electron microscopic (SEM) and immunohistochemical examinations.
In light microscopy and TEM examination the myelinated axons were small and medium sized. There was no grey matter in the examined area. In SEM examination, there were holes between the ependymal cells that showed transition zones between the inside of cavity and outside of it. In the immunohistochemical examination of the cryosections of the Sp, the ependymal lining cells were reactive with mAb B-F45. Significance and function of this antigen on ependymal cells remains to be clarified. The connection, relationships and function of the Sp is important in the human body. This detailed case study gives information for further research.

Key words: septum pellucidum, ultrastructure, immunohistochemistry, astrocytoma

Introduction

The septum pellucidum (septum gliosum [1-3], septum lucidum [4], postcomissural septum [5], supracomissural septum [6], Sp) is usually described as a thin (1.5-3 mm [7]), vertical partition composed of two laminae, largely separated by a narrow cavity (ventricule de la cloison [4], fifth ventricle [8-9], cavum septi pellucidi), an extrapial space, an therefore, not communicating with the brain's ventricular system. Seen from aside, the Sp is in the shape of a triangle, its base located anteriorly and apex posteriorly. It is attached above to the lower surface of the trunk of the corpus callosum (Cc), below and behind to the anterior part of the fornix, below and in front to the upper surface of rostrum of the Cc, and directly in front to the genu of the Cc. Each lamina forms part of the medial wall of the anterior horn and central part of the lateral ventricle, being therefore lined by ependymal cells on its ventricular side and pia on its medial aspect [10]. In most cases the two laminae are found to be attached to each other in midline, without a cavum septi pellucidi (CaSp) [11]. Classically each lamina of the Sp is made of neural tissue, a thin outer layer of gray matter, and an inner layer of white matter [12-13]. The laminal gray matter (ganglion du septum lucidum [4], nucleus septofimbrialis) is a dorsocaudal extention of the lateral septal nucleus which is located in precomissural septum (area parolfactoria of Broca, area precomissuralis, area paracomissuralis [5], septum verum [14], septum gangliosum [1-2]) [5]. Some antigenic and ultrastructural aspects of this structure are still unclear.

In this case study, first, we examined the light microscopic, and electron microscopic (transmission, scanning) appearance of the septum pellucidum. Secondly, we performed immunohistochemical staining which has not been previously reported in the literature.

Material and Methods

The tissue specimen was obtained from a 34 year-old male patient, who had undergone subtotal tumor excision operation by the neurosurgery department. This patient had a thalamic low grade astrocytoma and during the operation, transcortical – transventricular approach was performed (Figs.1a, b). Additionally, in the operation, a fenestration was performed to the septum pellucidum because of the hydrocephalus. The fenestrated part of the septum pellucidum was taken for routine light, transmission, scanning electron microscopic and immunohistochemical examinations.
The tissue specimen was immediately frozen in liquid nitrogen and stored at -24°C. Cryo sections (5-7μm thick) were taken and placed on gelatin-coated slides and kept in humidity-free containers at room temperature, and the staining was carried out within a few days.

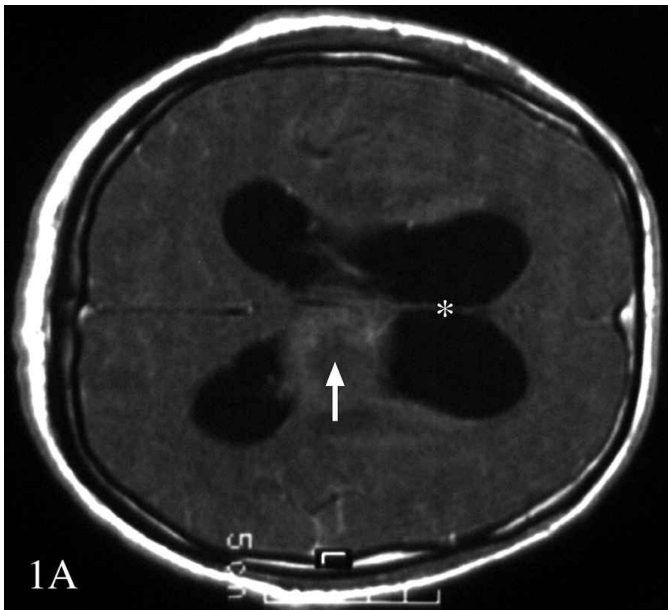

Figure 1a MR image of the patient with thalamic low grade astrocytoma. Arrow indicates the tumoral mass. Asterisk indicates the intact part of the septum pellucidum.

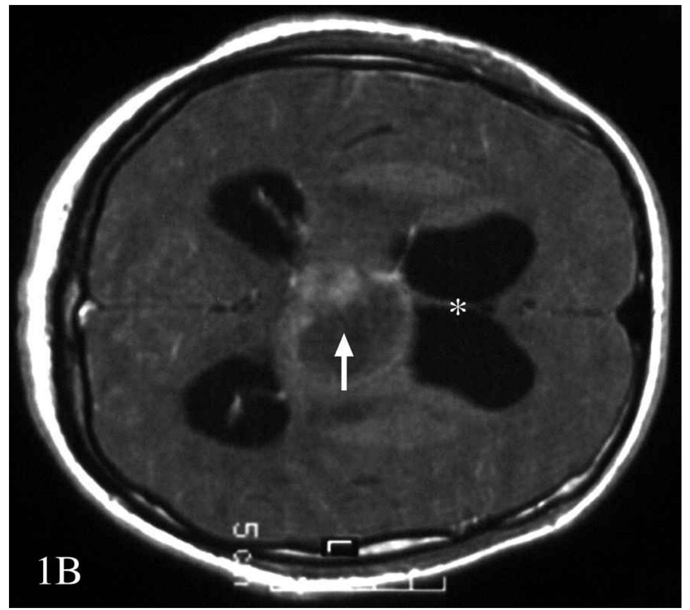

Figure 1b MR image of the patient with thalamic low grade astrocytoma. Arrow indicates the tumoral mass. Asterisk indicates the intact part of the septum pellucidum.

Primary monoclonal antibodies (mAb) used in this study, (originator Vermott-Desroches) were supplied from the Seventh HLDA Workshop Endothelial Panel, Harrogate 2000, England.

The indirect immunoperoxidase procedure used in the study has been described in detail by Dijkstra et al. [15]. Sections were fixed in acetone for 10 minutes and air-dried for at least 30 minutes. Sections were then incubated for 60 minutes with mAbs. After washing in 0.01 M phosphate buffered saline (PBS) pH 7.4, the sections were covered with rabbit anti-mouse Ig-G peroxidase conjugate (Sigma Cat No: A-9044, St. Louis) in PBS (dilution 1:200), containing 22% bovine serum albumin (Sigma Cat No: A-7034, St. Louis) (dilution 2.5:100) and normal human serum (dilution 1:100). After washing in PBS the slides were stained for peroxidase activity with 3.3'-diaminobenzidine-tetrahydrocloride (Sigma Cat No: D-5637, St. Louis) in 0.05 M Tris-HCL buffer pH 7.6 containing H_2O_2. Counterstaining was done with haematoxylin. Dual control staining was performed by omitting the first (primary antibody) step and using a mouse IgG. Stained sections were examined and photographed using Olympus BH2 microscope.

For light and transmission electron microscopy, the specimens were fixed in 2.5% glutaraldehyde for 24 hours, washed in phosphate buffer (pH: 7.4), post-fixed in 1% osmium tetroxide in phosphate buffer (pH: 7.4) and dehydrated in increasing concentrations of ethanol. Then, the tissues were washed with propylene oxide and embedded in epoxy-resin embedding media. Semi-thin sections about 2μm in thickness and ultrathin sections about 60nm in thickness were cut with a glass knife on a LKB-Nova (Sweden) ultramicrotome. Semi-thin sections were stained with methylene blue and examined by a Nikon

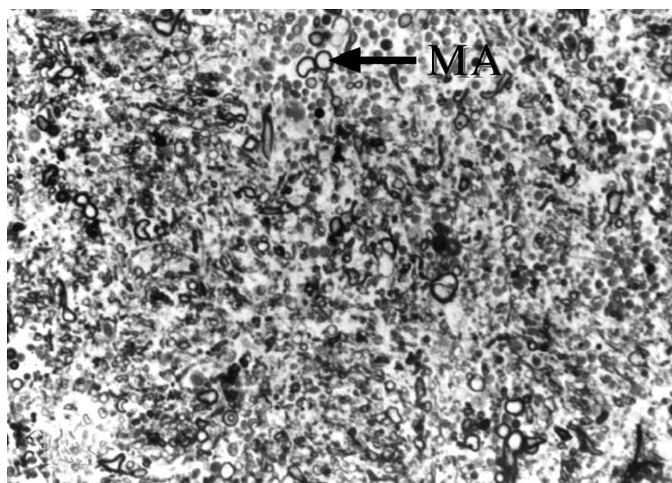

Figure 2 Myelinated axons (MA) in the light microscopic examination of Sp (X40).

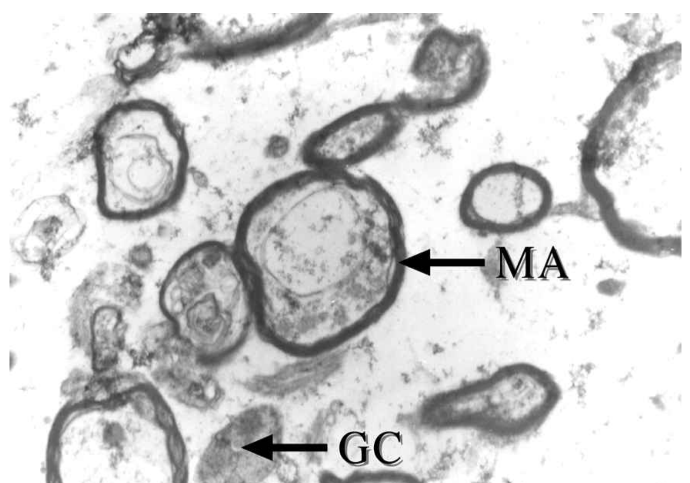

Figure 3 Myelinated axons (MA), and glial cells (GC) in TEM examination of Sp. (X5000).

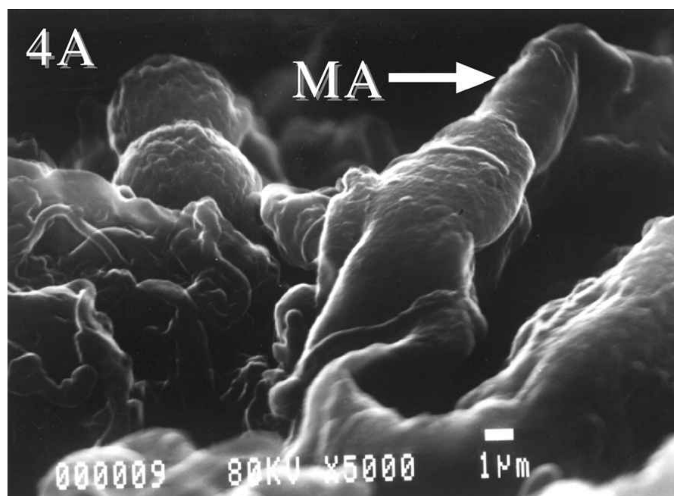

Figure 4a Regular myelinated axons (MA) in the SEM examination of Sp (X5000).

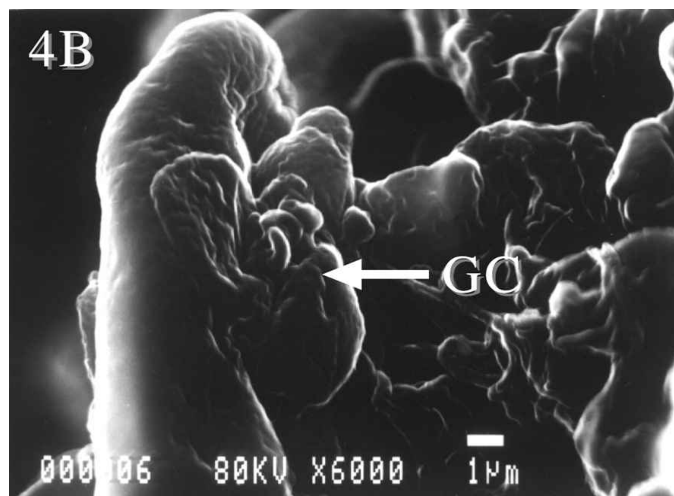

Figure 4b Glial cells (GC) between the myelinated axons in the SEM examination of Sp (X5000).

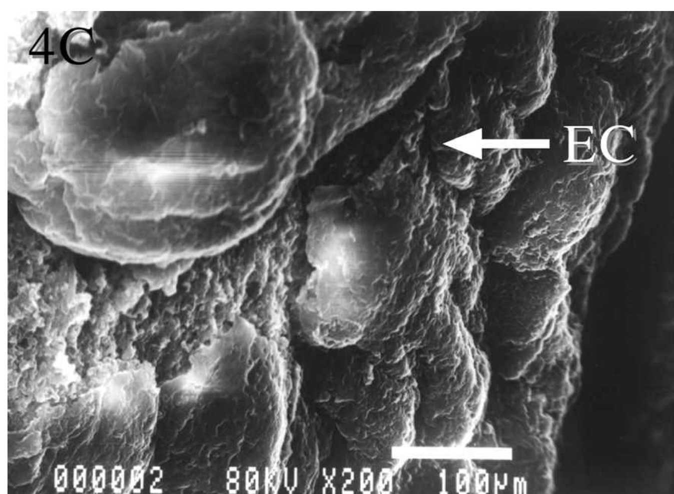

Figure 4c Ependymal cells (EC) in the SEM examination of Sp (X200).

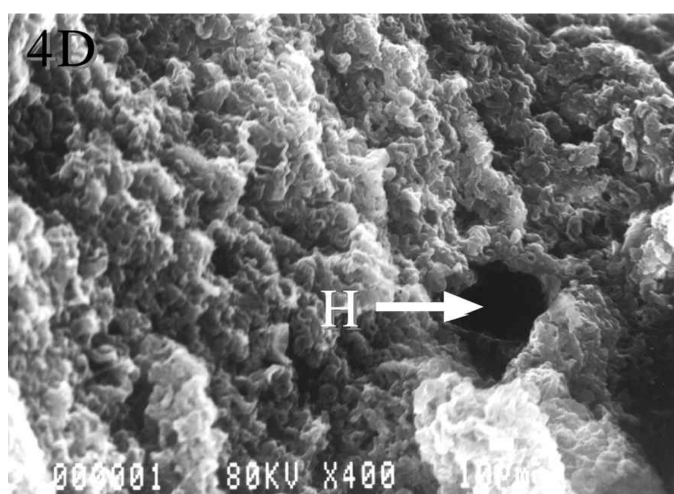

Figure 4d The hole (H) between the ependymal cells (X400).

Optiphot (Japan) light microscope. Ultrathin sections were collected on copper grids, stained with uranyl acetate and lead citrate and examined with a Jeol JEM 1200 EX (Japan) transmission electron microscope. For scanning electron microscopy, fixed samples were dehydrated in increasing concentrations of acetone and critical point dried, mounted on metal stubs with conductive silver paint and then sputtered with a 10 nm thick layer of gold in a BIO-RAD sputter apparatus (England). The tissue samples were examined with a Jeol scanning electron microscope (SEM ASID-10, Japan) at an acceleration voltage of 80 kV.

Results

In the light and transmission electron microscopic examination, myelinated axons, glial cells and blood vessels were observed. These myelinated axons were small and medium sized (Figs. 2, 3). The parts of the Sp which were examined did not contain any grey matter and for this reason no neuronal structures were found in these examined areas.

In the SEM examination, a large amount of myelinated axons were observed (Fig. 4a). The myelin composition in those axons were regular. Additionally, in some areas, glial cells were found around the myelinated axons (Fig. 4b). In addition to these structures ependymal cells were presented in the scanning electron microscopic examination (Fig. 4c). The most important finding observed in SEM was the holes found in between the ependymal cells (Fig. 4d). These holes were 10-60μm in diameter. The presence of these holes showed the transition zones between the inside of cavity and outside of it. In the immunohistochemical examination of the cryosections of septum pellucidum, by indirect immunoperoxidase technique, the ependymal lining cells were reactive with mAb B-F45 (Fig. 5).

Discussion

According to some authors, the Sp is a part of the limbic system [16-21]. The direct stimulation of the Sp causes the contractions of the urinary bladder [22]. In case of immunologic blockage of the Sp with antiseptal antibodies schyzophrenic manifestations can occur [23]. Additionally, there are a lot of information about its function [19, 24-28], connections [5, 20, 24], and relationships with psychiatric disorders [16-17, 28-35]. The antigenic structure of septum

pellucidum obtained by using immunohistochemical techniques is very important in pshychiatric disorders. Our study shows the importance of full-panel staining in larger series. The antigen recognized by B-F45 mAb is yet undefined. It is reactive with various endothelial cell types (artery, vein, high endothelial venule and umblical vessel endothelium) and other cell groups including cells of perineurium in peripheral nerves, synovial cells and some fibroblasts in different organs examined so far (our unpublished observation). Significance and function of this antigen on ependymal cells remains to be clarified. No reactivity was observed in the immunohistochemical stainings using antibodies CD 227 (BC2 and BC3), CD 146 (MUC18), BD 46 and VJ1/5. However, the staining of ependyma of septum pellucidum by B-F45 was an interesting finding.

The developmental abnormalities of the Sp are CaSp, cavum vergae (CaV), agenesis of the Sp, and a unique case of foramen formation on the Sp [16-17, 33]. It is reported that abnormalities of the Sp, especially CaV are more frequent in women [33]. On the other hand, some authors state that CaSp is more common in men [32]. Although a Sp syndrome caused by its abnormalities is described [20, 33], some authors state that an isolated absence of the Sp will not cause intellectual, neurologic, and behavioral defects [36-37]. The abnormalities of the Sp can be diagnosed prenatally using ultrasonography [38]. The comparison of antigenic structure of septum pellucidum in normal and abnormal cases is very important in the definition of molecular mechanisms of these abnormalities. This case study gives information to further research.

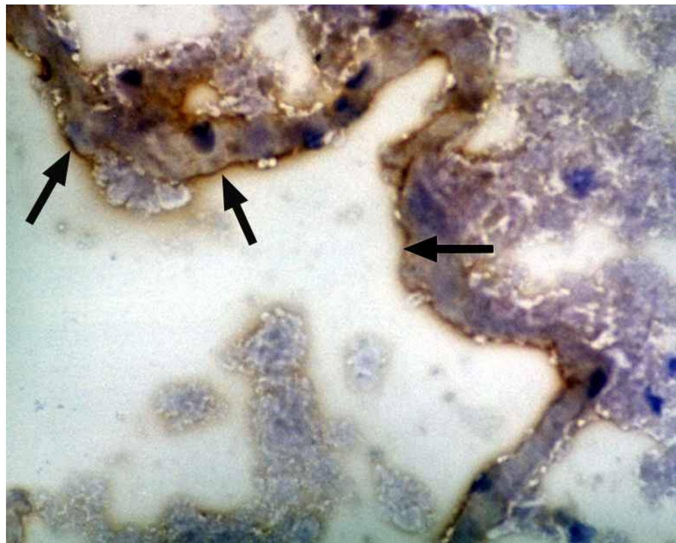

Figure 5 The ependymal lining cells were reactive with mAb B-F45 in the immunohistochemical examination of the serial cryosections of Sp. Arrows indicate the reactivity (X200).

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